

Cocoa Butter and Related Compounds

Editors

Nissim Garti
Neil R. Widlak



AOCS Mission Statement

To be a global forum to promote the exchange of ideas, information, and experience, to enhance personal excellence, and to provide high standards of quality among those with a professional interest in the science and technology of fats, oils, surfactants, and related materials.

AOCS Books and Special Publications Committee

M. Mossoba, Chairperson, U.S. Food and Drug Administration, College Park, Maryland
W. Byrdwell, USDA, ARS, BHNRC, FCMDL, Beltsville, Maryland
P. Dutta, Swedish University of Agricultural Sciences, Uppsala, Sweden
D.G. Hayes, University of Tennessee, Knoxville, Tennessee
V. Huang, Yuanpei University of Science and Technology, Taiwan
L. Johnson, Iowa State University, Ames, Iowa
H. Knapp, Big Sky Medical Research, Billings, Montana
G. Knothe, USDA, ARS, NCAUR, Peoria, Illinois
D. Kodali, Global Agritech Inc., Minneapolis, Minnesota
G.R. List, USDA, NCAUR-Retired, Consulting, Peoria, Illinois
J.V. Makowski, Windsor Laboratories, Mechanicsburg, Pennsylvania
A.G. Marangoni, University of Guelph, Ontario, Canada
T. McKeon, USDA, ARS, WRRRC, Albany, California
R. Moreau, USDA, ARS, ERRRC, Wyndmoor, Pennsylvania
P. White, Iowa State University, Ames, Iowa
N. Widlak, ADM Cocoa, Milwaukee, Wisconsin
R. Wilson, USDA, REE, ARS, NPS, CPPVS-Retired, Beltsville, Maryland

Copyright © 2012 by AOCS Press, Urbana, IL 61802. All rights reserved. No part of this book may be reproduced or transmitted in any form or by any means without written permission of the publisher.

Library of Congress Cataloging-in-Publication Data

Cocoa butter and related compounds / editors, Nissim Garti and Neil R. Widlak.

p. cm.

Includes bibliographical references and index.

ISBN 978-0-9830791-2-5 (alk. paper)

1. Cocoa butter--Analysis. 2. Cocoa butter--Microbiology. 3. Cocoa--Biotechnology.

4. Chocolate processing. I. Garti, Nissim, 1945- II. Widlak, Neil R.

TP640.C63 2012

664'.3--dc23

2012001673

Printed in the United States of America

15 14 13 12 11 5 4 3 2 1

The paper used in this book is acid-free, and falls within the guidelines established to ensure permanence and durability.

Preface

Chocolate is a delight and makes us happy; it is an indulgence everyone should have the opportunity to enjoy. While most people are aware of chocolate's enjoyment, most people are not aware of the nutritional and health benefits the minor components in chocolate provide.

Only a very few consumers, confectioners, and food scientists know much about the cocoa butter, which is a key component in chocolate and responsible for many of the desirable sensory attributes unique to chocolate.

It is cocoa butter's unique mixture of triglycerides that impart the desirable melting properties of chocolate that set the standard of quality for indulgent confections. However, this unique mixture of triglycerides has its unique problems. If not cooled properly or if it is stored for long periods of time and/or under fluctuating temperatures, the physical structure of the cocoa butter will change and impart an unappealing "bloom" on the chocolate's surface.

Cocoa butter must be tempered during cooling to obtain an optimal crystal state that will minimize the bloom effect. The complex physical changes, under complex tempering conditions, that occur remain still somewhat obscure. Many decades of research have passed and still the bloom and its relation to cocoa butter's unique polymorphic behavior, crystal development and growth, and interaction with other chocolate ingredients are not fully understood.

This book brings to readers the progress of the last 10 years' studies on cocoa butter. Descriptions of several aspects, including physical characteristics such as rheology, hardness, melt profiles, etc., studied by new and advanced techniques are included.

Similarly, the polymorphism of cocoa butter is reconsidered in light of studies done by synchrotron DSC, FTIR, and SAXS techniques. These data are complemented by new understandings on the cause of the crystallization and transitions of the polymorphs. Other aspects such as the effect of minor components, emulsifiers, and other fats are discussed in great detail in this book.

The first chapter, "Chocolate and Cocoa Butter—Structure and Composition," by Geoff Talbot, describes cocoa butter composition and its structure. This comprehensive review will familiarize readers with the complexity of the cocoa butter composition.

Chapter 2, "*Theobroma cacao*—An Introduction to the Plant, Its Composition, Uses, and Health Benefits," by César Vega and Catherine Kwik-Urbe, deals with minor components in cocoa beans that are bioactives. This chapter stresses the health benefits of cocoa butter and, as a result, also the chocolate that we are consuming.

Laurent Peno-Mazzarino describes the importance of the polyphenols present in cocoa beans' chocolate (chocolate mass), antioxidants, and other benefits in Chapter 3,

“Cocoa Polyphenols.” The editors found this chapter interesting and significant. It stresses yet again the significant benefits that chocolate provides.

In Chapter 4, “Phase Behavior of Saturated Triacylglycerides—Influence of Symmetry and Chain Length Mismatch,” Laziz Bouzidi and Suresh S. Narine introduce readers to the triacylglycerides (TAG) complexity of cocoa butter and deal with triglyceride phase behavior and its importance in cocoa butter,

In Chapter 5, “Molecular Composition Dynamics and Structure of Cocoa Butter,” Rodrigo Campos and Alejandro G. Marangoni introduce the molecular composition dynamics of cocoa butter. They present some very new and interesting findings related to cocoa butter microstructure.

In Chapter 6, “Polymorphism and Mixing Phase Behavior of Major Triacylglycerols of Cocoa Butter,” Mari Sasaki, Satoru Ueno, and Kiyotaka Sato introduce readers to the polymorphism of cocoa butter. They discuss the characterization, polymorphism, and mixing-phase behavior of major triglycerides in cocoa butter.

In Chapter 7, “Causes and Best Manufacturing Practices to Minimize Bloom in Confections,” by Neil R. Widlak and Richard W. Hartel, readers get more understanding of the bloom phenomena. Widlak and Hartel discuss the causes and best manufacturing practices for minimizing bloom in chocolate and compound coatings.

Chapter 8, “Morphology of Chocolate Fat Bloom,” by Yasuyoshi Kinta and Tamao Hatta, deals with various morphological aspects of bloom and brings to light some very interesting aspects related to it.

In Chapter 9, “Effect of Minor Components on Cocoa Butter Polymorphism and Kinetics of Crystallization,” Adam Lechter deals with new aspects related to some minor lipids naturally occurring in cocoa butter, such as fatty acids and mono and monody glycerides, on kinetics of crystallization.

In Chapter 10, “Noncocoa Ingredients in Cocoa Butter Crystallization,” Lina Svanberg discusses noncocoa ingredients affecting the crystallization of cocoa butter.

In Chapter 11, “Impact of Cocoa Butter Origin on Crystal Behavior,” the authors St  phanie Marty-Terrade and Alejandro G. Marangoni familiarize readers with the impact of cocoa butter origin on crystal behavior.

The effect of emulsifiers such as lecithin and PGPR as well as other emulsifiers on rheology and polymorphism are discussed in Chapter 12, “Effect of Emulsifiers on Cocoa Butter and Chocolate Rheology, Polymorphism, and Bloom,” by Nissim Garti and Abraham Aserin.

Chapter 13, “Methods of Studying Cocoa Butter and Bloom,” by Daniel Kalnin, comprehensively reviews current methods and analytical tools to study cocoa butter and bloom.

Satoru Ueno discusses a very novel approach for studying cocoa butter behavior in Chapter 14, “New Method to Study Molecular Interactions in Fats—Synchrotron Radiation Microbeam X-ray Diffraction.” The chapter explores novel XRD methods using synchrotron SAXS.

In Chapter 15, “Milk Fat and Cocoa Butter,” the authors, Serpil Metin and Richard W. Hartel, discuss the crystallization and microstructure of milk fat and cocoa butter blends.

Chapter 16, “Molecular Interactions of Triacylglycerides in Blends of Cocoa Butter with *trans*-free Vegetable Oils,” by Jorge F. Toro-Vazquez, Miriam A. Charó-Alonso, Juan A. Morales-Rueda, and J. David Pérez-Martínez, introduces readers to the behavior of cocoa butter in the presence of other triglycerides.

Orit Segman, Zeev Wiesman, and Leonid Yarmolinsky cover shea butter—a close relative of cocoa butter—in Chapter 17, “Methods and Technologies Related to Shea Butter Chemophysical Properties and to the Delivery of Bioactives in Chocolate and Related Products.”

In Chapter 18, “Enzymatic and Other Modification Techniques to Produce Cocoa Butter Alternatives,” Stefanie Verstringe, Nathalie De Clercq, Tuyet Mai Nguyen, Sheida Kadivar, and Koen Dewettink familiarize readers with enzymatic and other modification techniques to produce cocoa butter alternatives and optimize the physical properties of cocoa butter.

Kevin W. Smith details the topic of “Confectionery Fats” in Chapter 19.

And in Chapter 20, “Future of Cocoa Butter Research,” your editors share some thoughts on the future of the industry and directions that research on cocoa butter is likely to take.

We hope that the book will help technicians and scientists to familiarize themselves with the latest progress in this very fascinating research area. And to those newcomers in the field, we believe that this volume will be educational and informative.

Nissim Garti
Neil R. Widlak

Contents

Preface	vii
1: Chocolate and Cocoa Butter—Structure and Composition <i>Geoff Talbot</i>	1
2: <i>Theobroma cacao</i>—An Introduction to the Plant, Its Composition, Uses, and Health Benefits <i>César Vega and Catherine Kwik-Urbe</i>	35
3: Cocoa Polyphenols <i>Laurent Peno-Mazzarino</i>	63
4: Phase Behavior of Saturated Triacylglycerides—Influence of Symmetry and Chain Length Mismatch <i>Laziz Bouzidi and Suresh S. Narine</i>	73
5: Molecular Composition Dynamics and Structure of Cocoa Butter <i>Rodrigo Campos and Alejandro G. Marangoni</i>	103
6: Polymorphism and Mixing Phase Behavior of Major Triacylglycerols of Cocoa Butter <i>Mari Sasaki, Satoru Ueno, and Kiyotaka Sato</i>	151
7: Causes and Best Manufacturing Practices to Minimize Bloom in Confections <i>Neil R. Widlak and Richard W. Hartel</i>	173
8: Morphology of Chocolate Fat Bloom <i>Yasuyoshi Kinta and Tamao Hatta</i>	195
9: Effect of Minor Components on Cocoa Butter Polymorphism and Kinetics of Crystallization <i>Adam Lechter</i>	213
10: Noncocoa Ingredients on Cocoa Butter Crystallization <i>Lina Svanberg</i>	233
11: Impact of Cocoa Butter Origin on Crystal Behavior <i>Stéphanie Marty-Terrade and Alejandro G. Marangoni</i>	245
12: Effect of Emulsifiers on Cocoa Butter and Chocolate Rheology, Polymorphism, and Bloom <i>Nissim Garti and Abraham Aserin</i>	275

13: Methods of Studying Cocoa Butter and Bloom <i>Daniel Kalnin</i>	307
14: New Method to Study Molecular Interactions in Fats—Synchrotron Radiation Microbeam X-ray Diffraction <i>Satoru Ueno</i>	339
15: Milk Fat and Cocoa Butter <i>Serpil Metin and Richard W. Hartel</i>	365
16: Molecular Interactions of Triacylglycerides in Blends of Cocoa Butter with <i>trans</i>-free Vegetable Oils <i>Jorge F. Toro-Vazquez, Miriam A. Charó-Alonso, Juan A. Morales-Rueda, and J. David Pérez-Martínez</i>	393
17: Methods and Technologies Related to Shea Butter Chemophysical Properties and to the Delivery of Bioactives in Chocolate and Related Products <i>Orit Segman, Zeev Wiesman, and Leonid Yarmolinsky</i>	417
18: Enzymatic and Other Modification Techniques to Produce Cocoa Butter Alternatives <i>Stefanie Verstringe, Nathalie De Clercq, Tuyet Mai Nguyen, Sheida Kadivar, and Koen Dewettinck</i>	443
19: Confectionery Fats <i>Kevin W. Smith</i>	475
20: Future of Cocoa Butter Research <i>Nissim Garti and Neil R. Widlak</i>	497
Contributors	503
Index	515

1

Chocolate and Cocoa Butter— Structure and Composition

Geoff Talbot

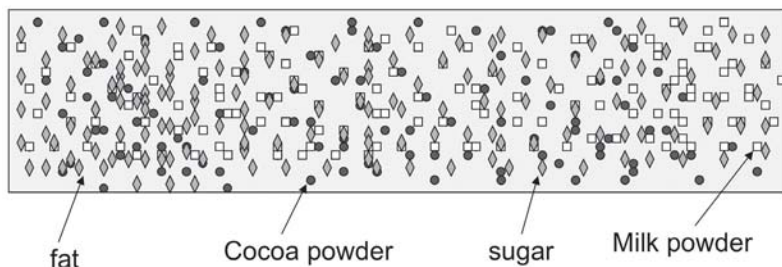
The Fat Consultant, Bedford, United Kingdom

Introduction

Chocolate is one of the most popular food indulgences throughout the world. In terms of per capita consumption, Switzerland tops the league with a consumption in 2008 of 10.77 kg/head, followed by the United Kingdom with 10.07 kg/head (CAOBISCO, 2010). In fact, in 2008, the ten countries consuming the most chocolate per capita were all in Europe (Austria was in 10th place with a per capita consumption of 6.52 kg. In 2005, according to Datamonitor (All Business, 2010) per capita consumption of chocolate in the United States was 12.31 pounds (or 5.60 kg). Why should this be? There are many reasons why people eat chocolate—energy, presence of healthy flavonoids in dark chocolate, and so on—but probably the greatest reason for eating chocolate is the pure pleasure that it brings. Much of that pure pleasure comes from the cocoa present in the chocolate, both the flavor of the cocoa powder, and the smooth meltdown of the cocoa butter. It's no wonder that the botanical name for the cocoa tree is *Theobroma cacao*—"food of the gods." Moving away from the hedonistic attributes of chocolate to its more scientific and technological aspects, what is chocolate? To answer that question we need to look at its composition, its structure, how it is produced, and how it is legally defined across the world.

Chocolate Composition and Structure

A basic definition of the structure of chocolate would be "a fat-continuous matrix within which are particles of cocoa powder, sugar, and, in the case of milk chocolate, milk powder" giving it a structure such as that schematically shown in Fig. 1.1. In terms of the fats, cocoa butter is the one constant in that this is present in the fat phase, whatever the type of chocolate. Indeed, in dark or plain chocolates, cocoa butter is usually the only fat that is present. The exceptions to this are when a small amount of milk fat is added to enhance the bloom resistance of the chocolate or when low levels of noncocoa vegetable fat are added in countries that permit the



The fat is the continuous phase within which all the non-fat solids are dispersed.

This means that the fat defines many of the qualities of the coating

Fig. 1.1. Schematic representation of the structure of chocolate.

use of these fats in chocolate. In milk chocolate, then the fat phase is boosted by the presence of milk fat. In this context, it is always milk fat from cows that is used. As already mentioned, some countries permit the use of vegetable fats in chocolate usually at a defined maximum level. These will be considered both as far as legislation is concerned in the section on legislative constraints and in compositional terms in the section on compositional similarities and differences between cocoa butter and cocoa butter equivalents. In the context of the term “vegetable fat” it should, though, be remembered that cocoa butter itself is a vegetable fat. Chocolates also contain some form of emulsifier and this too could be considered to be part of the lipid phase of the chocolate.

In terms of the nonfat components in chocolate, then for flavor the most important of these is cocoa powder, i.e., the nonfat part of cocoa. Cocoa processing will be summarised in the next section but it is sufficient to say at this stage that the cocoa components used in chocolate are cocoa butter (the fat phase), cocoa powder (the nonfat phase), or cocoa mass (also known as cocoa liquor) which is the combination of both cocoa powder and cocoa butter found naturally in the cocoa bean. Clearly, as cocoa powder is brown in color it is not used as a component in white chocolate. Also present in almost all chocolates is sugar (although there is a limited market for a 99% cocoa chocolate which is essentially all cocoa mass with some emulsifier and no sugar is present). In milk and white chocolates, milk powders and milk fat are also present. These can be introduced into the composition as either full-cream milk

powder containing about 25% milk fat or as skimmed milk powder to which the desired level of milk fat is added separately. A further component that is used in some chocolates (notably those produced in the United Kingdom, Ireland, Switzerland, the United States, and some British Commonwealth countries) is milk crumb or chocolate crumb. This is produced by blending whole milk, sugar, and cocoa liquor and spray-drying the mix. It was originally developed as a way of storing milk during times of the year when it was in short supply but, because it gives milk chocolate a distinctive flavor, it continues to be used simply because consumers have become used to and enjoy this particular flavor.

Cocoa Processing

The cocoa tree, *Theobroma cacao*, grows in a band between 20°N and 20°S of the Equator. In 2009/2010 the worldwide production was 3.596 million tonnes (ICCO, 2010). 68.4% of this was grown in Africa, 17.6% in Asia and Oceania, and 14.0% in South America. The largest individual growing countries were Côte d'Ivoire (33.1% of total production), Ghana (18.1% of total production), and Indonesia (14.9% of total production).

Cocoa pods are harvested when they change color, usually from green or red to orange or yellow. In West Africa this is usually carried out in the fourth quarter of the year. The harvested pods are cracked open using either a machete or a wooden club. Inside are a number (often between 30 and 45) of beans covered in a white mucilage (see Fig. 1.2). The beans are separated before fermentation and drying. Fermentation

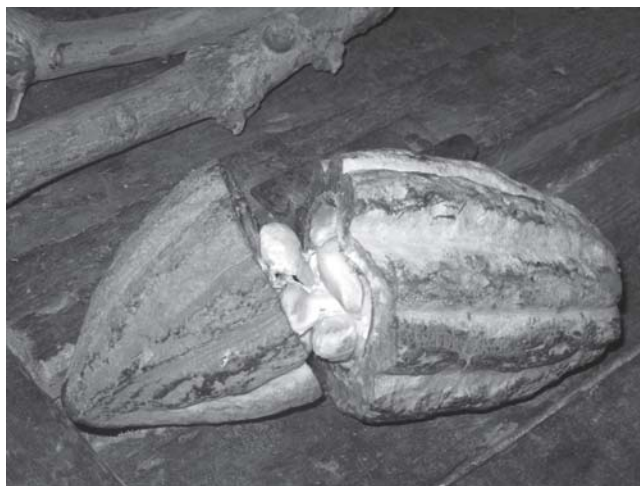


Fig. 1.2. Open cocoa pod showing cocoa beans surrounded by white mucilage.

is a simple process usually requiring only that the beans are left in piles or in large wooden boxes for up to 5 days. During that time the mucilaginous pulp surrounding the beans liquefies and drains away. It is during the fermentation process that the precursors of the cocoa flavor develop. These are components such as methylxanthines that give the bitterness to cocoa and flavonoids giving both color and astringency. Indeed if beans are dried without fermentation taking place then they have only a minimal chocolate flavor. Such beans are produced but are usually then used only for the production of cocoa butter (Fowler, 2009). After fermentation the beans are dried, often by laying them out on mats in the sun in layers about 100mm thick where climatic conditions allow this kind of drying. Otherwise, they can be artificially dried either in chambers containing a wood-burning fire beneath the drying platform or in forced air dryers in which the beans dry in air from heat exchangers. The problem with wood fire drying is the risk that the beans will take on a smoky flavor. It is usually at this stage that the beans are bagged and exported for further processing.

The first stage of processing of the beans is to remove any extraneous matter (stones, metal fragments, etc.) by sieving and magnets. Then the shells of the beans are separated from the interior (the “nib”) of the bean. This is done by a process of breaking and winnowing. After breaking the shells, often with a type of swing-hammer, the broken shell and nib pieces are separated by passing through a series of sieves. It is at this stage that alkalisation, if it is going to be carried out, is performed. Alkalisation is a process that mainly benefits cocoa powder although alkalisated cocoa liquor is sometimes used in chocolate. Its main purpose is to change the color of the cocoa powder, making it darker or more reddish-brown, and involves heating the nibs in an alkaline solution (for example, an aqueous solution of potassium carbonate).

At some stage during the process the cocoa is roasted. This can either be carried out on the whole beans before breaking the shell or it can be carried out on the nib itself. Roasting the whole bean makes it easier to remove the shell whereas roasting the nib, particularly after alkalisation, gives a better cocoa powder. Roasting conditions vary depending on the type of flavor required in the cocoa but temperatures between 110°C and 140°C are usually used. It is only after all of these processing stages that we get to the production of anything that is then directly used in chocolate—cocoa mass or cocoa liquor. This is produced by grinding the cocoa nibs. A range of different types of mill are used to achieve this. Cocoa mass is, then, a ground mix of cocoa powder and cocoa butter and should contain at least 53% fat although the Codex standard (Codex Alimentarius, 2003) has a range of 47–60% fat. The fat is separated from the powder by means of a cocoa press. This results in “press cocoa butter.” The reduced fat cake from the press can be further processed along with unpressed cocoa mass and nibs in an expeller to produce “expeller cocoa butter.” Finally, there is a grade of cocoa butter known as “refined cocoa butter.” This can have been produced by pressing, expelling, or by solvent fractionation. The common factor between them all in terms of “refined cocoa butter” is that after separation from the mass the fat is then refined using the normal fat refining techniques of neutralization, bleaching, and

deodorization. Normally press and expeller cocoa butters are used without refining as this process removes the flavor associated with cocoa butter. Because they are used without refining, they do contain measurable levels of free fatty acid, although these are limited by legislation to a maximum of 1.75% (European Union, 2000). The variabilities in composition that are found in different cocoa butters will be discussed in more detail in the section on cocoa butter composition.

The fat content of cocoa powder varies depending upon how well the cocoa butter has been removed but commercial varieties tend to come mainly in two types—10/12 cocoa powder containing 10–12% cocoa butter and 20/22 or 22/24 cocoa powder containing 20–22% or 22–24% cocoa butter respectively.

Chocolate Processing

Chocolate processing involves a number of stages which can be summarised as:

- refining
- conching
- tempering
- molding or enrobing
- cooling
- packing

The first stage of refining refers not to refining in the sense that oils and fats are refined but more to a grinding process to reduce the particle size of the chocolate. It is important that the nonfat particles in chocolate are reduced in size to below 25 microns because particles larger than this can be detected on the tongue as grittiness. The exception to this is for chocolates that are to be used to coat, for example, biscuits. The grittiness of the biscuit as it breaks down in the mouth is enough to mask grittiness in the chocolate and so particle sizes of greater than 25 microns can then be used. The most common refining process for chocolate uses a series of counter-rotating heavy cylinders. Industrially a five-roll refiner is usually used but this is often preceded by a two-roll refiner to begin the process of particle size reduction (Beckett, 2009). Each roller goes at a faster speed than the one before it allowing a very thin film of chocolate to be transferred from one roller to the next. This is scraped off the final roller with a knife. Usually, a low-fat paste is passed through the roll refiner, for example a mix in which only 20–23% fat is present, irrespective of the fat content of the final chocolate. The remaining fat is then added during conching. The exceptions to this are when either a ball mill or a Macintyre refiner are used. In those cases it is more usual to grind the full-fat chocolate mix. A ball mill is a vessel containing a large number of metallic or ceramic balls, often of different diameters. Central in the vessel is a rotating agitator. The chocolate mix is stirred with the balls, the grinding action

of the balls reducing the particle size. The temperature is such that the chocolate is fully liquid and so ball milling is often carried out at about 50°C. The Macintyre type of refiner is both a refiner and a conche in one unit. It consists of a large drum with a ridged inner surface. Central to the machine is a rotating shaft with spring loaded slats on its perimeter. The chocolate mix is introduced into the machine and the slats on the rotating shaft press against the ridges on the inside of the drum grinding the chocolate between them and thus reduce the particle size. Unlike roll refining this is a batch process. Because the chocolate is stirred and heated in this vessel and kept in there for a number of hours it is also conched as well as refined.

Conching essentially removes unwanted flavors and excess moisture from the chocolate. According to Ziegleder (2004) conching also redistributes the flavor in chocolate from initially being associated with the cocoa powder and cocoa butter to being almost equally distributed between these components and the sugar at the end of conching. Conching traditionally goes through three stages. In the first stage, the dry stage, the chocolate is in a flaky form direct from the roll refiners. In this stage the chocolate is thrown about in the conche so that unwanted flavor volatiles and moisture can be removed. It is also during this stage that the nonfat particles are largely coated in fat so it is important that the fat content should still be low enough to allow movement of the chocolate as a powder but high enough to allow a coating of fat on the particles. In the second, pasty stage, the particles become better coated with fat and the chocolate becomes more of a paste and less powdery. Because the fat content is still relatively low the paste is quite thick. This is important because it ensures that all of the particles are coated in fat, a factor that is important in defining the final viscosity of the chocolate. The remaining fat and the emulsifier are not added until the third stage, the liquid stage, of conching. This is because both of these additions change the paste from thick to thin. It is especially important if lecithin is being used as the emulsifier not to add it too soon as it attracts any moisture remaining in the chocolate and prevents its removal. Too much moisture in chocolate increases the viscosity. The amount of fat and the type and amount of emulsifier added during the final stage of conching are dependent on both the type of chocolate being produced and its final application, particularly whether it is to be used for molding or enrobing.

Up to this point the composition of the fat present in the chocolate has been less important than the amount of fat present at each stage. This changes with the next stage of chocolate processing—tempering—in which the composition, not only in terms of the relative amounts of cocoa butter, milk fat, and vegetable fat but also in terms of minor lipid components, can have a very large effect on temper and the conditions needed to temper the chocolate. This, though, will be covered in more detail in the section on the effect of chocolate fat phase composition on chocolate tempering and rheology. At this stage, the tempering process will be discussed in more general terms.

The reason why chocolate is tempered is because the fat phase of chocolate is essentially polymorphic. This means that it can crystallize in a number of crystal forms of different stabilities. Polymorphism of cocoa butter will be discussed in more detail

later in this chapter and in considerably greater detail in Chapter 6, Polymorphism in Cocoa Butter—Characterization of the Polymorphs. In outlining the tempering process it is sufficient, at this stage, to consider that when chocolate is cooled from liquid (say, 50°C to say, 26°C) it will crystallize in both stable and unstable crystal forms. If the chocolate were then to be molded or used to coat a centre product and passed through a cooling tunnel, the unstable crystal (and some of the stable crystal) forms would continue to grow as the chocolate cooled. When the product is then brought back to ambient temperatures and kept at such temperatures the crystals in the unstable form will fairly quickly transform into the stable form. This recrystallization of the fat phase will show itself as a layer of fat crystals on the surface of the chocolate (known as fat bloom) and a change in texture to the internal structure of the chocolate. If, though, when the chocolate is cooled as described above, its temperature is increased again to, say, about 30°C, then the unstable crystals will melt leaving only the stable ones. When the chocolate is then cooled after molding or enrobing there will be only stable crystals present to “seed” the rest of the chocolate which will then crystallize in this same stable form. Although this does not necessarily prevent a different form of fat bloom from growing on the chocolate it does ensure its long term stability when stored at 20°C or below.

Industrially, tempering is carried out by passing the chocolate through a series of heat exchangers which gradually cool and then re-heat the chocolate taking it through the stages needed to first crystallize both forms and then melt out the unstable form before the chocolate goes on to the next stage of processing.

After tempering, the chocolate can then be used in a variety of ways. It can be moulded into solid tablets. It can be moulded into empty shells that are then filled with different centres ranging from hard and soft pralines to very liquid centres such as liqueurs. It can also be used to coat or enrobe a wide range of products—biscuits, caramel, fudge, nuts, fruits, ice cream—the list is endless.

After molding or enrobing, the products pass through a cooling tunnel to solidify the chocolate. Industrial cooling tunnels have a number of stages within them that can be set to different temperatures. With chocolate it is important to start cooling at a fairly high temperature (15–18°C being typical). This allows more of the stable crystals to grow. If the chocolate was cooled quickly to lower temperatures then there would be the risk of again introducing unstable crystals that could go on to “seed” some of the chocolate in this form. By cooling gently this is avoided and only stable seeds are present. After this initial gentle cooling, the chocolate goes through cooler stages in the centre of the tunnel with temperatures of 10–12°C being common. This crystallizes and solidifies the bulk of the chocolate. Finally, as the chocolate exits the tunnel the temperature is raised again to about 15°C. This is to ensure that the exit temperature of the chocolate is above the dew point in the vicinity of the tunnel exit. If not, then there is the risk of condensation on the surface of the chocolate. At the very least this can lead to unsightly drying marks. Worse it can get trapped within subsequent packing leading to the risk of mold formation. It can also result in sugar

bloom in which a small amount of sugar in the chocolate can dissolve in the condensation and then recrystallize as sugar on the surface of the chocolate as the water evaporates. Sugar bloom and fat bloom can be easily distinguished. On the one hand, sugar bloom dissolves in water while fat bloom doesn't; on the other hand fat bloom usually melts on the fingers while sugar bloom doesn't.

After cooling, the products are then packed either by flow-wrapping, foil wrapping, or putting into boxes.

Chocolate Recipes

As so many chocolate companies have their own proprietary recipes for their chocolate (and often have more than one recipe for a particular type of chocolate) it is only possible to give guidelines and typical recipes here. A further constraint is that recipes have to meet specific national legislation. Chocolate can nominally be divided into three kinds—plain (or dark) chocolate, milk chocolate, and white chocolate. Generally speaking, plain chocolate is free from milk fat and nonfat milk solids while white chocolate is free from nonfat cocoa solids. The main components in chocolate are cocoa solids (i.e., cocoa butter, cocoa mass, cocoa liquor, cocoa powder) milk solids (i.e., milk fat, milk powders), and sugar. In some instances, e.g., the EU Chocolate Directive (European Union, 2000), these three groups of ingredients are termed “noble ingredients.” In addition to these noble ingredients, chocolate can contain noncocoa vegetable fat (where this is permitted), emulsifiers, and other edible substances (again where these are permitted). Examples of “other edible substances” are nuts, whey powder, vanilla or ethyl vanillin, salt, etc.

The emulsifiers used in chocolate are also usually defined in the chocolate legislation the most commonly used ones being soya lecithin, ammonium phosphatide (a synthetic type of lecithin produced from rapeseed oil and glycerol), and PGPR (polyglycerol polyricinoleate). Lecithin is the most commonly used emulsifier in chocolate with ammonium phosphatide being an alternative preferred by some manufacturers. PGPR has a marked effect on the yield value of chocolate and so is used (often in combination with lecithin) where particular flow characteristics are required in the chocolate. Chocolate is not an emulsion in the normal sense of the word and so these emulsifiers are not used in the usual sense of combining fatty and aqueous phases together. Rather, they are used to improve the flow characteristics of the chocolate by reducing its viscosity and/or yield value.

Using these ingredients together in different combinations produces different types of chocolate. A further variable factor in chocolate composition is the total fat content. This can range from 25% (often the minimum defined in chocolate legislation) to 29–30% as a more normal level up to 33–35% in some premium chocolates. In premium high cocoa solids dark chocolates the fat content can be quite high.

An example of a typical plain chocolate is shown in Table 1-A. Two milk chocolate formulations are shown in Table 1-B, one being low in milk fat (just above

Table 1-A. Typical Plain Chocolate Formulation.

Component	%
Cocoa mass	32.5
Cocoa butter	15.5
Sugar	51.6
Lecithin	0.4
Total fat	33.4

Table 1-B. Typical Milk Chocolate Formulations.

Component	Low milk fat %	High milk fat %
Cocoa mass	20.0	20.0
Cocoa butter	18.25	15.75
Full cream milk powder	15.0	25.0
Sugar	46.35	38.85
Lecithin	0.4	0.4
Fat composition:		
Cocoa butter	29.25	26.75
Milk fat	3.75	6.25
Total fat	33.0	33.0
Expressed as % of fat phase:		
Cocoa butter	88.6	81.9
Milk fat	11.4	18.1

the level permitted in EU and U.S. milk chocolates), the other being higher in milk fat (above the designation for “family milk chocolate” in the EU). Table 1-C shows a typical composition of white chocolate. In all cases the total fat content is about 33%. Where vegetable fat is permitted in chocolate it usually replaces part of the added cocoa butter in the recipe. In other words, the level of cocoa mass used remains the same. Some manufacturers produce what is often termed a “supercoating.” This is a coating that does not conform the chocolate regulations and cannot be labelled as such but which, in many ways, performs exactly like chocolate. In supercoatings all of the added cocoa butter is replaced by vegetable fat. Compara-

Table 1-C. Typical White Chocolate Formulation.

Component	%
Cocoa butter	27.0
Full cream milk powder	23.0
Sugar	49.6
Lecithin	0.4
Total fat	32.8

Table 1-D. Milk Chocolate and Milk Supercoating Recipes Using Vegetable Fat.

Component	Milk chocolate (no vegetable fat) %	Milk chocolate (with vegetable fat) %	Milk supercoating %
Cocoa mass	20.0	20.0	20.0
Cocoa butter	18.25	13.25	
Vegetable fat (CBE)		5.0	18.25
Full cream milk powder	15.0	15.0	15.0
Sugar	46.35	46.35	46.35
Lecithin	0.4	0.4	0.4
Fat composition:			
Cocoa butter	29.25	24.25	11.0
Milk fat	3.75	3.75	3.75
Vegetable fat		5.0	18.25
Total fat	33.0	33.0	33.0
Expressed as % of fat phase:			
Cocoa butter	88.6	73.5	33.3
Milk fat	11.4	11.4	11.4
Vegetable fat		15.1	55.3

tive recipes of a low milk-fat milk chocolate containing vegetable fat at the 5% level and as a supercoating are shown in Table 1-D. Further recipes for chocolates containing milk crumb and chocolates for particular applications (biscuits, mini rolls, hollow figures, and eggs, ice cream, chocolate chips, and chunks) are listed by Yates (2009).

Legislative Constraints

Reference has already been made on a number of occasions to the legislative constraints that are imposed on chocolate compositions. Rather than try to cover the different regulations defining chocolate compositions worldwide, three “regions” will be discussed here: (a) the United States, (b) the European Union, and (c) Codex Alimentarius. It should also be realised by the reader that the information given here is a snapshot of the legislation as it was at the time of writing. Regulations change and it is important when defining a new chocolate composition to refer to the regulations in force at that particular time and not assume that no changes have been made from those outlined here.

United States

The Standards of Identity for chocolate in the United States are defined in the Code of Federal Regulations (CFR), Title 21 (covering Food and Drugs), Part 163 (for cacao (chocolate) products). This defines the composition of a range of different chocolates. Sweet chocolate, for example must contain at least 15% of total dry cocoa solids and a maximum of 12% total dry milk solids. Semisweet or bittersweet chocolate must contain at least 35% of total dry cocoa solids. Milk chocolate is defined in a number of ways—as “milk chocolate,” “buttermilk chocolate,” “skim milk chocolate,” and “mixed dairy product chocolates.” Milk chocolate must contain at least 10% total dry cocoa solids, at least 12% total dry milk solids, and at least 3.39% milk fat. Buttermilk chocolate and skim milk chocolate have the same requirements as milk chocolate but differ in the source of the milk components. It should be noted that both buttermilk chocolate and skim milk chocolate contain less than 3.39% milk fat. White chocolate has to contain a minimum of 20% cocoa butter, a minimum of 14% total dry milk solids and a minimum of 3.5% milk fat. None of these chocolates are allowed to contain any noncocoa vegetable fat. However, there is a further group of products that can contain vegetable fat—“sweet cocoa and vegetable fat coating,” “sweet chocolate and vegetable fat coating,” and “milk chocolate and vegetable fat coating.” Taking the milk chocolate and vegetable fat coating as an example, it still needs to conform to the requirements for milk chocolate in terms of minimum cocoa solids, milk solids, and milk fat, but has the added option of including “safe and suitable vegetable derived oils, fats, and stearins other than cacao fat. The oils, fats, and stearins may be hydrogenated.”

European Union

The compositions of chocolate produced in the European Union are defined by EU Directive 2000/36/EC (European Union, 2000). Like the United States regulations this defines the limitations on a range of different chocolates the main ones of which are summarized in Table 1-E. In addition to these definitions there are also regulations covering both chocolate and milk chocolate with “quality” definitions. Up until these regulations were produced by the EU and came into force on August 3, 2003, each member state

Table 1-E. Compositional Limitations on Some EU Chocolate Descriptions.

	Chocolate	Milk chocolate	Family milk chocolate	White chocolate
Total dry cocoa solids	Min 35%	Min 25%	Min 20%	
Dry nonfat cocoa solids	Min 14%	Min 2.5%	Min 2.5%	
Cocoa butter	Min 18%			Min 20%
Total dry milk solids		Min 14%	Min 20%	Min 14%
Milk fat		Min 3.5%	Min 5%	Min 3.5%
Total cocoa butter + milk fat		Min 25%	Min 25%	Min 23.5%
Vegetable fat	Max 5%	Max 5%	Max 5%	Max 5%

had its own chocolate regulations. One of the main differences between these national regulations was whether noncocoa vegetable fat was permitted or not. Seven member states—UK, Ireland, Denmark, Sweden, Finland, Austria, and Portugal—permitted their use; the rest did not. The result of allowing their use throughout the EU was to put restrictions on their type, restrictions that did not apply previously in the countries that already allowed their use. In summary, these new restrictions limited them to being sourced from only six base oils (although coconut oil was allowed in ice cream coatings). These oils are considered in more detail in the section on composition of cocoa butter. In addition to this restriction a further limitation was imposed on the type of processing that is permitted. This is limited to fractionation and refining. The production of these fats by means of enzymic interesterification is specifically prohibited.

Codex Alimentarius

Although not specifically a “national” legislation the Codex standard for chocolate can be used as a guideline in those countries where there is no specific legislation for chocolate. The Codex standard is found in STAN 87-1981, Rev.1-2003 (Codex Alimentarius, 2003). As with the U.S. and EU regulations, a range of different types of chocolate is defined. The main types are summarised in Table 1-F. The limitations are very similar to those imposed by the EU apart from there being minimum ranges for milk solids and milk fat in milk and white chocolate. The imposition of a range for a minimum value does, at first sight, seem odd but the Codex standard goes on to say that the “minimum content for milk solids and milk fat shall be applied by the authority having jurisdiction in accordance with applicable legislation.”

Cocoa Butter Composition

Mention has already been made that cocoa grows in three main areas of the world—West Africa, Central and South Americas, and Asia. There are differences in fatty

Table 1-F. Compositional Limitations on Some Codex Chocolate Descriptions.

	Chocolate	Milk chocolate	Family milk chocolate	White chocolate
Total dry cocoa solids	Min 35%	Min 25%	Min 20%	
Dry nonfat cocoa solids	Min 14%	Min 2.5%	Min 2.5%	
Cocoa butter	Min 18%	Min 20%		Min 20%
Total dry milk solids		Min 12–14%	Min 20%	Min 14%
Milk fat		Min 2.5–3.5%	Min 5%	Min 2.5–3.5%
Vegetable fat	Max 5%	Max 5%	Max 5%	Max 5%

acid composition and, as a result of this, in triglyceride composition depending on the origin of the cocoa butter. Various workers over the past three decades or so have studied the fatty acid and triglyceride compositions of cocoa butter from these different origins. In the following section these studies will be summarised to show the differences and similarities between different cocoa butters.

Variation in Fatty Acid and Triglyceride Composition

Compared with many fats, cocoa butter has a very simple fatty acid composition being largely composed of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and a much lower level of linoleic acid (C18:2). This simple fatty acid composition results in an equally simple triglyceride composition with three triglycerides, POP, POST, and StOSt predominating.

One of the earliest surveys of the differences between cocoa butters from different origins was carried out by Chaiseri and Dimick (1989). They measured the triglyceride composition of 64 cocoa butters—15 from South America, 8 from North and Central Americas (the farthest north being one sample from Mexico), 17 from Africa, and 24 from Asia (20 of these being from Malaysia). The mean triglyceride compositions from these different origins is shown in Table 1-G. In terms of the main triglycerides, there is no significant difference in POP contents between the four main origins. POST is significantly higher in Asian cocoa butters than in butters from the other three areas; this triglyceride is also significantly lower in South American cocoa butters. StOSt is significantly higher in Asian cocoa butters than in the other three origins; this triglyceride is also significantly higher in African cocoa butters than in cocoa butters from the Americas. Turning to the more unsaturated triglycerides both POO and StOO differ significantly between the various origins with the amounts of these triglycerides being lowest in Asian cocoa butters, followed by African cocoa butters, and highest in cocoa butters from the Americas.

Timms and Stewart (1999) measured the fatty acid compositions of 140 cocoa butters of different origins. The palmitic acid level varied between 25.7% and 28.4%

Table 1-G. Triglyceride Compositions of Cocoa Butters from Different Origins.

	South America %	North and Central America %	Africa %	Asia %
POP	19.0	18.6	18.4	18.6
POSt	38.0	38.9	39.1	40.0
StOSt	26.0	26.9	28.2	30.8
AOSt	0.5	0.6	0.6	0.8
POO	3.4	2.7	2.2	1.2
StOO	5.7	5.3	4.7	2.9
PLiP	1.1	1.0	1.0	0.8
PLiSt	3.5	3.3	3.2	2.9
StLiSt	2.8	2.7	2.5	2.2

Source: Chaiseri & Dimick, 1989.

with a mean of 26.9% (SD 0.48). Stearic acid varied between 34.9% and 37.9% (mean 36.6%; SD 0.59) and oleic acid varied between 31.3% and 32.2% (mean 31.7%; SD 0.20). These statistics show just how little variability there is in cocoa butters. Foubert et al. (2004) measured both the fatty acid composition and the triglyceride compositions of 20 cocoa butters. Ten of these were of known origins, the remainder being from factory production batches in Belgium and The Netherlands. The fatty acid compositions and triglyceride compositions of those cocoa butters of known origins are shown in Table 1-H. As with Chaiseri and Dimick's studies, these too show that South American cocoa butters, particularly Brazilian cocoa butters contain less POP/POSt/StOSt and more POO/StOO.

Padley and Timms (1979) also studied cocoa butters from a range of origins but they did this to produce a baseline from which they could develop a method for determining the amount of cocoa butter equivalent that there was in chocolate. Having measured the triglyceride composition of a wide range of cocoa butters, some of known and some of unknown origins using the carbon number GC method they then normalised the C50, C52, and C54 values to P50, P52, and P54 values. So, for example P50 was calculated as follows:

$$P50 = \frac{\text{Percentage carbon number 50}}{\text{Percentage carbon number (50+52+54)}} \times 100\%$$

In this way the sum of P50, P52, and P54 was 100%. They then plotted P50 against P54 (see Fig. 1.3). Irrespective of the origin of the cocoa butter all natural unadulterated cocoa butters fell on the same straight line, a line with an equation of $P50 = 43.9 - 0.74P54$.

Table 1-H. Fatty Acid and Triglyceride Compositions of Cocoa Butters from Different Origins.

Origin	Fatty acids (%)				Triglycerides (%)				
	C16:0	C18:0	C18:1	C18:2	POP	POSt	StOSt	P00	St00
West Africa	26.2	36.6	33.6	2.7	17.7	41.3	25.5	2.3	3.0
Ivory Coast	25.6	36.5	34.1	2.8	18.3	41.7	25.2	2.4	2.9
Nigeria	26.5	37.1	33.1	2.3	18.3	43.0	25.7	1.8	2.1
Ivory Coast	26.4	36.5	33.5	2.7	18.1	41.9	25.1	2.4	2.7
Indonesia	26.1	37.3	33.3	2.4	17.5	41.8	25.8	2.4	2.8
Malaysia	25.7	37.1	33.7	2.4	17.8	40.7	25.9	2.4	2.8
San Domingo	26.9	34.4	34.8	2.9	19.4	41.4	23.2	3.1	3.5
Ecuador	27.1	35.4	33.7	2.6	18.9	41.0	25.2	2.4	2.9
Brazil	25.1	34.3	36.4	3.4	17.0	38.7	23.8	5.0	6.0
Brazil	24.9	32.9	37.6	3.7	16.6	38.6	23.1	6.0	7.0

Source: Foubert et al., 2004.

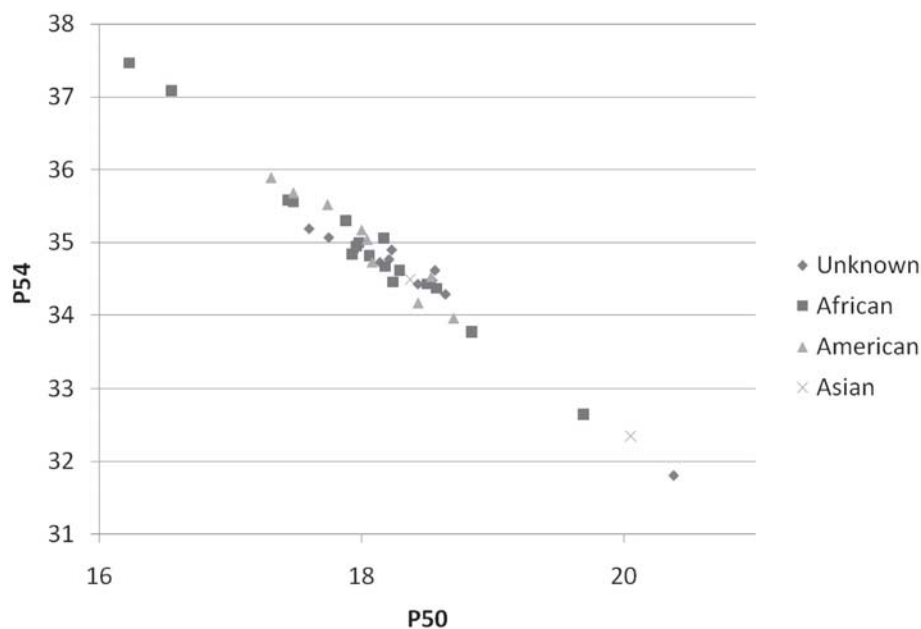


Fig. 1.3. Plot of P50 v P54 (i.e., normalized C50 and C54) for a range of cocoa butters. Based on data from Padley & Timms, 1979.

Minor Components in Cocoa Butter

From a lipid point of view the most important minor components in cocoa butter are (a) minor triglycerides, (b) partial glycerides, (c) free fatty acids, (d) phospholipids, and (e) unsaponifiable matter.

Minor Triglycerides

Minor triglycerides can, in some senses, be considered to be anything that is not one of the main SOS type of triglyceride present in the fat, i.e., anything other than POP, POSt, and StOSt. The presence of varying levels of more unsaturated triglycerides (e.g., POO, StOO etc) has already been mentioned, particularly in the context of variability dependent upon origin. What have not so far been considered but which are of tremendous importance in terms of rheology and tempering of chocolate are the presence (or absence) of trisaturated triglycerides. In cocoa butter these are mainly PPP, PPSt, PStSt, and StStSt. Timms and Stewart (1999) state that the typical level of trisaturated triglycerides present in a normally processed cocoa butter is 1.4% but that this can rise to 3.6% in a heat-damaged fat (i.e., one that has been heated in the laboratory for 1 hour at 250°C under vacuum). The implication is that this may be due to some interesterification occurring during the high-temperature treatment. Foubert et al. (2004) measured the levels of the above four individual trisaturated triglycerides in 20 different cocoa butters and found the following mean levels:

PPP	0.32 ± 0.09
PPSt	0.76 ± 0.15
PStSt	0.58 ± 0.29
StStSt	0.45 ± 0.22
Total	2.11 ± 0.57

The total SSS is above the level that Timms and Stewart (1999) define as being typical of a normally processed cocoa butter but well below that of a heat damaged cocoa butter which implies that perhaps there were some samples in the population of 20 cocoa butters that had been heat damaged in some way. One in particular had a total trisaturated triglyceride level of 3.45%. Four of the 20 had been identified as giving crystallization problems during production. The mean total SSS level of these four alone was 2.46%; the mean total SSS level of the remaining 16 samples was 2.02% suggesting that trisaturated triglycerides may be contributing to the crystallization problems.

Davis and Dimick (1989) isolated high-melting seed crystals present in cocoa butter that form during the early stages of crystallization. As well as being rich in gly-

Table 1-I. Fatty Acid and Trisaturated Triglyceride Compositions of the Simple Lipid Fraction of Whole Cocoa Butter and High-Melting Seed Crystals.

	Whole cocoa butter %	Seed crystal %
C16:0	26.5 ± 0.2	25.5 ± 0.3
C18:0	36.8 ± 0.2	63.4 ± 0.9
C18:1	33.3 ± 0.2	8.3 ± 0.3
C18:2	3.2 ± 0.1	0.5 ± 0.1
C20:0	0.3 ± 0.1	2.3 ± 0.6
PPSt (+OOA)	1.62	11.04
PStSt	1.77	28.33
StStSt	1.08	26.29

Source: Davis & Dimick, 1989.

colipids (11.08% compared to 0.89% in the whole cocoa butter) and phospholipids (6.55% compared to 0.037% in the whole cocoa butter), these high-melting seed crystals were also rich in trisaturated triglycerides. The fatty acid and trisaturated triglyceride compositions of the simple lipid fractions of the whole cocoa butter and the seed crystal are shown in Table 1-I. The total of the saturated fatty acids in the whole cocoa butter was 63.6%; in the seed crystals the total was 91.2%. The total of the three main trisaturated triglycerides in cocoa butter (PPSt, PStSt, and StStSt) was 4.47%; in the seed crystals the total was 65.66%.

Partial Glycerides

It is known that partial glycerides, particularly diglycerides, can have an adverse effect on the crystallization of fats. Siew and Ng (1999) showed that adding palm oil diglycerides to purified palm oil triglycerides inhibited nucleation and slowed down crystal growth rate. Tietz and Hartel (2000) suggest that at low levels partial glycerides act as nucleating sites and promote crystallization whereas at higher levels they start to interfere with crystallization. Timms and Stewart (1999) quote a maximum of 2.5% diglyceride in an acceptable cocoa butter. Foubert et al. (2004) measured the diglyceride content of 20 cocoa butters and found a mean level of 0.92% (SD 0.36) with the highest level in the 20 samples being 2.2%, i.e., all within the specification defined by Timms and Stewart (1999). Even the four cocoa butter samples identified by Foubert et al. (2004) as giving crystallization problems had a mean diglyceride content of 0.78% implying that whatever was causing the crystallization problems in these four samples it probably was not the diglyceride content.

Free Fatty Acids

Cocoa butter typically has a maximum specified free fatty acid level of 1.75% (Timms & Stewart, 1999; European Union, 2000; Folayan, 2010). The 20 cocoa butter samples analysed by Foubert et al. (2004) had a mean free fatty acid level (expressed as oleic acid) of 1.71% (SD 0.41) with eight of these samples having levels over the maximum specified level of 1.75%. Three of the four samples identified as giving crystallization problems had free fatty acid levels above this specified maximum while the fourth sample was only just inside the specification.

Phospholipids

The level of phospholipids in cocoa butter is not specified but the level of phosphorus was one of the measurements made by Foubert et al. (2004) on a series of 20 different cocoa butter samples. The phosphorus levels ranged from 2.3 ppm up to 63 ppm equating to a phospholipid range of 0.006% up to 0.16%. Davis and Dimick (1989) also measured the level of phospholipid in both whole cocoa butter (0.037%) and in the high-melting seed crystal fraction (6.56%) showing how this too is concentrated in this fraction and is therefore one of the first components to crystallize out from cocoa butter as it solidifies.

Unsaponifiable Matter

Unsaponifiable matter (higher aliphatic alcohols, sterols, pigments, hydrocarbons, etc.) is specified in the EU Chocolate Directive (European Union, 2000) as a maximum of 0.5%, except in the case of press cocoa butter when it can only be present up to a maximum of 0.35%. Codex Alimentarius (2001) specifies a maximum unsaponifiable matter of 0.7% in cocoa butter but also makes the exception for press cocoa butter where the maximum is 0.35%. Foubert et al. (2004) found unsaponifiable matter ranging from 0.31% up to 0.53%.

Compositional Similarities and Differences between Cocoa Butter and Cocoa Butter Equivalents

Although confectionery fats, cocoa butter substitutes, and cocoa butter equivalents (CBEs) will be discussed in more detail in later chapters it is useful, in the context of cocoa butter, to compare the similarities and differences between CBEs and cocoa butter. Reference has already been made to chocolate legislation that permits the use of vegetable fats in chocolate. These vegetable fats are generally referred to as cocoa butter equivalents because they have a chemical composition and physical properties that are very close to those found in cocoa butter. The EU Chocolate Directive (European Union, 2000) permits the use of vegetable fats from only six sources. These are:

Palm oil	<i>Elaeis guineensis</i> <i>Elaeis olifera</i>
Illipe, Borneo tallow or Tengkawang	<i>Shorea</i> spp.
Shea	<i>Butyrospermum parkii</i>
Sal	<i>Shorea robusta</i>
Kokum gurgi	<i>Garcinia indica</i>
Mango kernel	<i>Mangifera indica</i>

All of these oils are “rich” in some of the same type of symmetrical monounsaturated triglycerides (SOS) as are found in cocoa butter—POP, POST, StOST. “Rich” is a relative term—in some of these oils the SOS content is about 30–40%, in others it can be over 80%. These are not the only vegetable oil sources of SOS triglycerides—these triglycerides are also found in Allenblackia, Aceituno, Pentadesma, and Dhupa fats, among others. They are, though, the only oil sources permitted in vegetable fats in chocolate in the EU. Countries outside the EU that permit the use of vegetable fats in chocolate may well use oils from some of these other sources. They may also use oils produced via an enzymic interesterification route. For the sake of simplicity, though, only these six oil sources will be considered here.

As has already been mentioned the levels of SOS in these oils varies considerably from oil to oil (Table 1-J). It is clear that, from a total SOS point of view, only illipe butter, kokum gurgi, and, possibly, sal oil contain enough SOS to be able to match the levels found in cocoa butter. The other three oils have much lower levels of SOS. This means that the SOS needs to be concentrated by fractionation. Usually solvent fractionation is

Table 1-J. Typical Triglyceride Compositions of the Main CBE Base Oils Compared with Cocoa Butter.

	POP %	POST %	StOST %	Total SOS %
Cocoa butter ^a	16	37	26	79
Palm oil ^b	26	3	<1	29
Illipe butter ^b	7	34	45	80
Sal oil ^c	5	16	36	67*
Shea butter ^a	<1	6	30	36
Kokum gurgi ^a	<1	6	72	78
Mango kernel oil ^a	6	13	18	37

* Includes 9% StOA and 1% AOA.

Sources:

a. Talbot, 2006.

b. Jurriens, 1968.

c. Sridhar & Lakshminarayana, 1991.

Table 1-K. Typical Triglyceride Compositions of CBE Component Fats and Fractions.

	POP %	POSt %	StOSt %	Total SOS %
Cocoa butter	16	37	26	79
Palm mid-fraction	66	12	3	81
Illipe butter	7	34	45	80
Sal stearine	<1	10	60	81*
Shea stearine	1	7	74	82
Kokum gurgi	<1	6	72	78
Mango kernel stearine	1	16	59	76

*Includes 11% StOA.

Source: Talbot, 2006.

preferred for this to achieve a narrow enough fraction to concentrate the SOS. Because of the high level of trisaturated triglycerides in palm oil, this oil is fractionated twice, once to remove the high-melting triglycerides and a second time to remove the low melting oleine fraction. Therefore, a mid-fraction from palm oil is normally used in CBEs. Both shea butter and mango kernel oil (and often sal oil) are fractionated only once to remove the more unsaturated oleine fraction leaving the stearine to be used in CBEs. The SOS composition of the components used in CBEs is shown in Table 1-K.

None of the components or fractions by themselves match the triglyceride composition of cocoa butter, although illipe butter comes closest overall. This means that blends of these components need to be produced to match cocoa butter better. The main source of POP is palm mid-fraction; the main sources of StOSt are shea stearine, sal stearine, mango kernel stearine, and kokum gurgi. Only illipe butter contains a significant amount of POSt. A blend, then, of palm mid-fraction, illipe butter, and one of the StOSt sources would therefore enable a closer match to cocoa butter to be made. Even then, it is not possible to fully match the POSt level in cocoa butter. A further issue is that illipe butter is available in variable quantities and, in some years, is not available at all making a close match to the POSt content in cocoa butter even more difficult. This usually means that CBEs are produced from blends of palm mid-fraction and one or more of the StOSt sources. By introducing more POP and StOSt into the CBE than there is in cocoa butter a good approximation can be made in terms of matching cocoa butter both chemically and physically.

Effect of Compositional Differences in Cocoa Butter on Physical Properties

Tables 2-G and 2-H showed that cocoa butters from different origins had different fatty acid and triglyceride compositions. In particular they showed that cocoa but-

Table 1-L. Solid Fat Contents of Cocoa Butters from Different Origins.

	Malaysian cocoa butter %	West African cocoa butter %	Brazilian cocoa butter %
N20	81.2	76.2	66.3
N25	76.2	70.4	60.1
N30	54.8	45.1	36.9
N35	0.1	0.0	0.0

Source: Loders Croklaan, undated and reproduced with permission of Loders Croklaan.

Table 1-M. Effect of Heat Damage (Partial Interesterification) on Physical Properties of Cocoa Butter.

	Normal cocoa butter	Heat-damaged cocoa butter
Jensen cooling curve:		
Tmax (°C)	30.7	26.4
Tmin (°C)	25.8	23.9
Rise (°C)	4.9	2.5
Tmax (mins)	39	52
Solid fat content		
N20 (%)	77.7	52.7
N30 (%)	48.7	31.0
N35 (%)	1.3	5.2

Source: Timms & Stewart, 1999.

ter from the Americas generally contained slightly more unsaturates and slightly less saturates in total. This, in turn meant that these cocoa butters contained less SOS and more SOO/SLiS triglycerides. Furthermore, Asian cocoa butters contained, on average, 2–3% more StOSt and about 1% more POSt than did African cocoa butters. These differences will have an effect on the physical properties of the fat, notably on its melting profile (Table 1-L).

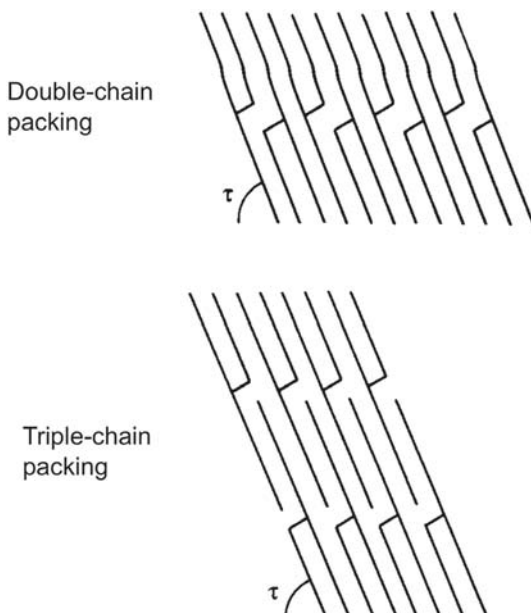
In addition to these naturally-occurring differences between cocoa butters, Timms and Stewart (1999) also showed that large differences can be seen between a normally processed cocoa butter and one that had been heat-damaged by holding for 1 hour at 250°C under vacuum. During that high temperature treatment some interesterification occurred increasing total SSS from 1.4% to 3.6% and reducing total SOS from 85.6% to 78.8%. The effect of this on the physical properties of the fat (Jensen cooling curve and solid fat contents) is shown in Table 1-M. More conventional deodorisation temperatures for cocoa butter (140–200°C), however, showed no such degradation in terms of the solid fat content.

Structure and Polymorphism of Cocoa Butter

Fats can crystallize in a number of different structures and hence are polymorphic. Cocoa butter and cocoa butter equivalents are amongst the most polymorphic of fats and much of this is down to their relatively simple triglyceride composition (about 80% of cocoa butter is made up of SOS triglycerides). The way in which fats crystallize is often defined in two ways. One is the “chain length packing;” the other is the crystal polymorphic form.

Triglycerides are triesters of glycerol and three fatty acid groups. When the structure of these are schematically drawn they are often shown as an “E” structure in which the glycerol backbone is the upright of the “E” and the three fatty acid groups are the three horizontal arms of the “E.” This, however, is wrong because it implies that the three fatty acid chains are all on the same side of the glycerol backbone. In reality, the fatty acid in the middle or *sn*-2 position comes off the glycerol backbone on the opposite side to the other two fatty acids. This is primarily for steric reasons—there is simply not enough room for three fatty acid chains to be together on the same side of the glycerol backbone. So, instead of having an “E” structure, triglycerides have a structure more akin to that of a tuning fork or a chair. Once that is realised then it is easy to go on to the next stage of deciding how individual triglyceride molecules pack together. In terms of “chain-length packing” there are two main ways—double chain packing and triple chain packing (Fig. 1.4).

Fig. 1.4. Schematic arrangement of triglycerides in the double-chain packing and triple-chain packing configurations. Reprinted with permission from Loders Crokiaan.

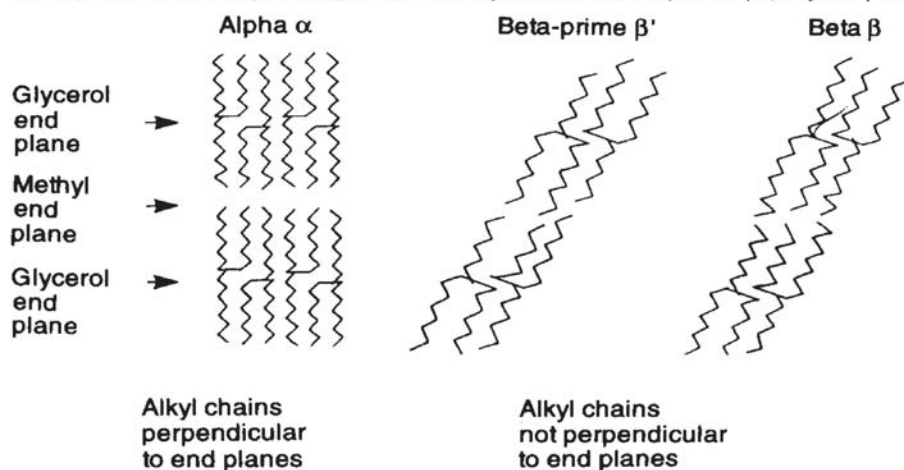


In double-chain packing the fatty acids in the sn-2 position (the middle position) of one triglyceride molecule are adjacent to fatty acids in the sn-1 and sn-3 positions of the next triglyceride molecule. In many fats this is a very stable structure. In cocoa butter and CBEs, though, it is not. This is because these fats have almost all of the oleic acid in the fat in the sn-2 position. Oleic acid is a *cis*-monounsaturated fatty acid and as such has a bend in the middle of the fatty acid chain. If it were to crystallize in a double-chain configuration then the bends would be next to straight chain saturated fatty acids in the sn-1 and sn-3 positions. This would give a “loose” structure and one that was not thermodynamically stable. In the triple-chain structure, however, all the fatty acids in the sn-2 position lie next to each other, as do all those in the sn-1 and sn-3 positions. Putting all the oleic acid groups next to each other allows all the bends in the chains to fit well together giving a much more stable configuration. For that reason, cocoa butter and CBEs all crystallize in a triple-chain configuration in their stable forms.

Polymorphism is very closely linked to this but is, nevertheless, somewhat different. As well as crystallizing in a particular chain length configuration, fats can crystallize in a variety of different crystal forms, polymorphic forms. The basic forms are called alpha (α), beta-prime (β'), and beta (β) but there are also sub-forms of these giving more than just three forms in some fats. As the polymorphic form changes from alpha to beta-prime to beta the crystal structure becomes more stable. Having said that, many fats are stable in the beta-prime form and rarely move into a beta form. Cocoa butter, though, is a beta-stable fat. The differences between the three basic forms are shown in Fig. 1.5. Cocoa butter can crystallize in more than these three basic forms. Exactly how many crystal forms cocoa butter has is the subject of some debate. For many years there were considered to be six polymorphic forms of cocoa butter. This was based on work done by Wille and Lutton (1966) and Larsson (1966). Work by van Malssen et al. (1999) suggested only five forms. Wille and Lutton (1966) gave the six polymorphic forms the number I through VI. Larsson (1966) and van Malssen et al. (1999) used the alpha, beta-prime, and beta nomenclature (as have most other studies). The polymorphic forms assigned by both Larsson (1966) and van Malssen et al. (1999) are compared with those of Wille and Lutton (1966) in Table 1-N along with the melting point of the various forms. The main differences are

- (a) van Malssen et al. (1999) found a lower melting form of cocoa butter than the earlier work in 1966 showed. They called this a gamma (γ) form. The lowest melting form found by Wille and Lutton (1966) and Larsson (1966) was the second least-stable form found by van Malssen et al. (1999).
- (b) van Malssen et al. (1999) found a range of beta-prime forms rather than just the two found by Wille and Lutton (1966)—forms III and IV—and the two found by Larsson (1966). In some ways, this would imply that there are many more than six polymorphs of cocoa butter. In other ways, it might suggest that there are only five forms if this range is considered to be just variants of a single form.

1. Projection showing arrangement of alkyl chains for α , β' and β polymorphs



2. Projection parallel to direction of alkyl chain (ie arrangement looking onto ends of chains)

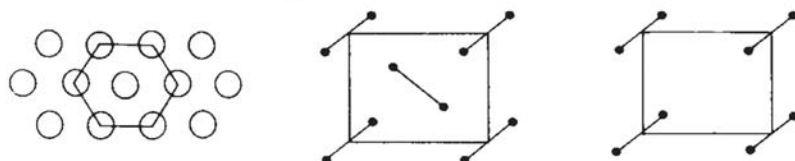


Fig. 1.5. Crystal packing of triglycerides. (1) Projection showing arrangement of alkyl chains for α , β' and β polymorphs. (2) Projection parallel to direction of alkyl chain (i.e., arrangement looking on to ends of chains). Reproduced with permission from Loders Croklaan.

Table 1-N. Nomenclature and Melting Points of Cocoa Butter Polymorphs.

Polymorphic form			Melting point ($^{\circ}\text{C}$)	
Wille and Lutton (1966)	Larsson (1966)	Van Malssen et al. (1999)	Wille and Lutton (1966)	Van Malssen et al. (1999)
I	β'_2	γ	17.3	-5 to +5
II	α	α	23.3	17–22
III	mixed	β' range	25.5	20–27
IV	β'_1		27.5	
V	β'_2	β -V	33.8	29–34
VI	β'_1	β -VI	36.3	

This is by no means the end of the story because work published after van Malssen et al. (1999) go back to suggesting there are six polymorphs (Sato & Koyano, 2001; Fessas et al., 2005). This, however, is meant purely as an introduction to the polymorphism of cocoa butter and the subject will be considered in much greater detail in Chapter 6.

Effect of Cocoa Butter Composition and Structure on Chocolate Composition and Structure

During the first part of chocolate production (mixing, refining, conching) all that is essentially required is to have some sort of fat present. The nuances of cocoa butter composition, be these differences in levels of unsaturation or in the presence of minor components, do not have a significant effect on these particular processes. In fact, coatings based on cocoa butter substitutes and containing very little cocoa butter go through exactly the same processes. Where the composition of cocoa butter and, indeed, in an even broader sense, the composition of the whole fat phase of the chocolate, plays a part is in the post-conching processes (tempering, flow characteristics, crystallization) and in sensory and storage characteristics of the chocolate.

Effect of Chocolate Fat Phase Composition on Chocolate Tempering and Rheology

Tempering is the process of inducing sufficient nucleation and crystallization of cocoa butter in form βV to “seed” the crystallization of the rest of the fat phase in this stable form. When chocolate is cooled very deeply and quickly, as it might be in an ice cream coating, it is likely that the cocoa butter will crystallize in one of the very unstable crystal forms (Forms I or II in Wille and Lutton (1966) nomenclature). Because the coating, assuming it is on ice cream, is then going to be held throughout its storage life at -18°C to -20°C no significant change will occur in the crystal form. Bearing in mind that there is still considerable debate about the exact number and types of the polymorphic forms in cocoa butter, if we stick with the original proposal of six polymorphic forms—Forms I to VI—then a range of transitions are possible within these six forms (Fig. 1.6). Various things are important to bear in mind when considering these transitions. One is that, although the βVI form is the most stable of the polymorphic forms it is not possible to crystallize cocoa butter directly from liquid into this form. It is possible to induce crystallization directly into βVI from solution in an organic solvent but that is hardly a viable process for the production of chocolate. The best that can be achieved is crystallization into βV . Since the transformation from βV into βVI is very slow under most storage conditions this is usually quite adequate for a stable chocolate (although when such a transformation does occur it is often accompanied by the formation of fat bloom).

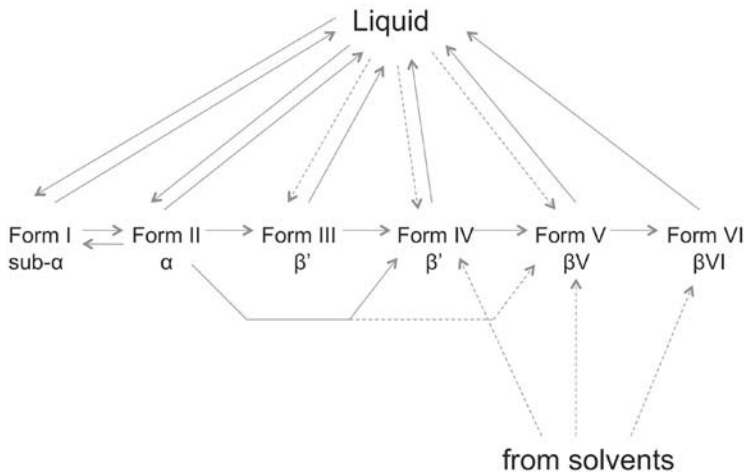


Fig. 1.6. Polymorphic transitions in cocoa butter.

When chocolate is tempered it is first cooled from molten, say at about 50°C, down to just over 30°C. At this temperature the chocolate is generally still fully liquid. However, if there are significant amounts of trisaturated triglyceride present in the chocolate then some of these may begin to crystallize out. They do not, however, contribute to temper as such. Cooling further to 26–27°C induces nucleation of both unstable β' forms and the stable βV form. Since the melting point of the unstable form is lower than that of the stable form, raising the temperature allows the unstable form to melt out again, leaving only the stable βV form as “seeds” for further crystallization of cocoa butter. The temperature to which the chocolate needs to be raised depends very much on the fat phase composition of the chocolate and, particularly how much milk fat is present. A plain (dark) chocolate will usually be raised in temperature to about 30–31°C depending on the origin of the cocoa butter (lower temperatures will be used for chocolate using South American cocoa butter than chocolate using Asian cocoa butter). A milk chocolate containing between 3.5% and 5% milk fat will be raised to a lower temperature (28–29°C) depending again on the origin of the cocoa butter and the actual level of milk fat in the chocolate.

It is not surprising—given the large differences in both composition and hardness between the two fats—that varying the ratio of cocoa butter to milk fat will have a large effect on tempering conditions. Smaller differences within the composition of the cocoa butter can also have a significant effect. Although the relative levels of POP and StOSt can vary from origin to origin in cocoa butter, adding a vegetable fat rich in POP can also change this ratio. Cebula et al. (1991) looked at coatings contain-

ing low, medium, and high levels of POP using both a batch tempering kettle and a three-stage continuous tempering unit. In the batch tempering kettle the coatings were held isothermally and seeded with polymorphically stable cocoa butter seeds. Samples were tested at regular intervals for state of temper until a plateau was seen on a tempermeter curve. As the POP content increased the time taken to achieve temper also increased from 22 minutes with the low POP system through 40 minutes for the medium POP system to 85 minutes for the high POP system. In the continuous tempering unit, the first stage was set to a fixed temperature ranging between 19°C and 24°C, the second stage was kept 1.5°C below the first stage temperature and the temperature of the third stage varied until temper was achieved. At a first stage temperature of 19°C, the third stage temperature needed to be adjusted to 30.5°C for the low POP system, 29.0°C for the medium POP system, and about 27.5°C for the high POP system in order to achieve temper. Changing the origin of cocoa butter and/or including vegetable fats with different POP levels can therefore mean that changes have to be made to tempering temperatures.

Varying diglyceride levels also had an effect on tempering, particularly batch tempering. Cebula et al. (1991) compared chocolate made with a particular fat phase with one in which the same fat phase had been passed through a silica column to remove all the polar material. The effect on batch tempering times was to reduce these from 51 minutes with the normal chocolate to 22 minutes with the one that had been silica treated. Effect on continuous tempering temperatures was less marked with the silica treated sample having a third stage temperature about 1°C higher than the normal chocolate.

Trisaturated triglyceride had less of an effect on batch tempering times (51 minutes for a moderate SSS chocolate; 56 minutes for a low SSS chocolate), and only minimal effect on continuous tempering temperatures (Cebula et al., 1991). Trisaturated triglycerides can, though have a marked effect on the rheology of chocolate. Unpublished work has shown that the effect on the yield value is greater than the effect on plastic viscosity. As the SSS of a supercoating (i.e., a coating containing a high level of CBE) is increased from 1.7% to 3.7% the effect on yield value of the coating untempered is negligible. However, at temper at 28°C, there is a large increase in yield value when the SSS level rises above about 2.7%.

Effect of Chocolate Fat Phase Composition on Chocolate Hardness and Other Sensory Properties

Various studies have been carried out on the relationship between the composition of chocolate and its hardness. Guinard and Mazzucchelli (1999) found a correlation between the hardness of chocolate and both sugar and fat levels:

Hardness =

$$50.72 + 0.54(\text{sugar}) + 2.32(\text{fat}) - 0.02(\text{sugar})(\text{fat}) + 0.01(\text{sugar})^2 + 0.02(\text{fat})^2$$

Talbot and Smith (2004) compared the hardnesses at 20°C of a range of chocolates with different fat phases with the solid fat contents at 20°C. The fat phases studied were (i) 100% cocoa butter, (ii) 90/10 cocoa butter/milk fat, (iii) 80/20 cocoa butter/CBI [A cocoa butter improver (CBI) is a CBE-like vegetable fat that is richer in StOSt than cocoa butter and so “improves” the performance of the cocoa butter in terms of hardness, heat resistance and bloom stability.], (iv) 80/20 cocoa butter/CBE, and (v) 80/20 cocoa butter/liquid vegetable oil. The relationship between chocolate hardness and N20 is shown in Fig. 1.7. The correlation is almost linear with an equation of:

$$\text{Hardness} = 1.37(\text{N20}) - 59.3 \quad (r^2 = 0.997)$$

An equally linear but different relationship was found between N20 of the fat phase and hardness of compound coatings based on nonlauric and lauric cocoa butter alternatives.

Afoakwa et al. (2008) studied the effects of both state of temper and particle size distribution, particularly the largest particle size (ranging from 18 microns to 50 microns). They found that chocolate hardness consistently decreased as the largest

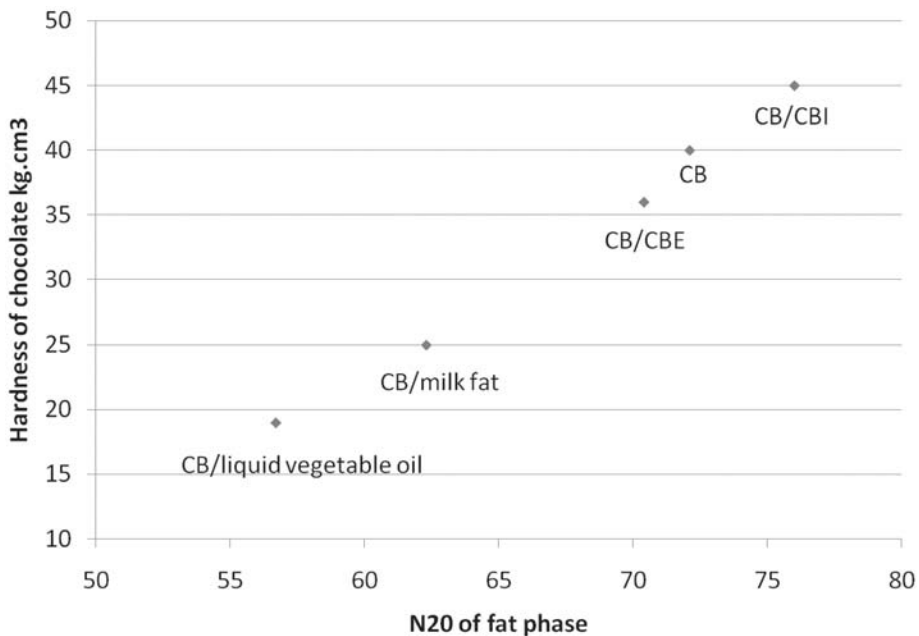


Fig. 1.7. Relationship between chocolate hardness and N20 of the fat phase. Source: Talbot & Smith, 2004.

particle size in the chocolate increased, irrespective of the state of temper. However, the state of temper of the chocolate had a marked effect on subsequent hardness measured at 20°C fourteen days after production. Undertempered chocolate (tempering unit set at 26–24–32°C) gave the hardest chocolate but bloomed. The next hardest chocolate was that produced by overtempering (tempering unit set at 18–16–32°C) with the least hard chocolate being the one that was optimally-tempered (tempering unit set at 21–19–32°C). As well as measuring hardness they also measured stickiness of the chocolate (the withdrawal force of the needle in a texture analyser) and found that the overtempered chocolate was stickiest and the undertempered chocolate the least sticky. As with hardness the stickiness decreased as particle size increased at all states of temper.

Vitova et al. (2009) studied the effect of the fat composition of dark chocolate on some of its sensory properties. They compared a dark chocolate containing only cocoa butter with one containing 5% vegetable fat and one in which all the cocoa butter had been replaced by vegetable fat. In terms of properties such as appearance and color, taste and aroma, texture, and overall evaluation there was no difference found between the samples without and with 5% added vegetable fat but the samples made without any cocoa butter were deficient in all of these parameters.

Effect of Chocolate Fat Phase Composition on Bloom Resistance and Formation

Fat bloom formation on chocolate will be considered in greater detail in Chapters 7, The Bloom Phenomenon—Physical Aspects, Morphology, and Problems Derived from the Bloom and 8, Morphology of Bloom. Suffice to say at this point that it is a phenomenon that is often linked to storage temperature and “mobility” or liquid oil content of the fat phase. In a well-tempered chocolate it is usually the result of a polymorphic change from βV to βVI . It occurs more rapidly at higher storage temperatures (where, as a result of partial melting, there is a greater proportion of liquid phase) or where liquid oil from a filling has migrated into a chocolate coating (again resulting in a greater proportion of liquid phase in the chocolate). Various fats can be added to chocolate to retard this polymorphic change. One of the simplest is to add milk fat and this is even added at low levels (up to 3% of the fat phase) to dark chocolate to help to give it some bloom resistance. Sonwai and Rousseau (2010) showed that chocolate containing 7.5% milk fat showed less than 20% transformation to βVI after four weeks 12-hourly cycling between 26°C and 29°C, whereas a chocolate containing no milk fat had completely transformed into βVI after this time. It is generally considered that it is the high-melting triglycerides in milk fat that inhibit bloom formation. Introducing the high-melting fraction into chocolate inhibited bloom compared with introducing the lower melting fractions which tended to accelerate bloom formation (Lohman & Hartel, 1994).

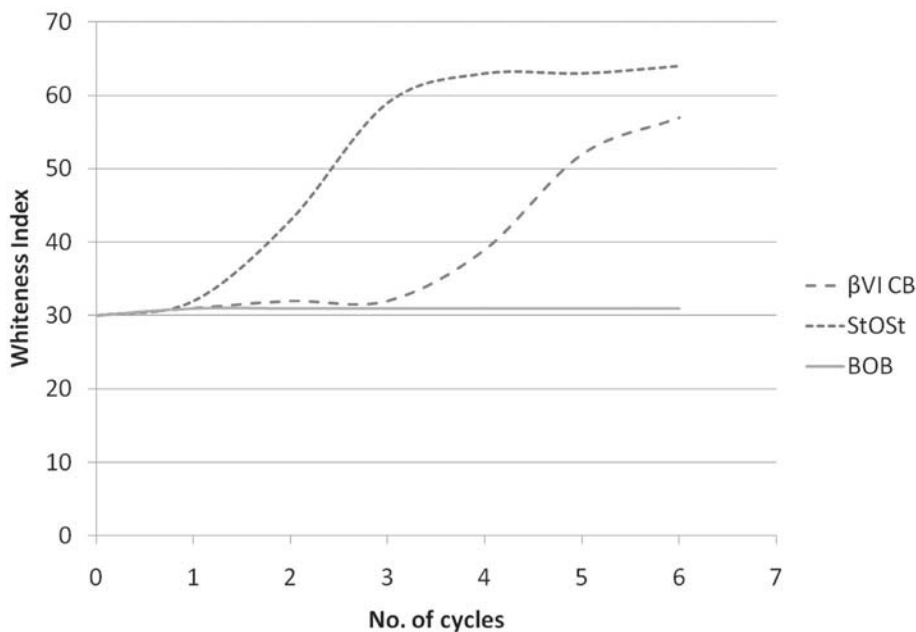


Fig. 1.8. Effect of seeding (2.5% level) on the whiteness index of dark chocolate cycled between 20°C and 32°C on a 12-hourly basis. Source: Hachiya et al., 1989.

Specific antibloom fats have also been developed that greatly retard the formation of fat bloom on chocolate. In the main, these have a composition based on H_2M and M_2H triglycerides, where H indicates a long-chain saturated fatty acid (C16 onwards) and M a medium-chain saturated fatty acid (C12–C14). These are produced by interesterifying a fat rich in C12–C14 (e.g., fully hydrogenated palm kernel oil) with one rich in C16–C18 (e.g., fully hydrogenated palm oil) (Cain et al., 1995). They can be added to chocolate at the 5% level as the vegetable fat in countries where these are allowed (but, note this can no longer be done in the EU because palm kernel oil is not on the list of permitted base oils for this application). Alternatively, they can be used in a filling fat and, as migration of liquid oil from the filling to the coating occurs they are partially carried along with it into the chocolate.

Sonwai and Rousseau (2006) compared chocolate made with cocoa butter and chocolate made with CBE in terms of their effects on bloom formation and polymorphic changes. They found no difference in the rate of formation of βVI whether CBE was present or not. It has been suggested, however, that CBEs containing higher melting types of SOS have a bloom-inhibiting effect and even a seeding effect on

chocolate. Hachiya et al. (1989) showed that adding 1,3-dibehenyl-2-oleoylglycerol (BOB) at a level of 2.5% to seed chocolate inhibited bloom much more effectively than adding the same level of β VI cocoa butter seed or StOst seed. After a week's storage at 20°C the chocolates were put through a week of cycling between 20°C and 32°C (12 hours at each temperature). The whiteness index, an indicator of fat bloom, shows the effectiveness of using BOB in this way (Fig. 1.8).

References

- Afoakwa, E.O.; Paterson, A.; Fowler, M.; Vieira, J. Effect of tempering and fat crystallization behavior on microstructure, mechanical properties and appearance in dark chocolate systems. *J. Food Eng.* 2008, 89, 128–136.
- Afoakwa, E.O.; Paterson, A.; Fowler, M.; Vieira, J. Fat bloom development and structure-appearance relationships during storage of under-tempered dark chocolate. *J. Food Eng.* 2009, 91, 571–581.
- All Business. Chocolate consumption increases. <http://www.allbusiness.com/retail-trade/food-stores/4489016-1.html> (accessed 10 November 2010).
- Beckett, S.T. Chocolate manufacture. In *Science and Technology of Enrobed and Filled Chocolate, Confectionery and Bakery Products*; Talbot, G., Ed. Woodhead Publishing: Cambridge, 2009; pp. 17–21.
- Cain, F.W.; Hughes, A.D.; Talbot, G. Chocolate-encapsulated fillings. U.S. Patent 5385744. 1995.
- CAOBISCO. Ranking of consumption: chocolate confectionery. http://www.caobisco.com/doc_uploads/Charts/ranking_of_consumption_chocolate_confectionery_2008.pdf (accessed 10 November 2010).
- Cebula, D.J.; Dilley, K.M.; Smith, K.W. Continuous tempering studies on model confectionery systems. *The Manufacturing Confectioner* 1991, 71(5), 131–136.
- Chaiseri, S.; Dimick, P.S. Lipid and hardness characteristics of cocoa butters from different geographic regions. *J. Am. Oil Chem. Soc.* 1989, 66(11), 1771–1776.
- Codex Alimentarius. Codex standard for cocoa butter. Codex Stan 86–1981, Rev 1-2001, 2001.
- Codex Alimentarius. Codex standard for cocoa (cacao) mass (cocoa/chocolate liquor) and cocoa cake. Codex Stan 141-1983, Rev. 1-2003, 2003.
- Davis, T.R.; Dimick, P.S. Lipid composition of high-melting seed crystals formed during cocoa butter solidification. *J. Am. Oil Chem. Soc.* 1989, 66(10), 1494–1498.
- European Union. Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption, 2000.
- Fessas, D.; Signorelli, M.; Schiraldi, A. Polymorphous transitions in cocoa butter—A quantitative DSC study. *J. Therm. Anal. Calorim.* 2005, 82, 691–702.
- Folayan, J.A. Nigerian cocoa and cocoa by-products: quality parameters, specification and the roles of stakeholders in quality maintenance. *Pakistan Journal of Nutrition* 2010, 9(9), 915–919.

- Foubert, I.; Vanbolleghem, P.A.; Thas, O.; Dewettinck, K. Influence of chemical composition on the isothermal cocoa butter crystallization. *J. Food Sci.* 2004, 69(9), E478–E487.
- Fowler, M.S. Cocoa beans: from tree to factory. In *Industrial Chocolate Manufacture and Use*, 4th Edition; Beckett, S.T., Ed.; Wiley-Blackwell: Oxford, 2009; p. 20.
- Guinard, J.-X.; Mazzucchelli, R. Effects of sugar and fat on the sensory properties of milk chocolate: descriptive analysis and instrumental measurements. *J. Sci. Food Agric.* 1999, 79, 1331–1339.
- Hachiya, I.; Koyano, T.; Sato, K. Seeding effects on solidification behavior of cocoa butter and dark chocolate. II. Physical properties of dark chocolate. *J. Am. Oil Chem. Soc.* 1989, 66(12), 1763–1770.
- ICCO. ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXVI, No. 3, Cocoa year 2009/2010. August 26, 2010.
- Jurriens, G. Analysis of triglycerides. In *Analysis and Characterisation of Oils and Fats and Fat Products*, Vol. 2; Boekennoogen, H.A., Ed.; Interscience Publishers: London, 1968.
- Larsson, K. Classification of glyceride crystal forms. *Acta Chem. Scand.* 1966, 20, 2255–2260.
- Loders Croklaan. Facts About Fats, No. 3. Wormerveer: The Netherlands, undated.
- Lohman, M.H.; Hartel, R.W. Effect of milk fat fractions on fat bloom in dark chocolate. *J. Am. Oil Chem. Soc.* 1994, 71(3), 267–276.
- Lonchampt, P.; Hartel, R.W. Surface bloom on improperly tempered chocolate. *Eur. J. Lipid Sci. Technol.* 2006, 108, 159–168.
- Padley, F.B.; Timms, R.E. Determination of cocoa butter equivalents in chocolate. *Chemistry and Industry* 1979, 2 December, 918–919.
- Sato, K.; Koyano, T. Crystallisation properties of cocoa butter. In *Crystallisation Processes in Fats and Lipid Systems*; Garti, N., and Sato, K., Eds.; Marcel Dekker: New York, 2001; pp. 429–456.
- Siew, W.-L.; Ng, W.-L. Influence of diglycerides on crystallisation of palm oil. *J. Sci. Food Agric.* 1999, 79(5), 722–726.
- Sonwai, S.; Rousseau, D. Structure evolution and bloom formation in tempered cocoa butter during long-term storage. *Eur. J. Lipid Sci. Technol.* 2006, 108, 735–745.
- Sonwai, S.; Rousseau, D. Controlling fat bloom formation in chocolate—Impact of milk fat on microstructure and fat phase crystallisation. *Food Chem.* 2010, 119, 286–297.
- Sridhar, R.; Lakshminarayana, G. Triacylglycerol compositions of some vegetable fats with potential for preparation of cocoa butter equivalents by high performance liquid chromatography. *J. Oil Tech. Assoc. India* 1991, 23, 42–43.
- Talbot, G. *Application of Fats in Confectionery*. Kennedy's Publications: London, 2006.
- Talbot, G.; Smith, K.W. Prediction of the hardness of chocolate and compound coatings. *Kennedy's Confection*, 2004, July, 22–24.
- Tietz, R.A.; Hartel, R.W. Effects of minor lipids on crystallization of milk fat–cocoa butter blends and bloom formation in chocolate. *J. Am. Oil Chem. Soc.* 2000, 77(7), 763–771.

- Timms, R.E.; Stewart, I.M. Cocoa butter: a unique vegetable fat. *Lipid Technology Newsletter*, 1999, October, 101–107.
- Van Malssen, K.F.; van Langevelde, A.J.; Peschar, R.; Schenk, H. Phase behavior and extended phase scheme of static cocoa butter investigated with real-time X-ray powder diffraction. *J. Am. Oil Chem. Soc.* 1999, 76, 669–676.
- Vitova, E.; Loupancova, B.; Stoudkova, H.; Macku, I.; Zamoanova, J.; Babak, L. Effect of fat composition on some physico-chemical parameters and sensorial evaluation of dark chocolate. *J. Food Nutr. Res.* 2009, 48(2), 72–79.
- Wille, R.L.; Lutton, E.S. Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* 1966, 43, 491–496.
- Yates, P. Formulation of chocolate for industrial application. In *Science and Technology of Enrobed and Filled Chocolate, Confectionery and Bakery Products*; Talbot, G., Ed.; Woodhead Publishing: Cambridge, 2009; pp. 17–21.
- Ziegler, G. A greater understanding of your conching process. *ZDS Chocolate Technology Conference 2004*, 14–15 December, Cologne, Germany.

2

Theobroma cacao—An Introduction to the Plant, Its Composition, Uses, and Health Benefits

César Vega and Catherine Kwik-Uribe

Mars, Incorporated, McLean, VA, USA

Introduction

Cacao played a pivotal role in the economic, social, religious, and political life of pre-Hispanic Maya. A frothy cacao drink was served to royal newlyweds and the seeds were offered to the bride's family as a sign of respect from the part of the groom. Cacao seeds were so valuable to Maya (just as jade beans and feathers from the quetzal bird) that they were a strong unit of currency. It follows that cacao was not accessible to society-at-large, being only available to those with power and influence. The scientific name of cacao, *Theobroma cacao* (i.e., food of the gods) was definitely appropriate and remains a testament to its past (Vail, 2009).

Today, cacao is more commonly referred to as cocoa—a widely traded commodity that continues to be key to the economic and social development of some regions in the world like Côte d'Ivoire, Ghana, or Indonesia where most of the world's annual crop comes from. However, the importance of cocoa to contemporary society goes well beyond the areas where it is cultivated, mainly because without cocoa, there wouldn't be chocolate. Except for sugar or milk solids, cocoa contains all the components that make chocolate what it is, although not all in the right proportions. Cocoa butter for example, makes up to 50% of the weight of dried cocoa beans and about 30% in regular dark chocolate (approx. 55% cocoa mass).

Cocoa's public image—mainly as chocolate—has also significantly and positively evolved over the past few decades as research has demonstrated its association with a range of health benefits. The aim of this chapter is to introduce the reader to the lengthy and chemically complex processes that cocoa undergoes to render the variety of raw materials that enable among others, the chocolate industry. In doing so, the focus will be on those steps that more significantly affect its chemical composition and functionality, particularly when it comes to the putative health benefits of cocoa (see below).

Cacao and Cocoa—From Tree to Factory

Cacao is at the heart of chocolate and it is the reason for its unique sought after flavor and melt-in-mouth characteristics. However, it takes a considerable amount of effort and chemistry, to transform an otherwise inedible cocoa bean into a delicious treat. The cocoa tree (*Theobroma cacao*, L.) originated from Central and South America (book) where it was cultivated and the seeds of the its fruit consumed by the Preclassic Mayan civilization as early as 600 BC (Hurst, Tarka et al., 2002). Olmec, Mayan, and Aztec peoples enjoyed cocoa as a drink seasoned with chili, vanilla, honey, and annatto (Dircks, 2009). The discovery of America and the subsequent era of colonialism facilitated the migration of the fruit to different parts of the world—in the mid 1500s the Dutch introduced cocoa to Indonesia; in the early 1600s Spain did the same in the Philippines and the West Indies and, in the 1800s, the Spanish, British, and French began cocoa production in West Africa (thesis). However, it wasn't until the 19th century that cocoa was consumed as a solid food by the general public. Before then, it was a liquid concoction, at times sweetened, and available only to the social elites.

The geographical expansion of cocoa meant that the distinctive attributes of the bean, such as size, flavor, color, chemical composition (e.g., fat, polyphenol, alkaloid content) vary considerably in beans of different origins. Historically, the two main types of cocoa are known as Criollo (*T. cacao* var. *cacao*) and Forastero (*T. cacao* var. *sphaerocarpum*). The Criollo cocoa variety is recognized for its light color and mild, nutty flavor, as well as its susceptibility to disease and low production yields. Today, this variety is very rare, with only a few plantations still surviving in places like Venezuela or Madagascar (Fowler, 2009). The Forastero variety of cocoa produces beans that are of dark brown color, more strongly flavored, slightly bitter, and possess a higher fat content compared to Criollo beans. The greater part of the world's cocoa crop has long been considered to be of the Forastero type, more specifically a sub-type known as Amelonado which migrated from Brazil to West Africa in the nineteenth century (Wood, 1991). A third type is known as "Trinitario" which is basically a hybrid of Criollo and Forastero crosses and consequently produces cocoas with special flavors. Last but not least is what is known as "Nacional" which is only grown in Ecuador which is recognized for having an enhanced floral character. Though the type of bean plays an important role in determining flavor, the ultimate flavor of chocolate is a consequence of a complex array of chemical reactions that start from the moment the beans is harvested through the time it is processed into finished chocolate.

The successful cultivation of cocoa requires a special climate that is mostly found within the area bounded by the Tropics of Cancer and Capricorn (10–20° north and south of the Equator). Most trees grow from sea level to an altitude of less than 300 meters. Optimal temperatures lie within the band of 18–30°C (65–86°F) and rainfall of a minimum of 1000–1500 mm must be evenly distributed during the year (Dircks 2009; Fowler 2009). Cocoa is a delicate crop and its trees need to be

Table 2-A. Global Production of Cocoa Beans (thousands of metric tonnes and %).

Region/ Years	1980/ 81	1990/ 91	2000/ 01	2007/ 08	2008/ 09	2009/ 10	2010/ 11
Africa	1010 (60)	1418 (57)	1948 (68)	2682 (72)	2442 (70)	2483 (68)	3100 (74)
Asia and Oceania	97 (6)	487 (17)	487 (17)	602 (16)	590 (17)	633 (17)	559 (13)
Central/ South America	542 (32)	611 (24)	369 (13)	400 (11)	383 (11)	517 (14)	536 (13)
Total world production	1696	2506	2857	3735	3467	3632	4195

Source: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXVII, No. 3, 2010/11.

protected from strong winds as it has a rather weak root system; the soils must be well aerated, and pests and diseases must be carefully controlled. The global production of cocoa has steadily increased in the last three decades, growing at a rate of approx. 2.2% from 1998 to 2010 and the largest cocoa-producing countries are Côte d'Ivoire and Ghana, as together they account for close to 70% of the world crop. Table 2-A describes global cocoa production in the last three decades (<http://www.worldcocoaoundation.org/learn-about-cocoa/cocoa-facts-and-figures.html>). However, the cultivation and harvesting of cacao beans is seriously threatened mainly by fungal diseases such as black pod, frosty pod, and witches' broom. Similarly, cocoa is an 'orphan crop' in the sense that governments where it is cultivated do not invest in sustainable agricultural programs that enable and incentivize farmers to increase the yields and profitability of their crops. However, the fact that the cocoa genome has been recently elucidated will accelerate the release of robust *T. cacao* cultivars (Risterucci, Grivet, et al., 2000; Argout, Salse, et al., 2011; Feltus, Saski, et al., 2011; Saski, Feltus, et al., 2011).

Today, it typically takes up to four years for cocoa trees to become productive (an improvement to the common six to seven years of the past) and they can stay productive for 25–30 years (Dircks, 2009; Meursing, 2009). Mature pods, those that measure between 10–15 cm at the center, and are 15–25 cm long, are harvested twice a year as pollination-to-maturity cycle takes about six months. The pod contains some 40 seeds or beans that, after fermentation and drying, make up about 40 g of beans, i.e., an average of 1 g per bean. Yields per hectare have risen over time from around 350 kg to above 500 kg. Today, cocoa trees are cultivated in more than 40 countries around the world, across an estimated area of 3.6 million hectares, producing an annual crop of more than 4.0 million tons of dried beans ready for processing.

Harvesting

When pods ripen, they change from a green or red color to a yellow or orange color, depending of the variety. The crop doesn't ripen at the same time and as such, pods are harvested every 2–4 weeks during the harvest period. Typically, the harvesting occurs in two major periods referred to as *main* and *mid* crops. The time of the year these occur varies from region to region, for example: in West Africa, the main crop occurs from early October to mid December whereas for Indonesia, it takes place from April to June. The main crop is larger and generally of higher quality. Pods are cut directly from the trunk of the tree and then cracked open to release the 30–45 beans contained within, along with the sweet and white mucilaginous pulp that surrounds them.

Each bean consists of two cotyledons and a small germ or embryo all enclosed in a skin or testa (the shell). The cotyledons serve both as the storage organs containing the food for the development of the seedling and as the first two leaves of the plant when the seed germinates. Much of the energy stored in the cotyledons is stored as fat, specifically cocoa butter which represents close to 50% of the weight of the dry seed. The composition of fresh cocoa beans and pulp is shown in Table 2-B.

Fermentation

In traditional cocoa processing, the immediate step following the harvest is fermentation. Fermentation is a critical period as it is when some of the flavors of cocoa begin to develop. This step relies on the action of the native microflora upon the fermentable sugars in the pulp and the action of enzymes on carbohydrates, proteins

Table 2-B. Proximate Analyses of Fresh Cocoa Beans.

Component	Average concentration (% w/w)	
	Pulp	Seed
Water	80–85	35–45
Lipids	<0.5	45–55
Sugars (sucrose, glucose and fructose)	10–16	0.5–2
Polysaccharides	1.5–3.0	14–20
Pectin	4–7	2.0
Protein	0.6	1.5
Organic acids	1–3	0.3–0.9
Inorganic salts	0.5–1.0	0.5–1.0
Polyphenols	<0.1	7–10
Alkaloids (theobromine and caffeine)	<0.1	3.5

Adapted from Ardhana & Fleet, 2003; Rosane & Alan, 2004; Camu, De Winter, et al., 2008; Dircks, 2009.

and polyphenols in the cocoa beans as to enable the future development of proper color and flavor (Camu, De Winter, et al., 2008). The actual set up of the beans for fermentation process is a simple one (the fermentation process *per se*, is rather complicated)—for example, in West Africa, the world's largest cocoa growing area, farmers place the pulp-covered beans on the ground, cover them with layers of leaves (often banana), and allow the heap to ferment for four to seven days, depending on the variety of the bean, and with intermittent stirring as to ensure even fermentation.

During the first stage of fermentation, the pulp surrounding the beans, rich in fermentable sugars and pectins, is broken down by the action of anaerobic yeasts which drops the pH to about 5.5 and facilitates the diffusion of oxygen into the bean mass (Dircks, 2009). As fructose and glucose from the pulp are converted into ethanol and acetic acid (with a concomitant increase in temperature), they diffuse into the seed breaking down the cellular structure of the cotyledon causing the death of the embryo. This triggers a series of reactions involving endogenous cocoa enzymes and the previously compartmentalized substrates (see Fig. 2.1) that will render many of the desired precursors of the cocoa and chocolate flavors and colors (Camu, De Winter, et al., 2008; Meursing, 2009). For example:

- a) Proteases break down storage proteins, forming flavor-active peptides and amino acids that in the presence of glucose and fructose, will enable the Maillard reaction (Voigt, 1995);
- b) Polyphenol oxidase (EC 1.14.18.1) decreases the bitterness and astringency from the bean by oxidizing what are known as cocoa polyphenols, in particular, flavanols and procyanidins (Misnawi, Jinap, et al., 2003);
- c) Glycosidases act on anthocyanins as to bleach off their purple color into the more characteristic brown of fermented beans (Hansen, del Olmo, et al., 1998; Dircks, 2009). Of note is that these stages are not discretely separated from one another, but rather they overlap and so do the type of microorganisms active at any given time during fermentation.

Related to the structural breakdown of the bean as the above processes occur, the report by (De Brito, 2000) is very illustrative. Cocoa seeds were observed through a light microscope as fermentation and roasting proceeded. The study revealed that anionic residues, such as pectin-like substances from the cell walls, as well as a high number of phenolic bodies were abundant at $t=0$ [Fig. 2.1(a)]. Within the first 48 h of fermentation, d, polyphenols were observed to have diffused throughout the whole cotyledon. After 72 h, no phenolic material could be detected [Fig. 2.1(b)]. Dried and roasted material still contained a large number of ruptured cells as compared to the material at the start of fermentation [Fig. 2.1(a) and 2.1(b)]. Proteinaceous material was quite dispersed in the seed cytoplasm and it diffused as proteolysis took its course and, after 72 h of fermentation, almost no protein material was detected [Fig. 2.1(c) and 2.1(d)]. The decrease in staining intensity was probably related to the

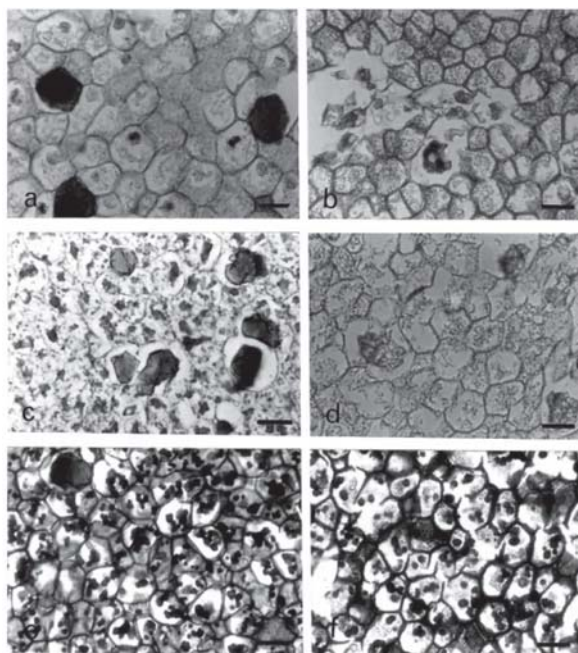


Fig. 2.1. Cocoa cotyledons at different rimes and processing stages. (a) at $t=0$, the presence of phenolic bodies is obvious; (b) roasted cocoa bean where the presence of damaged cells and the absences of phenolic bodies can be observed; (c) at $t=0$, note the proteinaceous material dispersed in the cytoplasm; (d) at $t=72\text{h}$ of fermentation, there is a lack of proteinaceous material; (e) at $t=0$, stained cytoplasmic material (starch and glycoproteins) and cell walls (cellulose); (f) dried cocoa, stained starch granules. Bar = 20 mm. Reproduced with permission from De Brito, 2000.

formation of smaller, more soluble peptides, not detectable by the assay. Seeds stained positive for cellulose, grains of starch and glycoproteins were seen in the cytoplasm [Fig. 2.1(e)]. Drying and roasting increased the number of damaged and disrupted cells [Fig. 2.1(f)]. PAS staining and polarised light microscopy showed the presence of starch at the beginning of fermentation. With the loss of protein material and cell disruption the starch grains became increasingly evident, particularly after roasting.

In spite of the critical role of fermentation in developing the flavor and determining the final quality of cocoa beans, it is usually performed in an uncontrolled manner, yielding unpredictable results (Dircks, 2009). Hence, there is a need to better correlate specific microbial activity to flavor development and quality of the beans.

Following fermentation, beans must be dried in order to extend their shelf life, a critical step given the distance that the beans must travel prior to their conversion to a range of ingredients and food products. This can be done either by sun or artificial drying methods. Sun drying is environmentally friendly, low cost, and produces beans of

Table 2-C. Changes in the Chemical Composition of Pulp and Bean Fractions During Cocoa Fermentation of Indonesian Forastero Type Beans.

Component	Concentration (mg/g)			
	Pulp		Bean	
	Before	After	Before	After
Fructose	62	11	1.0	0.4
Glucose	41	7.0	0.7	0.1
Sucrose	32	0.0	19	0.0
Ethanol*	0.5	0.1	0.2	0.4
Citric acid	24	11	9.0	4.0
Lactic acid	0.3	6.0	0.1	2.0
Acetic acid	0.4	12	1.0	25
pH	3.7	3.9	6.3	5.1

* (% w/w).

Adapted from Ardhana & Fleet, 2003.

good quality however, depending on the reigning climate; it cannot always be a reliable method. It usually takes about a week of sunny weather to reduce moisture content from over 60% to less than 8%, the water content needed to prevent mold growth during transport and storage. Care must be practiced not to over-dry the beans (below 6% moisture) as they become quite brittle and are easily damaged during subsequent handling (Table 2-C).

Industrial Processing of Cocoa

Cleaning, Breaking, and Winnowing

The transformation of the fermented and dried bean into cocoa powder and chocolate starts at the manufacturing site with the following three steps: cleaning, breaking, and winnowing. The objective of these steps is to obtain clean, broken, and de-shelled kernels (nibs). First, the beans are sieved, and foreign matter such as bamboo, twigs, string, stones, and magnetic materials is removed. The clean beans are then broken to loosen the shells from the nibs. After the breaking step, broken shell is removed by a stream of air (winnowing) (Meursing, 2009).

Sterilization and Alkalization

The microbiologically contaminated nib is sterilized in a batch or a continuous process by wetting and heating with steam. The total plate count (TPC) is normally reduced to less than 500 cfu/g, and pathogenic bacteria are killed. After sterilization, the nib can be immediately roasted (natural process) or alkalized (Dutch process).

Alkalizing or dutching consists of treating the cocoa nibs with an alkali solution such as potassium or sodium carbonate. It is practiced primarily to modify the color and flavor of cocoa powder or cocoa liquor; as the high pH promotes the formation of flavor and color. Alkalization can be conducted at various points in the production process. Depending on the stage at which alkalization takes place, different results will be obtained. Nib alkalization is often preferred, as it combines optimal flavor and color development with minimal alkali usage (Ziegleder, 2009).

Roasting

During roasting—exposing the nibs to temperatures ranging from 95–145°C (200–295°F) depending on the process, equipment, type of nib processed, and the end product required—the aim is to reduce the water content and continue developing flavor as to maximize the potential of the flavor precursors that developed during fermentation. Thanks to its relative high temperatures, roasting causes an additional reduction in the number of microorganisms ensuring the safety of the final ingredient.

Most of the various compounds found in the flavor of cocoa are generated by the Maillard reaction. This reaction(s) play a major role in all food preparations in which the flavor is developed by a heating process like baking, frying, or roasting (Lersch, 2012). Aldehydes and pyrazines in particular, are considered to be important for the characteristic flavor of cocoa. They form via complex reaction pathways that start with the condensation reaction between a *reducing* sugar like glucose or fructose and a compound with an amino group like a protein. The resulting compounds are known as Amadori compounds. As roasting proceeds, Amadori compounds further react and are converted into di-carboxylic compounds by removal of H₂O. These compounds reduce the α -amino acids into aldehydes, and during further dehydration, the heterocyclic components like pyrazines are formed. In cocoa, all these reactions are facilitated by the relative large amounts of glucose and fructose present in

Table 2-D. Changes in the Chemical Composition of Ghanaian and Ecuadorian Beans Before and After Roasting.

	Concentration (mg/g)			
	Ghanaian		Ecuadorian	
	Before	After	Before	After
Saccharide				
Fructose	4.2	0.6	1.7	0.6
Glucose	0.62	0.05	0.85	0.1
Sucrose	1.6	1.4	4.8	6.2
Raffinose	0.02	0.03	0.6	0.75
Stachyose	0.75	0.55	1.0	1.1

Adapted from Redgwell, 2003.

the bean (see Table 2-D). Generally, cocoa roasting conditions are rather mild. The product temperature at the end of the roasting process should not exceed 110–120°C (230–248°F), and the final moisture content should be between 1% and 2%. If the roasting is overdone, volatile components like aldehydes, esters, and low molecular acids like butyric acid will be removed, leaving only the pyrazines and the non-volatile acids. This results in a burnt and/or acidic flavor.

Nib Grinding

The roasted nib is typically ground in a multi-stage process where the broken kernels change from a solid to a fluid mass of cocoa particles suspended in cocoa butter. This is due to the high fat content of the bean: about half of the nib is fat. Grinding breaks up the cell structure of the cocoa nibs and releases the cocoa butter. This finely ground fluid mass is known as cocoa liquor, which when allowed to harden forms what is more commonly known as baking chocolate.

Pressing

Cocoa butter constitutes about half the weight of the cocoa nib. This fat is partially removed from the cocoa liquor by means of hydraulic presses applying pressures as high as 450 kg/cm². Depending upon the pressing time and the setting of the press, the resulting cakes may have a fat content of 10 to 24%, however most commercially available powders are between 10–12%.

Cocoa Cake

After pressing, the cakes are broken into kibbled cake. The pressing operation is susceptible to the attack of microorganisms as this is not a closed-environment process. Hygienic procedures are therefore of particular importance in the pressing department. Kibbled cake is typically stored by fat content and degree of alkalization and may be blended before pulverization to obtain the desired type of cocoa powder. The cocoa butter is filtered and stored in tanks.

Cocoa Powder

The powder grinding lines pulverize cocoa cake particles into a specified particle size distribution. After pulverization, the powder is cooled so that the fat of the cocoa powder crystallizes into its stable form. This prevents any discoloring (fat bloom) and/or lump formation during storage. Next, the free-flowing powder is passed through sieves and over magnets prior to packing in paper bags or in bulk containers. Cocoa powder accounts for approximately 29% of the weight of dry bean entering a cocoa processing plant (see Fig. 2.2).

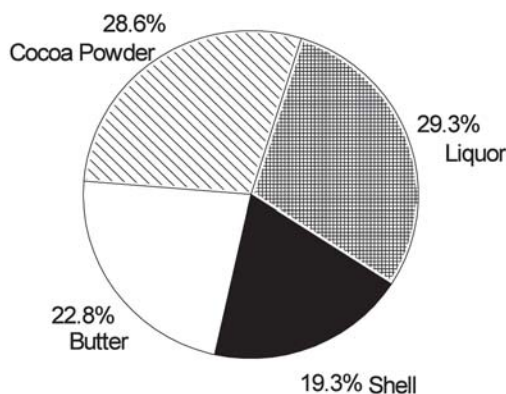


Fig. 2.2. Typical by-products of the cocoa processing operation. The numbers represent the % w/w of each by product from an input of fermented and dried cocoa beans. The % of cocoa butter seems small however, recall that cocoa powder and liquor are approx. 10 and 50% cocoa butter, respectively. Based on data obtained in Meursing, 2009.

Cocoa Butter

The cocoa butter from the presses is filtered and stored. Upon request, the butter can be partially or fully deodorized. Delivery of the various types of cocoa butter can be either in liquid form or in solid form. The *total* cocoa butter contained in the bean (approx. 50% of dry and de-shelled bean weight) get re-distributed after cocoa processing among the powder, the liquor and pure cocoa butter—approx. 8, 37, and 55%, respectively (see Fig. 2.2).

Cocoa–Chemical Composition and Health

Cocoa products, particularly chocolate, have long been enjoyed as decadent, indulgent treats. The quintessential organoleptic properties of chocolate—most notably its taste and melt are recognized to be a result of the special fatty acid composition of cocoa butter. What is less generally appreciated is the diverse and complex chemical composition of the cocoa bean, and of food products made from this tropical seed. While cocoa products, and chocolate itself, have been subject to criticism for being too calorically dense and of limited dietary value, several hundred scientific papers have been published over the past decade which closely examine the potential health benefits related to the consumption of chocolate products, specifically of some particular chemical constituents within cocoa. Though the science is still emerging, a review of the published scientific evidence by the 2010 Dietary Guidelines Advisory Committee concluded that there was moderate evidence in support of the idea that the “modest consumption of chocolate or cocoa is associated with health benefits in the form of reduced cardiovascular disease risk” (2010). This is certainly not a dietary

Table 2-E. General Composition of Minimally Fermented Cocoa Beans and Baking Chocolate.

Component	Minimally fermented cocoa beans*	Typical whole dried cocoa beans
Fat	54–63%	48–57%
Protein	18%	10–16%
Carbohydrate	9–20%	27–30%
Fiber	5.6–5.9%	15–17%
Moisture	5.0–5.3%	2–5%
Theobromine	2.7–2.9%	0.8–1.4%
Caffeine	n.d.	0.1–0.7%
Flavanols and procyanidins	9.5%	1.6%
Ash	3.2–4.2%	2.6–4.2%

*up to 3 days fermentation; n.d. not determined.

Sources: Aremu, Agiang, et al., 1995; Beckett, 2009; Gu, Kelm, et al., 2002; Bertazzo, Agnolin, et al., 2011; USDA Proanthocyanidin Database, 2004.

recommendation (or endorsement) for daily chocolate consumption; however, it is encouraging recognition for the complex nature of cocoa and the potential role that the particular constituents in cocoa based products may play in health.

Overall Chemical Composition

The cocoa seed itself contains a rich and complex mixture of macro-, micro-, and phyto-nutrients. In Table 2-E, the range in macronutrient and select phytonutrient composition of a *minimally* fermented cocoa bean and typical, fermented, whole dried bean is reported. As expected, fat makes up over half the weight of a typically cocoa bean, but in addition, the bean contains significant amounts of protein (10–15%) and carbohydrates (27–30%), of which, around half is fiber.

Lipids

The lipid component of cacao is known as cocoa butter, a solid fat at room temperature that contains a mixture of saturated and monounsaturated fats, and to a much lesser extent, polyunsaturated fats. The predominant saturated fat is stearic acid (C18:0), followed by palmitic acid (C16:0). Oleic acid (C18:1), the same fat found in olive oil, is the next most abundant fatty acid, making roughly 1/3 of the lipid composition. Polyunsaturated fats, primarily linoleic (C18:2 n6) and linolenic acid (C18:3 n3), account for typically 5% or less of the fat in cocoa butter. Plant sterols such as β -sitosterol, campesterol, and stigmasterol have also been found in cocoa, usually at levels in the range of 0.1% by weight.

Though high in saturated fat, the specific chemical composition of cocoa butter seems to lend itself to produce less detrimental effects on serum lipids in contrast to other hard fats (Mensink, 2005). Human studies on the effects of stearic acid (typically in the form of cocoa butter) on serum cholesterol have generally found that the consumption of foods containing stearic acid did not negatively affect serum cholesterol levels (Ding, Hutfless, et al., 2006). Relative to other hard fats, stearic acid-containing fats did not significantly raise serum cholesterol levels, and have not been shown to negatively impact HDL cholesterol levels. In this specific context, the 2010 Dietary Guidelines Advisory Committee acknowledged these special properties of stearic acid and recommended that it in fact be considered distinct from other saturated fats which are cholesterol raising (2010). As a result, stearic acid, and stearic acid-containing foods such as chocolate and cocoa powder are regarded as non-atherogenic, and cholesterol neutral, and thus the inclusion of moderate amounts of cocoa-based products in the context of a balanced diet would not be expected to be detrimental to health, and may in fact, help to support health relative to diets containing other forms of saturated fat.

Fiber

When considering the cocoa bean, both the husk and seed itself contain fiber constituents. The cocoa fiber is largely insoluble (Lecumberri, Goya, et al., 2007), but also contains a proportion of soluble fiber. Soluble fiber itself is known to prevent and slow down the absorption of cholesterol. Insoluble fibers have been reported to have a range of potential health effects including support weight maintenance/loss, ameliorating constipation, promoting gut health, including potential prebiotic effects (Anderson, Baird, et al., 2009). Taking as reference the fiber content of both cocoa powder and dark chocolate (60–69% cacao) products, 2 tablespoons of cocoa powder (~10 g, approximately 20 calories) or 20 g of dark chocolate (~110 calories) can provide 3 g and 2 g total fiber (8–10% of the DV).

Minerals

Cacao itself does contain several minerals important to overall human health. Examination of commercially available cocoa products, specifically cocoa powder and baking chocolate (100% cocoa liquor) demonstrates that in addition to these macronutrients, these cocoa products are a reasonable dietary source of a range of micronutrients (Table 2-F). Though one would be hard pressed to readily consume 100 g cocoa powder, the inclusion of 2 tablespoons of cocoa powder (10 g, 20 calories) could easily provide a good source of iron (1.4 mg, ~17% DRI), copper (0.4 mg, ~42% DRI), manganese (0.4 mg, ~17% DRI), phosphorus (73 mg, ~10% DRI), and magnesium (50 mg, ~13% DRI). Considering 20 g of dark chocolate (60–69% cacao), 110 calories would provide 1.2 mg of iron (15% DRI), 35 mg magnesium (9% DRI),

Table 2-F. Select Micronutrients and Alkaloids In Cocoa Powder and Baking Chocolate.

Component, per 100 g	Cocoa powder	Baking chocolate
Fat, g	13.7	52.3
Protein, g	19.6	12.9
Carbohydrates, g	57.9	29.8
Fiber, g	33.2	16.6
Potassium, mg	1524	830
Phosphorus, mg	734	400
Magnesium, mg	499	327
Iron, mg	14	17
Zinc, mg	6.8	9.6
Copper, mg	3.8	3.2
Manganese, mg	3.8	4.2
Caffeine, mg	230	80
Theobromine, mg	2057	1297

Data taken from USDA Nutrient database.

and 0.26 g copper (29% DRI). Thus, in the context of a balanced diet, these cocoa-derived foods not only contribute calories, but can add to the total dietary needs through the provision of essential nutrients as well.

Alkaloids

Cocoa contains several different alkaloid constituents. Most notable is the presence of theobromine, and to lesser extent, caffeine. Very low levels of theophylline have also been reported (Thomas, Yen, et al., 2004). Theobromine is reported to be present in the range of 1–2.9% by weight and, given its high stability to food processing operations, comparable levels are reported in cocoa powder (2.1% by weight) and baking chocolate (1.3% by weight) (USDA Nutrient Database). The name theobromine itself pays homage to the plant in which it was first discovered in 1841 by Alexander Woskresensky, *Theobroma cacao* (Bibra, 1995). Though theobromine is found in several plants, the primary sources of theobromine in the human diet are chocolate and cocoa powder. The abundance of this compound in cacao has also lent itself to anthropological studies as the use of cacao in food and life rituals in ancient cultures has been documented based on the detection of residual levels of theobromine in vessels (Powis, Cyphers, et al., 2011).

Relative to the much more recognized (and consumed) alkaloid—caffeine, theobromine is a much weaker bioactive. Theobromine is a vasodilator, a diuretic and

heart stimulant. Because of its diuretic effects and vasodilating capacity, it has been used to treat high blood pressure and other vascular conditions (Smit, 2011). However, it is important to consider the distinction between the effects of pure theobromine applied in a clinical context versus the effects of theobromine in foods. Multiple acute and short term published intervention studies have not found that the consumption of theobromine via cocoa-based products providing 50–150 mg theobromine (equivalent to what is contained in 1 tablespoon of cocoa powder or 20 g of dark chocolate) resulted in any consistent or significant changes in blood pressure or heart rate. A recent study however, did find that the consumption of highly enriched levels of theobromine in a cocoa matrix (979 mg; equivalent to nearly 50 g of cocoa powder or 150 g of dark chocolate) resulted in a significant increase in systolic blood pressure and heart rate (van den Bogaard et al., 2010). Thus, it appears unlikely that in the context of the conventional diet, that the theobromine found in cocoa based products will directly lead to any significant changes in cardiovascular function.

Caffeine is present at typically one tenth the level of theobromine, with cocoa powder and chocolate found to contain approximately 0.23% by weight (about 12 mg per tablespoon) and baking chocolate at 0.08% by weight (about 25 mg per 1 oz square). Caffeine is a stimulant, affecting the central nervous system. It is the stimulant properties of caffeine that are responsible for its ability to improve cognitive function, in particular alertness and focus, as well as improve physical performance (reviewed in (Glade, 2010). Furthermore, in recent years, there has been increasing evidence that the moderate consumption of caffeine containing products (most typically coffee, in the range of 1–2 cups per day) is associated with improved health outcomes, including reduced risk of stroke (Larsson, Virtamo, et al., 2011), diabetes (Goto et al., 2011), and dementia (Arab et al., 2011). The affects of caffeine are acute and short-lived (typically wearing off within 3–5 hours), but improvements in performance (cognitive and/or physical) have been noted in the range of 200–500 mg. In a comparative context, drinks such as black coffee, espresso, and tea provide 80–100 mg, 180–200 mg, and 30–50 mg, respectively per standard serving, while soda provides in the range of 30–60 mg/12 oz. For cocoa products, the levels of caffeine are generally substantially less, with milk chocolates providing 5–15 mg/1 oz, and dark chocolate products providing 15–25 mg/1 oz. A single tablespoon of cocoa powder would offer 12 mg. Thus, in the context of a typical diet, cocoa based products are unlikely to contribute a substantial amount of caffeine.

Flavonoids

Polyphenols are the products of the secondary metabolism of plants and constitute one of the most numerous and widely distributed groups of substances in the plant kingdom (Haslam, 1998). They can be divided into at least ten different classes depending on their central structure. The most important grouped is constituted by the flavonoids, which can be further divided into 13 classes, with more

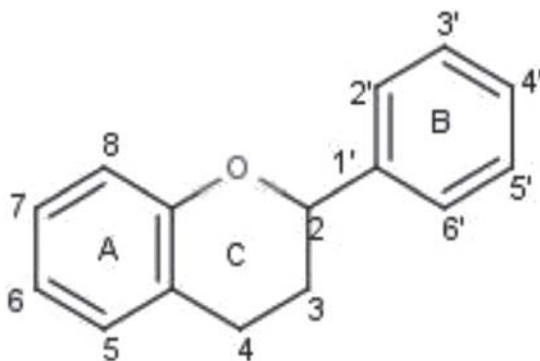


Fig. 2.3. Basic structure and numbering system for flavonoids.

than 5000 compounds catalogued (Wollgast & Anklam, 2000). They are characterized by so-called *diphenylpropane* structures (C6-C3-C6) that consist of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle (see Fig. 2.3). The phenylpropanoid pathway, which renders diphenylpropanes, is ubiquitously present in higher plants and is at the core of a series of biochemical pathways leading to biosynthesis of flavonoids and stilbens and appears essential to the survival of terrestrial plants by providing them with (a) lignin as an important mechanical and structural material; (b) signaling compounds within the plant; (c) factors controlling male sterility and regulating hormonal activity; and (d) defensive compounds against microorganisms or predators (Wollgast & Anklam 2000).

Individual differences within each group of flavonoids result from the variation in number and arrangement of the hydroxyl groups with the most commonly occurring being those with dihydroxylation in the 3' and 4' positions (Bravo, 1998). In the context of the human diet, there are 6 primary sub-classes of flavonoids—flavonols, flavones, flavan-3-ols, flavanones, anthocyanins, and isoflavones.

Among these classes, the flavan-3-ols are noteworthy as they are the only common dietary flavonoid sub-class that exists primarily in foods in non-glycosylated forms. In addition, flavan-3-ols can exist as simple monomeric forms, or can polymerize, forming derived tannins or condensed tannins. Derived tannins are formed during food handling and processing, and their formation is most commonly discussed in the context of black and oolong teas, beverage products which are the primary sources of derived tannins in the diet. Condensed tannins or proanthocyanidins (PACs) are high molecular weight polymers where the monomeric unit is a flavan-3-ol (e.g., epicatechin or galocatechin). When the monomeric unit involves non-gallated flavanols such as (epi)catechin, as it is the case in cocoa (Romanczyk, 1997), PACs can be referred to as *procyanidins*; if the flavanols are gallated, as the (epi)galocatechins commonly found in tea, PACs are referred to as *prodelphinidins*. Oxidative or enzy-

matic condensation, leading to the creation of polymers, occurs between carbon C-4 of the heterocycle and carbons C-6 or C-8 of adjacent units. PACs can have a degree of polymerization of 50 and more however, validated methods can only detect up to a degree of polymerization (DP) of 10 (Robbins, Leonczak, et al., 2009).

Cocoa Specific Flavonoids. Flavonoids are readily available in a wide variety of foods and beverages and, within plant based foods, different classes of flavonoids can co-exist in a single food. Considering the cocoa bean itself, the raw, unfermented cocoa bean can be particularly rich in flavanols and procyanidins (DP 2-10), with nearly 8–10% of the weight of the bean made up of these phytonutrient components. The monomeric flavanols in fresh cocoa bean are predominately (-)-epicatechin and to a much lesser extent, (+)-catechin, where the reported ratio of epicatechin to catechin could be as high as 100:1 (Niemenak, Rohsiusb, et al., 2006). Monomeric flavanols can make up 1–3% (by wt) of the cocoa bean, whereas the oligomeric fraction (procyanidins) are more abundant, being present at levels of 6–8% (Gu et al., 2002). However, as shown previously, these levels can dramatically change with the degree of processing. Conventional cocoa handling and processing has been shown not only to lead to an overall loss in cocoa flavanols and procyanidins (Miller et al., 2008), but also to stereochemical changes, specifically the formation of (-)-catechin from (-)-epicatechin (Cooper et al., 2007). Gallated flavanols have on occasion been reported [USDA Flavonoid Database (2011)], but this is far less consistent, particularly in the context of cocoa products such as cocoa powder and chocolate. This is in contrast to tea in which over 75% of the flavanols present exist in gallated forms (e.g., epigallocatechin gallate and epigallocatechin). Thus, in relation to cocoa and chocolate products, the non-gallated flavanols, in particular (-)-epicatechin, and the structurally related procyanidins are the key flavanol species present.

Cocoa, Chocolate, and Health—Insights from Epidemiological Studies

Since the early 1990's, there has been a growing body of evidence to suggest that the regular dietary inclusion of flavonoid-containing foods is associated with positive cardiovascular health outcomes, including reduced cardiovascular morbidity and mortality. Some of the earliest study examining the relationship between flavonoids, diet, and health focused on flavonoids from select dietary sources—most often tea, apples, and onions. This was in large part a result of the limited awareness of the types and amounts of flavonoids readily available in commonly consumed foods, a factor that today has been somewhat overcome by the availability of various flavonoid databases including the USDA's Flavonoid and Proanthocyanidin databases, as well as INRA's Phenol Explorer. Though today it is far more recognized within the scientific community that cocoa products can be significant dietary contributors to flavonoid intake

(specifically, flavanol and procyanidin intakes), this fact was largely under-appreciated until the 1990's (Lazarus et al., 1999).

While by the 1990s, several published studies had already demonstrated an inverse association between tea intake and risk factors for coronary heart disease, coronary heart disease, and total mortality (Stensvold, Tverdal, et al., 1992; Hertog, Feskens, et al., 1994), it was not until 2006 that the first study focused on cocoa intake, hypertension, and cardiovascular disease mortality was published. In this study, known as the Zutphen Elderly Study, involving 470 Dutch men—all free of cardiovascular disease and diabetes, the regular dietary intake of cocoa products (including chocolate) was inversely associated with systolic and diastolic blood pressure, even following adjustment for a variety of confounders (Buijsse et al., 2006). Furthermore, the intake of cocoa products was associated with significantly lower cardiovascular and all-cause mortality. Those in the highest tertile of intake—the group that experienced the greatest reduction in risk—consumed an average of 2.3 g/day (a fraction of a standard 40-g bar of chocolate).

Later in 2007, one of the largest studies assessing the relationship between flavonoid intake and cardiovascular disease mortality was published—the Women's Health Study. In this 16 years long study, involving nearly 35,000 women, the disease-intake relationship was examined for each of the major sub-classes of flavonoids, as well as for individual flavonoid-rich foods (Mink et al., 2007). Though no significant reduction in risk was found to be specifically associated with flavanol or proanthocyanidin intake, significant reductions in coronary heart disease and/or cardiovascular disease were associated with the intake of apples, pears, red wine, and chocolate. From the perspective of flavonoid sub-class, all of these foods contain flavanol and proanthocyanidins, thus lending support to the idea that consumption of these types of foods may be offering protective benefits.

Since the publication of this study, the positive association between regular chocolate consumption and improved cardiovascular health has been further studied. As part of the National Heart, Lung and Blood Institute Family Heart Study, a cross-sectional investigation involving nearly 5,000 men and women aged 25–93 years looked at the relationship between chocolate consumption and calcified atherosclerotic plaque formation in the coronary arteries (Djousse et al., 2011). Individuals who reported the consumption of chocolate 2 or more times per week were found to be 32% less likely to coronary artery calcification. Risk was further reduced by 57% with chocolate intakes of 5 times or more per week, leading the authors to suggest that chocolate consumption “is inversely related with prevalent coronary heart disease in a general US population.”

Several large studies published in 2010 and 2011, examining a total of over 80,000 adults, examined the relationship between dietary habits, including the regular consumption of chocolate and cardiovascular health outcomes. Among 19,357 men and women in Germany participating in the European Prospective Incidence into Cancer and Nutrition (EPIC) trial, incident cases of heart attack and stroke were reduced by 39% among men and women in Germany who reported a mean chocolate intake

of 7.5 g/day (Buijsse et al., 2010). In addition, mean systolic and diastolic blood pressure were 1.0 and 0.9 mm Hg lower among regular chocolate consumers. On a population basis, such a reduction in mean systolic blood pressure has been estimated to lead to the prevention of 10,000 coronary heart disease deaths each year (Lewington et al., 2002), thus such modest reductions in blood pressure induced by a dietary change has the potential for significant and meaningful improvements in the health of a population. Interestingly, records showed that of the chocolate consumed, 57% and 24% was milk and dark chocolate, respectively. The reduction in stroke incidence was recently confirmed as the consumption of 2.3 oz of chocolate per week was associated with a 20% reduction in stroke among this population of women (Larsson, Virtamo, et al., 2011). Similarly, after a period of 9 years, the rates of heart failure hospitalization and death were lower among middle age and elderly women who reported consuming up to 1–2 serving of chocolate per week relative to those who reported no chocolate consumption (Mostofsky, Levitan, et al., 2010). Interestingly, higher levels of chocolate consumption, either 3–6 serving per week or 1 or more servings per day were not associated with the same outcome; showing, for the first time in a study, that the boundaries of the protective benefits of chocolate may exist.

Cocoa Flavanols and Procyanidins and Cardiovascular Health

Epidemiological studies have approx. examined over 200,000 men and women from various geographic regions of the globe and have identified that the regular dietary inclusion of cocoa based products (e.g., chocolate) appears to be associated with a range of cardiovascular benefits. While these studies offer compelling data, they cannot establish true causality, merely associations. The fact that chocolate consumption was associated with these benefits may not be a result of some specific components in cocoa or chocolate, whether it be the fat, fiber, or even phytonutrient components. The positive associations may simply be related to the fact that the consumption of chocolate is merely representative of another behavior, such as eating more fruit and vegetables, exercising more, or an exemplification of a balanced diet and lifestyle. Though diet itself is well established as one of the most important lifestyle factors in influencing cardiovascular health (De Caterina et al., 2006), these epidemiological studies on their own cannot provide proof of chocolates benefits. This proof must be provided through proper randomized, controlled, dietary intervention trials. With this in mind, the following sections will briefly review the results of controlled intervention trials. The focus of the studies presented herein will be examination of the effects of cocoa flavanols and procyanidins on physiological endpoints related to cardiovascular function and health.

Platelet Function

Platelets are cell-like particles found in blood that function in response to injury, signaling the recruitment and activation of other cells that serve to repair damage. When

the cardiovascular system is healthy, platelets circulate in an inactivated state, ready to respond when injury occurs. In response to high blood pressure, high cholesterol, and smoking, more platelets circulate in an activated state, triggering unnecessary inflammatory responses (Gregg & Goldschmidt-Clermont, 2003). While the role of platelets in the formation of blood clots that lead to heart attacks, stroke, and peripheral occlusions is well-appreciated, what is less appreciated by most is that the activation of platelets and their subsequent interactions with the vascular environment can exacerbate inflammation and trigger the promotion of atherosclerosis. Thus, platelets are more than mediators of clots, but in fact, mediators of the progression of cardiovascular disease itself (Huo & Ley, 2004).

Specific to the effects of flavanols, several studies examined the impact of acute consumption of flavanol and procyanidin rich cocoa products on *ex vivo* platelet function. In these studies, the consumption of these products was associated with a reduction in platelet activation (Rein et al., 2000; Rein et al., 2000). In another study, the effect of ~900 mg of cocoa flavanols and procyanidins was compared to 81 mg of baby aspirin (Pearson et al., 2002). The effects of aspirin were as expected, a rapid and specific reduction in platelet activation. Interestingly, the acute consumption of cocoa flavanols/procyanidins also resulted in significant reductions in platelet activation, though to a far lesser extent than that seen with aspirin, a promising finding given that a food was being compared with a drug. In addition to suppressing platelet activation, studies in humans also demonstrated a suppression of leukocyte activation in response to acute cocoa flavanol/procyanidin consumption, suggesting that these cocoa components may work in multiple ways to reduce the activation and proliferation of inflammatory responses (Heptinstall et al., 2006). There is also evidence that these effects can be sustained as a 28-day study in healthy adults demonstrated that the daily consumption of approx. 230 mg of cocoa flavanols/procyanidins could lead to sustained reductions in platelet activation, as well as a reduction in the appearance of micro-particles in the circulation (Heptinstall et al., 2006). Circulating microparticles are small vesicle releases from cells such as platelets and their presence is strongly associated with vascular damage, platelet activation, and a propensity for clotting, so a reduction in microparticle formation would suggest an improvement in the inflammatory state of the cardiovascular environment (Shantsila, Kamphuisen, et al., 2010). Though improvements in platelet activation have been reported with other flavanol/procyanidin containing products, including grape-derived products, a systematic review of over 25 intervention studies with a wide variety of polyphenol-containing foodstuffs found that some of the strongest evidence in favor of platelet-inhibiting effects was for cocoa containing products (Ostertag et al., 2010).

Endothelial Function

The vascular system is composed of arteries, veins, and capillaries, each lined with a single layer of cells known as the endothelium. These cells that line the interior sur-

face of all blood vessels serve as critical regulators of vascular homeostasis, and the loss in the proper function of these cells is recognized to start even in the early stages of vascular disease, including atherosclerosis (Verma & Anderson, 2002). Impaired function, commonly known as endothelial dysfunction, measured by examining the relaxation response of arteries (vasodilation), has been shown to be predictive of future (Fathi et al., 2004; Deanfield et al., 2007) and recurrent (Yeboah et al., 2007; Yeboah et al., 2009) cardiovascular events. Furthermore, pharmaceutical interventions that have been shown to improve cardiovascular-related morbidity and mortality have also been shown to be accompanied by significant improvements in endothelial function (Davignon, 2011) supporting the idea that interventions that improve endothelial function may offer the strong potential to maintain and improve cardiovascular health.

The first demonstration that the acute consumption of a beverage containing cocoa flavanols could improve endothelial function was offered by Heiss et al. (2003). In a study of 20 adults with one or more cardiovascular risk factors (e.g., high cholesterol, smoking), the consumption of a cocoa drink containing 176 mg of cocoa flavanols and procyanidins was able to improve the vascular response within 2 h, with a return to baseline function after 6 h. In addition, an increase in the circulating pool of nitric oxide—the primary mediator of this vasodilation response—was also increased, suggesting that the mechanism by which these cocoa compounds exerted their effects was through influencing the nitric oxide pathway.

Since then, a number of studies using both dark chocolate (Engler et al., 2004; Faridi et al., 2008; Shiina et al., 2009), and cocoa-beverages (Fisher et al., 2003; Heiss et al., 2005; Faridi et al., 2008) have similarly demonstrated acute and short term benefits related to the consumption of these products rich in cocoa flavanols and procyanidins. Particularly noteworthy is the study by Schroeter et al. (2006) that established for the first time a direct causal link between cocoa flavanols and procyanidins, and specifically, (-)-epicatechin consumption and improvements in vascular function. Applying the Hill's Criteria of Causality, this study clearly demonstrated that the acute consumption of a product rich in cocoa flavanols and procyanidins resulted in a statistically significant increase in vascular function that was paralleled by an increase in: (1) the circulating levels of flavanols, and (2) the circulating pool of nitric oxide metabolites. The improvement of vascular function was completely blocked by the co-administration of an inhibitor of nitric oxide production, supporting the fact that the flavanols in cocoa were working through a nitric oxide dependent pathway. Importantly, the vascular effects of cocoa could be replicated by the acute administration of pure (-)-epicatechin (the primary flavanol in cocoa). Together, these data establish a direct connection between the consumption of (-)-epicatechin, both as a pure molecule and as a native constituent of cocoa, and improvements in vascular function. These vascular effects of (-)-epicatechin in humans have since been independently replicated (Loke et al., 2008).

In addition to studies in healthy adults, or adults with cardiovascular risk factors, work with flavanol and procyanidin rich cocoa products have also been conducted in mediated individuals with established cardiovascular disease. In a well-controlled longer term intervention study, the thrice daily consumption a drink rich in cocoa flavanols and procyanidins resulted in a sustained improvement in vascular function among medicated diabetics (Balzer et al., 2008). This vascular improvement was demonstrated within 8 days, and sustained over the 30-day period of the intervention with cocoa. Likewise a study in medically treated patients with established coronary artery disease, the twice daily consumption of a cocoa flavanol and procyanidin rich product improved vascular function (Heiss et al., 2010). Interestingly, this study also demonstrated a significant increase in the number of circulating angiogenic cells, cells responsible for the repair of endothelium itself, thus suggesting that the regular consumption of these rich cocoa products not only improves vascular function, but may also help to promote vascular repair. Importantly, the regular consumption of a nutrient-matched drink largely devoid of cocoa flavanols and procyanidins (produced through the addition of alkalized cocoa powder to the products) did not result in these significant improvements, indicating that the flavanols and procyanidins were critical drivers of the observed improvements.

Blood Pressure

The potential for the consumption of cocoa products to protect against the development of hypertension has been suggested in part as a result of epidemiological work with the Kuna Indians. The Kuna Indians are an indigenous tribe who live off the coast of Panama. While it had been recognized for some time that the Kuna appeared unique in that Indians living on the San Blas Islands did not develop hypertension with age. This population was originally thought to be genetically-protected from the development of hypertension, but in 1997, the demonstration that hypertension developed in the Kuna when they migrated to more urban areas of Panama (Hollenberg et al., 1997) revealed that it was not genetics but something environmental that appeared to be the key. An examination of the differences in dietary habits on the island versus those in the city identified that one key factor that was markedly different was the high consumption of a native drink preparation made from cocoa. When on the island, the Kuna were conservatively estimated to consume in the range of 8 cups a day of this beverage (McCullough et al., 2006), leading some to speculate that the cocoa itself was conferring these blood pressure benefits.

This study in the Kuna, along with work done in other populations (Buijsse et al., 2006) does suggest that the regular consumption of cocoa products may offer protection from the development of hypertension. However, much more direct and supportive evidence in favor of a potential anti-hypertensive effect comes from several human trials. Beginning in 2003, there was initial evidence that the short term

consumption of 100-g flavonoid-rich chocolate product could reduce systolic blood pressure by approximately 5 mmHg in an older population of hypertensive adults (Taubert et al., 2003). Additional independent studies with prehypertension, hypertension or established coronary artery disease confirmed these findings (Taubert et al., 2007; Grassi et al., 2008; Heiss et al., 2010). Interestingly, systematic reviews suggested that the pooled mean reduction in systolic and diastolic blood pressure following the consumption of flavanol-rich cocoa products or dark chocolate was 4.7 mmHg and 2.8 mmHg, and 5 mmHg and 2.7 mmHg, respectively (Taubert et al., 2007; Ried et al., 2010). Though these studies provide promising support for the blood pressure reducing benefits of flavanol-rich cocoa products, the data are yet incomplete to make general recommendations regarding the required intakes of cocoa flavanols and procyanidins to achieve these benefits.

Antioxidant Function

In the early years of flavonoid research, a great majority of the studies on the benefits of flavonoids focused on their suggested ability to function as antioxidants. Like much of the research in the field at the time, early studies with cocoa products focused on measuring their ability to disrupt free radical mediated reactions, with measurements of plasma antioxidant capacity following the consumption of cocoa flavanol/procyanidin rich products frequently studied. The fundamental chemical structure of flavanols and procyanidins supports their ability to function as modest antioxidants *in vitro*; however, within the body, this chemistry changes. The biotransformation of (-)-epicatechin, the primary flavanol in cocoa, has been studied and data available from these studies supports that (-)-epicatechin is extensively transformed once absorbed into the body (Schroeter et al., 2006; Mullen et al., 2009), as it undergoes a range of methylation, glucuronidation, and/or sulfation reactions, resulting in the formation of molecules with far lower redox activity (Spencer et al., 2001). The change in chemical activity, combined with the overall low level of flavanols in the circulation (low micromolar range in contrast to strong circulating antioxidants such as vitamin C and urate, both present at levels 10–100 times higher) lessens the likelihood that flavanols themselves can serve as effective antioxidants within the systemic circulation. Furthermore, a number of studies have failed to find consistent demonstration of antioxidant effects *in vivo* (Murphy et al., 2003; Heiss et al., 2005; Crews et al., 2008). A recent review focused on examining the science surrounding the potential for dietary polyphenols to exert beneficial effects on the cardiovascular system via their ability to serve as antioxidants *in vivo* found no direct evidence that these compounds, including flavanols, functioned as antioxidants (Hollman et al., 2011). Together these data provide strong support for the fact that the flavanols and procyanidins in cocoa do not function as antioxidants *in vivo*, and thus the health effects of these phytonutrients is attributed to other mechanisms.

Conclusions

Cocoa remains an important global commodity due to its fundamental role for the manufacture of chocolate and as an enabler (as a flavor or color component) in the production of many foods. A complex chemistry is involved in the transformation of an otherwise bitter, astringent and unpleasant raw material into the characteristic cocoa by-products such as cocoa powders, cocoa liquor and cocoa butter that enable the characteristic flavor and aromas of chocolate. Fermentation and roasting are of primary importance in this transformation.

Of more recent relevance is the fact that certain cocoa-derived products such as dark chocolate have demonstrated cardiovascular health benefits thanks to their relative abundant concentration of flavanols, specifically (-)-epicatechin. This fact has definitely secured a special place for cocoa in the diet of future generations, and once again, confirming that cocoa is indeed, the Food of the Gods.

References

- Anderson, J.W., P. Baird, et al. (2009). Health benefits of dietary fiber. *Nutr Rev* 67(4): 188–205.
- Arab, L., M.L. Biggs, et al. (2011). Gender differences in tea, coffee, and cognitive decline in the elderly: the cardiovascular health study. *J Alzheimers Dis*.
- Ardhana, M.M., and G.H. Fleet (2003). The microbial ecology of cocoa bean fermentations in Indonesia. *International Journal of Food Microbiology* 86(1–2): 87–99.
- Aremu, C.Y., M.A. Agiang, et al. (1995). Nutrient and antinutrient profiles of raw and fermented cocoa beans. *Plant Foods Hum Nutr* 48(3): 217–23.
- Argout, X., J. Salse, et al. (2011). The genome of *Theobroma cacao*. *Nat Genet* 43(2): 101–108.
- Balzer, J., T. Rassaf, et al. (2008). Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial. *J Am Coll Cardiol* 51(22): 2141–9.
- Beckett, S.T. (2009). *Industrial Chocolate Manufacture and Use*. Chichester, U.K.: Wiley-Blackwell.
- Bertazzo, A., F. Agnolin, et al. (2011). The protein profile of *Theobroma cacao* L. seeds as obtained by matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Commun Mass Spectrom* 25(14): 2035–42.
- Bibra, B.E.V. (1995). *Plant Intoxicants*. Rochester, Vt.: Healing Arts Press.
- Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews* 56(11): 317–333.
- Buijsse, B., E.J. Feskens, et al. (2006). Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Arch Intern Med* 166(4): 411–7.
- Buijsse, B., C. Weikert, et al. (2010). Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. *Eur Heart J* 31(13): 1616–23.

- Camu, N., T. De Winter, et al. (2008). Fermentation of cocoa beans: influence of microbial activities and polyphenol concentrations on the flavour of chocolate. *J Sci Food Agric* 88(13): 2288–2297.
- Cooper, K.A., E. Campos-Gimenez, et al. (2007). Rapid reversed phase ultra-performance liquid chromatography analysis of the major cocoa polyphenols and inter-relationships of their concentrations in chocolate. *J Agric Food Chem* 55(8): 2841–7.
- Crews, W.D., Jr., D.W. Harrison, et al. (2008). A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. *Am J Clin Nutr* 87(4): 872–80.
- Davignon, J. (2011). Pleiotropic effects of pitavastatin. *Br J Clin Pharmacol*.
- De Brito, E.S., Garcia, N., Gallao, M.I., Cortelazzo, A.L., Fevereiro, P.S., and Braga, M.R. (2000). Structural and chemical changes in cocoa (*Theobroma cacao* L.) during fermentation, drying and roasting. *J Sci Food Agric* 81: 281–288.
- De Caterina, R., A. Zampolli, et al. (2006). Nutritional mechanisms that influence cardiovascular disease. *Am J Clin Nutr* 83(2): 421S–426S.
- Deanfield, J.E., J.P. Halcox, et al. (2007). Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 115(10): 1285–95.
- Ding, E.L., S.M. Hutfless, et al. (2006). Chocolate and prevention of cardiovascular disease: a systematic review. *Nutr Metab (Lond)* 3: 2.
- Dirks, H.D. (2009). Investigation into the fermentation of Australian cocoa beans and its effects on microbiology, chemistry and flavour. School of Chemical Sciences and Engineering. Sydney, University of New South Wales.
- Djousse, L., P.N. Hopkins, et al. (2011). Chocolate consumption is inversely associated with prevalent coronary heart disease: the National Heart, Lung, and Blood Institute Family Heart Study. *Clin Nutr* 30(2): 182–7.
- Engler, M.B., M.M. Engler, et al. (2004). Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Nutr* 23(3): 197–204.
- Faridi, Z., V.Y. Njike, et al. (2008). Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial. *Am J Clin Nutr* 88(1): 58–63.
- Fathi, R., B. Haluska, et al. (2004). The relative importance of vascular structure and function in predicting cardiovascular events. *J Am Coll Cardiol* 43(4): 616–23.
- Feltus, F., C. Saski, et al. (2011). Sequencing of a QTL-rich region of the *Theobroma cacao* genome using pooled BACs and the identification of trait specific candidate genes. *BMC Genomics* 12(1): 379.
- Fisher, N.D., M. Hughes, et al. (2003). Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* 21(12): 2281–6.
- Fowler, M.S. (2009). Cocoa beans: From tree to factory. In *Industrial Chocolate Manufacture and Use*. S. Beckett, Ed. Chichester: John Wiley & Sons, 10–39.
- Glade, M.J. (2010). Caffeine—Not just a stimulant. *Nutrition* 26(10): 932–8.

- Goto, A., Y. Song, et al. (2011). Coffee and caffeine consumption in relation to sex hormone-binding globulin and risk of type 2 diabetes in postmenopausal women. *Diabetes* 60(1): 269–75.
- Grassi, D., G. Desideri, et al. (2008). Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr* 138(9): 1671–6.
- Gregg, D., and P.J. Goldschmidt-Clermont (2003). Cardiology patient page. Platelets and cardiovascular disease. *Circulation* 108(13): e88–90.
- Gu, L., M. Kelm, et al. (2002). Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J Agric Food Chem* 50(17): 4852–60.
- Hansen, C.E., M. del Olmo, et al. (1998). Enzyme activities in cocoa beans during fermentation. *J Sci Food Agric* 77(2): 273–281.
- Haslam, E. (1998). *Practical Polyphenols: From structure to molecular recognition and physiological action*. Cambridge: Cambridge University Press.
- Heiss, C., A. Dejam, et al. (2003). Vascular effects of cocoa rich in flavan-3-ols. *JAMA* 290(8): 1030–1.
- Heiss, C., S. Jahn, et al. (2010). Improvement of endothelial function with dietary flavanols is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease. *J Am Coll Cardiol* 56(3): 218–24.
- Heiss, C., P. Kleinbongard, et al. (2005). Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J Am Coll Cardiol* 46(7): 1276–83.
- Heptinstall, S., J. May, et al. (2006). Cocoa flavanols and platelet and leukocyte function: recent in vitro and ex vivo studies in healthy adults. *J Cardiovasc Pharmacol* 47 Suppl 2: S197–205; discussion S206–9.
- Hertog, M.G., E.J. Feskens, et al. (1994). Dietary flavonoids and cancer risk in the Zutphen Elderly Study. *Nutr Cancer* 22(2): 175–84.
- Hollenberg, N.K., G. Martinez, et al. (1997). Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension* 29(1 Pt 2): 171–6.
- Hollman, P.C., A. Cassidy, et al. (2011). The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *J Nutr* 141(5): 989S–1009S.
- Huo, Y., and K.F. Ley (2004). Role of platelets in the development of atherosclerosis. *Trends Cardiovasc Med* 14(1): 18–22.
- Hurst, W.J., S.M. Tarka, et al. (2002). Archaeology: Cacao usage by the earliest Maya civilization. *Nature* 418(6895): 289–290.
- Larsson, S.C., J. Virtamo, et al. (2011). Chocolate consumption and risk of stroke in women. *J Am Coll Cardiol* 58(17): 1828–9.
- Larsson, S.C., J. Virtamo, et al. (2011). Coffee consumption and risk of stroke in women. *Stroke* 42(4): 908–12.
- Lazarus, S.A., J.F. Hammerstone, et al. (1999). Chocolate contains additional flavonoids not found in tea. *Lancet* 354(9192): 1825.

- Lecumberri, E., L. Goya, et al. (2007). A diet rich in dietary fiber from cocoa improves lipid profile and reduces malondialdehyde in hypercholesterolemic rats. *Nutrition* 23(4): 332–41.
- Lersch, M. (2012). Maximizing food flavor by speeding up the Maillard reaction. In *The Kitchen as Laboratory: Reflections on the Science of Food and Cooking*. C. Vega, Ubbink, J.B., and van der Linden, Erik, Eds. New York: Columbia University Press, 91–99.
- Lewington, S., R. Clarke, et al. (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 360(9349): 1903–13.
- Loke, W.M., J.M. Hodgson, et al. (2008). Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* 88(4): 1018–25.
- McCullough, M.L., K. Chevaux, et al. (2006). Hypertension, the Kuna, and the epidemiology of flavanols. *J Cardiovasc Pharmacol* 47 Suppl 2: S103–9; discussion 119–21.
- Mensink, R.P. (2005). Effects of stearic acid on plasma lipid and lipoproteins in humans. *Lipids* 40(12): 1201–5.
- Meursing, E.H. (2009). *De Zaan Cocoa and Chocolate Manual*. Switzerland, ADM: 12–28.
- Miller, K.B., W.J. Hurst, et al. (2008). Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *J Agric Food Chem* 56(18): 8527–33.
- Mink, P.J., C.G. Scrafford, et al. (2007). Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am J Clin Nutr* 85(3): 895–909.
- Misnawi, S. Jinap, et al. (2003). Effects of incubation and polyphenol oxidase enrichment on colour, fermentation index, procyanidins and astringency of unfermented and partly fermented cocoa beans. *International Journal of Food Science and Technology* 38(3): 285–295.
- Mostofsky, E., E.B. Levitan, et al. (2010). Chocolate intake and incidence of heart failure: a population-based prospective study of middle-aged and elderly women. *Circ Heart Fail* 3(5): 612–6.
- Mullen, W., G. Borges, et al. (2009). Milk decreases urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol metabolites in humans. *Am J Clin Nutr* 89(6): 1784–91.
- Murphy, K.J., A.K. Chronopoulos, et al. (2003). Dietary flavanols and procyanidin oligomers from cocoa (*Theobroma cacao*) inhibit platelet function. *Am J Clin Nutr* 77(6): 1466–73.
- Niemenak, N., C. Rohsiusb, et al. (2006). Comparative study of different cocoa (*Theobroma cacao* L.) clones in terms of their phenolics and anthocyanins contents. *Journal of Food Composition and Analysis* 19: 612–619.
- Ostertag, L.M., N. O’Kennedy, et al. (2010). Impact of dietary polyphenols on human platelet function—a critical review of controlled dietary intervention studies. *Mol Nutr Food Res* 54(1): 60–81.
- Pearson, D.A., T.G. Paglieroni, et al. (2002). The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb Res* 106(4–5): 191–7.
- Powis, T.G., A. Cyphers, et al. (2011). Cacao use and the San Lorenzo Olmec. *Proc Natl Acad Sci U S A* 108(21): 8595–600.
- Redgwell, R.J., Trovato, V., and Curti, D. (2003). Cocoa beans carbohydrates: roasting induced changes and polymer interactions. *Food Chemistry* 80: 511–516.

- Rein, D., T.G. Paglieroni, et al. (2000). Cocoa and wine polyphenols modulate platelet activation and function. *J Nutr* 130(8S Suppl): 2120S–6S.
- Rein, D., T.G. Paglieroni, et al. (2000). Cocoa inhibits platelet activation and function. *Am J Clin Nutr* 72(1): 30–5.
- Ried, K., T. Sullivan, et al. (2010). Does chocolate reduce blood pressure? A meta-analysis. *BMC Med* 8: 39.
- Risterucci, A.M., L. Grivet, et al. (2000). A high-density linkage map of *Theobroma cacao* L. TAG Theoretical and Applied Genetics 101(5): 948–955.
- Robbins, R.J., J. Leonczak, et al. (2009). Method performance and multi-laboratory assessment of a normal phase high pressure liquid chromatography–fluorescence detection method for the quantitation of flavanols and procyanidins in cocoa and chocolate containing samples. *J Chromatogr A* 1216(24): 4831–4840.
- Romanczyk, J., Hammerstone, J., Buck, M., Post, L., Cipolla, G., Micccland, C., Mundt, J., and Schmitz, H. (1997). Cocoa extract compounds and methods for making and using the same. USA, Mars, Incorporated.
- Rosane, S., and W. Alan (2004). The microbiology of cocoa fermentation and its role in chocolate quality. *Crit Rev Food Sci Nutr* 44(4): 205–221.
- Saski, C., F. Feltus, et al. (2011). A genetically anchored physical framework for *Theobroma cacao* cv. Matina 1–6. *BMC Genomics* 12(1): 413.
- Schroeter, H., C. Heiss, et al. (2006). (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci U S A* 103(4): 1024–9.
- Shantsila, E., P.W. Kamphuisen, et al. (2010). Circulating microparticles in cardiovascular disease: implications for atherogenesis and atherothrombosis. *J Thromb Haemost* 8(11): 2358–68.
- Shiina, Y., N. Funabashi, et al. (2009). Acute effect of oral flavonoid-rich dark chocolate intake on coronary circulation, as compared with non-flavonoid white chocolate, by transthoracic Doppler echocardiography in healthy adults. *Int J Cardiol* 131(3): 424–9.
- Smit, H.J. (2011). Theobromine and the pharmacology of cocoa. *Handb Exp Pharmacol* (200): 201–34.
- Spencer, J.P., H. Schroeter, et al. (2001). Epicatechin and its in vivo metabolite, 3'-O-methyl epicatechin, protect human fibroblasts from oxidative-stress-induced cell death involving caspase-3 activation. *Biochem J* 354(Pt 3): 493–500.
- Stensvold, I., A. Tverdal, et al. (1992). Tea consumption. relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* 21(4): 546–53.
- Taubert, D., R. Berkels, et al. (2003). Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA* 290(8): 1029–30.
- Taubert, D., R. Roesen, et al. (2007). Effect of cocoa and tea intake on blood pressure: a meta-analysis. *Arch Intern Med* 167(7): 626–34.
- Thomas, J.B., J.H. Yen, et al. (2004). Determination of caffeine, theobromine, and theophylline in standard reference material 2384, baking chocolate, using reversed-phase liquid chromatography. *J Agric Food Chem* 52(11): 3259–63.

- U.S. Dept. of Agriculture. (2004). USDA Database for the Proanthocyanidin Content of Selected Foods. Nutrient Data Laboratory; Beltsville Human Nutrition Research Center; Agricultural Research Service; U.S. Department of Agriculture.
- U.S. Dept. of Agriculture. (2011). USDA Database for the Flavonoid Content of Selected Foods, Release 3.0., U.S. Department of Agriculture, Agricultural Research Service.
- U.S. Dept. of Agriculture and U.S. Dept. of Health and Human Services. (2010). Dietary Guidelines for Americans, 2010. U.S. Government Printing Office: Washington, DC.
- Vail, G. (2009). Cacao Use in Yucatan Among the Pre-Hispanic Maya. *Chocolate—History, Culture and Heritage*. L.E. Grivetti and H. Shapiro. Hoboken: John Wiley & Sons, 3–7.
- van den Bogaard, B., R. Draijer, et al. (2010). Effects on peripheral and central blood pressure of cocoa with natural or high-dose theobromine: a randomized, double-blind crossover trial. *Hypertension* 56(5): 839–46.
- Verma, S., and T.J. Anderson (2002). Fundamentals of endothelial function for the clinical cardiologist. *Circulation* 105(5): 546–9.
- Voigt, J., and B. Biehl (1995). Precursors of the cocoa-specific aroma components are derived from the vicilin-class (7S) globulin of the cocoa seeds by proteolytic processing. *Botanica Acta* (108): 283–289.
- Wollgast, J., and E. Anklam (2000). Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Res Int* 33(6): 423–447.
- Wood, G. (1991). A history of early cocoa introductions. *Cocoa Grower's Bulletin* 44: 13–17.
- Yeboah, J., J.R. Crouse, et al. (2007). Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation* 115(18): 2390–7.
- Yeboah, J., A.R. Folsom, et al. (2009). Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation* 120(6): 502–9.
- Ziegler, G. (2009). Flavour Development in Cocoa and Chocolate. In *Industrial Chocolate Manufacture and Use*. S. Beckett, Ed. West Sussex: Wiley-Blackwell, 169–191.

3

Cocoa Polyphenols

Laurent Peno-Mazzarino

Laboratoire BIO-EC, 1, Chemin de Saulxier, 91165 Longjumeau, France

Introduction

The Cacao contains numerous components, which some are considered as minor. However “minor” is a quantitative term that does not reflect their nutritional and biological benefits. Some of them contribute to the wealth of subtle flavors and odors that characterize the chocolate (Carlin, et al., 1998). Beyond this aspect of taste, others components, offer interesting perspectives in some fields such as nutrition, health and cosmetics.

Cocoa and cocoa derived products as cocoa butter contain a various number of polyphenols and particularly flavonoids. This kind of compounds is widely found in variety of vegetables or fruits, such as teas or grapes. If the presence of these components can be an explanation for the well-known “French paradox” provided by red wine, more healthy activities of polyphenols are well documented (Manach et al., 2004; Richelle et al., 2001). It is well noticed that cocoa and cocoa derived products are considered as a rich source of polyphenols (Ding et al., 2006).

Several thousands of molecules with a polyphenol structure have been identified in higher plants, and several hundred are found in edible plants. These molecules are secondary metabolites of plants and generally involved in defence against external stressors, like ultraviolet radiation or aggression by pathogens (Manach et al., 2004).

Polyphenols may be further classified into different groups as a function of the number of aromatic rings and the structural elements that bind these rings together. Distinctions are made between flavonoids, non-flavonoids and phenolic acids (see Fig. 3.1), with the first group being the largest one with more than 2,000 known compounds.

Cocoa is particularly rich in a subgroup of flavonoids, specifically the flavanols (flavan-3-ols). Flavanols are present as monomers epicatechin and catechin or as oligomers of epicatechin and/or catechin called procyanidins (Porter et al., 1991).

An increasing body of evidence supports the concept that dietary intake of polyphenols promotes health and attenuates or delays the onset of various diseases, including cardiovascular diseases, cancer, and other chronic diseases.

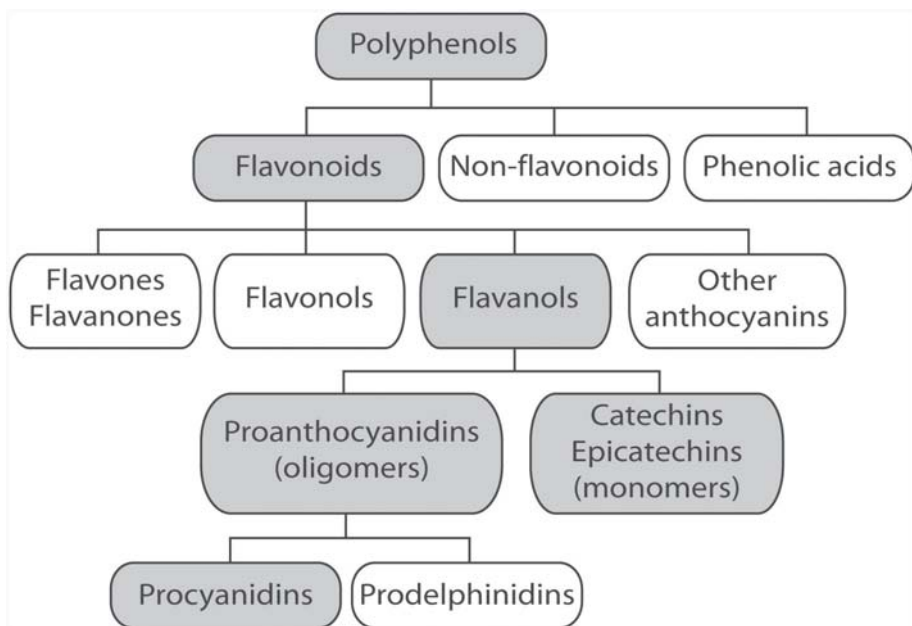


Fig. 3.1. Classification hierarchy of polyphenols with epicatechin, catechin and the procyanidins being the predominant class of polyphenols in cocoa (indicated in gray). Source: Ding et al., 2006.

Flavanols in cocoa and cocoa products exert some beneficial vascular effects (Schroeter et al., 2006; Engler et al., 2004), reduce the risk for cardiovascular morbidity and mortality (Buijsse et al., 2006), cancer (Yamagishi et al., 2003), and may help to prevent neurodegenerative diseases (Bayard et al., 2007).

Moreover a long-term ingestion of flavanols contributes to photoprotection UV-radiation, dermal blood flow increase, thickening of the skin and improvements of its structure (Heinrich et al., 2006). In another study from the same research group (Neukam et al., 2007), an increase in dermal blood flow and oxygen saturation of haemoglobin was detected within two hours after ingestion of a single-dose of flavanol-rich cocoa. In addition, cocoa butter is already widely used as an active excipient in skin care products; however, additional experiments are needed to fully explore the potential of cocoa polyphenols, particularly concerning moisturizing and anti-aging.

Cocoa Polyphenols on Skin Structure Experiment

In the following study, Gasser et al. (2008) and his colleagues demonstrated the activity of cocoa polyphenols on skin structure by histological techniques, through the examination of parameters such as the general skin morphology, the influence on

the expression of glycosaminoglycans (GAGs) and the influence on the expression of three collagen types. On the other hand, neutral GAGs close to the dermal-epidermal junction (DEJ) are known to be rich in growth factors. On the other hand, acidic GAGs in the epidermis and papillary dermis are essentially constituted of hyaluronic acid which is involved in skin moisture. Both of them constitute the major part of the extra cellular matrix. Concerning collagen, types I and III are components of the dermal fibrillary structure; type IV collagen is an important connection component of the DEJ. In vivo, GAGs are involved in skin elasticity and moisture (Carrino et al., 2000; Meyer & Stern, 1994; Willen et al., 1991), and collagens are responsible for skin tonus (Shuster et al., 1975).

The tested products were the cocoa polyphenols extract named Acticoa[™] cocoa, a natural material prepared from selected cocoa beans that are naturally rich in polyphenols. On top of this selective sourcing, a special process known as the Acticoa[™] process is applied to preserve the maximum amount of cocoa polyphenols in the cocoa bean. These beans are then used as a raw material for the extraction to produce a cocoa polyphenols extract, which polyphenols content is superior to 45%, with 5,17% epicatechin and 0,75% catechin. The cocoa butter used was Mycryo[®] from Barry Callebaut, a form of cocoa butter reduced to a dry powder by freezing at a temperature of -65°C . It was restored to a paste texture by gentle mixing. Cocoa butter in general does not contain a high amount of polyphenols.

These products were tested on an ex vivo model of living skin explants prepared from abdominoplasties of Caucasian women aged 39–68 years (three donors). Living skin explants, circular skin discus (10 mm in diameter) of epidermis and dermis were put in survival in classical cell culture conditions, in 2 mL BEM (BIO-EC's Explant Medium). The explants were maintained in survival for up to 12 days. Each batch comprised six explants (three for each sampling time), and each experiment comprised a control batch of nine explants (three for each sampling time, and three for the T0 control). Products were tested by topical application of 2 mg on each explant. The activities of the cocoa polyphenols extracts were investigated using histological techniques and microscopic examinations and images analysis.

Assessment of the Activity

The activity of cocoa polyphenols on skin structure was assessed by topical application of either 1.5% cocoa polyphenols blended into cocoa butter (CPP), or cocoa butter alone (CB) during 9 days. The skin explants were prepared from a 68-year-old donor. The results show that, after 5 days, cocoa polyphenols induce a slight increase in epidermal thickness as well as an increase in collagen density in the papillary dermis. The increase in collagen density is confirmed after 12 days. Regarding GAGs, no changes compared to the control batch are observed for the CB batch, whereas the CPP batch exhibits a slight increase in the amount of GAGs observed in the papillary dermis. A small increase in type I collagen expression is observed after 5 days for the CPP

batch. After 12 days, the increase is clear, while the CB batch displays only a moderate increase. Concerning both type III and type IV collagen, a clear increase is observed on day 5 for the CPP batch. After 12 days, the increase is even more marked. In the CB batch, for all three collagen subtypes, the increase observed after 5 days is weaker than that of the CPP batch. After 12 days, the increase is similar to that of the CPP batch.

These results demonstrate that cocoa polyphenols, when blended into cocoa butter, have a positive influence on the measured parameters, as early as 5 days after the start of the treatment. The activity of cocoa butter alone is similar to the above-mentioned effect, but it takes longer for it to exert the same influence on the skin structure. As cocoa butter does not contain any polyphenols, there is a strong evidence that cocoa polyphenols alone can induce a rapid improvement in parameters involved in skin tonus and elasticity.

Determinating the Effective Dose

A second experiment consisted in determining the dose at which cocoa polyphenols have the best restructuring activity. In this aim, topical application of cocoa polyphenols at different concentration (0.125%, 0.25%, 0.50%, 0.75% and 1.0%), blended into cocoa butter, were realised on the explants. The skin explants were prepared from a 43-year-old donor. The treatment was applied during 10 days, and three explants from each batch were sampled on day 6 and day 11. The results show that whatever the concentration tested, no harmful effects occur. This implies that overall product tolerance is very good. The observation of the general morphology shows that on the explants treated with CB alone, the epidermal structure is close to the one observed on the control explants. On the other hand, on the explants treated with CPP 0.75, the epidermal thickness is clearly increased (Fig. 3.2).

After examination of the general morphology, the further investigations (staining of GAGs and immunostaining of type I, III, and IV collagen) were focussed on the explants treated with the various concentrations of polyphenols for 11 days. After 11 days, the cocoa polyphenols concentrations induce an increase in GAG expression in the following order: 0.75 (very clear) > 0.50 (clear) > 0.125 (slight) > 1.0 (moderate) > 0.25 (very moderate). The ranking for type I collagen is 0.75 (very clear) > 0.25 = 0.50 = 0.125 (clear) > 1.0 (moderate), for type III collagen 0.50 = 0.25 =



Fig. 3.2. General morphology after Masson's trichrome staining. (a) Untreated explants (D 11); (b) explants treated with CB alone (D 11); (c) explants treated with 0.75% CPP (D 11).

0.125 (moderate, moderately dense) > 0.75 = 1.0 (moderate, slightly dense), and for type IV collagen 0.25 (very clear) > 0.75 = 0.50 = 1.0 = 0.125 (clear). In conclusion, the concentrations at which cocoa polyphenols blended into cocoa butter induce the best overall skin restructuring are 0.75% or 0.50%. The pictures of the explants treated with the polyphenols concentration that exhibit the best response for each parameter assessed, are presented in Fig. 3.3.

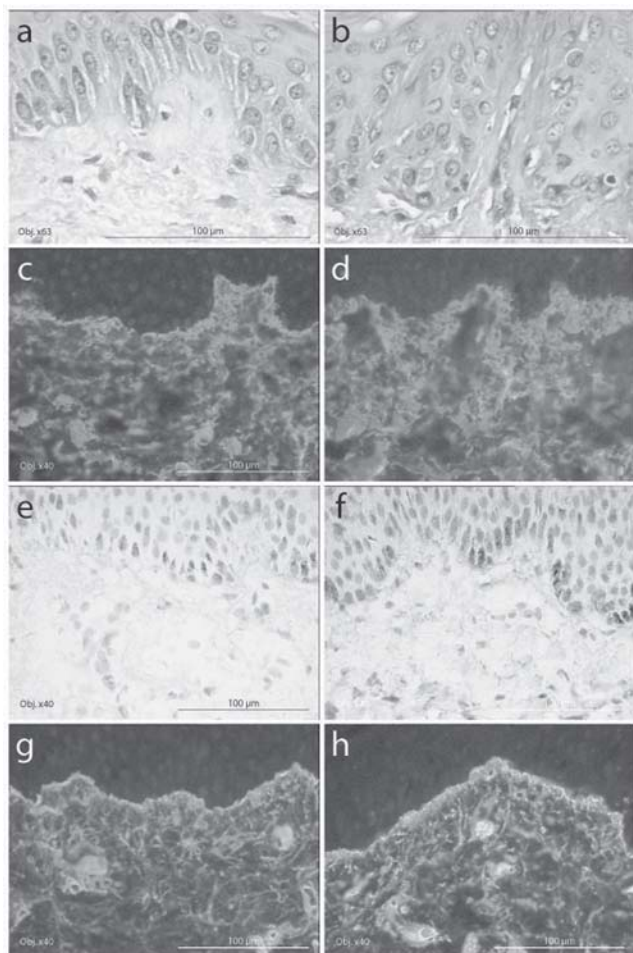


Fig. 3.3. Pictures of stainings and immunostainings. (a) GAGs, untreated explants (D 11); (b) GAGs, explants treated with 0.75% CPP (D 11); (c) collagen I, untreated explants (D 11); (d) collagen I, explants treated with 0.75% CPP (D 11); (e) collagen III, untreated explants (D 11); (f) collagen III, explants treated with 0.50% CPP (D 11); (g) collagen IV, untreated explants (D 11); (h) collagen IV, explants treated with 0.25% CPP (D 11).

Comparison with a Commercially Available Product

In order to assess the efficacy of the polyphenols-containing formulas in comparison with commercially available products, an anti-aging cream (Future Perfect, Estee Lauder) was tested in parallel with the various concentrations assessed in the previous experiment. The cream was applied during 10 days. After 11 days, the increase in GAGs expression is similar to that of explants treated with 1.0% CPP (moderate), in the case of type I collagen it corresponds to that of 1.0% CPP (moderate), for type III collagen to 0.125%, 0.25% and 0.50% CPP (moderate, moderately dense), and for type IV collagen to 0.25% CPP (very clear).

An image analysis was carried out on the sections from the following batches, sampled on day 11: untreated control (U), CPP 0.50% (CPP 0.50), CPP 0.75% (CPP 0.75), and reference cream (RC). The parameters assessed were: the percentage of surface occupied by type I or type III collagen in the papillary dermis, and the percentage of surface occupied by GAGs or type IV collagen close to the DEJ. A diagrammatical representation is shown in Fig. 3.4.

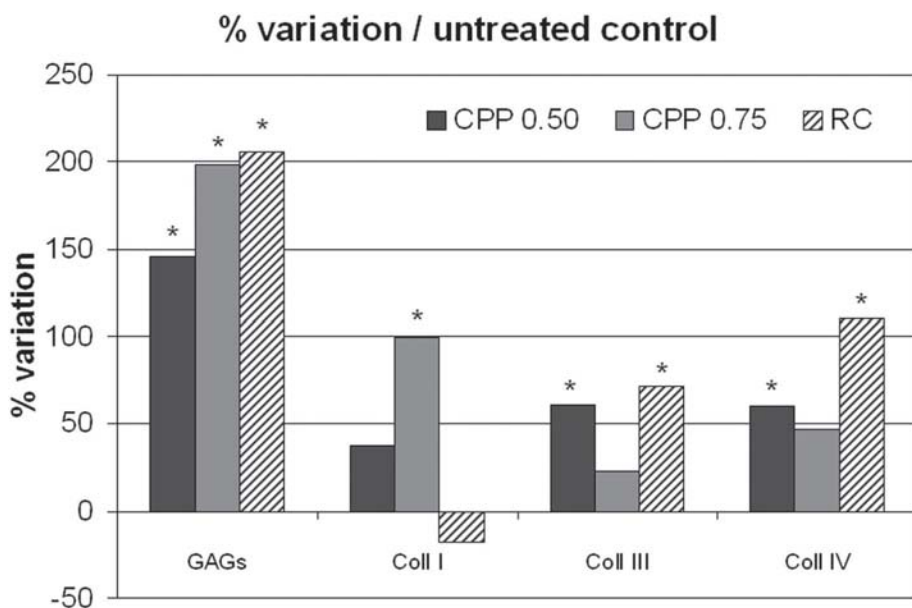


Fig. 3.4. Variation of the expression of GAGs, type I, type III and type IV collagen as a function of the product tested. CPP = cocoa polyphenols blended in cocoa butter, RC = reference cream. Asterisks indicate a statistical difference from the control ($p < 0.05$).

The qualitative results obtained in the previous experiments are substantiated by these results. A very clear increase in GAGs expression is observed for explants treated with CPP 0.75% (+198%) and CPP 0.50% (+145%). As for those treated with the reference cream, the increase (+206%) is level with that of CCP 0.75%. Concerning type I collagen, the only sample that is significantly different from the untreated control is the CPP 0.75% batch (+99%). As for type III and type IV collagen, only samples treated with CPP 0.50% (+60% and +59% respectively) or the reference cream (+71% and +110%) exhibit a significant increase compared to the control.

Enhancing Effect

The boosting effect of cocoa butter on cocoa polyphenols activity was assessed by topical application of formulas containing 0.5% cocoa polyphenols (CPP), 5% cocoa butter (CB), or both (CPP + CB). The skin explants were prepared from a 39-year-old donor. The formulas were applied topically during 11 days.

The parameters assessed were the general skin morphology and expression of GAGs, type I and type IV collagen. There is a moderate increase in GAGs expression near the DEJ after 5 days of treatment, for all three batches. After 11 days however, clear differences in GAGs expression for the CPP + CB batch appear while the increase is moderate for either CPP or CB alone. Concerning type I collagen, after 5 days, both CPP and CB batches show a slight increase in its expression, whereas the CPP + CB batch displays a clear increase. However, after 11 days, all three batches display the same very clear increase in type I collagen expression. As for type IV collagen, after 5 days of treatment, the increase in expression is observed only for the CB batch. After 11 days, there is a slight increase for the CPP batch, a clear increase for the CB batch, and the CPP + CB batch shows a very clear increase in its expression. Consequently, the enhancing effect of cocoa butter on cocoa polyphenols is highlighted, after 5 days in the case of type I collagen, or after 11 days in the case of GAGs and type IV collagen.

Conclusions

This study has shown that cocoa polyphenols exhibit a positive effect on skin structure when applied for at least 5 days. Cocoa butter also exhibits an activity, but its onset of action is longer (12 days). The dose at which cocoa polyphenols—when blended into cocoa butter—improve the most *ex vivo* parameters associated with skin tonus and elasticity is comprised between 0.50% and 0.75%. At such doses, the influence on parameters such as GAGs and collagen expression is comparable to (or better, in the case of collagen I) the activity of a commercially available anti-aging cream. Moreover, when applied in conjunction with cocoa polyphenols, cocoa butter exhibits an enhancing effect on the parameters assessed.

In a new stage, the exploration of cocoa polyphenols properties including the evaluation of their influence on skin moisturizing and restructuring *in vivo*, assessment of protective effect against UV exposure, and biochemical mechanism of action is currently in progress.

Acknowledgment

This study was funded jointly by Laboratoire BIO-EC and Barry Callebaut.

References

- Bayard, V.; et al. Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. *Int. J. Med. Sci.* 2007, 4(1), 53–58.
- Buijsse, B.; et al. Cocoa intake, blood pressure and cardiovascular mortality. The Zutphen Elderly Study. *Arch. Intern. Med.* 2006, 166, 411–417.
- Carlin, James T.; et al. Comparison of acidic and basic volatile compounds of cocoa butters from roasted and unroasted cocoa beans. *J. Am. Oil Chem. Soc.* 1986, 63(8), 1031–1036.
- Carrino, D.A.; et al. Age-related changes in the proteoglycans of human skin. *Arch. Biochem. Biophys.* 2000, Jan. 1, 373(1), 91–101.
- Ding, E.; et al. Chocolate and prevention of cardiovascular disease: a systematic review. *Nutr. & Met.* 2006, 3, 1–12.
- Engler, M.B.; et al., Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J. Am. Coll. Nutr.* 2004, 23(3), 197–204.
- Gasser, P.; et al. Cocoa polyphenols and their influence on parameters involved in ex vivo skin restructuring. *Int. J. Cosmet. Sci.* 2008, Oct., 30(5): 339–4. <http://onlinelibrary.wiley.com/doi/10.1111/j.1468-2494.2008.00457.x/abstract>.
- Heinrich, U.; et al. Long-term ingestion of high flavanol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women. *J. Nutr.* 2006, 136, 1565–1569.
- Manach, C.; et al. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 2004, 79, 727–747.
- Meyer, L.J.M.; Stern, R. Age-dependent changes of hyaluronan in human skin. *J. Invest. Dermatol.* 1994, 102, 385–389.
- Neukam, K.; et al. Consumption of flavanol-rich cocoa acutely increases microcirculation in human skin. *Eur. J. Nutr.* 2007, 46, 53–56.
- Porter, L.J.; et al. Cacao procyanidins: major flavanoids and identification of some minor metabolites. *Phytochemistry* 1991, 30, 1657–1663.
- Richelle, M.; et al., Comparison of antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J. Agric. Food Chem.* 2001, 49, 3438–3442.

- Schroeter, H.; et al. Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *PNAS* 2006, 103, 1024–1029.
- Shuster, S.; et al. The influence of age and sex on skin thickness, skin collagen and density. *Br. J. Dermatol.* 1975, 93(6), 639–643.
- Willen, D.M.; et al. Patterns of glycosaminoglycan/proteoglycan immunostaining in human skin during aging. *J. Invest. Dermatol.* 1991, 96, 968–974.
- Yamagishi, M.; et al. Chemoprevention of lung carcinogenesis by cocoa liquor proanthocyanidins in a male rat multi-organ carcinogenesis model. *Cancer Lett.* 2003, 191, 49–57.

4

Phase Behavior of Saturated Triacylglycerides—Influence of Symmetry and Chain Length Mismatch

Laziz Bouzidi and Suresh S. Narine

Trent Centre for Biomaterials Research, Departments of Physics and Astronomy and Chemistry, Trent University, Peterborough, Ontario, Canada

Introduction

Triacylglycerols (TAGs) are the most widely occurring form of lipids stored in plant and animal tissues (O'Brien, 2004). They are an important group of molecules present in natural fats and oils and used extensively in human nutrition. They are also increasingly used in the formulation of pharmaceuticals and cosmetics (Gunstone & Padley, 1997; O'Brien, 2004). TAGs are made up of a glycerol backbone esterified to three fatty acid (FA) moieties which allow for many different combinations of arranging the FA moieties and therefore for a large chemical variety and a broad range of physical properties (Sreenivasan, 1978).

The elaborate structural hierarchy in a typical lipid network is arranged in a fractal manner (Narine & Marangoni, 1999a). As the fat crystallizes from the melt, the TAGs form domains of particular polymorphism/polytypism which grow into larger microstructural elements (single crystallites then clusters). These microstructural elements then aggregate into larger microstructures until a continuous three-dimensional network is formed by the collection of microstructures (Narine & Marangoni, 1999b). The structure–function relationships in these materials is still a difficult and open field of study (Sato, 1996; Narine & Marangoni, 1999b; Walstra et al., 2001; Marangoni & Narine, 2002).

The complex structure, packing characteristics, and physical and thermodynamic properties of TAGs have been the subject of numerous publications and reviews (Mal'kin & Meara, 1939; Chapman, 1962; Timms, 1984; Larsson, 1986; Small, 1986; Hagemann, 1988; Ghotra et al., 2002; Sato & Ueno, 2005; Himawan et al., 2006). However, detailed conformational and packing information from crystallography and molecular modeling studies have only been published for some pure TAGs (Jensen & Mabis, 1966; van Soest et al., 1990; Birker et al., 1991; van Langevelde et al., 2000; Sato et al., 2001; Chandrasekhar & van Gunsteren, 2002).

TAGs exhibit diverse and complex patterns of phase polymorphism governed by complex inter-molecular interactions involving both the hydrocarbon chains and functional

groups close to the polar interface. TAG polymorphism depends strongly on the position of the FA on the glycerol unit and the chemical properties of the FA moieties, such as the chain length, parity (odd or even), unsaturation (*cis* or *trans*), etc. (Sato, 1999).

TAG polymorphism also depends strongly on the crystallization kinetics. Kinetic factors are important in determining the amount, composition and properties of the crystalline phase and which polymorph will form from the melt and should be considered in order to describe properly the behavior of fats (Los & Floter, 1999; Hollander et al., 2002). Crystallization of the equilibrium phase can be a complex transformation process in which symmetry and the conformational degrees of freedom of the molecules play an important role. The crystallization path depends strongly on the processing conditions, which are instrumental in determining the growth modes, the number of intermediate states between the beginning and final states and the phases and the polymorphic forms which appear in the material at the different stages of the growth process. During the growth process, the formation of each intermediate may occur concurrently or simultaneously en route to the equilibrium structure. This process has characteristic thermodynamic and kinetic parameters which are still very challenging to model (Sato, 1996; Narine & Marangoni, 1999b; Walstra et al., 2001; Marangoni & Narine, 2002). Therefore, understanding the pattern of phase polymorphism and the conditions to isolate each intermediate state is necessary if the properties of products containing TAGs are to be controlled and manipulated.

Two types of polymorphic transformations may occur: solid-state transformations and melt-mediated transformations. During a solid-state transformation, the TAG molecules in the metastable forms (α or β' for example) reorient their molecular orientations and hydrocarbon-chain subcell packing in the solid phase. In the melt-mediated transformation, the more stable forms crystallize through the melting of the metastable forms (Ueno et al., 1997).

The phase morphology and chain packing characteristics of the hydrocarbon chains can have profound consequences on the physical properties of TAG materials (Small, 1986; Sato & Garti, 2001). The study of the chemical and physical properties of the individual constituent TAGs and their mixtures is an effective method to gain insight into the thermal, structural and rheological properties of natural and modified oils and fats (Rossell, 1967; Timms, 1984; Humphrey & Narine, 2004; Himawan et al., 2006; Zhang et al., 2007). The comprehensive phase diagrams describing the transformation behavior of TAGs, and more generally of lipids, accumulated over the years (Timms, 1984; Koynova & Caffrey, 2002), are used to understand and help monitor the crystallization, fractionation, phase development and stabilization of TAG mixtures (Gibon et al., 1986).

Symmetry, CLM and Physical Properties

The thermophysical properties of TAGs, and more generally of organic compounds, depend strongly on molecular symmetry (Wei, 1999; Pinal, 2004) and other factors

such as the conformational degrees of freedom of the molecules, inter- and intramolecular forces (Dearden, 1991), crystal structure, and crystal packing (Chickos & Nichols, 2001; Katritzky et al., 2001). For example, with few exceptions which can be explained, crystals of symmetrical molecules always possess higher melting temperatures (Gavezzotti, 1995) and are less soluble than the crystals of less symmetrical molecules with similar structures (Gilbert, 2007). In fact, symmetrical molecules are less soluble because of the higher melting temperature of their crystals (Yalkowsky & Valvani, 1980). Polymorphism and intersolubility phenomena of TAG mixtures are closely linked and many of the important properties of TAG mixtures are controlled by the liquid-solid phase transitions (Braipson-Danthine & Gibon, 2007). Note that the relationships between the different parameters that describe the molecule or group of molecules and physical property is often non-linear and require sophisticated non-linear regression methods to model them (Bhat et al., 2008).

There are few studies of binary mixtures of symmetrical and asymmetrical mixed-acid TAGs (Timms, 1984; Yano et al., 1999; van Mechelen et al., 2006a; van Mechelen et al., 2006b; Zhang et al., 2007). It has been observed that TAGs containing two different FA, but having a symmetrical structure, demonstrate different properties (such as a higher melting point) when compared with their asymmetrical counterparts (van Soest et al., 1990). This suggests that asymmetry perturbs the crystallization process (Rousset & Rappaz, 1997; Rousset et al., 1998). Furthermore, it has been reported that the presence of symmetrical/asymmetrical isomeric species in the same sample can result in significant effects on texture, melting and polymorphic form (Rossell, 1967; Timms, 1984; Humphrey & Narine, 2004; Narine et al., 2007).

The phase behavior of symmetrical and asymmetrical mixed chain TAGs is strongly influenced by the CLM between FA moieties but also by other contributions, such as molecular weight and hydrogen bonding. These parameters play major if not predominant roles in the determination of critical properties (critical temperature, pressure and volume) as similarly reported in studies of organic molecules (see for example the studies of a set of 73 classes of hydrocarbons using structure-property relationship (SPR) models by (Godavarthy et al., 2006; Godavarthy et al., 2008)).

In mixtures of two tri-monosaturated TAGs, different phase behavior is frequently observed for different polymorphs. For example, PPP/SSS shows complete miscibility of the less stable forms (α and β') but a eutectic system for the β -form (MacNaughtan et al., 2006). Eutectic and monotectic behavior are observed in the β -form for the LLL/PPP and LLL/SSS systems, respectively, with the α form of SSS co-existing with the β -form of LLL (Takeuchi et al., 2003). After studying the phase diagrams of LLL/MMM, LLL/PPP, and LLL/SSS and also considering that of PPP/SSS, (Takeuchi et al., 2003) concluded that for binary monosaturated TAG mixtures, the metastable α - and β' -forms are miscible when CLM between two monosaturated TAGs is 2 or less, and that immiscibility of the metastable phases appears when CLM of 4 or 6 are present.

Polymorphism of Symmetrical and Asymmetrical Mixed-acid TAGs

Studies of binary mixtures of symmetrical and asymmetrical mixed-acid TAGs, which would compare to the systems examined in this chapter, are few, but are the subject of increasing interest (Timms, 1984). It is known that the β polymorph of asymmetrical TAGs is not readily formed and that the stability of the β' phase in symmetrical TAGs is increased compared to asymmetrical TAGs, a phenomenon which has been attributed to a closer and more homogenous packing of the glycerol groups (Elisabettoni et al., 1998a). The existence of different sub-forms of β' in the symmetrical and asymmetrical TAGs is well documented. The polymorphism of pure symmetrical saturated TAGs of the series $n.n + 2.n$, to which PSP belongs, and asymmetrical $n.n.n + 2$ -TAG series have been comprehensively investigated (Birker et al., 1991; van de Streek et al., 1999; van Langevelde et al., 2000). Two β' forms, with a DCL packing (coined β'_{-2} and β'_2 , higher and lower melting point phases, respectively) have been solved from single-crystal XRD data for 10.12.10 (CLC) (van Langevelde et al., 2000) and 16.16.14 (PPM) (Sato et al., 2001), and from high-resolution x-ray powder diffraction (XRPD) data for 14.16.14 (MPM) (van Langevelde et al., 2000) and 12.14.12 (LML) (Birker et al., 1991). Both the β'_{-2} modifications of these TAGs crystallize in a chair conformation in which the fatty acid chains on glycerol positions *sn*-1 and *sn*-2 are adjacent and the chain on the *sn*-3 position form the back rest of the chair (Birker et al., 1991; van de Streek et al., 1999). From the detailed crystallographic data collected by (Birker et al., 1991) for LML, (van de Streek et al., 1999) assembled and optimized by molecular dynamics a crystalline structure consistent with experimental data of the β'_{-2} polymorph observed in compounds of the $n.n + 2.n$ series. (van Mechelen et al., 2008) using DSC and time-resolved XRPD have determined the crystalline structure, polymorphic stability and phase transition behavior of PSP, PPS, PEP and PPE (E, elaidic acid). They have found evidence for the existence of the lower melting β'_2 for PSS and discovered a novel polymorph of PSS, coined β'_0 , that melts at a higher temperature than the β'_{-2} polymorph. Note that we use here the common nomenclature ($n.p.r$) in which each TAG molecule is denoted by the lengths of the three fatty acids making up the TAG molecule (for example 16.16.16 for tripalmitin).

Symmetrical and Asymmetrical Mixed-acid TAGs Containing Oleic Acid

More recently, interest has been focused on symmetrical and asymmetrical TAGs containing oleic acid (O: *cis*-9-octadecenoic acid), one of the most abundant unsaturated fatty-acid chains in natural fats and oils. One can cite the detailed structural and phase behavior studies performed by Sato's group (Yano et al., 1999; Zhang et al., 2007) and Shen's groups (van Mechelen et al., 2006a; van Mechelen et al.,

2006b). (Yano et al., 1999), for example, investigating the polymorphic transformations in SOS and OSO by XRD and Fourier transform infrared (FT-IR) spectroscopy, have reported for both TAGs a reversible phase transition between a hexagonal and a pseudo-hexagonal packings (α and sub- α , respectively), and a series of irreversible transitions ($\alpha \rightarrow \beta' \rightarrow \beta$). Thermodynamic and kinetic studies by DSC and XRD with a conventional generator and synchrotron radiation of OOP and its mixtures with POP showed that POP/OOP mixtures exhibited immiscible eutectic natures in both their metastable α - and their most stable β' -states (Zhang et al., 2007). Shen's group has solved from high-resolution (HR) laboratory and synchrotron powder diffraction (SPD) data the crystalline structures of the β -1 polymorphs of pure MOM, POP and SOS compounds, and of the 1:1 molar mixture of SOS and POP (van Mechelen et al., 2006a) and of the β -2 polymorphs of POP and SOS and have established a crystal structure model for the β -2 polymorph of SOS (van Mechelen et al., 2006b).

Binary Systems Examined in this Chapter

Our group has completed a series of investigations of lipid binary systems made of symmetrical (BAB) and asymmetrical (BBA) TAGs having neighboring chain length mismatch (CLM). All of the TAGs contain A= stearic acid (S, C18) and the other neighboring fatty acids B are: lauric acid (L, C12) (Bouzidi et al., 2010), capric acid (C, C10) (Boodhoo et al., 2009a), myristic acid (M, C14) (Boodhoo et al., 2008) and palmitic acid (P, C16) (Boodhoo et al., 2009b). Two dramatically different cooling rates, i.e. 0.1°C/min and 3.0°C/min were used to investigate the kinetics of the crystallization process. These will be referred to as the slow and rapid cooling experiments. The binary mixtures were examined using X-ray diffraction (XRD), differential scanning calorimetry (DSC), polarized light microscopy (PLM), and wide-line pulsed nuclear magnetic resonance (pNMR) measurements to investigate the crystal structure, phase transition behavior, microstructure, and solid fat content (SFC), respectively. The relationships between TAG chemical structure and their physical properties were examined with an emphasis on the thermodynamics, characteristic phase structures, and phase transition kinetics. In this chapter, we provide an analytical summary of the melting and phase behavior of the binary mixtures crystallized at the two widely different cooling rates, as a function of molecular symmetry, chain-length mismatch and molecular weight.

Materials and Methods

Sample Preparation

Pure TAGs were synthesized in our laboratory according to known procedures (Chandran & Bhatnagar, 1968; Bentley & McCrae, 1970). Their purity as determined by

GC-FID and HPLC exceeded 98.0%. In the GC-FID (range of 0–10 V), the sample was run as is in chloroform using a chromo pack-triglycerides analysis phase column (CP-TAP, Varian, USA), specifically designed for TAG analysis. Waters Alliance (Milford, MA) e2695 HPLC system fitted with a Waters ELSD 2424 evaporative light scattering detector was used for HPLC analysis. The purified TAGs were mixed in the desired molar fractions (in 0.1 molar fraction increments), equilibrated at 90°C for 5 min and stirred at this temperature for a further 5 min using a mechanical stirrer to obtain homogeneous mixtures. Intermediate compositions were prepared when detailed analysis was needed.

Thermal Processing

The cooling profiles were obtained by heating the sample to higher than 90°C and equilibrating for 5 min, a temperature and a time over which crystal memory is erased, then cooled at two widely different cooling rate of 0.1°C/min and 3.0°C/min, to a finish temperature T_f , where it was held isothermally for a time t_b . T_f and t_b were designed to ensure that the crystallization is complete at the measurement temperature and that no further thermal event is ongoing as confirmed by long flat baselines in the DSC cooling thermograms. For DSC analysis, the sample was processed in the cell of the instrument. Samples in the DSC were reheated immediately after the cooling process at a constant rate of 5.0°C/min to 90°C to obtain the melting profile. All measurement temperatures are reported to a certainty of better than $\pm 0.5^\circ\text{C}$.

Experimental Techniques, Data Analysis and Modeling

Differential Scanning Calorimetry

Approximately 5.0 to 10.0 (± 0.1) mg of fully melted and homogeneously mixed sample was placed in an aluminum DSC pan, which was then hermetically sealed. The DSC measurements were carried out using a DSC Q100 model (TA Instruments, New Castle, DE). An empty aluminum pan was used as a reference and the experiments were performed under a nitrogen flow of 50 mL/min. The “TA Universal Analysis” software coupled with a method developed by our group (Bouzidi et al., 2005) was used to analyze the data and extract the main characteristics of the peaks (temperature at maximum heat flow, T_m ; onset temperature, T_{On} ; offset temperature, T_{Off} ; enthalpy, ΔH ; and full width at half maximum, FWHM). The temperature window over which a thermal event occurs is defined as the absolute value of the difference between T_{Off} and T_{On} of that event. It is labeled ΔT_c for crystallization and ΔT_m for melting. Whenever possible, the resolved peaks and shoulders were decomposed into single thermal events assuming Gaussian shapes. The characteristics of non-resolved shoulders were estimated using a simple decomposition of the signal into its obvious

main components. The positions in this case were estimated using the first and second derivatives of the differential heat flow.

Thermodynamic Analysis of the Boundaries in the Phase Diagrams

The kinetic phase diagrams were constructed from the data generated in the DSC heating experiments. The point determined by the most stable crystal is much more suitable for studying equilibrium properties and therefore, T_p of the last endotherm was used to determine the liquidus line, as typically done in the study of binary lipid mixtures (Höhne et al., 2003; Inoue et al., 2004; MacNaughtan et al., 2006; Abes et al., 2007; Costa et al., 2007; Abes et al., 2008; Boodhoo et al., 2008; Boodhoo et al., 2009b; Boodhoo et al., 2009c). The solidus line is constructed from T_{On} of the last melting peak. T_M of the other peaks when present, is used to represent solid-solid transition lines and the solidus line, after correction for the transition widths of the pure components (Inoue et al., 1993).

A thermodynamic model based on the Hildebrand equation (Hildebrand, 1929) coupled with the Bragg-William approximation for non-ideality of mixing (Bragg & Williams, 1934) was used to simulate the phase boundaries in the phase diagram and to investigate the miscibility of the components. This model is a powerful tool commonly used to study lipid mixtures (Lee, 1977a; Lee, 1977b; Höhne et al., 2003; Inoue et al., 2004; MacNaughtan et al., 2006; Abes et al., 2007; Costa et al., 2007; Abes et al., 2008; Boodhoo et al., 2008; Boodhoo et al., 2009a; Boodhoo et al., 2009b).

In a system composed of components A and B, given the assumption that there is complete miscibility of components A and B in the melted phase and their complete immiscibility in the solid state. The equilibrium liquidus line can be described by either of the following equations depending on whether the composition is smaller or larger than the eutectic composition X_E (Lee, 1977b; Tenchov, 1985)

$$\ln X_A = -\frac{\Delta H_A}{R} \left(\frac{1}{T} - \frac{1}{T_A} \right) \quad \text{Equation 4.1}$$

$$\ln X_B = -\frac{\Delta H_B}{R} \left(\frac{1}{T} - \frac{1}{T_B} \right) \quad \text{Equation 4.2}$$

where R is the gas constant, X_A represents the mole fraction of A, ΔH_A and T_A are the molar heat of fusion and the melting point of component A, X_B , ΔH_B and T_B are those of component B.

The Bragg-Williams approximation attributes the origin of the non-ideality of mixing to the enthalpy term of the free energy of mixing and assumes the same entropy term as in the ideal mixing case (Moore, 1972). In this model the deviation from an

ideal behavior is described by a non-ideality of mixing parameter, ρ (J/mol), defined as the difference in the energy of mixed-pairs (A–B) and the average pair interaction energy between like pairs (A–A and B–B) formed in the mixture:

$$\rho = z \left(u_{AB} - \frac{u_{AA} + u_{BB}}{2} \right) \quad \text{Equation 4.3}$$

where z is the first coordination number, u_{AB} , u_{AA} and u_{BB} the interaction energies for AB, AA and BB pairs, respectively.

For ideal mixing, the intermolecular interaction of like-pairs is equal to that of mixed-pairs and consequently $\rho = 0$. An ideality parameter different from zero was explained by (Knoester et al., 1972) as being due to the formation of a solid solution between the high-melting and low-melting components. A positive ρ is obtained when mixed-pair formation is energetically less favorable and reflects a tendency of like molecules to cluster, which beyond some critical value leads to phase separation. A negative ρ is obtained when the formation of AB pairs is energetically more favorable compared with the formation of AA or BB pairs and reflects a tendency for order (Lee, 1977a; Lee, 1977b).

According to this approximation, the two branches of the liquidus line delimited by the eutectic composition are given by:

$$\ln X_A + \frac{\rho(1 - X_A)^2}{RT} = -\frac{\Delta H_A}{R} \left(\frac{1}{T} - \frac{1}{T_A} \right) \quad \text{Equation 4.4}$$

$$\ln X_B + \frac{\rho(1 - X_B)^2}{RT} = -\frac{\Delta H_B}{R} \left(\frac{1}{T} - \frac{1}{T_B} \right) \quad \text{Equation 4.5}$$

Melting Behavior and Phase Development of the Symmetrical/Asymmetrical TAG Binary Systems

The pattern of thermal behavior of the PSP-PPS binary system, CLM = 2, (Boodhoo et al., 2009b) is relatively complex and is influenced by the rate at which the samples were cooled, but not to the extent of changing the sequence of the phase transitions observed. The thermograms obtained after slow (0.1°C/min, Fig. 4.1a) and rapid (3.0°C/min, Fig. 4.1b) cooling show several thermal events with at least two or three prominent endotherms depending on PSP content. Recrystallization mediated by melt (mixtures with $X_{PSP} < 0.90$) and direct recrystallization (mixtures with $X_{PSP} \leq 0.90$) from the preexisting α -phase of the more stable β' -phase are observed for this system. The effect of the kinetics on phase development can be appreciated through the variation of the enthalpy of the different endotherms. As can be seen

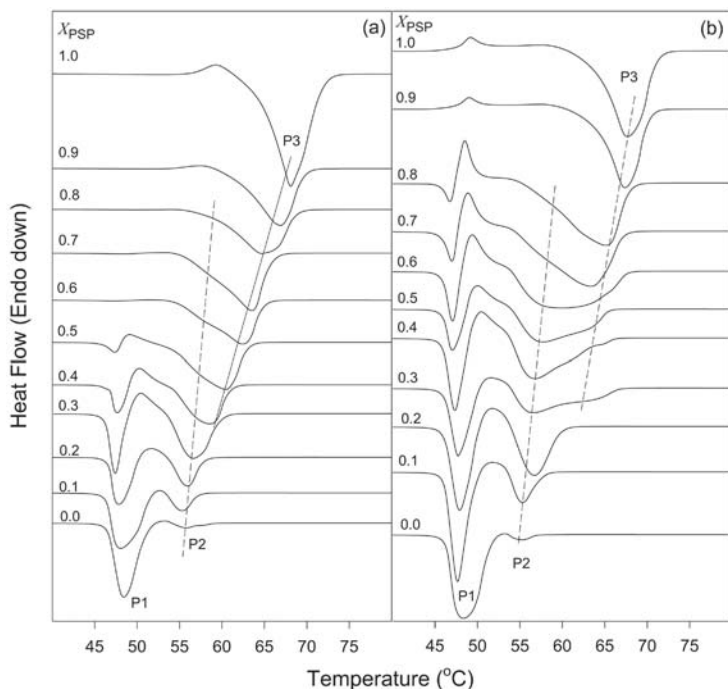


Fig. 4.1. Heating thermograms of PSP-PPS mixtures obtained using a heating rate of 5°C/min for samples cooled at rates of (a) 0.1°C/min and (b) at 3.0°C/min. Molar fractions are reported above each curve at the left-hand side of the figure. The lines are guide for the eye.

in both experiments (open and filled triangles, respectively in Figs. 4.2a and 4.2b), the enthalpy of the leading endotherm, ΔH_{M1} , decreased almost exponentially with increasing PSP content, whereas, the enthalpy of the combined last endotherms, ΔH_{M23} , increased accordingly.

The heating thermograms obtained for the MSM-MMS binary system, CLM= 4, (Boodhoo et al., 2008) when the mixtures were cooled slowly showed polymorphic transformations only for the 0.0 and 0.1_{MSM} mixtures (Fig. 4.3a). For mixtures with higher MSM content, only a single melting peak is observed, indicating a resistance to melt mediated transformation. This effect is probably due to MSM, which as claimed for other symmetric TAGs, may have singular chain–chain interactions and achieve an alignment of the glycerol moiety that prevent the transformation (Kellens M., 1991; Elisabettini et al., 1998b). When cooled rapidly, however, marked recrystallization mediated by melt of the pre-existing α -form into the thermodynamically more stable β' -form was observed for compositions up to 0.6_{MSM} (Fig. 4.3b). The higher cooling

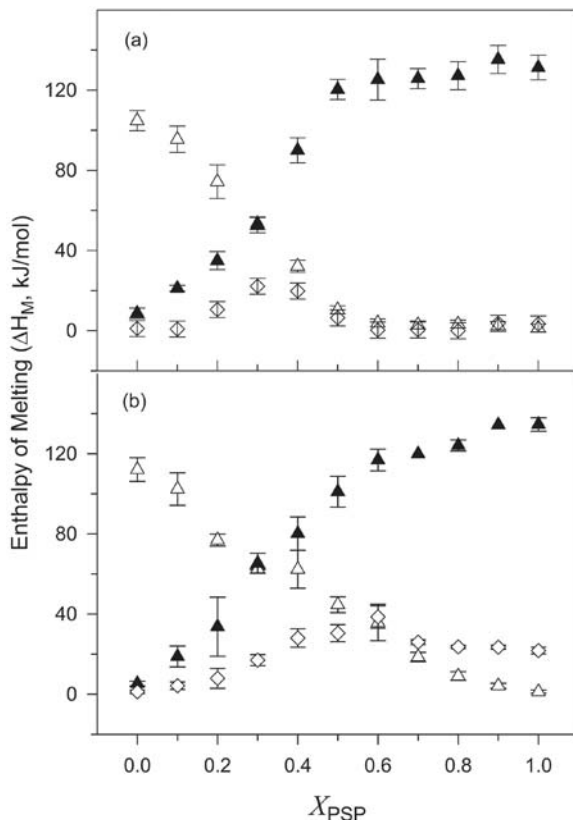


Fig. 4.2. Estimated enthalpies of melting (H_M , kJ/mol) as a function of the PSP molar fraction for the PSP-PPS binary mixtures for (a) the 0.1°C/min and (b) the 3.0°C/min cooling rate (leading peak, Δ ; combined last endotherms, $\blacktriangle$; and exotherm, $\diamond$).

rate (3.0°C/min) has in fact mitigated the halting effect of the symmetric MMS, at levels as high as 0.6_{MSM}. The cooling rate did not influence very significantly the overall enthalpy of melting. Furthermore, regardless of the modifications displayed by the individual peaks, the total enthalpy of melting remained almost the same for all the MSM/MMS mixtures, confirming the resistance to transformation outlined above.

The heating thermograms of the LSL/LLS mixtures cooled slowly (0.1°C/min) displayed a single endotherm with a shoulder (Fig. 4.4a). Assuming Gaussian shapes, the peak has been successfully decomposed into its two components (dashed curves P1 and P2 in Fig. 4.4a). Except for the pure LSL, where the peaks are at high temperatures corresponding to the melting of β -phases, all the other peaks are the record-

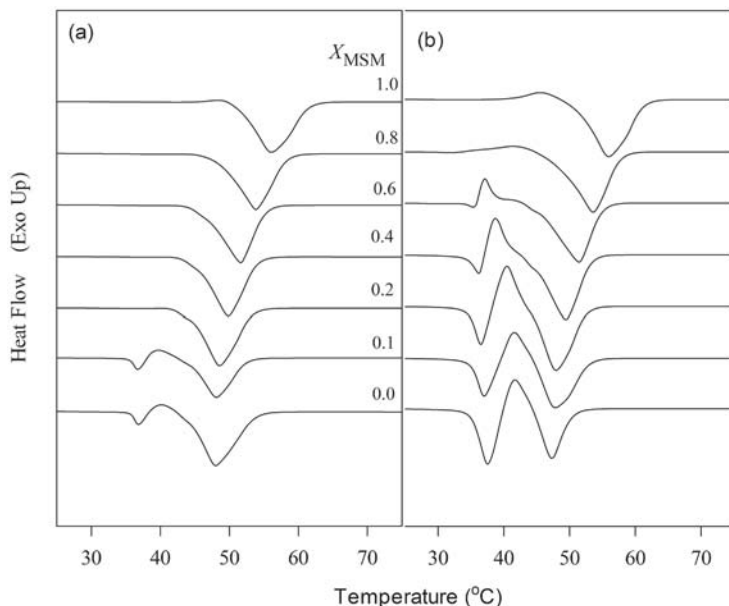


Fig. 4.3. DSC heating (5.0°C/min) thermograms of MSM/MMS obtained using a heating rate of 5°C/min for samples cooled at rates of (a) 0.1°C/min and (b) at 3.0°C/min. Molar fractions are reported above each curve at the right-hand side of the panel (a).

ing of the melting of β' -phases. A larger number of endotherms and exotherms, and hence polymorphic transformations, are recorded on reheating the samples previously cooled rapidly at 3°C/min (Fig. 4.4b). The successive peaks and shoulders and the exotherms are the signature of phase development driven by recrystallizations mediated by the melt. The transformation sequence is simple starting with the melting of the pre-existing α -phase, its recrystallization to a β' - then to a β -form. This analysis has been confirmed by DSC and XRD data collected for β' - and β -phases which have been isolated in both LSL and LLS.

The DSC data of the CSC-CCS binary system, CLM= 8, (Boodhoo et al., 2009a) unraveled a complex pattern of thermal behavior. Crystallization and subsequent polymorphic transformations revealed by the heating thermograms of the CSC/CCS mixtures are strongly dependent on cooling rate. A relatively simple heating thermogram was demonstrated in the slow cooling experiment (0.1°C/min) with one or two melting peaks depending on CSC content (Fig. 4.5a) compared to the rapid cooling experiment (3.0°C/min), where a complex thermal behavior with multiple endotherms is observed, particularly for mixtures with molar ratio lower than 0.50_{CSC} (Fig. 4.5b), highlighting again the strong halting effect of the symmetrical TAG on phase development.

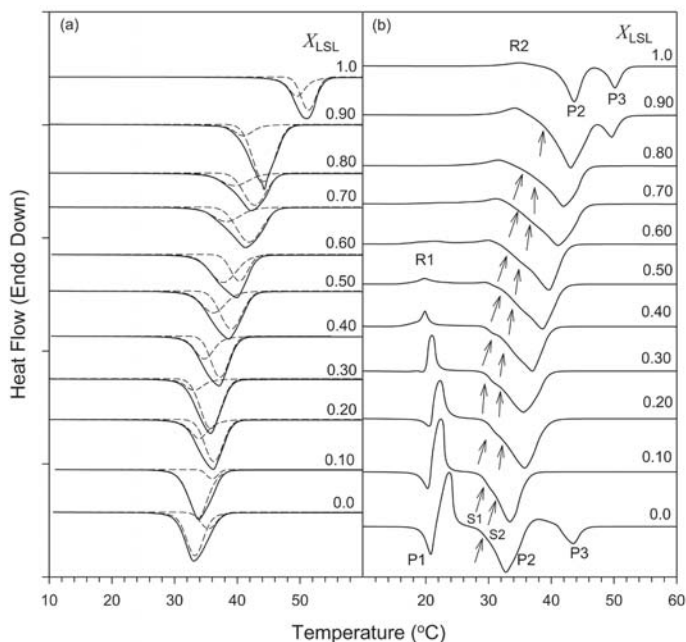


Fig. 4.4. Heating curves of LSL-LLS mixtures obtained using a heating rate of 5°C/min for samples cooled at rates of (a) 0.1°C/min and (b) at 3.0°C/min. Molar fractions are reported above each curve at the right-hand side of the figure. The dotted lines in (a) are Gaussian peaks from the decomposition of the endotherms and the arrows in (b) indicate the positions of the shoulders. Molar fractions are reported above each curve at the right-hand side of the figure.

Phase Diagrams of the Symmetrical/Asymmetrical TAG Binary Systems

The liquidus line in the phase diagram of the binary system was simulated using the thermodynamic model described in section 2.3.2 with values of (ΔH_A , T_A), and (ΔH_B , T_B) determined from the DSC heating curves of the purified (A) and (B) samples. The non-ideality of mixing parameter, ρ , was adjusted first manually in small steps to obtain a liquidus line which lies closest to the experimental boundaries. This line was then refined to calculate the curve that has the least sum of squares of the difference between experimental and calculated temperatures over the whole experimental composition range. Recall that the Bragg-Williams approximation attributes the origin of the non-ideality of mixing to the enthalpy term of the free energy of mixing and assumes the same entropy term as in the ideal mixing case (Moore, 1972).

The calculated liquidus line of the binary system assuming an ideal fluid phase using Eq. (1) and (2) did not reproduce the experimental phase boundary for any

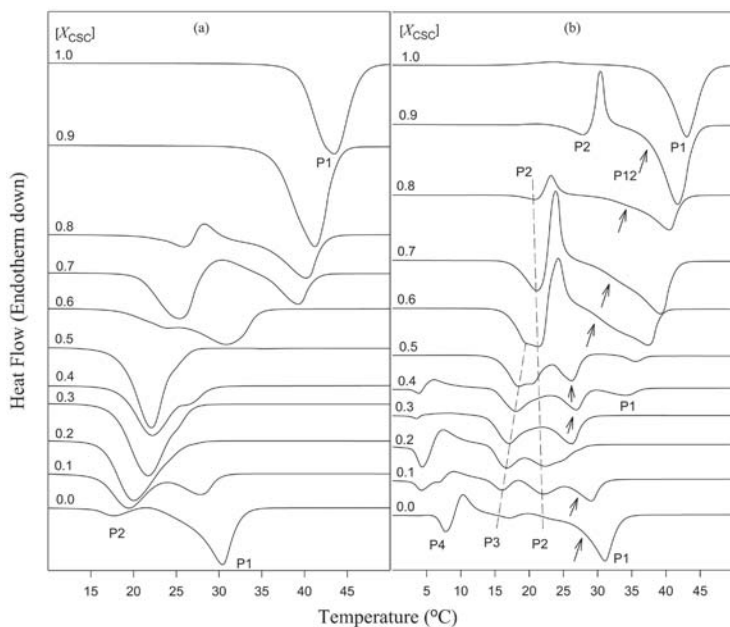


Fig. 4.5. DSC heating curves of CSC/CCS mixtures obtained using a heating rate of 5°C/min for samples cooled at rates of (a) 0.1°C/min and (b) at 3.0°C/min. Molar fractions are reported above each curve at the left-hand side of the figure.

binary system studied and is therefore not shown. The simultaneous simulation of the liquidus and solidus lines, for the whole phase diagram as well as for the eutectic region and monotectic region alone, have been inconclusive due to the simplicity of the model which does not take into account the dependence of the non-ideality parameter with molar fraction.

The fit of the experimental liquidus line has been very satisfactorily achieved by applying the Eq. (4) and (5) to all regions delimited by the eutectic and any singularity if present. Overall, the calculated values of ρ for the systems presented here are comparable to published values for other binary lipid systems (Nibu & Inoue, 1995; Inoue et al., 2004; Garidel et al., 2005; Abes et al., 2007; Abes et al., 2008).

Note that the reported position of the phase diagram features (eutectic point as well as transformation lines) depends on the thermal procedure used to identify phase transformation and development. The thermal protocol (cool and heat at constant rates) used to construct the phase diagrams do not produce equilibrium states. However, they allow the study of TAG solubility and may be extrapolated to describe equilibrium states. They are also interesting from an applied view point as the thermal protocol is close to that/those used in industry.

PSP-PPS with CLM = 2 (Boodhoo et al., 2009b)

The PSP/PPS binary kinetic phase diagram exhibits a singularity in the liquidus line at the 0.5_{PSP} concentration indicative of the formation of a 1:1 (mol:mol) compound (Minato et al., 1997b) for both the slow (Fig. 4.6a) and fast cooling experiments (Fig. 4.6b). The singularity divides the experimental kinetic phase diagram into two regions: a eutectic region from 0.0_{PSP} to 0.5_{PSP} and a monotectic region afterwards. The eutectic behavior is more marked in the slow cooling compared to the rapid cooling experiment with a eutectic located at the same concentration, X_b , of approxi-

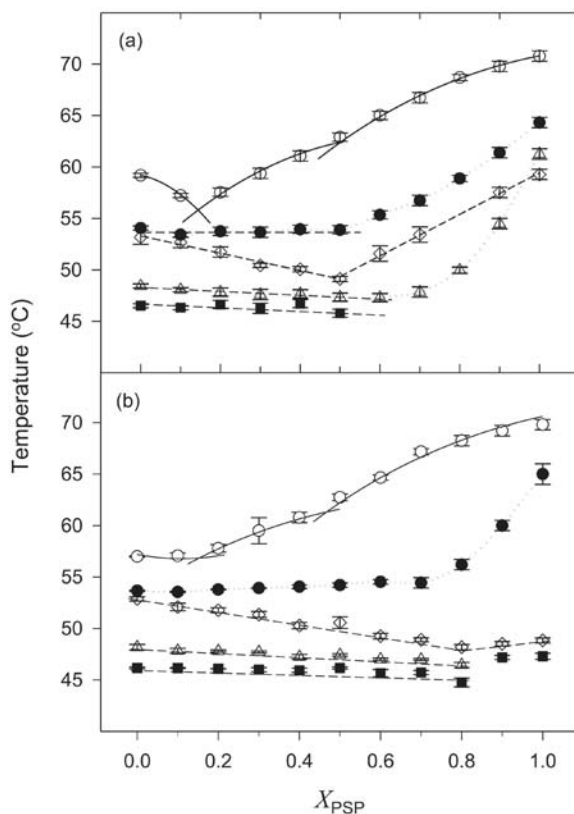


Fig. 4.6. PSP/PPS binary phase diagram constructed from the DSC heating runs of the samples cooled at (a) $0.1^{\circ}\text{C}/\text{min}$ and (b) $3.0^{\circ}\text{C}/\text{min}$. Offset (T_{off}), $\circ$; and onset (T_{on}), $\blacksquare$ temperature of melting. Peak temperature of the exotherm (T_R), $\diamond$. All temperatures are in $^{\circ}\text{C}$. The solid lines are calculated segments of the liquidus line. The dashed lines are linear fits of the experimental points through which they pass. The dotted lines are guides for the eye.

mately 0.15_{PSP} . The solidus line of the phase diagram obtained in the slow cooling experiment extends horizontally in the $X_{PSP} = [0.1 - 0.5]$ concentration range and the solid-solid transition line extends linearly to the $X_{PSP} = 0.6$ composition with a slight shift to lower temperature with increasing PSP molar fraction. The same qualitative characteristics were observed in the case of the rapid cooling experiment where both lines extend to $X_{PSP} = 0.7$ (dashed lines passing through the experimental data points ??? and ???, respectively, in Fig. 4.6a and 4.6b).

The fit of the experimental liquidus line has been very satisfactorily achieved by considering the singularity at 0.5_{PSP} and applying Eq. (4.4) and (4.5) to both the eutectic and monotectic regions which it delimits. The singularity has been confirmed at 0.5_{PSP} in both experiments. The simulated three segments of the liquidus line are represented by solid lines in Figs. 6a and 6b for the $0.1^\circ\text{C}/\text{min}$ and $3.0^\circ\text{C}/\text{min}$ cooling experiments, respectively. The simulation yielded negative values of ρ for all segments, except for the $X_{PSP} \leq X_E$ segment in the $3.0^\circ\text{C}/\text{min}$ cooling experiment where it is positive. The calculated values of ρ , X_E and T_E are listed in Table 4-A.

The PSP/PPS binary phase diagram obtained when the mixtures were cooled slowly ($0.1^\circ\text{C}/\text{min}$) is comparable to that less detailed obtained earlier using differential thermal analysis (DTA) measurements (Perron et al., 1971) or that established using an extensive and complex thermal procedure so-called “thermal conditioning” involving slow heating ($0.1^\circ\text{C}/\text{min}$) (Ollivon & Perron, 1979). Both phase diagrams

Table 4-A. Thermodynamic parameters ΔH (kJ/mol), the molar heat of fusion and T_m ($^\circ\text{C}$), the melting point of purified PSP (A), purified PPS (B) and 0.50_{PSP} mixture (C) used to model the liquidus line in the phase diagram for the $0.1^\circ\text{C}/\text{min}$ and $3.0^\circ\text{C}/\text{min}$ cooling rates. T_E , eutectic temperature ($^\circ\text{C}$); and X_E , eutectic composition (PSP molar fraction) determined by the intersection of the calculated segments of the liquidus line. The non-ideality parameter ρ (kJ/mol) is derived from the simulation of the phase diagram boundaries in the $0 \leq X_{PSP} \leq X_E$ range, the $X_E \leq X_{PSP} \leq 0.50_{PSP}$ range and the $0.50_{PSP} \leq X_{PSP} \leq 1$ range.

	T_m ($^\circ\text{C}$)			ΔH (kJ/mol)		
	A	B	C	A	B	C
$0.1^\circ\text{C}/\text{min}$	70.8	59.2	62.4	130	113	124
$3.0^\circ\text{C}/\text{min}$	70.6	57	62.6	114	116	115

	Singularity	X_E	T_E ($^\circ\text{C}$)	ρ (kJ/mol)		
				$0.0_{PSP} - X_E$	$X_E - 0.50_{PSP}$	$0.50_{PSP} - 1.0_{PSP}$
$0.1^\circ\text{C}/\text{min}$	0.50_{PSP}	0.14	55.8	-35.0	-8.0	-5.0
$3.0^\circ\text{C}/\text{min}$	0.47_{PSP}	0.15	56.8	+14.0	-4.0	-3.5

show a singularity at the 0.50_{PSP} mixture as well as a eutectic, albeit at different points ($\sim 0.1_{PSP}$ and 0.38_{PSP} for the first and second work, respectively). The authors suggested a probable peritectic transformation for the PSP/PPS binary system at $\sim 63^\circ\text{C}$ spanning from 0.55_{PSP} to 0.85_{PSP} , without a direct backup with experimental data. A eutectic type phase diagram ($X_E = 0.3$ and $T_E \sim 61^\circ\text{C}$) for the PSP/PPS binary system was obtained by (Knoester et al., 1972) using an extensive cycling stabilization procedure and interpreted in terms of ideal miscibility in the liquid phase and immiscibility in the solid-state. The differences in values obtained for the different phase diagrams features (eutectic point as well as transformation lines) depends on the thermal procedure used, as discussed earlier in the section.

The experimental kinetic phase diagram of the PSP/PPS binary system was well described by the introduction of negative values of ρ for all the segments considered, apart from the $X_{PSP} \leq X_E$ region in the $3.0^\circ\text{C}/\text{min}$ cooling experiment (Table 4-A). In this region, a large positive value was necessary to fit the data, and the calculated line has a relatively flat minimum centered at 0.14_{PSP} . Even if the uncertainty attached to the calculated ρ for the $X_{PPS} \leq X_E$ concentration region is larger than in the others, the positive value of ρ calculated in the $3.0^\circ\text{C}/\text{min}$ cooling experiment, contrast strikingly with the large negative value calculated in the $0.1^\circ\text{C}/\text{min}$ cooling experiment. Recall that positive ρ reflects a tendency of like molecules to cluster, which beyond some critical value, ρ_c , leads to a phase separation. The molecular interactions, as depicted by the negative ρ -values, are strong and tend to favor the formation of unlike pairs in the liquid state. Recall that a negative ρ reflects a tendency for order (Lee, 1977a).

The effect of the cooling rate on mixing in the PSP-PPS system is quite interesting. The kinetic effects are evident, particularly dramatically in the $X_{PPS} \leq X_E$ side of the phase diagram. The miscibility in the $X_{PPS} \geq X_E$ concentration region as inferred from the model in both experiments didn't change dramatically with the processing condition. Other key parameters obtained from the phase diagram of the PSP-PPS binary system, such as the solid–solid transformation lines, did not show any dependence on the kinetics. The effect of cooling rate is, however, obviously revealed in the shift of the maximum of the estimated enthalpy of recrystallization, ΔH_R , versus X_{PSP} from 0.3_{PSP} to 0.5_{PSP} (Figs. 2a and 2b) and in the blurring of the eutectic composition when the cooling rate is increased (Figs. 6a and 6b).

The marked kinetic effects seen in the $X_{PSP} \leq X_E$ concentration region, as reflected by the flattening of the liquidus line at the eutectic composition in the fast cooling experiment, is probably due to the halting effect of the asymmetrical PPS which combined to a rapid crystallization prevented the formation of the most stable packing and conformation possible.

MSM-MMS with CLM = 4 (Boodhoo et al., 2008)

The MMS/MSM binary kinetic phase diagrams obtained in the slow cooling ($0.1^\circ\text{C}/\text{min}$) and rapid cooling experiment ($3.0^\circ\text{C}/\text{min}$) closely overlay and displayed a typi-

cal monotectic behavior. The MMS/MSM binary kinetic phase diagrams obtained in the slow cooling ($0.1^\circ\text{C}/\text{min}$) is displayed in Fig. 4.7. In this system the solidus line is almost horizontal between 0.0 and 0.6_{MSM} , indicating the occurrence of phase separation. The experimental liquidus and solidus lines have been very well reproduced assuming non-ideal mixing in both liquid and solid phases and using expressions of the Bragg-Williams model originally derived by Lee and co-workers (Lee, 1977b). The best fit (solid lines in Fig. 4.7) was achieved using $p_L = +1.5 \text{ kJmol}^{-1}$. The introduction of positive values for p_L suggests that the MSM–MMS attraction is less favored than the MSM–MSM and MMS–MMS attractions in the liquid phase.

LSL–LLS with CLM = 6 (Bouzidi et al., 2010)

The liquidus line in both kinetic phase diagrams of the LSL–LLS system obtained with mixtures cooled slowly (Fig. 4.8a) and rapidly (Fig. 4.8b) is obviously not one of a simple eutectic. It displayed a singularity at the 0.5_{LSL} composition indicating the formation of a 1:1 (mol:mol) molecular compound. The simulation of the liquidus line in the kinetic phase diagram boundaries (solid lines passing through T_{Off} in Figs. 8a and 8b) revealed two distinct behaviors of the LSL–LLS mixtures: a eutectic in the $X_{\text{LSL}} \leq 0.5$ and a monotectic in the $X_{\text{LSL}} \geq 0.5$ concentration region. In samples cooled at $0.1^\circ\text{C}/\text{min}$, X_E is $\sim 0.15_{\text{LSL}}$ and $T_E \sim 37.5^\circ\text{C}$ (Fig. 4.8a) and displayed a clear eutectic line running from 0.0_{LSL} to 0.2_{LSL} and a horizontal phase separation line spanning from approximately 0.5_{LSL} to 0.8_{LSL} (dashed lines passing through ● in Fig. 4.8a). In the $3.0^\circ\text{C}/\text{min}$ crystallization cooling rate experiment, X_E is $\sim 0.1_{\text{LSL}}$ and $T_E \sim 36^\circ\text{C}$

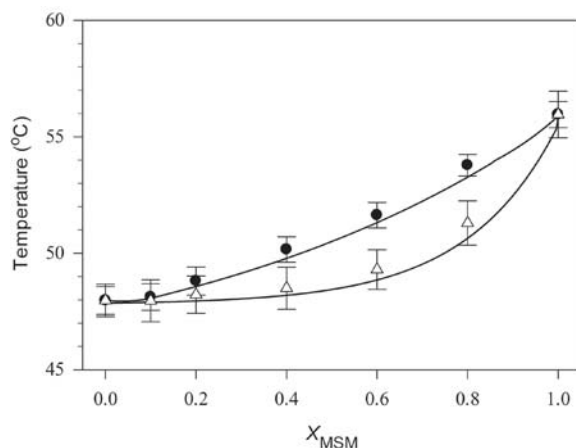


Fig. 4.7. MSM/MMS kinetic phase diagram constructed from the DSC heating runs of the samples cooled at $0.1^\circ\text{C}/\text{min}$. The solid lines are obtained from the simulation of the experimental liquidus line (●) and solidus (Δ) lines.

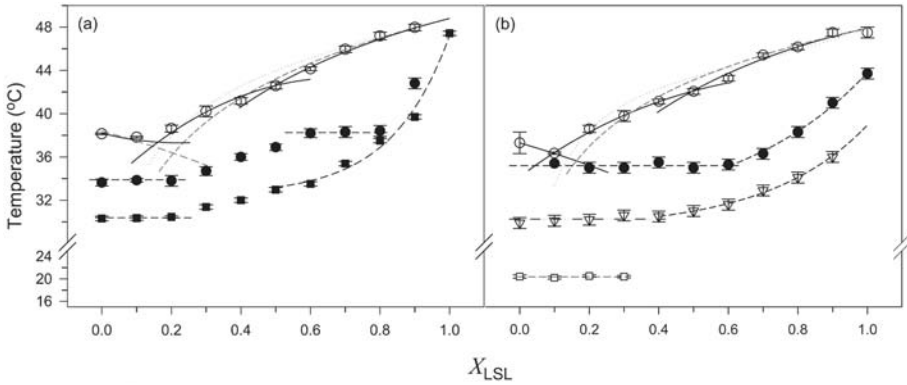


Fig. 4.8. LSL/LLS kinetic phase diagram constructed from the DSC heating runs of the samples cooled at (a) 0.1°C/min and (b) at 3.0°C/min. The symbols are as follows: Offset temperature of melting, $\square$; temperature at position of shoulder S (T_3), $\bullet$; temperature at maximum heat flow (T_m) of main peak, ∇ ; onset temperature of melting, $\blacksquare$. (Dashed lines are calculated liquidus segments assuming an ideal fluid phase; dotted line passing are calculated liquidus line segments considering a simple eutectic behavior; solid lines are calculated liquidus line segments considering a singularity at 0.50 $_{LSL}$, a eutectic and a monotectic). All temperatures are in °C.

(Fig. 4.8b) and displayed a eutectic line running from 0.0 $_{LSL}$ to 0.6 $_{LSL}$ (dashed line passing through $\bullet$ in Fig. 4.8b). The horizontal transformation segments indicate phase separation as commonly observed for other binary mixtures of TAGs (Rossell, 1967; Knoester et al., 1972).

The effect of the cooling rate on mixing in the LSL-LLS system was not dramatic. In both experiments, the simulation yielded small negative values of the non-ideality parameter of mixing, ρ , for all the segments considered, except for the $X_{LSL} \leq X_E$ region where it is slightly positive (Table 4-B). The miscibility in the liquid state, as inferred from the model in both experiments, is almost ideal and doesn't change with the processing conditions. Moreover, T_E decreases only by approximately 1°C, a low value which suggests the formation of the same phase constituents for the eutectic mixture. The effect of cooling rate is revealed by the shift of X_E from 0.16 (for 0.1°C/min) to 0.10 (for 3.0°C/min) and by the appearance of solid–solid transformation lines when the samples are cooled rapidly. This is not surprising as a difference in behavior is not to be excluded, when there are large differences in the time available for crystal growth (Chen et al., 2005). The marked kinetic effects are probably due to the halting effect of the asymmetrical LLS combined to a rapid crystallization, such that the molecules do not have enough time to form the same molecular arrangements and the most stable packing and conformation possible. Phase separation is also detected in both kinetic phase diagrams albeit within a larger concentration range in the 3.0°C/min cooling rate experiment.

Table 4-B. Thermodynamic parameters ΔH (kJ/mol), the molar heat of fusion and T_m (°C), the melting point of purified LSL (A), purified LLS (B) and 0.50_{LSL} mixture (C) used to model the liquidus line in the phase diagram for the 0.1°C/min and 3.0°C/min cooling rates. X_E eutectic composition (LSL molar fraction); T_E eutectic temperature (°C) determined by the intersection of the calculated segments of the liquidus line. The non-ideality parameter ρ (kJ/mol) derived from the simulation of the phase diagram boundaries in the $0 \leq X_{LSL} \leq X_E$ range, the $X_E \leq X_{LSL} \leq 0.50_{LSL}$ range and the $0.50_{LSL} \leq X_{LSL} \leq 1$ range.

	T_m (°C)			ΔH (kJ/mol)		
	A	B	C	A	B	C
0.1°C/min	48.8	38.2	42.6	105	98	105
3.0°C/min	47.9	37.3	42.1	122	86	95

	Singularity	X_E	T_E (°C)	ρ (kJ/mol)		
				$0.0_{LSL} - X_E$	$X_E - 0.50_{LSL}$	$0.50_{LSL} - 1.0_{LSL}$
0.1°C/min	0.50 _{LSL}	0.16	37.2	3 ± 3	-6.0 ± 0.1	-0.8 ± 0.1
3.0°C/min	0.50 _{LSL}	0.10	36.1	1 ± 1	-2.5 ± 0.1	-1.5 ± 0.1

CSC-CCS with CLM = 8 (Boodhoo et al., 2009a)

The kinetic phase diagram of the CSC-CCS system cooled at 0.1°C/min (Fig. 4.9a) and 3.0°C/min (Fig. 4.9b) displayed typical eutectic behaviors. The eutectic composition, X_E , shifted dramatically from 0.50 for the 0.1°C/min cooling rate to 0.25 for the 3.0°C/min, and the eutectic temperature decreased by ~2.3°C. The shift of the eutectic composition with cooling rate, as well as the presence of solid–solid transformations that are different for the two cooling rates, demonstrates the strong dependence of the phase diagram of this lipid system on the thermodynamics as well as the kinetics.

The experimental kinetic phase diagram of the CSC/CCS binary system was well described (solid lines in Figs. 9a and 9b for the 0.1°C/min and 3.0°C/min, respectively) by the introduction of negative values of non-ideality of mixing parameter, ρ , (Table 4-C) which indicates that, in the liquid state, the formation of a CSC–CCS pair is energetically more favorable compared with CSC–CSC and CCS–CCS pairs. The calculated ρ -values are large and illustrate the peculiarity of the molecular interactions of this system. The solubility of CSC in CCS as depicted by ρ shows a strong dependence on the cooling rate and CSC content. The effect of the cooling rate on ρ , and therefore on mixing, is quite interesting. For the 3.0°C/min cooling rate measurements, the segment $X_{CSC} < X_E$ yielded a ρ -value three times that of the $X > X_E$ segment, the inverse of what was displayed by ρ for the 0.1°C/min experiment.

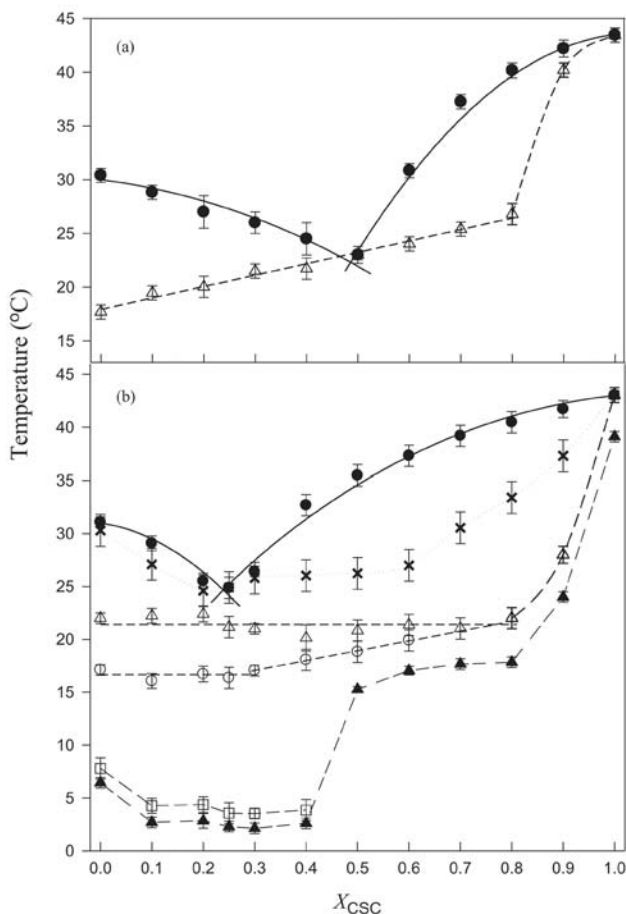


Fig. 4.9. CSC/CCS binary phase diagram constructed from the heating runs of the samples cooled at (a) 0.1 °C/min and (b) 3.0 °C/min. The solid lines are calculated liquidus line segments. The dashed line is a guide for the eye.

Molecular Compounds

The presence of a singularity in the liquidus line and the formation of a 1:1 (mol:mol) compound has been suggested in the LSL/LLS (Bouzidi et al., 2010) and PSP/PPS binary systems studied in our laboratory (Boodhoo et al., 2009b). The formation of such a compound is not unusual for binary TAG systems even if only little work has been reported on the molecular structures and kinetic properties of systems which form molecular compounds. The formation of a 1:1 molecular compound has been suggested in mixed saturated TAG system such as SPS/PSS and PPP/PPS (Knoester

Table 4-C. Thermodynamic parameters ΔH_A (kJ/mol), the molar heat of fusion and T_A (°C), the melting point of purified CSC, and ΔH_B (kJ/mol), the molar heat of fusion and T_B (°C), the melting point of purified CCS used to model the liquidus line in the phase diagram for the 0.1°C/min and 3.0°C/min cooling rates. The non-ideality parameter ρ (kJ/mol) derived from the simulation of the phase diagram boundaries in the $X_E \leq X_{CSC} \leq 1$ range and the $0 \leq X_{CSC} \leq X_E$ range. T_E (°C) and X_E are the eutectic temperature and the eutectic composition, respectively, determined by the intersection of the calculated segments of the liquidus line.

Scan rate (°C/min)	ΔH_A (kJ/mol)	ΔH_B (kJ/mol)	T_A (°C)	T_B (°C)	X_E	T_E (°C)	ρ (kJ/mol)	
							$X_{CSC} < X_E$	$X_{CSC} > X_E$
0.1	156	128	43.5	30.4	0.49	22.4	-6.5	-32.5
3.0	145	115	43.1	31.0	0.24	24.7	-30	-9.4

et al., 1972). The formation of a 1:1 molecular compound has been suggested in both systems to explain the singularity of the clear-point of the 1:1 mixture which is well above the liquidus curve (Knoester et al., 1972). The phase diagram showed clearly a eutectic behavior in each side of the 1:1 (mol:mol) composition for both systems. The formation of a 1:1 molecular compound in these systems was justified by conformational considerations. The authors suggested that the shape of the TAG molecules is such that a very dense packing becomes possible with equal amounts of both molecules, though the crystals of each of the pure components can accommodate only a small amount of the other component. It is however, possible that due to specific interactions (molecular interactions of acyl chain packing, glycerol conformation, and methyl end stacking) as is generally accepted (Timms, 1984; Engstrom, 1992; Koyano et al., 1992), symmetrical and asymmetrical TAGs can display a synergistic compatibility and pack more easily together than on their own to form a molecular compound.

The formation of a 1:1 molecular compound is also observed in systems of two TAGs which both contain an unsaturated fatty acid (oleic acid) such as POP/OPO (Rossell, 1967), SOS/OSO (Koyano et al., 1992), POP/PPO and POP/OPO (Minato et al., 1997a; Minato et al., 1997b), and SOS/SSO (Engstrom, 1992; Takeuchi et al., 2002). The formation of such compound in SOS/SSO (Engstrom, 1992) and SOS/OSO (Koyano et al., 1992) for example, is explained by specific molecular interactions through the acyl chain moieties. It has been suggested that the arrangement in these systems is less problematic, as like chains from either TAG can arrange themselves together, than in mixtures where monosaturated and mixed-acid saturated/unsaturated TAGs are combined, such as the PPP/POP system (Minato, 1996), where there is a pronounced steric effect. Such a mechanical interlocking of the TAGs cannot fully explain the formation of the compound in the case of our samples. Note

that as reported by (Zhang et al., 2007), the formation of a molecular compounds of a DCL structure in POP/PPO and POP/OPO mixtures appears to be most influenced by the contribution of the glycerol conformation. More experimental and modeling work is needed to understand this behavior.

Comparison of the Phase Behavior of the Symmetrical/Asymmetrical Binary Systems with CLM = 2, 4, 6, and 8

CLM between FA moieties strongly influence the overall phase behavior of mixed chain symmetrical and asymmetrical TAGs but its relative importance compared to other contributions is difficult to predict.

Overall, the asymmetrical TAGs demonstrated lower melting and crystallization points, whether the sample is cooled at 0.1°C/min or 3°C/min. The observed differences in melting temperature between the symmetrical and asymmetrical TAGs are ~18.0°C, 7.8°C, 10.3°C, and 13.1°C in the 0.1°C/min cooling experiment for PSP-PPS with CLM = 2 (Boodhoo et al., 2009b), MSM-MMS with CLM = 4 (Boodhoo et al., 2008), LSL-LLS with CLM = 6 (Bouzidi et al., 2010) and CSC-CCS with CLM = 8 (Boodhoo et al., 2009a), respectively. Except for PSP and PPS TAGs which have the highest temperature difference, an increase in CLM of 2 carbon atoms increases this temperature difference by ~2.5°C. Note that, compared to the other binary systems, and under the thermal conditions used, the PSP/PPS binary system, with a CLM of 2, demonstrates unique behavior, attributed to its large molecular weight and the disturbances introduced at the methyl-end level in the solid state (Boodhoo et al., 2009b). The difference in melting as well as crystallization temperature between the symmetrical and asymmetrical TAGs, in essentially the same polymorphic form, can be explained by differences in packing at the methyl end-plane and the position of the step plane which may have an important influence on the melting point of the TAGs (de Jong and van Soest, 1978).

Similar to systems found in the literature, such as PPP/SSS (Himawan et al., 2007), the more ordered phases crystallize in mixtures with larger CLM, with the phases crystallized using the slower cooling rate being relatively more stable. The PSP-PPS mixtures pack in the metastable α -form whether cooled at 0.1°C/min or 3.0°C/min. All MSM-MMS mixtures crystallize in the β' -form when cooled at 0.1°C/min and in the α -form when cooled at 3.0°C/min (Boodhoo et al., 2008). The polymorphism of the LSL-LLS and CSC-CCS mixtures depend strongly on concentration as well as thermal processing. The LSL-LLS mixtures pack exclusively in the β' -form when they are cooled slowly, whereas a mix of α - and β' -phases form when they are cooled rapidly. In this case, the β' -form is promoted by LSL and predominates largely when LSL molar fraction ≥ 0.7 . CSC-CCS mixtures present related forms of the β' -form

for all compositions as well as a β -form for mixtures with CSC molar fraction ≥ 0.7 whether cooled at $0.1^\circ\text{C}/\text{min}$ or $3.0^\circ\text{C}/\text{min}$ (Boodhoo et al., 2009a).

The LSL/LLS system presents a relatively complex phase diagram. Similarly to the PSP/PPS mixtures, the LSL-LLS mixtures form a so-called “molecular compound” with a 1:1 molar ratio of the two components and display two distinct behaviors: a eutectic in the $X_{LSL} \leq 0.5$ and a monotectic in the $X_{LSL} \geq 0.5$ concentration region. The CSC-CCS binary system with $\text{CLM} = 8$ presents a eutectic behavior whereas, the MSM-MMS with $\text{CLM} = 4$ is monotectic and both do not form molecular compounds. In both the solid and liquid states of MSM-MMS ($\text{CLM} = 4$) binary mixtures, unlike pair interactions are favored over like pair interactions resulting in the monotectic behavior whereas, in the case of LSL-LLS ($\text{CLM} = 6$) and CSC-CCS ($\text{CLM} = 8$) binary mixtures, like-pair interactions are favored over unlike-pair interactions and result in limited miscibility and a eutectic behavior (Boodhoo et al., 2009a).

The eutectic composition, X_E , is dependent on CLM and strongly affected by processing. In both experiments (slow or rapid cooling) X_E increased with CLM. For PSP/PPS, MSM/MMS, LSL/LLS and CSC/CCS systems, X_E is 0.15, 0, 0.15 and 0.50, respectively, in the slow cooling experiment ($0.1^\circ\text{C}/\text{min}$) and 0.15, 0, 0.10 and, 0.25, respectively, in the fast one ($3.0^\circ\text{C}/\text{min}$). Note that except in the case of the PSP/PPS system, X_E shifts to smaller values with increasing cooling rate.

The changes due to kinetics effects are more pronounced in mixtures containing higher amounts of asymmetrical than symmetrical TAG, with the effect being stronger for larger molecular weights and larger CLM. The crystals obtained with slow cooling ($0.1^\circ\text{C}/\text{min}$) are more homogeneous and pack more closely than those obtained with rapid cooling ($3.0^\circ\text{C}/\text{min}$). This is not unusual as rapid crystallization is known to result in poorly packed crystals (Dafler, 1977; Gibon et al., 1986; Timms, 1991). Imperfect crystals may persist for years in the absence of a liquid phase but can easily recrystallize into well packed crystals via the liquid phase, if a liquid phase is present (Hernqvist, 1988). The thermal properties of such crystals depend on their “degree of imperfection” and can deviate significantly from those of well-ordered ones (Hagemann, 1988).

Our work highlights the critical effect of the conformation of the molecules on the phase behavior of TAG mixtures and shows that even small differences in total chain length, or a simple rearrangement of the chains have dramatic effect on the mutual solubility of long-chain saturated TAGs. The particular behavior under the thermal conditions used in our studies of the PSP/PPS system (Boodhoo et al., 2009b) compared to the other binary systems, is attributed to its large molecular weight and the disturbances introduced at the methyl-end level in the solid state. This suggests that melting temperature and other thermo-physical properties, which are bulk properties in many respects, are determined, not only by molecular symmetry (Wei, 1999), but also by other parameters, such as molecular weight, H-bonding and van der Waals interactions (Godavarthy et al., 2006; Godavarthy et al., 2008).

References

- Abes, M.; Bouzidi, L.; Narine, S.S. Crystallization and phase behavior of 1,3-propanediol esters II. 1,3-Propanediol distearate/1,3-propanediol dipalmitate (SS/PP) and 1,3-propanediol distearate/1,3-propanediol dimyristate (SS/MM) binary systems. *Chem. Phys. Lipids* 2007, 150, 89–108.
- Abes, M.; Bouzidi, L.; Narine, S.S. Crystallization and phase behavior of fatty acid esters of 1,3 propanediol III: 1,3 propanediol dicaprylate/1,3 propanediol distearate (CC/SS) and 1,3 propanediol dicaprylate/1,3 propanediol dipalmitate (CC/PP) binary systems. *Chem. Phys. Lipids* 2008, 151, 110–124.
- Bentley, P.H.; McCrae, W. Efficient synthesis of symmetrical 1,3-diglycerides. *J. Org. Chem.* 1970, 35, 2082–2083.
- Bhat, A.U.; Merchant, S.S.; Bhagwat, S.S. Prediction of melting points of organic compounds using extreme learning machines. *Ind. Eng. Chem. Res.* 2008, 47, 920–925.
- Birker, P.; Dejong, S.; Roijers, E.C.; Vansoest, T.C. Structural investigations of β' triacylglycerols—an X-ray-diffraction and microscopic study of twinned β' crystals. *J. Am. Oil Chem. Soc.* 1991, 68, 895–906.
- Boodhoo, M.V.; Bouzidi, L.; Narine, S.S. The binary phase behavior of 1,3-dicaproyl-2-stearoyl-*sn*-glycerol and 1,2-dicaproyl-3-stearoyl-*sn*-glycerol. *Chem. Phys. Lipids* 2009a, 157, 21–39.
- Boodhoo, M.V.; Bouzidi, L.; Narine, S.S. The binary phase behavior of 1, 3-dipalmitoyl-2-stearoyl-*sn*-glycerol and 1, 2-dipalmitoyl-3-stearoyl-*sn*-glycerol. *Chem. Phys. Lipids* 2009b, 160, 11–32.
- Boodhoo, M.V.; Kutek, T.; Filip, V.; Narine, S.S. The binary phase behavior of 1,3-dimyristoyl-2-stearoyl-*sn*-glycerol and 1,2-dimyristoyl-3-stearoyl-*sn*-glycerol. *Chem. Phys. Lipids* 2008, 154, 7–18.
- Bouzidi, L.; Boodhoo, M.; Humphrey, K.L.; Narine, S.S. Use of first and second derivatives to accurately determine key parameters of DSC thermographs in lipid crystallization studies. *Thermochim. Acta* 2005, 439, 94–102.
- Bouzidi, L.; Boodhoo, M.V.; Kutek, T.; Filip, V.; Narine, S.S. The binary phase behavior of 1,3-dilauroyl-2-stearoyl-*sn*-glycerol and 1,2-dilauroyl-3-stearoyl-*sn*-glycerol. *Chem. Phys. Lipids* 2010, 163, 607–629.
- Bragg, W.L.; Williams, E.J. The effect of thermal agitation on atomic arrangement in alloys. *Proc. R. Soc.* 1934, 145, 699–730.
- Braipson-Danthine, S.; Gibon, V. Comparative analysis of triacylglycerol composition, melting properties and polymorphic behavior of palm oil and fractions. *Eur. J. Lipid Sci. Technol.* 2007, 109, 359–372.
- Chandran, D.V.; Bhatnagar, R.K. A method for synthesis of α -monoricinolein. *J. Am. Oil Chem. Soc.* 1968, 45, 581–582.
- Chandrasekhar, I.; van Gunsteren, W.F. A comparison of the potential energy parameters of aliphatic alkanes: molecular dynamics simulations of triacylglycerols in the alpha phase. *Eur. Biophys. J. Biophys. Lett.* 2002, 31, 89–101.

- Chapman, D. Polymorphism of glycerides. *Chem. Rev.* 1962, 62, 433–456.
- Chen, Y.P.; Tang, M.; Kuo, J.C. Solid-liquid equilibria for binary mixtures of N-phenylacetamide with 4-aminoacetophenone, 3-hydroxyacetophenone and 4-hydroxyacetophenone. *Fluid Phase Equilib.* 2005, 232, 182–188.
- Chickos, J.S.; Nichols, G. Simple relationships for the estimation of melting temperatures of homologous series. *J. Chem. Eng. Data* 2001, 46, 562–573.
- Costa, M.C.; Rolemberg, M.P.; Boros, L.A.D.; Krahenbuhl, M.A.; de Oliveira, M.G.; Meirelles, A.J.A. Solid-liquid equilibrium of binary fatty acid mixtures. *J. Chem. Eng. Data* 2007, 52, 30–36.
- Dafler, J. Polymorphism behavior in fully hydrogenated mono-acid triglycerides. *J. Am. Oil Chem. Soc.* 1977, 54, 249–254.
- de Jong, S.D.; van Soest, T.C. Crystal-structures and melting points of saturated triglycerides in β -2 phase. *Acta Crystallogr. Sect. B-Struct. Commun.* 1978, 34, 1570–1583.
- Dearden, J.C. The Qsar prediction of melting-point, a property of environmental relevance. *Sci. Total Environ.* 1991, 109, 59–68.
- Elisabettni, P.; Lognay, G.; Desmedt, A.; Culot, C.; Istasse, N.; Deffense, E.; Durant, F. Synthesis and physicochemical characterization of mixed diacid triglycerides that contain elaidic acid. *J. Am. Oil Chem. Soc.* 1998a, 75, 285–291.
- Elisabettni, P.; Lognay, G.; Desmedt, A.; Culot, C.; Istasse, N.; Deffense, E.; Durant, F. Synthesis and physicochemical characterization of mixed acid triglycerides that contain elaidic acid. *J. Am. Oil Chem. Soc.* 1998b, 75, 285–291.
- Engstrom, L. Triglyceride systems forming molecular compounds. *Fett Wiss. Technol.-Fat Sci. Technol.* 1992, 94, 173–181.
- Garidel, P.; Johann, C.; Blume, A. The calculation of heat capacity curves and phase diagrams based on regular solution theory. *J. Therm. Anal. Calorim.* 2005, 82, 447–455.
- Gavezzotti, A. Molecular symmetry, melting temperatures and melting enthalpies of substituted benzenes and naphthalenes. *J. Chem. Soc.-Perkin Trans.* 1995, 2, 1399–1404.
- Ghotra, B.S.; Dyal, S.D.; Narine, S.S. Lipid shortenings: a review. *Food Res. Int.* 2002, 35, 1015–1048.
- Gibon, V.; Durant, F.; Deroanne, C. Polymorphism and intersolubility of some palmitic, stearic and oleic triglycerides—PPP, PSP and POP. *J. Am. Oil Chem. Soc.* 1986, 63, 1047–1055.
- Gilbert, A.S. Molecular symmetry depresses the entropy of fusion of organic molecules with regard to their expected values when comparing structural isomers. *Thermochim. Acta.* 2007, 452, 135–139.
- Godavarthy, S.S.; Robinson, R.L.; Gasem, K.A.M. An improved structure-property model for predicting melting-point temperatures. *Ind. Eng. Chem. Res.* 2006, 45, 5117–5126.
- Godavarthy, S.S.; Robinson, R.L.; Gasem, K.A.M. Improved structure-property relationship models for prediction of critical properties. *Fluid Phase Equilib.* 2008, 264, 122–136.
- Gunstone, F.D.; Padley, F.B. *Lipid Technologies and Applications*. Marcel Dekker: New York, 1997; p. 848.

- Hagemann, J. Thermal Behavior and Polymorphism of Acylglycerols. In *Crystallization and Polymorphism of Fats and Fatty Acids*; Garti, N., and Sato, K., Eds.; Marcel Dekker, New York, 1988; pp. 9–95.
- Hernqvist, L. Crystal Structures of Fats and Fatty Acids. In *Crystallization and Polymorphism of Fats and Fatty Acids*; Garti, N., and Sato, K., Eds.; Marcel Dekker, New York, 1988; pp. 97–137.
- Hildebrand, J.H. Solubility XII. Regular solutions. *J. Am. Chem. Soc.* 1929, 51, 66–80.
- Himawan, C.; MacNaughtan, W.; Farhat, I.A.; Stapley, A.G.F. Polymorphic occurrence and crystallization rates of tristearin/tripalmitin mixtures under non-isothermal conditions. *Eur. J. Lipid Sci. Technol.* 2007, 109, 49–60.
- Himawan, C.; Starov, V.M.; Stapley, A.G.F. Thermodynamic and kinetic aspects of fat crystallization. *Adv. Colloid Interface Sci.* 2006, 122, 3–33.
- Höhne, G.W.H.; Hemminger, W.F.; Flammersheim, H.-J. *Differential Scanning Calorimetry*, 2nd ed. Springer-Verlag: Berlin, Heidelberg, New York, 2003; pp. 298.
- Hollander, F.F.A.; Kaminski, D.; Duret, D.; van Enckevort, W.J.P.; Meekes, H.; Bennema, P. Growth and morphology of thin fat crystals. *Food Res. Int.* 2002, 35, 909–918.
- Humphrey, K.L.; Narine, S.S. Lipid Phase Behavior. In *Fat Crystal Networks*. Marcel Dekker, New York, 2004; pp. 83–114.
- Inoue, T.; Hisatsugu, Y.; Yamamoto, R.; Suzuki, M. Solid-liquid phase behavior of binary fatty acid mixtures 1. Oleic acid/stearic acid and oleic acid/behenic acid mixtures. *Chem. Phys. Lipids* 2004, 127, 143–152.
- Inoue, T.; Motoda, I.; Hiramatsu, N.; Suzuki, M.; Sato, K. Phase-Behavior of binary mixture of palmitoleic acid (*cis*-9-hexadecenoic acid) and asclepic acid (*cis*-11-octadecenoic acid). *Chem. Phys. Lipids* 1993, 66, 209–214.
- Jensen, L.H.; Mabis, A.J. Refinement of structure of β -tricaprin. *Acta Cryst.* 1966, 21, 770–781.
- Katritzky, A.R.; Jain, R.; Lomaka, A.; Petrukhin, R.; Maran, U.; Karelson, M. Perspective on the relationship between melting points and chemical structure. *Cryst. Growth Des.* 2001, 1, 261–265.
- Kellens M.; M.W.; Gehrke R.; Reynaers H. Synchrotron radiation investigations of the polymorphic transitions in saturated monoacid triglycerides. Part 2: Polymorphism study of a 50:50 mixture of tripalmitin and tristearin during crystallization and melting. *Chem. Phys. Lipids* 1991, 58, 145–158.
- Knoester, M.; Vandente, M.; Debruijn, P. Solid-liquid equilibrium of binary-mixtures of triglycerides with palmitic and stearic chains. *Chem. Phys. Lipids* 1972, 9, 309–319.
- Koyano, T.; Hachiya, I.; Sato, K. Phase behavior of mixed systems of SOS and OSO. *J. Phys. Chem.* 1992, 96, 10514–10520.
- Koynova, R.; Caffrey, M. An index of lipid phase diagrams. *Chem. Phys. Lipids* 2002, 115, 107–219.
- Larsson, K. Physical Properties—Structural and Physical Characteristics. In *The Lipid Handbook*; Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds.; Chapman and Hall: London, 1986; pp. 335–377.

- Lee, A.G. Lipid phase-transitions and phase-diagrams. 1. Lipid phase-transitions. *Biochim. Biophys. Acta.* 1977a, 472, 237–281.
- Lee, A.G. Lipid phase-transitions and phase-diagrams. 2. Mixtures involving lipids. *Biochim. Biophys. Acta.* 1977b, 472, 285–344.
- Los, J.; Floter, E. Construction of kinetic phase diagrams. *PCCP Phys. Chem. Chem. Phys.* 1999, 1, 4251–4257.
- MacNaughtan, W.; Farhat, I.A.; Himawan, C.; Starov, V.M.; Stapley, A.G.F. A differential scanning calorimetry study of the crystallization kinetics of tristearin-tripalmitin mixtures. *J. Am. Oil Chem. Soc.* 2006, 83, 1–9.
- Malkin, T.; Meara, M.L. An x-ray and thermal examination of the glycerides. Part IV: Symmetrical mixed triglycerides, CH(O COR')(CH₂ O COR)(2). *J. Chem. Soc.* 1939, 103–108.
- Marangoni, A.G.; Narine, S.S. Identifying key structural indicators of mechanical strength in networks of fat crystals. *Food Res. Int.* 2002, 35, 957–969.
- Minato, A. Synchrotron radiation X-ray diffraction study on phase behaviour of PPP-POP binary mixtures. *J. Am. Oil Chem. Soc.* 1996, 73, 1567–1572.
- Minato, A.; Ueno, S.; Smith, K.; Amemiya, Y.; Sato, K. Thermodynamic and kinetic study on phase behavior of binary mixtures of POP and PPO forming molecular compound systems. *J. Phys. Chem. B.* 1997a, 101, 3498–3505.
- Minato, A.; Ueno, S.; Yano, J.; Smith, K.; Seto, H.; Amemiya, Y.; Sato, K. Thermal and structural properties of *sn*-1,3-dipalmitoyl-2-oleoylglycerol and *sn*-1,3-dioleoyl-2-palmitoylglycerol binary mixtures examined with synchrotron radiation x-ray diffraction. *J. Am. Oil Chem. Soc.* 1997b, 74, 1213–1220.
- Moore, W.J. *Physical Chemistry* 4th ed. Prentice-Hall: Englewood Cliffs, New Jersey, 1972; pp 229–278.
- Narine, S.S.; Humphrey, K.L.; Kincs, F.F. Shortenings and Methods of Making and Using Thereof. Application#: 60/953,609, United States, 2007.
- Narine, S.S.; Marangoni, A.G. Fractal nature of fat crystal networks. *Phys. Rev. E.* 1999a, 59, 1908–1920.
- Narine, S.S.; Marangoni, A.G. Relating structure of fat crystal networks to mechanical properties: a review. *Food Res. Int.* 1999b, 32, 227–248.
- Nibu, Y.; Inoue, T. Miscibility of binary phospholipid mixtures under hydrated and non-hydrated conditions. 2. Phosphatidylethanolamines with different acyl-chain lengths. *Chem. Phys. Lipids* 1995, 76, 159–169.
- O'Brien, R.D. *Fats and Oils: Formulating and Processing for Applications*, 2nd ed. CRC Press: Boca Raton, Fla., 2004; pp. 529.
- Ollivon, M.; Perron, R. Study of binary mixtures of triglycerides derived from palmitic and stearic acids. *Chem. Phys. Lipids* 1979, 25, 395–414.
- Perron, R.; Petit, J.; Mathieu, A. Etude des triglycerides derives des acides palmitique et stearique et de leurs melanges par analyse thermique differentielle II—etude des quinze melanges binaires—1° et 2° parties. (Study of triglycerides derived from palmitic and stearic acids and their

- mixtures by differential thermal analysis. II. Study of fifteen binary mixtures 1 and 2). *Chem. Phys. Lipids* 1971, 6, 58–94.
- Pinal, R. Effect of molecular symmetry on melting temperature and solubility. *Org. Biomol. Chem.* 2004, 2, 2692–2699.
- Rossell, J.B. Phase diagrams of triglyceride systems. *Adv. in Lip. Res.* 1967, 5, 353–408.
- Rousset, P.; Rappaz, M. α -melt-mediated crystallization of 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol. *J. Am. Oil Chem. Soc.* 1997, 74, 693–697.
- Rousset, P.; Rappaz, M.; Minner, E. Polymorphism and solidification kinetics of the binary system POS-SOS. *J. Am. Oil Chem. Soc.* 1998, 75, 857–864.
- Sato, K. Polymorphism of Pure Triacylglycerols and Natural Fats. In *Advances in Applied Lipid Research*; Padley, F.B., Ed.; JAI Press Inc.: London, 1996; pp. 213–268.
- Sato, K. Solidification and phase transformation behaviour of food fats—a review. *Fett-Lipid.* 1999, 101, 467–474.
- Sato, K.; Garti, N. *Crystallization Processes in Fats and Lipid Systems*; Marcel Dekker: New York, 2001; p. 533.
- Sato, K.; Goto, M.; Yano, J.; Honda, K.; Kodali, D.R.; Small, D.M. Atomic resolution structure analysis of β' polymorph crystal of a triacylglycerol: 1,2-dipalmitoyl-3-myristoyl-*sn*-glycerol. *J. Lipid Res.* 2001, 42, 338–345.
- Sato, K.; Ueno, S. Polymorphism of Fats and Oils. In *Edible Oils and Fat Products: Chemistry, Properties, and Health Effects*; Shahidi, F., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2005, pp. 77–120.
- Small, D.M. Glycerides. In *The Physical Chemistry of Lipids, from Alkanes to Phospholipids*; Hanahan, D.J., Ed.; Plenum Press: New York, 1986; pp. 345–372.
- Sreenivasan, B. Interesterification of fats. *J. Am. Oil Chem. Soc.* 1978, 55, 796–805.
- Takeuchi, M.; Ueno, S.; Sato, K. Crystallization kinetics of polymorphic forms of a molecular compound constructed by SOS (1,3-distearoyl-2-oleoyl-*sn*-glycerol) and SSO (1,2-distearoyl-3-oleoyl-*rac*-glycerol). *Food Res. Int.* 2002, 35, 919–926.
- Takeuchi, M.; Ueno, S.; Sato, K. Synchrotron radiation SAXS/WAXS study of polymorph-dependent phase behavior of binary mixtures of saturated monoacid triacylglycerols. *Crys. Growth & Des.* 2003, 3, 369–374.
- Tenchov, B.G. Non-uniform lipid distribution in membranes. *Prog. Surf. Sci.* 1985, 20, 273–340.
- Timms, R.E. Phase-behavior of fats and their mixtures. *Prog. Lipid. Res.* 1984, 23, 1–38.
- Timms, R.E. Crystallization of fats. *Chem. Ind.* 1991, 342–345.
- Ueno, S.; Minato, A.; Seto, H.; Amemiya, Y.; Sato, K. Synchrotron radiation x-ray diffraction study of liquid crystal formation and polymorphic crystallization of SOS (*sn*-1,3-distearoyl-2-oleoyl glycerol). *J. Phys. Chem. B.* 1997, 101, 6847–6854.
- van de Streek, J.; Verwer, P.; de Gelder, R.; Hollander, F. Structural analogy between β' triacylglycerols and *n*-alkanes. Toward the crystal structure of $\beta'-2$ p.p+2.p triacylglycerols. *J. Am. Oil Chem. Soc.* 1999, 76, 1333–1341.

- van Langevelde, A.; van Malssen, K.; Driessen, R.; Goubitz, K.; Hollander, F.; Peschar, R.; Zwart, P.; Schenk, H. Structure of $C_nC_n+2C_n$ -type ($n = \text{even}$) β' -triacylglycerols. *Acta Crystallogr. Sect. B-Struct. Sci.* 2000; 56, 1103–1111.
- van Mechelen, J.B.; Peschar, R.; Schenk, H. Structures of mono-unsaturated triacylglycerols. I. The β -1 polymorph. *Acta Crystallogr. Sect. B-Struct. Sci.* 2006a, 62, 1121–1130.
- van Mechelen, J.B.; Peschar, R.; Schenk, H. Structures of mono-unsaturated triacylglycerols. II. The β -2 polymorph. *Acta Crystallogr. Sect. B-Struct. Sci.* 2006b, 62, 1131–1138.
- van Mechelen, J.B.; Peschar, R.; Schenk, H. Structures of mono-unsaturated triacylglycerols. IV. The highest melting $\beta'-2$ polymorphs of *trans*-mono-unsaturated triacylglycerols and related saturated TAGs and their polymorphic stability. *Acta Crystallogr. Sect. B-Struct. Sci.* 2008, 64, 249–259.
- van Soest, T.C.; Dejong, S.; Roijers, E.C. Crystal-structures and melting-points of saturated triacylglycerols in the β -3 phase. *J. Am. Oil Chem. Soc.* 1990, 67, 415–423.
- Walstra, P.; Kloek, W.; van Vliet, T. Fat Crystal Networks. In *Crystallization Processes in Fats and Lipid Systems*; Garti, N., and Sato, K., Eds.; Marcel Dekker: New York, 2001; pp. 289–328.
- Wei, J. Molecular symmetry, rotational entropy, and elevated melting points. *Ind. Eng. Chem. Res.* 1999, 38, 5019–5027.
- Yalkowsky, S.H.; Valvani, S.C. Solubility and partitioning. 1. Solubility of non-electrolytes in water. *J. Pharm. Sci.* 1980, 69, 912–922.
- Yano, J.; Sato, K.; Kaneko, F.; Small, D.M.; Kodali, D.R. Structural analyses of polymorphic transitions of *sn*-1,3-distearoyl-2-oleoylglycerol (SOS) and *sn*-1,3-dioleoyl-2-stearoylglycerol (OSO): assessment on steric hindrance of unsaturated and saturated acyl chain interactions. *J. Lipid Res.* 1999, 40, 140–151.
- Zhang, L.; Ueno, S.; Miura, S.; Sato, K. Binary phase behavior of 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol. *J. Am. Oil Chem. Soc.* 2007, 84, 219–227.

5

Molecular Composition Dynamics and Structure of Cocoa Butter

Rodrigo Campos¹ and Alejandro G. Marangoni^{2*}

¹Mars Chocolate North America, Hackettstown NJ, USA and ²Department of Food Science, University of Guelph, Guelph Ontario, Canada.

Introduction

Chocolate is a smooth suspension of particulate sugar, cocoa and milk solids in a continuous fat phase composed of cocoa butter and a minor portion of milk fat (Beckett, 2008; Sato & Koyano, 2001). Upon cooling of a chocolate melt, this continuous fat phase will solidify into an ordered crystalline structure, directly affecting chocolate's functional and sensory characteristics, ultimately impacting consumer acceptance. Its constituent lipid species will define different levels of structure within its crystal network and determine essential attributes (Narine & Marangoni, 2002; Campos et al., 2002). For example, the chemical composition and crystal habit of cocoa butter dictate the distinctive texture, snap, gloss, and melting character of chocolate. Crystal habit includes polymorphism, crystallite size and shape, and spatial distribution of network mass. Furthermore, heat, mass, and momentum transfer conditions during the tempering process used in the manufacturing/crystallization of chocolate greatly affect the solid fat fraction and crystal habit of cocoa butter.

Cocoa butter has been widely studied in terms of its composition (Dimick, 2000; Lipp et al., 2001), polymorphism (Wille & Lutton, 1996; van Malssen et al., 1996a; van Malssen et al., 1996b; van Malssen et al., 1999), and microstructure (Manning & Dimick, 1985; Marangoni & McGauley, 2003) and a number of authors have undertaken the challenge of relating cocoa butter's chemical composition to different levels of structure. Polymorphism, molecular compatibility, and phase behavior of pure triacylglycerols (TAG) found in cocoa butter have been reported by different research groups (Ng, 1989; Sato et al., 1989; Koyano et al., 1989; Minato et al., 1996; Miura & Konishi, 2001; Sato & Koyano, 2001; Smith et al., 2005). Alternative examinations considered cocoa butter and other natural edible fats (i.e., milk fat and vegetable oils) as distinct lipid components rather than complex mixtures of different TAGs (Lovegren et al., 1976a; Lovegren et al., 1976b, Gordon et al., 1979; Dibildox-Alvarado & Toro-Vazquez, 1989; Fairley et al., 1995; Toro-Vazquez & Gallegos-Infante, 1996; Martini et al., 2001; Humphrey et al., 2003; Timms, 2003; Perez-Martinez et al.,

2005; and Perez-Martinez et al., 2007). In addition to functional properties dictated by cocoa butter's constituent lipid species, its crystal network can be influenced by heat and mass transfer during processing conditions such as crystallization temperature (van s et al., 1999; McGauley, 2001; Brunello et al., 2003; Marangoni & McGauley, 2003), cooling rate (Rousset & Rappaz, 2001), and shear (Bolliger, et al., 1998; Stapley et al., 1999; Beckett, 2008; Rousset & Rappaz, 2001; Sato & Koyano, 2001; Briggs & Wang, 2004; Mazzanti et al, 2005; Dhonsi & Stapley, 2005; Sonwai & Mackley 2006; Maleky & Marangoni, 2008).

In this work, it is hypothesized that TAG molecules progressively attach to the crystal surface in decreasing order of their melting point during the crystallization of cocoa butter, influencing the crystal habit and macroscopic properties of the resulting crystal network. We put forward that this sequential process can be manipulated by changes in the molecular makeup of cocoa butter as well as through processing conditions. Thus, the present study aims to examine how processing conditions and minor changes in the molecular composition of cocoa butter affect a fat crystal network, where the attachment of lipid species onto growing seed crystals influences different levels of structure and ultimately dictates the functionality of cocoa butter. The first part of this chapter will focus on the effects of mechanical work on different levels of structure. This will elucidate how different crystallization conditions affect the attachment of lipid species onto the growing seed crystals of cocoa butter cooled at different rates in the presence and absence of shear to a temperature of 24°C. Shear will then be continually applied (with its static counterpart) until after nucleation when early crystal growth has taken place. Different levels of structure will be analyzed as well as the composition and thermal properties of the initial crystal mass which develops in the melt. Meanwhile, in the second part of this chapter, we added up to 5% wt/wt 1,2,3-tristearoyl-glycerol (SSS) or 1,2,3-trilinoleoyl-glycerol (LLL) to natural commercially available cocoa butter. This allowed us to modify the concentration of saturated/unsaturated lipid species and subsequently study different levels of structure in the cocoa butter's crystal network, illustrating how small changes in its composition affect the different levels of structure, from crystal habit and crystallization behavior to microstructure and mechanical properties.

Materials and Methods

Materials

For all experiments we used refined cocoa butter (Callebaut, Qzina Specialty Foods Inc., Toronto, ON, Canada). To study the effects of small changes in composition on different levels of crystal structure, we made blends (w/w) of cocoa butter and 1% or 5% either LLL (99% purity, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) or SSS (91% purity, Acros Organics, NJ, USA). For all analytical determinations, we melted samples at 80°C for 30 minutes in order to erase all crystal memory prior to their crystallization.

Processing Conditions

To study the effect of shear work on crystallization, we processed cocoa butter both statically (absence of shear) and dynamically (presence of shear) at three cooling rates (0.3, 1, and 4°C/min). For dynamic processing of cocoa butter, we used a Lightning Lab Master Mixer (Lightning, Rochester, NY, USA) equipped with a radial flow impeller (2" R100 5/16"), operated at a speed of 400RPM to apply shear during cooling. We chose the radial flow impeller as it provides a high shear field. The experimental set up used (illustrated in Fig. 5.1) provided a maximum shear rate of 120s^{-1} . We melted cocoa butter samples in stainless steel beakers at 80°C for 30 min in order to erase all crystal memory. We then transferred the beakers to a temperature-controlled Neslab RTE-11 water bath (Neslab, Portsmouth, NH, USA) for cooling from 60 to 24°C at 0.3, 1, or 4°C/min. We held samples at 24°C until nucleation and early crystal growth took place, evidenced as the melt became cloudy and by a temperature increase of 0.2–0.4°C. We defined the sampling point as that when the cooled cocoa butter had reached a solid fat content (SFC) of 1.5%. At 1.5% SFC, we took samples for characterization, and considered this as the starting time ($t = 0$ minutes). SFC was measured by pulsed nuclear magnetic resonance (pNMR)—measurement details provided later in this section. This SFC was the highest level of solids in which it was practically possible to sample. The viscosity of the cooled cocoa butter at higher concentration of solids became too high to be sampled or analyzed. Fig. 5.2 depicts the temperature profile of cooled cocoa butter samples and the sampling points at each cooling rate. Depending on the process treatment, we performed either two or three experimental runs.

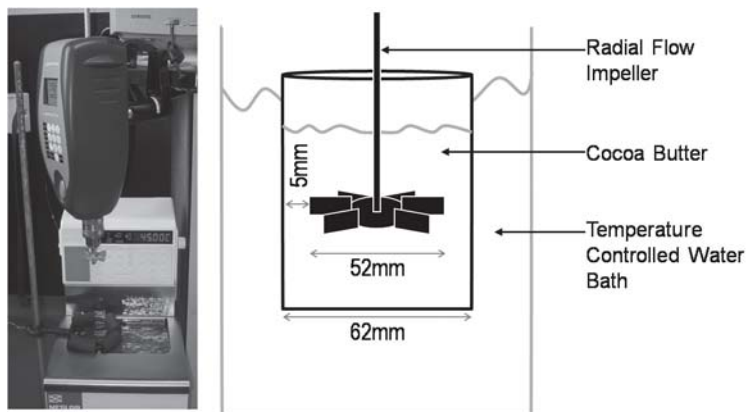


Fig. 5.1. Photograph of the LabMaster Mixer used in this study, along with a schematic representation of the experimental set up used, illustrating the radial flow impeller used, and the dimensions of the system.

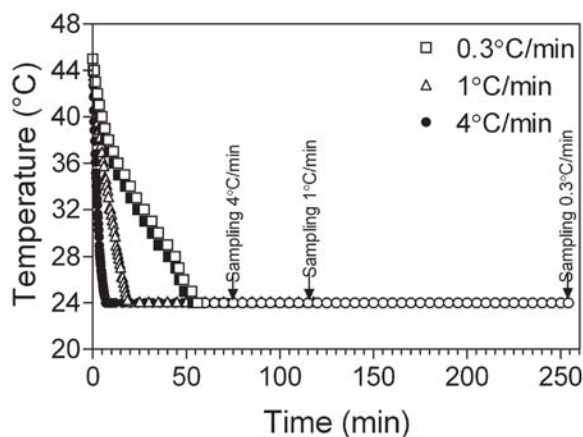


Fig. 5.2. Cocoa butter cooling profiles used in this study. Marked are the sampling points (where SFC $\approx$ 1.5%) for each cooling rate. For each treatment, the sampling point corresponds to time = 0 minutes where the SFC $\approx$ 1.5%.

Seed Crystal Isolation

With the aim of understanding the effects of processing at a molecular level, we isolated the crystalline material present in cocoa butter cooled under static and dynamic conditions with a SFC of $\approx$ 1.5%. We placed aliquots of processed cocoa butter in 2 ml Eppendorf snap-cap microcentrifuge tubes (Fisher Scientific, Ottawa ON, Canada) and centrifugated using an Eppendorf microcentrifuge 5410 (Brinkman Instruments Inc., Mississauga, ON, Canada). We decanted the liquid fraction and quickly washed the crystalline mass three times with cold (5°C) isobutanol (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada), separating the solvent by decantation after each washing. We allowed the remaining solvent to evaporate overnight in a fume hood. We then melted the crystalline mass and analyzed for its chemical composition and thermal properties.

Fatty Acid Profiles

The fatty acid profiles were determined by gas chromatography (GC). We prepared fatty acid methyl esters (FAME) according to Bannon et al., (1985) by dissolving 25 mg of cocoa butter in 1 ml of isooctane (Fisher Scientific, Ottawa ON, Canada) and adding 200 μ l of 2N potassium hydroxide (KOH) in methanol (Fisher Scientific, Ottawa ON, Canada). We vortexed samples for 1 minute, incubated at room temperature for 5 minutes, neutralized with 2N hydrochloric acid (HCl) using methyl orange as an indicator (Fisher Scientific, Ottawa ON, Canada) and removed the aqueous phase (red in colour). We purified FAMES by thin layer chromatography (TLC)

using silica Gel G TLC redi-plates (20 cm × 20 cm × 250 μm, Fisher Scientific, Ottawa ON, Canada) and a developing solvent mixture of hexane/ethyl ether/acetic acid (85:15:1 v/v/v) (Fisher Scientific, Ottawa ON, Canada). We scraped off and collected the band corresponding to FAME. We eluted the silica gel with hexane (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and analyzed the extracted FAME by GC. We used a Hewlett-Packard Model 5890 Series II, GC (Palo Alto CA, USA) with a splitless injection port flushed after 0.3 seconds, a flame ionization detector (FID), auto sampler (Hewlett-Packard, Model 7673), 100 m CP-Sil 88 fused capillary column (Varian Inc., Mississauga, ON Canada), and Hewlett-Packard ChemStation A.07 software system. The injector and detector were both kept at 250°C. We used hydrogen as the carrier gas (1 mL/min) and for the FID (40 mL/min), nitrogen as the make up gas (100 mL/min), and purified air (250 mL/min). We used the following temperature program: initial temperature of 45°C held for 4 minutes, heating at 13°C/minutes to 175°C and held for 27 minutes, heating at 4°C/min to 215°C and held for 35 minutes (Cruz-Hernandez, 2003). We identified the FAME by comparison with the GC reference FAME Nu-Check-Prep standard #463 (Nu-Check-Prep Inc, Elysian MN USA) spiked with long-chain saturated FAME C23:0.

Triacylglycerol Profiles

The triacylglycerol (TAG) profiles were determined by high performance liquid chromatography (HPLC). We prepared 5% (w/w) solutions of cocoa butter in HPLC grade acetone (Fisher Scientific, Ottawa ON, Canada). We heated and vortexed the solutions to ensure their dissolution; and filtered to remove foreign debris using a reusable glass syringe (5 cc capacity, BD Brand, Fisher Scientific, Ottawa ON, Canada) equipped with a syringe filter holder (13 mm diameter, Fisher Scientific, Ottawa ON, Canada) and Osmonics magma nylon membrane filters (13 mm diameter, 0.45 μm pore size, Fisher Scientific, Ottawa ON, Canada). We injected the filtered solutions into a Beckman 110B solvent delivery module (Beckman Coulter Canada Inc., Mississauga, ON Canada) connected to an Econosil (C18) column (Alltech, Associates Inc., Deerfield, IL USA) tempered at 36°C, and a differential refractometer R40 (Watters Associates, Mississauga, ON Canada). We used an acetone–acetonitrile (60:40 v/v, both HPLC grade, Fisher Scientific, Ottawa ON, Canada) mixture as the mobile phase. We previously filtered the mobile phase using a glass vacuum filter holder (Millipore Canada Ltd., Cambridge, ON Canada) with Osmonics magma nylon membrane filters (47 mm diameter, 0.455 μm pore size, Fisher Scientific, Ottawa ON, Canada) and subsequently deaerated under vacuum. With the experimental conditions used, we achieved separation by polarity but we could not resolve positional isomers (e.g., PPO and POP). We integrated peaks corresponding to each separated glycerol ester molecule using a Shimadzu C-R5A Chromatopac Integrator (Shimadzu Corporation, Kyoto, Japan). We identified TAG species by comparison with standards (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada and Larodan Lipids–Fine chemicals AB, Malmö Sweden).

Crystallization Kinetics

We monitored the development of solid crystalline material from the melt as a function of crystallization time or temperature using two different analytical techniques: pNMR and differential scanning calorimetry (DSC).

SFC by pNMR

pNMR was used to measure the SFC as a function of time. We placed approximately 3 grams of sample in pre-tempered glass pNMR tubes (10 mm diameter, 1 mm thickness, and 180 mm height), and immediately transferred to a water bath set at the temperature of study. We obtained SFC readings at determined time intervals using a Bruker PC/20 series pNMR analyzer (Bruker, Milton, ON, Canada) until readings plateaued and we obtained an equilibrium SFC. We plotted the SFC data as a function of time to construct crystallization curves, and fitted these plots to the Avrami equation using non-linear regression with the assistance of data fitting software (Prism 4, Graph Pad Software Inc., San Diego CA, USA). The Avrami model, as applied to fats (Wright et al., 2000; Campos, 2005), has the following form:

$$\frac{SFC(t)}{SFC_{\infty}} = 1 - e^{-kt^n} \quad \text{Equation 5.1} \\ \text{(Avrami, 1939)}$$

where SFC(t) is the % SFC as a function of time, SFC_{∞} is the limiting % SFC as time approaches infinity or equilibrium, the Avrami constant (k) represents the crystallization rate constant, and the Avrami exponent or index of crystallization (n) indicates the crystal growth mechanism. We calculated half times of crystallization ($t_{1/2}$), which reflect the magnitude of the crystallization rate constants, represent the time required for 50% conversion of the material, using the following relationship derived from Equation 5.1:

$$t_{1/2} = \left(\frac{0.693}{k} \right)^{1/n} \quad \text{Equation 5.2}$$

DSC

A DSC 2910 instrument (DuPont Instruments, Willington DE, USA) was used to measure the exothermic heat evolved as a function of temperature during static crystallization. We placed five to ten milligrams of sample in DSC pans, sealed hermetically, transferred to the DSC cell, and run the following method: melt at 80°C for 30 minutes, cool to 0 or 5°C at a rate of 5°C/min, hold isothermally for 30 minutes, and melt at a rate of 5°C/min to 60°C. From the resulting thermograms, we obtained the following parameters: onset crystallization temperatures (T_{onset}) and peak melting

temperature. From the resulting thermograms, we obtained the temperature at which the heat flow deviated from the baseline, which corresponds to the temperature at which the first crystallites formed (T_{onset}), using TA Instruments Universal Analysis 2000 V4.2E software (TA Instruments, Mississauga ON, Canada). Additionally, we determined the peak crystallization temperature, defined as the temperature at which the sample has the highest heat flow and is related to the temperature at which the largest proportion of lipid species crystallize, considered the average crystallization point of the samples. Alternatively, we measured the heat flow as a function of time that results from crystallization events at a specific storage temperature with a Microcalix [DSC coupled with an X-Ray diffraction (XRD)], as described further.

Microstructure

Polarized light microscopy (PLM) was used to image the crystalline microstructure. We placed one droplet of sample on a pre-tempered microscope glass slide, using a pre-tempered capillary tube before carefully placing a pre-tempered glass cover slip over the sample. We placed the cover slip parallel to the plane of the microscope slide and centered on the sample drop to ensure a uniform thickness and prevent the formation of air bubbles. We placed the prepared sample slides on a Linkam LTS 350 temperature-controlled microscope stage (Linkam Scientific Instruments, Surrey, UK), set at the temperature of study. We used an Olympus BHA light microscope (Olympus America Ltd., Melville NY, USA) to view the microstructures formed and recorded images at defined time points using a Sony XC-75 CCD video camera (Sony Corporation, Japan) with the gain switch in auto position. We digitized images using Scion Image software (Scion Corporation, Fredrick, MD, USA). For longer time experiments, we transferred sample slides to temperature controlled incubators set at the temperature of study.

We made qualitative observations as well as quantitative analysis on the acquired images. We quantified microstructural features (i.e., number of features, equivalent particle diameter, and nearest neighbor distance) using Photoshop 7.0 software (Adobe Systems Inc., San Jose CA, USA) on inverted thresholded (black features on a white background) images representative of the original imaged microstructure. We quantified the fractal dimension (D_b) from thresholded images using Benoit 1.3 software (TruSoft International Inc, St. Petesburg, FL, USA) with the box counting dimension method. The fractal dimension calculated using the box-counting method is sensitive to the degree of fill, crystal size and shape of the network (Awad & Marangoni, 2005).

Thermal Properties

DSC was used to determine peak melting temperatures of cocoa butters at different times of crystallization with a 2910 DSC (DuPont Instruments, Willington DE,

USA). We placed five to ten milligrams of sample in pre-tempered DSC pans, sealed hermetically, and quickly transferred to temperature controlled incubators for static crystallization. At appropriate time points, we quickly transferred the samples pans to the DSC cell, previously pre-tempered at the temperature of study. We allowed the DSC cell to stabilize for 1 minute and melted the samples at a rate of 5°C/min to 60°C. We obtained peak melting temperatures using TA Instruments Universal Analysis 2000 V.4.2E (TA Instruments, Mississauga ON, Canada). Peak melting temperatures are defined as the temperature at which the sample had the lowest heat flow and are related to the temperature at which the largest proportion of lipid species in the fat sample melt, considered an average of the melting points of the sample.

Mechanical Properties

The mechanical strength of the fat blends was measured by breaking sample disks and obtaining the force necessary to cause such fracture. We prepared sample disks (20 mm diameter and 3.2 mm height) by pouring the melted blends into pre-tempered PVC molds and allowing them to crystallize statically in temperature controlled incubators. We used an 1122 Instron machine (Instron Canada, Burlington, ON, Canada) with a three point bending geometry to measure the breaking force of the crystallized samples. We positioned the sample disk over two points while a third point impinged a constant force in the middle of the sample disk to the point of fracture. We calculated the force (F) necessary to break the sample disks at a speed of 50 mm/min, as well as the deformation of the disks to the point of fracture (D), with the Instron Series IX Automated Materials Tester V.9.09.00 software (Instron Canada, Burlington, ON, Canada). With the obtained experimental data, along with the height (h) and diameter (b) of the disk, and the separation between the two supporting points (a), we calculated the bending elastic modulus (E_B) using Equation 5.3 (Steffe, 1996).

$$E_B = \frac{Fa^3}{4dbh^3} \quad \text{Equation 5.3} \\ \text{(Steffe, 1996)}$$

Crystal Structure

A Microcalix (a high resolution XRD transmission instrument coupled with a DSC) was used to study polymorphism, while measuring the heat flux that resulted from the crystallization process. The Microcalix was developed in the Laboratory for Physical Chemistry of Polyphasic Systems, at the University of Paris-South in Châtenay – Malabry, France (Ollivon et al., 2006). The coupled XRD recorded simultaneously at both small ($q = 0 - 0.45 \text{ \AA}^{-1}$) and wide ($q = 1.1 - 2.1 \text{ \AA}^{-1}$) angles through two position sensitive gas linear detectors placed at 177 and 30 cm respectively from the sample.

We calibrated the detector channels to express the collected XRD data in the scattering vector q ($\text{\AA}^{-1}$), where

$$q = \frac{4\pi \sin \theta}{\lambda} = \frac{2\pi}{d} \quad \text{Equation 5.4}$$

$\theta(^{\circ})$ is the angle of incidence of X-rays relative to the crystalline plane, λ is the X-ray wavelength, and $d(\text{\AA})$ is the repetition distance between two planes. We calibrated the detectors with high-purity tristearin and the DSC with lauric acid.

We filled glass capillary tubes (1.4 ± 1 mm diameter and 80 mm long) with molten samples with the aid of a specially developed syringe in order to fill the lower 15 mm of the capillary. This level of filling corresponds to an average sample size of 20 mg. We melted samples in an oven at 80°C for 30 minutes before placing them in the sample holder pre-set to 60°C . Immediately after sample insertion, we ran the samples using the following method: cool to either 20 or 24°C at a rate of $5^{\circ}/\text{min}$ and hold isothermally at the temperature of study to allow for crystallization to take place. For each run, we obtained a total of 32 consecutive XRD patterns after acquiring diffraction data for a period of 200 seconds in the case of samples crystallized at 20°C , and 1200 seconds for samples crystallized at 24°C . Throughout the duration of the experiment, the DSC acquired data every 3 seconds. We also analyzed samples after 1, 2, 3, and 7 days of isothermal storage at each temperature by acquiring XRD data for 1200 seconds under isothermal conditions.

Shear Work

Crystallization

Crystallization is the phase transition from the liquid state to the solid state. It is a complex process in which, once an energy barrier is overcome, TAG molecules in random thermal motion aggregate and form stable nuclei. These nuclei will subsequently grow and arrange into clusters, eventually forming a three-dimensional fat crystal network. We studied this complex process by generating crystallization curves, which chart the development of crystalline material—SFC—as a function of time. Fig. 5.3 shows crystallization curves for cocoa butter cooled to 24°C at different cooling rates both under static and dynamic conditions, with the resulting Avrami analysis in Table 5-A. Crystallization of cocoa butter at 24°C is an inherently variable process, evidenced by the high variability in the SFC at different crystallization times. The application of shear reduced the variability of the crystallization dynamics, as seen by a reduction in the variability in SFC at any given time point, relative to statically processed samples. Keeping in mind that at the time of sampling the SFC is roughly at 1.5%, the analysis in Fig. 5.3 provided insight on secondary nucleation and crystal growth events, rather than the initial isothermal nucleation from the melt.

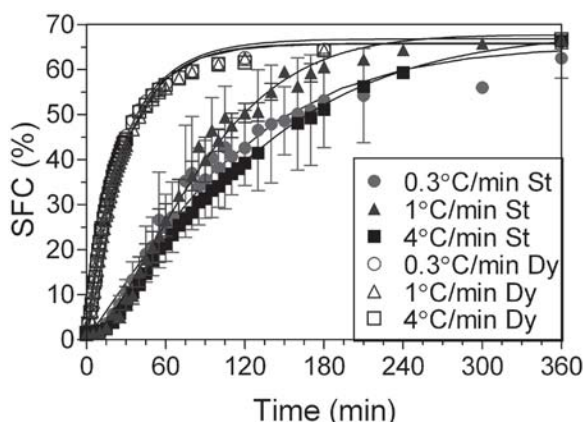


Fig. 5.3. Crystallization curves of shear-worked cocoa butter samples cooled at 0.3 (●), 1 (▲), and 4°C/min (■) from 80°C to 24°C under both static (open symbols) and dynamic (closed symbols) conditions. Samples were obtained when the processed cocoa butter samples reached a SFC $\approx$ 1.5%—considered $t = 0$ min—after which they were allowed to crystallize statically at 24°C and the SFC was measured as a function of time. The experimental data was fitted to the Avrami equation. The resulting fits are indicated by the solid lines.

Table 5-A. Kinetic parameters obtained by the Avrami analysis of crystallization curves constructed from SFC (%) as a function of time data of shear worked cocoa butter samples cooled at different rates from 80°C to 24°C under both static and dynamic conditions. Samples were obtained when the processed cocoa butter samples reached a SFC $\approx$ 1.5%—considered $t = 0$ min—after which they were allowed to statically crystallize at 24°C and the SFC (%) was measured as a function of time.

Cooling Rate	k (min^{-n})		n		$t_{1/2}$ (min)	
	Static	Dynamic	Static	Dynamic	Static	Dynamic
0.3°C/min	0.004 ^A	0.053 ^B	1.3 ^A	0.9 ^C	112.6 ^A	17.6 ^B
1°C/min	0.001 ^A	0.037 ^C	1.5 ^A	1.0 ^{BC}	80.70 ^A	19.4 ^B
4°C/min	0.002 ^A	0.046 ^B	1.2 ^{AB}	0.9 ^{BC}	102.3 ^A	17.8 ^B

* Letters indicate significance between treatments ($P < 0.05$).

** Values represent the average of at least two experimental runs (crystallizations) for each treatment. For every experimental run, two different NMR tubes are used to monitor SFC (%) development as a function of time.

All treatments reached comparable equilibrium SFC values (in the range of 63.4 to 68.7%), however we observed an effect of shear on the kinetics of crystallization. Mechanically worked cocoa butter crystallized faster, evidenced by shorter times to reach this equilibrium SFC relative to statically processed samples. Upon examination of the crystallization rate, k , we observed an almost tenfold increase for samples

processed dynamically compared to those cooled statically. Due to the fact that the units of k are min^{-n} , it is somewhat inadequate to compare varying k values, as their units are different. Alternatively, half times of crystallization, $t_{1/2}$, which represent the time required for half of the crystallization to take place and have units of time, more accurately reflect the magnitude of k and n (Marangoni, 2005a). We observed significant differences (at least a fourfold difference) between $t_{1/2}$ in statically and dynamically processed samples (Table 5-A). The observed outcome on crystallization kinetics is a result of secondary nucleation events and enhanced heat and mass transfer in the melt (Stapley et al., 1999). During nucleation and early crystal growth, shear applied to the crystalline mass results in dissipation of heat of crystallization and increased contact between existing growing crystals, as well as between crystals and the impeller or beaker surfaces, promoting crystal disruption (Grall & Hartel, 1992). The newly formed crystals are redistributed in the melt and serve as nucleation sites for subsequent growth (Hartel 2001; Metin & Hartel, 2005; Mazzanti et al., 2005). As a result, samples processed dynamically crystallize faster, as indicated by higher k and lower $t_{1/2}$ relative to statically processed sample. This was consistent with published studies in which increased crystallization rates were found for cocoa butter (Rousset & Rappez, 2001) and butterfat (Grall & Hartel, 1992) when cooled under shear.

The Avrami index (n) indicates the crystal growth mechanism and is a combined function of time dependence of nucleation and the number of dimensions in which growth takes place (Sharples, 1966; Wright et al., 2000). Considering that all treatments have similar crystal morphologies (imaged with PLM, shown in Fig. 5.4), the

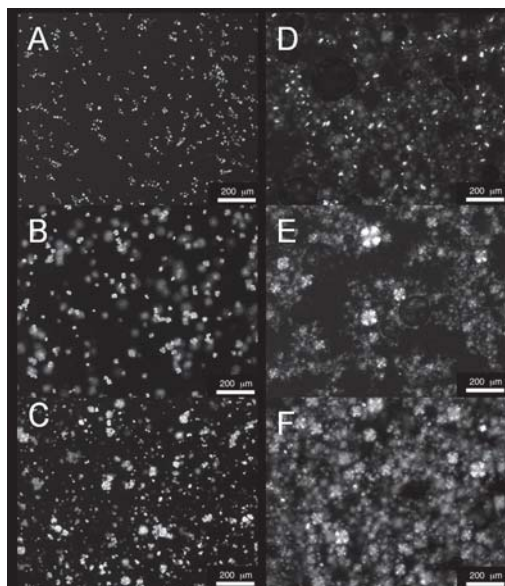


Fig. 5.4. Polarized light micrographs of cocoa butter cooled statically at 0.3°C/min (A), 1°C/min (B), and 4°C/min (C), and dynamically at 0.3°C/min (D), 1°C/min (E), and 4°C/min (F) from 80°C to 24°C. Samples were obtained when the cocoa butter reached a SFC $\approx$ 1.5%, after which they were placed on a microscope slide and transferred to a cold stage set at 24°C. Images were acquired 1 minute after sampling. Magnification bar represents 200 μm .

higher n values observed under static conditions suggest that secondary nucleation is sporadic in nature (nuclei appearing over time), while the application of shear promotes instantaneous nucleation (nuclei appearing all at once early in the process) reflected by lower n values the presence of shear. Secondary nucleation events are induced from the breakage and distribution of crystals promotion further nucleation sites (Hartel 2001; Mazzanti et al., 2005) and consequently resulting in a more instantaneous secondary nucleation.

Microstructure

Fig. 5.4 illustrates the microstructure of cocoa butter cooled at different rates under static and dynamic conditions, one minute after sampling. We observed considerable amounts of crystalline mass after only 1 minute of storage. Table 5-B includes quantitative image analysis results. We observed a higher number of crystals of smaller size under dynamic conditions relative to statically processed cocoa butter. The mechanical work of shear applied during cooling and early crystal growth influenced crystal size by disruption of existing crystals. This led to a higher number of small crystals that serve as nucleation sites during secondary nucleation (Grall & Hartel, 1992; Stapley et al., 1999). Additionally, shear impedes agglomeration and interlocking during crystal growth (Foley & Brady, 1984). Under static conditions, there is no fracture or disruption of crystals, nor enhanced mass transfer in the melt, translating to a smaller number of bigger crystals. Additionally, cooling under static conditions yielded a wider crystal size distribution (Fig. 5.5) for statically processed samples relative to samples cooled under shear. We also noted a considerable difference in the equivalent diameter's standard error (roughly three orders of magnitude difference) between samples cooled under different shear regimes. Other studies have reported the same effect of agitation on crystal number and size for butterfat (Grall & Hartel, 1992), and blends of high and low melting fractions of milk fat (Herrera & Hartel, 2000a).

Table 5-B. Microstructural analysis parameters of shear-worked cocoa butter samples cooled at different rates from 80 to 24°C under both static and dynamic conditions. Sampling was performed when the processed cocoa butters reached a SFC $\approx$ 1.5%. The analyzed microstructural features were imaged by polarized light microscopy after 1 minute of crystallization at 24°C subsequent to sampling.

Cooling Rate	Number of Features		Equivalent Diameter (mm)	
	Static	Dynamic	Static	Dynamic
0.3°C/min	278 (142) ^{A,I} $n = 2$	3410 (292) ^{A,II} $n = 2$	6.3 (1.6) ^{A,I}	2.1 (0.1) ^{A,I}
1°C/min	113 (67) ^{A,I} $n = 2$	2819 (81) ^{A,II} $n = 2$	33.5 (5.2) ^{B,I}	2.2 (0) ^{A,II}
4°C/min	291 (170) ^{A,I} $n = 3$	2916 (0) ^{A,II} $n = 2$	16.5 (6.4) ^{A,B,I}	1.9 (0) ^{A,I}

* Letters indicate significance between cooling rates, while roman numerals indicate significance between static and dynamic conditions ($P < 0.05$).

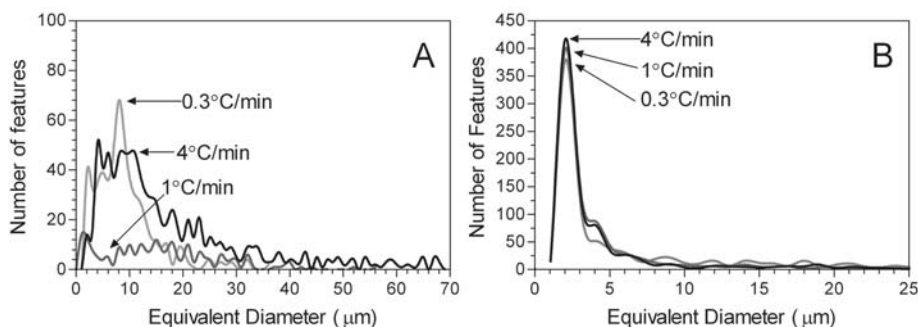


Fig. 5.5. Histograms reflecting crystal size distributions of samples cooled statically (A) and dynamically (B) at different cooling rates from 80 to 24°C. Samples were obtained when cocoa butter reached a SFC $\approx$ 1 5%. Images were acquired 1 minute after sampling and holding at 24°C. Number of features and equivalent diameter data was obtained from image analysis of thresholded PLM images.

Thermal Behavior

Different research groups have widely reported on the polymorphic forms of cocoa butter (Wille & Lutton, 1996; Merken & Vaack, 1980; van Malssen et al., 1996a; Marangoni & McGauley 2003). For the purposes of this work, we related the melting ranges of the six crystalline phases (identified by Roman numerals I to VI) published by van Malssen et al., (1996a) to the polymorphic state of the studied samples.

Fig. 5.6 shows the peak melting temperatures for processed cocoa butter samples as a function of time. With the experimental set up used in this study, the crystalline

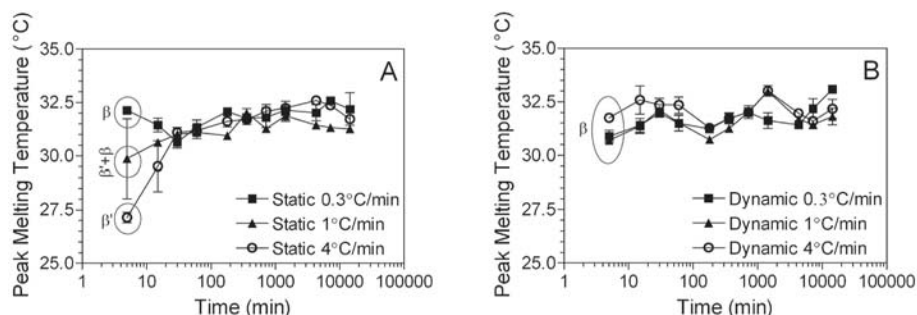


Fig. 5.6. Peak melting temperatures as a function of storage time ($T = 24^\circ\text{C}$) of shear-worked cocoa butter samples cooled under different rates from 80°C to 24°C under static (A) and dynamic (B) conditions. Samples were obtained and DSC pans prepared after the processed cocoa butters had reached a SFC $\approx$ 1 5%—considered $t = 0$ min. DSC pans were stored for different time periods (from 5 minutes to 10 days) in incubators set at 24°C prior to melting in the DSC at a rate of 5°C/min.

structure of cocoa butter cooled in the presence of shear corresponded to the high melting stable β form (with peak melting temperatures in the range from 30.7 to 33.1°C) regardless of the cooling rate used. XRD data—discussed in detail further in this paper—showed that when cocoa butter is cooled statically at 5°C/min, the α form followed by the β' form crystallize directly from the melt, subsequently transforming into the stable β form. The mechanical work applied to the samples accelerated transformation of metastable form to the stable form. Stapley and co-workers found that high shear rates will result in high melting forms in the crystallization of chocolate melts (Stapley et al., 1999). It is then possible to manipulate the degree of molecular packing of a fat crystal network through the application of shear, thereby improving cooling protocols in the production of chocolate.

When processed statically, cocoa butter samples had peak melting temperatures in the range of 27 to 32°C, corresponding to both the β' and β forms. Cooling conditions affected the rate at which metastable forms transformed into the stable β form. Five minutes after sampling, samples statically cooled at 4°C/min had a peak melting temperature of $27.14 \pm 0.56^\circ\text{C}$, corresponding to the β' form. Conversely, samples cooled statically at 0.3°C/min had a peak melting temperature of $32.12 \pm 0^\circ\text{C}$, evidence that the crystal structure had transformed into a stable β form only 5 minutes after sampling and subsequent storage at 24°C. Slow cooling allows TAG molecules to arrange in the melt and acquire an efficient spatial packing geometry, thus forming stable crystal structures—high peak melting temperatures observed. On the other hand, fast cooling results in a rapid decrease of viscosity, limiting molecular diffusion in the melt. This translates into molecules aggregating in disorganized conformations, thus leading to crystalline forms of lower melting point—lower stability. Similar trends have been reported for other edible fat systems. Anhydrous milk fat, lard (Campos et al. 2002), palm oil based shortening (Litwinenko et al. 2002), and palm stearin–palm kernel oil blends (Johansson & Bergenstahl, 1995) have all been found to crystallize in more stable crystalline forms when slowly cooled relative to fast cooling. When cooling at an intermediate rate, we believe that a mixture of $\beta' + \beta$ forms is present, indicated by the variable peak melting temperatures ($29.87 \pm 1.86^\circ\text{C}$) observed for samples cooled at 1°C/min. In time, the observed differences in peak melting temperatures between different cooling rates disappeared due to transformation of metastable to stable forms ($\beta' \rightarrow \beta$). After only 30 minutes and for the duration of the study (up to 10 days), all samples had peak melting temperatures in the range of 30.1 and 33.6°C which corresponds to the β polymorph.

Mechanical Properties

We measured the bending elastic modulus (E_B) of the cocoa butter samples after 6 and 24 hours of storage at 24°C. E_B at these two storage times were not significantly different, and thus we pooled averages for each processing variable. Fig. 5.7 shows

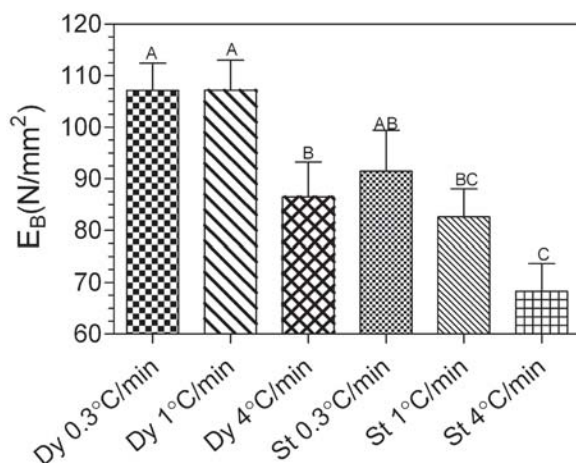


Fig. 5.7. Bending elastic modulus (E_B) of shear-worked cocoa butter samples cooled at different rates from 80°C to 24°C under both static and dynamic conditions. Samples were obtained when processed cocoa butter samples reached a SFC $\approx$ 1.5%, after which they were allowed to statically crystallize at 24°C for 6 and 24 hours prior to analysis. Each bar represents pooled values of E_B after 6 and 24 hours of storage ($n = 24$). Letters denote significance between treatments ($P < 0.05$).

pooled averages for the different processing conditions. Even when all treatments have similar SFC ($67.1 \pm 0.88\%$), we found that processing conditions (both shear and cooling rate) affect the mechanical properties of cocoa butter, observing higher E_B when we subjected samples to shear and cooled at slow rates. This confirms that SFC is not the only parameter which will determine the mechanical properties of fat crystal networks (van Aken & Visser, 2000; Campos et al, 2002). The existence of both primary and secondary structural bonds, as well as crystal structure, will influence the hardness of the resulting solid fat.

In fat crystal networks, microstructures can be connected by primary and secondary bonds (van den Tempel, 1961; van den Tempel, 1979; Johansson & Bergenstahl, 1995). Primary bonds are solid connections formed when growing crystals or nuclei touch and merge. Secondary bonds—weak compared to the former—are van der Waals attractive interactions between neighboring microstructures (van den Tempel, 1961). Mechanical deformation disrupts the primary bonds, leading to softening (Heertje et al., 1988; Heertje, 1993), and subsequent restructuring of the microstructure. In certain cases there is a restoration of primary bonds through recrystallization that can sometimes lead to an increase in firmness (van Aken & Viser, 2000). The weaker secondary bonds survive mechanical work (Johansson & Bergenstahl, 1995) and are greatly responsible for the aggregation of the microstructures in a crystal network (Haighton, 1965; Chaseri & Dimick, 1995). The interactions between the

microstructural features (both primary and secondary) greatly affect the structural strength of a fat crystal network as well as the size of crystal structures present and the solid fat content of the network (van Aken & Visser, 2000; Campos & Marangoni, 2010).

Previous research reports that the application of shear to vegetable shortenings (Narine & Humphrey, 2004) and milk fat (van Aken & Visser, 2000; Herrera & Hartel 2000b) yields softer products due to the rupture of strong primary bonds (Heertje 1993), and restriction of crystal growth (Foley & Brady, 1984). In this study, however, we observed higher E_B values with the application of shear, which can be accounted for by the observed differences in microstructure. One minute after sampling, we observed a higher number of small crystals, in the presence of shear relative to samples processed statically (Fig. 5.4, Table 5-A). At a fixed SFC, a smaller crystal size translates to a higher elastic modulus (Marangoni, 2000; Marangoni & Rogers, 2003).

Previous research studied the effect of cooling rate on the mechanical strength of a fat crystal network for blends of high and low melting fractions of milk fat (Herrera & Hartel, 2000b). Similar to our results, the authors reported that slow cooling rates led to more elastic crystal networks relative to fast cooling. This can be explained in terms of the effect of cooling rates on the crystallization process. Fast cooling forces molecules in the melt to crystallize onto the growing nuclei without enough time to orient into proper conformations. Conversely, slow cooling of a molten fat allows for proper re-arrangement of the TAG molecules and hence an efficient molecular packing (Rye et al, 2005). In such crystalline structures, it is likely that a higher number of interactions will form between neighboring microstructures yielding a stronger network. This is reflected by higher E_B observed at slow cooling rates for both statically and dynamically processed samples.

Isolated Crystalline Phase

We isolated the crystalline phase present in processed cocoa butter melts at the time of sampling and characterized the sample for its chemical composition and thermal behavior.

Chemical Makeup of the Isolated Crystalline Material

After examination of the composition of native cocoa butter and the isolated crystalline masses, we found the same lipid species. However, we observed differences in the concentration of specific fatty acids and TAGs between processing conditions. There were no significant differences in the concentration of the three major fatty acids of cocoa butter (C16:0, C18:0, and C18:1*c*) for all treatments, with the exception of cocoa butter cooled rapidly under static conditions, for which we observed 10% enrichment in C18:0. We observed the following differences in the

concentration of minor fatty acids between native cocoa butter and the isolated crystalline material: enrichment in medium chain fatty acids (15:0, 18:1*t*, 19:0), along with depletion of long chain fatty acids (20:1, 22:0, 23:0, 24:0). These differences were significant for isolated crystals from rapidly cooled cocoa butter under static conditions.

The analysis of fatty acids alone is not sufficient to yield any conclusive information regarding differences in chemical composition between the isolated crystals and native cocoa butter, as fatty acids are not present in a free form in natural edible fats. Fatty acids are predominantly esterified to a glycerol backbone in TAG molecules. Only less than 2% of cocoa butter is in the form of free fatty acids (Dimick, 2000). Hence it is important to examine the TAG profile of the studied samples. Table 5-C shows the TAG composition for native cocoa butter and the isolated crystalline phases. We observed a depletion of mono- and diunsaturated TAG (i.e., POP, POS, POO, and SOO) along with enrichment of saturated (i.e., PPS, SSP, SSS), and the stearic acid rich monosaturated TAG SOS in the isolated crystals from cocoa butter melts cooled under static conditions, relative to native cocoa butter. Other literature reports similar trends. Researchers have found cocoa butter crystals isolated from statically crystallized melts to be rich in SOS (Dimick & Manning, 1987; Chaiseri & Dimick, 1995a), saturated TAG such as PPS, PSS, and SSS (Davis & Dimick, 1989a; Dimick 2000), phospholipids (Arruda & Dimick, 1991) and glycolipids (Davis & Dimick, 1989b). Furthermore, lower concentrations of POP (Dimick & Manning, 1987) and POS (Dimick, 2000) were found in seed crystals relative to cocoa butter. Similar differences were also observed in seed crystals isolated from dynamically crystallized cocoa butter (Savage & Dimick, 1995; Chaiseri & Dimick, 1995b).

In addition to the described changes in TAG between native cocoa butter and the isolated crystals, we observed enrichment of a fraction of lipid species which eluted prior to the unsaturated TAG OOL for cocoa butter statically cooled. Fig. 5.8 illustrates the TAG profile of isolated crystalline material from cocoa butter cooled statically at 1°C/min along with the standards used to identify each peak. We observe in Fig. 5.8 that a peak which eluted prior to OOL has the same retention time as LLL, however, we did not observe significant enrichment in linoleic (18:2) acid when analyzing it for its fatty acid profile. We isolated this fraction by HPLC (using the same experimental setup described in the materials and methods section) from the cocoa butter sample cooled rapidly under static conditions. Table 5-D shows its fatty acid profile. The absence of significant concentrations of linoleic acid confirmed that the fraction was not LLL, despite having the same retention time. The analyzed fraction is composed of roughly 70% saturated and 30% monounsaturated fatty acids. We propose that this fraction is not composed of a single molecule but is rather a mixture of highly saturated DAG that co-elute in between the region of DAGs and TAGs.

Table 5-C. TAG composition of cocoa butter and isolated crystalline fractions from cocoa butter melts subjected to different shear and cooling conditions. Isolation was performed when SFC $\approx$ 1.5%.

TAG	Cocoa Butter % Area	0.3°C/min			
		Static		Dynamic	
		% Area	Δ	% Area	Δ
DAG	1.70 (0.744)	2.03 (0.769)		1.56 (0.225)	
SatDAG	0.05 (0.087)	0.42 (0.100)		0.00	S
OOL	0.61 (0.098)	0.35 (0.307)		0.56 (0.039)	
PLP	1.69 (0.092)	1.40 (0.279)		1.68 (0.100)	
OOO	0.26 (0.096)	0.19 (0)		0.25 (0.051)	
P00	2.60 (0.260)	1.71 (0.162)	-34.38	2.61 (0.244)	
POP	18.73 (0.473)	16.65 (0.432)	-11.08	18.98 (0.064)	
PPP	0.16 (0.100)	0.35 (0.041)		0.18	
SOO	3.43 (0.181)	2.78 (0.295)	-18.82	3.33 (0.004)	-0.03 S
POS	40.90 (0.710)	39.15 (1.667)	-4.28	40.68 (0.592)	
PPS	0.36 (0.298)	1.58 (0.478)	345.78	0.00	
D	0.22 (0.105)	0.46 (0)		0.28	
SOS	27.53 (0.551)	28.07 (0.707)		27.41 (0.122)	
SSP	0.77 (0.167)	2.37 (1.011)	209.71	0.89 (0.021)	S
F	1.44 (0.151)	1.60 (0.073)		1.55 (0.150)	
SSS	0.29 (0.050)	1.42 (0.836)		0.26 (0.372)	

* Separation between P00 and LOS was not achieved. The % area of P00 corresponds to the area of both P00 + LOS.

n = 2 for all samples except for static -0.3°C/min for which n = 3.

When significant difference ($P < 0.05$) was found between the isolated crystals and native cocoa butter, the magnitude of the difference is indicated ($\Delta\%$).

Positive $\Delta\%$ indicates enrichment, while negative $\Delta\%$ indicates depletion of the particular fatty acid.

S indicates significant difference between shear treatments at the same cooling rate ($P < 0.05$).

Significant difference ($P < 0.05$) between cooling rates was only observed for the concentration of SSP in crystals isolated from cocoa butter processed under static conditions. No other cooling rate effect was observed.

1°C/min				4°C/min			
Static		Dynamic		Static		Dynamic	
% Area	Δ	% Area		% Area	Δ	% Area	
2.14 (0.344)		1.56 (0.290)		1.39 (0.214)		2.66 (1.755)	
0.50 (0.101)	893.63	0.00	S	0.29 (0.416)	483.34	0.00	S
0.38		0.62 (0.010)	S	0.23 (0.327)		0.50 (0.204)	
1.15 (0.113)		1.73 (0.069)		1.25 (0.108)		1.55 (0.304)	
0.14 (0)		0.17 (0.021)		0.22 (0)		0.23 (0.045)	
1.85 (0.261)	-29.01	2.42 (0.033)		2.03 (0.445)		2.49 (0.151)	
16.19 (0.341)	-13.56	18.34 (0.098)		17.13 (0.023)	-8.53	18.15 (0.637)	
0.35		0.23 (0.329)		0.16 (0.227)		0.23 (0.095)	
2.90 (0.111)	-15.43	3.34 (0.315)		2.92 (0.131)	-14.85		
37.89 (1.576)	-7.37	39.67 (0.519)		39.09 (0.641)	-4.42	39.75 (0.925)	
1.77 (0.090)	398.75	1.00 (0.049)	S	1.42 (0.116)	299.74	0.48 (0.447)	S
		0.33 (0.144)		0.20 (0)		0.26 (0.143)	
27.75 (1.229)		27.63 (0.391)		28.71 (0.170)	4.28	28.11 (0.836)	
3.03 (0.044)	295.10	0.95 (0.085)	S	1.99 (0.681)	159.06	1.21 (0.390)	
0.75 (1.028)	-47.67	1.38 (0.085)		1.62 (0.048)		1.60 (0.160)	
2.44 (0.443)	747.64	0.36 (0.035)	S	1.09 (0.552)		0.98	

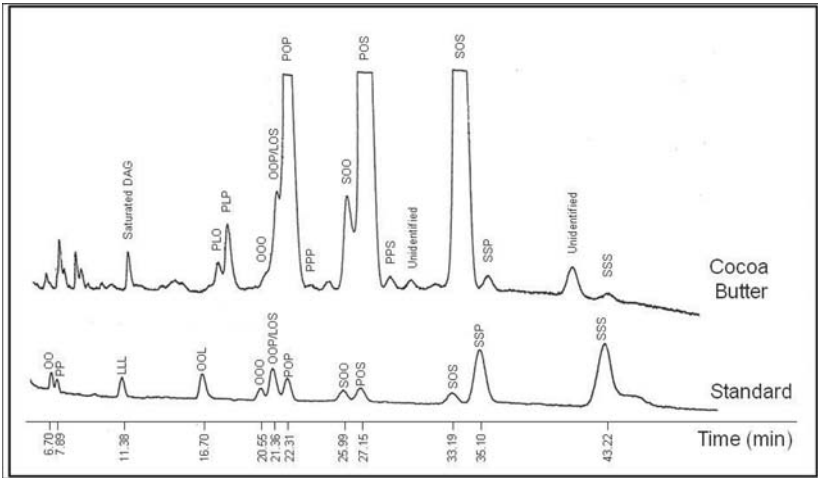


Fig. 5.8. HPLC chromatogram of standard TAG, and crystalline material isolated from cocoa butter cooled statically. Separation was performed using a Econosil C18 column tempered at 36°C, using an acetone:acetonitrile (60:40 v/v) mixture as the mobile phase.

Table 5-D. FA profile of a fraction isolated by reverse phase HPLC from the crystalline mass isolated from statically crystallized cocoa butter.

FA	% Area
16:0	30.4
16:1	8.1
18:0	34.5
18:1	19.9
20:0	7.1
Saturated	72
Monounsaturated	28

Crystallization of the Isolated Crystalline Material

The observed changes in composition affected the crystallization behavior of the isolated crystalline material. Fig. 5.9 shows crystallization curves of native cocoa butter and the crystallized solids isolated at the time of sampling when there is roughly 1.5% SFC. For all samples, we observed a major crystallization event with a peak melting temperature between 12 and 13°C, corresponding to the crystallization of the bulk of

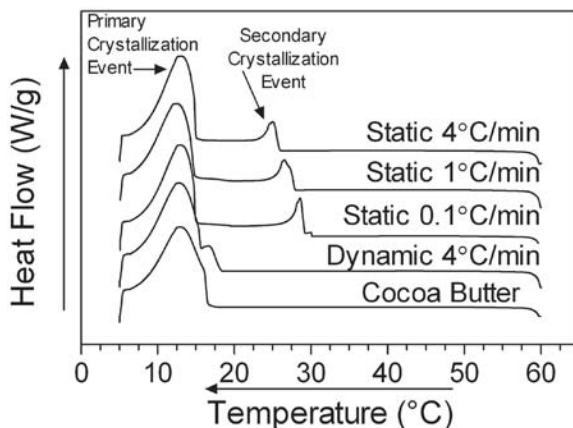


Fig. 5.9. Crystallization thermograms of cocoa butter and isolated crystalline fractions from cocoa butter melts subjected to different shear and cooling conditions. Samples were isolated from the melt when SFC $\approx$ 1.5%. Isolated samples were melted after their isolation and subsequently cooled from 60 to 5°C at 5°C/min.

the lipid species. We observed a small peak between 29.9 and 16.2°C that preceded this main crystallization event in isolated material from cocoa butter cooled under static conditions. This small peak is evidence of the fractional crystallization of a high melting fraction. During cooling, high melting point molecules—such as SOS and saturated TAGs—become undercooled, forming crystalline compounds with other TAG of similar chain length and conformation. As cocoa butter's crystallization process continues, different molecules will crystallize onto the surface of the growing high melting nuclei, thus changing its physical properties. Alongside, the solubility and concentration of the remaining TAG in the melt will change, as will the thermal characteristics of the growing nuclei. Cooling rate affected this minor crystallization event, with slow cooling leading to higher peak crystallization temperatures (25.6°C) relative to rapid cooling (21.6°C). When native cocoa butter is cooled slowly, there is more time for TAG molecules to associate with more structurally compatible (i.e., similar chain length and saturation) molecules resulting in the crystallization of high melting point fractions. Conversely, when cocoa butter is cooled rapidly, the driving force for crystallization at any given time interval is higher, hence TAG molecules of different molecular weights and conformations form mixed crystals containing higher and lower melting point species.

While we observed a distinct secondary crystallization event for the crystalline phases isolated from statically cooled samples, we only noted a shoulder for samples cooled under shear. In the presence of shear, mass transfer of TAG molecules is enhanced in the melt, and thus can incorporate into the crystalline network more

readily (Rousset & Rappez, 2001). We believe that the application of shear led to formation of mixed crystals, in turn reducing the lipid segregation observed in the absence of shear.

We propose to explain the fractionation observed during the crystallization of the isolated crystals by changes in chemical composition. Fig. 5.10 shows correlations between the concentrations of trisaturated (SatSatSat), monounsaturated (SatUnsatsat) and diunsaturated (SatUnsatsat) TAG and the highest peak melting temperature obtained with DSC. Higher peak crystallization temperatures result from samples with a higher content of SatSatSat, alongside lower concentrations of SatUnsatsat and SatUnsatsat TAG molecules. SatSatSat TAG have a high melting point relative to the rest of the species in cocoa butter. As the temperature of the melt decreases, these become undercooled (as the temperature of the system is below their melting temperature). Their solubility is diminished, and consequently become saturated in the melt. This leads to their preferential crystallization in the melt forming high melting point

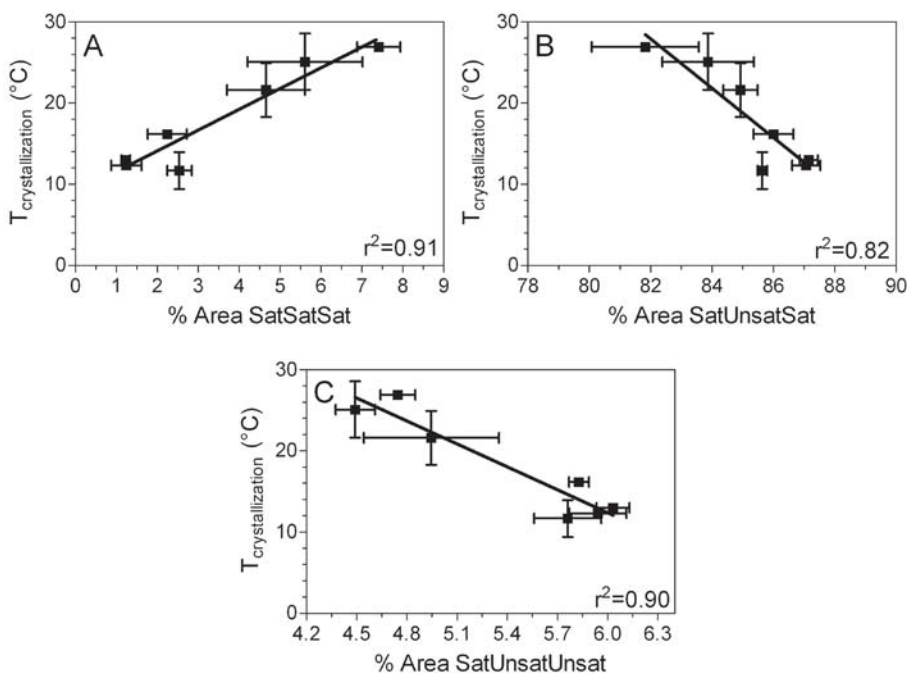


Fig. 5.10. Correlations between the concentration of trisaturated TAG (PPS+SSP+SSS) (A), monounsaturated TAG (POP+POS+SOS) (B), and diunsaturated TAG (POO+SOO) (C) and the highest peak crystallization temperature observed by DSC of native cocoa butter and isolated crystalline samples from cocoa butter cooled at 0.1, 1, and 4°C/min in the presence and absence of shear.

stable nuclei on top of which other lipid species will crystallize. The presence of unsaturated TAG molecules will lower the crystallization point, as a result of their lower crystallization temperatures. Additionally, we believe that the SatUnsaturated TAG will interfere with the attachment of SatUnsaturated TAG to the growing crystal (even though their concentration is less than 10% of the latter). This will result in lower crystallization temperatures.

Chemical Makeup and Structure

During the analysis of the mass initially crystallized from the melt under static conditions, we found significant enrichment of stearic rich TAG's along with a peak initially identified as the unsaturated LLL. We proceeded to design an experiment that aimed to understand how a change at the molecular level of cocoa butter would affect different levels of structure and ultimately functionality. Our approach was to enrich cocoa butter with highly saturated SSS and a highly unsaturated LLL, and further study the crystallization kinetics, crystal habit, microstructure, and mechanical properties of such blends.

Crystallization Kinetics

Cocoa butter is composed primarily of TAGs containing predominantly palmitic (C16:0), stearic (C18:0), and oleic C18:1 *cis*9 fatty acids. The majority of TAGs in cocoa butter are symmetrical mono-unsaturated, with POP, SOS, and POS being the most predominant (Lip et al., 2001). Melting points for these glycerides range between 23.5–43.0°C for SOS and 15.2–36.7°C for POP, depending on the polymorphic form (Sato et al., 1989). In addition, cocoa butter contains minor quantities of trisaturated as well as di- and tri-unsaturated TAGs such as OOO, PPP, and SSS. In the α polymorphic form, their melting points are –31°C, 44.7°C, and 54.9°C respectively (Small, 1986). With such chemical makeup, cocoa butter has a melting range of 29–34°C when crystallized in the stable β polymorph (van Malssen et al., 1996b; Marangoni & McGauley, 2003). Thus, the addition of specific TAGs to cocoa butter—such as highly saturated SSS and highly unsaturated LLL in this study—can potentially alter the saturation conditions of the melt and consequently affect its crystallization behavior and structure.

Fig. 5.11 shows the T_{onset} of cocoa butter and cocoa butter enriched with 1% of LLL or SSS. Cocoa butter has an T_{onset} of 17°C. The addition of only 1% liquid oil in the form of low melting LLL did not affect the crystallization behavior of cocoa butter as reflected by no significant change in T_{onset} . On the other hand, the addition of only 1% highly saturated SSS had a considerable effect on cocoa butter's crystallization behavior. T_{onset} increased by roughly 5°C. We also observed a second crystallization event at 15.6°C. We believe that the undercooled molecules of SSS induce fractional crystallization by forming a mixed crystal with other high melting point molecules

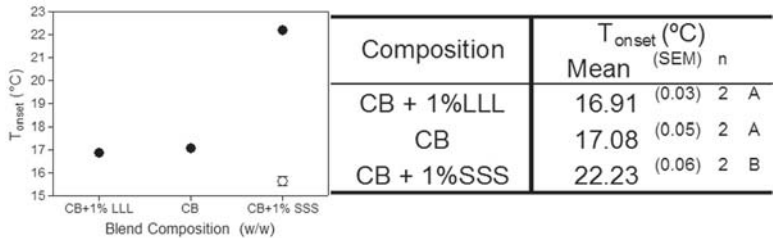


Fig. 5.11 Onset crystallization temperature (T_{onset}) of cocoa butter, and cocoa butter samples enriched with 1% (w/w) of LLL or SSS determined by DSC. Samples were melted to 80°C for 30 minutes and subsequently cooled to 0°C at a rate of 5°C/min. The temperature at which the heat flow deviates from baseline corresponds to T_{onset} . Full symbols correspond to an initial crystallization event; empty symbols correspond to the crystallization of a second fraction. Letters indicate significance ($P < 0.05$).

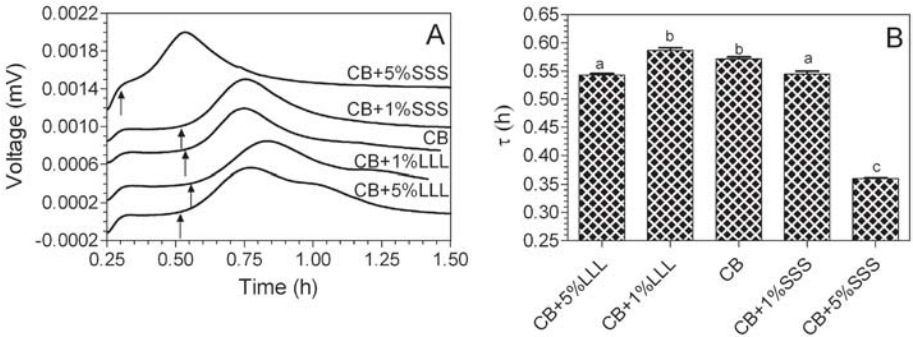


Fig. 5.12. Crystallization of cocoa butter blends at 20°C studied by DSC. The crystallization curves (A) were obtained by melting the samples at 80°C for 30 minutes and subsequently cooling to 20°C at 5°C/min. The crystallization curves were constructed with data collected once the DSC cell had reached thermal stability at 20°C. Induction times of crystallization (τ) (B) were obtained from the crystallization curves by extrapolating the linear increase in voltage to the signal baseline (indicated by arrows in the crystallization curves). Letters denote significant differences ($P < 0.05$).

naturally present in cocoa butter, producing a high melting fraction which crystallizes at 22°C. With further cooling, a second lower melting point fraction subsequently crystallizes at 15.6°C.

Fig. 5.12 illustrates the changes in isothermal heat flow as a function of time that result from the crystallization of the studied cocoa butter blends at 20°C. From the curves, we calculated the induction time for crystallization (τ) by extrapolating the linear increase in voltage to the signal baseline. Acquired XRD data yields informa-

tion relevant to the samples' polymorphic dynamics. We will discuss this data in detail later in the Crystal Structure section; however, for the interpretation of Fig. 5.12, we will briefly describe the polymorphic forms present during the first hour of crystallization at 20°C. There is evidence that upon static crystallization of cocoa butter at 20°C, the unstable α polymorph initially forms between 2 and 5 minutes after the sample reached isothermal conditions. After 37 minutes of isothermal storage, a second crystallization event takes place, corresponding to the formation of the β' phase via solid state transformation of the α phase (van Malssen et al., 1999). This indicates that the values of τ obtained from the thermograms correspond to the crystallization of the β' form, rather than the initial nucleation events. We could not observe the crystallization of the α form with calorimetry as it takes place early on during the crystallization, when—under the conditions used in this experiment—the DSC cell first reaches isothermal equilibrium.

At 20°C, all blends had τ in the range of 0.55 and 0.60 hours, with the exception of cocoa butter with added 5% SSS which had a considerably lower τ of 0.36 hours. The addition of 5% SSS accelerates the overall rate of crystallization, as well as the formation of the β' polymorph. One can notice in Fig. 5.12 that there are two different fractions crystallizing, observed as a change of slope of the cocoa butter plus 5% SSS peak between 0.35 and 0.45 hours. We believe that SSS may co-crystallize with high melting fractions of cocoa butter at early time points. Further in the crystallization process, we also observed a change in slope for samples containing LLL, evidenced in Fig. 5.12 as a shoulder to the right side of the crystallization peak (between 0.90 and 1.20 hours of crystallization). Such change in heat flow is thought to be indicative of either the crystallization of a second—lower melting—fraction or recrystallization of the existing crystalline phase in a higher liquid fraction environment.

Fig. 5.13 shows the isothermal crystallization behavior of the studied blends at 24°C. At this temperature, pure cocoa butter will crystallize fractionally, as evidenced by the presence of two distinct crystallization peaks. The first peak, corresponding to a high melting point fraction, had an onset time of 2.2 hours. The second peak, corresponding to a low melting point fraction, had an onset time of 6 hours. Upon addition of 1% LLL fractional crystallization is evident as a shoulder to the right of the main crystallization peak forms after roughly 3.9 hours. This shoulder corresponds to the crystallization of a lower melting fraction. Further addition of LLL prevented fractionation, as we observe only a single peak. We believe the enrichment of a liquid TAG (i.e., LLL) increases molecular mobility in the melt, promoting the formation of mixed crystals and thus preventing the fractionation. Cocoa butter enriched in SSS also crystallizes in a single event when stored isothermally at 24°C (Fig. 5.13). We found the effect of SSS addition on τ to be somewhat proportional to the amount of SSS added. As the concentration of SSS increases, so does the number of high melting point molecules, which become undercooled (with a melting point of 54.9°C, at 24°C SSS has a ΔT of roughly 30°C), thus leading to a larger driving

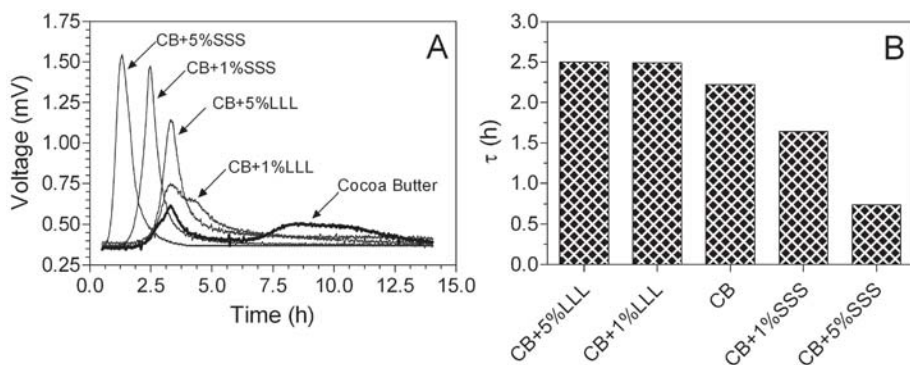


Fig. 5.13. Crystallization of cocoa butter blends at 24°C studied by DSC. The crystallization curves (A) were obtained by melting the samples at 80°C for 30 minutes and subsequently cooling to 24°C at 5°C/min. The crystallization curves were constructed with data collected once the DSC cell had reached thermal stability at 24°C. Each curve was normalized with respect to its total area. Induction times of crystallization (τ) (B) were calculated by extrapolating the linear increase in voltage to the signal baseline.

force for nucleation. This resulted in shorter values of τ , as well as in co-crystallization of SSS with the bulk of the TAG in cocoa butter, thereby preventing fractional crystallization.

Crystal Structure

When liquid oil is cooled to a temperature below its melting point, it will undergo a phase change. The TAGs, which are in random thermal motion in the liquid oil, will orient and align with each other in characteristic lateral packing and longitudinal stacking forming lamellae upon undercooling. Series of lamellae form domains, which in turn stack to form single crystallites (Fairley et al., 1995; Mazzanti, 2004). Crystallites aggregate with each other to form clusters, further interacting with each other to create flocs which ultimately form three dimensional networks (Mazzanti, 2004; Marangoni, 2005b). Fig. 5.14 depicts such a hierarchy of structure in a fat crystal network. In this study, we examined the crystalline structure formed from cooling cocoa butter blends at 20 and 24°C by measuring some of these structural levels using powder X-ray diffraction. We used wide angle reflections to determine the characteristic “short spacings,” which provide information on the lateral packing of fatty acid chains within the lamella. Similarly, we used small angle reflections to obtain the long spacings, which correspond to the 001, and higher order planes of the unit cell, which are a function of the size and polymorphic form of the TAGs forming the lamellae. Additionally, we used the full width half maximum (FWHM) to determine the size of the domains (ξ) (Fig. 5.15). FWHM is the width of the long spacing peak at 50% of

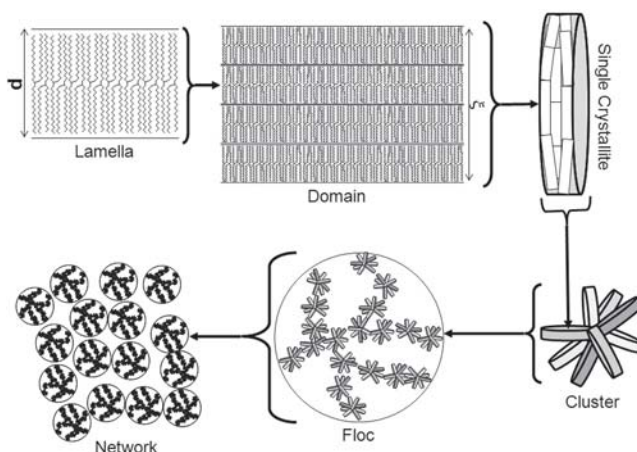


Fig. 5.14. Schematic representation of the different levels of structure in crystallized fats. A single crystallite may have one or more domains of a thickness ξ , composed in turn of several lamellae of thickness d . Each lamella is formed by TAG organized with a characteristic longitudinal stacking and lateral packing. Source: Mazzanti, 2004.

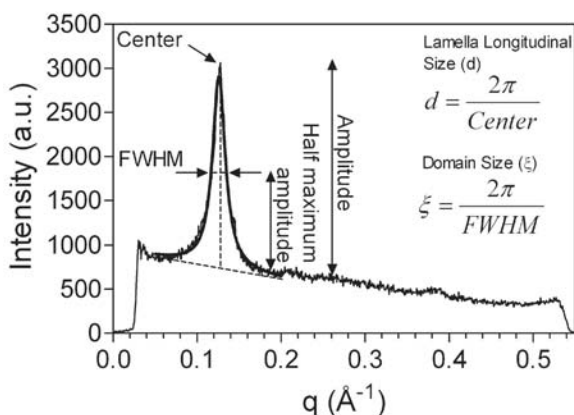


Fig. 5.15. A short angle X-ray diffraction pattern, illustrating the parameters obtained which characterize the crystal structure.

the total peak amplitude. Furthermore, we used polarized light microscopy to image the morphology and distribution of the clusters or flocs.

Fig. 5.16 shows the development of crystalline structures during the initial 52 minutes of cocoa butter's crystallization at 20°C. For each blend, we observed an initial peak with a short spacing around 4.2Å and a long spacing around 49Å.

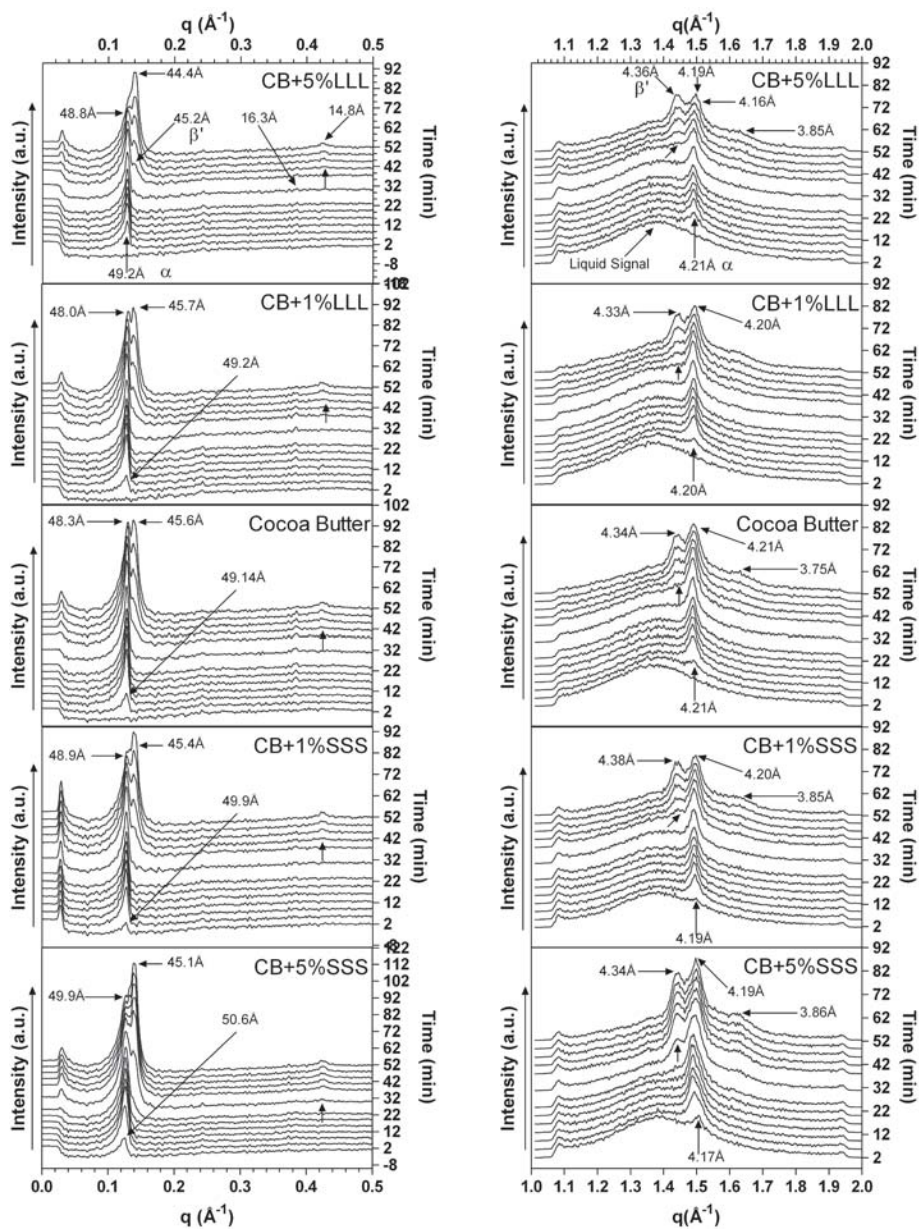


Fig. 5.16. XRD patterns of cocoa butter blends crystallized isothermally at 20°C. Scans were performed every 200 seconds during a 52 minute period. Plots on the left column correspond to the long spacings, while plots on the right column correspond to the short spacings.

This crystalline form corresponds to the unstable α polymorph (Form II) (Sato et al., 1989; Sato & Koyano, 2001), characterized by a double chain length structure conformation with no tilt (Mazzanti, 2004). This crystalline form continues to grow as time progresses. We observed a second peak with a short spacing of 4.3Å, and a long spacing of 45Å after roughly 30 minutes (indicated by the arrows in Fig. 5.16). These spacings point to the crystallization of the metastable β' phase in an inclined (with respect to the lamellar interface) double chain length structure. These peaks grow over time, both in the small and the large spacings, indicating an increase in the amount of the β' polymorph. The α and the β' forms coexist as evidenced by the presence of both peaks in the small and large spacing regions. For some blends (cocoa butter enriched with 5%LLL, 1 and 5% SSS), the α peak merges with the β' in the large spacing region indicating a possible $\alpha \rightarrow \beta$ polymorphic transformation.

All studied blends crystallized in the same polymorphic forms; however we observed differences in the time of their appearance. In the case of cocoa butter enriched with SSS, we observed a peak as early as 2 minutes after the sample reached 20°C, while for the rest of the samples we observed no evidence of crystallinity until after 5.3 minutes. Similarly, we observed the second peak with a short spacing of 4.3Å after 28.6 minutes for cocoa butter + 5%SSS, while it was not observed until after 35 minutes for the rest of the blends. This is consistent with the shorter values of τ (determined by DSC) previously reported for blends containing the high melting point SSS.

Further analysis of small angle reflections generated structural information regarding the crystals formed. We fitted peaks to a Lorentzian curve using Prism 4 software (Graph Pad Software Inc, San Diego CA, USA). We obtained and utilized the center of the peak, as well as the FWHM, to calculate the size of the lamellae (d) and the domain size (ξ) (Fig. 5.15). Additionally, we recorded the number of lamellae per domain (d/ξ). Fig. 5.17 shows the results from the diffraction patterns acquired during the first 35 minutes, when only the α form is present. The crystallization of the β' polymorph complicated the analysis, due to the difficulty in resolving each polymorph's corresponding peaks. As shown in Fig. 5.17, the long spacing for cocoa butter crystallized at 20°C throughout the first 35 minutes of crystallization is 49.2Å. When cocoa butter is enriched with SSS, the lamellae are larger as evidenced by larger long spacings. After 2 minutes, long spacings of 50.3Å and 51.2Å for blends with 1% and 5% SSS, respectively, characterized the initial crystal structure. One can visualize the lateral packing of TAG molecules during crystallization and the impact that the chain length and conformation of its constituent lipid species have on the overall interlamellar distance. TAGs in cocoa butter are mainly monounsaturated (i.e., POP, POS, SOS). The double bond of the oleic acid in the sn-2 position of the glycerol backbone, translates to a kink in their structure. On the other hand, the fully saturated SSS is spatially a longer molecule (absence of kinks or bends in the stearic acid moieties). As nuclei form, which we believe to be rich in high melting point SSS molecules, the size of the lamellae will be somewhat

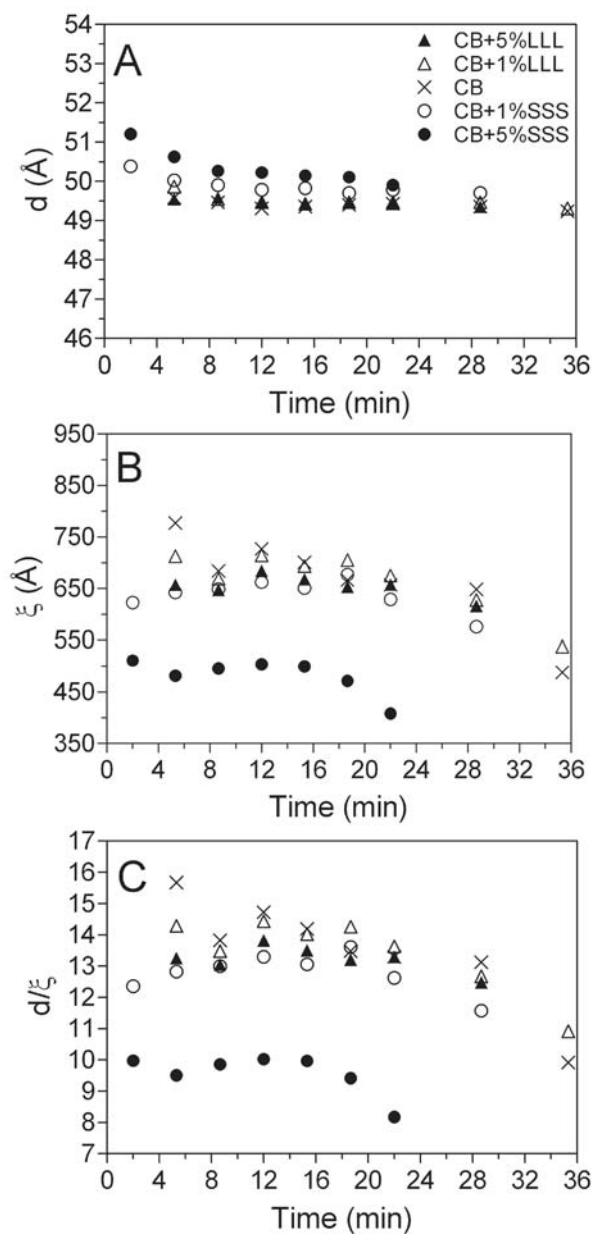


Fig. 5.17. Lamellae (d) (A), domain (ξ) (B) size, and number of lamellae per domain (C) obtained from long spacing peak parameters (peak position and FWHM) of cocoa butter blends crystallized statically at 20°C for 36 minutes.

longer relative to nuclei rich in monounsaturated TAGs. In time, the interlamellar distance of SSS containing blends shortens as a result of subsequent inclusion of monounsaturated (shorter) TAGs from the melt, being comparable to that of cocoa butter's 49Å after 8 minutes for 1% SSS and 22 minutes for 5% SSS. On the other hand, at these early time points the low melting unsaturated LLL molecules will most likely exist in the melt, having no impact on the crystal structure, as evidenced by the long spacings of blends with LLL comparable to those of pure cocoa butter.

SSS also affected the size of the domains, evidenced by a dramatic decrease in ξ for blends of cocoa butter + 5% SSS (Fig. 5.17B). Blends containing 5% SSS have ξ values almost 200Å smaller than the rest of the samples, which corresponds to a crystalline domain with 10 vs. 14 stacked lamellae (Fig. 5.17C). It appears that the inclusion of SSS in the crystal structure infringes significantly upon the order of the domain. For the rest of the samples, we observed only small differences in ξ , with cocoa butter having the highest values. For all three parameters plotted in Fig. 5.17, there is a relatively constant value from 6 to 20 minutes, time period where the α phase is present. As time reaches the crystallization of the β' phase, the existing crystal structure changes as a result of the formation of this new phase from the melt, along with possible transformation of the α into the β' forms. A dramatic decrease in ξ , as well as the number of lamellae per domain, points to this possible transformation.

After initial crystallization studies, we stored samples isothermally at 20°C for further analysis. Fig. 5.18 shows the diffraction patterns of the studied blends stored for 1, 2, 3, and 7 days. After 1 day of storage, we observed two short spacings at 4.36 and 4.18Å, and a long spacing at 43.9Å, for cocoa butter. This crystalline structure corresponds to cocoa butter's β' (IV) polymorph (Mazzanti et al., 2005). After 2 days of storage, we observed the same spacings, along with a small peak at 4.58Å, indicating the presence of the stable β form (Wille & Lutton, 1996; Sato & Koyano, 2001). In time, this 4.58Å peak grows, while the peaks at 4.36 and 4.18Å decrease in size. At 20°C, cocoa butter reaches an equilibrium SFC after only 120 minutes (Campos, 2006), thus the formation and growth of the newly observed β phase is thought to be at the expense of the existing β' through solid state polymorphic transformations (van Malssen et al., 1999). The transformation of the β' to the more efficiently packed β results from a structural stabilization of the crystal structure in which the unsaturated fatty acid (oleic) segregates into one chain layer and the remaining two saturated fatty acids (palmitic and/or stearic) are located in a different layer (Larsson, 1994; Sato, 2001), creating a triple chain length structure. A change in the long spacing peaks from 43.9 to 63.9Å after 3 days, which correspond to a triple chain length conformation (Mazzanti, 2004) typical of the β form, evidences this transformation in our work. After one week, the β' peaks have disappeared, and we observe a large β peak (with a short spacing at 4.58Å).

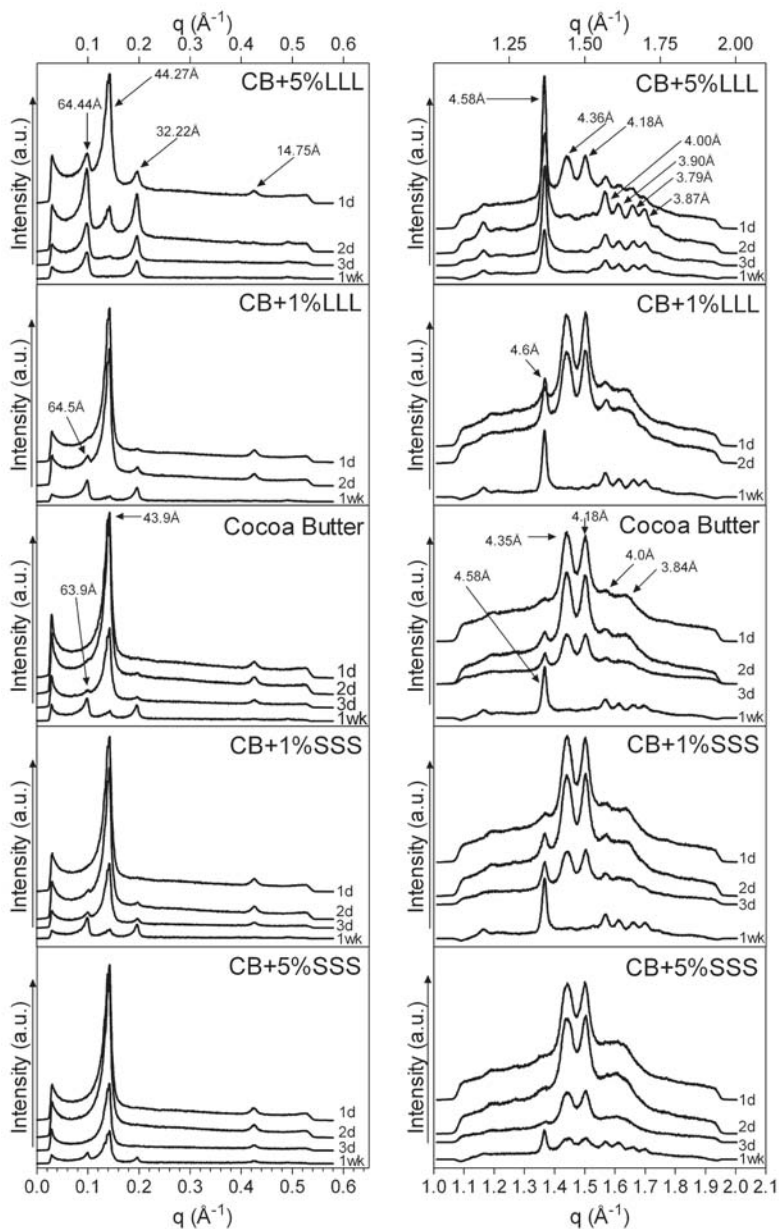


Fig. 5.18. XRD patterns of cocoa butter blends crystallized isothermally and stored at 20°C. Scans were performed after 1, 2, 3 and 7 days of storage. Plots in the left column correspond to the long spacings, while plots on the right column correspond to the short spacings. Signal intensities have been reduced and shifted to avoid overlap.

Addition of the low melting point LLL molecules to cocoa butter accelerates the formation of the stable β form at 20°C. The characteristic long spacing of the triple chain length conformation at 64.5Å and the short spacing at 4.6Å of the stable β form became apparent after 1 day of storage, and were very pronounced after only 2 days of storage for cocoa butter with 1% LLL. Furthermore, upon addition of 5% LLL, we observed a long spacing at 64.4Å and a short spacing at 4.58Å after only 1 day of storage at 20°C, along with the reflections associated with the β' form. After only 2 days, we observed a diffraction pattern typical of the $\beta(V)$ form in the short spacings region. We believe that LLL promotes the transformation of metastable forms to the stable β form. Our results are consistent with those reported by Smith et al. (2007), which reported that addition of only 1% hazelnut oil to tempered cocoa butter accelerated $\beta' \rightarrow \beta$ transformation. Additionally, previous research suggests that the presence of liquid oil aids in the polymorphic transformations of other fat systems such as lard (Rousseau et al., 1998) and anhydrous milk fat (Wright et al., 2005). Similarly, the presence of a low melting fraction in milk fat induced polymorphic transformations in higher melting point fractions (Timms, 1980; Cisneros et al., 2006). Our findings confirm that the presence of liquid oil either entrapped in the crystalline network or on the crystal surface accelerate polymorphic transformations, as molecular diffusion is altered, allowing for TAG to adopt higher stability arrangements (Lovegren et al., 1976b; Smith et al., 2005).

SSS also affected the polymorphic transformation of the studied blends. Although SSS promoted the early appearance of the metastable forms α and β' , it decreased the rate of transformation into the stable β form. Cocoa butter and cocoa butter enriched with 1% SSS crystallized in the same forms throughout the duration of this study. However, upon addition of 5% SSS, we observed no spacings corresponding to the stable β polymorph during the first 3 days of storage. We did not observe a diffraction pattern typical of a β form until after one week of storage. As previously mentioned, we think that LLL promotes polymorphic transformations as it increases the volume fraction of liquid in the system, thus decreasing viscosity and enhancing molecular mobility. The opposite occurs with addition of SSS. Previous data shows an increase in viscosity of model chocolate melts in the presence of trisaturated TAG's (Cebula et al., 1991) where high melting molecules, such as SSS, become undercooled and crystallize out. The proposed effect of SSS on melt viscosity results in hindered molecular mobility and thus delayed polymorphic transformations.

Fig. 5.19 illustrates the polymorphism of cocoa butter blends at 24°C during the first 12 hours of crystallization. For all studied blends, the first peak observed in the small angle region had a long spacing between 47.9 and 50.6Å, corresponding to the metastable α phase (Mazzanti 2004). Consistent with observations at 20°C, blends with SSS had the highest long spacings for the α form (49.5 and 50.6Å for 1% and 5% added SSS respectively) relative to the rest of the sample blends (between 48.3Å for pure cocoa butter and 47.9Å for 5% LLL enriched cocoa butter). In time we observed a second peak with a long spacing between 44.2 and 45.4Å, corresponding

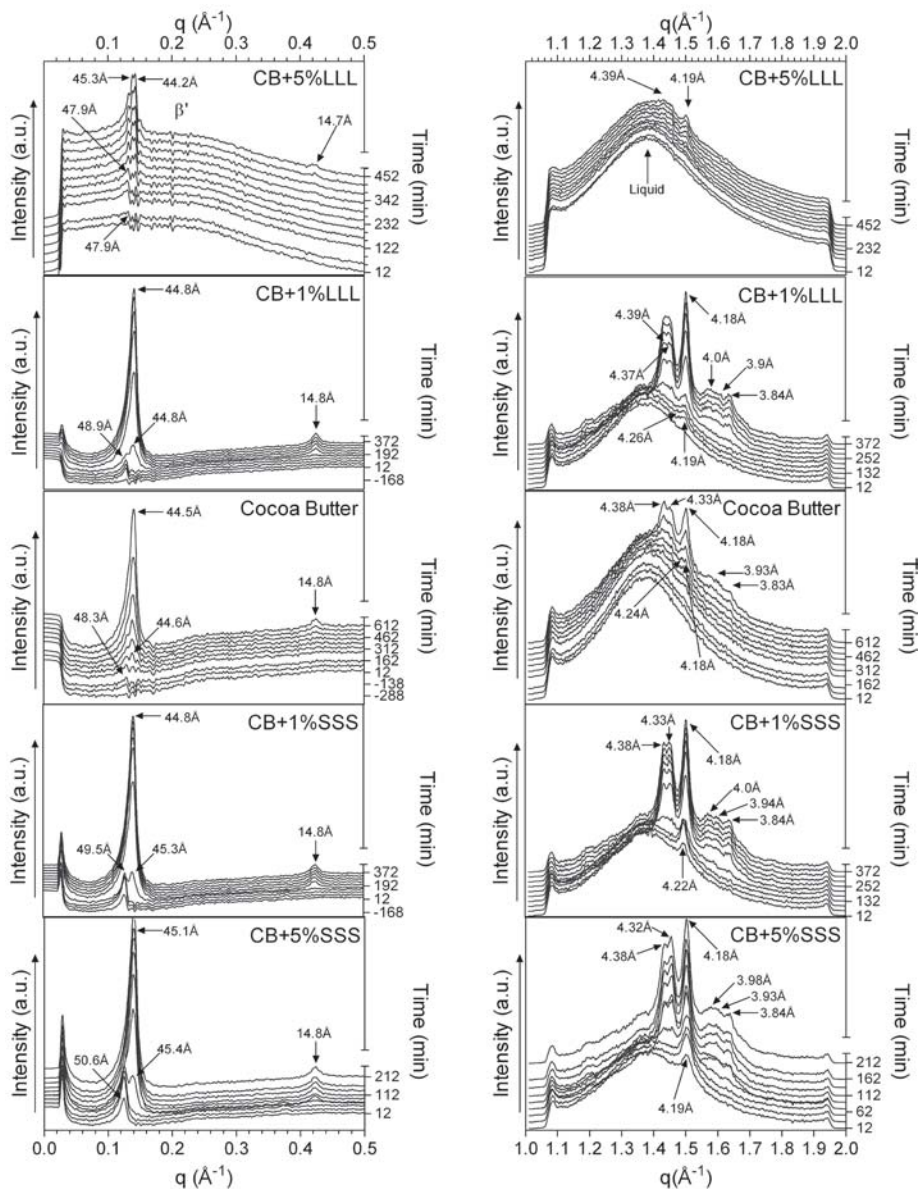


Fig. 5.19. XRD patterns of cocoa butter blends crystallized isothermally at 24°C. Plots on the left column correspond to the long spacings, while plots on the right column correspond to the short spacings.

to the β' form (Ollivon et al., 2006). The times at which the β' form appeared differed with composition. For blends containing SSS, we observed the β' form after 80 minutes; for pure cocoa butter after 100 minutes; and for blends containing LLL after 100–120 minutes of crystallization. As the β' peak grew in time, the α peak correspondingly disappeared.

In the wide angle region, we observed small spacings at 4.39, 4.18, 3.9, and 3.8 Å for all samples. Based on the work published by Sato and Koyano (2001), the β' (IV) of cocoa butter displays short spacings at 4.35 and 4.15 Å, and a long spacing at roughly 44 Å. Thus, we believe that all samples are in the β' form. We once again found differences between the samples with regards to the times at which we observed the spacings. Blends containing SSS crystallized more rapidly, as we observed peaks at earlier times relative to blends with LLL. During the first 12 hours of crystallization there was no evidence of the existence of the stable β form.

After 24 hours of isothermal storage, we further analyzed the polymorphism of blends crystallized at 24°C. Fig. 5.20 shows the resulting XRD scans. For all blends except cocoa butter + 5%SSS, we observed a very large peak with spacings at 4.59 Å, along with smaller spacings at 3.99, 3.88, 3.77, and 3.67 Å. This diffraction pattern corresponds to the stable β (V) form of cocoa butter (Koyano et al., 1989). On the other hand, cocoa butter + 5%SSS had spacings at 4.38 and 4.18 Å that correspond to a metastable β' form. Consistent with the results observed at 20°C, we found the addition of high melting SSS to delay polymorphic transformations of the less stable forms to the stable β form in spite of it reducing the induction times of crystallization of the unstable forms.

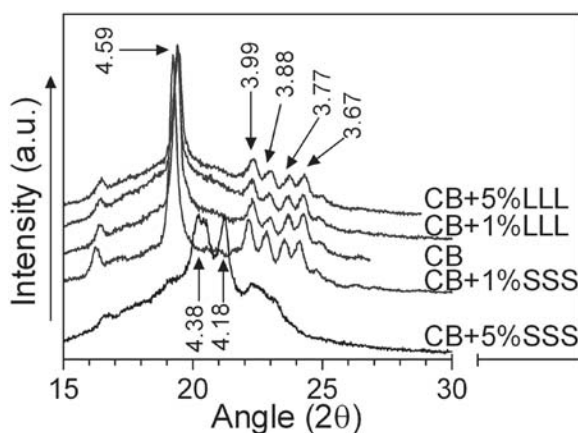


Fig. 5.20. XRD patterns of cocoa butter blends crystallized isothermally at 24°C for 1 day.

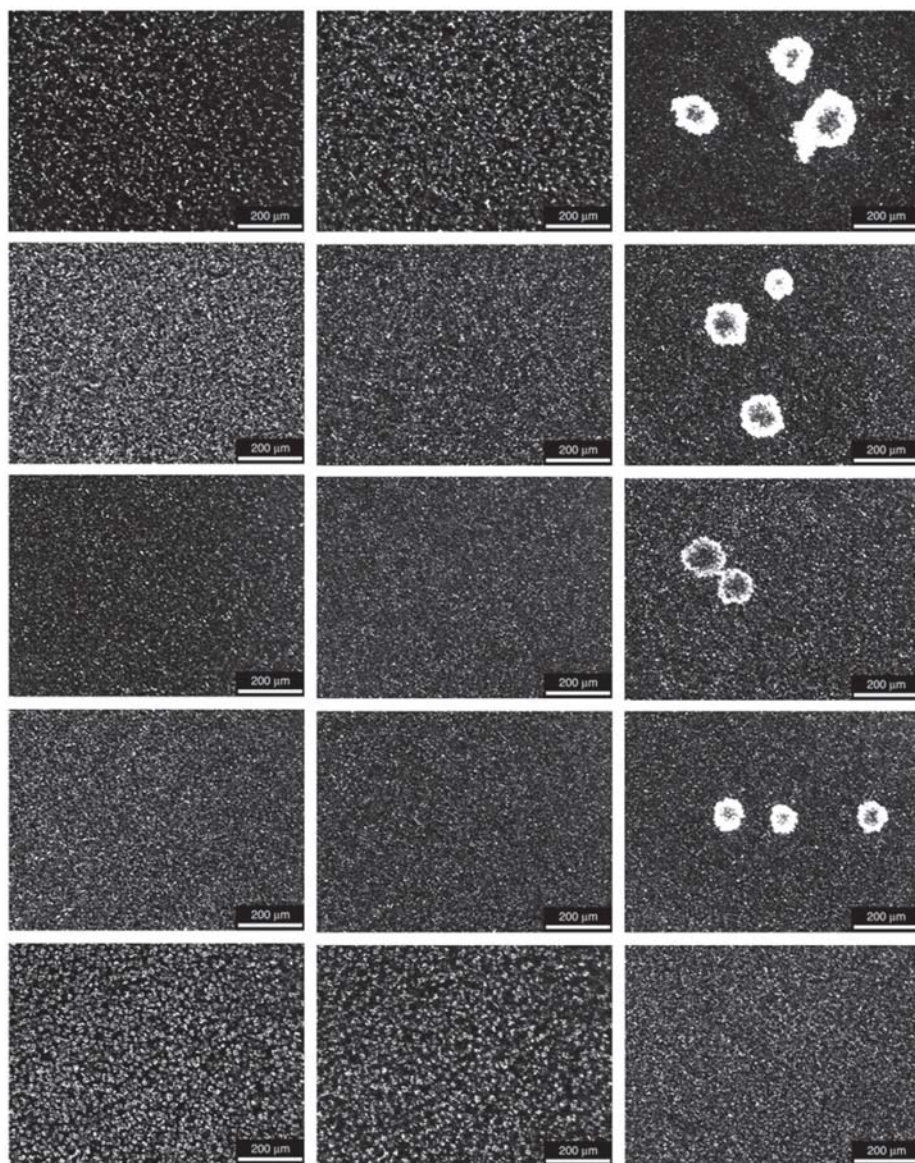


Fig. 5.21. Polarized light micrographs of cocoa butter blends crystallized isothermally at 20°C. Prior to imaging, samples were heated to 80°C for 20 minutes, followed by cooling at a rate of 5°C/min. The microstructure was imaged at different time points.

Microstructure

PLM allows the study of crystalline microstructures, yet at a much larger length-scale relative to XRD, enabling the visualization of the aggregation of domains into single crystallites and their further aggregation into clusters. We studied the microstructure of fat crystal networks formed at 20 and 24°C by using PLM.

Fig. 5.21 shows a large number of crystallites after only 2 minutes of crystallization in all blends at 20°C. The observed crystal morphology can be described as a high number of very small needle-like crystallites. At this time-temperature condition, Marangoni and McGauley (2003) report that cocoa butter crystallizes in the α polymorph, forming needle-like features. During the first hour of crystallization, microstructural features are comparable between the studied blends. After 24 hours of storage at 20°C we observed differences in the microstructure. The presence of relatively big crystal clusters, which are 100–200 μm in size, was evident for all sample blends except for cocoa butter + 5% SSS. We observed aggregation of crystallites into clusters for cocoa butter in the β' polymorph, which previous research indicates form via the α phase between 15 and 22°C (Marangoni & McGauley, 2003). The presence of clusters is evidence of a polymorphic transformation and XRD results show that SSS delays polymorphic transformation, therefore cocoa butter + 5%SSS is expected to be the last blend to display such changes in crystal morphology.

Fig. 5.22 shows the imaged microstructures of samples crystallized at 24°C. After 3 hours of crystallization, the morphology of cocoa butter crystals consisted of very small, needle-like structures. The addition of LLL and SSS impacted the microstructures under observation. Relative to cocoa butter, we observed a smaller number of larger features surrounded by substantial amounts of liquid oil with the addition of LLL. With time, the solid fraction increased as evidenced by an increase in the number of features. After 24 hours of storage, considerable areas void of solid mass were still present in blends of LLL. In some cases, liquid oil filled the areas void of crystalline mass within these microscope slides. In other instances, segregation of oil and empty space was evident to the naked eye. Conversely, the addition of SSS led to an increased number of smaller features, resulting from increased crystallization rates. For all blends, we observed no significant changes in the crystal morphology in the time frame of this study (i.e., 3–24 hours).

Mechanical Properties

The effects of SSS and LLL addition to cocoa butter's mechanical strength by quantifying the elastic bending modullae (E_B) for samples crystallized at 20 and 24°C (Fig. 5.23). The addition of SSS increased the solid fraction of cocoa butter, yet resulted in a softening effect; while LLL increased its mechanical strength.

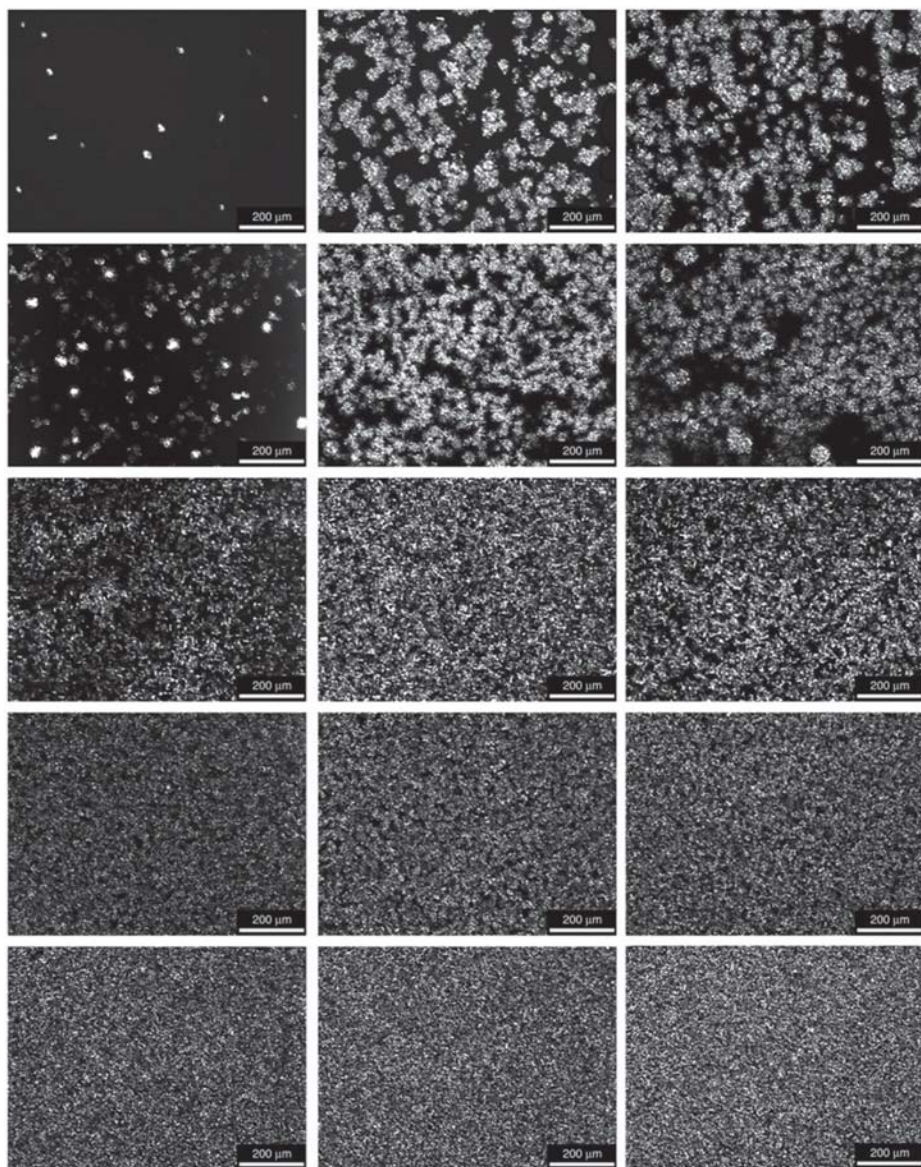


Fig. 5.22. Polarized light micrographs of cocoa butter blends crystallized isothermally at 24°C. Prior to imaging, samples were heated to 80°C for 20 minutes, followed by cooling at a rate of 5°C/min. The microstructure was imaged at different time points.

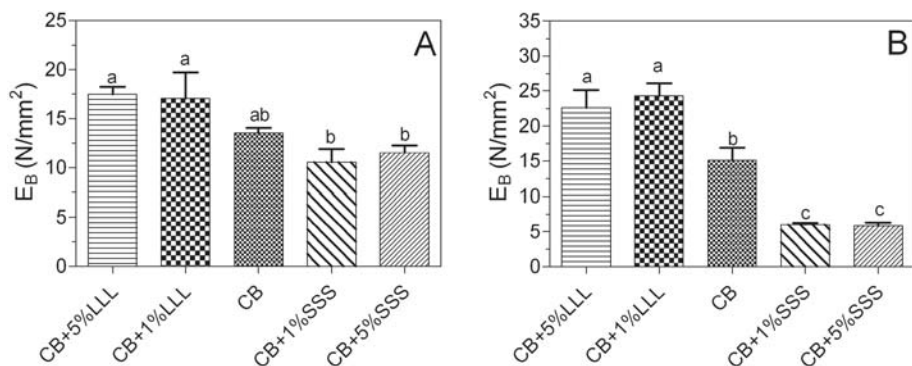


Fig. 5.23. Elastic bending modulus (E_B) of cocoa butter blends crystallized at 20°C for 24 hours (A) and 24°C for 48 hours (B). Bars represent an average of 4 replicates. Letters denote significance between samples at each crystallization temperature ($P < 0.05$).

We propose the observed outcome on E_B to be a result of the effect that the addition of different molecules has on the crystal–melt interfacial tension (δ). We obtained a theoretical estimation of δ from a derivation of the Fisher–Turnbull equation (Marangoni, 2005b), in its following form:

$$\delta = \left(\frac{3mk_B\Delta H_f^2}{16\pi(V_m^s)^2T_f^2} \right)^{1/3} \quad \text{Equation 5.5}$$

where m (K^3) is the slope of a $\ln(\tau T)$ vs $1/[T(\Delta T)^2]$ plot constructed with τ values obtained from experimental calorimetric data (Fig. 5.12 and 8.13); k_B is the Boltzman constant (1.38×10^{-23} J/K); ΔH_f (J/mol) is the enthalpy of fusion obtained from experimental calorimetric data; V_m^s (m^3/mol) is the molar volume of solid fat calculated as the ratio of cocoa butter's average molecular weight divided by its density; and T_f (K) is the melting temperature also obtained experimentally from calorimetric data. We considered the molecular weight of POS (861.4 g/mol) to be the average molecular weight of cocoa butter. Table 5-A shows the calculated δ as well as free energies of nucleation (ΔG_n) at 24°C for all studied blends. We calculated ΔG_n values using the following (Marangoni, 2005b):

$$\Delta G_n = \frac{mk_B}{\Delta T^2} \quad \text{Equation 5.6}$$

The model previously proposed by our group (Marangoni, 2000; Marangoni & Rogers, 2003) expresses the relationship between δ and the mechanical strength of

fat crystal networks and describes the estimation of the modulus of elasticity (E) with the following equation:

$$E = \frac{\sigma^*}{\varepsilon} \sim \frac{6\delta}{a\varepsilon} \Phi^{\frac{1}{d-D}} \quad \text{Equation 5.7}$$

where ζ^* and ε^* are the critical stress and strain respectively; a is the size of the particles that form the network; Φ is the solid fraction; d is the Euclidian dimension of the embedding space ($d = 3$); and D is the fractal dimension. The above expression illustrates that the elastic properties of a fat crystal network will depend on the amount of mass present (indicated by Φ), the particle properties (a), and the interaction between these particles (associated with δ). Throughout this work, we experimentally obtained a series of structural indicators, such as the SFC (%); parameters that describe the microstructure [i.e., equivalent particle diameter (a) and fractal dimension (D_b)]; and the large deformation properties of the studied blends (displacement to point of fracture and E_B). These are also shown in Table 5-E.

The addition of high melting point SSS to cocoa butter changes the saturation conditions in the melt, which result in lower ΔG_n , translating into lower inductions times (Figs. 5.11–Fig. 5.13). We observed slight increases (up to 7% at 24°C) in the SFC, alongside decreased particle size (roughly 16%) with small changes in D_b . This would point towards an expected increase in the elasticity of the network. However, we observed a reduction of approximately 20% in the δ at higher SSS contents. We simulated the effect of δ on ζ^* of cocoa butter over a range of solids' fractions according to Equation 5.5, using the structural parameters obtained experimentally throughout this work. We used averages of the equivalent particle diameter (2.09 μm) and D_b (1.89) for all studied blends for the purposes of the simulation. We plotted ζ^* as a function of Φ at the highest (1% LLL), intermediate (pure cocoa butter), and lowest (5% SSS) values for δ . Fig. 5.24 illustrates that at a

Table 5-E. Structural parameters obtained experimentally or theoretically from cocoa butter blends.

Blend	24°C								
	δ (J/m ²)	τ (h)	ΔG_n (kJ)	SFC _{max} (%)	D_b	Eq. Diam (μm)	Displace- ment (mm)	F_{fracture} (N)	E_B (N/mm ²)
CB+5%LLL	0.019	2.50	4.14	49.08	1.79	2.33	0.25	8.58	22.62
CB+1%LLL	0.021	2.49	3.88	52.14	1.76	2.33	0.26	9.17	24.29
CB	0.018	2.20	3.54	52.75	1.86	2.16	0.47	10.27	15.09
CB+1%SSS	0.015	1.64	2.94	50.74	1.90	1.82	0.42	3.80	5.97
CB+5%SSS	0.014	0.74	2.26	56.18	1.88	1.82	0.42	3.69	5.84

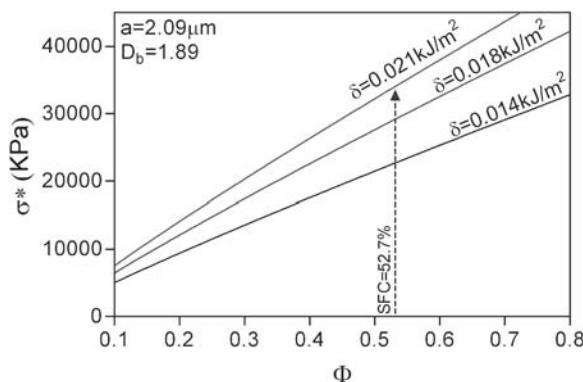


Fig. 5.24. Simulation of the effect of surface free energy on the critical stress (ζ^*) of a plastic disperse system over a range of solids' volume fraction, which is based on the model proposed by Marangoni and Rogers (2003). The primary particle diameter (a) and fractal dimension (D_b) used for this simulation were the average of the equivalent particle diameter and D_b (obtained from the analysis of polarized light micrographs acquired at 24°C) of the five studied cocoa butter sample blends. The SFC (%) of pure cocoa butter crystallized isothermally at 24°C is indicated. The chosen surface free energy values for this simulation correspond to that of pure cocoa butter (0.0017 J/m²), as well as cocoa butter enriched with LLL (0.0019 J/m²) and SSS (0.0013 J/m²).

particular solid fraction (i.e., 52.7% SFC for cocoa butter) the ζ^* decreases proportionally (from 2,645 Pa to 2,084 Pa) when δ decreases roughly 20% upon addition of SSS. Such a decline in ζ^* will result, according to Equation 5.4, in a decrease in the elasticity of the system, as observed in experimental values of E_B . Likewise, the calculated increases in δ for blends containing LLL will result in higher ζ^* , yielding a crystal network with higher E_B .

In addition to the structural parameters previously discussed, polymorphism also affects the mechanical properties of the studied blends. The addition of LLL accelerated the $\beta' \rightarrow \beta$ transformation; while SSS slowed down such transformations. Brunello et al. (2003) demonstrated that polymorphism strongly influences the mechanical properties of cocoa butter. The β polymorph is not only the most stable, but also the most efficiently packed and most dense crystal form. As SSS delays the $\beta' \rightarrow \beta$ transformation, we expect that samples which contain SSS will be softer relative to those in which the transformation into the β form has been completed.

Conclusions

In this work, we examined the effects of extrinsic (i.e., processing) and intrinsic (i.e., molecular make-up) conditions on the crystallization process and different structural levels of cocoa butter. The application of shear during the crystallization of cocoa butter strongly influenced different levels of structure in the resulting crystal network.

The experimental evidence in this study demonstrates that mechanical work applied to cocoa butter at 24°C impacts the crystallization process from molecular to macroscopic structural levels. When cocoa butter is crystallized statically, there is a selective crystallization of TAG molecules onto the growing crystal. This is observed in our work as the crystalline material that is initially formed is rich in saturated and stearic acid rich TAGs (i.e., PPS, SSP, SSS, and SOS) along with what we proposed to be a high melting point mixture of DAGs. Such changes in composition translate to early crystallization events that are fractional in nature.

The application of shear affected the crystallization process of cocoa butter. Shear allows dissipation of heat during cooling and early nucleation events. Moreover, shear increases contact between crystals, promoting crystal disruption and enhancing secondary nucleation. Unlike the statically processed samples, we found the composition of dynamically processed samples to be identical to native cocoa butter when analyzing the crystalline material that initially formed. This suggests that shear affects primary nucleation and early crystal growth at a molecular level. We propose that shear affects TAG mobility in the melt, promoting formation of mixed crystals over the fractional crystallization of high melting saturated molecules observed under static conditions. Secondary nucleation thus becomes more instantaneous in nature, which translates to faster crystallization processes. A larger number of small crystals with form the resulting crystal structures, which result in a stronger network.

This work also proves that significant alteration to the crystal structure and therefore the functionality of cocoa butter can be achieved through minor changes in its fatty acid composition, as depicted in Fig. 5.25. The addition of SSS changed the saturation conditions of the melt, consequently affecting crystallization and the resulting crystal network. The high melting point saturated TAGs become rapidly undercooled as the temperature of cocoa butter decreases, providing the energy required for nucleation and crystal growth (observed as lower onset crystallization temperatures and onset times for crystallization). Fully saturated SSS molecules are spatially bigger (i.e., straight) relative to monounsaturated TAGs which have bends in the unsaturated oleic acid moieties. Their inclusion into the crystal lattice of nuclei introduces a certain degree of disorganization during the stacking of lamella in the very early stages of crystallization, thus resulting in differences in crystal lamella and domain size. Subsequently, different levels of structure are affected. Despite the lower induction times for crystallization observed, SSS delayed polymorphic transformation from metastable forms into the stable β form. Lastly, we found cocoa butter with added SSS to be less elastic, as a result of the effect SSS had on the crystal–melt interfacial tension.

Changes in the TAG profile of cocoa butter in the opposite direction (i.e., addition of fully unsaturated LLL) also resulted in structural differences. With a very low melting point, LLL will not be undercooled at the studied temperatures, and will only slightly affect induction times and temperatures of crystallization. We observed no significant changes in the size of the lamellae. This, along with LLL's structural

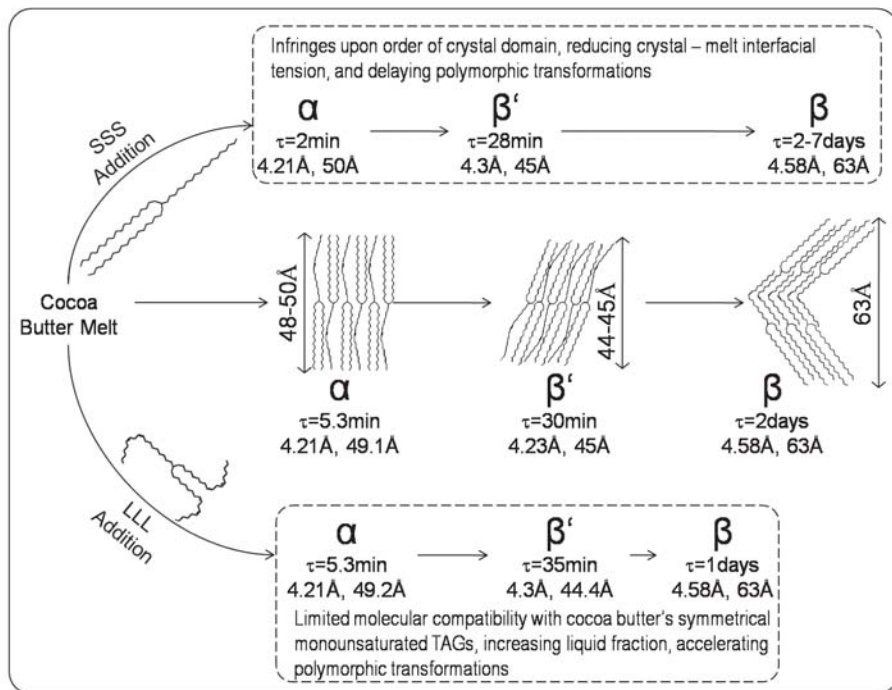


Fig. 5.25. Schematic representation of cocoa butter's crystallization, in its native state and upon addition of either saturated tristearin (SSS) or unsaturated trilinoleate (LLL) at 20°C. Indicated are times (τ) in which each polymorphic form was observed with X-ray diffraction, as well as their short and long spacings.

incompatibility with the crystal surface suggests that LLL does not co-crystallize with the bulk of cocoa butter's TAGs, but rather surrounds crystal domains, increasing the crystal network's liquid fraction. Consequently, LLL enhances molecular mobility and the rearrangement of TAG molecules into highly dense, efficiently packed, stable crystalline structures (in other words, accelerates $\beta' \rightarrow \beta$ transformation).

References

- Arruda, D.H.; Dimick, P.S. Phospholipid composition of lipid seed crystals isolates from Ivory Coast cocoa butter. *J. Am. Oil Chem. Soc.* 1991, 68, 385–390.
- Avrami, M. Kinetics of phase change. I. General theory. *J. Chem. Phys.* 1939, 7, 103–1112.
- Awad, T.S.; Marangoni, A.G. Comparison between image analyses methods for the determination of the fractal dimension of fat crystal networks. In *Fat Crystal Networks*, 1st Ed.; Marangoni, A.G. CRC Press: Boca Raton, FL, 2005, 381–412.

- Becket, S.T. *The Science of Chocolate*, 2nd Ed.; Royal Society of Chemistry Publishing: Cambridge, UK; 2008.
- Bolliger, S.; Breitschuh, B.; Stranzinger, M.; Wagner T.; Windhab, E.J. Comparison of precrystallization of chocolate. *J. Food Eng.* 1998, 35, 281–297.
- Briggs, J.L.; Wang, T. Influence of shearing and time on the rheological properties of milk chocolate during tempering. *J. Am. Oil Chem. Soc.* 2004, 81, 117–121.
- Brunello, N.; McGauley, S.E.; Marangoni, A.G. Mechanical properties of cocoa butter in relation to its crystallization behavior and microstructure. *Lebensm. Wiss. Technol.* 2003, 36, 525–532.
- Campos, R.; Narine, S.S.; Marangoni, A.G. Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Res. Int.* 2002, 35, 1971–1982.
- Campos, R. Experimental methodology. In *Fat Crystal Networks*, 1st Ed.; Marangoni, A.G. CRC Press: Boca Raton, FL, 2005, 267–348.
- Campos, R. Effects of processing conditions on the crystallization of cocoa butter; Doctoral Thesis; University of Guelph: Guelph, ON, Canada, 2006.
- Campos, R.; Ollivon, M.; Marangoni, A.G. Molecular composition dynamics and structure of cocoa butter. *Cryst. Growth Des.* 2010, 10, 205–217.
- Cebula, D.J.; Dilley, K.M.; Smith, K.W. Continuous tempering studies on model confectionery systems. *The Manufacturing Confectioner*. 1991, 71, 131–136.
- Chaiseri, S.; Dimick, P.S. Dynamic crystallization of cocoa butter I. Characterization of simple lipids in rapid- and slow-nucleating cocoa butters and their seed crystals. *J. Am. Oil. Chem. Soc.* 1995a, 72, 1491–1496.
- Chaiseri, S.; Dimick, P.S. Dynamic crystallization of cocoa butter II. Morphological, thermal and chemical characteristics during crystal growth. *J. Am. Oil Chem Soc.* 1995b, 72, 1495–1504.
- Cisneros, A.; Mazzanti, G.; Campos, R.; Marangoni, A.G. Polymorphic transformation in mixtures of high- and low-melting fractions of milk fat. *J. Agric. Food Chem.* 2006, 6030–6033.
- Cruz-Hernandez, C. Compositional analysis of dairy fats, phospholipids and positional distribution on TAG from cows fed fish meal. Doctoral Thesis; University of Guelph: Guelph, ON, Canada, 2003.
- Davis, T.R.; Dimick, P.S. Isolation and thermal characterization of high-melting seed crystals formed during cocoa butter solidification. *J. Am. Oil Chem. Soc.* 1989a, 66, 1488–1493.
- Davis, T.R.; Dimick, P.S. Lipid composition of high-melting seed crystals formed during cocoa butter solidification. *J. Am. Oil Chem. Soc.* 1989b, 66, 1494–1498.
- Dhonsi, D.; Stapley A.G.F. The effect of shear rate, temperature, sugar, and emulsifier on the tempering of cocoa butter. *J. Food Eng.* 2005, 77, 936–942.
- Dibildox-Alvarado, E.; Toro-Vazquez, J.F. Evaluation of tripalmitin crystallization in sesame oil through a modified Avrami equation. *J. Am. Oil Chem. Soc.* 1989, 75, 73–76.
- Dimick P.S.; Manning D.G. Thermal and compositional properties of cocoa butter during static crystallization. *J. Am. Oil Chem. Soc.* 1987, 64, 1663–1669.
- Dimick, P.S. Compositional effect on crystallization of cocoa butter. In *Physical Properties of Fats, Oils, and Emulsifiers*, 1st ed.; Widlak, N., Ed.; AOCS Press: Champaign IL, 2000; pp 140–162.

- Fairley, P.; Krochta, J.M.; German J.B. Crystal morphology of mixtures of tripalmitin and but-terfat. *J. Am. Oil Chem. Soc.* 1995, 72, 693–697.
- Foley, J.; Brady, J. Temperature-induced effects on crystallization behaviour, solid fat content, and the firmness values of milk fat. *J. Dairy Res.* 1984, 51, 579–589.
- Gordon, M.H.; Padley, F.B.; Timms, R.E. Factors influencing the use of vegetable fats in chocoalte. *Fette Seifen Anstrichmittel*. 1979, 81, 116–121.
- Grall, D.S.; Hartel, R.W. Kinetics of butterfat crystallization. *J. Am. Oil Chem. Soc.* 1992, 69, 741.
- Haighton, A.J. Work softening of margarines and shortenings. *J. Am. Oil Chem. Soc.* 1965, 42, 27–30.
- Hartel, R.W. *Crystallization in Foods*. Aspen Publishers: Gaithersburg, MD, 2001.
- Heertje, I.; van Eendenburg, J.; Cornelisson, J.M.; Juriaanse, A.C. The effect of processing on some microstructural characteristics of fat spreads. *Food Microstructure* 1998, 7, 189–193.
- Heertje, L. Microstructural studies in fat research. *Food Structure* 1993, 12, 77–94.
- Herrera, M.L.; Hartel, R.W. Effect of processing conditions on physical properties of a milk fat model system: rheology. *J. Am. Oil Chem. Soc.* 2000a, 77, 1189–1195.
- Herrera M.L.; Hartel, R.W. Effect of processing conditions on physical properties of a milk fat model system: microstructure. *J. Am. Oil Chem. Soc.* 2000b, 77, 1197–1204.
- Humphrey, K.L.; Moquin, P.H.L.; Narine, S.S. Phase behavior of a binary lipid shortening system: From molecules to rheology. *J. Am. Oil Chem. Soc.* 2003, 80, 1175–1182.
- Johansson, D.; Bergenstahl, B. Sintering of fat crystal networks in oil during post-crystallization processes. *J. Am. Oil Chem. Soc.* 1995, 72, 911–920.
- Koyano T.; Hachiya, I.; Arishimo, T.; Sato, K.; Sagi, N. Polymorphism of POP and SOS. II. Kinetics of melt crystallization. *J. Am. Oil Chem. Soc.* 1989, 66, 675–679.
- Larsson, K. *Lipids: Molecular Organization, Physical Functions and Technical Application*, 1st ed.; The Oily Press Ltd.: Dundee, Scotland, 1994.
- Lipp, M.; Simoneau, C.; Ulberth, F.; Anklam, E.; Crews, C.; Brereton, P.; de Greyt, W.; Schwack, W.; Wiedmaier, C. Composition of genuine cocoa butter and cocoa butter equivalents. *J. Food Compos. Anal.* 2001, 14, 399–408.
- Litwinenko, J.W.; Rojas, A.M.; Gerschenson, L.N.; Marangoni, A.G. Relationships between crystallization behaviour, microstructure, and mechanical properties in a palm oil-based shortening. *J. Am. Oil Chem. Soc.* 2002, 79, 647–654.
- Lovegren, N.V.; Fray, M.S.; Feuge, R.O. Polymorphic changes in mixtures of confectionery fats. *J. Am. Oil Chem. Soc.* 1976a, 53, 83–88.
- Lovegren, N.V.; Gray, M.S.; Feuge, R.O. Effect of liquid fat on melting point and polymorphic behavior of cocoa butter and a cocoa butter fraction. *J. Am. Oil Chem. Soc.* 1976b, 53, 108–112.
- Maleky, F.; Marangoni, A.G. Process development for continuous crystallization of fat under laminar shear. *J. Food Eng.* 2008, 89, 399–407.
- Manning, D.M.; Dimick, P.S. Crystal morphology of cocoa butter. *Food Microstruc.* 1985, 4, 249–265.

- Marangoni, A.G. Elasticity of high volume-fraction fractal aggregate networks: A thermodynamic approach. *Phys. Rev. B*. 2000, 62, 13951–13955.
- Marangoni, A.G.; Rogers, M.A. Structural basis for the yield stress in plastic disperse systems. *Appl. Phys. Lett.* 2003, 82, 3239–3241.
- Marangoni, A.G.; McGauley S.E. Relationship between crystallization behavior and structure in cocoa butter. *Crys. Growth Des.* 2003, 3, 95–108.
- Marangoni, A.G. Crystallization kinetics. In *Fat Crystal Networks*, 1st ed.; Marangoni, A.G., Ed.; CRC Press: Boca Raton FL, 2005a; pp. 21–82.
- Marangoni, A.G. The yield stress and elastic modulus of a fat crystal network. In *Fat Crystal Networks*, 1st ed.; Marangoni, A.G., Ed.; CRC Press: Boca Raton FL, 2005b; pp. 255–266.
- Martini, S.; Herrera, M.L.; Hartel, R.W. Effect of cooling rate on nucleation behavior of milk fat–sunflower oil blends. *J. Agric. Food Chem.* 2001, 49, 3223–3229.
- Mazzanti, G. X-Ray diffraction study on the crystallization of fats under shear; Doctoral Thesis; University of Guelph: Guelph, ON, Canada, 2004.
- Mazzanti, G.; Guthrie, S.E.; Sirota, E.B.; Marangoni, A.G.; Idziak, S.H.J. Crystallization of bulk fats under shear. In *Soft Materials: Structure and Dynamics*, 1st ed.; Dutcher, J.R.; Marangoni, A.G., Eds.; Marcel Dekker: New York, 2005; pp. 279–298.
- McGauley, S. The relationship between polymorphism, crystallization kinetics, and microstructure of statically crystallizes cocoa butter. Master in Science Thesis. University of Guelph: Guelph, ON, Canada, 2001.
- Merken, G.V.; Vaeck S.V. A study of the polymorphism of cocoa butter by means of SDC calorimetry. *Lebensmittel – Wissenschaft & Technologie*. 1980, 13, 314–317.
- Metin, S.; Hartel R.W. Crystallization of fats and oils. In *Bailey's Industrial Oil and Fat Products*, 6th ed.; Shahidi, F., Ed.; John Wiley & Sons Inc.: New York, 2005, Vol 1: 45–76.
- Minato, A.; Ueno, S.; Yano, J.; Wang, Z.H.; Seto, H. Amemiya, Y.; Sato, K. Synchrotron radiation X-ray diffraction study on phase behavior of PPP-POP binary mixtures. *J. Am. Oil Chem. Soc.* 1996, 73, 1567–1572.
- Miura, S.; Konishi, H. Crystallization behavior of 1,3-dipalmitoyl-2-oleoyl-glycerol and 1-palmitoyl-2,3-dioleoyl-glycerol. *Eur. J. Lipid Sci. Technol.* 2001, 103, 804–809.
- Narine, S. S.; Marangoni, A.G. Structure and mechanical properties of fat crystal networks. In *Physical Properties of Lipids*, 1st ed.; Marangoni, A.G.; Narine, S.S., Eds.; Marcel Dekker: New York, 2002; pp. 63–84.
- Narine, S.S.; Humphrey K.L.; A comparison of lipid shortening functionality as a function of molecular ensemble and shear: microstructure, polymorphism, solid fat content and texture. *J. Am. Oil. Chem. Soc.* 2004, 37, 28–38.
- Ng, W.L. Nucleation behavior of tripalmitin from a triolein solution. *J. Am. Oil Chem. Soc.* 1989, 66, 1103–1106.
- Ollivon, M.; Keller, G.; Bourgaux, C.; Kalnin, D.; Villeneuve, P.; Lesieur, P. DSC and high resolution X-ray diffraction coupling. *J. Ther. Anal. Calorim.* 2006, 85, 219–224.

- Perez-Martinez, D.; Alvarez-Salas, C.; Morales-Rueda, J.A.; Toro-Vazquez, J.F.; Charo-Alonso, M.; Dibildox-Alvarado, E. The effect of supercooling on crystallization of cocoa butter–vegetable oil blends. *J. Am. Oil Chem. Soc.* 2005, 82, 471–479.
- Perez-Martinez, D.; Alvarez-Salas, C.; Charo-Alonso, M.; Dibildox-Alvarado, E.; Toro-Vazquez, J.F. The cooling rate effect on the microstructure and rheological properties of blends of cocoa butter with vegetable oils. *Food Res. Int.* 2007, 40, 47–62.
- Rousseau, D.; Marangoni, A. G.; Jeffrey, K. R. The influence of chemical interesterification on the physicochemical properties of complex fat systems. 2. Morphology and polymorphism. *J. Am. Oil Chem. Soc.* 1998, 12, 1833–1839.
- Rousset, Ph.; Rappaz M. Experimental study and computer modeling of the dynamic and static crystallization of cocoa butter. In *Crystallization and Solidification Properties of Lipids*, 1st ed.; Widlak, N.; Hartel, R.W.; Narine, S.S., Eds.; AOCS Press: Champaign, IL, 2001; pp. 96–109.
- Rye, G.G.; Litwinenko, J.W.; Marangoni, A.G. Fat crystal networks. In *Bailey's Industrial Oil and Fat Products*, 6th ed.; Shahidi, F., Ed.; John Wiley & Sons Inc.: New York, 2005, Vol 1: 121–158.
- Sato, K. Molecular aspects in fat polymorphism. In *Crystallization and Solidification Properties of Lipids*, 1st ed.; Widlak, N., Hartel, R.W., and Narine, S.S., Eds.; AOCS Press: Champaign IL, 2001; pp. 1–15.
- Sato, K.; Arishima, T.; Wang, Z.H.; Ojima, W.K.; Sagi, N.; Mori, H. Polymorphism of POP and SOS. I. Occurrence and polymorphic transformation. *J. Am. Oil Chem. Soc.* 1989, 66, 664–674.
- Sato, K.; Koyano, T. Crystallization properties of cocoa butter. In *Crystallization Processes in Fats and Lipid Systems*, 1st ed.; Garti, N.; Sato, K., Eds.; Marcel Dekker: New York, 2001; pp. 429–456.
- Savage, C.M.; Dimick, P.S. Influence of phospholipids during crystallization of hard and soft cocoa butters. *The Manufacturing Confectioner*, 1995, 75, 127–132.
- Sharples, A. Introduction to Polymer Crystallization. Edward Arnold Ltd.: London, 1966, 44–59.
- Small, D.M. Handbook of Lipid Research: The Physical Chemistry of Lipids: From Alkanes to Phospholipids. Vol. 4. Plenum Press: New York, 1986.
- Smith, K.; Cain, F.W.; Talbot, G. Crystallisation of 1,3-dipalmitoyl-2-oleoylglycerol and tripalmitoylglycerol and their mixtures from acetone. *Eur. J. Lipid Sci. Technol.* 2005, 10, 683–593.
- Smith, K.; Cain, F.; Talbot, G. Effect of nut oil migration on polymorphic transformation in a model system. *Food Chemistry*, 2007, 102, 656–663.
- Stapley, A.G.F.; Tewkesbury, H.; Fryer, P. The effects of shear and temperature history on the crystallization of chocolate. *J. Am. Oil Chem. Soc.* 1999, 76, 677–85.
- Steffe, J.F. *Rheological Methods in Food Process Engineering*, 2nd ed.; Freeman Press: East Lansing, MI, 1996; pp. 9–10.
- Timms, R.E. The phase behavior and polymorphism of milk fat, milk fat fractions, and fully hardened milk fat. *Aust. J. Dairy Technol.* 1980, June, 47–53.
- Timms, R.E. *Confectionery Fats Handbook: Properties, Production and Application*, 1st ed.; Lipid Technology: Bridgwater, England, 2003; pp. 255–269.

- Toro-Vazquez, J.F.; Gallegos-Infante, A. Viscosity and its relationship to crystalization in a binary system of saturated triacylglycerides and sesame seed oil. *J. Am. Oil Chem. Soc.* 1996, 73, 1237–1246.
- van Aken, G.A.; Visser, K.A. Firmness and crystallization of milk fat in relation to processing conditions. *J. Dairy Sci.* 2000, 83, 1919–1932.
- van den Tempel, M. Mechanical properties of plastic-dispersed systems at very small deformations. *J. Colloid Sc.* 1961, 16, 284–296.
- van den Tempel, M. Rheology of concentrated suspensions. *J. Colloid and Interface Science.* 1979, 71, 18–20.
- van Malssen, K.F.; Peschar, R.; Schenk, H. Geheugeneffect en stolling van cacaoboter. *Voedings Middelen Technologie* 1995, 30, 67–69.
- van Malssen, K.; Peschar, R.; Schenk H. Real-time X-ray diffraction Investigations on cocoa butter. I. Temperature dependent crystallization behavior. *J. Am. Oil Chem. Soc.* 1996a, 73, 1209–1215.
- van Malssen, K.; Peschar R.; Schenk H, Real-time X-ray powder diffraction investigations on cocoa butter. II. The relationship between melting behavior and composition of cocoa butter. *J. Am. Oil Chem. Soc.* 1996b, 73, 1217–1223.
- van Malssen, K.; van Langevelde, A.; Peschar, R.; Schenk. Phase behaviour and extended phase scheme of static cocoa butter investigated with real-time X-ray powder difraction. *J. Am. Oil Chem. Soc.* 1999, 76, 669–676.
- Wille, R.L.; Lutton, E. S. Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* 1966, 43, 491–496.
- Wright, A.J.; Hartel R.W.; Narine S.S.; Marangoni A.G. Effects of minor components on crystallization behaviour of milk fat. *J. Am. Oil Chem. Soc.* 2000, 77, 463–475.
- Wright, A.J.; Batte, H.D.; Marangoni, A.G. Effects of canola oil dilution on anhydrous milk fat crystallization and fractionation behavior. *J. Dairy Sci.* 2005, 1955–1965.

6

Polymorphism and Mixing Phase Behavior of Major Triacylglycerols of Cocoa Butter

Mari Sasaki*, Satoru Ueno, and Kiyotaka Sato

Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, 739-8528 Japan

* Present address, Kao Co. Sumida, Tokyo, 131-8501 Japan

Introduction

Chocolate consists of a solid system in which fine particles of cocoa powder, sugar, food emulsifiers and milk powder (for milk chocolate) are dispersed in polycrystalline particles of cocoa butter (Timms, 2003). The production of chocolate requires the following physical controlling technology: rheological properties of chocolate liquor, crystallization of cocoa butter, and morphology and network structure of cocoa butter crystals. In particular, cocoa butter crystals influence the quality of chocolate, such as gloss, snap, heat resistance, and fat-bloom stability.

One of the most influential factors of crystallization of cocoa butter is its polymorphic behavior, which greatly influences the crystallization and melting properties of cocoa butter crystals (Cebula & Smith, 1991, van Malssen et al., 1996a, 1996b, Loisel et al., 1998a, 1998b, Sato & Koyano, 2001, van Langevalde et al., 2001, Rousset, 2002). The peculiarity in the polymorphism of cocoa butter is that cocoa butter is not a single fat system, but is composed of different TAGs containing different types of fatty acid moieties. Therefore, the polymorphic behavior of cocoa butter must be understood by taking into account of the interactions of its major component TAGs. Cocoa butter is composed of major TAGs containing three fatty acids: palmitic acid (25%), stearic acid (35%), and oleic acid (35%) (Timms, 2003). Oleic acid is esterified at the stereo-specific numbered second (*sn*-2) position in the TAG molecule, while palmitic and stearic acids occupy the *sn*-1,3 positions. In considering the TAGs composition, a stereospecific analysis by HPLC has shown that 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol (*sn*-POS) and 1-stearoyl-2-oleoyl-3-palmitoyl-*sn*-glycerol (*sn*-SOP) are present in equal amounts, i.e. a racemic mixture (POS) in cocoa butter (Takagi & Ando, 1995). Consequently, three TAGs (POP, POS, and SOS) represent between 80–90% of the total amount of cocoa butter as the major cocoa butter-component TAGs.

The ratios of the three TAGs depend on average temperature of the places of productions of cocoa butter in a way that the concentration of SOS increases with

increasing temperature (Chaiseri & Dimick, 1989). For example, the cocoa butter grown in West Africa areas contain 16% of POP, 40% of POS, 27% of SOS and 17% of other TAGs.

The polymorphism of cocoa butter is influenced by the mixing behavior of POP, POS, and SOS. Therefore, the polymorphic structures of cocoa butter must be understood based on the mixing phase behavior of its major component TAGs. In general, the physical chemical analyses of multi-component systems of TAGs begin with individual TAG molecules, and move to the mixed systems such as binary systems, ternary systems, etc. It is thus important to study the phase behavior of binary systems and ternary systems of POP, POS, and SOS for understanding the polymorphic behavior of cocoa butter as briefly summarized by Timms (2003). The understanding of the polymorphism in such a view of molecular interactions is still open to question.

In this chapter, we first discuss the polymorphic behavior of cocoa butter and its three major component TAGs of POP, POS and SOS. Then, we consider the mixing behavior of the three TAGs in binary and ternary systems to deeply understand the polymorphic behavior of cocoa butter. To begin with, we make a brief review of polymorphism and mixing phase behavior of fats in relation to cocoa butter and related fats.

Polymorphism and Mixing Behavior of Fats

Polymorphism

Polymorphism is defined as the ability of a chemical compound to form different crystalline or liquid crystalline structures and three forms of α , β' and β are typical polymorphic forms of fats as illustrated in Fig. 6.1. The melting and crystallization behavior differ from one polymorph to others. The α , β' and β forms are defined in accordance with the subcell structure. The α polymorphs have a hexagonal subcell (H); β' polymorphs have an orthorhombic-perpendicular subcell ($O_{\perp}$); and β polymorphs have a triclinic-parallel subcell ($T_{//}$). The subcell structures can be determined most clearly by measuring X-ray diffraction (XRD) short spacing patterns of polycrystalline samples. The structures of fats also differ in chain length structure, which illustrates the repetitive sequence of the acyl chains involved in a unit cell lamella along the long-chain axis. The chain length structure varies from double (DCL) to triple (TCL), quarto (QCL) or hexa (HCL) structures, as the fatty acid moieties of the fats become more complicated.

In addition to the subcell and chain length structures, glycerol group conformations are also important, as it affects mixing behavior of fats. Two typical glycerol conformations of tuning fork type and chair type have been observed in TAG crystals (Fig. 6.2). Depending on the fatty acid compositions of the TAGs, the glycerol conformation varies from chair-type to tuning fork-type, and such conversion influences the mixing behavior of the TAGs having different types of glycerol conformations, as discussed later.

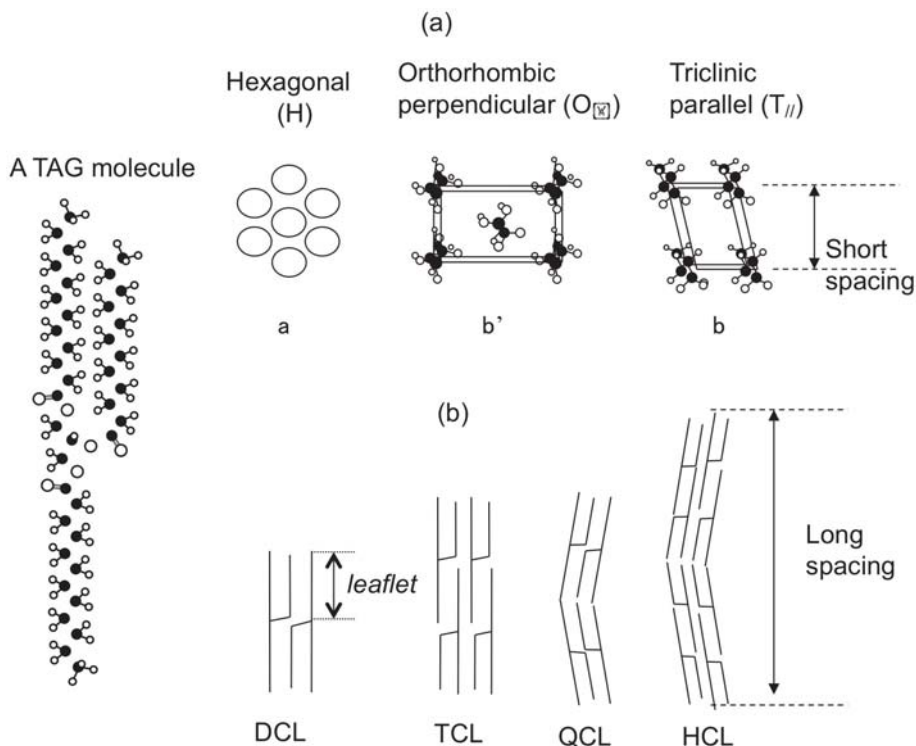


Fig. 6.1. (a) Subcell structures and (b) chain length structures of triacylglycerol crystals.

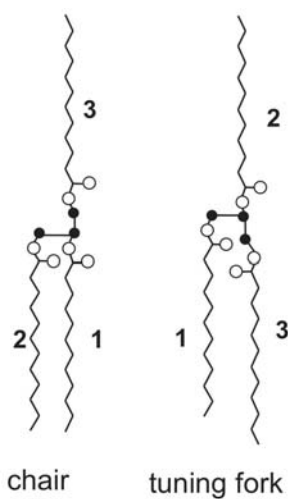


Fig. 6.2. Conformation of glycerol groups in triacylglycerol crystals. White circles, black circles and zigzag lines mean oxygen atoms, glycerol carbon atoms and hydrocarbon chains, respectively.

In food fats, transformation from β' to β often causes deterioration of the end product, mostly because of changes in crystal morphology and network. As bulky large crystals of β form deconstruct fine crystal network formed by β' crystals, rheological and textural properties of the end products are damaged, for example lowered spreadability and sandy taste may occur. The β -type polymorph has highest density among the three polymorphs due to its highest subcell packing.

Fig. 6.3 illustrates three models of the crystal structures of TAGs containing saturated acid and unsaturated acid acyl chains, in which, for example, α -2 and β -3 mean α form of DCL structure and β form of TCL structures, respectively (Kaneko et al., 1998). As a typical unsaturated acid chain, oleic acid is presented in the figures.

Fig. 6.3(a) presents five polymorphic forms of 1,3-disaturated-2-unsaturated-glycerols, such as 1,3-distearoyl-2-oleoyl-glycerols (SOS). As the saturated acids are connected to the 1st and 3rd carbon positions of glycerol group, such TAGs are called symmetric mixed acid TAGs. In these TAGs, we may assume that the glycerol conformation is of tuning fork type. As will be discussed below, cocoa butter

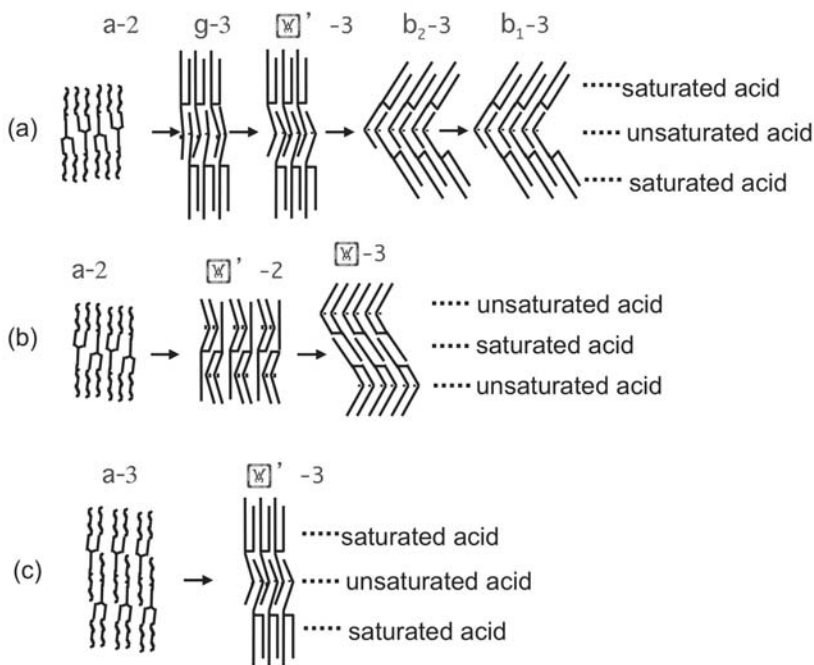


Fig. 6.3. Structure model of polymorphism of saturated-unsaturated mixed acid triacylglycerols. (a) 1,3-disaturated-2-unsaturated-glycerols, (b) 1,3-diunsaturated-2-saturated-glycerols and (c) 1,2-disaturated-3-oleoylunsaturated-glycerols.

and its major component TAGs may reveal the polymorphic structures of this type. Fig. 6.3(b) shows three polymorphic forms of 1,3-disaturated-2-unsaturated-glycerols, such as 1,3-dioleoyl-2-palmitoyl-glycerol (OPO). Similarly to Fig. 6.3(a), such symmetric mixed-acid TAGs may reveal the tuning fork-type glycerol conformation in the all polymorphic forms (Minato et al., 1997a, Bayes-García et al., 2011). By contrast, Fig. 6.3(c) shows asymmetric mixed-acid TAGs, in which two saturated acids are connected to the 1st (or 3rd) and the 2nd carbon positions, and unsaturated acid is connected to the 3rd (or 1st) carbon position of a glycerol group. Typical examples are 1,2-distearoyl-3-oleoyl-glycerols (SSO) (Takeuchi et al., 2002a) and 1,2-dipalmitoyl-3-oleoyl-glycerols (PPO) (Minato et al., 1997b and 1997c). Due to asymmetry in the fatty acid structures of SSO and PPO, we may assume that the glycerol conformation may by chair type.

Mixing Phase Behavior

When the two TAGs are mixed in the all proportions in a liquid phase, three types of general mixing phases can occur: solid solution phase, eutectic phase and molecular compound formation. The T - c phase diagrams for the mixtures of A and B are illustrated in Fig. 6.4.

Fig. 6.4(a) illustrates a solid solution phase, in which two components are miscible at all concentration ratios. Only two phases can exist in such a system: a homogeneous liquid-solution and a solid-solution. In this mixture system, a binary mixture is cooled but neither component solidifies without containing some of the other component. That is, both components are crystallized simultaneously. All mixtures of the two components have melting points intermediate between the melting points of the pure components. This phase property is formed when the TAGs are very similar in melting point, molecular volume and polymorphism.

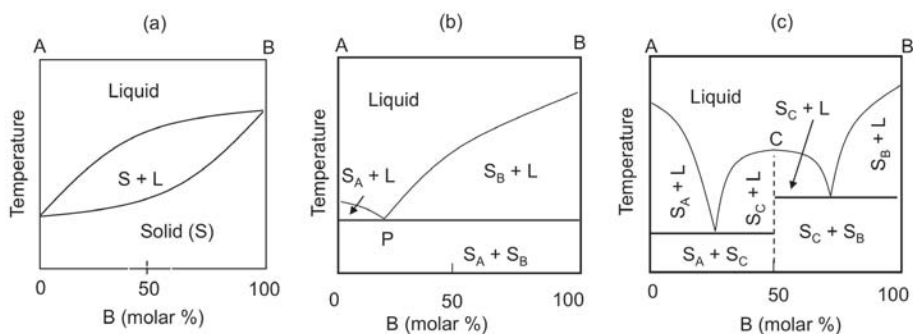


Fig. 6.4. Typical three phase diagrams of binary mixtures of two components A and B: (a) solid-solution phase formation; (b) eutectic phase formation; (c) molecular compound formation. S, solid; C, molecular compound.

A eutectic phase exhibits that the two components are miscible in liquid, but not miscible in solid phases, as shown in Fig. 6.4(b). The straight line, named as solidus, represents the temperature at which solid mixtures of A and B begin to melt, or temperature below which mixtures of these two components are completely solid. The two liquidus curves represent the temperature, above which homogeneous liquid-solution of two components are completely liquid. The eutectic point P is the lowest melting or solidification temperature. When the eutectic point shifts along the solidus to endpoint, this phase is called monotectic.

As the third typical mixing behavior, Fig. 6.4(c) shows the phase diagram of a molecular compound formation, which demonstrates two eutectic phases. The total phase diagram can be split into two phase diagrams of its sub-eutectic mixtures of A-C and C-B, in which the molecular compound is noted by C.

We have recently studied the mixing behavior of various TAGs including cocoa butter–component TAGs. Fig. 6.5 summarizes typical cases showing three mixture systems. The solid solution system is formed when the TAGs are very similar in melting point, molecular volume and polymorphism. For example, mixtures of LLL/MMM (L; lauric acid, M; myristic acid) and PPP/MMM (P; palmitic acid) (Takeuchi et al., 2003), α and β' forms of PPP/SSS (S; stearic acid) (Kellens et al., 1991), and SOS/SLS (L; linoleic acid) (Takeuchi et al., 2002b). Among the cocoa butter component TAGs, SOS/POS showed the solid solution mixture, as will be elaborated below.

A eutectic phase is most commonly found in the binary mixtures of TAGs, e.g. β form in PPP/SSS (Kellens et al., 1991), PPP/LLL (Takeuchi et al., 2003), PPP/POP (Minato et al., 1996), POP/OOP (Zhang et al., 2007) and SOS/OOS (Zhang et al., 2009). A molecular compound formation is based on a possibility to achieve specific close-packing arrangements of the molecules in the solid state. The occurrence of

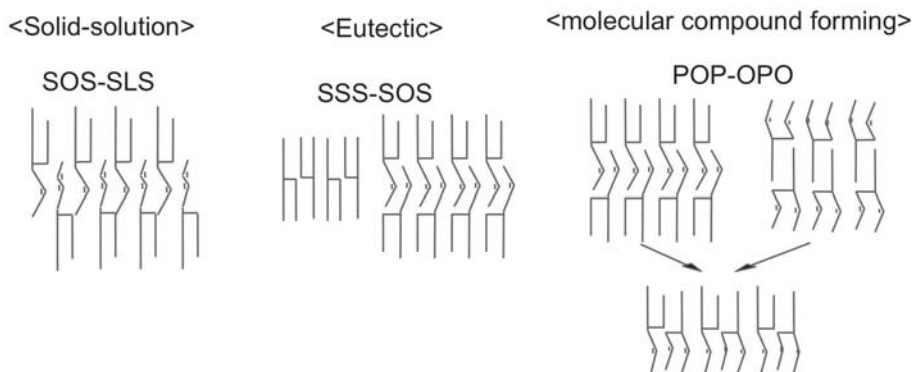


Fig. 6.5. Structure models of mixing behavior of triacylglycerols. S: stearoyl chain, P: palmitoyl chain, O: oleoyl chain and L: linoleoyl chain.

the molecular compound formation in particular combinations of TAGs has been observed in SOS/OSO (Koyano et al., 1991), SOS/SSO (Engstrom, 1992, Takeuchi et al., 2002a), POP/OPO (Minato et al., 1997b, 1997c, Ikeda et al., 2010) and POP/PPO (Minato et al., 1997a). The presence of oleic acids moiety is commonly observed in the TAGs forming molecular compound.

Molecular Interactions and Mixing Phase Behavior

The variety of the exhibition of the mixing behavior of different types of saturated-unsaturated mixed acid TAGs can partly be understood by taking into account of the molecular interactions through acyl chains and glycerol conformation. In particular, we should consider the contribution of the glycerol groups when we explain the formation of eutectic phase in the mixtures SOS/OOS and POP/OOP. This is because, the mixtures of SOS/OSO and POP/OPO form the molecular compound crystals, as depicted in Fig. 6.5.

In general, the molecular interactions which influence the stabilization of the crystal structures of TAGs containing saturated and unsaturated fatty acid chains are aliphatic chain packing, glycerol conformation, and methyl end stacking (Sato et al., 1999). The aliphatic chain packing results from the molecular interactions between saturated and unsaturated fatty acid chains, determining the subcell structures, olefinic conformations, and chain-length structures. The glycerol conformation among the glycerol groups determines the overall configuration of the TAG molecules; methyl-end stacking may play an important role in organizing the chain inclination and chain-length structure.

The formation of a double-chain-length structure in the molecular compounds of POP/OPO and POP/PPO may be due to combined effects of the above interactions, and the inability to form a molecular compound in the POP/OOP mixture can be explained by the same consideration. Therefore, we first discuss the glycerol conformations observed in the stable polymorphs of representative TAG crystals.

Fig. 6.6(a) illustrates the different types of glycerol conformations of two asymmetric units of TAG molecules present in a unit cell of the most stable forms of the TAGs, whose atomic-level crystal structures were determined by X-ray methods. We symbolized the glycerol conformation using the direction of the glycerol group indicated by an arrow, which is defined as the direction between the middle point of two glycerol carbons at the first and third positions and a glycerol carbon at the second position.

The directions of the glycerol groups of neighboring molecules in the β form of tricaproyl-glycerol (CCC) lay parallel to the chain axis with the opposite turn (Jensen & Mabis, 1963, 1966). The same arrangements were observed in the asymmetric units of the β'_2 form of 1,2-dipalmitoyl-3-myristoyl-sn-glycerol (PPM), although there were two combinations in the directions of the glycerol groups with an opposite turn and parallel turn (Sato et al., 2001). The directions of the glycerol groups of

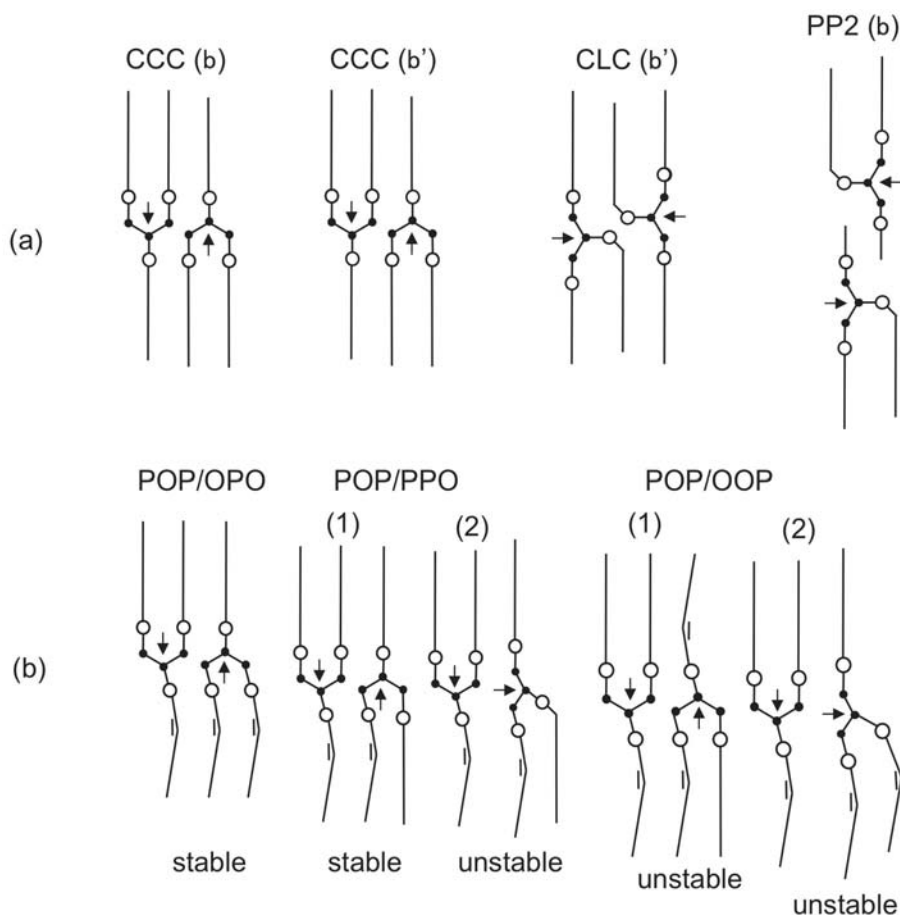


Fig. 6.6. Structure models of acyl chain interactions and glycerol group interactions in single crystals and binary mixtures.

two TAGs in the β' form of 1,3-dicaproyl-*sn*-2-lauroylglycerol (CLC) were parallel with an opposite turn, both making a right angle with respect to the chain axis (van Langevelde et al., 2000). The same arrangements were observed in the β form of 1,2-dipalmitoyl-3-acetyl-*sn*-glycerol (PP2) (Goto et al., 1992).

The most important property that was commonly observed in the four TAG crystals was the lack of arrangement of unparallel directions of adjacent glycerol groups making right angles to each other. This indicates that such an arrangement must cause instability of glycerol conformations.

Fig. 6.6(b) illustrates possible glycerol conformations in the molecular compound crystals of mixtures of POP/OPO, POP/PPO, and POP/OOP with double-chain-length structures. The neighboring glycerol groups in a parallel arrangement were directed along the chain axis with an opposite turn in the POP/OPO mixture. This glycerol conformation enables the oleoyl and palmitoyl chains to make separate leaflets with stabilized aliphatic interactions, which may be destabilized when the oleoyl and palmitoyl chains are placed in the same leaflet owing to steric hindrance. This structure may be revealed in molecular compound crystals in the metastable and stable states of POP/OPO. It is inevitable in POP/PPO mixtures that the palmitoyl and oleoyl chains will be located in one leaflet in addition to being present in another leaflet composed of all the palmitoyl chains in the double-chain-length structure.

Two situations may occur; in case 1, the adjacent glycerol groups would be directed along the chain axis with an opposite turn, similar to those in the POP/OPO mixture. In case 2, the directions of adjacent glycerol groups would make right angles to each other. Destabilization in the aliphatic chain packing may be caused in both cases by coexistence of the oleoyl and palmitoyl chains in the two leaflets. However, destabilization may be more enhanced in the structures in case 2, in which the glycerol conformation may be destabilized due to the unparallel directions of the glycerol groups. Furthermore, the methyl end stacking may also be destabilized due to unequal chain lengths of the neighboring molecules of POP and PPO, which would again be caused by the unparallel directions of the glycerol groups. Therefore, the molecular compound structure of the POP/PPO mixture observed in the metastable and most stable states [8] may be that of the structural model described in case 1.

We have considered two cases for POP and OOP mixtures. The model of case 1 assumes a parallel direction for the glycerol groups, which may be more stabilized than the unparallel direction in case 2. However, the structural model of case 1 causes destabilization of the acyl chain packing because of the coexistence of oleoyl and palmitoyl chains in the two leaflets. This situation differs significantly from the structural model of case 1 of the POP/PPO mixture, in which the coexistence of oleoyl and palmitoyl chains are limited in either leaflet.

An alternative model for POP/OOP is described for case 2, in which the oleoyl and palmitoyl chains are separately placed in their own leaflets. This structure was postulated by Moran (1963). However, this model must cause additional destabilization of the glycerol conformation and unequal chain lengths of the neighboring molecules of POP and OOP, as discussed for case 2 of the POP/PPO mixture.

Consequently, neither of the two possible models of the molecular compound structure for a POP/OOP mixture may alleviate destabilization of the acyl chain packing, glycerol conformation, and methyl end stacking. Therefore, no molecular compound has been observed in either the metastable or stable states in the POP/OOP mixture, as concluded in the present study.

Polymorphism of Cocoa Butter and Its Component TAGs

So far, the polymorphism of cocoa butter has been discussed by many researchers as summarized in a monograph (Timms, 2003). To avoid unnecessary repetition, we very briefly describe the polymorphism of cocoa butter and compare it with the polymorphism of POP, POS, and SOS.

Cocoa butter crystals exhibit several polymorphic forms. The nomenclature of cocoa butter polymorphism varies from one researcher to others, but Table 6-A compares two representative nomenclatures given by Vaeck (1960) and Wille & Lutton (1966). Fig. 6.7 shows wide-angle X-ray diffraction (WAXD) patterns of the six forms of cocoa butter, whose nomenclature is based on Wille and Lutton's. Despite of the fact that cocoa butter is composed of various TAGs, each polymorph of cocoa butter exhibits a unique melting point and WAXD pattern. This indicates that cooperative interactions of the three major TAGs of cocoa butter, SOSO, POS and POP may occur. We assume that the key polymorphic structure underlying the molecular interactions of the three TAGs is the polymorphism of a series of Sat-O-Sat TAGs, in which saturated fatty acid moieties are placed at the 1st and 3rd carbons and oleic acid is placed at the 2nd carbon of a glycerol group (Sato & Koyano, 2001, Sato & Ueno, 2005).

Fig. 6.3(a) illustrates the structure model of the five polymorphic forms of SOS (Sato et al., 1989). Taking into account of XRD (Sato et al., 1989, Mykhaylyk & Hamley, 2004, Mykhaylyk et al., 2004, 2007) and spectroscopic studies such as FT-IR methods (Yano et al., 1993, Yano & Sato, 1999, Yano et al., 1999), we summarize the details of each polymorph in the following.

α form: Melting point (T_m) is 23.5°C. It is of DCL structure as verified by long spacing value of 4.83 nm, indicating the coexistence of the stearyl and oleoyl chains in the same leaflet. The hexagonal subcell having a single WAXD short spacing value

Table 6-A. Cocoa butter polymorphs based on the definition of Vaeck (1960) and Wille and Lutton (1966) (Melting points refer to cocoa butter produced in West Africa).

Vaeck	Wille and Lutton	Melting points (°C)	Subcell structure	Chain length structure
γ	I	17.3	n.d*	double
α	II	23.3	H	double
β'	III	25.5	$O_{\perp}$	double
β'	IV	27.5	$O_{\perp}$	double
β	V	33.8	$T_{//}$	triple
β	VI	36.3	$T_{//}$	triple

* not determined

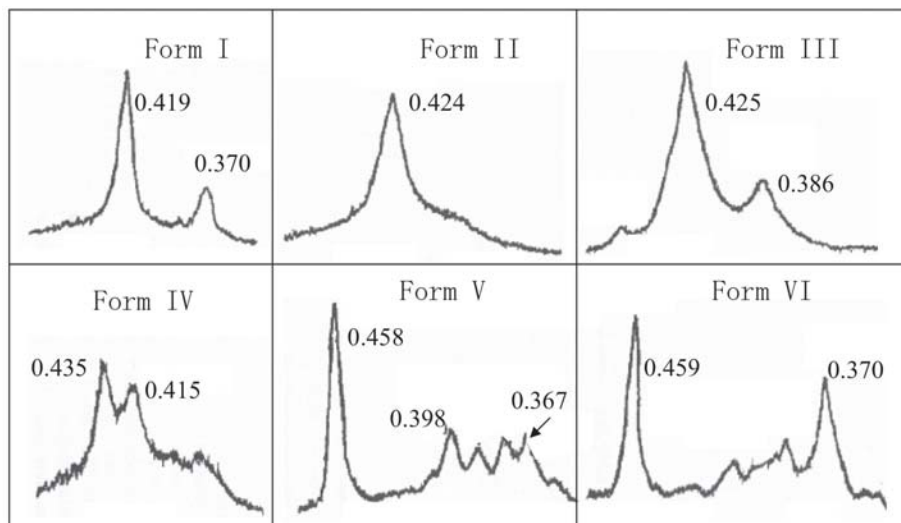


Fig. 6.7. Wide-angle X-ray diffraction patterns (short spacing) of six polymorphs of cocoa butter.

of 0.42 nm is revealed. This form was observed in POS (Arishima et al., 1991, Rousset & Lappaz, 1997) and POP (Sato et al, 1989), both are in the DCL structure. The WAXD pattern of α form is identical to that of Form II of cocoa butter.

γ form: $T_m = 35.4^\circ\text{C}$. The long spacing value of 7.05 nm represents the TCL structure, in which the stearyl and oleoyl leaflets are separated as a result of chain sorting during the $\alpha \rightarrow \gamma$ transformation. The subcell structures of the stearyl and oleoyl leaflet can therefore be different in each leaflet. The stearyl leaflet assumes specific parallel packing and the oleoyl leaflet may retain a hexagonal subcell structure. The long chains are arranged almost normal to the lamellar interface. This form in the TCL structure was observed in POP (Sato et al, 1989). In cocoa butter, there is no form showing the same WAXD pattern as that of γ form of SOS.

β' form: $T_m = 36.5^\circ\text{C}$. The long spacing value of 7.00 nm represents the TCL structure, in which the stearyl leaflet assumes an $O_\perp$ subcell, whereas hexagonal subcell was retained by the oleoyl leaflet. In the case of POP, however, two β' forms having the DCL structure were observed, and another form having the TCL structure, named δ , was observed. The three forms, two β' and δ of POP, exhibited very similar WAXD patterns, indicating the presence of $O_\perp$ subcell structure.

Two β forms: $T_m = 41.0^\circ\text{C}$ (β_2) and 43.0°C (β_1). The long spacing values are 6.75 nm (β_2) and 6.60 nm (β_1), indicating a very inclined chain arrangement against the lamellae interface, compared to the other forms. The WAXD patterns of β_2 and β_1 forms are quite similar to those of Form V and Form VI of cocoa butter. The subcell structure is $T_{//}$ for both the stearyl and oleoyl leaflets in β_1 . There is subtle difference

in the subcell structure between β_1 and β_2 , yet no convincing data are available. Van Mechelen et al. (2006 a and b) reported the different lamellae arrangement of the TCL structures between β_1 and β_2 . The form β_2 is formed by solvent crystallization at a rapid rate of cooling, whereas the β_1 form crystallized at a very slow rate of cooling (Arishima & Sato, 1989). The two β forms were observed in POP, whereas POS showed one β form, whose WAXD pattern is similar to β_1 of SOS.

In addition to the above five forms, there is another low-temperature polymorph called sub- α (Yano et al., 1999), whose WAXD pattern is quite similar to that of Form I of cocoa butter.

To summarize the comparison of the polymorphic behavior of cocoa butter and its component TAGs, we assume that the forms of I, II, V and VI of cocoa butter may correspond to sub- α , α , β_2 and β_1 of SOS and POP, respectively, based on the WAXD patterns and the chain length structure. Unclear correspondence is about Form III and IV of cocoa butter, whose WAXD patterns are similar to β' of SOS, but chain length structure is different (TCL for β' of SOS, and DCL for III and IV of cocoa butter). As the β' forms of POP are of DCL structure, the contribution of POP may be influencing in the structure formation of the meta-stable forms of III and IV of cocoa butter.

Mixing Behavior of POP, POS, and SOS

Several researchers have studied the phase behavior of binary and ternary mixtures of cocoa butter-component TAGs and related fats. As for the binary mixtures, Rousset et al. studied phase behavior of POS/SOS binary mixtures in the stable and meta-stable forms (Rousset et al., 1998). For this system, the most stable β phase formed solid-solution with complete miscibility. As to the POP/POS and POP/SOS mixtures, it was reported to be eutectic (Timms, 1984).

As for ternary POP/POS/SOS mixture systems, Koyano et al. (1993) studied the phase behavior of the crystals grown from acetone solution. This study dealt with the most stable β phase because chocolate needs the β form (Form V) of cocoa butter crystals. In this study, they found that ternary phase is composed of solid-solution and eutectic phases depending on the proportion of POP/POS/SOS, and a solid-solution phase was observed at the ratio of POP/POS/SOS corresponding to cocoa butter. The ternary iso-solids diagrams of POP/POS/SOS mixtures were also reviewed (Timms, 2003). However, precise analysis about the regions of solid-solution or eutectic phase in the POP/POS/SOS ternary mixtures has not completed.

Considering quite diversified phase behavior of the mixture systems of cocoa butter-component and related TAGs, we may conclude that the molecular interactions acting for stabilization of binary mixtures can be categorized in the following: (i) aliphatic chain packing, (ii) glycerol conformation, and (iii) methyl end stacking. The combination of the three interactions may explain the binary and ternary phase behavior of the cocoa butter-component TAGs, in which saturated (palmitic and

stearic) and unsaturated (oleic) fatty acid chains are present in the *sn*-1, 3 positions and *sn*-2 positions, respectively.

In this section, we discuss the melting behavior of the binary mixtures of SOS/POS, POP/SOS, and ternary mixtures of POP/POS/SOS in their most stable states.

SOS/POS Mixture

Rousset et al. (1998) studied the binary system of SOS and POP by using DSC and polarized light microscopy. They examined the phase behavior of various polymorphic forms of sub- α , intermediate forms and stable β form. As for the stable state of β forms, the phase behavior is illustrated in Fig. 6.8. The results indicate that the β phase seems to be a solid solution with complete miscibility at all temperatures.

SOS/POP Mixture

We constructed the phase diagram of the mixture of SOS/POP by measuring the melting points and polymorphic structures for the mixture samples, which were subjected to the following two-stage thermal incubation of the crystallized mixture samples to reach the equilibrium: (1) the crystallized samples were held at 27°C for 1 day, (2) incubation at 30°C was carried out over 180 days to stabilize the mixtures. The heating rate was 0.5°C/min or 0.2°C/min for DSC and synchrotron radiation X-ray diffraction (SR-XRD).

Fig. 6.9 shows the phase diagram of the most stable β form of SOS/POP mixtures. In the concentration ratio range of SOS/POP from 0/100 to 50/50, single melting peak was observed by DSC. This indicates the miscible nature of this mixture in the concentration range of SOS up to 50%. However, separated eutectic property is revealed when the concentration of SOS exceeded over 50%. Fig. 6.10 shows the DSC heating thermopeaks of the SOS/POP mixtures examined at 5% concentration intervals, which were employed to construct the phase diagram shown in Fig. 6.9.

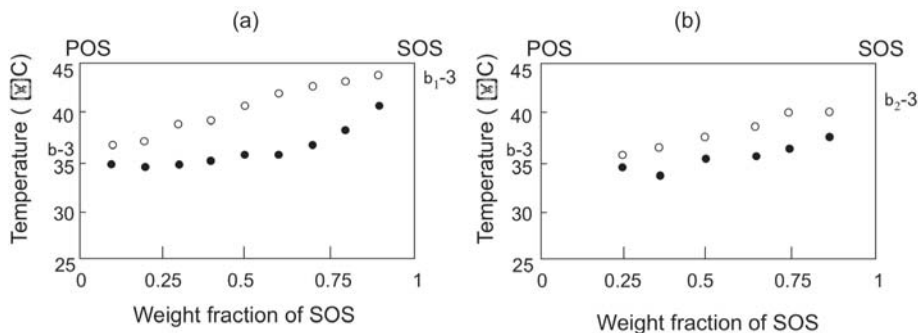


Fig. 6.8. Phase behavior of binary mixture of SOS and POS. Closed and open circles correspond to solidus line (crystallization temperature) and liquidus line (melting temperature).

Fig. 6.9. Phase diagrams of stable forms of SOS/POP mixtures. bSS means b form in a solid-solution phase of POP and SOS.

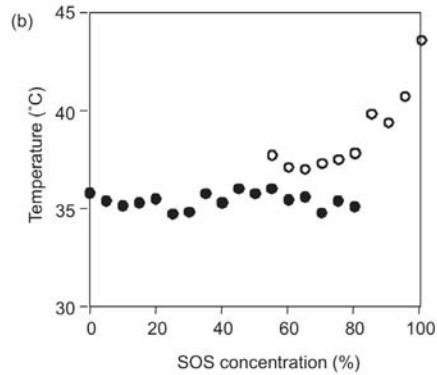
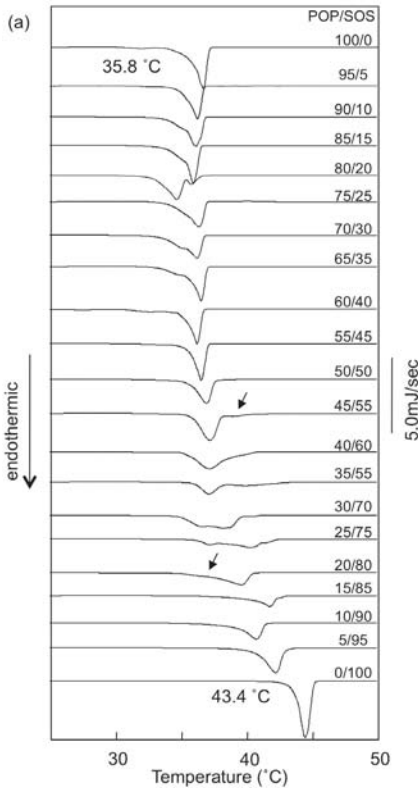
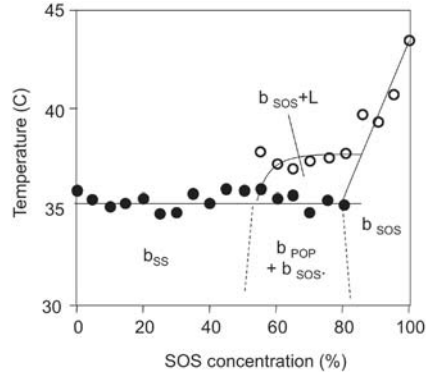


Fig. 6.10. (a) DSC heating thermograms of the most stable forms of POP/SOS mixtures at a heating rate of 0.5 °C/min. (b) Onset temperatures of lower endothermic peaks (filled circles) and higher peaks (open circle).

In order to show the structural changes of the mixtures in the immiscible region above the SOS concentration of 50% more precisely, Fig. 6.11 presents the results of the simultaneous SR-XRD and DSC heating measurements for the most stable form of the SOS/POP = 70/30 mixture. At 30°C, two (002) reflection peaks of 3.26 nm and 3.18 nm in the small angle X-ray diffraction (SAXD) patterns, which correspond to the long spacing values of 6.52 nm and 6.36 nm, were clearly observed. During the heating process, the SAXD peak of 3.18 nm disappeared at 38°C in the first, then the SAXD peak of 3.26 nm disappeared at 40°C. Correspondingly, the DSC measurement showed multiple melting peaks which were separated into three independent peaks. It is clear that $T_m = 34.8^\circ\text{C}$ and $T_m = 37.3^\circ\text{C}$ correspond to the melting of crystal fraction having the long spacing values of 6.36 nm and 6.52 nm, respectively. The WAXD patterns of the two crystal fractions are β_2 form. The very small third peak at 40.0°C corresponds to the melting of the most stable β form of the SOS-rich fraction whose SAXD peak is very weak. Fig. 6.11(c) shows temperature variation of the relative SAXD peak intensity of the POP-rich and SOS-rich crystal fractions. It is clear that $T_m = 40.0^\circ\text{C}$ corresponds to the melting of the most stable form of the SOS-rich fraction, most probably β_1 form.

The same results as those shown in Fig. 6.9 were observed in the mixtures at the SOS concentration ranges of 55–80%. In the range of SOS > 80%, no separation into

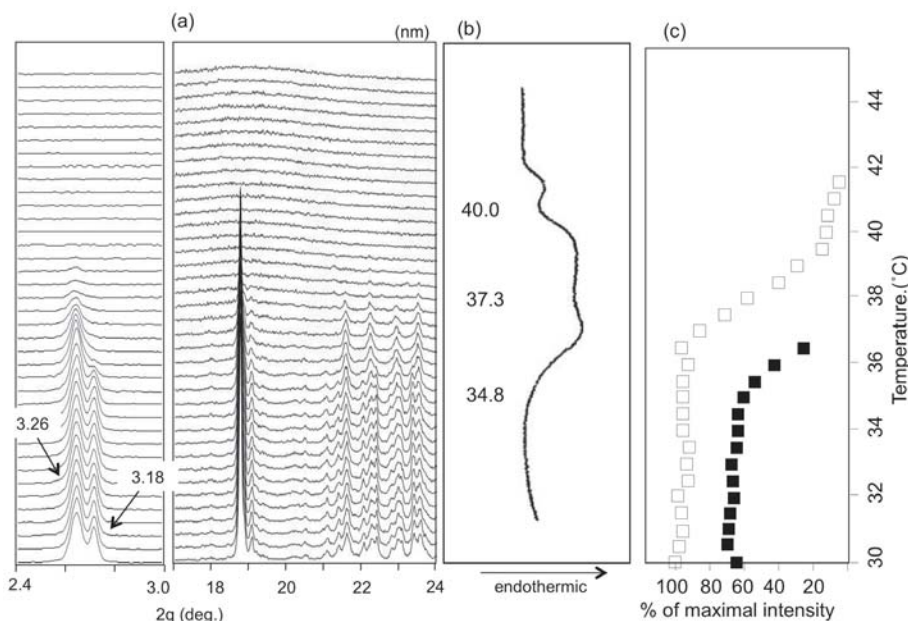


Fig. 6.11. Simultaneous (a) SR-XRD and (b) DSC patterns of the most stable form of the POP/SOS = 30:70 mixture at a heating rate of $0.5^\circ\text{C}/\text{min}$. (c) Relative intensities of SR-SAXD peak of 3.26 nm (open square) and 3.18 nm (filled square) respectively,

SOS and POP fraction was observed. This means that POP molecules are incorporated into the SOS crystal phases.

From these results, we may conclude that the mixing behavior of SOS/POP is summarized in the following.

- (1) The mixture exhibits the solid-solution type properties when the SOS concentration is lower than 50%.
- (2) Eutectic mixture is revealed at the SOS concentration ranges of 55–80%.
- (3) POP is incorporated into SOS crystal phase when the POP concentration becomes lower than 20%.

SOS/POS/POP Mixture

Fig. 6.12 summarizes the phase diagram of the ternary mixtures of POP/POS/SOS at the concentration ratios near the point of cocoa butter having the general ratio of POP/POS/SOS = 22/46/32 (black circle in Fig. 6.12). The ratio of cocoa butter is placed in the region of solid-solution mixture, which has only one T_m and one long spacing value. Solid-solution regions were obtained below 30% of SOS concentration, whereas eutectic phases were observed above 35% of SOS and 15% of POP concentration. It is clear that the solid-solution phases change to the eutectic phases with increasing the SOS concentration except the ratio of 10% of POP concentration.

To confirm the phase diagram shown in Fig. 6.10, two precise data will be presented. Fig. 6.13 presents the results of the simultaneous SR-XRD and DSC heating measurements for the most stable form of the POP/POS/SOS = 22/46/32. At 30°C, one (002) reflection peaks of 3.17 nm, which corresponds to the long spacing value of 6.34 nm was observed. The WAXD patterns are β_1 form. During the heating pro-

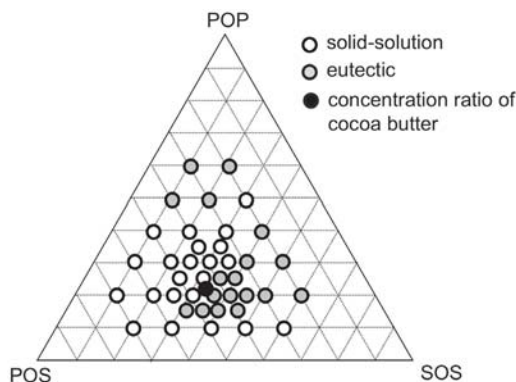


Fig. 6.12. Phase diagram of the most stable b form in the ternary mixture of POP/POS/SOS.

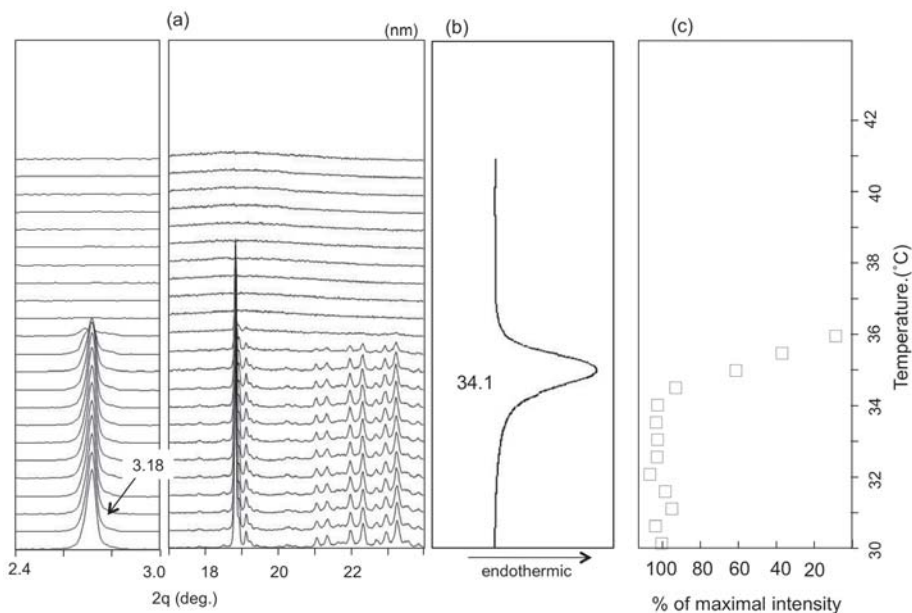


Fig. 6.13. Simultaneous (a) SR-XRD and (b) DSC patterns of the most stable form of the POP/POS/SOS = 22:46:32 mixture at a heating rate of 0.5°C/min. (c) Relative intensities of the SR-SAXD peak of 3.17 nm.

cess, the SAXD peak of 3.17 nm disappeared at 36.5°C. Correspondingly, the DSC measurement showed 34.1°C of single sharp melting behavior. Fig. 6.13(c) shows the relative intensities of the SR-SAXD of 3.17 nm as a function of temperature. It is obvious that this crystal melt smoothly around 34.5°C which directly corresponds to DSC melting behavior.

In order to bring out the structural changes of the mixtures in the immiscible region above the SOS concentration of 50% more precisely, Fig. 6.14 presents the results of the simultaneous SR-XRD and DSC heating measurements for the most stable form of the POP/POS/SOS = 30/20/50 mixture. At 30°C, large (002) reflection peaks of 3.16 nm and shoulder of 3.24 nm, which correspond to the long spacing values of 6.32 nm and 6.48 nm, were observed. During the heating process, the SAXD peak of 3.16 nm disappeared at 38.5°C in the first, then the SAXD peak of 3.24 nm disappeared at 40.5°C. Correspondingly, the DSC measurement showed double melting peaks which were separated into two peaks. It is clear that $T_m = 34.5^\circ\text{C}$ and $T_m = 37.5^\circ\text{C}$ correspond to the melting of crystal fractions having the long spacing values of 6.32 nm and 6.48 nm, respectively. We think that the crystal fractions having the long spacing value of 6.32 nm with $T_m = 34.5^\circ\text{C}$, and the long spacing values of 6.48 nm with $T_m = 37.5^\circ\text{C}$ may comprise of solid-solution TAGs

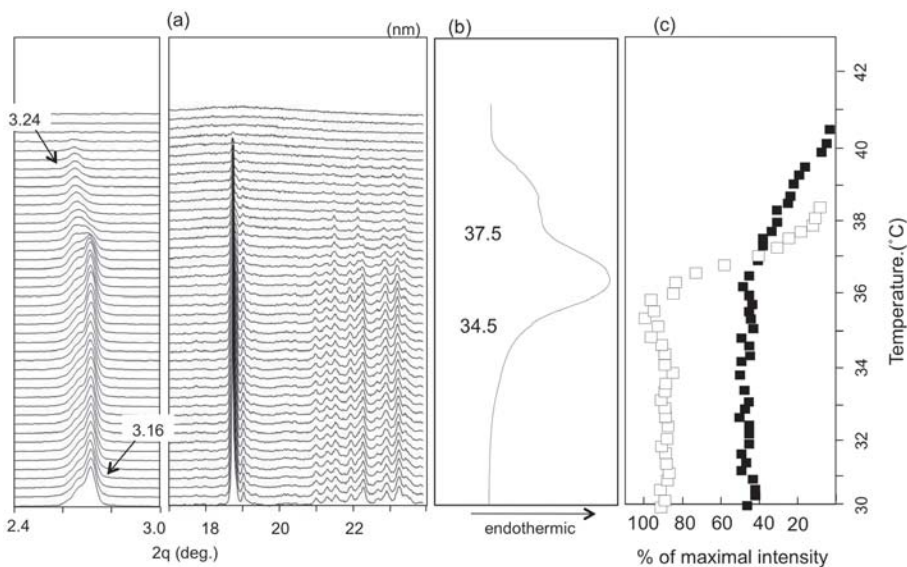


Fig. 6.14. Simultaneous (a) SR-XRD and (b) DSC patterns of the most stable form of the POP/POS/SOS = 30:20:50 mixture at a heating rate of 0.5°C/min. (c) Relative intensities of the SR-SAXD of 3.16 nm (open square) and 3.24 nm (filled square) respectively.

and SOS-rich TAGs, respectively. The WAXD patterns are β_1 form. Fig. 6.14(c) shows the relative intensities of the SR-SAXD of 3.16 nm fractions and 3.24 nm fractions.

Conclusions

This chapter discussed the polymorphic structures of the TAGs constructing cocoa butter and cocoa butter itself, and the mixing phase behavior of principal TAGs whose molecular structures are similar to cocoa butter-constructing TAGs. Particular attention was paid to the polymorphism of saturated-unsaturated mixed acid TAGs, since cocoa butter is composed of such mixed-acid TAGs, in which saturated acids (stearic and palmitic) are placed at the 1st and 3rd carbon positions and oleic acid is placed at the 2nd carbon position. Such types of TAGs are so unique that materials design technology for cocoa butter equivalents (CBE) and cocoa butter improvers (CBI) has worked so much on the polymorphism and phase behavior of TAGs and cocoa butter.

In this chapter, we have concluded that the polymorphism of cocoa butter is basically the same as that of its component TAGs, SOS, POS, and POP. In addition, the mixing behavior of SOS, POS and POP at the same ratio as that in cocoa butter

exhibited solid-solution type. This indicates that the polymorphism of cocoa butter can be interpreted based on that of its component TAGs. However, some details are contradictory. For example, it was rather difficult to explain the metastable forms of cocoa butter, Form II and Form IV, based on the polymorphic structures of SOS. We assume that such discrepancy may come from the mixing behavior of the three major TAGs in the metastable states, which were not fully considered in this chapter. We may expect that future research may give defined answers to this.

Atomic-level understanding of the polymorphic structures have been attempted by using powder X-ray diffraction and molecular modeling (van Mechelen et al., 2006a and b). Despite of its interesting information, there still remains a lot of uncertainty in the details of molecular structures in particular the differences between Form V and Form VI. We again must rely on future work to solve this problem.

References

- Arishima, T.; Sagi, N.; Mori, H.; Sato, K. Polymorphism of POS. I. Occurrence and polymorphic transformation. *J. Am. Oil Chem. Soc.* 1991, 68, 710–715.
- Arishima, T.; Sato, K. Polymorphism of POP and SOS. III. Solvent crystallization of #2 and #1 polymorphs, *J. Am. Oil Chem. Soc.* 1989, 66, 1614–1617.
- Bayes-García, L.; Calvet, T.; Cuevas-Diarte, M.; Ueno, S.; Sato, K. In situ synchrotron radiation X-ray diffraction study of crystallization kinetics of polymorphs of 1,3-dioleoyl-2-palmitoyl glycerol (OPO). *Cryst. Eng. Comm.* 2011, 13, 3592–3599.
- Cebula, D.; Smith, K. Differential scanning calorimetry of confectionery fats. Pure triglycerides: effects of cooling and heating rate variation, *J. Am. Oil Chem. Soc.* 1991, 68, 591–595.
- Chaiseri, S.; Dimick, P.S. Lipid and hardness characteristics of cocoa butters from different geographic regions. *J. Am. Oil Chem. Soc.* 1989, 66, 1771–1776.
- Engstrom, L. Triglycerides systems forming molecular compounds. *J. Fat Sci. Technol.* 1992, 94, 173–181.
- Goto M.; Kodali D.R.; Small D.M.; Honda K.; Kozawa K.; Uchida T. Single crystal structure of a mixed-chain triacylglycerol: 1,2-dipalmitoyl 1-3-acetyl-sn-glycerol. *Proc. Natl. Acad. Sci. USA* 1992, 89, 8083–8086.
- Ikeda, E.; Ueno, S.; Miyamoto, R.; Sato, K. Phase behavior of a binary mixture of 1,3-dipalmitoyl-2-oleoyl-sn-glycerol and 1,3-dioleoyl-2-palmitoyl-sn-glycerol in n-dodecane solution. *J. Phys. Chem. B* 2010, 114, 10961–10969.
- Jensen L.H.; Mabis A.J. Crystal structure of β -tricaprin. *Nature* 1963, 197, 681–682.
- Jensen L.H.; Mabis A.J. Refinement of the structure of β -tricaprin. *Acta Cryst.* 1966, 22, 770–781.
- Kaneko, F.; Yano, J.; Sato, K. Diversity in the fatty-acid conformation and chain packing of cis-unsaturated lipids. *Curr. Opin. Struct. Biol.* 1998, 8, 417–425.
- Kellens, M.; Meeussen, W.; Hammersley, A.; Reynaers, H. Synchrotron radiation investigation of the polymorphic transitions. Part 2: Polymorphic study of 50:50 mixture of tripalmitin and tristearin during crystallization and melting. *Chem. Phys. Lipids*, 1991, 58, 145–158.

- Koyano, T.; Hachiya, I.; Sato, K. Phase behavior of mixed systems of SOS and OSO. *J. Phys. Chem.* 1991, 96, 716–718.
- Koyano, T.; Kato, Y.; Hachiya, I.; Umemura, R.; Tamura, K.; Taguchi, N. Crystallization behavior of ternary mixtures of POP/POS/SOS. *J. Jpn. Oil Chem. Soc. (Yukagaku)* 1993, 42, 453–457.
- Loisel, C.; Keller, C.G.; Lecq, G.; Bourgaux, C.; Ollivon, M. Phase transformations and polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* 1998a, 75, 425–439.
- Loisel, C.; Lecq, G.; Keller, G.; Ollivon, M. Dynamic crystallization of dark chocolate as affected by temperature and lipid additives. *J. Food Sci.* 1998b, 63, 73–79.
- Minato, A.; Ueno, S.; Yano, J.; Wang, Z. H.; Seto, H.; Amemiya, Y.; Sato, K. Synchrotron radiation X-ray diffraction study on phase behavior of PPP–POP binary mixtures. *J. Am. Oil Chem. Soc.* 1996, 73, 1567–1572.
- Minato, A.; Yano, J.; Ueno, S.; Smith, K.; Sato, K. FT–IR study on microscopic structures and conformations of POP–PPO and POP–OPO molecular compounds. *Chem. Phys. Lipids*, 1997a, 88, 63–71.
- Minato, A.; Ueno, S.; Smith, K.; Amemiya, Y.; Sato, K. Thermodynamic and kinetic study on phase behavior of binary mixtures of POP and PPO forming molecular compound systems. *J. Phys. Chem.* 1997b, B 101, 3498–3505.
- Minato, A.; Ueno, S.; Yano, J.; Smith, K.; Seto, H.; Amemiya, Y.; Sato, K. Thermodynamic and structural properties of sn-1,3-dipalmitoyl-2-oleoylglycerol and sn-1,3-dioleoyl-2-palmitoylglycerol binary mixtures examined with synchrotron radiation X-ray diffraction. *J. Am. Oil Chem. Soc.* 1997c, 74, 1213–1220.
- Moran, D.P. J. Phase behavior of some palmito-oleo triglyceride systems. *J. Appl. Chem.* 1963, 13, 91–100.
- Mykhaylyk, O.O.; Casteletto, V.; Hamley, I. W.; Povey, M. J. W. Structure and transformation of low-temperature phases of 1,3-distearoyl-2-oleoyl-sn-glycerol. *Eur. J. Lipid Sci. Technol.* 2004, 106, 319–324.
- Mykhaylyk, O.O.; Hamley, I.W. The packing of triacylglycerols from SXAS measurements: Application to the structure of 1,3-distearoyl-2-oleoyl-sn-glycerol crystal phases. *J. Phys. Chem.* 2004, B108, 8069–8083.
- Mykhaylyk, O.O.; Smith, K.W.; Martin, C.M.; Ryan, A.J. Structural models of metastable phases occurring during the crystallization process of saturated/unsaturated triacylglycerols. *J. Appl. Cryst.* 2007, 40, s297–302.
- Rousset, P. Modeling crystallization kinetics of triacylglycerols. In *Physical Properties of Lipids*; Marangoni, A.G., and Narine, S.S., Eds. Marcel Dekker, Inc.: New York, 2002; pp. 1–36.
- Rousset, P.; Rappaz, M. α -Melt-mediated crystallization of POS. *J. Am. Oil Chem. Soc.* 1997, 74, 1693–1697.
- Rousset, P.; Rappaz, M.; Minner, E. Polymorphism and solidification kinetics of the binary system POS–SOS. *J. Am. Oil Chem. Soc.* 1998, 75, 857–864.
- Sato, K.; Arishima, T.; Wang, Z.H.; Ojima, K.; Sagi, N.; Mori, H. Polymorphism of POP and SOS. I. Occurrence and polymorphic transformation. *J. Am. Oil Chem. Soc.* 1989, 66, 664–674.

- Sato, K.; Goto, M.; Yano, J.; Honda, K.; Kodali, D.R.; Small, D.M. Atomic resolution structure analysis of β' polymorph crystal of a triacylglycerol: 1,2-dipalmitoyl-3-myristoyl-sn-glycerol. *J. Lipid Res.* 2001, 42, 338–345.
- Sato, K.; Koyano, T. Crystallization properties of cocoa butter. In *Crystallization Processes in Fats and Lipid Systems*; Garti, N., and Sato, K., Eds. Marcel Dekker: New York, 2001, pp. 429–456.
- Sato, K.; Ueno, S. Molecular interactions and phase behavior of polymorphic fats. In *Crystallization Processes in Fat and Lipid Systems*; Garti, N., and Sato, K., Eds. Marcel Dekker: New York, 2005, 177–210.
- Sato, K.; Ueno, S.; Yano, J. Molecular interactions and kinetic properties of fats. *Prog. Lipid Res.* 1999, 38, 91–116.
- Takagi, T.; Ando, Y. Stereospecific analysis of monounsaturated triacylglycerols in cocoa butter. *J. Am. Oil Chem. Soc.* 1995, 72, 1203–1206.
- Takeuchi, M.; Ueno, S.; Sato, K. Crystallization kinetics of polymorphic forms of a molecular compound constructed by SOS (1,3-distearoyl-2-oleoyl-sn-glycerol) and SSO (1,2-distearoyl-3-oleoyl-rac-glycerol). *Food Res. Intern.* 2002a, 35, 919–926.
- Takeuchi, M.; Ueno, S.; Floeter, E.; Sato, K. Binary phase behavior of 1,3-distearoyl-sn-2-oleoylglycerol (SOS) and 1,3-distearoyl-sn-2-linoleoylglycerol (SLS). *J. Am. Oil Chem. Soc.* 2002b, 79, 627–632.
- Takeuchi, M.; Ueno, S.; Sato, K. Synchrotron radiation SAXS/WAXS study of polymorph dependant phase behavior of binary mixtures of saturated monoacid triacylglycerols. *Cryst Growth Des.* 2003, 3, 360–374.
- Timms, R.E. Phase behavior of fats and their mixtures. *Prog. Lipid Res.* 1984, 23, 1–38.
- Timms, R.E. *Confectionary Fats Handbook*; The Oily Press: Bridgwater, UK, 2003.
- Vaeck, S.V. Cocoa butter and fat bloom. *Manufacturing Confectioner* 1960, 40, 35–36 & 71–744.
- van Langevelde A.; van Malssen K.; Dressen R.; Goubits K.; Hollander F.; Peschar R.; Zwart P.; Schenk H. Structure of $C_nC_{n+2}C_n$ -type (n =even) β' -triacylglycerols. *Acta Crystallogr. B.* 2000, 56, 1103–1111.
- van Langevelde, A.; van Malssen, K.; Peschar, R.; Schenk, H. Effects of temperature on recrystallization behavior of cocoa butter. *J. Am. Oil Chem. Soc.* 2001, 78, 919–925.
- van Malssen, K.; Peschar, R.; Schenk, H. Real-time X-ray powder diffraction investigations on cocoa butter. I. Temperature-dependent crystallization behavior. *J. Am. Oil Chem. Soc.* 1996a, 73, 1209–1215.
- van Malssen, K.; Peschar, R.; Schenk, H. Real-time X-ray powder diffraction investigations on cocoa butter. II. The relationship between melting behavior and composition of beta-cocoa butter. *J. Am. Oil Chem. Soc.* 1996b, 73, 1217–1223.
- van Mechelen, J. B.; Peschar, R.; Schenk, H. Structures of mono-unsaturated triacylglycerols. I. The β_1 polymorph. *Acta Cryst.* 2006a, B62, 1121–1130.
- van Mechelen, J.B.; Peschar, R.; Schenk, H. Structures of mono-unsaturated triacylglycerols. II. The β_2 polymorph. *Acta Cryst.* 2006b, B 62, 1131–1138.

- Wille, R.L.; Lutton, E.S. Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* 1966, 43, 491–496.
- Yano, J.; Ueno, S.; Sato, K.; Arishima, T.; Sagi, N.; Kaneko, F.; Kobayashi, M. FT-IR study of polymorphic transformations in SOS, POP and POS. *J. Phys. Chem.* 1993, 97, 12967–12973.
- Yano, J.; Sato, K. FT-IR studies on polymorphism of fats: molecular structures and interactions. *Food Res. Int.* 1999, 32, 249–259.
- Yano, J.; Sato, K.; Kaneko, F.; Small, D.M.; Kodali, D.R. Structural analyses of polymorphic transitions of sn-1,3-distearoyl-2-oleoylglycerol (SOS) and sn-1,3-dioleoyl-2-stearoylglycerol (OSO): assessment on steric hindrance of unsaturated and saturated acyl chain interactions. *J. Lipid Res.* 1999, 40, 140–151.
- Zhang, L.; Ueno, S.; Miura, S.; Sato K. Binary phase behavior of 1, -dipalmitoyl-2-oleoyl-glycerol and 1, 2-dioleoyl-3-palmitoyl-rac-glycerols. *J. Am. Oil Chem. Soc.* 2007, 84, 219–227.
- Zhang, L.; Ueno, S.; Sato, K.; Adlof, R.O.; List, G.R. Thermal and structural properties of binary mixtures of 1, 3-distearoyl-2-oleoyl-glycerol (SOS) and 1, 2-dioleoyl-3-stearoyl-sn-glycerols (sn-OOS). *J. Therm. Anal. Calorim.* 2009, 98, 105–111.

7

Causes and Best Manufacturing Practices to Minimize Bloom in Confections

Neil R. Widlak¹ and Richard W. Hartel²

¹ADM Cocoa Division, Archer Daniels Midland Company, Milwaukee, Wisconsin, USA, and ²University of Wisconsin, Madison, Wisconsin, USA

Introduction

The Olmec civilization (1500 BC) was the first to transform the cacao bean into a form of chocolate. For a long time the cacao bean has been used as a foodstuff as well as currency. It also has had both religious and divine aspects. In the eighteenth century, the Europeans found it had some aphrodisiac properties (Coe & Coe, 1996). Although chocolate is no longer reserved for the elite, from a scientific point of view, chocolate still retains some mysteries. For example, bloom in chocolate, perhaps a less poetic notion but just as much exciting, is still somewhat of a mystery.

Fat bloom is directly related to the fat in chocolate products, either cocoa butter (CB) or vegetable oils. Its commercial impact is significant. Although records of its economic impact due to lost sales and rework are not revealed, most confectioner manufacturers and food manufacturers consider it an important quality defect with a significant impact on profits. There is definitely a commercial incentive to better control, minimize, or eliminate fat bloom in confections.

In this chapter, we intend to review the current knowledge of fat bloom, what it is, what causes it, and what manufacturers can do to avoid it. It is our intention to apply our current understanding of the chemical and physical properties of cocoa butter and confectionery fats, as presented in previous chapters, to understanding the possible mechanisms that may provide an actionable explanation of fat bloom formation.

More specifically, this chapter will attempt to identify causes and/or contributing factors for bloom from formulation/composition (interaction between different sources of fats, minor lipids, and interactions with non-lipid ingredients), processing (blending, tempering, depositing, cooling, packaging), and storage/distribution, and it will attempt to provide a bridge between research and commercial perspectives to gain an improvement in manufacturing practices and identify technical gaps that require additional research.

Definition and Characterization

A glossy to matte surface is universally accepted as an important attribute associated with quality chocolate and confectionery coatings, “glossy” most likely having a greater acceptance by consumers than matte, but matte being within consumers’ range of acceptability. The crystalline properties of the fat system directly impact structural properties that impact surface appearance. Generally, small crystals that provide a homogeneous crystalline network, or structure, impart a reflective surface to confections. A change in structure which imparts a visual change in appearance from glossy/matte to dull and rough is what is known as bloom. A change in sugar structure can also impart a bloomed appearance in confections, however this chapter will cover only bloom related to changes in structure related to fat.

Bloom can vary in appearance from dull to gray to white. Bloom may appear as spots, streaks, irregular shapes, or marbled. The bloom areas may be dark brown or light brown in addition to white. Kinta’s chapter on *The Morphology of Chocolate Fat Bloom* provides an excellent overview on characterizing and classifying different types of bloom as well as identifying their underlying causes.

In addition, Fig. 7.1 provides examples of Type 1, and Fig. 7.2 examples of Type 2 Bloom.

The products with Type 1 are compound coatings. Sample A bloom development after 1 year storage, Sample B is bloom development from scuffing. Fig. 7.2 shows a sample of cocoa liquor, or mass, cooled slowly after 1 year storage at room temperature.

While there is no data to support which bloom has the greatest commercial impact, an informal survey of industrial and retail confectioners encounter Type 1 more frequently. Type 1 bloom is generally associated or developed with incompatible fats (either from the product formula or inadvertent comingling of fats or products during processing), oil migration, mild temperature abuse during distribution, and oil migration. These factors will be discussed more thoroughly in the chapter.

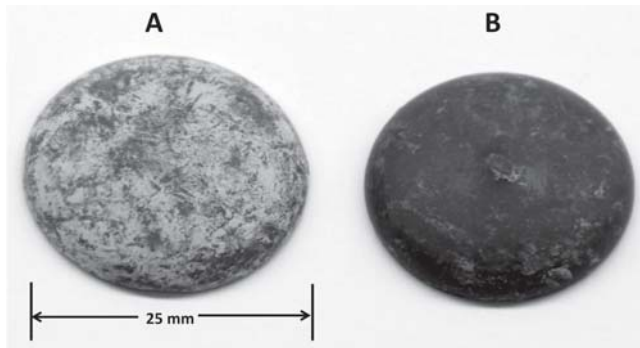


Fig. 7.1. Confectionery wafers with bloom.

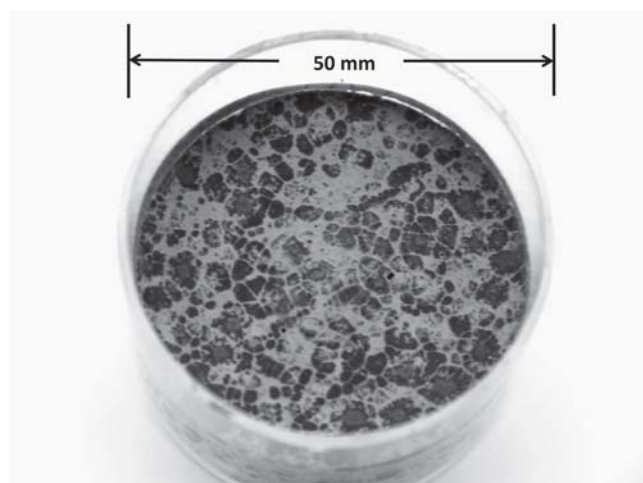


Fig. 7.2. Chocolate liquor bloom.

Generally any change in surface appearance, any change consumers believe to be unacceptable, which is due to a change in fat structure, is considered bloom.

Our analysis indicates less than 1.5% of the formula fat is in the bloomed fraction in heavily bloomed confections and chocolates, in which the confection surface is 90% covered in a Type 1 bloom (Sample A in Fig. 7.1). Very little of fat needs to be altered or participate to have a significant impact on structure and product appearance.

Type 1 bloom observed by the naked eye appears to a variation of intensity of the same event—dull surface to definite white crystalline material. However, scanning electron microscopy (SEM) reveals bloom to have many forms, generally related to the mechanism that formed the bloom conditions which is influenced by composition of the confection, processing, and/or storage conditions.

Fig. 7.3 (Lechter, 2010) is an SEM of compound coating at 300 $\times$ with no apparent bloom to the naked eye.

Fig. 7.4 (Lechter, 2010) is an SEM of a compound coating stored over a long period of time under undocumented conditions which developed a heavy bloom surface. The difference between surface structure in Figs. 7.3 and 7.4 is obvious. At higher magnification [Fig. 7.5 (Lechter, 2010)] some of the structures, observed in Fig. 7.4, resemble volcano tubes. This observation supports Kleinert's description of bloom in 1962 as a migration of liquid fat to the surface by capillary action and recrystallization.

Similar observations of this type of bloom mechanism were observed by Rousseau & Smith (2008) and Wang et al. (2010), contributing both diffusion and nan-pores, inherent to the confections microstructure, as mechanisms for transferring liquid fats to the surface.

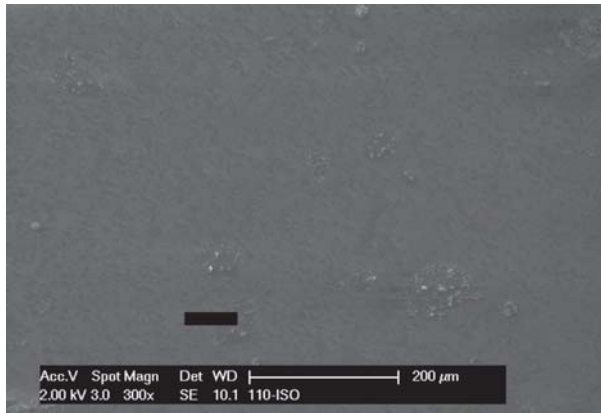


Fig 7.3. Confectionary fat with no visible bloom (Lechter, 2010).

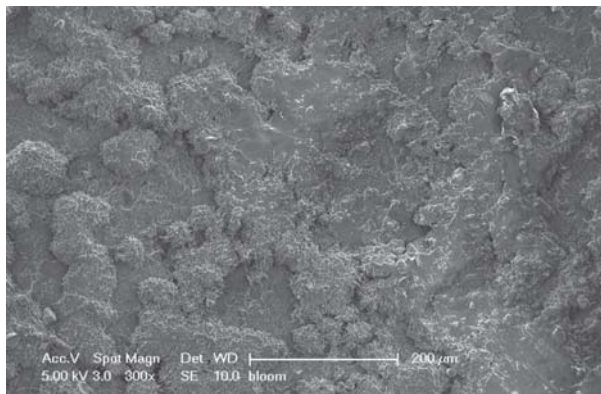


Fig. 7.4. Compound bloom (with cocoa butter present). Long storage time uncontrolled conditions. Heavy bloom on surface 300x.

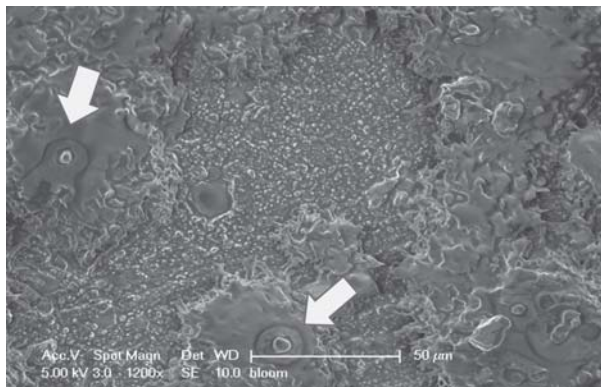


Fig. 7.5. Compound coating (with <4% cocoa butter present). Long storage time uncontrolled conditions. Heavy bloom on surface 1200x.

How and why liquid fat migrated to the surface can be explained by many factors which will be discussed later in this chapter.

The SEM of bloom on fully hydrogenated palm kernel (FHPK) is shown in Fig. 7.6 (Lechter, 2010), and bloom on fractionated palm kernel oil (FPK) is shown in Fig. 7.7 and Fig. 7.8 (Lechter, 2010).

Both products were cycled under controlled conditions between 65°F and 80°F for 1.5 months (six days at 65 and one day at 80°F). To the naked eye the bloom in all three samples, compound with cocoa, FHPK, and FPK appear the same. Under SEM magnification, the morphologies are significantly different. Since both fats were chilled and cycled under identical conditions, it may be safe to infer the surface

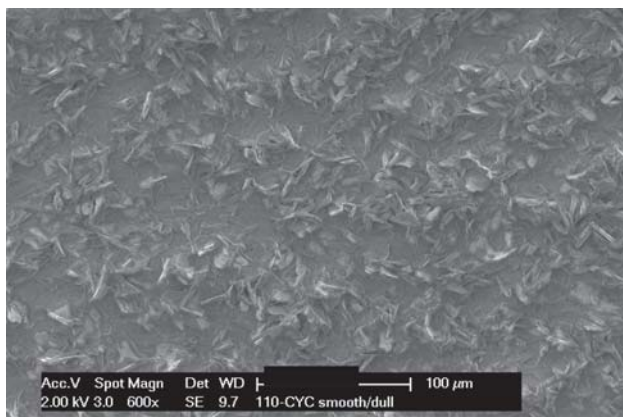


Fig. 7.6 Fully hydrogenated palm kernel oil following 2 months cycling. Visible bloom, smooth to dull surface 600x.

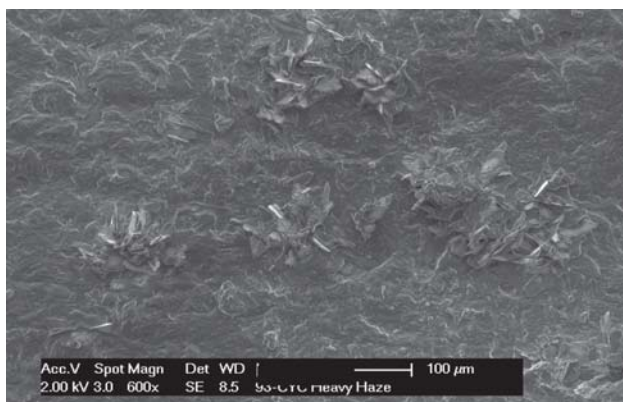


Fig. 7.7. Fractionated palm kernel oil following 2 months cycling. Visible bloom, heavy haze on surface 600x.

structures developed are characteristic of these fats. In Fig. 7.9 (Lechter, 2010) the temperature cycle is boxed over the SFC of each fat.

While neither fat completely melted during temperature cycling, a significant level of partial melt most likely occurred, allowing sufficient liquid to accumulate at the surface and recrystallize upon re-cooling. Through each cycle the TAG population of

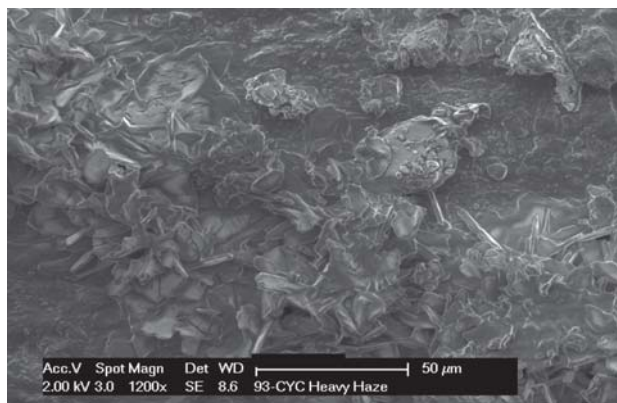


Fig. 7.8. Fractionated palm kernel oil following 2 month cycling conditions. Visible bloom heavy haze on surface 1200 $\times$.

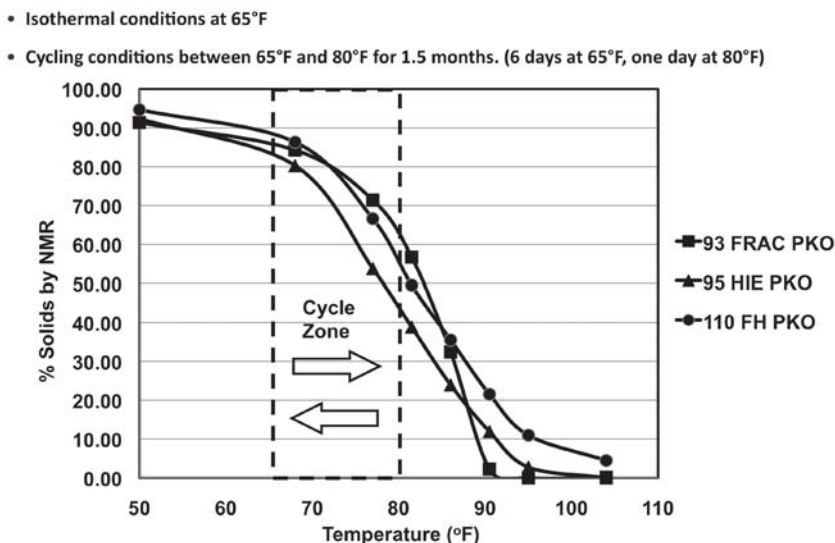


Fig. 7.9. Storage conditions and cycling conditions.

the melted phase changes based on the TAG population available to participate in the partial melt. Likewise, the TAG population that is recrystallized on the surface also based on the pool of TAG in the liquid phase available to recrystallize on the surface. It appears that crystallization occurring after each cycle builds onto the crystals developed after the first cycle, versus developing new groups of crystals.

Compositional Analysis of Bloom

Comparison between the fatty acid composition analysis of the base fats and bloom of FHPK (Fig. 7.6) and FPK (Fig. 7.7) indicate a modest increase in shorter chain fatty acids in the bloom fat versus base fats and slight decrease in longer chain fatty acids (Table 7-A).

Results suggest the bloom is the result of the melting and recrystallizing of lower melt TAG generated by the recycling storage temperatures. Although the change in fatty acid composition was modest, the impact on microstructure and bloom was significant. This example is to reinforce that only minor changes can have a dramatic impact on microstructure and visual appearance.

It should be emphasized that most commercial bloom is not the result of a polymorphic transition. Specifically, chocolate bloom is not the result of the transformation from Form V to Form VI. Rather, polymorphic change is the consequence of a physical change in the microstructure that preceded the polymorphic transition. Bricknell & Hartel (1998), Wang et al. (2010), and others have demonstrated that while bloom crystals may be in a more thermodynamically stable state, it is the result and not the cause for the change in the physical structure. Form VI polymorph can exist in chocolate without bloom.

Table 7-A. Comparison between Fatty Acid Composition Analysis of the Base Fats and Bloom of FHPK and FPK.

% FAC:	FHPK			FPK		
	Base	Bloom	Difference	Base	Bloom	Difference
C8:0	3.5	4.7	1.2	1.9	3.1	1.2
C10:0	3.3	3.8	0.5	2.8	3.3	0.5
C12:0	46.3	46.1	0.2	55.4	53.9	-0.5
C14:0	15.6	15.3	-0.3	21.8	20.9	-0.9
C16:0	8.6	8.3	-0.3	8.6	8.4	-0.2
C18:0	21.4	19.8	-1.6	2.2	2.3	0.1
C18:1	0.3	0.2	-0.1	6.0	6.0	0.0
C18:2	0.0	0.2	0.4	0.9	1.3	0.4
Total	99.0	98.4	0.0	99.6	99.2	0.6

Overview of Key Attributes in Confectionery Fats

Before discussing the causative factors for bloom, it may be important to review why confectioners use the fats they do use in chocolates and compound coatings. Why use fats that have a potential to bloom?

What does fat provide in confections (chocolate and compound coatings)? What are the important attributes/properties of a fat or fat system in confections? Not necessarily in the order of importance they include the following:

1. Melting properties—the ability for a fat to go from a solid to liquid quickly at body temperature is a key attribute. This imparts desirable sensory sensations of an audible snap, mouthfeel, and quick flavor release. Only a few fats deliver this unique property, cocoa butter recognized as having ideal melting profile for confections and setting the traditional standard for confectionery fats. Like butter set the standard for table spreads, cocoa butter established the melting standards most people associate with fine confections.
2. Solidification properties—as important as melting is to the consumer of chocolates, solidification properties are as equally important to the confectioner. Instant solidification is not necessarily a desired attribute. Especially if the confection is to be formed in mold or enrobed onto another material. There is a need for confections to solidify within an optimum time, that time being dependent on the application. The rate of solidification is key to delivering confections in a preferred form (bars, truffles, bunnies, enrobed snack cakes) is extremely important. Confections that solidify too quickly may not provide an even coating over a truffle, or may not completely fill all the crevices in a novelty mold to capture the detail of the novelty item. Confections that solidify too slow may flow off a truffle and not provide an even coating or take too long to solidify in a mold increasing production costs by slowing down production rates.
3. Crystal size/shape—crystal size is important from a sensory requirement to impart a smooth mouthfeel. Small fat crystals develop a microstructure that imparts a glossy and smooth surface appearance, also a critical attribute for meeting consumer acceptance and expectations. Small crystals and resulting microstructure are important for capturing and stabilizing small amounts of liquid fat and other solid particles dispersed in the confection matrix (sugar, cocoa, and dairy solids).
4. Level of solids—minimum level of solids, in addition to optimum size and shape, are required to provide sufficient structure to capture and hold the dispersed solids, provide sufficient rigidity to maintain structural integrity through distribution, deliver a “snappy” texture, and provide a pleasant mouthfeel.

5. Stability—ability to not change over time, or minimize changes that effect microstructure is critical and highly desirable. Most bloom is a result of two stability issues. One, not achieving an optimum or critical level of stable crystals during manufacturing. Two, intolerant to fluctuations in temperatures during storage and distribution (melting and recrystallizing into an undesirable crystal form within a narrower range than the range of temperatures encountered during distribution).
6. Marketing/Economics—the fats must be within defined or implied standards of identity for a confection, must have sufficient supply to be sustainable and economical, and must meet consumer's perceived requirements and government's regulatory requirements for health, nutrition and safety.

Meeting all these attributes at some minimum level is required for a fat to be used in confections. All of the attributes are interrelated. Changing one most likely impacts another. From a commercial perspective it would be difficult to assign priorities.

No fat in their natural form excels in all the attributes. While cocoa butter sets standard for melting and textural attributes, it is not tolerant to temperature change during distribution and has this polymorphic property that makes maintaining structural integrity over time a challenge.

Causes

Fat bloom is a broad term to cover a multitude of changes in appearance in confections related to structural changes in the fat system. Fat bloom is not always the consequence of a single factor or single mechanism. It can be due to a specific factor and it can be due to multiple factors and interactions of multiple factors. This is why identifying a definitive mechanism for bloom and controlling bloom has been a mystery for as long as we have had chocolate confections.

Very broadly and in very general terms, bloom development is related to several internal and external factors.

The internal factors may include the following:

1. Interaction and incompatibility between fats or major TAG populations within the confectionery fat composition. Negative interactions from using/ blending fats not completely compatible with each other, that is, fats that disrupt each other's crystallization/solid behavior (from super saturation, nucleation, growth, networking, and development of microstructures) may be considered incompatible fats. In reality, all fats and major TAGs within a fat influence behavior or other fats, it is a matter of degree which separate compatible and incompatible fats. Also an area which requires further clarification and more exact science based definitions.

2. A fat systems inherent sensitivity to changes in temperature and fluctuations in temperatures, resulting in melting and recrystallization or changes in polymorphic form. Both resulting in a change in the overall crystal structure and microstructure and development of bloom on the product surface.
3. Ineffective or excessive levels of minor lipids, emulsifier, or crystal modifiers and/or ineffective methods for incorporating minor lipids into the product during blending and melting.
4. Improper or sub-optimal heating and cooling during blending, tempering, depositing, or final cooling that produces a sub-optimal crystal network/structure which reorganize itself over shelf life, alter the microstructure or surface structure of the confection which develops into bloom.
5. Scuffing, physical damage, to the cooled confection or product application. A disruption to the confectionery surface stimulating changes in crystal growth behavior leading to changes in microstructure and surface structure which develops into bloom with time.

The external factors may include

1. Physical properties of the non-lipid ingredients; size, shape, and surface chemistry of sugar, dairy solids, cocoa solids.
2. Oil from fats or oils within other components of the confection (fillings) or other fats or oils from other components in a food made with the confection (enrobed snack cake) that migrate and recrystallize on the surface as bloom, or co-mingle with confectionery fats and recrystallize on the surface as bloom.
3. Storage temperatures and fluctuating temperatures.

Any factor, chemical, physical, or environmental, that disrupts the structural integrity of the chocolate/confection lipid microstructure over time has the potential to create a bloom condition.

Because more than one factor can be responsible for a bloom situation, discussions of each session will require some overlap. It is nearly impossible to discuss factors independently and without being redundant.

Key Factors Contributing to Bloom from Composition to Oil Migration

Composition

Composition will include the ingredients commonly used in confections: cocoa butter (CB), cocoa butter substitutes (CBS), minor lipids (milk fats), non-lipid particles (sugar, cocoa powder, dairy ingredients).

Two main composition problems tend to induce bloom. The first is when two “incompatible” fats are used in a chocolate or coating. The second composition problem occurs when the coating comes in contact with a filling, inclusion or nut having a lower solid composition allowing for fat migration.

Use of “Incompatible” Fats

It is important to differentiate incompatibility of fats due to dilution effects from problems due to eutectic mixing behavior (Bigalli, 1988). When two fats are compatible but have different melting points, a dilution effect is observed where the decrease in solid fat content is proportional to the amount of low-melting component added. An example of two compatible fats used in chocolate are a Malaysian cocoa butter with a “high” disaturated TAG content and solid fat content (SFC) and a Bahian cocoa butter with a “low” disaturated TAG and SFC content (for cocoa butters). When blended together the SFC of the blend is very close to the values contributed in proportion to the parent butters (Fig. 7.10).

In this example rate of solidification (ROS) was used to measure crystal behavior and monitor compatibility or incompatibility between fats. More commonly melt points, solid fat content or DSC melt curves are used to monitor compatibility. The DSC being the preferred method, and best method of the three to determine a true eutectic condition.

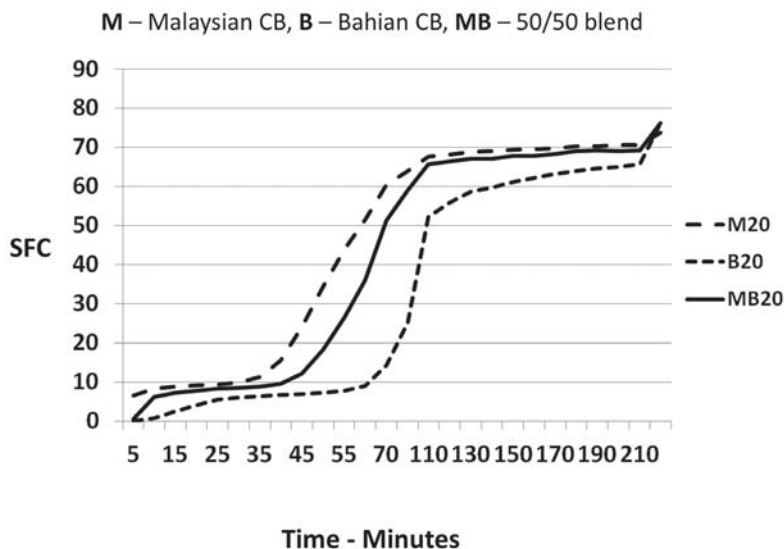


Fig. 7.10. Rate of solidification curves of cocoa butter from Malaysia, Bahia and a 50/50 blend.

However, from a commercial perspective, monitoring crystal behavior by ROS provides the confectionery manufacturer valuable information on rate of solidification development in addition to melt and total solids at a given temperature. From a commercial perspective ROS can be as important as SFC and melt point because solidification rate impacts processing time and capabilities. A fat with a slow ROS may require reduce production rates to ensure crystallization is complete through processing, even though the fat after complete crystallization will contain a minimum level of solids deliver sufficient structure to the finished product.

In contrast to the compatible fats, when truly incompatible fats are mixed together, they tend to separate from each other and the solid fat content decreases below that of either individual fat. The concentration at which the two fats separate determines the limits of solubility. Below this concentration, the fats are compatible, but above this concentration, the fats are incompatible and tend to separate. To observe a true incompatibility, both fats must have a certain SFC (at the studied temperature). Two such blends can contribute to bloom formation in chocolates and coatings. Fig. 7.11 is an ROS of blend of fats that incompatible and the theoretical ROS based on the solids

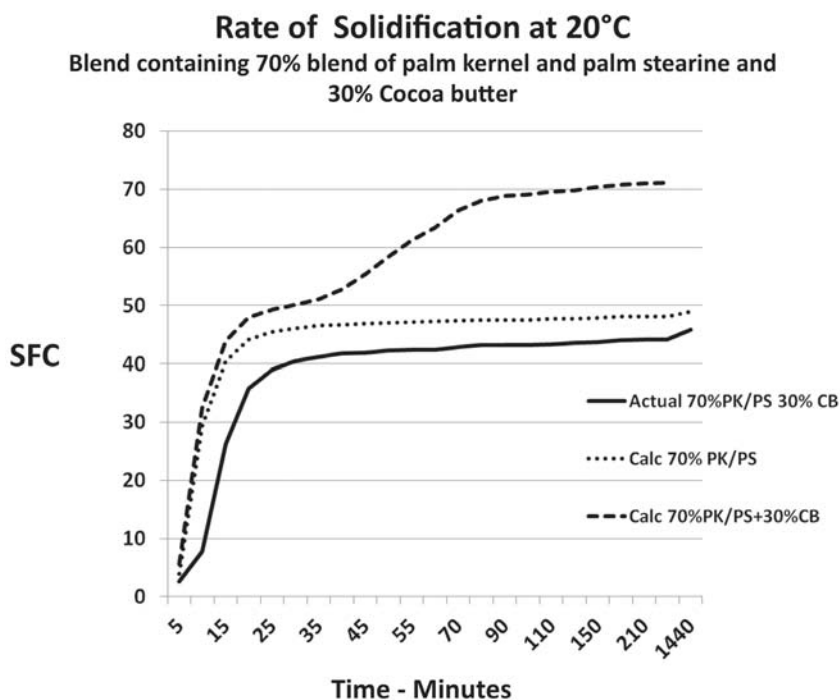


Fig. 7.11. Rate of solidification curves.

from each component fat in proportion to their percentage in the blend. In this example the sample is a blend of 70% fractionated palm kernel oil with palm mid fraction and 30% pressed cocoa butter from Ghana (FFA under 1.5%, and DAG under 2.0%).

The data shows the ROS of the actual blend is significantly lower than the calculated contribution from each component, but also lower than the solids from the contribution of the PKS/PMF component. Most likely these fats are forming a eutectic and meet the commercial criteria for being incompatible.

Cocoa Butter (CB) with Cocoa Butter Substitutes (CBS).

Coatings made with hard lauric butter, or combination of hard lauric butter and palm oil fractions (may include palm stearines and or palm mid fractions), are very sensitive to the presence of cocoa butter (Hogenbirk, 1988; Noorden, 1982). A eutectic is formed at very low addition levels of CB to CBS. In addition to softening of the mixture, the blend of these two fats promote bloom formation. A cocoa butter concentration above 4% can result in a bloomed product in a few months and that time drops to less than a week when the concentration is around 10% (Hogenbirk, 1988). If the proportion of C in CBS is very high, bloom could appear in less than two days (Sequine, 2001). Moreover, in a study comparing fully hydrogenated palm kernel oil/cocoa butter, FHPKO/CB and fractionated palm kernel oil/cocoa butter, FPKO/CB blends, Williams et al. (1997) showed that blends with higher SFC had faster bloom formation. The softest compound coatings, so the most affected by the fat incompatibility, were not the most affected by bloom. This effect remains to be explained.

Key Factors from Other Components/Ingredients

I highly recommend reviewing each of the following chapters in this book for greater detail on other components in confections and their potential impact on bloom:

Talbot – *Chocolate and Cocoa Butter—Structure and Composition*

Smith – *Confectionery Fats*

Garti, Asein – *Effect of Emulsifiers on Cocoa Butter and Chocolate Rheology, Polymorphism and Bloom*

Metin, Hartel – *Milk Fat and Cocoa Butter*

Lechter – *Effects of Minor Components on Cocoa Butter Polymorphism and Kinetics of Crystallization*

Svanberg – *Non-Cocoa Ingredients in Cocoa Butter Crystallization*

In addition to the components discussed in other chapters, Nakae (2000) demonstrated glycolipid fractions (mono-, di-, and tri-galactosyldiacylglycerols) added at 2% in place of lecithin could inhibit bloom in dark and milk chocolates in accelerated

tests (25 cycles, 12 hours at 32 and 20°C, per cycle). While the inclusion of these materials are not compliant with standards of identity of chocolate in many countries, and may not provide a commercial solution for bloom within chocolate standards, the impact of glycol lipids on bloom of non-standard products and compounds may provide an economic advantage and should be studied further.

Summary Compositional Factors that Impact Bloom

The goal in making a traditional chocolate-like confection is to entrap in a fat crystal matrix, sugar, cocoa powder, dairy solids (optional), and a little vanilla or other flavoring, with emulsifiers added to improve the rheological properties and/or reduce fat. The physical, structural, melting properties of the final product are a result of the interactions of all the ingredients and how they impact the crystal/solidification behavior of the primary TAGs.

Not only do minor changes in TAG profiles impact crystal behavior, by blending fats together, the minor ingredients inherent in these fats (the phospholipids, diacylglycerols, fatty acids), the emulsifiers, and solids (amount, particle size, distribution and surface chemistry) found in the composition of the confection influence and impact crystal behavior. And their influence/impact may take place at different points along the sequence of crystallization/solidification—supersaturation, nucleation, growth and microstructure. And any change to a fat's supersaturation, nucleation, growth, and microstructure can impact its response to tempering, cooling, and storage conditions, resulting in a change in structure and potential bloom development.

Advice to the confectioner: proceed with caution when making changes in composition based upon conclusions from research that examines single ingredients or variables.

Processing

In this section we shall review processing steps after the confectionery ingredients are refined and conched or liquefied, and ready to convert from a liquid state to a solid state. The process I will review can be followed in Fig. 7.12, beginning with blend tank, supply tank, tempering and/or dynamic cooling, depositing, final static cooling/solidification and packaging.

Blending and Supply Tanks

Maintaining minimum temperatures to avoid pre-crystallization is critical. Generally, maintaining confection slurries above 50°C for compounds and above 43°C provides sufficient temperature to inhibit crystallization of the base fats and minor lipids (crystal modifiers, stearines) over 24 hours, provided the minor lipids were fully melted prior to incorporation into confectionary fat or confectionery slurry. Best manufac-

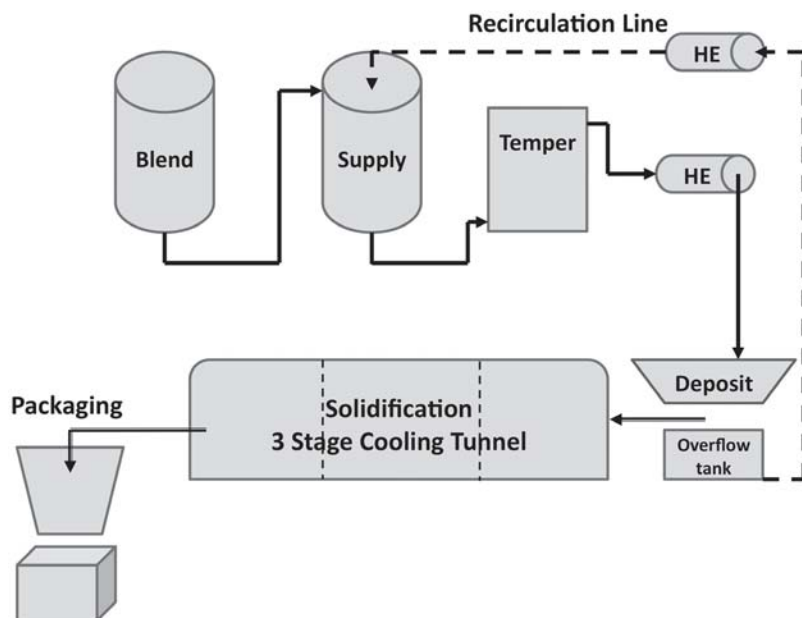


Fig. 7.12. Common process flow for chocolate and compound coatings

turing practices generally require blending higher melting lipids together with a portion of the confectionery fat (no less than 50/50) prior to incorporating the minor lipid into the remaining confectionery fats or confectionery slurry.

Crystal modifiers and stearines are most effective if they are completely melted and incorporated into the fat phase prior to blending with other confectionery ingredients. Failure to completely melt the crystal modifiers and/or stearines can influence development of sub-optimal crystallization during cooling and solidification, which may lead to early blooming.

Some manufacturers add rework to the supply tank, which is generally an acceptable practice providing sufficient time and temperature are applied to thoroughly melt, erase the crystal memory or any of the lipid components. The minimal time temperatures must be determined by the manufacturer based on the product components and heat transfer efficiencies of their equipment. It will vary from formula to formula and from process to process.

Tempering/Dynamic Cooling

Tempering chocolate. Chocolate must be tempered to control the polymorphism of cocoa butter (Dimick, 1991; Gerhard, 1979; Jocanovic et al., 1995; Kempf, 1949).

The melted chocolate undergoes a temperature cycle to induce the formation of nuclei in Form V (and also to destroy the other unstable forms). The addition of seed is also used to induce the crystallization step. New tempering methods used Form VI cocoa butter seed. It facilitated greatly this step that is less sensitive to temperature fluctuation, quicker and gives even better final quality (Zeng et al., 2002). The seed crystals formed during tempering permit the surrounding liquid TAG to crystallize quickly in the right polymorphic form (Kleinert, 1965, 1970). Effectively, it is more favorable for TAG to attach to a growing crystal face than to find sufficient energy to create new nuclei (Seguine, 1991). When the seed crystal concentration is sufficiently high (0.5 to 2% total mass), the chocolate can be used for the production of deposited free-form, molded, coated, or enrobed products.

Under-tempering chocolate. Chocolate is considered to be under-tempered when the concentration of nuclei is not large enough to ensure a good crystallization of chocolate mass upon cooling. In this case, the crystallization time increases drastically since nucleation must occur rather than crystal growth. Furthermore, if new seeds are spontaneously generated during cooling, they form in an unstable polymorph, and re-crystallization problems can occur. In such cases, bloom occurs relatively quickly, often in less than two days, and causes a drastic modification of the surface, which appears as large white spots and /or white rings surrounding a black and glossy center.

Over-tempering chocolate. The term over-tempered is used when the seed concentration in the melted chocolate mass is too high. The seed concentration may increase due to excessive tempering time. In this case, the extent of crystallization in the mold is not sufficient to produce the desired mass contraction. The molded surface is not bright and the unmolded surface turns a gray dull very quickly (Hettich, 1966).

Cooling compounds. Since compound coatings made with CBS crystallize directly in the most stable form, beta prime, they do not need to be tempered prior to cooling. However, some companies choose either to add a nucleating agent (like fully hydrogenated oils) or temper to ensure complete and proper crystallization of lauric-based coatings.

Because it is becoming popular to make compound fats from combinations of lauric fats and palm mid fractions, tempering to optimize crystal stability maybe required. This is dependent on the ratio of the component fats and compatibility of the resulting TAG population.

Depositing

Impact on bloom during depositing is generally a result of inadequate melting of recirculated product back to the supply tank, or developing a build-up of solids on the depositing equipment which comes in contact with fresh product.

Generally, chocolate and compounds are deposited slightly above their crystallizing temperature to ensure that crystallization occurs immediately upon static cooling. As a result, some material that is either not deposited, or during enrobing or bottoming, is the excess material required for adequate coverage but does not remain on the product being covered. It is the overrun material which is recirculated back to the supply tank.

The crystals formed in the recirculated product and residual “build-up” is not likely to be in a stable crystalline form and may influence undesirable, sub-optimal crystal behavior, eventually resulting in bloom after packaging and well into distribution. Recirculated product must be thoroughly melted upon return to the supply tank, and residual build up on equipment must be monitored and controlled.

Static Cooling/Solidification

The product is cooled statically through a cooling tunnel before storage to ensure complete solidification of the confectionery fat. Proper temperature control during cooling is critical to growth of seed crystals in tempered chocolate.

For chocolate, the cooling rate is also an important issue in preventing bloom formation. Cooling too fast may induce crystallization of unstable crystal polymorphs as well as formation of hair cracks and pores on the surface. Both effects could promote further bloom. Homogeneous heat release, which occurs through the inside, as well as from the outer layer, is the best way to avoid any tension on the chocolate surface and consequently, reduce subsequent bloom [79].

Additionally, if the cooling tunnel temperature is too low it may shift the crystallization to favor nucleation of undesirable, unstable polymorphs, versus crystallization and growth influenced by the desirable polymorphic crystals developed during tempering.

In general, trying to increase production by decreasing the tunnel temperature is not a good idea. It will promote unstable crystals that are more likely to bloom.

Common practice is to begin cooling under moderate cooling (60–65°F) during the first stage of the cooling tunnel (generally the first third), followed by lower temperatures (45–50°F) during the second stage, and slightly warmer cooling temperature (55–60°F) during the third stage to avoid condensation upon exiting the tunnel. Generally 12 to 15 minutes is adequate for cooling.

However, optimum cooling conditions is really product and equipment-design dependent, and a best operating conditions and procedure must be developed for each product with the equipment manufacturers.

Changes in formulation/composition that change supersaturation, nucleation, growth, or microstructure (crystallization behavior) will change tempering and cooling parameters and conditions.

For coatings, the cooling rate must be sufficient to insure complete crystallization (Hogenbirk, 1988; Sequine, 2001). Generally the lower the tunnel temperature and the shorter the cooling time, the better the bloom resistance (Wennermark & Carlsson, 1994).

Packaging

Although most of the heat of crystallization is removed during the static cooling, the product surface is very vulnerable to scuffing or smearing. This is especially a concern when demolding bars or novelty figurines, and conveying wafers, drops, chips, and chunks.

Scuffed product will bloom and must be avoided or minimized during the packaging process. Even product to product contact is sufficient to scuff the outer layer and initiate bloom. The wafer B in Fig. 7.1 is an example of bloom that can occur from scuffing. Some manufacturers will apply a mild heat treatment to the cooled product prior to packaging to increase bloom resistance. The heat treatment will be dependent on the melting characteristics of the confectionery fat, but generally sufficient heat is applied for a minimal time to eliminate the cracks and crevices generated from scuffing. A more detailed explanation to the mechanics is provided in the next section.

Distribution and Storage

Keeping storage temperatures below the melting point of the confectionery fat is not sufficient to prohibit bloom. Because of the narrow melt range of confectionery fats and the significant impact of slight changes in temperature on solid content it is difficult to make recommendations of maximum temperatures for confections.

Generally colder is better; keeping chocolates and compounds under 65°F is recommended to minimize bloom.

Chocolate Storage

For chocolate, three ranges of storage temperature can be distinguished, each with different bloom propensity. The same temperature ranges were also applicable for filled chocolates (Adenier et al., 1975; Ali et al., 2001; Wooton et al., 1970).

Low Temperature Chocolate Storage (<18°C)

Cebula and Ziegleder [88] reported that storage below 18°C inhibited storage bloom in chocolate over 1 year. The storage of chocolate at low temperature generally minimizes bloom formation; however, even if the storage temperature is low, bloom can occur after more than one year.

Medium Temperature (18 < T < 30°C)

In this range of temperature, which is below the melting point of Beta V crystals, bloom occurs more quickly with an increase in temperature.

High Temperature (32–34 °C)

When temperature goes sufficiently high, the cocoa butter is partially melted. Upon subsequent cooling, the cocoa butter crystallizes uncontrolled, in unstable polymorphic forms. This is a similar cause for bloom as in under and untempered chocolate: low seed concentration and unstable polymorph. Thus bloom will occur very quickly after crystallization and the chocolate will exhibit large white spots.

Compound Coatings

For compound coatings, the effects of temperature storage is more confusing. It has been suggested that storage temperatures slightly lower than room temperature, about 18°C, promotes the most rapid bloom formation in compound coatings. Storage temperatures either higher or lower give better bloom stability.

A study by Smith (Smith et al., 2004) indicates compound coatings made with fractionated palm kernel (FPK) and fractionated and hydrogenated palm kernel (FHPK), both containing 10% cocoa butter in the fat phase, developed bloom significantly faster when stored at 15°C versus 20 and 25°C. The FPK developed bloom within 4 weeks at 15°C, compared to 23 weeks at 20 and 25°C. And FHPK developed bloom in 10 weeks at 15°C compared to 23 weeks at 20 and 25°C. They demonstrated that bloom is not due simply to separation of the PK and CB components and is temperature dependent. The bloom developed at 15 and 20°C, contained more CB TAG, whereas the bloom developed at 25°C was predominantly from lauric fat.

Fluctuating Storage Temperatures

Fluctuating temperatures, even if the range of temperatures is within a “high” solid content of a confectionery fat, can change the microstructure sufficiently over repeated cycles to induce bloom. Temperature gradients within the confection (the surface see higher temperature fluctuations, which are dampened in the interior) lead to a driving force for liquid TAG to move to the surface. As temperature increases, the amount of liquid TAG increases as does the volume of the system (in reverse, there is a contraction as more fat crystallizes upon cooling). The liquid TAG are pushed (or pumped) through the confection to the surface due to this dilation effect. When the temperature decreases again, not all of the liquid fat is reabsorbed back into the chocolate matrix, leaving a “pool” of liquid fat near the surface. One of the key points in bloom formation is the surface state and whether the liquid fat actually crosses to the surface. As long as the surface of the confection remains smooth, compact and free of defects like scratches or crevices (scuffing), the liquid fat does not cross the surface, as seen in the NMR results of Guiheneuf et al. (1997), and bloom may be delayed. Once the surface loses its impermeability, liquid fat easily crosses to the exterior of the piece and re-crystallizes.

Oil Migration

Migration of liquid fat from a center into a chocolate or coating occurs due to the concentration difference in specific TAG between the coating and center (the same driving force as for any mass transfer situation). Diffusion of TAG, from center to coating and from coating to center, occurs due to this concentration driving force. However, capillary forces may also draw liquid TAG from center to coating. Once the liquid TAG from the center are absorbed into the coating, the two fats mix according to their phase behavior. Whether due to dilutional softening or a eutectic formation, the solid fat content within the coating occurs as some of the fat crystals in the coating dissolve in the liquid TAG. The dilution of CB crystals leads to a softer coating with more liquid TAG with greater mobility that are more likely to re-crystallize into a more stable polymorph, initially at the surface, and appear as visible bloom. Once fat migration and softening occur, the bloom mechanism is similar to that which occurs in storage bloom.

The rate of migration of liquid TAG may be influenced by numerous factors, including the hardness of the fat (change in liquid fat with temperature), the particulate structure (sugar, cocoa solids, milk solids, etc.), emulsifier interactions and the porosity of the confection (cracks, crevices providing access to the surface and capillary forces) (Ziegler, 1997). Thus, increased porosity of the chocolate due, for example, to crack and crevice formation from cooling too quickly, results in immediate migration of liquid fat to the surface and rapid onset of bloom.

Conclusions

There are many internal and external factors that contribute to bloom in confections. Generally not a single factor is responsible but generally a combination of factors in commercial processes that influence a sufficient change in microstructure to develop a bloom condition.

Based upon the multiple possible factors for bloom, when trying to identify a cause, it is important to review the entire process, from formulation through distribution to identify the causes and take appropriate corrective action.

From a commercial perspective, we tend to view fat bloom as a product defect, although it is not a defect of the fat, it is simply the fat reacting to its environment to reach a preferred state of equilibrium.

My greatest learning from writing this chapter is fat bloom cannot be studied as an independent event that occurs in confections. Each ingredient and process step influences crystal behavior (supersaturation to nucleation to growth and microstructure). While we understand the influence of many individual components, there is still more to learn about the interactions between components and their interaction and influence on the confectionary fat.

I would like to thank Mike Schaff and Frank OKorn for their input and direction.

References

- Adenier, H., M. Ollivon, R. Perron, H. Chaveron. Le blanchiment gras. I Observations et commentaires. *Choc. Confiserie Fr.* 1975, 315, 7–23.
- Ali, A, J. Selamat, Y. Che Man, A. Suria. Effect of storage temperature on texture, polymorphic structure, bloom formation and sensory attributes of filled dark chocolate. *Food Chem.* 2001, 72, 491–497.
- Bigalli, G. Practical aspects of the eutectic effect on confectionery fats and their mixtures. *Manuf. Confect.* 1988, 68, 65–80.
- Bricknell, J., R. Hartel. Relation of fat bloom in chocolate to polymorphic transition of cocoa butter. *J. Am. Oil Chem. Soc.* 1998, 75 1609–1615.
- Coe, S.D., S M.D. Coe. *The true history of chocolate.* Thames & Hudson: New York (USA), 1996.
- Dimick, P. Principles of cocoa butter crystallization. *Manuf. Confect.* 1991, 71, 109–114.
- Gerhard, N. Tempering, coating and cooling chocolate. *Manuf. Confect.* 1979, 59, 48–56.
- Guiheneuf, T.G., P. Couzens, H. Wille, L. Hall. Visualization of liquid triacylglycerol migration in chocolate by magnetic resonance imaging. *J. Sci. Food* 1997, 73, 265–273.
- Hettich, A.H. Experimental basis for the definition of “proper” chocolate temper. *Manuf. Confect.* 1966, 46, 29–36.
- Hogenbirk, G. Compatibility of specialty fats with cocoa butter. *Manu. Confect.* 1988, 64, 65–80.
- Jocanovic, O., D. Karlovic, J. Jakovljevic. Chocolate pre-crystallization: A review. *Acta Aliment.* 1995, 24, 225–239.
- Kempf, N. Crystallization of cocoa butter and its effects on properties of chocolate. In *Industrial Chocolate Manufacture and Use.* Blackwell Science Publisher: York (UK), 1949.
- Kleinert, J. Studies on the formation of fat bloom and methods of delaying it. *Rev. Int. Choc.* 1962, 16, 201–219.
- Kleinert, J. The relationship between temper and cocoa butter constants. *Rev. Int. Choc.* 1965, 20, 42–57.
- Kleinert, J. Cocoa butter and chocolate: The correlation between tempering and structure. *Rev. Int. Choc.* 1970, 25, 386–399.
- Laustsen, K. The nature of fat bloom in molded compound coatings. *Manuf. Confect.* 1991, 71, 137–144.
- Lechter, A. Fat bloom in palm kernel oil based confectionery coatings. Paper presentation at AOCS annual meeting, May 19, 2010.
- Nakae, T., T. Kometani, T. Nishimura, H. Takii, S. Okada. Effect of glycolipid fraction on fat bloom in dark and milk chocolate. *Food Sci. Technol. Res* 2000, 6, 269–274.
- Noorden A.C. Fat bloom—causes and prevention when using lauric hard butters. *Susswaren Tech. u. Wirtschaft* 1982, 26, 318–322.
- Rousseau, D., P. Smith. Microstructure of fat bloom development in plain and filled chocolate. *Royal Society of Chemistry* 2008, 4, 1706–1712.

- Seguine, E. Tempering—the inside story. *Manufact. Confect.* 1991, 71, 118–125.
- Sequine, E. Diagnosing chocolate bloom. *Manuf. Confect.* 2001, 81, 45–50.
- Smith, K., F. Cain, G. Talbot. Nature and composition of fat bloom from palm kernel stearin and hydrogenated palm kernel stearin compound chocolates. *J. Agric. Food Chem.* 2004, 52, 5539–5544.
- Wang, F., Y. Liu, L. Shan, Q. Jin, Z. Meng, X. Wang. Effect of fat composition on texture and bloom of lauric compound chocolate. *European Journal of Lipid Science and Technology*, 2010, 112, 1270–1276.
- Wennermark, M., M. Carlsson: How processing conditions affect the appearance of fat bloom. Paper presented at the Specialty Fats Seminar, Orlando, FL (USA), 1994.
- Williams, S., K. Ransom-Painter, R. Hartel. Mixtures of palm kernel oil with cocoa butter and milk fat in compound coatings. *J. Am. Oil Chem. Soc.* 1997, 75, 357–366.
- Wooton, M., D. Weeden, N. Munk. Mechanism of fat migration in chocolate enrobed goods. *Chem. Ind.* 1970, 32, 1052–1054.
- Zeng, Y., P. Braun, E. Windhab: Tempering, continuous pre-crystallization of chocolate with seed cocoa butter crystal suspension. *Manufact. Confect.* 2002, 82, 71–80.
- Ziegler, G. Fat migration and bloom. *Manuf. Confect.* 1997, 7, 43–44.

8

Morphology of Chocolate Fat Bloom

Yasuyoshi Kinta¹ and Tamao Hatta²

¹Research Institute, Morinaga & Co., Ltd. 2-1-1, Shimosueyoshi, Tsurumi-ku, Yokohama, Kanagawa 230-8504, Japan, and ²Japan International Research Center for Agricultural Sciences (JIRCAS), 1-1 Ohwashi, Tsukuba, Ibaraki 305-8686, Japan

Introduction

Chocolate is a suspension in which solid particles of sugar and cocoa powder are dispersed at high concentrations in a continuous phase of cocoa butter. Many of chocolate's physical properties are thus determined by the behavior of cocoa butter, which plays numerous essential roles in providing chocolate with a pleasing appearance, such as a shiny surface, snap at room temperature, and smooth melting in the mouth. On the other hand, the behavior of cocoa butter is complicated and makes chocolate difficult to handle. Chocolate requires strict tempering (temperature control) during production and must be stored under the right temperature conditions. Producing good chocolate thus takes considerable effort and planning. A bloom appears on chocolate when the unfavorable properties of cocoa butter are manifested.

The bloom can be classified as sugar bloom or fat bloom. When chocolate is produced under the appropriate conditions, both sugar and fat are present in fine texture and are dispersed uniformly at the macroscopic level. This allows the cocoa butter to melt smoothly and pleasurably in the mouth, releasing the taste and aroma of the solid particles dispersed in it. Bloom is a condition in which the fine texture of sugar and fat crystals is lost for some reason and the chocolate becomes nonuniform. Sugar bloom is caused by changes in the morphology of the sugar crystals, and is mediated by water, such as high humidity or contact with water. Fat bloom is caused by fat melting due to high temperatures and/or dissolution of fat in oil. Both are attributable to changes in crystal morphology. Fat crystals are more sensitive to environmental conditions and are thus less stable than sugar crystals. Under ordinary daily conditions, therefore, fat bloom is overwhelmingly more frequent than sugar bloom. This chapter discusses the fat bloom of chocolate.

Chocolate fat bloom is a major problem in the confectionery industry. Even items that have been properly designed and manufactured suffer fat bloom if they are stored inappropriately after shipping. Fat bloom makes chocolate look as if mold has developed on it, but is in fact unrelated to any growth of microorganisms and has no

detrimental effects on human health. However, fat bloom is crucial, as it adversely affects the overall organoleptic experience, such as mouth feel, scent, and taste, as well as having a damaging appearance. A number of studies have been conducted on chocolate fat bloom over the years, but its detailed morphology and developing mechanism are still not clear in many respects. Chocolate fat bloom is classified into various types by the form of chocolate it is found on and the storage conditions that cause it (Hartel, 1999; Seguíne, 2001; MacCarthy et al., 2003; Lonchampt & Hartel, 2004). However, the relationships between the causes and results of bloom are diverse, and the classification of chocolate fat bloom is complicated. In terms of morphology, a specific form of chocolate fat bloom has been known for years (Martin; 1987; Schlichter-Aronhime & Garti, 1988; Hartel, 1998; Sato & Koyano, 2001). More recently, new forms have been reported (Lonchampt & Hartel, 2004; Kinta & Hatta, 2005a and b; Lonchampt & Hartel, 2006; Kinta & Hatta, 2007; Kinta & Hartel, 2010). In this article, we classify chocolate fat bloom according to bloom morphology. Organizing the morphological states can help us to understand the developing mechanism, which gives a complicated flow chart showing the dependence on the type of chocolate item and its storage conditions. Although there are chocolate-like compounds that use cocoa butter replacer (CBR) or cocoa butter substitute (CBS) instead of cocoa butter, this chapter discusses only the bloom observed on chocolates that use mainly cocoa butter.

Classification by Morphology of Chocolate Fat Bloom

Changes in the morphology of chocolate items and the preservation environment are discussed below for each fat bloom type, classified by morphology.

Type 1 Bloom

Morphology and Developing Mechanism of Type 1 Bloom

Type 1 bloom has separated fat components on the surface of chocolate (Fig. 8.1). Separated fat forms euhedral (idiomorphic), needlelike, thin platelike or foliated crystals (Fig. 8.2). β -crystals of triacylglycerol (TAG) at equilibrium have been reported to be flat and needle-like (Bennema et al., 1992). This type has been widely reported and frequently observed, and it can be said to be a readily understood form.

Type 1 bloom is seen in plain chocolate that has been stored for a long time and filled chocolate confections. Plain chocolate that is preserved at temperatures lower than the melting point of cocoa butter develops a bloom faster when stored at higher temperatures. At temperatures below 18°C, bloom can be prevented for 1 year or even longer (Cebula & Ziegler, 1993). In chocolate confections with fillings that contain oils with low melting points and/or nuts, bloom develops mainly due to the oils mov-



Fig. 8.1. External appearance of Type 1 bloom on plain chocolate after long time storage.

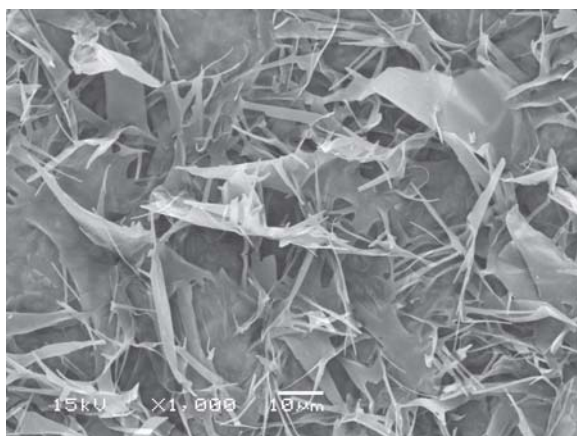


Fig. 8.2. SEM image of Type 1 bloom on plain chocolate after long time storage.

ing from the filling to the chocolate cover (Hartel, 1999; Seguire, 2001; McCarthy et al., 2003; Lonchampt & Hartel, 2004). The temperature at which bloom most readily develops varies according to the item.

The morphology of Type 1 bloom was first reported by Whymper and Bradley (1925). Based on a report by Hartel (1998), development of bloom in long-stored plain chocolate can be summarized as described later. Chocolate that is prepared under the appropriate conditions contains minute and stable β -V polymorphic crystals of fat (Wille & Lutten, 1966; van Malssen et al., 1999), which give the chocolate

a smooth and shiny appearance. At the initial stage of bloom development, small needlelike crystals of fat appear around holes and along cracks on the surface of the chocolate. Adenier et al. (1993) determined the size of these crystals, by microscopic observation, to be 2 to 3 μm . At this stage, little difference is visible. Chocolate just appears slightly duller than immediately after production. In one month in an environment that promotes bloom, a number of needlelike crystals of fat develop that project from the surface. These separated fat crystals, observed under an electron microscope, have been reported to be about 10 μm long (Jewell, 1972; Adenier et al., 1993). At this stage, the bloom looks whitish since the separated fat disperses light and is whitish itself.

In this type of bloom, fat separates, deposits and forms euhedral crystals on the surface of the chocolate. Certain combinations of environmental conditions, such as storage temperature, the TAG composition of the chocolate, and the composition of adjacent oily parts, lead to this form of bloom, since it is thermodynamically stable under these sets of conditions. Fat bloom is caused by a recrystallization process in which the crystalline structure undergoes processes to minimize either internal energy or surface free energy (Hartel, 1998). This results in the development of euhedral crystals, and crystal separation takes place at the interface between chocolate and air if there is any. Therefore, crystallization occurs not only on the chocolate surface but also in spaces inside the chocolate if there are air bubbles, etc. (Rousseau & Smith, 2008).

There have been many reports on the TAG composition of separated fat. Separated fat has been reported to have the same composition as that of cocoa butter that constitutes chocolate (Chapman et al., 1971), and it has been reported that the composition of separated fat from filled chocolate and plain chocolate is the same (Chaveron et al., 1976). The TAG components of the fillings of filled chocolates were found to not affect the TAG components of the separated fat: instead, it determined the speed of fat separation (Cerbulis et al., 1957). On the other hand, there have been studies on the fatty acid composition of separated fat reporting that the saturated fatty acid component (high melting point component) was slightly larger than its counterpart (Nevill et al., 1950; Cerbulis et al., 1957) and that the amounts of palmitic acid and oleic acid were somewhat high (Chaveron et al., 1976; Adenier et al., 1993). It has also been reported that the component balance of sn-1, 3-saturated acyl, sn-2-oleoyl glycerols (Sat-O-Sat type TAGs) in separated fat showed a drop in POP (P, palmitate) (Steiner & Bonar, 1961). The composition of separated fat differed among the studies, which is likely attributable to differences in type of chocolate and storage conditions. Fat separates and deposits on the chocolate's surface to achieve the most stable state under a specific set of conditions. Thus, the composition of the most stable euhedral crystals varies according to the conditions.

There are also quite a few reports on the relationship between the development of Type 1 bloom, which involves separation and deposition of fat on the chocolate surface, and the polymorphic transition of cocoa butter. Crystals of separated fat on

the chocolate surface are reported to be β -VI polymorphic (Chapman et al., 1971). Sato and Koyano (2001) mention that fat separation occurs at the same time as the polymorphic transition of cocoa butter, which may involve transition from unstable crystals to β -V as well as from β -V to β -VI, and that fat separation does not depend on specific polymorphic transition. Polymorphic transition does not always result in fat separation, as there have been cases in which polymorphic transition from β -V to β -VI was not accompanied by bloom (Bricknell & Hartel, 1998).

Identifying Type 1 Bloom

Type 1 bloom results from the phase separation of cocoa butter from the main body of the chocolate and formation of cocoa butter crystals on the chocolate surface. It is therefore possible, in principle, to identify Type 1 bloom by observation. Type 1 bloom has a dull and whitish external appearance. It is sometimes possible to see with the naked eye that cocoa butter crystals have separated and deposited on the surface, but it is easier and more reliable to use a microscope to obtain an enlarged view.

Stereomicroscopes are useful for observing and evaluating fat bloom. They have a lower magnification than ordinary optical microscopes but have a deeper depth of field and are easy to focus on samples that have rough surfaces. Because there is a long distance between the sample and the objective lens, it is also easy to investigate parts of a specimen under the microscope. Care must be taken, however, not to allow the tungsten light normally used for illumination to heat the sample specimens. LED lighting is preferable.

Optical microscopes in reflection mode are also useful. They can provide high magnifications and enable detailed observations. However, due to their shallow depth of field, they can focus only on specific points in a specimen at a time. An entire view of a specimen can be obtained by combining multiple of images taken while changing the focus.

Other than optical microscopes, scanning electron microscopes (SEMs) are widely used. SEMs do not provide color data, since the images are taken using electrons, but they have great depth of field. Secondary electron images, which create contrast by tilting the specimens, are especially sharp. They reveal surface unevenness very precisely, and are thus useful for identifying Type 1 bloom, which has characteristic irregularities on its surface. Low to high magnifications are also usable. However, SEMs have several disadvantages. Chocolate, which is an electrical insulator, readily accumulates charge, leading to poor images, and also tends to melt under irradiation with electrons. This causes a tradeoff between observation at high acceleration voltages, which generally give high resolution, and length of observation.

These microscopes can be used for external observation. There are analytical instruments available for analyzing components. Fourier-transform infrared (FT-IR) spectroscopy is useful for analyzing the components of substances deposited on the chocolate surface. When an object is irradiated with infrared light, the object selectively

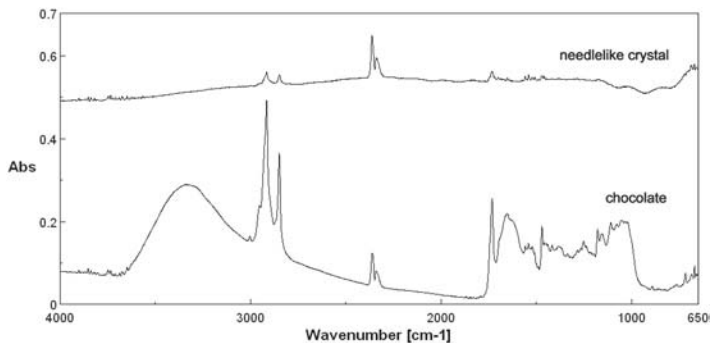


Fig. 8.3. Infrared spectra of main part of chocolate and needlelike crystals on chocolate surface of Type 1 bloom.

absorbs light at specific wavelengths. Because the wavelength range of infrared absorption is known for each material, the chemical structure of an unknown object can be determined from its infrared spectrum. An FT-IR microscope is needed to analyze Type 1 bloom, since the fat crystals on the chocolate surface are small. A stereomicroscope as described above is useful for obtaining specimens. Fig. 8.3 is an example of FT-IR microscope analysis. Type 1 needlelike crystals on the chocolate surface and the main part of the chocolate were analyzed using the transmission method. Unlike the chocolate part, the needlelike crystals showed absorption characteristics unique to fats (e.g. 1,750 and 2,900 cm^{-1} , which correspond to the C and O double bond of an ester and a C and H single bond, respectively) showing that they are fat crystals.

Type 2 Bloom

Morphology and Developing Mechanism of Type 2-A Bloom

Recently, bloom has been reported that has different morphology from that of the widely seen Type 1 (Lonchampt & Hartel, 2004; Kinta & Hatta, 2005a and b; Lonchampt & Hartel, 2006; Kinta & Hartel, 2010). It involves a lightening of the color in parts with low fat content, and is caused by uneven distribution of fat content. This occurs when there are no seed crystals: for example, when chocolate is exposed to high temperatures until the cocoa butter melts completely, and then is allowed to solidify.

Unlike Type 1, which is usually rather whitish, Type 2-A has the appearance of mixed patches of dark brown and light brown in most cases. The external appearance of chocolate produced without tempering is shown in Fig. 8.4. Non-tempered chocolate, melted at 60°C and cooled to 30°C, was poured into a polycarbonate mold and left at 20°C for 24 hours. The demolded sample was then stored at 20°C. One month later, a bloomed and untempered chocolate sample was obtained. The dark brown patches consisted of spheres and aggregates of spheres, and the light



Fig. 8.4. External appearance of Type 2-A bloom on chocolate prepared without tempering.

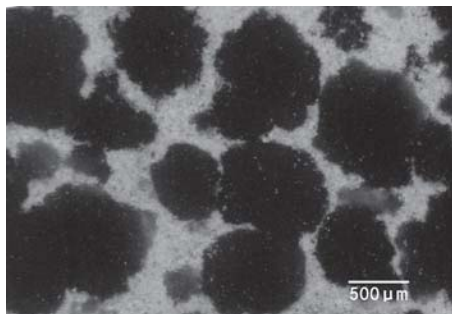


Fig. 8.5. Micrograph using a stereomicroscope of a cross section of bloomed chocolate sample of Type 2-A without tempering.

brown sections had spread to fill the remaining spaces. Unlike Type 1, Type 2-A does not show needlelike fat crystals on its surface (Fig. 8.5). The results of a component analysis of dark brown and light brown sections are shown in Table 8-A. The fat content in the light brown section was definitely lower than in the dark brown part, which is likely the cause of the former's pale appearance. This is the same phenomenon as that observed when the fat content in cocoa liquor is reduced, which results in a lighter color.

The development process of bloom in chocolate produced without tempering is shown in Fig. 8.6. Non-tempered chocolate was melted at 60°C, cooled to 30°C, poured into a polycarbonate mold, and left at 20°C for 24 hours. The demolded sample was then stored at 20°C. The external appearance was the same as correctly produced chocolate immediately after preparation, but light brown patches gradually developed and spread until the light brown sections surround dark brown spheres. The spherical dark brown parts are reported to be β -V polymorphs of cocoa butter (Kinta & Hatta, 2005). From this observation and the fact that the dark brown spherical sections have a high fat content, particularly Sat-O-Sat, the major component of cocoa butter (Table 8-A), the developing mechanism of Type 2-A can be deduced as follows.

Table 8-A. Fat and TAG^a Concentrations of Chocolate Before Developing Bloom, Dark and Light Brown Parts in Type 2-A Untempered Bloomed Chocolate (wt%).

	Chocolate before bloom	Dark brown part	Light brown part
Fat content	38.0	39.4	21.2
PLO	0.8	0.8	1.4
PLP	1.7	1.8	3.3
OOO	0.5	0.6	1.0
SLO	0.6	0.6	1.2
POO	3.0	3.1	6.3
PLS	3.1	3.2	5.2
POP	15.2	15.4	15.9
PPP	0.4	0.3	0.4
SOO	4.2	4.2	8.4
SLS	1.9	1.9	2.5
POS	35.9	36.1	25.5
PPS	0.4	0.3	1.7
A00+ALS	0.4	0.3	0.2
SOS	25.4	25.1	11.7
SPS	0.5	0.4	2.5
SOA	1.4	1.3	0.8
other	4.6	4.6	12.0
Total	100.0	100.0	100.0
Sat-O-Sat ^b	77.9	77.9	53.9

^aA: arachidic acid; L: linoleic acid; O: oleic acid; P: palmitic acid; S: stearic acid

^bSat-O-Sat: POP, POS, SOS, SOA

**Fig. 8.6.** Development of Type 2-A bloom on chocolate prepared without tempering. (A) Immediately after preparation, (B) After 12 days, (C) After 30 days.

(i) Cocoa butter crystals in chocolate melt and disappear completely if the temperature rises above the melting point of cocoa butter. (ii) As the temperature drops, very few β crystal nuclei gradually form. (iii) Fat, especially Sat-O-Sat, crystallizes around the β crystal nuclei, grow while reducing their volume and incorporating nearby components (such as cocoa power and sugar), and form spherical anhedral (xenomorphic) crystals in preference to euhedral crystals. (iv) Components with low fat content remain and turn light brown.

While Type 1 bloom is characterized by euhedral separation of components during the crystallization of fat, Type 2-A is characterized by anhedral separation of components during the crystallization of fat. It is chiefly fat that undergoes the spatial movement that leads to component separation. Solid particles other than fat generally remain in place. It can thus be stated that only melted fat moves through the spaces between static non-fat solid particles. During the observations shown in Fig. 8.6, the first visible change was the development of small light brown dots, which gradually grew larger. This visible change on the chocolate surface was simply the process in which light brown sections spread as a result of the spherical growth of fat crystals around β crystal nuclei. As aggregates of fat crystals developed around nuclei, dark brown spheres grew while incorporating nearby cocoa powder particles. The driving force was crystal formation of fat around β crystal nuclei.

The size of a dark brown sphere, which is formed within a light brown section, depends inversely on the number of β crystal nuclei from which it grew. The smaller the number of crystal nuclei, the larger the sphere grows; and the larger the number, the smaller the sphere (Kinta & Hartel, 2010). The number of β crystal nuclei is mainly determined by the environment, chiefly the thermal environment, in which the nuclei develop from a state in which there are no seed crystals (van Malssen et al., 1999).

Identification of Type 2-A Bloom

The simplest method is observation with the naked eye. If there are dark brown mottles in a light brown substrate, it is Type 2-A bloom. The shape and size of the mottles vary according to the development process, and are difficult to observe by eye when they are small. In this case, a microscope is helpful, preferably an optical microscope to obtain color information.

Type 2-A bloom is caused by uneven distribution of fat content. Low fat content results in a light brown color. Serious bloom of this type is easy to identify, since the fat content in the light brown section is extremely low, making that section powdery and dry. To allow detailed and reliable identification, the fat content of the dark brown and light brown sections should be measured. However, care must be taken when there is little difference in fat content between the sections, since Type 3 bloom, which is described below, may also show lower fat content in the light brown section than in the dark brown parts. Component analysis methods include chemical

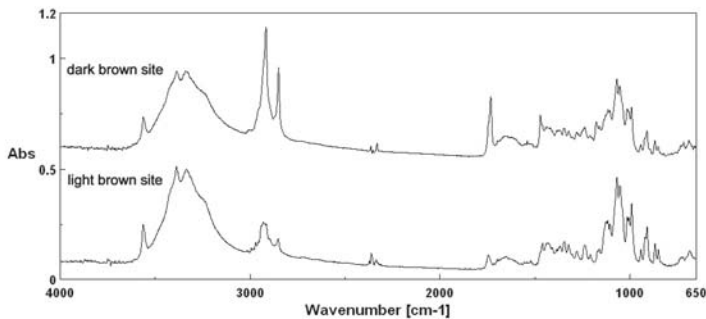


Fig. 8.7. Infrared spectra of dark brown and light brown parts of Type 2 bloom.

analysis, FT-IR and differential scanning calorimetry (DSC). Chemical analysis of fat content, using Soxhlet extraction, has been reported (Kinta & Hatta, 2005), and can be used to determine Type 2-A based on the low fat content in the light brown part. TAG component analysis is one way to definitively discriminate Type 2-A from Type 3 bloom. It can determine Type 2-A bloom by showing the content of Sat-O-Sat, which are the main component of cocoa butter, is high in the dark brown parts and low in the light brown section (Table 8-A). FT-IR microscopes can analyze small specimens and determine Type 2-A by showing that the absorbance is low at 1,750 and 2,900 cm^{-1} , which correspond to the C and O double bond in esters and the C and H single bonds, respectively, of fats, in the light brown section (Fig. 8.7) (Kinta & Hartel, 2010). DSC can also be used to determine the components of the dark brown and light brown sections. DSC determines fat and sugar content by measuring fat and sugar melting enthalpies. Its use has been reported by Lonchampt and Hartel (Lonchampt & Hartel, 2006).

Type 2-B Bloom

Morphology and Developing Mechanism of Type 2-B bloom

Like Type 2-A bloom, Type 2-B bloom is caused by uneven distribution of fat content, which results in light color in areas of low fat content. Because the process of development is slightly different from that of Type 2-A, Type 2-B has a different appearance (Fig. 8.8). It appears that the development of bloom is determined by the numbers of β crystal nuclei of fat generated during the tempering process (Schlichter-Aronhime & Garti, 1988). Type 2-A develops when chocolate solidifies from a state in which there are no β crystal nuclei. On the other hand, Type 2-B develops when β crystal nuclei are present, but their numbers are insufficient to allow full tempering (Kinta & Hartel, 2010). The former is mainly observed when chocolate is exposed to high temperatures until its cocoa butter completely melts,



Fig. 8.8. External appearance of Type 2-B bloom on poorly tempered chocolate.

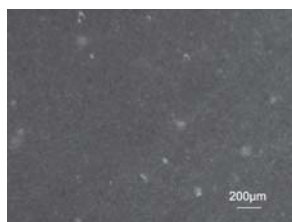
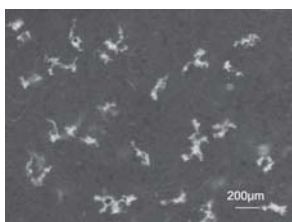
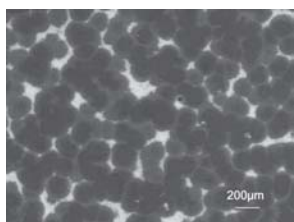


Fig. 8.9. Stereomicroscopic images of the surface of chocolates made with different levels of seed addition. (A) 5.5 ppm seeds in fat (2 ppm in chocolate), (B) 270 ppm seeds in fat (100 ppm in chocolate), (C) 1370 ppm seeds in fat (500 ppm in chocolate).

and is then solidified. The latter is observed when tempering has been inadequate and the process poorly tempered.

Crystallization of fat progresses around β crystal nuclei. The spherical crystallized sections of relatively high fat content become dark brown, and the matrix of relatively low fat content appears light brown, as in Type 2-A. While the development process of Type 2-A involves the formation of spherical crystals from a state in which there are no β crystal nuclei via spontaneous generation of β crystal nuclei, there are a certain number of β crystal nuclei in Type 2-B. During simple cooling, spontaneous generation of β crystal nuclei usually takes place rarely and slowly; and crystallization around the nuclei is also slow when there are few β crystal nuclei (Kinta & Hartel, 2010). Type 2-A thus takes a relatively long time to develop and has relatively large dark brown spheres. On the other hand, Type 2-B does not depend on nucleus formation because β crystal nuclei are already present. It undergoes fast crystal growth around the nuclei, develops bloom fast, showing small dark brown spheres. If tempering fails, the resultant poorly tempered chocolate develops bloom just after cooling and solidification. As the number of β crystal nuclei increases, the dark brown spheres that grow around the nuclei increasingly overlap with each other, and the light brown sections with low fat content shrink and finally disappear (Fig. 8.9) (Kinta & Hartel, 2010). This is the state under which the tempering of chocolate should ideally be performed.

Identifying Type 2-B Bloom

In principle, Type 2-B bloom can be determined using the same analytical methods as Type 2-A, because the bloom is caused by uneven distribution of fat content and shows low fat content sections of a lighter brown color. Because the phases (dark brown mottles and light brown sections with low fat content) are more finely dispersed than in Type 2-A, it is difficult to obtain samples from each separate section. FT-IR microscopes and DSC are thus effective tools, since they can analyze even very small samples.

Type 3 Bloom

Morphology and Developing Mechanism of Type 3 Bloom

A new morphology of fat bloom has been reported, which does not belong to Type 1, which is caused by deposition of separated fat on the surface; or Type 2, which is caused by uneven distribution of fat content and has light brown parts of low fat content (Kinta & Hatta, 2007). The new type (Type 3) has sections of different colors (dark brown and light brown) as in Type 2; but the light brown sections are either in streamlined form or entirely light brown (Fig. 8.10). It has been frequently and daily observed in chocolate that is repeatedly exposed to cyclic temperature changes up to a temperature not exceeding the melting point of cocoa butter. The sample in Fig. 8.10 was prepared by exposing a demolded sample to a cycle of temperatures between 20 and 32°C at 12-hour intervals to induce development of bloom by partial liquefaction of fat. In one week, the chocolate sample developed bloom surrounded by a light brown color. The components of the dark brown and lighter brown sections, shown in Table 8-B, were analyzed. As in Type 2, the fat content was lower in the light brown section than in the darker brown section. However, a fat content of 31.0% should still give a dark brown color. When a light brown piece was removed and heated to 32°C in an undisturbed condition, the color did not change. On the other hand, when light brown sections in contact with dark brown parts

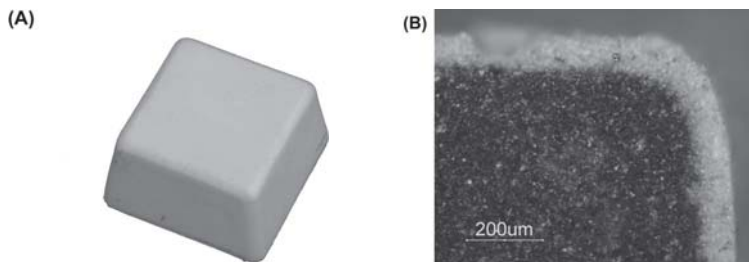


Fig. 8.10. (A) External appearance of Type 3 bloomed chocolate due to the partial liquefaction of fat and (B) a micrograph of a cross section of the sample by optical microscopy.

Table 8-B. Fat and TAG^a Concentrations in Chocolate Before Developing Type 3 Bloom as a Result of The Partial Liquefaction of Fat; Dark and Light Brown Parts of Bloomed Chocolate; and Light-Brown Parts of Bloomed Chocolate Heated to 32°C in Contact with the Dark Brown Parts That Have Turned Dark Brown (wt%).

	Chocolate before bloom	Dark brown part	Light brown part	Light brown part at 32°C
Fat content	38.0	38.1	31.0	38.8
PLO	0.8	0.8	0.3	0.8
PLP	1.7	1.7	1.2	1.7
OOO	0.5	0.6	0.6	0.9
SLO	0.6	0.6	0.4	0.6
POO	3.0	3.1	1.9	3.3
PLS	3.1	3.0	2.2	3.1
POP	15.2	15.1	13.0	14.6
PPP	0.4	0.5	0.3	0.3
SOO	4.2	4.5	2.5	4.3
SLS	1.9	1.8	1.4	1.8
POS	35.9	35.5	37.1	34.6
PPS	0.4	0.4	0.5	0.4
AOO+ALS	0.4	0.3	0.5	0.4
SOS	25.4	24.8	30.7	25.6
SPS	0.5	0.5	0.6	0.5
SOA	1.4	1.4	1.6	1.4
other	4.6	5.4	5.2	5.7
Total	100.0	100.0	100.0	100.0
Sat-O-Sat ^b	77.9	76.8	82.4	76.2

^aA: arachidic acid; L: linoleic acid; O: oleic acid; P: palmitic acid; S: stearic acid

^bSat-O-Sat: POP, POS, SOS, SOA

were heated, they turned dark brown, the same color as the darker parts (Fig. 8.11). The TAG components of the part that was light brown prior to heating and turned ordinary dark brown after heating are shown in Table 8-B. It contained more oil components than before heating. This bloom occurred because oils moved from the dark brown parts to the light brown section. The developing mechanism of this type of bloom involves the exterior turning light brown due to movement of oils toward the center of the chocolate.

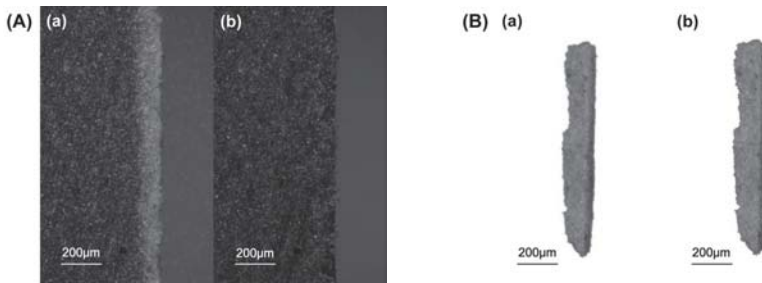


Fig. 8.11. Optical micrographs of (A) a cross section of a Type 3 chocolate sample that has bloomed due to partial liquefaction of fat and (B) the light-brown part separated from the bloomed sample, (a) at room temperature and (b) at 32°C.

It has been reported that crystals of cocoa butter are β -VI polymorphic in both dark and light brown sections of bloomed chocolate (Kinta & Hatta, 2007).

Judging by these results and the fact that the light brown portion contains a great deal of Sat-O-Sat, the main component of cocoa butter (Table 8-B), the developing mechanism of Type 3 bloom involves the following processes.

(i) Crystals of cocoa butter partially melt when they are heated to just below their melting temperature. Polymorphic transition from β -V to β -VI gradually takes place mainly via oil-mediated transformation (Sato & Koyano, 2001). (ii) Cooling causes recrystallization as β -V or β -VI because β seed crystals are present. Contraction occurs and draws the oils toward the center of the chocolate. (iii) Repetition of this series of processes leads to the formation of a high melting point framework and increases the quantity of oils separated from the component. (iv) Condensation is induced by solidification, which further draws oils toward the center of the chocolate. It leaves a high melting point framework on the surface, which turns light brown and forms a visible bloom.

Repetitive partial melting and recrystallization caused by temperature cycles triggers not only the polymorphic transition of cocoa butter from β -V to β -VI but also the separation of TAG components into high melting point components and low melting point fractions (Manning & Dimick, 1984), causing a coarsening of the fat crystal structures. These phenomena are likely to increase the amount of separated oils, facilitate the movement of oils toward the center of the chocolate during cooling crystallization, and increase the roughness and/or porosity of the microstructure (which affects the light scattering properties of the high melting point framework resulting from the movement of the oils), thus giving it a light brown appearance. Loisel et al. (1997) mentioned that there is a porous matrix partly filled with liquid cocoa butter fractions in chocolate and that the vacant spaces account for 1 to 4% of total volume in chocolate with a 31.9% cocoa butter con-

tent. It is likely that the coarsening of the fat crystal structure and movement of oil components rearrange the spatial distribution of the vacant spaces and thus affect the color. The surface morphology of Type 3 bloom has been investigated using instruments for measuring surface irregularities. The results showed that the bloom increased the irregularity of the surfaces (Hodge & Rousseau, 2002; Rousseau et al., 2010). It can be concluded that the visible color change in this type of bloom is not due to component separation on the surface but is caused by a change in the irregularity of the surface itself.

In general, Type 3 bloom is not caused by temperature cycles in which the peak temperature is low. This is because only a small percentage of oils melt even at the highest temperature, resulting in little separation of TAG components and little coarsening of the fat crystal structures. However, polymorphic transition of cocoa butter from βV to βVI may take place under these conditions. Other than cyclic temperature changes, conditions that induce separation of TAG components and coarsening of fat crystal structures may also cause Type 3 bloom.

Identification of Type 3 Bloom

Like Type 2 bloom, the light brown part of Type 3 bloom contains less fat than in the dark brown counterpart, but this difference is not the direct cause. It can, however be used to identify Type 3 bloom. In Type 3, the light brown section turns dark brown, or the ordinary color of the chocolate, when it is excised alone, heated, stirred and solidified again. On the other hand, the light brown parts of Type 2 remain light brown.

For definitive discrimination from Type 2 described above, TAG component analysis can be used. Type 3 bloom can be identified by confirming that the content of Sat-O-Sat, which are the major component of cocoa butter, are higher in the light brown portion compared with its dark brown counterpart (Table 8-B).

Other Types

There are other types of bloom for which the conditions under which bloom developed (or which directly caused the bloom) have been reported, but whose resultant morphologies are not known. Lonchampt & Hartel (2006) cooled over-tempered chocolate slowly to develop bloom and investigated its composition. However, they found no clear difference in composition from ordinary chocolate without bloom.

Summary

The characteristics of chocolate fat bloom are summarized in Table 8-C, with each type classified according to its morphology.

Table 8-C. Characteristics of Chocolate Fat Bloom for Each Type of Morphology.

	Type 1	Type 2	Type 3
Color of the discolored part	White	Light brown	Light brown
Fat content in the discolored part	Almost 100%	Lower than before bloom development	Slightly lower than before bloom development
Fat composition in the discolored part	Same or slightly higher melting point than before bloom development	Less Sat-O-Sat ^a than before bloom development	More Sat-O-Sat ^a than before bloom development
Reasons for discoloration	The color of separated fat on the surface and/or scattering of light by the fat	Roughness and/or porosity of the microstructure caused by reduced fat content	Roughness and/or porosity of the microstructure
Developing mechanism	Euhedral component separation on the chocolate surface accompanying crystallization of fat	Anhedral component separation accompanying crystallization of fat	Coarsening of fat crystal network and liquid fat migration

^aSat-O-Sat: POP, POS, SOS, SOA

Conclusions

All transitions occur as a move toward states with lower free energy. Chocolate bloom is no exception. Chocolate undergoes phase separation, or bloom, via polymorphic transition and Ostwald ripening. The resultant bloom morphology can vary depending on the form of the sample and the preservation conditions. Bloom with the same morphology may have a different appearance with respect to size and extent, possibly due to very small changes in conditions. Thus, care is needed when identifying different types of bloom. Chocolate is a man-made foodstuff prepared to be as uniform as possible in composition, but it is thermodynamically unstable. It will move towards and ultimately reach its most stable energy state. Because it is impossible to stop this process, the only recourse is to delay the transition by modifying its kinetics. A full understanding of chocolate's morphology is anticipated to lead to kinetic control of the routes and changes that lead to its lowest energy state that will delay the development of bloom.

References

- Adenier, H.; Chaveron, H.; Ollivon, M. Mechanism of fat bloom development on chocolate. In *Shelf Life Studies of Foods and Beverages*; Elsevier Science: London, 1993, 353–389.

- Bennema, P.; Vogels, L.J.P.; de Jong, S. Morphology of β phase monoacid triacylglycerol crystals: theory and observations. *J. Cryst. Growth* 1992, 123, 141–162.
- Bricknell, J.; Hartel, R.W. Relation of fat bloom in chocolate to polymorphic transition of cocoa butter. *J. Am. Oil Chem. Soc.* 1998, 75, 1609–1615.
- Cebula, D.; Ziegler, G. Studies of bloom formation using X-ray diffraction from chocolates after long-term storage. *Fat Sci. Technol.* 1993, 95, 340–343.
- Cerbulis, J.; Clay, C.; Mack, H. The composition of bloom fat in chocolate. *J. Am. Oil Chem. Soc.* 1957, 34, 533–537.
- Chapman, G.M.; Akehurst, E.E.; Wright, W.B. Cocoa butter and confectionery fats. Studies using programmed temperature X-ray diffraction and differential scanning calorimetry. *J. Am. Oil Chem. Soc.* 1971, 48, 824–830.
- Chaveron, H.; Ollivon, M.; Adenier, H. Blanchiment gras. Migration des matières grasses dans les produits composites. *Chocolaterie-confiseries de France* 1976, 328, 3–11.
- Hartel, R.W. Phase transitions in chocolate and coatings. In *Phase/State Transition in Foods*; Marcel Dekker: New York, 1998, 217–251.
- Hartel, R.W. Chocolate: fat bloom during storage. *Manuf. Confect.* 1999, 79 (5), 89–99.
- Hodge, S. M.; Rousseau, D. Fat bloom formation and characterization in milk chocolate observed by atomic force microscopy. *J. Am. Oil Chem. Soc.* 2002, 79, 1115–1121.
- Jewell, G. G. Some observations on bloom on chocolate. *Rev. Int. Choc.* 1972, 27, 161–162.
- Kinta, Y.; Hartel, R.W. Bloom formation on poorly-tempered chocolate and effects of seed addition. *J. Am. Oil Chem. Soc.* 2010, 87, 19–27.
- Kinta, Y.; Hatta, T. Composition and structure of fat bloom in untempered chocolate. *J. Food Sci.* 2005a, 70, S450–S452.
- Kinta, Y.; Hatta T. Morphology of fat bloom in chocolate. *J. Am. Oil Chem. Soc.* 2005b, 82, 685.
- Kinta, Y.; Hatta, T. Composition, structure, and color of fat bloom due to the partial liquefaction of fat in dark chocolate. *J. Am. Oil Chem. Soc.* 2007, 84, 107–115.
- Loisel, C.; Lecq, G.; Ponchel, G.; Keller, G.; Ollivon, M. Fat bloom and chocolate structure studied by mercury porosimetry. *J. Food Sci.* 1997, 62, 781–788.
- Lonchampt, P.; Hartel, R.W. Fat bloom in chocolate and compound coatings. *Euro. J. Lipid Sci. Technol.* 2004, 106, 241–274.
- Lonchampt, P.; Hartel, R.W. Surface bloom on improperly tempered chocolate. *Euro. J. Lipid Sci. Technol.* 2006, 108, 159–168.
- Manning, D.M.; Dimick, P.S. Cocoa butter crystallization. P.M.C.A. Proceedings of the 38th Annual Production Conference, P.M.C.A. Production Conference Committee: Drexel Hill, 1984; 29–33.
- Martin, R.A., Jr. Chocolate. In *Advances in Food Research* Volume 31; Academic Press: San Diego, 1987, 211–342.
- McCarthy, M.J.; Reid, D.S.; Wei, D. Fat bloom in chocolate. *Manuf. Confect.* 2003, 83 (9), 89–93.
- Neville, H.A.; Easton, N.R.; Bartron, L.R. The problem of chocolate bloom. *Food Technol.* 1950, 4, 439–441.

- Rousseau, D.; Smith, P. Microstructure of fat bloom development in plain and filled chocolate confections. *Soft Matter* 2008, 4, 1706–1712.
- Sato, K.; Koyano, T. Crystallization properties of cocoa butter. In *Crystallization Processes in Fats and Lipid Systems*; Marcel Dekker: New York, 2001, 429–456.
- Schlichter-Aronhime, J.; Garti, N. Solidification and polymorphism in cocoa butter and the blooming problems. In *Crystallization and Polymorphism of Fats and Fatty Acids*; Marcel Dekker: New York, 1988; 363–393.
- Seguine, E. Diagnosing chocolate bloom. *Manuf. Confect.* 2001, 81 (11), 45–50.
- Steiner, E.; Bonar, A. Separation of some glycerides of cocoa butter by paper chromatography. *J. Sci. Food Agric.* 1961, 12, 247–250.
- van Malssen, K.; van Langevelde, A.; Peschar, R.; Schenk, H. Phase behavior and extended phase scheme of static cocoa butter investigated with real-time X-ray powder diffraction. *J. Am. Oil Chem. Soc.* 1999, 76, 669–676.
- Whymper, R.; Bradley, A. The setting of cocoa butter with special reference to the development of bloom on the chocolate. *J. Soc. Chem. Ind.* 1925, XLIV, 77–86.
- Wille, R.; Lutton, E. Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* 1966, 43, 491–496.

9

Effect of Minor Components on Cocoa Butter Polymorphism and Kinetics of Crystallization

Adam Lechter

ADM Cocoa, 12500 W Carmen Ave., Milwaukee WI 53225

Introduction

Many factors affect the chemical composition of a natural vegetable fat, and cocoa butter is no different. Climate, tree genetics, disease stress, and cocoa bean handling post-harvest and physical refining are some of the major factors that influence both the triglyceride composition and minor lipid content and profiles of a given cocoa bean and its fat. In addition, post processing steps such as oil refining and deodorization can have dramatic effects on the minor components of cocoa butter. This chapter will explore the minor components in cocoa butter and the influence which some of those components have on the crystallization of the overall cocoa butter. Many minor components used as additives have been studied in chocolate for their ability to change flow properties, retard bloom, or change crystallization behavior. This chapter will only focus on those minor components which are indigenous to cocoa butter.

Minor Components

Plant systems contain many different types of fatty material that serve different biological purposes. During oil expression from a seed such as cocoa butter, much of this non-polar material is expressed with the bulk of the fat (triglycerides). There are many types of non-triglyceride or partial glyceride material carried in the bulk, some of the more important chemicals of interest from both an oil stability and crystallization point of view are the mono (MAG) and di-glycerides (DAG), free fatty acids (FFA), phospholipids (PL), hydrocarbons, sterol and sterol-esters, lipid peroxides, tocopherols, soaps of fatty acids, and many more. Nonlipid material can also be present in cocoa butter. These minor components include minerals, metals, moisture, and non-fat cocoa solids.

As Smith et al. (2011) explain there is no firm definition of what concentration level defines a minor component. Depending on the mechanism by which a given minor component acts will help to define when its concentration will affect

crystallization. Herrera and Rocha (1996) explain that if a minor component is incorporated into the crystal network, it will likely require 0.5–5wt% concentrations; where if a minor component only participates in nucleation, a concentration of less than 0.1% may be significant. Minor components may also hinder crystal growth by blocking sites on crystal surfaces for further growth. Triglyceride profile (Shukla 1995) is probably the most important factor in determining much of a cocoa butter's crystallization behavior. However oftentimes, cocoa butters with nearly identical triglyceride profiles exhibit remarkably different crystallization behavior, such as changes in time to nucleate, crystallization kinetics, equilibrium solid fat contents, and microstructure differences. One then must look to understand the chemical differences in the minor lipid profiles to help explain these crystallization differences.

Cocoa butter, unlike many other vegetable fats available to the baker or confectioner, is typically expressed from the ground cocoa bean (nib) by mechanical means. Most other commercially available vegetable fats utilize a solvent extraction step. This type of non-solvent extracted cocoa butter is typically referred to as prime, pure, and pressed. Following the expression of the fat from the non-fat material the cocoa butter is typically only filtered to remove particulate and then could be directly used in chocolate production. However, some further processing steps, common to other commercial fat and oil production, can also be used to modify the cocoa butter in terms of flavor, color, and composition. This refining may include deodorization, bleaching, and degumming. These treatments serve as a way to typically remove non-triglyceride material from the bulk. So, unlike most other vegetable fats used in the confectionery industry which are always fully refined to remove much of the minor lipid component, cocoa butter may still have a complex mixture of fatty material because it typically is utilized in chocolate production without undergoing further refining. In understanding crystallization in most other vegetable fat systems that are fully refined to remove polar lipids, free fatty acids, and phospholipids a scientist is basically studying a mixture of triglycerides. The study of cocoa butter crystallization is much more complex in that all of this other non-triglyceride material may influence crystallization as well.

Table 9-A. Minor Lipid Composition by Commercially Available Cocoa Butters by Geography.

Component	Malaysia	Ivory Coast	Ecuador	Brazil (Bahia)
DAG (%wt)	2.05	1.67	1.41	2.01
MAG (%wt)	0.02	0.02	0.00	0.06
FFA (%wt)	1.59	2.13	0.77	1.62
Phosphorus (ppm)	81.5	45.3	33.0	42.4
Total Sterols (%wt)	0.19	0.21	0.20	0.19

Free Fatty Acids

Deodorization is the most common form of refining practiced on cocoa butter, if any refining is practiced at all. In Europe, cocoa butter must comply with the European Union Directive 2000/36/EC, which states that cocoa butter must contain less than 1.75% FFA (European Union, 2000). In the United States no such legal limit exists, however most commercial specifications still utilize this 1.75% threshold. In cocoa butter, deodorization is quite typical to lower the level of FFA in butters above this level, and also reduce flavor. However, deodorization needs to be done with care. Deodorization can lead to darkening of the oil due to phospholipid content, reduction in desirable flavors of cocoa butter, intra or inter-esterification of triglycerides, creation of trans fatty acids, and polymerization reactions. Keeping these negative outcomes from happening, especially preventing intraesterification, while removing FFA typically means the industry uses lower deodorization temperatures than might be found in other vegetable oils. Typical temperatures used in deodorization are 104–110°C (Minifie 1989), and 130–180°C (Timms & Stewart, 1999). In addition to temperature, steam and vacuum conditions also effect the removal of FFA from cocoa butter during deodorization. Deodorization is very effective at removing FFA from cocoa butter, but because FFA present is typically present due to postharvest hydrolysis, there is also a relatively equal amount of DAG present. We do not typically find high levels of MAG in cocoa butter. Thus, even a good deodorization step to reduce FFA, will still yield a cocoa butter with higher DAG content than the final FFA concentration. Impacts of DAG content will be discussed later in this chapter. Additionally, like any oil refining step, each step will produce product losses as the product is further refined. Given that cocoa butter is one of the most expensive edible fats, oftentimes refining oil losses can cause significant financial loss to the producer.

As described previously, FFA found in cocoa butter is predominately due to degradation of beans during transport and storage by fungal lipase enzymes. Therefore in some cases high levels of FFA can be an indicator for poor quality beans as these beans may have been mishandled and contained higher amounts of broken beans, moldy, or diseased beans.

Recent research shows that FFA content affects crystallization kinetics and equilibrium solid fat contents (SFC) of cocoa butter. According to Ayala et al. (2007), solid fat contents at 20, 25 and 30°C increased as a function of a decrease in FFA. Strong linear correlation is also noted for SFC v. FFA measured at these temperatures. The authors also note that differences in solid fat is simply not mathematically equal to that of uncrystallized FFA, but that it also is attributed to additional TAG that is not crystallized as well. It has been found in palm oil that a eutectic mixture of TAG and FFA forms (Jacobsberg, 1976). This work was later confirmed in another study by Calliauw et al. (2008), where the authors find SFC values increased in between 20–30°C as deodorization conditions were more severe. Also, no SFC differences existed above 35°C, which would be an indication of the presence or formation of

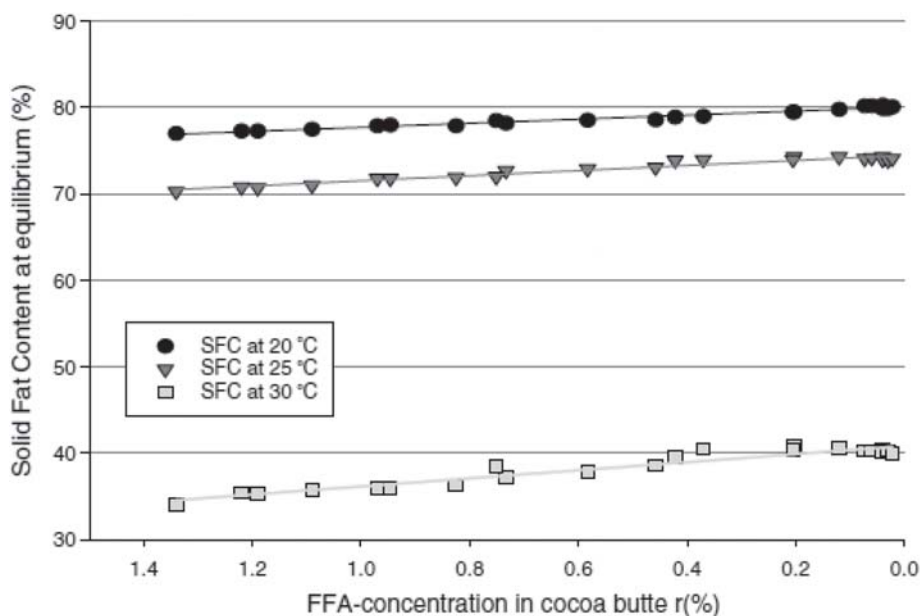


Fig. 9.1a. Solid fat content of cocoa butter, as a function of FFA. Source: Calliauw et al., 2008.

trisaturated triglycerides during high temperature deodorization. Again, the authors note the strong linear increase in SFC as FFA decrease (Fig. 9.1). Solid fat content is a very important attribute for the confectioner. Total fat solids help to increase heat stability of chocolate, increase hardness, and possibly help to delay onset of age related fat bloom. In addition, cocoa butter with higher total solids will also be able to withstand additional amounts of milkfat, which depresses total solid fat when added to chocolate.

In addition to the equilibrium effects that FFA displays on total solid fat, it also has effects on crystallization kinetics. Ivory Coast cocoa butter deodorized at 160°C and 200°C to reduce FFA from 2.01% in the natural cocoa butter to 0.96% and 0.07% respectively helped to increase crystallization kinetics and equilibrium solid fat contents between 20 and 30°C. At 22°C crystallization rates for the deodorized samples were faster than that of the feed material. This can be noted by both isothermal NMR and DSC results shown (ref. Figure 2-6 from Lechter & Widlak, 2011).

Initial crystallization at 22°C seems to be happening at relatively equal levels as shown in the isothermal NMR curves. However, there is a much greater delay until the main crystallization event occurs in the feed material that is not seen in both deodorized samples. This shows that at 22°C the FFA present at about 2% both delays time till the main crystallization and once that event does occur it occurs at a slower rate.

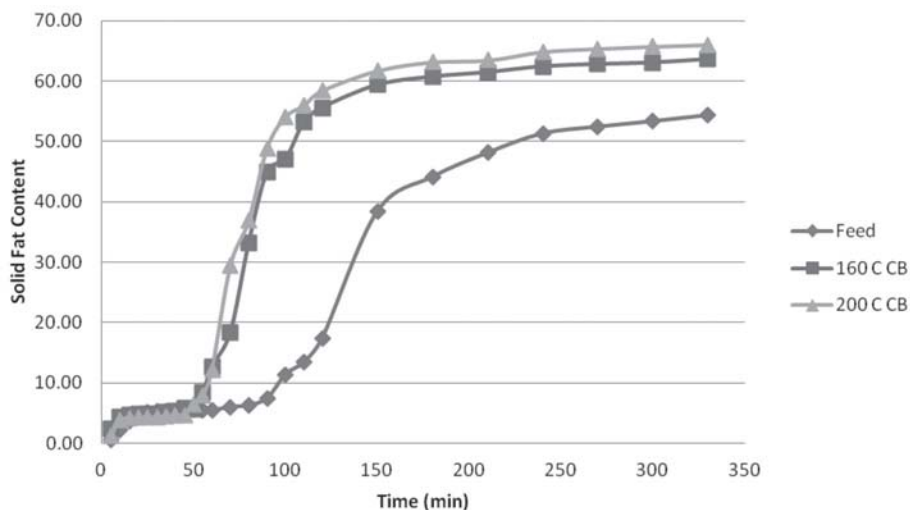


Fig. 9.1b. Solid fat content vs. time during static crystallization at 22°C. Feed (natural Ivory Coast cocoa butter FFA = 2.01%), 160 C CB (cocoa butter deodorized at 160°C, FFA = 0.96%), 200 C CB (cocoa butter deodorized at 200°C, FFA = 0.07%).

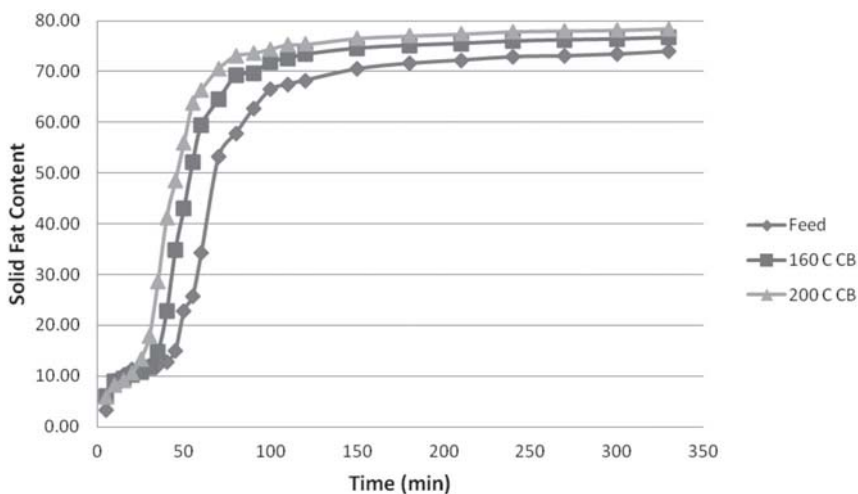


Fig. 9.2. Solid fat content vs. time during static crystallization at 20°C. Feed (natural Ivory Coast cocoa butter FFA = 2.01%), 160 C CB (cocoa butter deodorized at 160°C, FFA = 0.96%), 200 C CB (cocoa butter deodorized at 200°C, FFA = 0.07%).

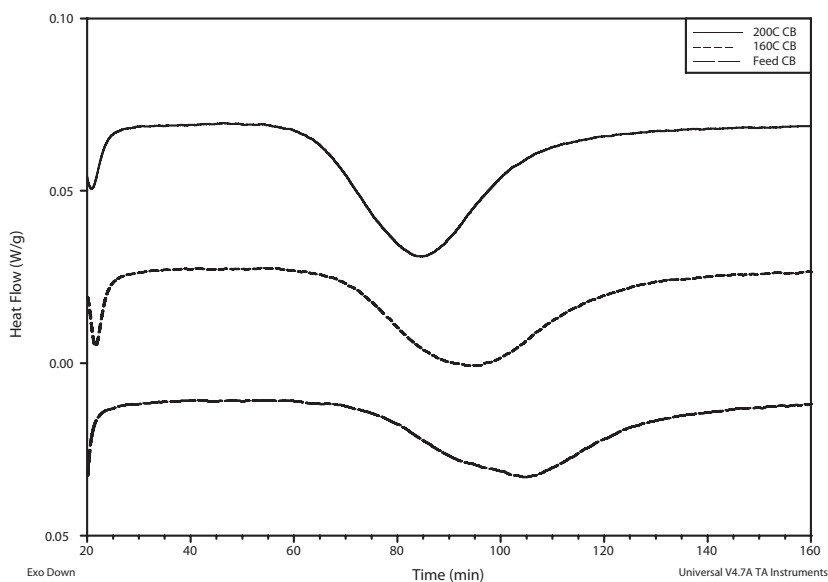


Fig. 9.3. DSC isothermal crystallization thermograms at 22°C. Feed (natural Ivory Coast cocoa butter, FFA = 2.01%), 160 C CB (cocoa butter deodorized at 160°C, FFA = 0.96%), 200 C CB (cocoa butter deodorized at 200°C, FFA = 0.07%).

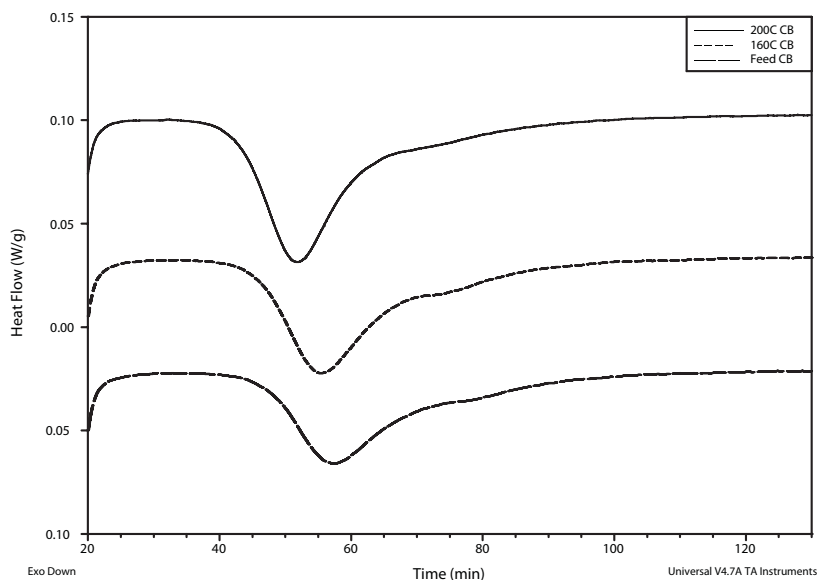


Fig. 9.4. DSC isothermal crystallization thermograms at 20°C. Feed (natural Ivory Coast cocoa butter, FFA = 2.01%), 160 C CB (cocoa butter deodorized at 160°C, FFA = 0.96%), 200 C CB (cocoa butter deodorized at 200°C, FFA = 0.07%).

In this study, after measuring crystallization for 5.5 hours (330 minutes) the various cocoa butters were allowed to remain at the given isotherm for 24 hours. After measuring solids again following 24 hours, one can see in Figs. 9.5 and 9.6 that much greater amounts of the total solids developed later in the samples that have not been deodorized. From this and inspection of the micrographs, one can assume that much greater amounts of liquid fat are being trapped in crystal network during initial crystallization and causing this delayed crystallization or post hardening. Polymorphism changes of the samples were not measured, so it has not been determined if any polymorphism differences occurred. Delayed crystallization as was observed in the feed material with elevated FFA would favor crystal growth, larger crystals and greater ability for liquid fat to migrate to the surface creating potential for bloom formation during storage. This would need to be confirmed with further study.

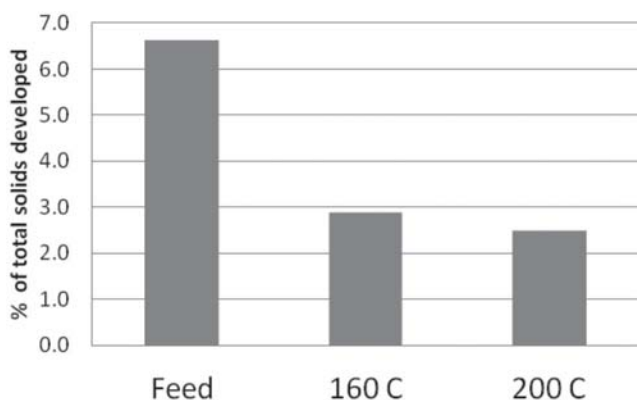


Fig. 9.5. Percentage of total solids developed in solids between 5.5 hours and 24 hours held at 20°C.

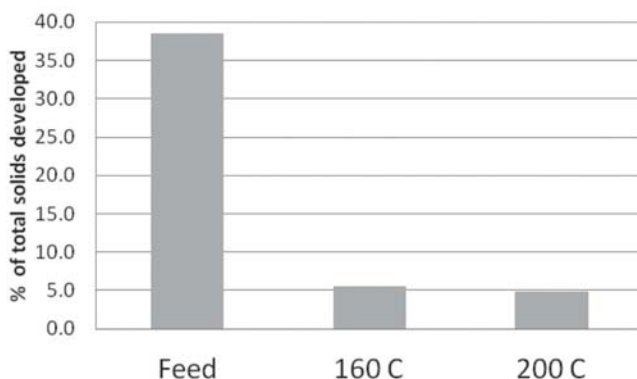


Fig. 9.6. Percentage of total solids developed in solids between 5.5 hours and 24 hours held at 22°C.

Calliauw et al. (2008) also studied the effects of steam refining on polymorphism and crystallization kinetics. During isothermal crystallization at 20°C, initial α crystallization occurred at a higher degree relative to β' in the sample which was strongly deodorized (0.02% FFA) compared to a more mildly deodorized sample containing 1.2% FFA. All samples during the time frame of the experiment recrystallize to β' , or further crystallize from melt directly to β' . It has been shown that isothermal crystallization of cocoa butters at 20°C result in initial formation of α crystals, followed by a transformation of these crystals to the β' polymorph (Dewettinck et al., 2004) (Van Malssen et al., 1999). The authors believe that it is possible that FFA is forming eutectics in cocoa butter, and that 20°C isotherm may result in a deeper subcooling for the deodorized samples. Also, the authors believe that FFA is incorporated into the crystal lattice, as they note increased intensity and consistently more compact long spacings for α crystals in lower FFA cocoa butter. This is most likely due to the interference of the free carboxyl group on the FFA causing interruption in the network and longer longitudinal arrangements.

In addition, Calliauw et al. (2008) described the isothermal β' kinetics of the deodorized cocoa butter of varying FFA contents using the Foubert Model. The rate constant (K) at FFA levels above 0.7% increased inversely proportional to the FFA level. However, at levels less than 0.6% the value of K the rate constant drops to that consistent with levels of higher FFA. However, this is due to a fixed value of n (the reverse order of the reaction) in the model, and is not necessarily due slower β' crystallization, but rather a relatively slower secondary part of the crystallization curve. Looking at the DSC data for the crystallization events of all the cocoa butters, the authors observed that the maximum slope of the crystallization event increases inversely to FFA at both levels of FFA. So initial crystallization from α to β' does happen more quickly as more FFA is removed.

Induction times of the β' event are generally decreased with lower levels of FFA. A strong linear correlation is found when induction time is plotted against FFA concentration. This helps to prove that removal of FFA helps to remove molecules that interfere in organization or reorganization of cocoa butter crystallization (Calliauw et al., 2008) (Lechter & Widlak, 2011).

FFA Effects on Microstructure

Microstructure of the cocoa butter crystal network also is an important attribute when assessing quality of cocoa butter. It is believed that a network with robust nucleation and fine well packed crystals is desired. This type of network would help to create a chocolate with high solids that would have good shine, strong snap and would help to inhibit liquid migration of fat through the crystal matrix. Initial crystallization differences were noted at 20°C (Lechter & Widlak, 2011), highly refined cocoa butter formed more numerous and finer needle like crystals. In samples with higher amounts of FFA, we found less nuclei and crystals that were more spherulitic in nature with

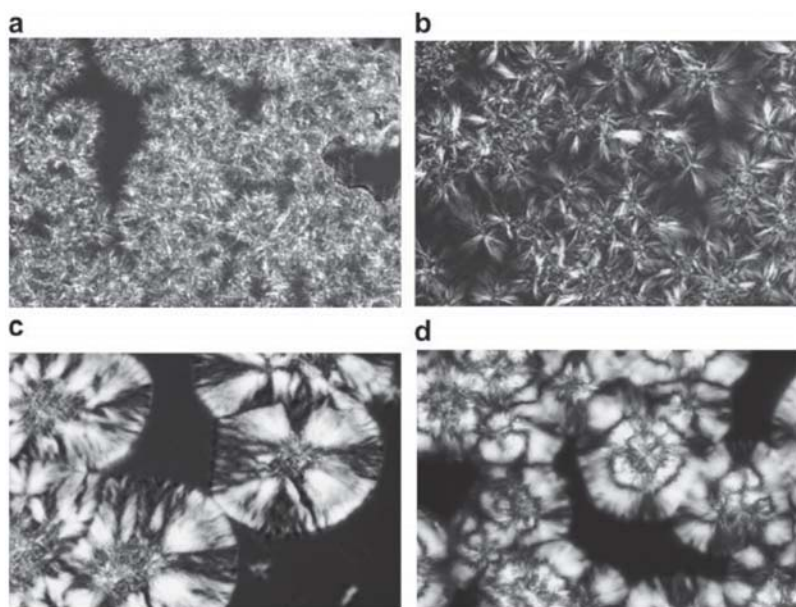


Fig. 9.7. Polarized light micrographs of cocoa butters after 40 hr at 24°C. FFA contents 1.3% (a), 0.87% (b), 0.5% (c), and 0.04% (d). Source: Calliauw et al., 2008.

more liquid fat. Structural differences were also noted after 40 hours at 24°C (Figs. 9.7 and 9.8). Lower FFA content seemed to promote conversion of needlelike spherulites to platelet like structures. It was also confirmed with WAXD patterns that these microstructural changes were not due to differences in cocoa butter polymorphs, as all were found to predominately exist in the βV form (Calliauw et al., 2008).

Diglycerides

As discussed previously, FFA levels can be quite significant in cocoa butter due to disease stress and poor segregation of diseased beans and pods or poor post bean handling practices in origin countries. In addition to the formation of FFA in beans, DAG is also formed. DAG levels in cocoa butter are well correlated with FFA levels in cocoa butter which has not been deodorized. Liberation of fatty acids from TAG in cocoa butter does not seem to drive towards generation of MAG, but only DAG. Whereas removal of FFA is fairly easy as is shown above with deodorization of cocoa butter, DAG levels remain unchanged following such a treatment. Therefore, a high level of DAG in your crude cocoa butter, regardless of basic processing will produce a finished cocoa butter with a relatively equal amount of DAG. So it is necessary to understand if and how DAG is affecting crystallization of cocoa butter.

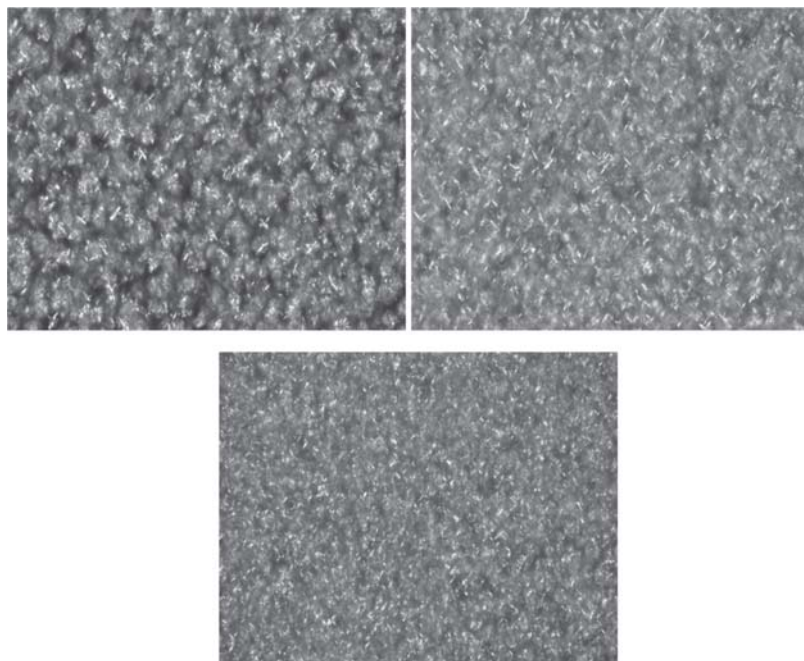


Fig. 9.8. Polarized Light Micrographs. Initial crystallization after 2 minutes at 20°C, upper left 2.01% FFA, upper right 0.96% FFA, bottom center 0.04% FFA. Source: Lechter & Widlak, 2011.

The effects of DAG on crystallization has been studied in many different fat systems including cocoa butter and related fats. Cebula and Smith (1992) studied the effects of trisaturated TAG and DAG content in Coberine, a commercially available cocoa butter equivalent. They found that both added DAG, purified from a silica column treatment of the Coberine, and trisaturated TAG increased temperature of onset for crystallization during a cooling regime on a DSC, however DAG also slowed down the rate of crystallization following this initial nucleation (Fig. 9.9).

Foubert et al. (2004) investigated the influence of overall chemical composition on cocoa butter crystallization. Twenty samples of cocoa butter were analyzed for a variety of chemical components and crystallization attributes and a model was used to understand the influences each component may have on crystallization. In terms of DAG, samples that were analyzed contained between 0.59% to 2.2%. The induction times for the primary crystallization event at 20°C isotherm were shown to be explained mostly by the percentage of total saturated fatty acids and monounsaturated triglycerides. However DAG, FFA, and phosphorus all increased induction times. During the 20°C isothermal crystallization it is the induction time of the second crys-

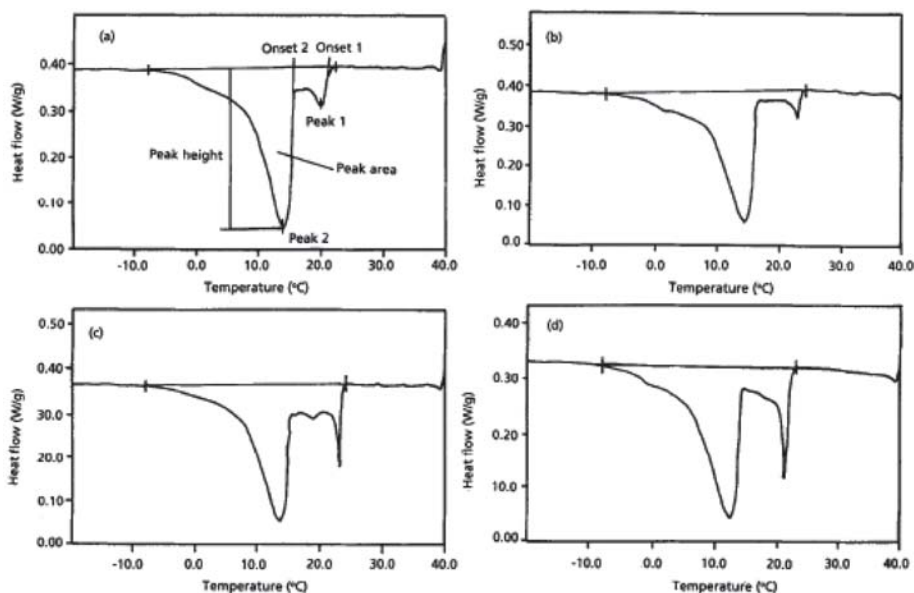


Fig. 9.9. DSC cooling thermograms of Coberine (a), Coberine + 4.4% DAG (b), Coberine + 3.9% Trisaturated TAG (c), and Coberine + 4.4% DAG + 3.9% Trisaturated TAG (d). Cooling rate 2°C/min. Source: Cebula & Smith, 1992.

tallization event that is measured. This is a β' crystallization event, however this event depends on an α to β' polymorphic transition. A small α peak can be noted on the DSC thermogram previous to the β' peak, and has been proven in previous work to be an α peak (Dewettinck et al., 2004) (Van Malssen et al., 1999). The authors note that structural irregularities in the crystal network by DAG may explain the stabilization in the α form and a retardation of the polymorphic shift. Cebula and Smith (1992) in the work on Coberine also noted the effect of DAG slowing polymorphic transitions in the TAG.

Foubert et al. (2004) found that the only crystallization parameter not affected most by ratio of saturated to unsaturated fatty acids, and monounsaturated to diunsaturated TAG was the rate constant, K . K is an explanation of the rate or kinetics of crystallization. FFA, DAG, and soaps were shown to have the most influence on this parameter. Both FFA and DAG have a negative influence on K , meaning that their presence slows the rate of crystallization. It has been explained in previous studies that DAG creates irregularities in growing crystals because of the absence of a fatty acid. The polar nature of the hydroxyl group and irregularity caused by the absence of the fatty acid chain may help to prevent TAG incorporation into the crystal lattice due to potential site blocking for further incorporation (Wright et al., 2000).

The effects of adding stearic acid, distearin, and tristearin to dark chocolate have also been studied. Chocolate was crystallized under dynamic conditions at 28.2°C to allow chocolate to directly crystallize into the β polymorph. Crystallization was quantified by measuring torque variation in a scraped surface heat exchanger. Additions of tristearin dramatically shortened the initial induction time (time of first increase in torque) of the chocolate. The authors believe that this initial response is fractionation of the trisaturated TAG crystallizing out first. Stearic acid and distearin did not dramatically affect the initial time for a torque increase; however both slowed the second increase in viscosity or the main crystallization event. This shows that while stearic acid and distearin did not affect nucleation and initial crystallization, it did slow kinetics of crystallization for the chocolate (Loisel et al., 1988).

Phospholipids

Often referred to generally as lecithin, phospholipids are similar to TAG except the third hydroxyl group is esterified with a phosphate group and a variety of different molecules. These different molecules and the fatty acids also attached to the glycerol backbone distinguish different phospholipids in terms of how they act as emulsifiers or their impacts on crystallization. Lecithin and phospholipids are often terms which are used interchangeably. Lecithin is commercially extracted from a variety of oilseeds, most predominately from soybeans. Lecithin is actually a complex mixture of phospholipids, and commercial fluid lecithins also contain added vegetable fats.

Native cocoa butter contains a mixture of phospholipids. Phospholipid content and profiles are dependent on origin and genetics of the cocoa beans and physical processing of the cocoa butter following extraction.

Much work was done in the 1990's to understand the role of phospholipids in cocoa butter crystallization and the chemical makeup of the initial crystallizing material. The initial seed crystals formed during the very first crystallization were determined to have melt points much above that of the bulk of the cocoa butter. The isolated crystals had a melting point of 62°C where the bulk material had a melting point of 33°C measured by DSC (Davis & Dimick, 1989a). The composition of the material in these initial seed crystals was also dramatically different than that of the bulk. The seed crystals contained 11% glycolipids, 6% phospholipids, and only 82% simple lipids by weight. The beginning Ivory Coast cocoa butter used in this experiment only contained 0.89% glycolipids, 0.37% phospholipids, and 98.75% simple lipid material. In addition, to this dramatic increase in polar lipid material in the initial crystals it was also noted that TAG and fatty acid composition was also different than in the bulk. This initial seed material was 50% trisaturated TAG, and total saturated fatty acids increased from 63% to 89%. Initial trisaturated TAG material was measured at 4.5% in the cocoa butter (Davis & Dimick 1989b).

The seed crystals contained the same phospholipids as were present in the original cocoa butter, however phosphatidylethanolamine (PE) and phosphatidylcholine (PC)

Table 9-B. Cocoa Butter Seed Composition.

Sample	Melting Point (°C)	Composition (% wt)		
		TAG	PL	Glycolipids
Cocoa butter	32–34	96.2	0.4	0.9
Dynamic crystallization				
Seed crystal	34–37	92.1	2.0	2.6
Static crystallization				
Seed crystal	55–62	87.7	4.0	6.0
Seed crystal	62–72	82.2	6.6	11.1

Source: Dimick, 1999.

were the major phospholipids found in the seed crystals. Further characterization of a variety of cocoa butters from different countries of origins was conducted in the same manner as the Ivory Coast cocoa butter. Again, increased phospholipids were found in five of the six cocoa butter samples (Ghana not showing an increase) seed crystals (Savage & Dimick, 1994). The authors also separated the samples into fast and slow nucleating cocoa butters. The class that was termed the slower nucleating cocoa butters had twice the phospholipid content in the seed crystals as that of the fast nucleating cocoa butter's seeds. It was found that the slower nucleating cocoa butters were richer in lyso-phosphatidylcholine (LPC) and phosphatidylinositol (PI) and low in PC. The faster nucleating butters had lower LPC and PI and higher PC. The more polar phospholipids were associated more with the slower crystallizing cocoa butters, such as LPC and PI, and the less polar phospholipids, such as PC and PE were associated with the more rapidly crystallizing cocoa butters (Savage & Dimick, 1994).

TAG differences were also observed in cocoa butters that are faster or slower nucleating in addition to phospholipids. The butters that were slow nucleating, Brazil, Ecuador, and Dominican Republic, had higher amounts of di-unsaturated TAG, and the faster nucleating cocoa butters, Ivory Coast, Ghana, and Malaysia had higher amounts of symmetrical di-saturated TAG. This is consistent for what we find in cocoa butters, African and Asian butters are more rich in "symmetrical" mono-unsaturated TAG, and the butters from South America have higher concentrations of di-unsaturated TAG (Fig. 9.10). No other differences besides the TAG and phospholipids were found in regards to MAG, FFA, or DAG content, except that DAG in the faster nucleating cocoa butters had higher stearic acid concentrations.

A simpler way to understand if phospholipids are affecting crystallization kinetics of a cocoa butter is to understand the differences between a sample of cocoa butter that is untreated and one which is degummed to remove the phospholipid material. Dimick's lab also conducted this experiment on Bahian (Brazilian) and Ivory Coast cocoa butters, and found that degummed cocoa butters also had significantly slower crystallization rates studied using absorbance techniques. In addition, when

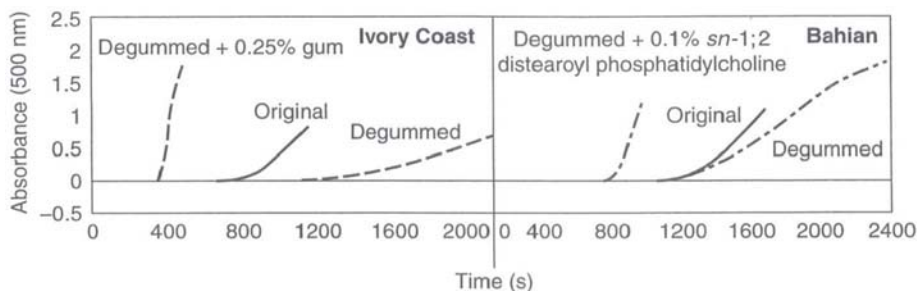


Fig. 9.10. Absorbance (crystallization) curves of Ivory Coast and Bahian cocoa butters and following different treatments. Source: Dimick, 1999.

the degummed material or sn-1,2-distearoyl phosphatidylcholine was added back to the degummed cocoa butter samples an increase crystallization kinetics and higher equilibrium solids were found at 30°C (Dimick, 1999).

Dimick later proposed an ordered structure where certain phospholipids favored inverse hexagonal phases and certain favored normal hexagonal phases. Inverse hexagonal phases favor reverse micelle type conformations where polar head groups face inwards, orienting around small amounts of moisture that may be present in cocoa butter. The outward facing non-polar tails of phospholipids or glycolipids in this conformation would help to create order or a network for which TAG to crystal from. This type of conformation would be favored by PC and PE, the phospholipids found to be more closely associated with the fast nucleating cocoa butter. In normal phase packing, micelle structures are formed with the non-polar tails face inwards creating a tube-like structure and the polar head groups are facing the bulk of the fat. This type of structure would be favored by LPC and PI in fat systems. This type of structure would not provide the ordered structure of inverse hexagonal phases to crystallize from, thus creating cocoa butters with longer induction times. The LPC and PI which create this type of geometry were found to be associated more closely with the slower-nucleating cocoa butters (Dimick, 1999).

Sterol and Sterol Esters

Sterol lipids in vegetable fats can exist as free sterols, sterol esters, sterol glucosides and acylated sterol glucosides. Sterols and their effects on cocoa butter crystallization have not been studied thoroughly, most research regarding sterols in other fat systems is in regards to their extraction and use for blood cholesterol reducing properties. As noted previously, Sidisky and Dimick (Sidisky & Dimick, 1993) found a dramatically higher concentration in glycolipid content in initial cocoa butter seed crystals. These were identified primarily as sterol glucosides and esterified sterol glucosides. In the biodiesel industry, the presence of sterol glucosides has caused some production

Table 9-C. Sterol Content of Cocoa Butter.

Sterol	Percentage			
	Free	Sterol ester	Sterol glucoside	Acylated steryl glucoside
Cholesterol	1.1	2.7	1.2	1.0
Brassicasterol		1.4	0.3	0.9
Campesterol	12.3	10.7	8.6	8.2
Stigmasterol	23.4	12.6	16.9	17.0
Sitosterol/ Stigmastanol	60.3	67.2	70.7 ^a	70.5 ^a
Isofucosterol	2.9	4.6	2.3	2.4
$\Delta 7$ -Stigmastanol		0.8	Trace	Trace

^a Sitosteryl acetate only.

Source: Staphylakis & Gegiou, 1985.

issues, namely filter plugging. Steryl glucosides have an extremely high melting point of about 240°C, so in effect these are simply “dispersed fine particles” which may help in promoting nucleation. The concentration of these compounds in the initial seed crystals may help to explain the increased melting point of the initial seeds and also to show that these may serve as a surface for TAG nucleation (Lee et al., 2007). Further work is certainly needed to fully understand how these components impact nucleation and initial crystal growth of cocoa butter.

Staphylakis and Gegiou (1985) measured sterol contents of cocoa beans using gas chromatography and gas chromatography-mass spectrometry. It was found that free sterols comprise the main fraction of sterols in cocoa butter, where sterol esters accounted for 11.5% of total sterols and glucosidic sterols for 16.3% of total sterols. It was also noted that fatty acids of sterol esters and acylated steryl glucosides were richer in unsaturated fatty acids than the total fatty acid composition of the bulk cocoa butter. Total sterol content was 339.4 mg/100g cocoa butter.

Total Minor Lipids

Other papers have looked simply at all minor lipid material, in some cases defined as non-TAG material, and how that group of material affects crystallization kinetics.

Using Mexican cocoa butter, unrefined cocoa butter was compared to cocoa butter treated in a diol column to remove polar lipids. The native cocoa butter contained $97.94 \pm 0.80\%$ TAG, $1.71 \pm 0.64\%$ MAG and DAG, and $0.35 \pm 0.15\%$ PL, following the diol treatment no MAG, DAG or PL were detected by HPLC. In addition no differences to TAG profiles were noted following treatment. It was found that shorter times were needed to complete crystallization at temperatures between 18.5 and 20°C for the cocoa butter with polar lipids removed than the native cocoa butter.

The authors attribute this “delaying effect” of the polar lipids to a retardation of α to β' transition (Toro-Vazquez et al., 2005).

The elimination of polar lipids decreases the amount of time for the transition from α to β' , and reduces time for crystallization to be completed as interpreted from rheometry data. Overall, all events involving the cocoa butter without polar lipids, both nucleation and transitions, occurred at a faster rate than the native cocoa butter (Toro-Vazquez et al., 2005). Because this study did not differentiate the minor lipid components it is difficult to understand which minor lipids are affecting nucleation differences and which may be effecting polymorphic transitions.

Foubert et al. (2004) investigated the influence of chemical composition on cocoa butter crystallization. The authors used total energy released (J/g) for each sample to indicate relative amounts of solids formed from each sample. An increase in energy released was influenced most by unsaturated fatty acid and diunsaturated TAG content. This is explained by lower melting points of β' polymorphs for POO and SOO (Hageman, 1988) and also that diunsaturated TAG causes interruption in symmetrical monounsaturated TAG packing. Additionally, FFA and DAG also had an influence on the energy released. Higher amounts of FFA and DAG had a negative effect, meaning that these constituents were also reducing solids formed, and potentially creating a metastable weaker crystalline form. Soaps were also quantified in this study. Soaps were associated with a lower rate constant, meaning they slowed the rate at which crystallization occurred. Soaps are known to be present in cocoa butter, especially following strong alkalization reactions of cocoa nibs, however their effects on crystallization of cocoa butter has not been well studied.

The effects of minor lipids from milkfat and cocoa butter in cocoa butter crystallization were also studied by Tietz and Hartel. It was found that when minor lipids were removed from milkfat using a Florisil purification or when purified minor lipids were added back at double the original content; it resulted in longer nucleation times, slower crystallization rates, and rapid bloom onset in chocolates made with 10% added milkfat. They concluded that even at the low levels which minor lipids exist naturally in milkfat affect the microstructure and crystallization of chocolate or cocoa butter-milkfat blends. Confocal imaging of crystals showed that the removal of minor lipids resulted in significantly different microstructure, without changes to polymorph (Fig. 9.11). Removal of minor lipids resulted in more irregular shaped spherulites with potentially more liquid fat included in the spherulite. The authors believed that it may be possible that the minor lipids help to organize primary crystal structure. Below are confocal micrographs comparing Ivory Coast cocoa butter crystal to pure TAG Ivory Coast cocoa butter (Tietz & Hartel, 2000).

Conclusions

Crystallization is a complex process involving nucleation, growth and polymorphism. As has been shown in this chapter, minor lipids can have an effect on all of these.

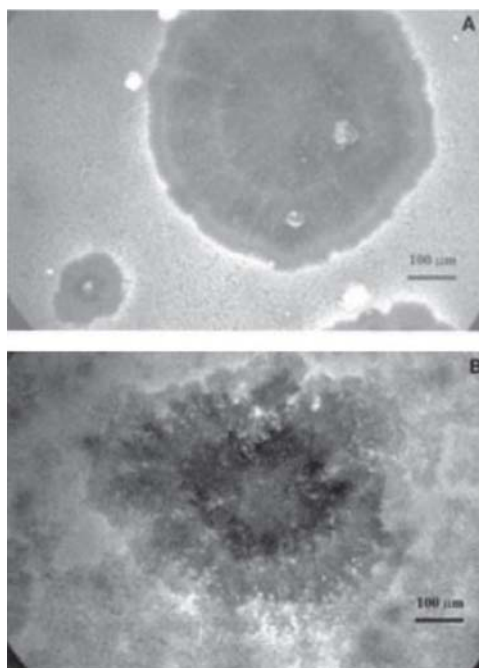


Fig. 9.11. Confocal laser scanning micrographs of Ivory Coast cocoa butter (A) and purified TAG of Ivory Coast cocoa butter (B). Sample crystallized at 25°C for 1 hr, and 10°C for 16 hrs
Source: Tietz & Hartel, 2000.

Understanding the chemical makeup of cocoa butter is important to understand how the cocoa butter will behave from a crystallization standpoint. Many cocoa butters sourced from principal growing regions that the chocolate industry relies on have quite similar TAG profiles as plant types and climates are similar. However, disease stress and post-harvest handling will significantly affect the minor lipid contents and profiles of cocoa butter. Understanding the mechanisms that these minor lipids play in crystallization and at what levels they exist in a specific cocoa butter may determine if further refining of a cocoa butter is needed or if a specific cocoa butter may not be suitable for an application. Minor lipids can influence nucleation by increasing sites for nucleation, changing temperature at which nucleation occurs, or interfering with nuclei formation. Also minor lipids can affect the rate of crystal growth, either retarding or accelerating it until the system reaches equilibrium.

Diglycerides are a great example of the complexity of minor lipids effects on crystallization. It is shown that DAG may help to increase nucleation in a cocoa butter system or give response on instrumentation that nucleation is occurring at elevated temperature. This can simply be because DAG may be crystallizing out of the TAG

bulk. However, remaining DAG in the bulk or DAG incorporated in these initial crystals inhibits crystal growth, as DAG makes a disruption in the TAG matrix that creates the crystal lattice. So one must understand the effects that a minor lipid plays in both initial crystallization and growth, in some cases a minor lipid at a given concentration may have both positive and negative effects on different aspects of crystallization.

It is noteworthy that many of the studies used in this chapter to explain the influence of minor lipids on cocoa butter crystallization use isotherms, which cocoa butter directly crystallizes into α forms and subsequently crystallizes into β' . Cocoa butter undergoes a commercial tempering process, subcools the fat, then reheats the fat to drive towards a β polymorph. The influence of minor lipids on those conditions and if it affects those conditions has not been studied thoroughly. While isotherms at colder temperatures than those employed in commercial chocolate tempering and use certainly give us an indication of the crystallization characteristics of a cocoa butter, the effects of minor lipids may be enhanced or diminished at higher temperatures or when driving to different polymorphic forms straight from melt. Minor lipids have been shown to strongly influence kinetics in many studies. However, the subcooling in chocolate tempering may help to diminish these effects by overcoming these kinetic differences.

Equilibrium conditions also can be affected. We have seen that FFA concentration is directly correlated with decreases in solid fat content in cocoa butter at certain temperatures. This can be because of differences in crystal structuring, trapping additional liquid fat, or changing thermodynamics at equilibrium conditions.

Much of the research into minor lipids and lipid additives has also proven that a minor lipid's ability to alter growth or polymorphic transitions is dependent on its similarity to the bulk fat. Similarity, especially in terms of fatty acid chain length, allows easier incorporation into the crystal matrix. Because much of cocoa butter's minor lipid content is formed post-harvest and made directly from bulk TAG, it will be very similar in structure to that of the bulk fat. Even though much research has been done to prove that many crystal modifiers or additives have tremendous effects on cocoa butter crystallization and polymorphism it is very important to also understand the indigenous minor content to understand how these also are affecting crystallization as well.

References

- Ayala, J.V.; Calliauw, G.; Foubert, I.; Dewettinck, K.; Dyer, B.; Greyt, W.D. Impacts of bleaching and packed column steam refining on cocoa butter properties. *J. Am. Oil Chem. Soc.* 2007, 84, 1069–1077.
- Calliauw, G.; Ayala, J.V.; Gibon, V.; Wouters, J.; Greyt, W.D.; Foubert, I.; Dewettinck, K. Models for FFA-removal and changes in phase behavior of cocoa butter by packed column steam refining. *J. of Food Engin.* 2008, 89, 274–284.

- Cebula, D.J.; Smith, K.W. Differential scanning calorimetry of confectionery fats. Part II: Effects of blends and minor components. *J. Am. Oil Chem. Soc.* 1992, 69, 992–998.
- Davis, T.R.; Dimick, P.S. Isolation and thermal characterization of high-melting seed crystals formed during cocoa butter solidification. *J. Am. Oil Chem. Soc.*, 1989a, 66, 1488–1493.
- Davis, T.R.; Dimick, P.S. Lipid composition of high-melting seed crystals formed during cocoa butter solidification. *J. Am. Oil Chem. Soc.* 1989b, 66, 1494–1498.
- Dewettinck, K.; Foubert, I.; Basiura, M.; Goderis, B. Phase behavior of cocoa butter in a two step isothermal crystallization. *Crystal Growth and Design* 2004, 4(6), 1295–1302.
- Dimick, P.S. Compositional effect on crystallization of cocoa butter. In *Physical Properties of Fats, Oils, and Emulsifiers*; Widlak, N.; AOCS Press, Champaign, IL, 1999; pp. 140–163.
- Foubert I.; Vanrolleghem, P.A.; Thas, O.; Dewettinck, K. Influence of chemical composition on the isothermal cocoa butter crystallization. *J. Food Sci.* 2004, 69(9), 478–487.
- Hageman, J.W. Thermal behavior and polymorphism of acylglycerides. In *Crystallization and Polymorphism of Fats and Fatty Acids*; Sato, K., and Garti, N., Eds.; Marcel Dekker, New York, 1988; pp. 9–95.
- Herrera, M.; Rocha, F.M. Effects of sucrose ester on the kinetics of polymorphic transition in hydrogenated sunflower oil. *J. Am. Oil Chem. Soc.* 1996, 73, 321–326.
- Jacobsberg B.; Oh, O.C. Studies in palm oil crystallization. *J. Am. Oil Chem. Soc.* 1976, 52, 609–617.
- Lechter, A.; Widlak, N.; Sikorski, D.; Karcher, G. Impact of Deodorization on the Crystallization Properties and Solidification Behavior of Cocoa Butter. *AOCS Conference*. Cincinnati, OH, 2011.
- Lee, I.; Pfalzgraf, L.M.; Poppe, G.G.; Powers, E.; Haines, T. The role of steryl glucosides on filter plugging. *Biodiesel Mag.* 2007, 4, 105–112.
- Loisel, C.; Lecq, G.; Keller, G.; Ollivon, M. Dynamic crystallization of dark chocolate as affected by temperature and lipid additives. *J. Food Sci.* 1988, 63, 73–79.
- Minifie, B.W. *Chocolate, Cocoa, and Confectionery: Science and Technology*; Aspen Publishers: Gaithersburg, MD, 1989.
- Savage, G.M.; Dimick, P.S. Influence of phospholipids during crystallization of hard and soft cocoa butters. *Proc. Penn. Manuf. Conf. Assoc.* 1994, 49, 54–59.
- Shukla, V.K.S. Cocoa butter properties and quality. *Lipid Technol.* 1995, 7(3), 54–57.
- Sidisky L.M.; Dimick P.S. Further characterization of steryl glucosides and esterified steryl glucosides in cocoa butter and seed crystal nuclei. *Inform* 1993, 4, 504.
- Smith, K.W.; Bhagga, K.; Talbot, G.; van Malssen, K.F. Crystallization of fats: Influence of minor components and additives. *J. Am. Oil Chem. Soc.* 2011, 88, 1085–1101.
- Staphylakis, K.; Gegiou, D. Free, esterified and glucosidic sterols in cocoa butter. *Lipids* 1985, 20, 723–728.
- Tietz, R.A.; Hartel, R.W. Effects of minor lipids on crystallization of milkfat-cocoa butter blends and bloom formation in chocolate. *J. Am. Oil Chem. Soc.* 2000, 77, 763–771.

- Timms, R.E.; Stewart, I.M. Cocoa butter, a unique vegetable fat. *Lipid Technol.* 1999, 10, 101–107.
- Toro-Vazquez, J.F.; Rangel-Vargas, E.; Dibildox, E.; Charo-Alonso, M.A. Crystallization of cocoa butter with and without polar lipids evaluated by rheometry, calorimetry and polarized light microscopy. *Eur. J. Lipid Sci. Technol.* 2005, 107, 641–655.
- Union, European. Directive 2000/36/EC of the European Parliament and of the council relating to cocoa and chocolate products intended for human consumption. *Official Journal of European Communities*, 2000, 19–25.
- Van Malssen, K.; Van Langevelde, A.; Peschar, R.; Schenk, H. Phase behavior and extended phase scheme of static cocoa butter investigated with real-time X-ray powder diffraction. *J. Am. Oil Chem. Soc.* 1999, 76(6), 669–676.
- Wright, A.J.; Hartel, R.W.; Narine, S.S.; Marangoni, A.G. The effect on minor components on milk fat crystallization. *J. Am. Oil Chem. Soc.* 2000, 77, 463–475.

10

Noncocoa Ingredients on Cocoa Butter Crystallization

Lina Svanberg

SIK—the Swedish Institute for Food and Biotechnology, Gothenburg, Sweden

Introduction

The vast majority of the cocoa butter produced worldwide is used in chocolate and confectionery products. Compared with the pure fat system, chocolate has a more complex structure in which sugar crystals and cocoa particles are surrounded by a continuous phase of crystalline and liquid cocoa butter. Because of the hydrophilic nature of the sugar crystals, a small proportion of emulsifier, e.g., soy lecithin or polyglycerol polyricinoleate (PGPR), is often added to improve mixing with the hydrophobic cocoa butter (Beckett, 2000). The six different polymorphic forms of cocoa butter, often denoted by the Roman numerals I to VI and the Greek letters α , β , and β' , each exhibit different thermodynamic stability and melting points (Timms, 1984; Wille & Lutton, 1966). This makes cocoa butter a complex ingredient in chocolate-based products, because only one of the polymorphic forms (β V) is desirable and associated with good sensory and visual attributes (Timms, 2002). The crystallization process of cocoa butter in chocolate is crucial to achieving the correct crystal form. This is generally controlled during the pre-crystallization step by tempering, i.e. subjecting the chocolate to a well-defined temperature program under the influence of shearing, or by seeding technology, homogeneously mixing 0.2%–2% (w/w) of cocoa butter crystals in the most stable β VI form with pre-cooled chocolate (Talbot, 1999; Zeng, 2000). Besides processing, the crystallization behavior of cocoa butter in chocolate is also affected by particulate solids, foreign fats (e.g. milk fat), and emulsifiers, which offer additional nucleation sites and/or steric hindrance to crystal nucleation and growth. This chapter covers the effect of noncocoa ingredients in chocolate, i.e. sugar, milk powder, and emulsifiers, on the crystallization of cocoa butter.

Effect of Sugar with or without Emulsifier on Cocoa Butter Crystallization

The sugar mixed with cocoa butter in chocolate is the disaccharide sucrose, i.e. glucose and fructose linked by an ether bond. It can exist amorphously or in the crystal state most frequently used in chocolate manufacture. The sugar crystals have an inert internal structure and a hydrophilic surface. During production the crystals are first refined to a particle size of 5–35 μm before being mixed with the cocoa butter and remaining chocolate ingredients (Beckett, 2000).

If undercooled, i.e., brought below its melting temperature, cocoa butter, like any other fat, starts to crystallize, first through nucleation followed by crystal growth (Garside, 1987; Walstra, 1998). Nucleation involves the formation of stable nuclei on which the remaining liquid fraction solidifies during crystal growth. A typical crystallization curve is illustrated in Fig. 10.1.

In general there are three types of nucleation: primary homogeneous, primary heterogeneous, and secondary. Primary homogeneous nucleation occurs in pure solution in the absence of foreign surfaces or impurities and requires significant undercooling because no surfaces are available to reduce the surface free energy. Primary heterogeneous nucleation occurs in the presence of foreign particles. In this case, the surface free energy is reduced by the foreign surfaces and consequently the same amount of undercooling is not required. Secondary nucleation occurs on the surface of crystals after primary nucleation has occurred (Rogers et al., 2008). In chocolate production, when sugar is mixed with cocoa butter the sugar crystals' surfaces can act as sites for primary heterogeneous nucleation, thus improving the crystallization rate over that of the pure fat system.

Dhonsi and Stapley (2006) compared the crystallization of pure cocoa butter, cocoa butter + sugar, and cocoa butter + sugar + lecithin) using a viscometer combined with differential scanning calorimetry (DSC). Fig. 10.2 illustrates the measured

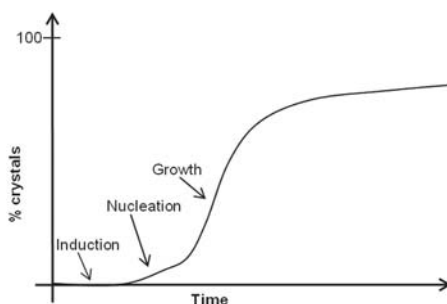


Fig. 10.1. Typical solidification curve of cocoa butter with the different phases of crystallization highlighted.

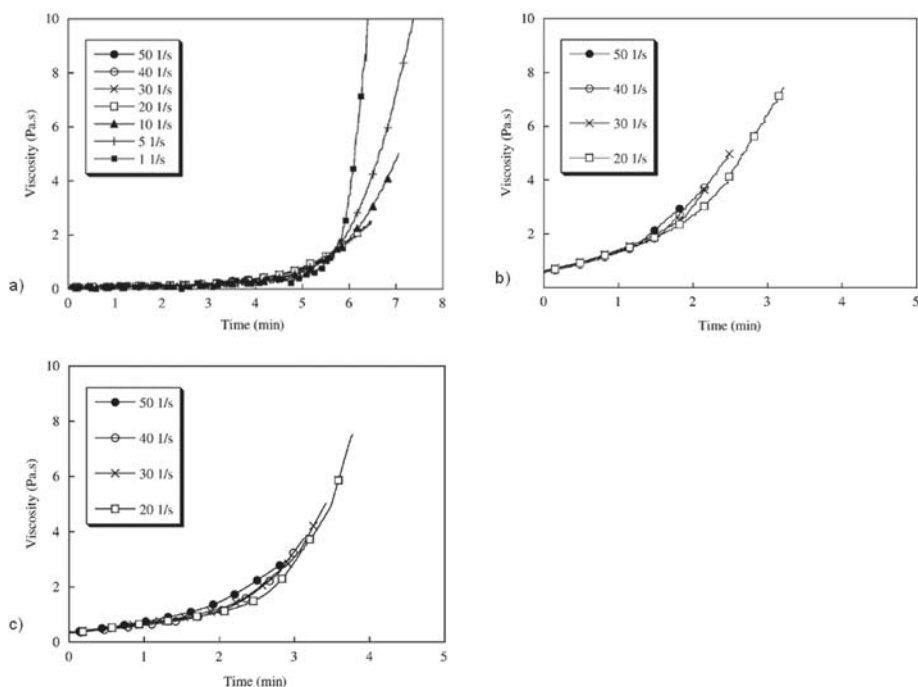


Fig. 10.2. Viscosity time curves of (a) plain cocoa butter, (b) cocoa butter + sugar, and (c) cocoa butter + sugar + lecithin cooled at 13°C at different shear rates. Adapted from Dhonsi & Stapley, 2006.

increase in viscosity, i.e. cocoa butter crystallization, for the mixtures cooled at 13°C at different shear rates.

As can be seen in Fig. 10.2, the induction times for crystallization, observed as fast changes in viscosity, were significantly reduced when sugar was added to the cocoa butter. Furthermore, the shear rate proved to have less effect on crystallization kinetics in the samples containing sugar than in the pure cocoa butter. The more rapidly induced crystallization caused by adding sugar particles can be explained by heterogeneous nucleation on the sugar particles' surfaces. When lecithin was added, it tended slightly to increase the induction time for crystallization over the times for the cocoa butter and cocoa butter + sugar samples, especially at higher crystallization temperatures (23°C). The authors suggested two hypotheses to explain this. First, because the lecithin is located on the surface of the sugar, with its hydrophilic end facing the sugar particle and its hydrophobic tail extended towards the cocoa butter phase, it creates a less favorable environment for heterogeneous nucleation to occur. Second, lecithin may affect the flow properties of the mixture by creating a greater slip between the cocoa butter and the particles, thus reducing the shear rate within the fat

phase. Although crystallization occurred at a higher rate in the mixtures than in the pure fat system, the latter proved to generate polymorphs with higher melting points. The authors therefore concluded that the heterogeneous nucleation that occurs on the surface of sugar particles favors the formation of less stable polymorphic forms (Dhonsi & Stapley, 2006).

Microscopy techniques, such as polarized light microscopy (PLM) and confocal laser scanning microscopy (CLSM), can successfully be applied to monitor fat crystallization under dynamic conditions (Bowser, 2006; Kinta & Hartel, 2010; Svanberg et al., 2011; Tietz & Hartel, 2000). This offers an extra dimension for investigations of the impact of sugar and other non-fat ingredients on cocoa butter crystallization, because it provides information about crystal morphology and could also prove visually whether heterogeneous nucleation occurs on the sugar surface. In a study by Svanberg et al. (2011), sugar or sugar + lecithin was added to cocoa butter and subjected to either an optimized or a sub-optimized pre-crystallization process before being placed under a CLSM for monitoring of the cocoa butter crystallization. If the cocoa butter was optimally pre-crystallized, the addition of sugar showed little or no effect on the induction time for crystallization. However, if the cocoa butter was not properly processed, addition of sugar particles tended to slightly reduce the induction time. Special attention, therefore, was paid to whether the sugar surfaces in these samples acted as nucleation sites, but the authors were not able to capture any heterogeneous nucleation originating from the sugar particles. However, once lecithin was added, the phenomenon was observed as illustrated in Fig. 10.3, which shows CLSM micrographs of cocoa butter crystallization on cooling in pure cocoa butter, cocoa butter + sugar, or cocoa butter + sugar + lecithin. When lecithin was added, the induction time for crystallization was shorter than in the other samples.

In 2006, Bowser investigated the crystallization of cocoa butter with nuclear magnetic resonance and microscopy and monitored the effect of adding sugar or sugar + emulsifier (lecithin or PGPR—polyglycerol polyricinoleate). The NMR measurements indicated that sugar alone did not decrease induction time, but with an emulsifier, particularly PGPR, the crystallization rate was significantly enhanced. The author concluded that the sugar surface in itself is not a suitable environment for heterogeneous nucleation and an emulsifier needs to be added to induce nucleation.

Thus, microscopy studies show that sugar crystals require the addition of an emulsifier to enhance the crystallization of cocoa butter (Bowser, 2006; Svanberg et al., 2011), while rheological studies show that with the application of shear sugar without an emulsifier was sufficient to reduce the induction time (Dhonsi & Stapley, 2006). One explanation for this could be that different techniques were applied to monitor the crystallization kinetics. Both the difference in experimental conditions (static versus dynamic) pure viscosity changes unrelated to crystallization during the rheological measurements could have influenced the results. Additional studies using analytical techniques to measure crystal mass and/or structure during solidification combined with microscopy techniques have great potential to further clarify this relationship.

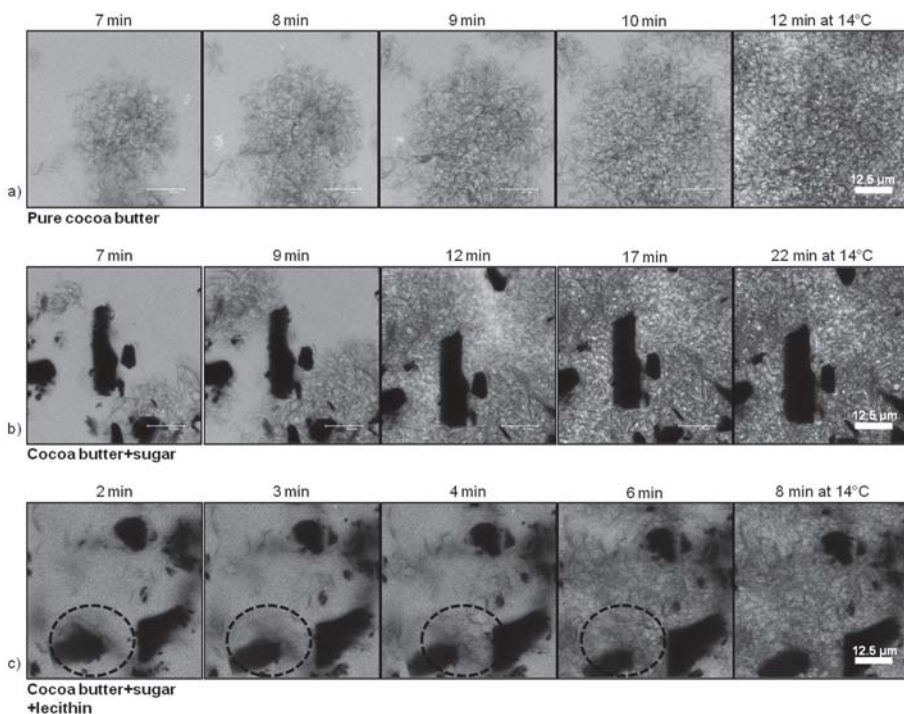


Fig. 10.3. CLSM micrographs showing time-dependant crystal growth in (a) pure cocoa butter, (b) cocoa butter + sugar, and (c) cocoa butter + sugar + lecithin. Indication of time above each micrograph corresponds to cooling time at 14°C. The background light grey represents liquid fat, connective dark grey shows areas of fat crystals, and angular black shows areas of sugar crystals. For the cocoa butter + sugar + lecithin mixture, heterogeneous nucleation is indicated by a dotted circle.

Lecithin

Emulsifiers are added to improve flow properties and promote mixing of ingredients, which influence the product's viscosity. The most frequently applied emulsifier in chocolate is lecithin (amount range 0.1–0.5% w/w), which is extracted from soybean oil and contains a mixture of natural phospholipids, predominantly phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) (Beckett, 2000).

Previous work has identified the seeding effect of the phospholipid PC (~15% w/w content in lecithin), which due to its tendency to form micelles in the H_{II} mesophase (i.e., with polar heads facing the centre and non-polar parts oriented towards the exterior region), provides an appropriate foundation for the nucleation of fat crystals

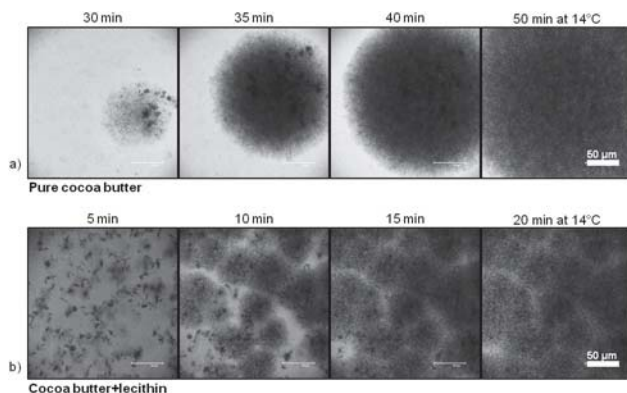


Fig. 10.4. CLSM micrographs of the crystallization of (a) pure cocoa butter and (b) cocoa butter + lecithin. The time indicated above each image correlates to minutes kept at 14°C. The light grey background represents liquid fat, while the fat crystals are identified by connective dark grey areas. The seeding effect from the lecithin in (b) is most evident in the left micrograph where small dots are visible, from which the remaining fat solidifies throughout the image series.

(Savage & Dimick, 1995). This phenomenon has also been observed in microscopy studies in which the seeding effect of lecithin on cocoa butter crystallization was pronounced in the absence of correct pre-crystallization processing (Svanberg et al., 2011). This is illustrated in Fig. 10.4, in which CLSM micrographs show the crystallization of pure cocoa butter and of cocoa butter mixed with 0.5% (w/w) lecithin.

The significant effect of phospholipids in general and PC in particular, has been thoroughly investigated by Lawler (1997), who examined the crystallization of cocoa butter using visible light spectroscopy. The author compared the crystallization rate of original cocoa butter with samples that had phospholipids either added or removed (degummed cocoa butter). The added phospholipids consisted of pure saturated PC, unsaturated PC, or PC-enriched lecithin, and two different cocoa butters, one hard and one soft, were used. The degummed cocoa butter showed significantly slower crystallization than both the original hard and soft cocoa butters, which further supports findings of the positive effect on crystallization of phospholipids up to a certain concentration. However, if more phospholipids were added to the original cocoa butter, the crystallization was generally retarded, except when >0.3% (w/w) unsaturated PC was added to original hard cocoa butter. The authors propose that at this concentration the unsaturated PC forms inverted micelles, which then act as crystal nuclei and aggregate with the monounsaturated triacylglycerides (TAGs) in the hard cocoa butter. This stabilizes the system and enhances crystallization rate. The soft cocoa butter contained a higher amount of polyunsaturated TAGs, which allowed for greater solubility with the unsaturated PC, and thereby minimized the PC's self-association into micelles.

Based on the results presented by Savage & Dimick (1995) and Lawler (1997) it can be concluded that the interaction between the phospholipids in lecithin and unsaturated TAGs in cocoa butter are highly depend on the chemical composition, volume ratio and compatibility between both systems. This could help explain the somewhat contradictory results of the impact of lecithin on cocoa butter crystallization in chocolate products.

Milk Powder

Milk chocolate can consist of up to 20% (w/w) milk powder, whose major components are lactose, milk fat, and proteins (Beckett, 2008). The TAG distribution in milk fat differs significantly from the majority of long-chained monounsaturated TAGs found in cocoa butter, and instead contains significant quantities of short-chained fatty acids, particularly butyric acid and myristic acid. Due to its great variety, milk fat is often separated into high-, middle- or low-melting fractions. The long-chained saturated TAGs are mainly found in the high-melting region, while short-chained TAGs together with 1,2-diacylglycerols (DAGs) are in the middle- and low-melting fractions (Metin, 1997). Besides the large distribution of TAG molecules, milk fat also contains ~2.5% minor lipids, DAGs, monoacylglycerols (MAGs), and cholesterol (Tietz & Hartel, 2000).

During cocoa butter crystallization the presence of milk fat increases the induction time for crystallization and generates the formation of less stable polymorphic forms (β' forms) (Chapman et al., 1971; Hartel, 1996; Metin & Hartel, 1998). There is also a significant difference in induction time between high- and low-melting fractions, and the latter has a pronounced delaying effect on cocoa butter crystallization. The reason for this is believed to be the larger difference in chemical composition between the short-chain TAGs present in the low-melting fraction and the long-chained TAGs in the cocoa butter (Metin, 1997). Tietz and Hartel (2000) showed that the concentration of minor lipids in milk fat affects the crystallization process. The nucleation time for cocoa butter crystallization was fastest when the amount of minor lipids was kept at the natural level of ~2.5%. Increasing and decreasing the amount of minor lipids from the natural value each proved to have a delaying effect on the induction time. This is illustrated in Fig. 10.5, which shows the induction time for the crystallization of cocoa butter and various milk fat fractions.

Although the nucleation time was influenced by the minor lipids found in milk fat, the crystal growth rate was not. The authors therefore hypothesize that certain DAG or MAG molecules in the milk fat could act as nucleation sites, similar to previous reports for phospholipids by (Savage & Dimick, 1995). Using X-ray diffraction and CLSM microscopy, the authors also concluded that the milk fat in general, but especially the minor lipids, affected the microstructure during storage and delayed the polymorphic transition of βV form into βVI form.

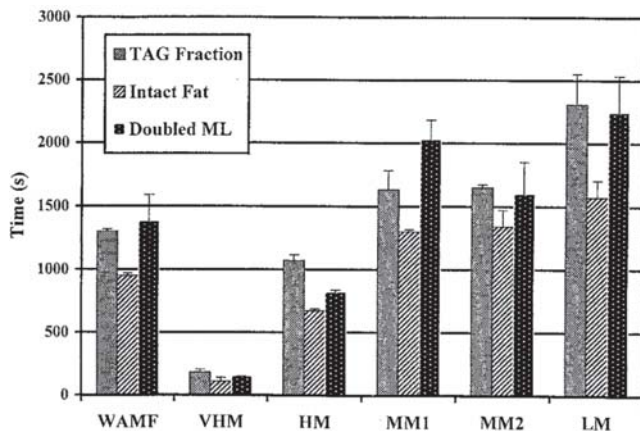


Fig. 10.5. Crystallization induction times for 10% milk fat-cocoa butter blends with varying levels of minor lipids (ML): no milk-fat ML [TAG fraction], normal levels of milk-fat ML (intact fat) and twice the level of milk-fat ML (doubled ML). Winter anhydrous milk fat (WAMF) and milk-fat fractions; very high-melting (VHM), high-melting (HM), middle-melting 1 (MM1), middle-melting 2 (MM2), and low-melting (LM) milk-fat fractions. Source: Tietz & Hartel, 2000.

During storage, milk fat also affects the microstructure of cocoa butter and prevents the development of fat bloom (Kaylegian, 1997; Lohman & Hartel, 1994). The small TAG molecules found in milk fat are difficult to incorporate into the crystal lattice of cocoa butter, where long-chained monounsaturated TAGs predominate. Rousseau & Sonwai (2008) investigated the surface microstructure of milk chocolate and pure cocoa butter with atomic force microscopy (AFM) and X-ray diffraction during 4 weeks of storage. They found that the milk chocolate compared to the pure fat samples had a more heterogeneous fat phase where some areas developed into disordered crystal agglomerates during storage. However, in the pure cocoa butter significantly more of the most stable β VI polymorph was developed than in the milk chocolate. The authors suggested that the observed differences could be caused by the particulate microstructure in the milk chocolate, which hinders lamellar rearrangement. However, possible effects from the pre-crystallization protocols used for the two sample groups could not be excluded (Rousseau & Sonwai, 2008).

The different fractions (high-, middle-, or low-melting) of milk fat affect the final microstructure of the cocoa butter in various ways and result in different product properties, such as softer chocolate texture, reduced melting point, and altered viscosity (Auty et al., 2001; Dimick et al., 1996; Jeffery, 1991). The softening is mainly caused by the low- and middle-melting fractions of the milk fat, which respectively dissolve the cocoa butter or form eutectic mixtures with high-melting fractions (Lohman & Hartel, 1994). The high-melting fractions, on the other hand, are associated with delaying the ongoing transformation rate to higher forms, i.e. from β V

to β VI, during storage and with anti-bloom properties (Bricknell & Hartel, 1998; Chapman et al., 1971; Hartel, 1996; Lohman & Hartel, 1994).

Thus, it can be concluded that the various melting fractions of milk fat and different proportions of minor lipids have a pronounced effect on both cocoa butter crystallization and the final microstructure, although the exact underlying mechanisms are yet not fully understood.

Conclusions

The noncocoa ingredients in chocolate, i.e. sugar, lecithin, and milk powder (milk fat), all influence the crystallization of cocoa butter. Of the three ingredients, the effect of milk fat is the best documented. Although the exact mechanisms remain unknown, milk fat has been found to retard cocoa butter crystallization, delay transformation into higher melting polymorphic forms, and exhibit anti-bloom effects. Microscopy studies have shown that sugar particles do not reduce the induction time for static cocoa butter crystallization except in the presence of an emulsifier, while rheological investigations have reported the opposite effect of plain sugar enhancing crystallization. Future studies using analytical techniques to measure crystal mass and/or structure during solidification, combined with microscopy techniques, have great potential to clarify this mechanism in more detail. The enhancing or retarding effect of lecithin on cocoa butter crystallization has also been debated in the literature. Based on fundamental studies of pure cocoa butter and lecithin mixtures it can be concluded that the interaction and subsequent nucleation between the phospholipids in lecithin and unsaturated TAGs in cocoa butter are highly dependent on the chemical composition, volume ratio, and compatibility between both systems. This could help explain the somewhat contradictory results of the impact of lecithin on cocoa butter crystallization in chocolate products.

References

- Auty, M.A.E.; Twomey, M.; Guinee, T.P.; Mulvihill, D.M. Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. *Journal of Dairy Research* 2001, 68, 417–427.
- Beckett, S.T. *The Science of Chocolate*; The Royal Society of Chemistry, Cambridge, 2000.
- Beckett, S.T. *The Science of Chocolate*: 2nd Ed.; The Royal Society of Chemistry, Cambridge, 2008.
- Bowser, A. Crystallization of cocoa butter. *The Manufacturing Confectioner* 2006, 86, 115–118.
- Bricknell, J.; Hartel, R.W. Relation of fat bloom in chocolate to polymorphic transition of cocoa butter. *Journal of the American Oil Chemists' Society* 1998, 75, 1609–1615.
- Chapman, G.M.; Akehurst, E.E.; Wright, W.B. Cocoa butter and confectionery fats. Studies using programmed temperature X-ray diffraction and differential scanning calorimetry. *Journal of the American Oil Chemists' Society* 1971, 48, 824–830.

- Dhonsi, D.; Stapley, A.G.F. The effect of shear rate, temperature, sugar and emulsifier on the tempering of cocoa butter. *Journal of Food Engineering* 2006, 77, 936–942.
- Dimick, P.S.; Ziegler, G.R.; Full, N.A.; Yella Reddy, S. Formulation of milk chocolate using milk fat fractions. *The Australian Journal of Dairy Technology* 1996, 51, 123–126.
- Garside, J. In *Food Structure and Behaviour*; Blanshard, J.M.V., and Lillford, P., Eds.; Elsevier, London, 1987, 35–49.
- Hartel, R.W. Applications of milk-fat fractions in confectionery products. *Journal of the American Oil Chemists' Society* 1996, 73, 945–953.
- Jeffery, M.S. The effect of cocoa butter origin, milk fat and lecithin levels on the temperability of cocoa butter systems. *The Manufacturing Confectioner* 1991, 71, 76–82.
- Kaylegian, K.E. Milkfat fractions in chocolate. *The Manufacturing Confectioner* 1997, 77, 79–84.
- Kinta, Y.; Hartel, R.W. Bloom formation on poorly-tempered chocolate and effects of seed addition. *Journal of the American Oil Chemists' Society* 2010, 87, 19–27.
- Lawler, P.J. (1997). *Kinetic and Polymorphic Advantages During Cocoa Butter Solidification from Phosphatidylcholine and Fractionated Lecithin*. Phd Thesis. Department of Food Science, The Pennsylvania State University.
- Lohman, M.H.; Hartel, R.W. Effect of milk fat fractions on fat bloom in dark chocolate. *Journal of the American Oil Chemists' Society* 1994, 71, 267–276.
- Metin, S. (1997). *Crystallization Behavior and Kinetics of Blends of Cocoa Butter and Milk Fat or Milk Fat Fractions*. Phd Thesis. Food Science, University of Wisconsin–Madison.
- Metin, S.; Hartel, R.W. Thermal analysis of isothermal crystallization kinetics in blends of cocoa butter with milk fat or milk fat fractions. *Journal of the American Oil Chemists' Society* 1998, 75, 1617–1624.
- Rogers, M.; Tang, D.; Ahmadi, L.; Marangoni, A. In *Food Materials Science: Principles and Practice*. Aguilera, J.M., and Lillford, J., Eds.; Springer: New York, 2008; pp. 369–414.
- Rousseau, D.; Sonwai, S. Influence of the dispersed particulate in chocolate on cocoa butter microstructure and fat crystal growth during storage. *Food Biophysics* 2008, 3, 273–278.
- Savage, C.M.; Dimick, P.S. Influence of phospholipids during crystallization of hard and soft cocoa butters. *The Manufacturing Confectioner* 1995, 75, 127–132.
- Svanberg, L.; Ahrné, L.; Lorén, N.; Windhab, E.J. Effect of sugar, cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems. *Journal of Food Engineering* 2011, 104, 70–80.
- Talbot, G. In *Industrial Chocolate Manufacture and Use*; Blackwell Publishing: Oxford, UK, 1999; pp. 218–230.
- Tietz, R.A.; Hartel, R.W. Effects of minor lipids on crystallization of milk fat-cocoa butter blends and bloom formation in chocolate. *Journal of the American Oil Chemists' Society* 2000, 77, 763–771.
- Timms, R.E. Oil and fat interactions: theory, problems and solutions. *The Manufacturing Confectioner* 2002, 82, 50–64.

- Timms, R.E. Phase behaviour of fats and their mixtures. *Progress in Lipid Research* 1984, 23, 1–38.
- Walstra, P. Secondary nucleation in triglyceride crystallization. *Progress in Colloid Science* 1998, 108, 4–8.
- Wille, R.L.; Lutton, E.S. Polymorphism of cocoa butter. *Journal of the American Oil Chemists' Society* 1966, 43, 491–496.
- Zeng, Y. (2000). Impf- Und Scherkristallisation Von Schokoladen. PhD Thesis. Laboratorium für Lebensmittelverfahrenstechnik. Zürich, ETH-Eidgenössische Technische Hochschule PhD Thesis.

11

Impact of Cocoa Butter Origin on Crystal Behavior

Stéphanie Marty-Terrade and Alejandro G. Marangoni

Professor and Canada Research Chair, Food and Soft Materials Science, Dept. of Food Science, University of Guelph, Guelph, ON N1G2W1, Canada

Introduction

Cocoa (liquor or powder) and cocoa butter are products derived from cocoa beans, the seeds of the cacao tree (*Theobroma cacao* L.). Native to South and Central America, these trees are now found between 20° north or south of the equator in several countries, Ivory Coast, Ghana, Nigeria, Cameroon, Brazil, Ecuador, Indonesia and Papua New Guinea being the largest producing countries (Fig. 11.1, Source: ICCO website) (Cook, 1972; Beckett, 2000c). However, due to cocoa tree varieties, pests, pathogens, weather variability and, social and political changes, cocoa production continually evolves (Fig. 11.2, Source: ICCO website) and efforts still need to be made to make it sustainable. Concomitantly, the pressure of the international cocoa markets and the variations of the cocoa product quality, are often forcing raw cocoa material suppliers and chocolate manufacturers to use cocoa beans from various origins (Fowler, 2008).

As explained in the preceding chapters of this book, cocoa butter is the most important and the most expensive component of chocolate products. Its complex crystallization behavior explains the very attractive characteristics of chocolate products as they specifically melt just below body temperature, thus providing a pleasant mouthfeel. Despite the fact that cocoa butter crystallization has been extensively studied during the last 50 years (Vaack, 1960; Wille & Lutton, 1966; Chapman et al., 1971; Dimick & Manning, 1987; Schlichter-Aronhime et al., 1988; Schlichter-Aronhime & Garti, 1988; van Malssen et al., 1996a; van Malsen et al., 1996b; van Malssen et al., 1996c; Loisel et al., 1998; van Malssen et al., 1999; Sato & Koyano, 2001; Rousset & Rappaz, 2001; Marangoni & McGauley, 2003), the fascinating complexity of the cocoa butter world has still not been completely elucidated. As an example, several researchers have worked on the effects of cocoa bean origin on cocoa butter crystallization, yet a lot remains to be understood (Chaiseri & Dimick, 1987; Savage & Dimick, 1995; Chaiseri & Dimick, 1995a; Chaiseri & Dimick, 1995b; Foubert et al., 2004; Marty & Marangoni, 2009).

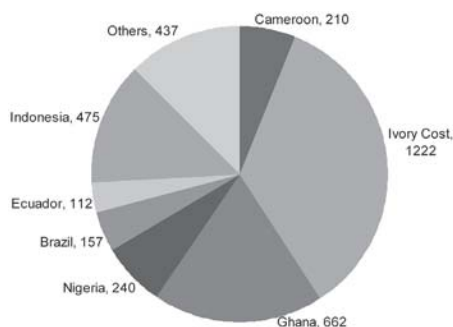


Fig. 11.1. Production of cocoa beans (thousand tonnes) by country in 2008/2009 with permission from the International Cocoa Organisation (ICCO, www.icco.org. Reproduced with permission), London.

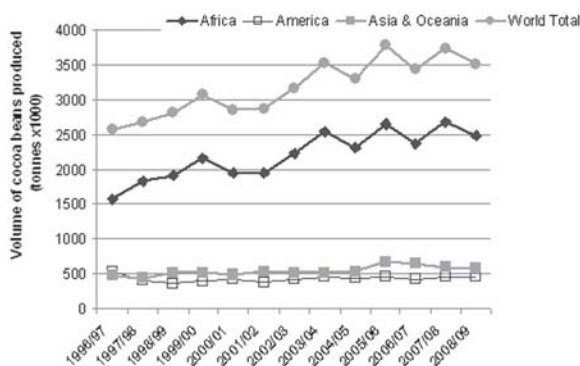


Fig. 11.2. World cocoa production by continents with permission from the International Cocoa Organization (ICCO, www.icco.org. Reproduced with permission), London.

Thus, the objective of this book chapter is to review the different factors influencing the cocoa butter composition due to environmental conditions (e.g. geographic region), agronomic practices (e.g. harvest frequency), post-harvest treatments (e.g. fermentation) and final processes in chocolate manufacture (e.g. roasting) and to relate them to its crystallization behavior.

Characteristics of Cocoa Butter from Different Origins

The very unique triacylglycerol (TAG) composition of cocoa butter is responsible for the physical properties of chocolate like snap, gloss, flavor delivery, hardness or resistance to melting. Like any other natural fat, cocoa butter is mainly composed

Table 11-A. Average Values of Component of Cocoa Butter.

Component	% (average value)
Triglycerides	96.6
Free fatty acids	1.2
Mono- and diglycerides	1.0
Unsaponifiable matter	0.5
Phospholipids	0.3
Glycolipids	0.3
Minerals, metals and vitamins	0.1

Source: Hanneman, 2000. Reproduced with permission.

of TAG (about 96%) and contains minor concentrations of mono- and diacylglycerols (MAG and DAG, respectively), polar lipids (i.e. glycolipids and phospholipids), unsaponifiable matter (e.g. sterols), free fatty acids (FFA), vitamins (e.g. tocopherols) and volatile compounds (Table 11-A) (Chaiseri & Dimick, 1987; Hanneman, 2000).

However, unlike other naturally occurring fat, the 2oleo-disaturated glycerides (1,3dipalmitoyl-2oleoylglycerol (POP), 1palmitoyl-2oleoyl-3stearoyl glycerol (POSt) and 1,3distearoyl-2oleoylglycerol (StOSt)) represent more than 70% of the total TAG of cocoa butter. While all researchers agree these symmetrical TAG explain the overall polymorphism of cocoa butter, any chemical composition variation (on both major and minor components) could greatly affect the crystallization stages such as the nucleation, crystal growth, crystal network formation and modification. As an example, Table 11-B shows the large variation in composition (TAG profile and FFA content) that could be found in cocoa butters from different countries. However, as illustrated in Fig. 11.3, any variable due to the environment, agronomic practices, post-harvest treatments (on cocoa bean and cocoa butter) and the final processes at the factory can influence cocoa butter composition and thus its physical properties.

Effect of Environmental Conditions and Agronomic Practices on Cocoa Butter Composition

Most of the research conducted on this topic looked at the impact of the country origin of cocoa butter on its chemical composition and/or physical properties but a very few only selected a precise number of parameters such as the temperature during pod growth or maturation time and the cocoa bean variety. The results of these studies will be reviewed in the following section; however, one must also consider an unimaginable amount of data from the industries remain, to date, unpublished.

Table 11-B. Triacylglycerol profile and free fatty acid content of cocoa butters from various origins.

	Cocoa butter origin	Triacylglycerol (%)									Free fatty acid in % oleic acid*
		PLiP	POO	PLiS	POP	S00	SLiS	POS	SOS	SOA	
Central and South America	Bolivia	1.1	3.3	3.5	22.6	4	2.1	40.4	22.8	0.5	0.84 (1)
	Brazil	0.9	3.9	3.7	17.9	6.7	3.2	37.1	26	0.04	0.91 ± 0.05 (4)
	Colombia	1.1	3.3	3.6	20.4	4.4	2.3	39.4	25	0.6	1.07 ± 0.29 (2)
	Ecuador	1.2	3	3.2	19.2	5.4	2.3	38.4	26.9	0.4	1.42 ± 0.73 (2)
	Peru	1.5	4.3	3.9	18.3	7.4	3.7	35.8	24.6	0.4	3.11 (1)
	Venezuela	0.9	1	3.1	20.4	2.8	1.9	40.4	28.8	0.8	n.a.
	Costa Rica	1	2.6	3.5	17.8	5.5	3	38.7	27.4	0.4	0.72 (1)
	Dominican Republic	1.1	3.3	3.2	18.4	6.1	2.7	38.2	26.5	0.6	0.88 ± 0.01 (2)
	Guatemala	1	2.3	3.4	19.3	4.9	2.2	39	27.5	0.4	n.a.
	Mexico	1.1	2.4	3.5	19.1	4.1	3	38.8	27.8	0.6	0.42 (1)
Africa	Panama	1	1.5	3	19.1	3.1	2.7	41.4	27.3	0.8	1.07 (1)
	Cameroon	1	3	3.4	17.9	5.8	2.5	38.3	27.7	0.5	n.a.
	Gabon	0.9	3.7	3.5	17.5	7.3	3	37.1	26.5	0.4	n.a.
	Ghana	1.2	2.2	3.4	17.8	4.9	2.2	39	27.5	0.4	n.a.
	Ivory Coast	1	1.9	3	19	3.9	2.5	39.6	28.5	0.6	1.36 ± 0.18 (5)
	Nigeria	1	2.3	3.6	17.9	5.2	3	38.8	27.8	0.5	1.21 (1)
Oceania	Indonesia	1.1	1.6	3	19.9	3.6	1.7	40.6	28.1	0.5	n.a.
	Malaysia	0.7	1.2	2.8	18.4	2.9	2.2	40	31.1	0.8	1.44 ± 0.42 (3)
	Solomon Island	1	0.9	3	19.3	2.8	2	40.7	29.5	0.7	n.a.

n.a.: data not available

*Number in brackets indicates the number of sample analyzed to determine the free fatty acid content of the corresponding cocoa butter.

Adapted from Chaiseri & Dimick, 1989.

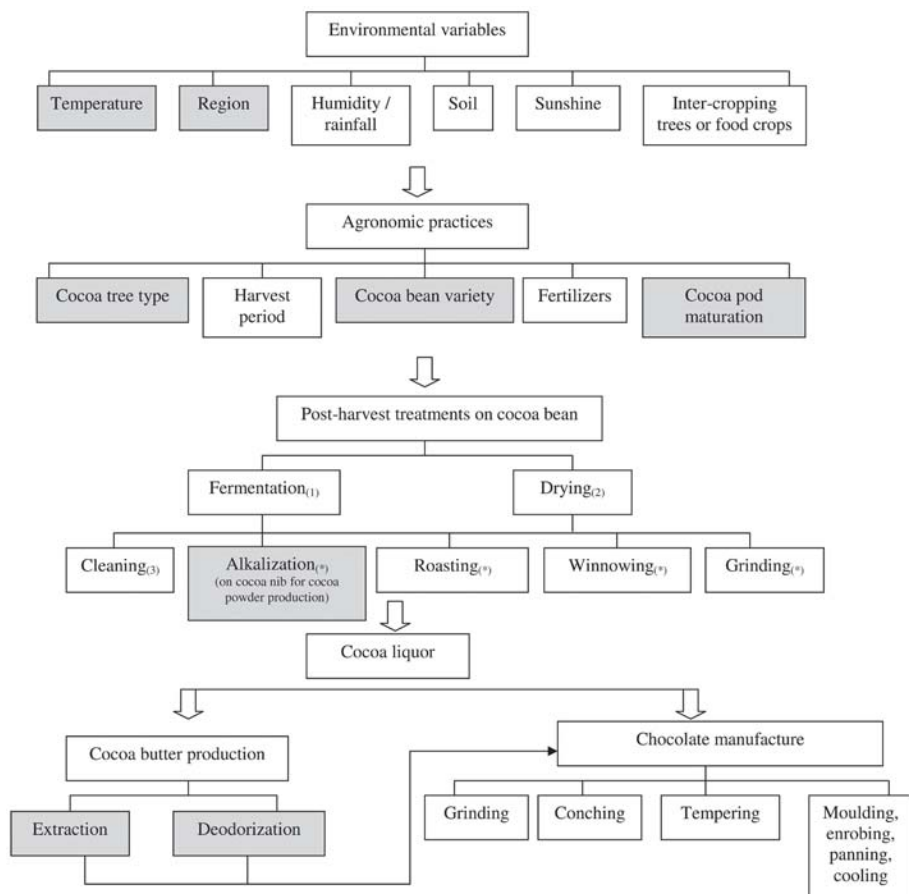


Fig. 11.3. Cocoa butter: from cocoa tree to chocolate. Numbers in brackets indicate the step order and asterisk in brackets indicate processes that can be done in different orders depending on the final application. Factors that have been thoroughly studied and reported in literature are identified by gray text boxes.

Temperature

Among these factors, temperature was unquestionably the most influential parameter on cocoa butter composition. Lehrian et al. (1980) reported higher temperatures increased the level of palmitic acid, stearic acid and monounsaturated TAG (SUS) and decreased the level of diunsaturated TAG (SUU). Moreover, a study of the enzymes involved in TAG synthesis (McHenry & Fritz, 1987) showed temperature higher than 30°C affected the selectivity of acyltransferases by increasing

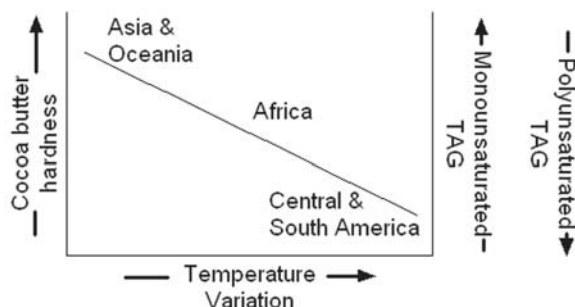


Fig. 11.4. Schematic representation of the relationship between cocoa butter hardness, mono- and polyunsaturated TAG, country of origin and temperature variation during pod maturation.

the ratio of saturated to unsaturated fatty acid incorporation into TAGs, this being responsible for the increased synthesis of disaturated TAGs. More importantly, Chai-seri and Dimick (1989) proposed “hard” cocoa butters originate from regions with uniform and warmer climate throughout the year (temperature varying from 20 to 30°C and constant humidity) such as in Asia and Oceania. On the contrary, cocoa butters from Central and South America will often be softer as temperatures can drop to about 10°C during cold winter nights. According to these observations, a schematic representation (Fig. 11.4) was drawn to give the overall picture connecting cocoa butter hardness, TAG composition, temperature variation and country of origin. Nevertheless, one must also consider temperature can greatly change from one year to another or between regions of a same country (due to different elevation for example).

Maturation Time of Cocoa Pod

The lipid composition of the bean was shown to change as the cocoa pod matures (Lehrian & Keeney 1980; Wright et al., 1982). As most of the lipid components (MAG, FFA, glycolipids, and sterols), the level of monounsaturated TAG (POSt, StOSt, and POSt) increased at all stages during maturation. However, the level of mono- and diunsaturated FA (linoleic and linolenic acids), diunsaturated TAG (POO or 1-palmitoyl-2,3-dioleoylglycerol, StOO or 1-stearoyl-2,3-dioleoylglycerol, and PLP or 1,3-dipalmitoyl-2-linoleoylglycerol), DAG and phospholipids increased at the beginning of maturation and then rapidly decreased in the last stages. It is relevant to note that this variation in composition as cocoa pods do not ripen at the same time (Fowler, 2008). Harvested pods with different degree of ripeness could thus introduce variability in cocoa butter composition.

Variations within a Cocoa Bean Variety

All the conclusions made in this section must take into consideration that genetic variations could also induce significant fatty acid profile or total phospholipid content differences within the same bean variety (Parsons et al. 1969; Liendo et al., 1997).

Country of Origin

A large number of studies reported the impact of cocoa butter origin on its composition and physical properties. Considering what has been explained above, the effect of temperature remains the main factor influencing cocoa butter characteristics. Thus, it appears uniformly through these publications that Brazilian cocoa butter is always softer than Malaysian cocoa butter. The generalization summarized in Fig. 11.4 reflects the main conclusion: cocoa butter hardness increases in the following order: South and Central America, then West Africa and finally Asia and Oceania. It was unanimously accepted that hard cocoa butters contained higher amount of StOSt, POSt, and POP while softer butters contained more polyunsaturated TAG (POO, StOO, PLP, PLSt or 1-palmitoyl-2-linoleoyl-3-stearoylglycerol, StLSt or 1,3-distearoyl-2-linoleoylglycerol).

On the other hand, Parsons et al., (1969), Parson and Keeney (1969), Dimick and coworkers (1989; 1991; 1995; 1995a; 1995b) and Shukla (1995) were the first to identify and quantify minor lipid compounds bearing in mind they could have an effect on chocolate viscosity or cocoa butter crystallizing/melting properties. These studies as well as the others that followed (Gutshall-Zakis & Dimick, 1993; Foubert et al., 2004; Vila Ayala et al., 2007; Marty & Marangoni, 2009) often included FFA, phospholipid, MAG, DAG, and sterol content analyses together with FA and TAG profile determinations. As post-harvest treatments can greatly modify the level of these components, their origin and their role on cocoa butter crystal behavior will be discussed in the two next sections.

Other Factors

As one could extrapolate from the studies of the temperature effects, Chaiseri and Dimick (1989) reported sunlight increased palmitic acid content while high rainfall led to higher stearic acid, oleic acid and free fatty acid.

Although studying single factor effects (e.g. cocoa tree variety on cocoa butter composition) appears extremely valuable it will also be very challenging. The complicated experimental setup as well as the confidentiality requested by the industries performing such analyses probably explain the very limited amount of information available on these aspects. However, the recent advances on cocoa genetics and breeding should definitively contribute to a better understanding of cocoa genome and its effect on cocoa butter variability (Seguine, 2011).

Effect of Post-Harvest Treatments and Chocolate Manufacture on Cocoa Butter Composition

For a complete understanding of cocoa bean processing from cocoa tree to chocolate product, the readers should refer to dedicated books such as *The Science of Chocolate* or the *Industrial Chocolate Manufacture and Use* (S. Beckett), for example.

Fermentation and Drying

Freshly harvested beans are usually fermented and dried by cocoa farmers. While the former is essential to prevent bean from germination and to develop flavor precursors, the latter is necessary to avoid mould growth during beans storage and transport (Beckett, 2000c; Fowler, 2008). Very little information is available with regard to their effect on cocoa butter composition, however certain authors suggested fermentation may affect the amount of FFA and the type of phospholipids yet no obvious trend was observed.

Roasting, Alkalization and Conching

Kattenberg (1981) and Chaiseri and Dimick (1989) studied the impact of cocoa nib roasting on cocoa butter composition and reported there was no effect on the FFA, DAG and TAG content but yet the solid fat content was slightly increased.

In addition, alkalization (used for color and cocoa flavor development) is another step that needs to be considered as most of the time, cocoa nib chosen for cocoa powder production will be alkalized (Beckett 2000b). Thus, the cocoa butter extracted from these nibs could also be modified by the alkali solution used in this process. In fact, it is well known that drastic alkalization conditions will damage the composition and the crystallization characteristics of cocoa butter as saponification will occur (formation of soap with the fatty acids and release of the glycerol molecules). Thus, as it could be expected, Kattenberg (1981) noted a decrease in FFA, an increase in DAG and a rearrangement of TAG (saturated acid on *sn*-2 position) and, also reported a slight solid fat content increase. More recently, Sulistyowati and Misnawi (2008) found a correlation between alkali concentration and conching temperature on milk chocolate hardness and fat melting point. They found harder chocolate and higher fat melting point with higher alkali concentration. While conching did not affect the chocolate hardness, there was an interaction between conching temperature and alkali concentration with regard to the melting point. Low fat melting point was observed for high conching temperature and high alkali concentration. While chocolate remained hard at those conditions it is difficult to consent with authors' explanation of milk fat and cocoa butter TAG hydrolysis at temperature ranging from 40 to 80°C. However, it is indisputable that interaction between chemical and thermal treatments can occur and influence cocoa butter and thus chocolate properties.

Extraction and Refining of Cocoa Butter

Pressed cocoa butter from cocoa liquor is considered as a prime butter and is commonly used in the chocolate industry. However, cocoa butter can also be obtained by an expeller process or a solvent extraction technique. While the former can soften the cocoa butter by incorporating components of shell fat (mainly due to C18:2) (El-Saied et al., 1981; Timms & Stewart, 1999; Beckett, 2000b), hence creating eutectic mixtures, the latter can significantly increase the phospholipid, pesticide and shell fat levels in cocoa butter (Parsons & Keeney 1969; Timms & Stewart, 1999). Timms and Stewart (1999) reported solvent-extracted butters are significantly softer (about 3% less solid at 30°C) than pressed ones.

Depending on the quality of the raw material (cocoa beans and nibs), the extraction process and the final application, cocoa butter can be further treated (Timms & Stewart, 1999; Beckett, 2000b; Timms, 2003a; Kamphuis, 2008). Two possibilities may occur:

- Pure prime pressed cocoa butter is usually only filtered and mildly deodorized (at temperature <180°C).
- Cocoa butter obtained from lower quality beans (e.g. poorly fermented) via expeller pressing or solvent extraction or to be used in white chocolate is fully refined. In this case, full refining includes neutralization with an alkali, bleaching and mild deodorization.

Degumming with water or dilute citric acid solution can also be occasionally used on cocoa butter (pressed or solvent extracted) in order to remove phospholipids (Gutshall-Zakis & Dimick, 1993; Timms, 2003a).

Research conducted on the effect of these processes on cocoa butter composition showed its FA and TAG profiles are not (or rarely) altered and usually no *trans* isomers are formed under mild deodorization (Timms & Stewart, 1999; Lipp et al., 2001; Vila Ayala et al., 2007). Timms and Stewart (1999) reported only heat-damaged cocoa butter (1 hour at 250°C, under vacuum) exhibited different melting/crystallization behavior due to TAG rearrangements (Table 11-C). They suggested the reduction in symmetrical TAG as well as the increase in diunsaturated TAG were the reason of a lower solid fat content. In addition, they highlighted the rise in trisaturated TAG could lead to a poorer chocolate temperability as they will crystallize at a much higher temperature (crystallizing peak at 25°C instead of 15°C in normally processed cocoa butter as measured by differential scanning calorimetry).

On the contrary, the type and the quantity of cocoa butter minor components (especially phospholipids, FFA and tocopherols) could be completely modified during refining and deodorization. While the FFA and tocopherol levels drop as deodorization temperature increases (Lipp et al., 2001; Timms, 2003a; Vila Ayala et al., 2007), the whole refining process can influence the phospholipid content as they can be

Table 11-C. Effect of Deodorization Temperature on the Properties of Cocoa Butter.

Temp. (°C)	Pure-prime pressed					Solvent-extracted				
	FFA (%)	Color (Red 1")	SFC (%)		Pesticides (ppb)	FFA (%)	Color (Red 1")	SFC (%)		Pesticides (ppb)
			20°C	30°C				20°C	30°C	
Crude	1.10	-	-	-	11	1.54	-	-	-	77
140	-	-	-	-	5.5	1.34	2.7	77.0	48.1	-
150	1.07	3.7	79.6	54.3	-	1.33	2.7	77.5	48.7	47
180	0.94	3.7	79.9	52.2	0	1.10	2.4	77.6	48.9	7
200	0.59	3.7	79.2	53.6	0	-	-	-	-	-

Temp. = temperature, FFA = free fatty acids, SFC = solid fat content, ppb = parts per billion

Source: Timms & Stewart, 1999. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

removed (or at least partly) during degumming, bleaching or caustic neutralization (Gutshall-Zakis & Dimick, 1993; Vila Ayala et al., 2007). On the other hand, there is no clear conclusion regarding the MAG and DAG content: depending on the deodorization conditions they could be either decreased or not affected at all (Chaiseri & Dimick, 1987; Vila Ayala et al., 2007). Although the concentration of these compounds is relatively low (e.g. FFA <1.75% as oleic acid in good quality crude cocoa butter (Kamphuis 2008), slight variations can significantly alter the cocoa butter melting and crystallizing properties. Thus, Vila Ayala and coworkers (2007) reported a shorter induction time, higher crystallization rate and solid fat content in cocoa butters containing less FFA. Similarly, Gutshall-Zakis and Dimick (1993) suggested the reduced crystallization induction time observed in all the refined cocoa butters was due to FFA, phospholipids, mucilages and gums removal. However, as they did not quantify their reductions, it is impossible to have a clear correlation between the concentration of these minor compounds and the modification of the cocoa butter crystallization behavior.

The above sections outlined that variation in both major (TAG) and minor (e.g. FFA, MAG, DAG, and phospholipids) lipid components due to cocoa butter origin (from tree growing area to chocolate manufacture) can either improve or on the contrary, be deleterious to cocoa butter quality. The objective of the next part is to explain how small changes in cocoa butter chemical composition can dramatically change its crystallization behavior.

Crystallization Habit of Cocoa Butter from Different Origins

The very special taste and physical attributes of cocoa butter make it a unique and valuable ingredient for the food and cosmetic industries. However, the specific melting characteristics of cocoa butter originate from its complex polymorphic behavior. As the preceding chapters (Chocolate and Cocoa Butter, Polymorphism and Mixing Phase Behavior of Major Triacylglycerols of Cocoa Butter, and Phase Behavior of Saturated Triacylglycerols) have discussed the cocoa butter polymorphism, only the effect of variable intrinsic composition on crystallization will be presented here. The effect of both major components (FA and TAG) and minor compounds (especially FFA and phospholipids) on cocoa butter crystal behavior will be reviewed.

Cocoa Butter Crystallization

Fat crystallization is related to the molecular conformation and lateral packing of acyl chains of TAG molecules as temperature is decreased below the temperature of melting (T_m). Chain length, parity of carbon atoms, unsaturation of acyl chains and processing conditions (e.g. temperature and shear) are some of the factors that influence crystal structures. Nucleation, crystal growth, formation of a crystal network

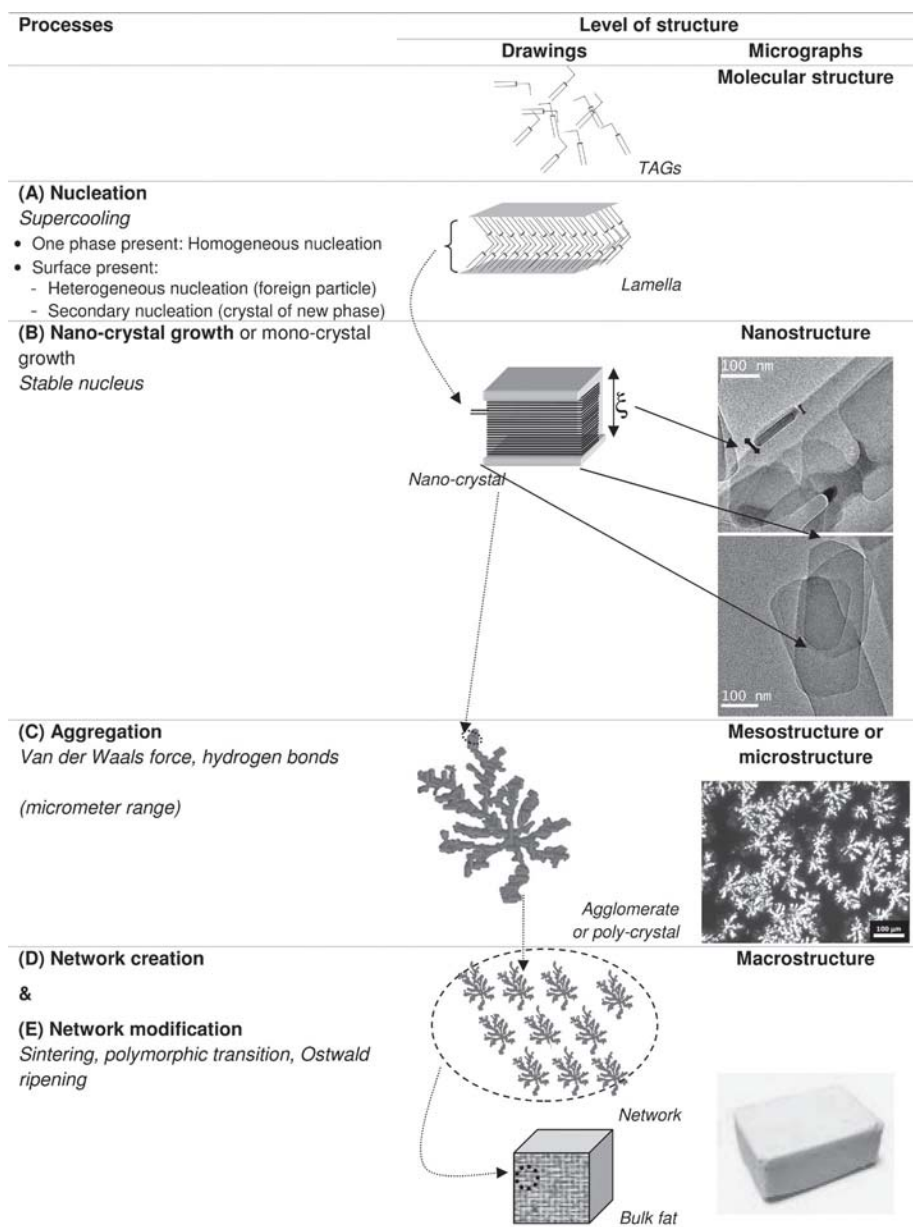


Fig. 11.5. Main processes involved in fat crystallization network formation and schematic representation of the associated different levels of structure. Adapted from Acevedo & Marangoni, 2010.

and network modification/alteration are the main stages that successively occur during fat crystallization (Walstra, 2003b; Walstra, 2003c). Fig. 11.5 gives a schematic representation of the different levels of structure in bulk fat and the main crystallization processes involved. The driving force for nucleation is the degree of supercooling or undercooling (ΔT), i.e. when the melt is cooled below T_m . Depending on the degree of supercooling and the presence (or not) of impurities heterogeneous or homogeneous nucleation may occur (Fig. 11.5A). In almost all bulk liquids, especially when the degree of supercooling is not high enough, foreign surfaces like dust particles, reverse micelle or air bubbles, will catalyze crystal nucleation. This process is called heterogeneous nucleation. On the contrary in pure systems and at a high degree of supercooling homogeneous nucleation may occur (Walstra, 1987; Boistelle, 1988). Once stable nuclei have been created, they grow as more TAG molecules are incorporated into their crystal lattice and form crystals (Fig. 11.5B).

In fat, three major polymorphs, α , β' and β , corresponding to different types of molecular packing exist (Kaneko et al., 1999; Marangoni, 2005). The least stable polymorph (α) has also the lowest free energy of activation, and thus will form more readily. However, a more stable polymorph can be formed via melt or solid-mediated polymorphic transitions as shown in Fig. 11.6.

The growth rate and crystal morphology depend on external factors (supercooling, impurities, changes in viscosity of the melt) and internal factors (structure, bonds). As the crystallization process continues (Fig. 11.5C and D), growing crystals will aggregate with each other due to van der Waals forces and hydrogen bonds and form a continuous three dimensional fat crystal network that can be described as fractal aggregates (Johansson 1995; Narine & Marangoni 1999; Walstra et al., 2001; Walstra, 2003b; Walstra, 2003c). In time, as the thermodynamic equilibrium between the solid and the liquid phases is rarely reached after crystallization, the initially formed network will be altered by various changes due to polymorphic transitions and Ostwald ripening (Fig. 11.5E). Small unstable crystals are dissolved to the benefit of larger ones in this latter process. Moreover, the recrystallization into more stable polymorphs may also lead to an overall contraction of the fat crystal network (Walstra, 2003a; Timms, 2003b).

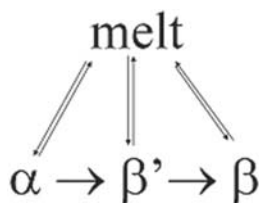


Fig. 11.6. Possible melt-mediated or solid-state polymorphic transformations in fats. Source: Marangoni, 2005. Reproduced with permission.

Like most of the fat containing foods, crystallization of the fat phase (here, cocoa butter) is an important phenomenon that determines the mechanical, visual and eating properties as well as the shelflife of chocolate products.

Effect of Cocoa Butter Origin on Crystallization Kinetics

Marty and Marangoni (2009) used the Avrami model to study the early crystallization event of cocoa butter from various origins based on the non linear regression (Equation 11.1):

$$SFC_t = SFC_{\infty} \left(1 - e^{-kt^n}\right) \quad \text{Equation 11.1}$$

where SFC_t is the SFC (%) as a function of time, SFC_{∞} describes the limiting SFC as time approaches infinity, k represents the Avrami rate constant and n is the Avrami exponent.

There, SFC of cocoa butter crystallized under static and isothermal conditions (20°C) was measured every 30 s for about an hour and plotted as a function of time (Fig. 11.7). As illustrated in this figure, cocoa butter origin strongly affects cocoa butter crystallization behavior. Here, the authors identified four groups with decreasing SFC_{∞} (NG; CN and MY, EC and CI; BR). Interestingly, this result was comparable to the SFC determined for stable polymorphs (Form V) of tempered and nontempered cocoa butters except no distinction could be made between Nigerian, Chinese and Malaysian butters.

Furthermore, the Avrami exponent and the Avrami rate constant determined in Marty and Marangoni's work (2009) (Fig. 11.8) illustrate the early crystallization events (nucleation and crystal growth) are highly dependant on cocoa butter origin. Thus, the significantly different Avrami exponent of Nigerian butter (~ 1 instead of ~ 2 for the others) could indicate it nucleates in a more instantaneous fashion and/or the

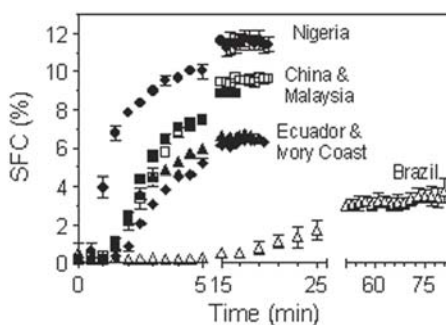


Fig. 11.7. Initial crystallization event of Nigerian (●), Chinese (□), Malaysian (■), Ecuadorian (▲), Ivorian (◆) and Brazilian (△) butters crystallized statically and isothermally at 20°C. Means and standard error of the means of 3 replicates are reported. Adapted from Marty & Marangoni, 2009.

growing crystals have different geometries compared to the other cocoa butters. Similarly, visualization of isothermal crystallization of cocoa butter through time-lapse micrographs confirmed the unique crystallization behavior of Nigerian butter (Marty & Marangoni, 2009). This observation is further explained in the “Microstructure difference of cocoa butter from various origins” section.

Effect of Different FA and TAG Profiles on Cocoa Butter Physical Properties

Constituting more than 90% of the fat phase, any profile variation (species and concentration of each species) of TAGs has a considerable effect on cocoa butter physical properties.

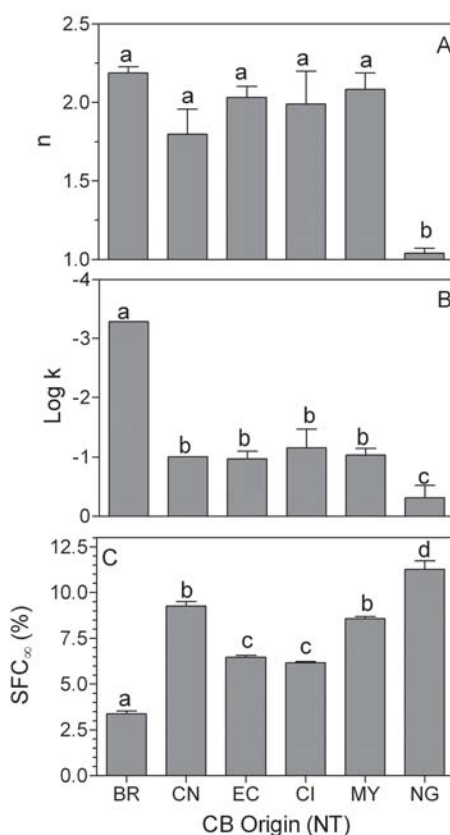


Fig. 11.8. Avrami parameters of nontempered (NT) cocoa butters from different origins determined from the initial part of the crystallization curve (α polymorph) of Fig. 11.7. Graphs A, B, and C show the Avrami exponent (n), the Avrami rate constant (k) and the limiting SFC as time approaches infinity (SFC_{∞}), respectively as a function of cocoa butter origin. Means and standard error of the means of 3 replicates are reported. Adapted from Marty & Marangoni, 2009.

Table 11-D. Characteristics of Six Samples of Cocoa Butter from Various Origins.

Charac- teristics ^c	Origin					
	Brazil	China	Ecuador	Ivory Coast	Malaysia	Nigeria
FA (%) [*]						
C16:0	23.9 ^a	24.3 ^a	25.1 ^a	24.7 ^a	23.6 ^a	24.0 ^a
C18:0	33.4 ^a	35.8 ^b	33.8 ^{a,c}	34.5 ^{a,c,d}	35.5 ^{b,d}	34.7 ^{b,c,d}
C18:1	35.7 ^a	33.3 ^b	33.9 ^b	33.6 ^b	33.4 ^b	33.8 ^b
C18:2	4.5 ^a	3.8 ^a	4.0 ^a	4.4 ^a	4.2 ^a	4.3 ^a
C18:3 + C20:0	1.6 ^a	1.8 ^a	1.8 ^a	1.7 ^a	1.9 ^a	1.9 ^a
TAG (%) ^{**}						
OOL	0.5±0.1 ^a	1.0±0.0 ^b	1.1±0.5 ^b	0.6±0.2 ^{a,b}	1.0±0.3 ^b	1.8±0.3 ^c
PLP	1.5±0.1 ^a	1.1±0.0 ^a	1.3±0.5 ^a	1.5±0.5 ^a	1.2±0.4 ^a	1.1±0.3 ^a
OOO	5.1±0.5 ^a	0.8±0.7 ^b	1.9±0.9 ^b	1.8±0.5 ^b	1.4±0.3 ^b	1.2±0.3 ^b
POP	22.8±0.4 ^{a,c}	23.5±0.8 ^{a,b,c}	24.1±1.1 ^{b,c}	24.3±0.6 ^b	22.7±0.5 ^a	23.1±0.4 ^{a,b,c}
PPP	0.0±0.0 ^a	0.1±0.1 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.1±0.2 ^a
St00	7.0±0.2 ^a	1.7±0.1 ^{b,c}	2.1±0.8 ^b	2.1±0.5 ^b	1.8±0.4 ^{b,c}	1.2±0.1 ^c
POSt	34.1±0.5 ^a	37.0±0.3 ^b	37.3±2.7 ^b	37.3±1.8 ^b	36.9±1.9 ^b	37.3±0.7 ^b
PPSt	0.1±0.1 ^a	0.2±0.1 ^a	0.1±0.1 ^a	0.1±0.1 ^a	0.1±0.1 ^a	0.2±0.1 ^a
St0St	28.3±0.2 ^a	33.1±0.3 ^{b,d}	31.1±1.8 ^c	31.4±0.8 ^{c,d}	33.4±0.6 ^b	32.5±1.4 ^{b,c,d}
StStP	0.1±0.1 ^a	0.4±0.1 ^b	0.3±0.2 ^{a,b}	0.3±0.1 ^{a,b}	0.4±0.1 ^b	0.5±0.1 ^b
StStSt	0.6±0.1 ^a	1.0±0.1 ^a	0.8±0.5 ^a	0.7±0.3 ^a	1.1±0.5 ^a	1.1±0.4 ^a
FFA (% oleic acid)						
	1.2 ^a	1.5 ^b	1.5 ^b	1.6 ^b	1.4 ^b	2.7 ^c
Phosphorus content (ppm)						
	75.0	40.0	4.3	8.0	11.6	8.5
T _m (°C) [*] when crystallized at 22°C						
	31.4±0.0 ^a	33.2±0.0 ^{c,d}	32.2±0.3 ^{a,b}	32.4±0.0 ^b	33.4±0.7 ^d	32.5±0.2 ^{b,c}

Superscript letters represent statistically significant differences between cocoa butters ($P < 0.05$). O = oleic acid, P = palmitic acid, St = stearic acid, L = linoleic acid. TAG designations include positional isomers, i.e. OOL = OOL + OLO + LOO. T_m was obtained from the phase transition analyzer used to determine the free energy of nucleation. Means of two (*) or three (**) replicates are reported.

Source: Marty & Marangoni, 2009. Reproduced with permission.

Table 11-D illustrates the level of di- and triunsaturated TAG found in Brazilian cocoa butter, is significantly higher than the ones measured in cocoa butters from other origins. As unanimously reported in other studies (Chaiseri & Dimick, 1989; Shukla, 1995; Chaiseri & Dimick, 1995a; Chaiseri & Dimick, 1995b; Foubert et al., 2004; Marty & Marangoni, 2009), this high content of polyunsaturated TAG is always correlated to a lower solid fat content. In addition, the determination of cocoa butter hardness (Chaiseri & Dimick, 1989; Jeffrey, 1991) or elastic constant (Marty & Marangoni, 2009) showed Brazilian cocoa butter is significantly softer than the others (Fig. 11.9). In contrast, Marty and Marangoni (2009) reported smaller SFC differences for tempered and nontempered

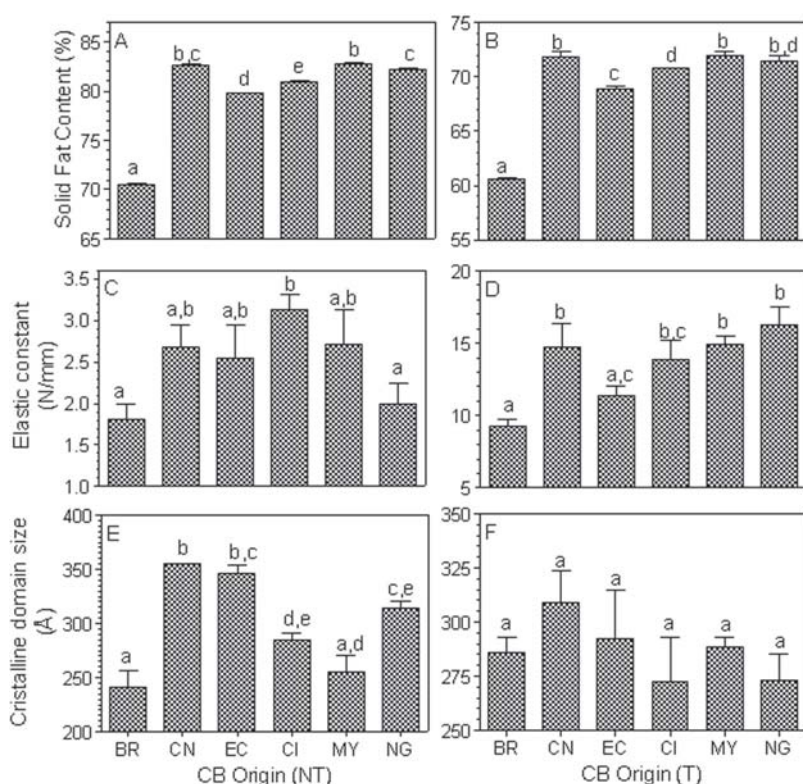


Fig. 11.9. Solid fat content (A and B), elastic constant (C and D) and crystalline domain size (E and F) of nontempered (A, C and E) and tempered (B, D and F) cocoa butters from different origins. Measurements were performed on nontempered (NT) cocoa butters after 3 days at 20°C (statically and isothermally crystallized) and after 3 hours at 20°C on tempered (T) cocoa butters (step 1: cooled down to 30°C under shear and seeded with 5% w/w of the cocoa butter crystallized in Form V (or β_2), step 2: static and isothermal crystallization at 20°C). Means and standard error of the means are reported. Letters denote statistically significant differences between cocoa butters ($P < 0.05$). Adapted from Marty & Marangoni, 2009.

Chinese, Ecuadorian, Ivorian, Malaysian and Nigerian butters yet statistically significant differences could also be observed (especially for cocoa butter from Ecuador). Moreover, Fig. 11.9A and B, Fig. 11.9C and D, and Fig. 11.9E and F, respectively, illustrate the crystallization conditions (tempered *vs.* nontempered) greatly influenced the physical characteristics of cocoa butters. As expected, high crystallization rate obtained by tempering increased the crystalline network strength (higher elastic moduli) yet significantly lowered the SFC. This demonstrates SFC does not necessarily fully explain bulk fat hardness (Dibildox-Alvarado et al., 2004; Marty & Marangoni, 2009). It is also interesting to note the tempering procedure eliminated the effect of cocoa butter origin on the crystalline domain size (Figs. 11.9 E and F).

The majority of the cocoa butter TAGs are of SUS-type, the unsaturated fatty acid being almost exclusively the oleic acid at the *sn*-2 position. Sato and co-workers (1989; 1989; 2001; 2002) systematically studied the crystal behavior of such TAG and confirmed stable (β_2 , β_1) polymorphs (POP, POST and StOSt) have a triple chain length structure as illustrated in Fig. 11.10 where the monounsaturated TAG at the *sn*-2 induce a molecular kinking.

However, higher level of di- and triunsaturated TAG such as those found in Brazilian cocoa butter may disrupt this molecular packing as they will lead to extra kinks in the molecule at the *sn*-1 or *sn*-3 position or both (Fig. 11.11). Beside the lower melting point of these TAGs, the decreased molecular compatibility during crystal-

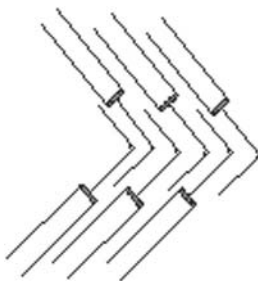


Fig. 11.10. Beta polymorph structure of SUS-type TAG (e.g. POP, StOSt, POST).

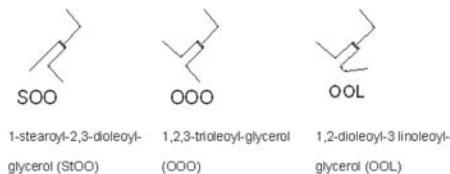


Fig. 11.11. Molecule structure of diunsaturated (StOO) and triunsaturated (OOO and OOL) TAG.

lization due to the presence of double bonds can almost certainly explain the softness of these cocoa butters (Chaiseri & Dimick, 1989; Foubert et al., 2004; Marty & Marangoni, 2009).

On the other hand, Marty and Marangoni (2009) recently reported the linear correlations of the linoleic acid (L) content with the crystalline domain size ($r = -0.82$, $P < 0.05$) and the averaged particle size ($r = 0.82$, $P < 0.05$). However, better correlations could be found when result of Chinese or Malaysian butters (Fig. 11.12A or B, respectively) is excluded. In this case, the relationship between the linoleic acid concentration and the microstructure remains linear (higher concentration of linoleic acid associated with larger averaged particle size). On the contrary, a new non linear model (Equation 11.2) is proposed to better represent the effect of linoleic acid concentration on the nanostructure:

$$\xi = \frac{\xi_0 - 83[L]}{1 - 0.21[L]} \quad \text{Equation 11.2}$$

where x is the crystalline domain size ($\text{\AA}$) at a specific linoleic acid concentration $[L]$ (%) and ξ_0 is a characteristic domain size dependant of the model system. This model predicts smaller crystalline domain size for higher linoleic acid concentration

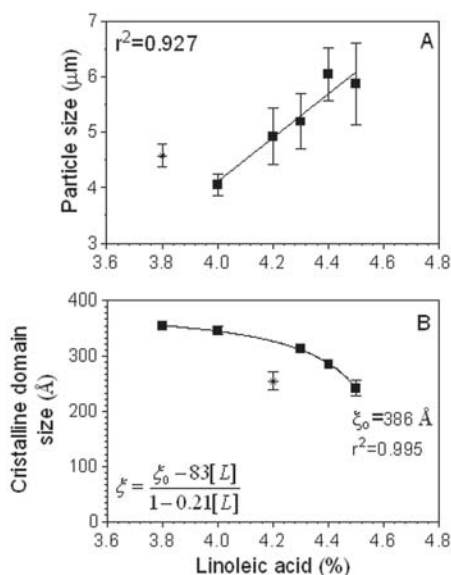


Fig. 11.12. Correlation analysis of averaged particle size (A) and crystalline domain size with linoleic acid content in cocoa butters from different origins. Values represent means and standard error of the means of 12 (A) or 2 (B) replicates.

however, it is important to note for $[L] > 4.2\%$, the domain size decreases more importantly (~ 70 Å) compare to the decrease observed for $3.8\% < [L] < 4.2\%$ (~ 20 Å). Of course, this observation must be pondered as the cocoa butter matrices could not be all included, which could suggest another chemical component may interfere more than L during crystal growth in Malaysian cocoa butter. Nevertheless, this result incontestably illustrates the strong relationship between molecular composition, nano- and mesostructure. Moreover, their study showed these three parameters were strongly correlated with nontempered cocoa butter matrix permeability. Thus, they found permeability coefficients were higher with larger squared average particle size ($r = 0.98$, $P < 0.01$), higher amounts of linoleic acid ($r = 0.86$, $P < 0.05$) and smaller crystalline domain size ($r = -0.98$, $P < 0.01$). Therefore, this work proves not only monounsaturated fatty acid (C18:1) acid but also diunsaturated fatty acid (C18:2) can strongly affect cocoa butter crystallization by limiting the molecular compatibility and hence modifying the whole network characteristics. Similarly, Campos et al., 2010 (Campos et al. 2010) showed triunsaturated TAG (LLL or 1,2,3-trilinoleoylglycerol) had a very limited molecular compatibility with typical SUS-type TAG of cocoa butter, significantly increased the liquid fraction of the blend and promoted the $\beta' \rightarrow \beta$ polymorphic transformation. Interestingly, these authors also reported trisaturated TAG (StStSt or 1,2,3-tristearoylglycerol) promoted nucleation and crystal growth yet delaying polymorphic transformation ($\beta' \rightarrow \beta$). In addition, they found StStSt decreased the network elasticity as it induced disorganization during lamellae stacking.

Effect of Minor Components on Cocoa Butter Physical Properties

Savage and Dimick (1995) as well as more recent studies (Foubert et al., 2004; Vila Ayala et al., 2007; Marty & Marangoni, 2009) have correlated the effect of intrinsic minor compounds such FFA and phospholipids to nucleation and crystal growth events. Both Foubert and co-workers (2004) and, Marty and Marangoni (2009) found lower phospholipid content shorten the crystallization or the nucleation induction time, respectively. In addition, a higher level of phospholipid was related to a smaller number of crystalline particles (Marty & Marangoni 2009). Unfortunately, the identification and quantification of the different phospholipid species were not reported and thus, based on previous work (Savage & Dimick 1995), it can only be speculated that phospholipids with large polar head groups may be predominant. Both lysophosphatidylcholine and phosphatidylinositol may form micelles with acyl moieties in interior regions and thus slow down nucleation (Fig. 11.13A). In contrast, phosphatidylcholine will form reversed micelle (acyl moieties in exterior regions, Fig. 11.13B) and hence, may reduce nucleation induction time by acting as seeds (Savage & Dimick, 1995; Sato & Koyano, 2001).

While there is little information on the effect of cocoa butter intrinsic MAG/DAG on its crystallization, higher FFA level was significantly correlated to a longer crys-



Fig. 11.13. Schematic representation of the H_I mesophase or micelle (A) and of the H_{II} mesophase or reversed micelle (B). Adapted from Savage & Dimick, 1995, and Sato & Koyano 2001.

tallization induction time, a slower crystallization rate and a lower solid fat content (Gutshall-Zakis & Dimick, 1993; Foubert et al., 2004; Vila Ayala et al., 2007). The incorporation of more polar molecules into the crystal lattice may impede the TAG packing and thus slow down the crystallization (Jacobsberg & Oh, 1976; Wright et al., 2000).

Marty and Marangoni (2009) reported cocoa butters from different origin and crystallized under the same conditions ((1) cooled from 70 to 20°C at 30°C/min and (2) held at 20°C for about 2 hours) display various microstructures. Fig. 11.14 and Fig. 11.15 illustrate number and size of particles varied greatly between cocoa butters. Brazilian, Chinese, Malaysian and Nigerian microstructures was relatively coarse (small number of large crystals) while Ecuadorian and Ivorian cocoa butters had a finer microstructure with a large number of small crystals. One could argue the degree of undercooling (ΔT) may be responsible for such variations. However, ΔT of Brazilian, Chinese, Malaysian, Nigerian, Ecuadorian and Ivorian butters is 11.4, 13.3, 13.4, 12.5, 12.2 and 12.4°C, respectively. This shows that for both the highest and the lowest degree of undercooling a coarse microstructure could be observed and thus the effect of the chemical factors described above (e.g. phospholipids, unsaturated FAs) were the most significant here.

In addition, it is particularly interesting to emphasize the peculiar crystallization behavior of Nigerian cocoa butter (Marty & Marangoni, 2009). In their study, Marty and Marangoni (2009) reported a very short induction time (about 17 min) with a rapid appearance of a small number of nuclei (about 1000 particles after 2 hours of crystallization at 20°C) (Fig. 11.16). A short induction time is usually associated with the rapid appearance of a large number of nuclei, which should lead to a finer microstructure. Such result was also described by Chaiseri and Dimick (1995a; 1995b). They crystallized cocoa butter under dynamic conditions at 26.5°C and determined the induction period, the nucleation and, the primary and secondary crystal growth using the light-scattering properties of crystals. Out of six cocoa butters, Ecuadorian butter had short induction time but slow crystal growth rate while other butters had either long induction time and slow crystal growth rate or short induction time and rapid crystal growth rate. They explained their observation by the fact that the Δ absorbance measurement could be increased

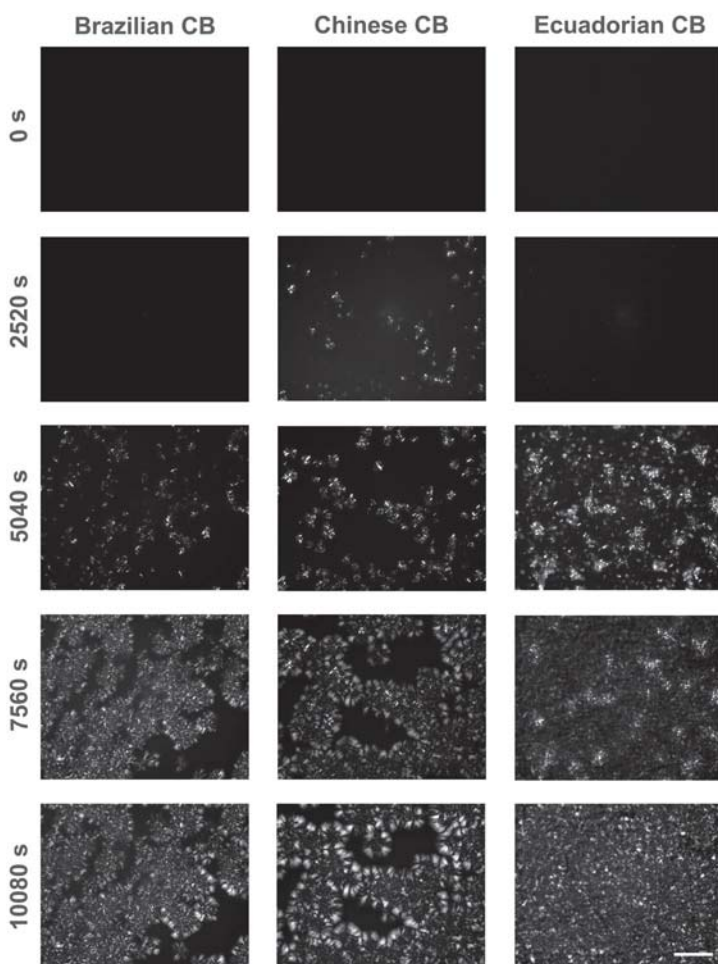


Fig. 11.14. Polarized light micrographs of Brazilian, Chinese and Ecuadorian cocoa butters (CB) crystallized isothermally at 20°C at different time points. Prior to imaging, samples were held at 70°C for 10 min, followed by cooling from 70 to 20°C at 30°C/min. The scale bar represents 50 μm . From time-lapse micrographs from Marty & Marangoni, 2009. Reproduced with permission.

by the formation of more crystals but also by the modification of existing crystals (structure or composition).

On the other hand, the higher level of FFA in Nigerian cocoa butter could explain its shorter nucleation induction time as these molecules could act as template and

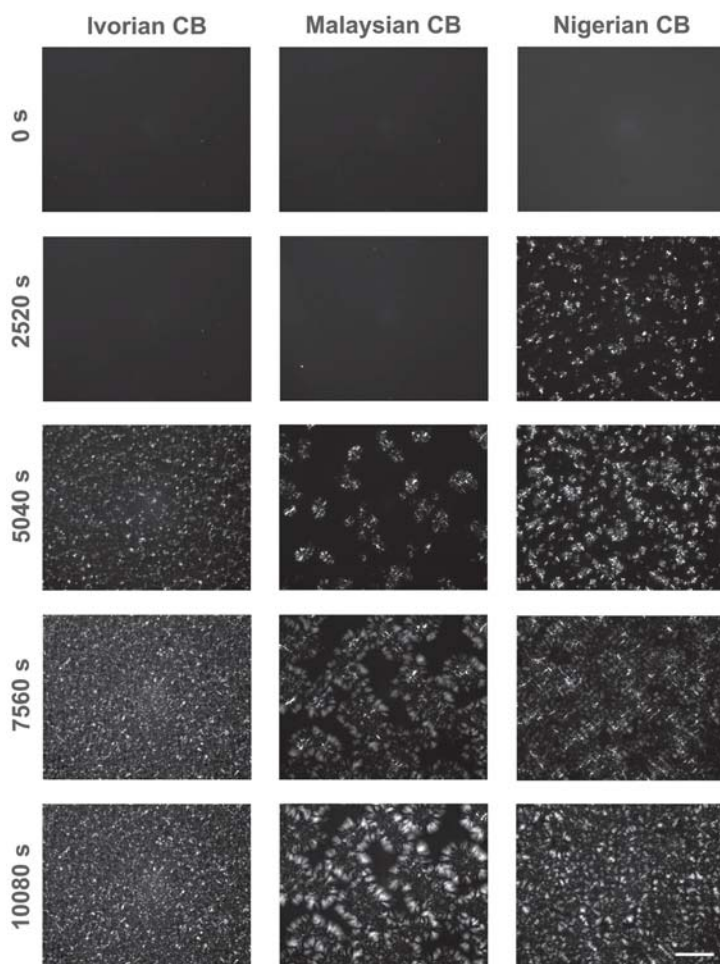


Fig. 11.15. Polarized light micrographs of Ivorian, Malaysian and Nigerian cocoa butters crystallized isothermally at 20°C at different time points. Prior to imaging, samples were held at 70°C for 10 min, followed by cooling from 70 to 20°C at 30°C/min. The scale bar represents 50 μm . From time-lapse micrographs from Marty & Marangoni, 2009. Reproduced with permission.

promote nucleation. However, as crystallization progresses, FFA may slow down the crystal growth rate as they could interact with the TAG molecules. As discussed in the previous section, minor compounds such FFA, MAG, DAG, and phospholipids could significantly influence the crystallization process; it seems reasonable to assume

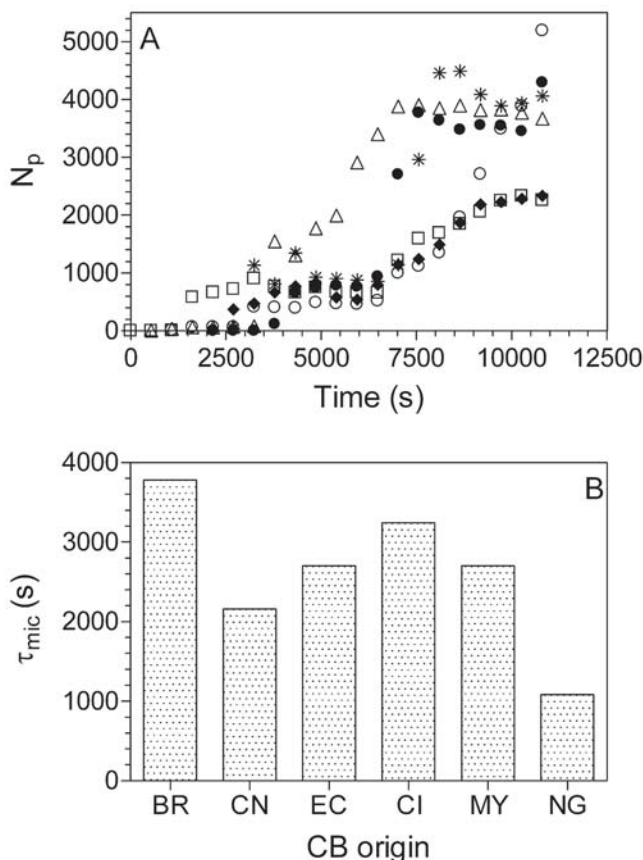


Fig. 11.16. Number of particles (N_p) as a function of time of Brazilian (●), Chinese (◆), Ecuadorian (✱), Ivorian (△), Malaysian (○) and Nigerian (□) cocoa butters (CB) statically cooled from 70 to 20°C at 30°C/min and held at 20°C for 2 hours (A). Induction time (τ_{mic}) for the first crystalline phase (or onset for the first N_p increase) as a function cocoa butter origin (B). N_p and τ_{mic} were extracted from the time-lapse microstructural analysis. Source: Marty & Marangoni, 2009. Reproduced with permission.

these molecules could impact cocoa butter crystallization differently depending on the stages (induction time, nucleation, crystal growth, formation of a crystal network and network modification). Unfortunately, there is no information with regard to the type and the concentration of FFA, MAG, DAG, and phospholipids, and their effect on all the cocoa butter crystallization stages.

Relevance for the Chocolate Industry

Tempering and Performance Variability

Tempering is one of the most critical steps during chocolate making as it impacts the final overall quality of the chocolate. During tempering, cocoa butter is crystallized into a specific triclinic polymorphic form referred to as Form V (or β_2). Out of the six known polymorphic forms (I to VI) (Wille & Lutton, 1966; Dimick, 1991), it is recognized that cocoa butter crystallized in Form V gives the proper gloss, snap, texture and melting characteristics in good and shelf-stable chocolates (Beckett, 2000a). This complex crystallization process could be summarized as successive heating and cooling steps during which proper stable β_2 seed crystals are formed and initial unstable crystals (Form I to IV) are melted out. In industries, scraped-surface, temperature-controlled unit is used for chocolate tempering. Once 1 to 3% of cocoa butter has crystallized into the stable polymorph, the chocolate mass is finally cooled and solidified (Lechter, 2009). Besides the influence of the chocolate recipes (especially the use of milkfat in milk chocolate) on cocoa butter crystallization, the chemical composition (both major and minor components) of cocoa butter itself must be taken into consideration to adapt temperature, shear and time during chocolate tempering. As it was discussed above, not only the origin due to environmental conditions but also agronomic practices and post-harvest treatments can especially modify the minor lipid component of cocoa butter. In order to minimize such effects, proper selection and mixing of cocoa butter from various origins are often recommended to standardize the cocoa butter characteristics (Chaiseri & Dimick, 1987). However, there is an increasing demand for “fine” chocolates with specific geographic origin for example. In this case, the use of blending to homogenize cocoa butter properties would be improbable.

Oil Migration and Cocoa Butter Physicochemical Properties

Oil migration is one of the main factors responsible for quality loss in confectionery products (Wootton et al., 1970; Talbot, 1990; Timms, 2003c). This phenomenon is observed in composite chocolate containing two or more fat phases in contact with each other, cocoa butter being often the fat component of at least one of these phases. Thus, the physicochemical properties of the cocoa butter matrix are also particularly important to consider in order to avoid too significant oil migration. As recently published, Marty and Marangoni (2009) reported the effect of cocoa butter origin on oil migration. Table 11-E illustrates the two highest oil migration rates (OMR) were found for Brazilian and Nigerian butters. Interestingly, these cocoa butters had both the lowest and one of the highest solid fat content (60.6 and 71.5°C, respectively).

Table 11-E. Oil Migration Rates from Plots of I_{10} vs. Time or Square Root of Time of Non-Tempered and Tempered Cocoa Butters (CB). Mean and Standard Deviation of N Replicates (When N > 2) Are Reported.

CB origin	Non-tempered			Tempered		
	mm/day	mm/day ^{1/2}	n	mm/day	mm/day ^{1/2}	n
BR	3.96 ^a	10.71 ^a	1	0.11±0.02 ^a	1.24±0.22 ^a	3
CN	0.36 ^b	2.57 ^b	2	0.02±0.02 ^b	0.23±0.17 ^b	3
EC	0.23 ^b	1.76 ^b	2	0.03±0.00 ^b	0.32±0.04 ^b	3
CI	0.29 ^b	3.02 ^b	2	0.03±0.02 ^b	0.37±0.19 ^b	3
MY	0.82 ^c	5.67 ^c	2	0.04±0.01 ^b	0.39±0.05 ^b	3
NG	2.81 ^d	10.59 ^a	2	0.06±0.01 ^c	0.67±0.07 ^c	3

Superscript letters correspond to statistically significant differences within a given column ($P < 0.05$).

Source: Marty & Marangoni, 2009. Reproduced with permission.

The strong correlations between the molecular composition (linoleic acid), the mesostructure (particle size), the nanostructure (crystalline domain size) and matrix permeability discussed earlier illustrate well the influence of cocoa butter origin on crystal network properties (Marty & Marangoni, 2009). However, they did not find a significant interaction between permeability coefficient and OMR. The authors proposed that the driving force responsible of the oil flow was significantly different between the different origins or the modification of the matrix structural parameters due to oil migration would have to be taken into account.

In contrast, two other chemical characteristics (level of oleic acid and total triunsaturated triglycerides) were significantly correlated with OMR. Higher C18:1 and UUU concentrations were associated with higher OMRs ($r = 0.93$ and $r = 0.94$, $P < 0.01$, respectively in tempered cocoa butters).

Conclusions

This review illustrates the “origin” of cocoa butter can significantly impact its crystallization behavior. Indeed not only environmental conditions and agronomic practices could induce cocoa butter chemical composition variability but also any step of the cocoa bean post-harvest treatments, of the cocoa butter production or of the chocolate manufacture may modify cocoa butter overall quality. Thus, extremely small changes in major or minor lipid components can dramatically change cocoa butter crystallization. Fig. 11.5 represents well the impact of molecular structure and composition on higher structural hierarchy levels by modifying nucleation and/or crystallization processes and hence affecting the crystal network properties (e.g. strength, permeability). However, as it remains difficult to know

the complete history of cocoa butter samples or to fully describe their whole chemical composition, further investigation would be required to draw more precise conclusions.

References

- Acevedo, N. and Marangoni, A.G. Characterization of the nanoscale in triacylglycerol crystal networks. *Cryst Growth Des* 2010, 10, 3327–3333.
- Arruda, D. and Dimick, P. Phospholipid composition of lipid seed crystal isolates from Ivory Coast cocoa butter. *J Am Oil Chem Soc* 1991, 68, 385–390.
- Beckett, S.T. Crystallizing the fat in chocolate. In *The Science of Chocolate*, The Royal Society of Chemistry: Cambridge, 2000a; 85–103.
- Beckett, S.T. Cocoa bean processing. In *The Science of Chocolate*, The Royal Society of Chemistry: Cambridge, 2000b; 31–48.
- Beckett, S.T. Chocolate ingredients. In *The Science of Chocolate*, The Royal Society of Chemistry: Cambridge, 2000c; 8–30.
- Boistelle, R. Fundamentals of nucleation and crystal growth. In *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. & Sato, K., Eds.; Marcel Dekker: New York, 1988; 189–226.
- Campos, R., Ollivon, M. and Marangoni, A.G. Molecular composition dynamics and structure of cocoa butter. *Cryst Growth Des* 2010, 10, 205–217.
- Chaiseri, S. and Dimick, P. Lipid and hardness characteristics of cocoa butters from different geographic regions. *J Am Oil Chem Soc* 1989, 66, 1771–1776.
- Chaiseri, S. and Dimick, P. Dynamic crystallization of cocoa butter. II. Morphological, thermal, and chemical characteristics during crystal growth. *J Am Oil Chem Soc* 1995b, 72, 1497–1504.
- Chaiseri, S. and Dimick, P. Dynamic crystallization of cocoa butter. I. Characterization of simple lipids in rapid- and slow-nucleating cocoa butters and their seed crystals. *J Am Oil Chem Soc* 1995a, 72, 1491–1496.
- Chaiseri, S. and Dimick, P.S. Cocoa butter—its composition and properties. *The Manufacturing Confectioner* 1987, 67, 115–122.
- Chapman, G.M., Akehurst, E.E. and Wright, W.B. Cocoa butter and confectionery fats, Studies using programmed temperature X-ray diffraction and differential scanning calorimetry. *J Am Oil Chem Soc* 1971, 48, 824–830.
- Cook, L.R. (1972). *Chocolate production and use*. Books For Industry, Inc., subsidiary of Magazines For Industry, Inc., 777 Third Avenue, New York, N.Y. 10017, USA.
- Dibildox-Alvarado, E., Rodrigues, J.N., Gioielli, L.A., Toro-Vazquez, J.F. and Marangoni, A.G. Effects of crystalline microstructure on oil migration in a semisolid fat matrix. *Cryst Growth Des* 2004, 4, 731–736.
- Dimick, P.S. Principles of cocoa butter crystallization. *The Manufacturing Confectioner* 1991, May, 109–114.
- Dimick, P. and Manning, D. Thermal and compositional properties of cocoa butter during static crystallization. *J Am Oil Chem Soc* 1987, 64, 1663–1669.

- El-Saied, H.M., Morsi, M.K. and Amer, M.M. Composition of cocoa shell fat as related to cocoa butter. *Zeitschrift für Ernährungswissenschaft* 1981, 20, 145–151.
- Foubert, I., Vanrolleghem, P.A., Thas, O. and Dewettinck, K. Influence of chemical composition on the isothermal cocoa butter crystallization. *J Food Sci* 2004, 69, 478–487.
- Fowler, M.S. Cocoa beans: from tree to factory. In *Industrial chocolate manufacture and use*, Beckett, S.T., Ed.; Willey-Blackwell: Ames, 2008; 10–47.
- Gutshall-Zakis, A. and Dimick, P.S. Influence of various refining treatments on the crystallization behavior of hard and soft cocoa butters and formulated dark chocolate. *The Manufacturing Confectioner* 1993, 73, 117–123.
- Hanneman, E. The complex world of cocoa butter. *The Manufacturing Confectioner* 2000, September, 107–112.
- Jacobsberg, B. and Oh, O.C. Studies in palm oil crystallization. *J Am Oil Chem Soc* 1976, 52, 609–617.
- Jeffrey, M.S. The effect of cocoa butter origin, milk fat and lecithin levels on the temperability of cocoa butter systems. *The Manufacturing Confectioner* 1991, 71, 76–82.
- Johansson, D. Weak gels of fat crystals in oils at low-temperatures and their fractal nature. *J Am Oil Chem Soc* 1995, 72, 1235–1237.
- Kamphuis, H.J. Production quality standards of cocoa mass, cocoa butter and cocoa powder. In *Industrial chocolate manufacture and use*, Beckett, S.T., Ed.; Willey-Blackwell: Ames, 2008; 121–141.
- Kaneko, N., Horie, T., Ueno, S., Yano, J., Katsuragi, T. and Sato, K. Impurity effects on crystallization rates of n-hexadecane in oil-in-water emulsions. *Journal of Crystal Growth* 1999, 197, 263–270.
- Kattenberg, H.R. The quality of cocoa butter. *The Manufacturing Confectioner* 1981, 27, 519–525.
- Koyano, T., Hachiya, I., Arishimo, T., Sato, K. and Sagi, N. Polymorphism of POP and SOS. II. Kinetics of melt crystallization. *J Am Oil Chem Soc* 1989, 66, 675–679.
- Lechter, A. Tempering chocolate—a review. *The Manufacturing Confectioner* 2009, 53.
- Lehrian, D.W. and Keeney, P.G. Changes in lipid components of seeds during growth and ripening of cacao fruit. *J Am Oil Chem Soc* 1980, 57, 61–65.
- Lehrian, D.W., Keeney, P.G. and Butler, D.R. Triglyceride characteristics of cocoa butter from cacao fruit matured in a microclimate of elevated temperature. *J Am Oil Chem Soc* 1980, 57, 66–69.
- Liendo, R., Padilla, F.C. and Quintana, A. Characterization of cocoa butter extracted from Criollo cultivars of *Theobroma cacao* L. *Food Res Int* 1997, 30, 727–731.
- Lipp, M., Simoneau, C., Ulberth, F., Anklam, E., Crews, C., Brereton, P., de Greyt, W., Schwack, W. and Wiedmaier, C. Composition of genuine cocoa butter and cocoa butter equivalents. *Journal of Food Composition Analysis* 2001, 14, 1–10.
- Loisel, C., Keller, G., Lecq, G., Bourgaux, C. and Ollivon, M. Phase transitions and polymorphism of cocoa butter. *J Am Oil Chem Soc* 1998, 75, 425–439.
- Marangoni, A.G. Crystallization Kinetics. In *Fat Crystal Network*, Marangoni, A., Ed.; Marcel Dekker: New York, 2005; 21–82.

- Marangoni, A.G. and McGauley, S.E. Relationship between crystallization behavior and structure in cocoa butter. *Cryst Growth Des* 2003, 3, 95–108.
- Marty, S. and Marangoni, A.G. Effects of cocoa butter origin, tempering procedure, and structure on oil migration kinetics. *Cryst Growth Des* 2009, 9, 4415–4423.
- McHenry, L. and Fritz, P. Cocoa butter biosynthesis: Effect of temperature on *Theobroma cacao* acyltransferases. *J Am Oil Chem Soc* 1987, 64, 1012–1015.
- Narine, S. and Marangoni, A.G. Fractal nature of fat crystal networks. *Physical Review* 1999, 59, 1908–1920.
- Parsons, J.G. and Keeney, P.G. Phospholipids concentration in cocoa butter and its relationship to viscosity in dark chocolate. *J Am Oil Chem Soc* 1969, 46, 425–427.
- Parsons, J.G., Keeney, P.G. and Patton, S. Identification and quantitative analysis of phospholipids in cocoa beans. *J Food Sci* 1969, 34, 497–499.
- Rousset, P. & Rappaz, M. Experimental study and computer modeling of the dynamic and static crystallization of cocoa butter. In *Crystallization and Solidification Properties of Lipids*, Widlak, N., Hartel, R.W. and Narine, S., Eds.; AOCS Press: Champaign, IL, 2001; 96–109.
- Sato, K., Arishima, T., Wang, Z.H., Ojima, K., Sagi, N. and Mori, H. Polymorphism of POP and SOS. I. Occurrence and polymorphic transformation. *J Am Oil Chem Soc* 1989, 66, 664–674.
- Sato, K. & Koyano, T. Crystallization properties of cocoa butter. In *Crystallization Process in Fats and Lipid Systems*, Garti, N. & Sato, K., Eds.; Marcel Dekker, Inc.: New York, 2001; 429–456.
- Savage, C.M. and Dimick, P.S. Influence of phospholipids during crystallization of hard and soft cocoa butters. *The Manufacturing Confectioner* 1995, 11, 127–131.
- Schlichter-Aronhime, J. and Garti, N. Solidification and polymorphism in cocoa butter and the blooming problems. *Surfactant Science Series* 1988, 31, 363–393.
- Schlichter-Aronhime, J., Sarig, S. and Garti, N. Reconsideration of polymorphic transformations in cocoa butter using DSC. *J Am Oil Chem Soc* 1988, 65, 1140–1143.
- Seguine, E. Cocoa genetics and breeding. *The Manufacturing Confectioner* 2011, February, 54–62.
- Shukla, V.K.S. Cocoa butter properties and quality. *Lipid Technology* 1995, May, 54–57.
- Sulistyowati and Misnawi, J.S. Effects of alkali concentration and conching temperature on antioxidant activity and physical properties of chocolate. *International Food Research Journal* 2008, 15.
- Takeuchi, M., Ueno, S., Flöter, E. and Sato, K. Binary phase behavior of 1,3-distearoyl-2-oleoyl-sn-glycerol (SOS) and 1,3-distearoyl-2-linoleoyl-sn-glycerol (SLS). *J Am Oil Chem Soc* 2002, 79, 627–632.
- Talbot, G. Fat migration in biscuits and confectionery systems. *Confectionery Production* 1990, 56, 265–272.
- Timms, R.E. Physical chemistry. In *Confectionery Fats Handbook: Properties, Production and Application*, Barnes, P.J.&A., Ed.; The Oily Press: Bridgwater, UK, 2003b; 9–62.
- Timms, R.E. Production and characteristic properties. In *Confectionery Fats Handbook: Properties, Production and Application*, Barnes, P.J.&A., Ed.; The Oily Press: Bridgwater, UK, 2003a; 191–254.

- Timms, R.E. Interactions between fats, bloom and rancidity. In *Confectionery Fats Handbook: Properties, Production and Application*, Barnes, P.J.&A., Ed.; The Oily Press: Bridgwater, UK, 2003c; 255–294.
- Timms, R.E. and Stewart, I.M. Cocoa butter, a unique vegetable fat. *Lipid Technology Newsletter* 1999, October, 101–107.
- Vaeck, S.V. Cocoa butter and fat bloom. *The Manufacturing Confectioner* 1960, June, 35–74.
- van Malssen, K., Peschar, R., Brito, C. and Schenk, H. Real-time X-ray powder diffraction investigations on cocoa butter. 3. Direct beta-crystallization of cocoa butter: Occurrence of a memory effect. *J Am Oil Chem Soc* 1996a, 73, 1225–1230.
- van Malssen, K., Peschar, R. and Schenk, H. Real-time X-ray powder diffraction investigations on cocoa butter. 2. The relationship between melting behavior and composition of beta-cocoa butter. *J Am Oil Chem Soc* 1996b, 73, 1217–1223.
- van Malssen, K., Peschar, R. and Schenk, H. Real-time X-ray powder diffraction investigations on cocoa butter. 1. Temperature-dependent crystallization behavior. *J Am Oil Chem Soc* 1996c, 73, 1209–1215.
- van Malssen, K., van Langevelde, A., Peschar, R. and Schenk, H. Phase behavior and extended phase scheme of static cocoa butter investigated with real-time X-ray powder diffraction. *J Am Oil Chem Soc* 1999, 76, 669–676.
- Vila Ayala, J., Calliauw, G., Foubert, I., Dewettinck, K., Dyer, B. and De Greyt, W. Impacts of bleaching and packed column steam refining on cocoa butter properties. *J Am Oil Chem Soc* 2007, 84, 1069–1077.
- Walstra, P. Changes in dispersity. In *Physical Chemistry of Foods*, Walstra, P., Ed.; Marcel Dekker, Inc.: New York, 2003a; 476–546.
- Walstra, P. Fat crystallization. In *Food Structure and Behavior*, Blanshard, J.M.V. & Lillford, P., Eds.; Academic Press: Orlando, FL, 1987; 67–85.
- Walstra, P. Crystallization. In *Physical Chemistry of Foods*, Walstra, P., Ed.; Marcel Dekker, Inc.: New York, 2003b; 583–649.
- Walstra, P. Nucleation. In *Physical Chemistry of Foods*, Walstra, P., Ed.; Marcel Dekker, Inc.: New York, 2003c; 548–582.
- Walstra, P., Kloeck, W. and van Vliet, T. Fat crystal network. In *Crystallization Process in Fats and Lipid Systems*, Garti, N. & Sato, K., Eds.; Marcel Dekker, Inc.: New York, 2001; 289–328.
- Wille, R.L. and Lutton, E.S. Polymorphism of cocoa butter. *J Am Oil Chem Soc* 1966, 43, 491–496.
- Wootton, M., Weeden, D. and Munk, N. Mechanism of fat migration in chocolate enrobed goods. *Chemistry and Industry* 1970 1052–1053.
- Wright, A., Hartel, R., Narine, S. and Marangoni, A. The effect of minor components on milk fat crystallization. *J Am Oil Chem Soc* 2000, 77, 463–475.
- Wright, D., Park, W., Leopold, N., Hasegawa, P. and Janick, J. Accumulation of lipids, proteins, alkaloids and anthocyanins during embryo development in vivo of *Theobroma cacao* L. *J Am Oil Chem Soc* 1982, 59, 475–479.

12

Effect of Emulsifiers on Cocoa Butter and Chocolate Rheology, Polymorphism, and Bloom

Nissim Garti^{*1} and Abraham Aserin¹

^{*}The Ratner Chair of Chemistry, and ¹Casali Institute of Applied Chemistry, The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

Introduction

Chocolate is a very complex system in which cocoa fat (butter), sugar, and cocoa solids (in some cases also milk fat) particles are dispersed in molten liquid cocoa butter to form, upon controlled cooling, a solid-like dispersion into a small fraction of liquid cocoa butter (Afoakwa et al., 2007b).

Particle sizes are reduced and adjusted by a roll refining machine to $<40\ \mu\text{m}$. A typical classical dark chocolate will consist of 3–32 wt% cocoa butter (CB), 6 wt% carbohydrates, 6 wt% fibers, and 52–54 wt% sugars, along with ca. 4 wt% proteins. An average milk chocolate contains 12 g cocoa mass, 19 g whole milk powder, 48.5 g sugar, and, additionally, 20 g added cocoa butter per 100 g chocolate (Cook, 1984). Cocoa beans consist of 54 wt% fat, 31 wt% carbohydrates, 11 wt% proteins, and 3 wt% polyphenols along with ca. 1 wt% minerals. All the values are average and might change significantly from product to product.

Cocoa butter is complex mixture of fatty acids grafted onto a glycerol skeleton to form the so-called triacylglycerols (TAG) of fatty acids. Most of the triglycerides ($>75\%$) are symmetrical, in which the saturated palmitic (P) and/or stearic (S) fatty acids occupy positions sn1 and/or sn3 (i.e., POP, SOS, and POS) with some unsymmetrical TAGs such as PPO, PSO, SSO (Segall et al., 2004) (Fig. 12.1A and B), 1.3% diacylglycerides or diglycerides (DAG), 0.03% monoacylglycerides or monoglycerides (GMS), 1.17% free fatty acids (FFA), and 0.12% sterols. In dark chocolate pressed CB is used, while in white chocolate refined (deodorized) CB is preferred (Fig. 12.1).

Amphiphilic molecules have a strong tendency to migrate from a bulk solution to liquid or solid interfaces, and while adsorbing can change the surface properties of the dispersing phase, the surface properties of these amphiphiles were extensively investigated and applied in many fields including pharmaceuticals, cosmetics, and food. Some of these amphiphiles, mainly lecithin, are essential ingredients in chocolate manufacture, controlling and modifying the rheology and polymorphism of the cocoa butter and the chocolate (Afoakwa et al., 2008, 2009a; Lee et al., 2002; Svanberg et al., 2011).

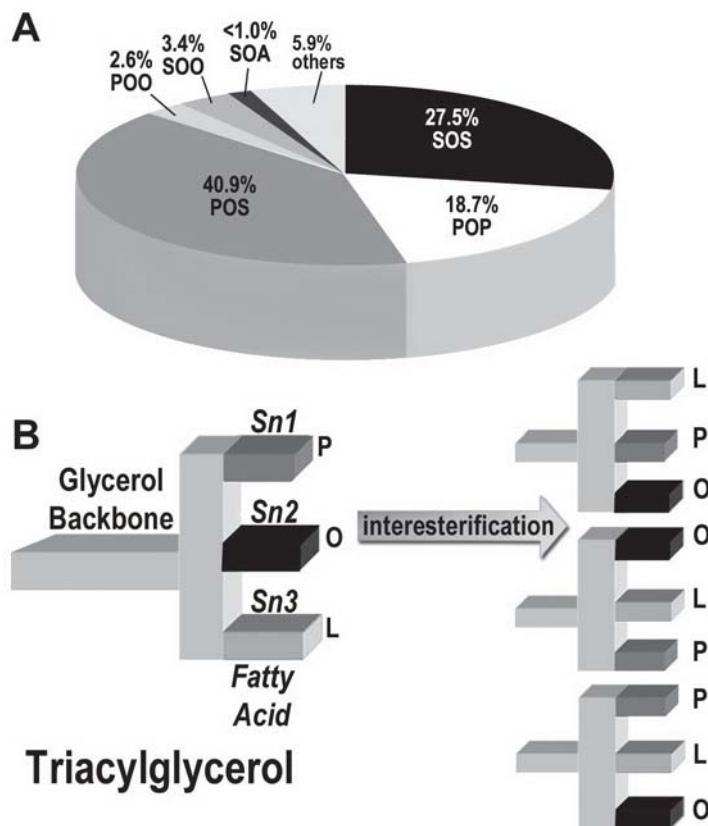


Fig. 12.1. Triacylglycerols: (A) composition of cocoa butter, and (B) example of positional distribution of the fatty acids. Source: Karupaiah & Sundram, 2007.

If an amphiphile (termed also surface active molecule or surfactant) is adsorbing onto an oil-water interface, it stabilizes the emulsion and retards or prevents its tendency to cream, aggregate, flocculate, and coalesce. Surfactants can also adsorb onto solid particles or crystals that are dispersed in liquid continuous phase. They stabilize the dispersion and retard the aggregation, precipitation, and separation of the solid phase from the liquid continuous phase (Garti et al., 1998).

The production of chocolate includes a step in which, in very general terms, the producers are micronizing solid sugar crystals and cocoa powder, adding them to a molten cocoa butter phase, and cooling the mixture after mixing and seeding with CB particles until most of the cocoa fat is crystallized and solid dispersed particles form a strong viscoelastic network that progressively hardens when completing the cooling process. The two very sensitive and crucial steps in the formation of the

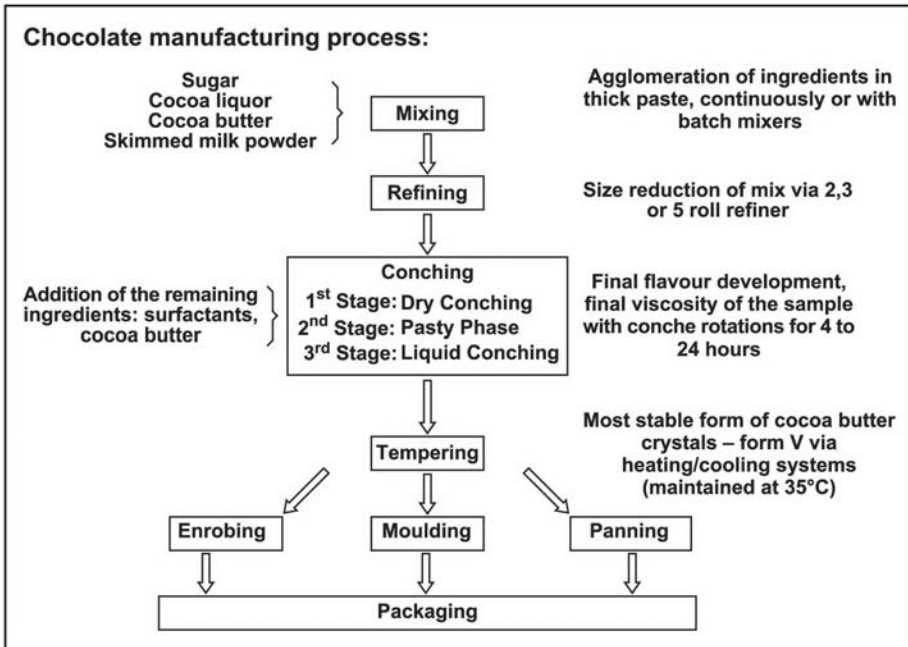


Fig. 12.2. General illustration of the steps in the production of chocolate. Source: Afoakwa et al., 2007.

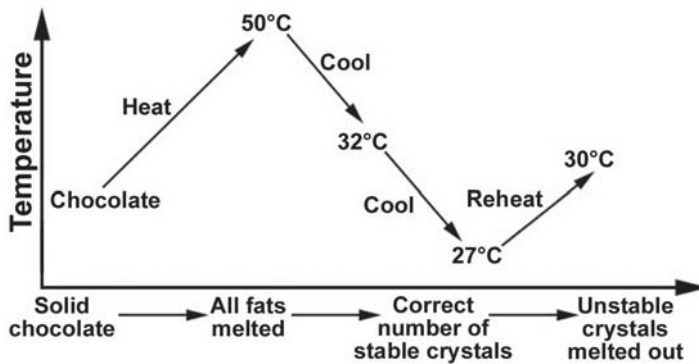


Fig. 12.3. The cooling profile in chocolate production.

final chocolate are known as conching and tempering (Martin, 1987; Prawira & Barringer, 2008). Figs. 12.2 and 12.3 demonstrate, in brief, the chocolate making steps. The effect of the emulsifiers is also indirectly manifested in the post-crystallization stage.

The production process in all its stages always requires the addition of one or more additives—viscosity controllers. The major additive, because of its low price and very unique properties, is lecithin (Vannieuwenhuyzen, 1981). Commercial lecithin that is used in chocolate manufacturing is a crude and complex physical mixture of phospholipids (PL) produced as a byproduct, mainly from soybeans, in the extraction and treatment process of vegetable oils and soy proteins (Ceci et al., 2008). For simplicity we will term this fraction lecithin, although its more accurate name will be lecithins. Only in some specific cases are other types of food-grade emulsifiers added to the mass as rheology or crystallization controllers. The other additives are added as co-viscosity reducers or polymorphism controllers to minimize the bloom phenomenon.

All the studied surfactants (to be termed in some cases emulsifiers) are, in general, lipophilic and dissolve or disperse well and uniformly in the molten fat phase but adsorb in part onto sugar particles, solid particles of fats (CB or milk fat), and cocoa solids.

Emulsifiers have long been used to modify the flow properties of chocolate masses. These surface-active ingredients lower the interfacial tension between the dispersed and the continuous phases. In addition, these additives affect a number of other properties such as the sensitivity to moisture and temperature, and the tempering behavior (Minifie, 1980; Schantz et al., 2001). Additionally, the emulsifiers may influence some properties such as stability to fat migration from fillings and oxidation (Hasenhuettl & Hartel, 1997; Schantz & Rohm, 2005; Schuster, 1985; Schantz & Rohm, 2005). In this chapter we will deal mostly with the two major phenomena—viscosity and bloom.

Note that even if we recognize the overall effect of certain emulsifiers under specific conditions (temperature and ingredient composition), their effect is often different and unpredictable if the crystallization conditions change before or during tempering, or raw material composition varies significantly. Moreover, even if we know to predict their general activity it is difficult to completely understand the mechanism of their action mainly because of the complexity of the system. It should also be noted that the theories on their activities have changed with the years of research, mainly because of the development of new advanced analytical tools that were lacking in the past. The reader must be careful when exploring old studies that in many cases are non-relevant in their interpretation.

This dynamic action of surfactants is still somewhat obscure and it seems that it will take more years and efforts of research to establish in full these interfacial activities.

In this chapter we will deal with two major activities of emulsifiers on cocoa butter and chocolate: rheology and polymorphism.

Rheology

Viscosity is the resisting force of a fluid matter to shear and depends on the speed of motion, expressed as the shear rate. Viscosity is a tensorial quantity that can be

decomposed in different ways to two independent components—shear viscosity and volume viscosity. Shear viscosity, referred to as simply viscosity, describes the reaction to applied shear stress; simply put, it is the ratio between the pressure exerted on the surface of a fluid, in lateral or horizontal directions, to the change in velocity of the fluid as you move down in the fluid (this is what is referred to as a velocity gradient). Volume viscosity (bulk viscosity) becomes important only for such effects where fluid compressibility is essential.

Shear stress, denoted τ , is defined as the component of stress coplanar with a material cross-section. Shear stress arises from the force vector component parallel to the cross-section. Normal stress, on the other hand, arises from the force vector component perpendicular or antiparallel to the material cross-section on which it acts ($\tau = F/A$).

Shear strain, σ , is a change in displacement with respect to distance. A strain is a normalized measure of deformation representing the displacement between particles in the body relative to a reference length. Deformation has units of length.

$$\sigma = dx_x/dy = X_0/H$$

Viscosity for Newtonian fluids is defined as the shear strain, σ , over the shear rate, γ .

$$\eta = \sigma/\gamma$$

Certain fluids are non-Newtonian and exhibit rheology properties for which simple viscosity does not describe the flow and deformation properties of the fluid. For Non-Newtonian fluids, there are different constitutive laws depending on the fluid, which relate the stress tensor to the shear rate tensor. In such systems in general terms, a shear modulus or modulus of rigidity better characterizes the viscosity behavior of the fluid. The term modulus is used, denoted by G (or sometimes S or μ), which is defined as the ratio of shear strain over the shear rate ($G = \sigma_0/\gamma_0$).

Elasticity is the property of an object or material that causes it to be restored to its original shape after distortion. It is said to be more elastic if it restores itself more precisely to its original configuration. This restoring force (like in any spring) is generally proportional to the amount of stretch, as described by Hooke's Law. The elasticity is generally described in terms of the amount of deformation (strain) resulting from a given stress (Young's modulus). Young's modulus describes the material's response to linear strain (like pulling on the ends of a wire). The shear modulus is one of several quantities for measuring the stiffness of materials. All of them arise in the generalized Hooke's law:

The storage modulus (elasticity) is defined as:

$$G' = (\sigma_0/\gamma_0)\cos\delta.$$

The loss modulus (viscous) is defined as:

$$G'' = (\sigma_0/\gamma_0)\sin\delta.$$

From the two terms one can derive

$$\tan \delta = G''/G'.$$

Most scientists use the parameters G' and G'' to describe the rheology properties of non-Newtonian fluids, but the manufacturers and technologies adopted a simpler model to evaluate, determine, and control the rheology behavior of chocolate in terms of the engineering and cost effect in the production stages—shear viscosity and yield stress determined in the Casson equation (Casson, 1959) and other models.

Shear viscosity describes the reaction to applied shear stress. It is the ratio between the pressures exerted on the surface of a fluid, in the lateral or horizontal direction, to the change in velocity of the fluid as you move deeper in the fluid.

The yield stress is a critical value of shear stress, below which a viscoplastic material behaves like a solid; above this value, a plastic material deforms and a viscoplastic material flows.

The three most important models that have been used to characterize chocolate rheology include the Herschel-Bulkley, Casson, and Bingham forms (Beckett, 2000; Chevalley, 1999; Sokmen & Gunes, 2006).

Herschel-Bulkley	$\tau - \tau_0 = \tau_{PL} (\dot{\gamma})^n$
Casson	$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} \sqrt{\dot{\gamma}}$
Bingham	$\tau_B = \tau_n + \mu_B + \dot{\gamma}$

In these equations, τ is the shear stress; τ_0 – yield stress; μ_{PL} – plastic (shear) viscosity; τ_{CA} – Casson yield value; τ_{CA} – Casson plastic viscosity; τ_B – Bingham yield value; μ_B – Bingham plastic viscosity; $\dot{\gamma}$ – shear rate; μ – viscosity of the suspension; n – flow viscosity index (Figs. 12.4 and 12.5).

The Casson model was the standard confectionery industry strategy to quantify rheological properties of molten chocolate until 2000, when the International Confectionery Association recommended the use of interpolation data to describe viscosity (Afoakwa et al., 2009b). The two values are used in some places while in others only the Casson model is exercised.

Based on the Casson model, shear viscosity and shear stress are important parameters in the manufacturing process and are directly influenced by product particle size distribution (PSD) and composition. The authors of many studies defined, compared, and correlated the rheological properties of molten dark chocolates prepared using different PSD, fat content, and lecithin content. Rheological parameters were determined using a shear rate-controlled rheometer, and data were examined using correlation, regression, and principal component analyses to establish their interrelationships. The results show the importance in taking into account the particle sizes and distribution in controlling the flow properties of molten chocolate (Afoakwa et al., 2009b).

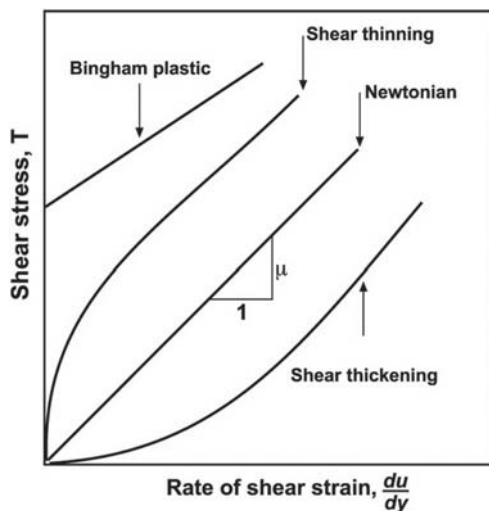


Fig. 12.4. Plot of shear stress against shear strain describing several types of fluids.

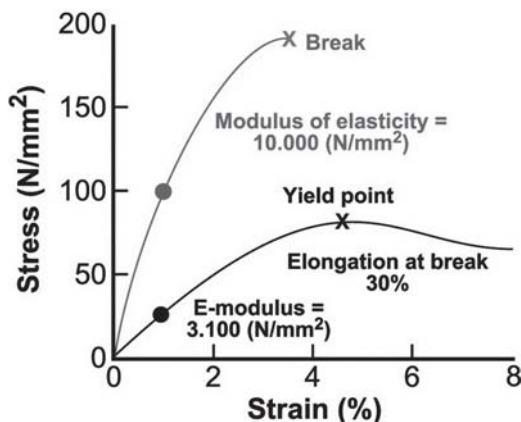


Fig. 12.5. The dependence of the shear rate on the viscosity for various types of fluids. Chocolate and cocoa butter can undergo deformations.

Since 1973, the International Confectionery Association (ICA) has accepted rheological measurement of molten chocolate using rotational viscometers with concentric cylinders (bob and cup geometry) and Casson equation calculation of the parameters (IOCCC, International Office of Cocoa, Chocolate and Confectionary, 1973) (Bouzas & Brown, 1995).

In 2000, ICA recommended measurement of stress and viscosity at shear rates between 2 s^{-1} and 50 s^{-1} using up and down curves in shear rate, preceded by a pre-shear at 5 s^{-1} lasting $\geq 5 \text{ min}$. The change in 2000 was based on the results from an inter-laboratory study (Aeschlimann & Beckett, 2000), which concluded that Casson's mathematical model employing only a small set of parameters was limited in accuracy as, at lower shear rates, rheology data do not fit the Casson equation well. The outcome was a low degree of repeatability in inter-laboratory analyses, and ICA thus recommended the use of interpolation data for chocolate viscosity. The newer International Confectionery Association technique gave higher correlation and regression coefficients than the Casson model, but multivariate principal component analysis showed that the two models were strongly related and either could effectively quantify dark chocolate viscosity parameters. In spite of these comments and since most studies were done using the Casson equation, we will describe in this chapter the effect of the emulsifiers in terms of the Casson equation and model.

The chocolate manufacturers are interested in the yield stress since it reflects the amount of energy required to initiate fluid flow. In products exhibiting plastic viscosity the energy required to keep fluid in motion is measured and controlled (Afoakwa et al., 2007; Beckett, 1999; Ziegler & Hogg, 1999). Plastic viscosity is important for defining the pumping characteristics, filling of rough surfaces, and coating properties, yield stress to shape retention, pattern holding, inclined surface coating, and bubbles in processing (Afoakwa et al., 2007a; Seguiné, 1988). These rheological properties are important in chocolate manufacturing for quality-control purposes and final product characteristics, and can be related to composition, processing strategy, and solid particle size distribution. Rheological parameters often give information related to sensory character in chocolate.

For better understanding of the rheology terms the reader should acquire some basic knowledge.

Lecithins as a Rheology Modifier

Lecithin was discovered in 1845–1874 by Théodore Nicolas Gobley (Gobley, 1845a, 1845b, 1847, 1850, 1874a, 1874b, 1874c), who isolated it in egg yolks and identified it as the substance that allowed oil and water to mix. The name is derived from the Greek word *lekithos*, which means “yolk of egg.” Lecithin is a naturally occurring fatty substance found in several foods including soybeans, whole grains, and egg yolks. It is often used as an emulsification agent in processed foods. It can be taken in various forms as a nutritional supplement, often derived from soybeans. The body breaks lecithin down into its component parts: choline, phosphate, glycerol, and fatty acids. The body's greatest concentration of lecithin is found in the vital organs, where it makes up about 30% of the dry weight of the brain. Lecithin acts as an emulsifier and helps the body in the absorption of fats.

The lecithin that is used in chocolate is a commercial product containing ca. 50–65 wt% phospholipids (PL); the rest is oil with some minor components. The PL is composed mainly from phosphatidylcholine (PC, ca. 21%) along with other PL derivatives such as phosphatidylethanolamine (PE, 22%), phosphatidylinositol (PI, 19 wt%), phosphatidic acid (PA, ca. 6%), phosphatidylglycerol (PG), phosphatidylserine (PS), and some other minor components (Fig. 12.6).

In addition to soy lecithin, in recent years some producers have offered lecithin from rapeseed oil (Canola) and from sunflower oil (Smiles et al., 1988). The Canola lecithin has very similar composition to soy lecithin, but its content in the rape seeds and crude oil is lower. The main advantage might be that it is not necessarily genetically modified (non-GMO). The sunflower is somewhat different and is very rich in PC (52%), PE (20%), and PI (26%), with a small amount of PA (only 2.2%). PA (which is not easily hydrated in water) in both lecithins can be extracted using new advanced enzymatic degumming methods.

The fatty acid compositions of the two major sources of lecithin are summarized in Table 12-A. The performance of rapeseed lecithin is similar to that of soy lecithin.

The unusual activity of lecithin in chocolate puzzled many technologies and scientists and to date there are no good lecithin replacers performing as well as lecithin in the major rheology parameters. Years of research were devoted to understanding the

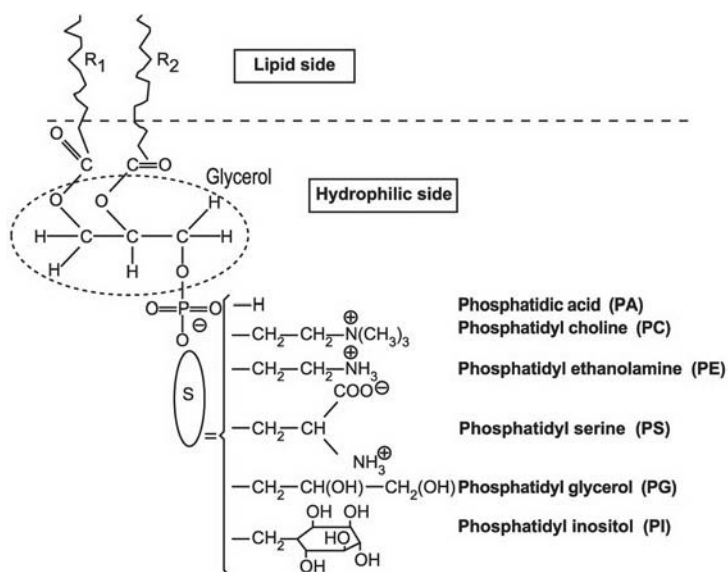


Fig. 12.6. Chemical structure of lecithin.

Table 12-A. Comparison between Fatty Acid Composition of Lecithin from Soybeans and Rapeseeds.

	Soybean (wt%)	Rapeseeds (wt%)
Palmitic acid (C16)	17	4
Stearic acid (C18)	5	1
Oleic acid (C18:1)	19	65
Linoleic acid (C18:2)	53	15
Linolenic acid (C18:3)	6	11

role of lecithin in chocolate and probably more are needed to understand its molecular activity in the bulk and on the different dispersed particles in the viscoelastic matrix.

It is now clear that lecithin plays a combined very significant role in the bulk (the molten oil) and on the interface of the solid dispersed particles (fats, sugar, and cocoa solids).

The main factors in chocolate that affect rheology are particle size distribution of the various components, ingredient composition, temperature, shear conditions, and the nature and concentration of the added rheology modifier (lecithin). Lecithin addition significantly changes the Casson yield value and plastic viscosity when added at between 0.1 wt% and 0.3 wt% (Fig. 12.5). It reduces chocolate's viscosity and also enhances toleration of higher moisture levels. The results indicate that the adsorption of emulsifier increases the lipophilicity of the sucrose particles via a strong decrease in the acidic character of the surface. This induces a decrease in the sucrose–sucrose interactions and thus explains the increased fluidity of the fat-based suspension. In a good study Rousset et al. have confirmed that the effect of lecithin is via its coating on the surface properties of standard and milled sucrose (Rousset et al., 2002). As expected, milling significantly increased the dispersive component of sucrose due to the creation of more, new energetic surfaces. Interestingly, when jet-milled sucrose was coated with a layer of phospholipids, the dispersive component of the surface energy was significantly reduced to the value of granulated sucrose. The dispersive component was not altered when granulated sucrose was coated with lecithin (Table 12-B). The solid surface energy is γ^D_s : the values in Table 12-B are calculated based on the classical theory of surface free energy. K_A and K_B are, respectively, the acidic and basic constants characterizing the surface properties of the solid particles (Rousset et al., 2002).

Lecithin greatly decreased the acidic (acceptor) character of sucrose originating from the hydroxyl groups of the molecule. It also slightly changed its basic characteristics (Fig. 12.7). The decrease in acidic character may be explained by the apolar chain of the phospholipids in lecithin. The increase in the basic character may be due to phospholipids such as phosphatidylethanolamine. In general, adsorbed lecithin

Table 12-B. Effect of Adsorbed Lecithin at the Sucrose Surface.

	Granulated sucrose		Jet milled sucrose	
	Without lecithin	With lecithin	Without lecithin	With lecithin
γ_D^S (mJ/m ²)	30.3	30.6	36.5	30.9
$K_A \cdot 10^{-2}$	18	15	19	16
$K_B \cdot 10^{-2}$	23	28	22	26

Source: Rousset et al., 2002.

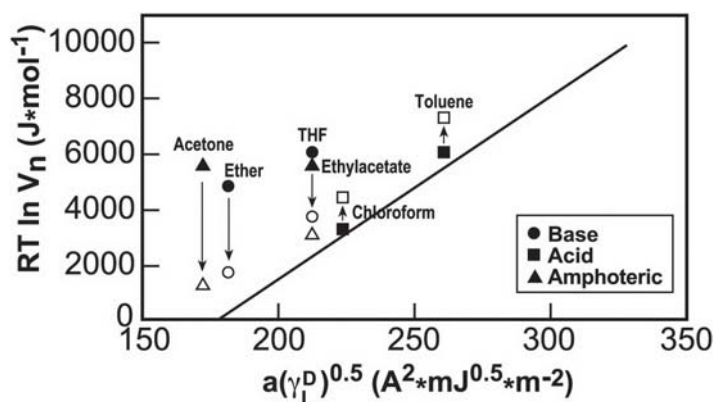


Fig. 12.7. Free energy of adsorption ($RT \ln V_n$) as a function of the dispersive surface energy of the vapor probe: effect of adsorbed lecithin at the granulated sucrose surface. (●) Without lecithin, (○) with lecithin. Source: Rousset et al., 2002.

increases the lipophilicity of sugar particles, thus inducing an increase in the fluidity of fat-based suspensions such as chocolate. Another observation (Table 12-B) is that particle size does not influence the polar properties of the material. This is demonstrated by the similar K_A and K_B values of granulated and jet-milled sucrose with and without lecithin.

At more than 0.5 wt% lecithin, yield value increases while plastic viscosity continues to fall (Afoakwa et al., 2007; Chevalley, 1999; Rector, 2000; Schantz & Rohm, 2005). Based on one of the possible explanations, the increase in yield value at elevated levels of lecithin is linked to reverse micelle formation in the continuous oily phase. The authors speculate that lecithin can possibly form multilayers around the sugar particles, resulting in hindrance of the flow. Alternatively, reverse micelles may form in the continuous phase and interact with fully covered sugar particles, consequently increasing yield value (Vernier, 1998). The effect of the lecithin in systems containing

sucrose is more significant in comparison to systems made from isomalt. The systems made with isomalt as sugar replacers have much lower Casson viscosity even without lecithin and its effect at low content (up to 0.5 wt%) is small. The viscosity increase in the presence of milk fat particles is significantly more pronounced than in dark chocolate, meaning that the milk fat particles also play a role in the rheology behavior. Particle size distributions vary, as smaller particles require more lecithin to coat sugar surfaces. Lecithin can only be added up to 1%, but will always be present in chocolate as traces from both cocoa and milk (Figs. 12.8 and 12.9) (Nebesny & Żyżelewicz, 2005; Wilson et al., 1998).

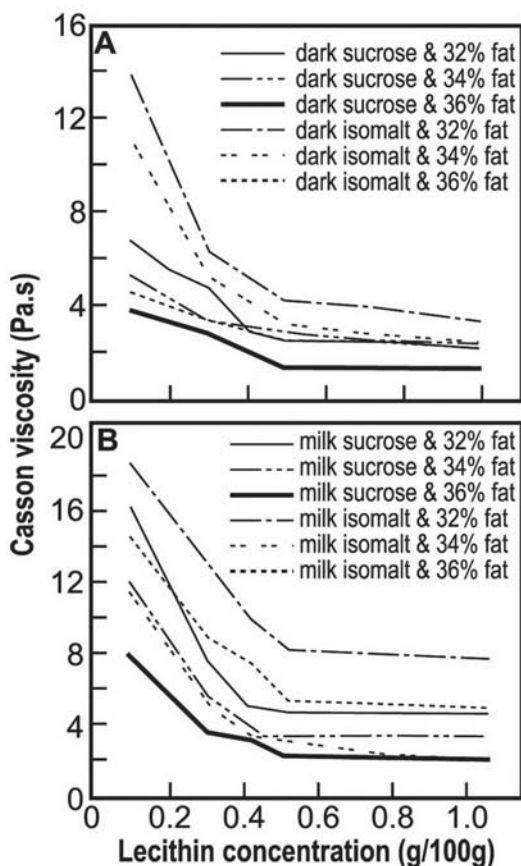


Fig. 12.8. Casson viscosity as a function of lecithin concentration in sucrose-free and sucrose-containing chocolate masses. (A) Dark chocolate masses (B) Milk chocolate masses. Source: Nebesny & Żyżelewicz, 2005.

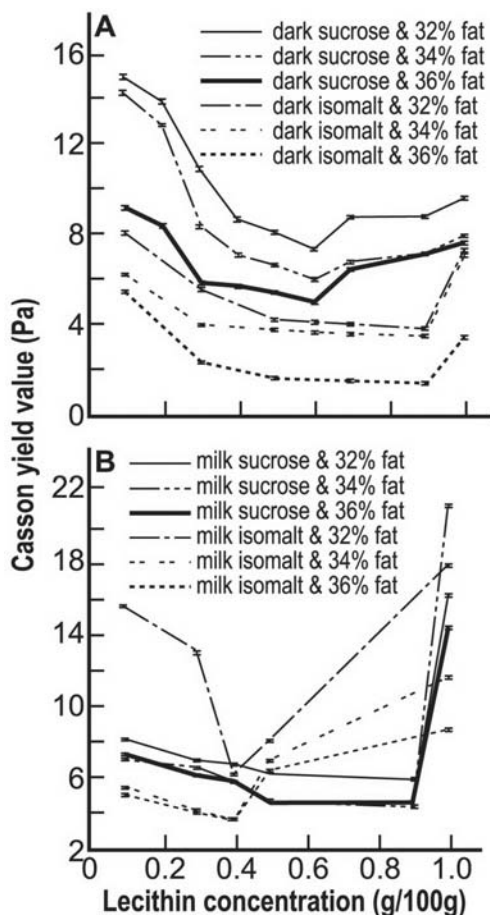


Fig. 12.9. Casson yield value as a function of lecithin concentration in sucrose-free and sucrose-containing chocolate. (A) Dark chocolate masses (B) Milk chocolate masses.

The chocolate scores in the presence of lecithin showed similarly that chocolate containing 0.3 wt% lecithin displays desirable features and scored a relative value of 4.87–5.78. Chocolate with 0.5 wt% lecithin scored values of 4.78–4.83, and chocolate with 1 wt% lecithin scored values as low as 3.30–3.33 along with unpleasant taste and aroma. This unique viscosity reduction in the Casson and yield is very typical of lecithin and no other emulsifiers are capable of doing it in the same quantitative or absolute levels.

Effect of Polyglycerol Polyricinoleate (PGPR)

PGPR is prepared by partial esterification of condensed castor oil fatty acids with polyglycerol (Rousset et al., 2002; Wilson et al., 1998). PGPR is a complex mixture with polyglycerol component dominated by di-, tri-, and tetraglycerols (Fig. 12.10) (Vernier, 1998).

PGPR does not have large effects on plastic viscosity but can reduce yield value by 50% at 0.2% or negate the plastic viscosity at about 0.8% (Rector, 2000; Schantz & Rohm, 2005), turning chocolate into a Newtonian liquid so that it flows more readily and settles rapidly. A similar outcome can be achieved by adding more cocoa butter at greater cost as PGPR coats solid particles, displacing cocoa butter to the continuous phase, decreasing yield value. Chocolate with 35% cocoa butter content has a similar yield value to that containing 32% cocoa butter and 0.1% PGPR. Polyglycerol polyricinoleate coats solid particles and, with greater molecular weight, extends further into the lipid continuous phase, producing better steric stabilization (Rector, 2000; Schantz & Rohm, 2005; Vernier, 1998).

The advantages of PGPR may be increased by combining it with lecithin. Depending on the application, the optimum lecithin:PGPR blends are given with 2:1 (Schuster, 1985), 2.5:1 (Hasenhuettl & Hartel, 1997), and 3:1 (Höfs et al., 2002). By using two different standard chocolate masses, the objective of the current project was to determine the effects of different, systematically varied mixtures of lecithin and PGPR

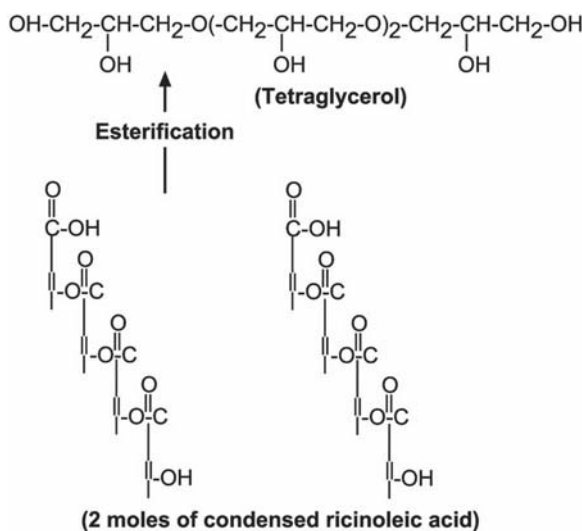


Fig. 12.10. PGPR is made from tetraglycerol esterified with 2 moles of condensed ricinoleic acid.

on the flow parameters of melted chocolate to obtain information on the optimum performance of the emulsifier blend.

In contrast to lecithin, PGPR in chocolate does not structure within the suspension, but increases the continuous phase volume fraction and binds residual water in the chocolate, making it unavailable to hydrate and swell the solid particles (Rector, 2000; Schantz & Rohm, 2005).

In recent developments, many chocolate manufacturers use PGPR and lecithin in combination for a desirable yield value and plastic viscosity, balancing out viscosity-reducing effects (Fig. 12.11) (Schantz & Rohm, 2005).

The detailed work of Schantz and Rohm (Schantz & Rohm, 2005) is summarized in this paragraph. Some of their results have already been depicted in previous studies, but it is worth re-bringing them because in some cases the intensities of the viscosity reduction are not identical. The yield stress and equilibrium viscosities of milk chocolate as a function of emulsifier addition are seen in Table 12-C. With increasing quantities of lecithin, the yield stress decreased drastically and showed a minimum at a lecithin concentration of 0.4 wt%; a further increase of the lecithin concentration then resulted in a minor increase (not a significantly large effect) of the yield stress. In line with results from literature (Beckett, 2000; Rector, 2000), PGPR appeared to be much more effective on the yield stress, and minimum yield stress corresponded to the highest PGPR concentration. As regards equilibrium viscosity, the reduction proved to be more efficient for lecithin, especially for low and intermediate quantities of added emulsifier. Generally, viscosity decreased continuously with increasing emulsifier content.

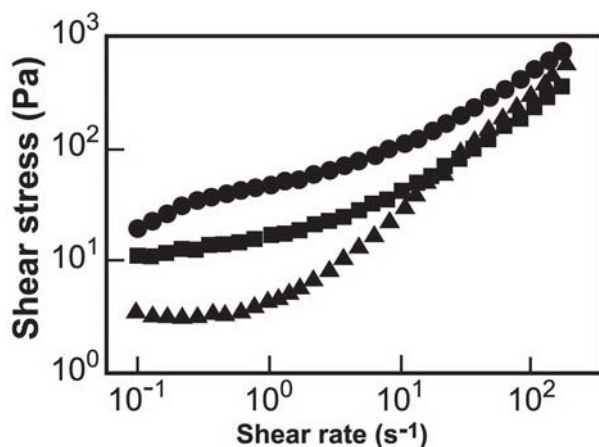


Fig. 12.11. Flow curves of dark chocolate masses (40°C) without emulsifier (circles) and with addition of 4 g/kg lecithin (squares) and 4 g/kg PGPR (triangles). Only every fifth data point is depicted in the graph (Schantz & Rohm, 2005).

Table 12-C. Effect of Emulsifier Concentration on Yield Stress (τ_0) and Equilibrium Viscosity (η_e) of Milk Chocolate Masses.

Emulsifier (g/kg)	Lecithin		PGPR	
	τ_0 (Pa)	η_e (Pa s)	τ_0 (Pa)	η_e (Pa s)
0	79.6	12.3	79.6	12.3
1	19.5	4.18	16.1	7.20
2	11.8	3.63	7.49	5.89
3	10.2	3.28	4.23	4.86
4	9.00	3.04	2.31	4.67
5	9.15	2.89	1.62	4.23
6	9.59	2.79	1.44	3.98
7	10.3	2.71	1.09	3.76

Source: Schantz & Rohm, 2005.

The effects of lecithin and PGPR, after varying both quantities and fractions of the emulsifiers, on the flow properties of dark chocolate mass are shown in Fig. 12.12. Starting with a yield stress of the plain mass of 41.0 Pa, the addition of lecithin led to a sufficient reduction of the yield stress. A minimum yield stress of 12.0 Pa was observed when 0.4 wt% lecithin was added to the system. Increasing quantities of PGPR caused a continuous decrease of the yield stress down to 2.28 Pa (0.4 wt%) and, further, to 0.95 Pa (0.7 wt%). In systems with emulsifier mixtures, the yield stress diminished exponentially with an increasing fraction of PGPR in the mixture. However, this effect was only significant up to 0.4 wt% PGPR, and larger PGPR additions caused only minor effects. On the other hand, a quantity of lecithin as small as 0.1 wt% added to a dark chocolate system with a PGPR content between 0.2 wt% and 0.6 wt% enhanced the yield stress reduction effects. As regards equilibrium viscosity, the addition of lecithin and PGPR led to reductions from 4.23 Pa for the base chocolate mass to 2.22 Pa after adding 0.7 wt% lecithin, and to 2.61 Pa after adding 0.7 wt% PGPR (Schantz & Rohm, 2005).

Despite the largely differing rheological properties of the emulsifier-free milk chocolate mass, mainly caused by differences in the total fat content, similar effects of emulsifier blends on yield stress and viscosity of dark and milk chocolate masses were observed. Fig. 12.13 shows that both yield stress and viscosity decrease with increasing quantity of emulsifier. Irrespective of the total concentration of the emulsifier, a minimum yield stress was achieved in systems with 70% PGPR and 30% lecithin. Equilibrium viscosity for milk chocolate with 0.4 wt% emulsifier and a PGPR to lecithin ratio of 70:30 was approx. 20% higher than milk chocolate with pure lecithin at 0.4 wt%. This difference diminished to 10% when considering a total emulsifier content of 6 g/kg. Independent

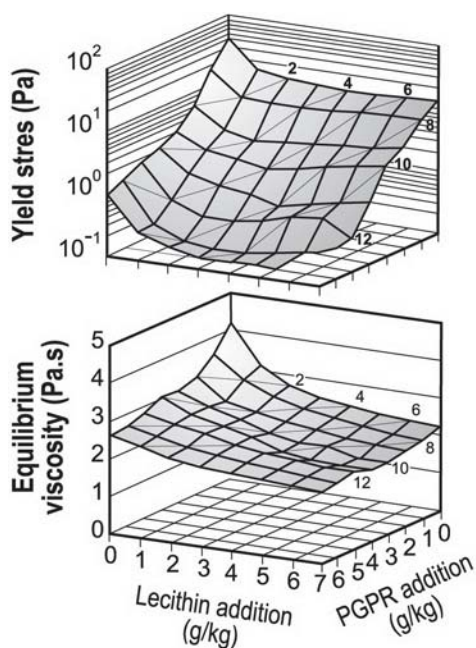


Fig. 12.12. Yield stress and equilibrium viscosity of dark chocolate mass as a function of lecithin and PGPR addition. Dotted lines refer to equal total quantities of emulsifier (g/kg) in the system, which are indicated by numbers in the plot. Source: Schantz & Rohm, 2005.

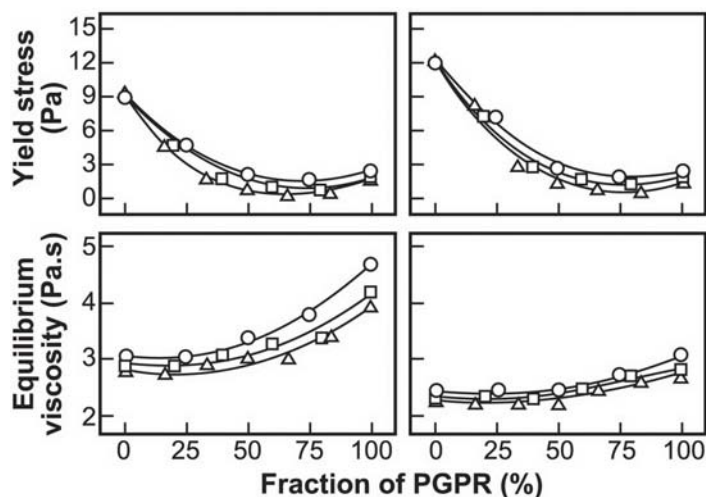


Fig. 12.13. Yield stress and equilibrium viscosity of milk chocolate (left) and dark chocolate (right) with varying emulsifier content (circles, 4 g/kg; squares, 5 g/kg; triangles, 6 g/kg) as a function of emulsifier blending. Source: Schantz & Rohm, 2005.

of the emulsifier concentration, a fraction of up to 25% PGPR in the mixture was possible without significantly increasing the equilibrium viscosity of milk chocolate. For the dark chocolate mass, this value proved to be 50% PGPR (Schantz & Rohm, 2005).

While the dispersive component and acidic character for both emulsifiers were comparable, the basic character exhibited a slight difference. It is hypothesized that this is linked to the presence of oxygen atoms in poly-condensed ricinoleic acid (Rousset et al., 2002).

Glycerol Monostearate and Mixtures

Glycerol monostearate (GMS), widely used in confectionery industries, is formed by the incomplete esterification of hydroxyl groups of glycerol stearic acid (Fig. 12.14) (Heath, 1982).

Vernier (1998) reported glycerol fatty acid esters were inefficient at reducing yield value and increased plastic viscosity since they produce less efficient coverage of sugar particles, thus leading to greater friction. A mixture of sorbitan and glycerol esters of fatty acids give yield values similar to lecithin but higher plastic viscosity.

Other Emulsifiers and Additives

Ammonium salts of phosphatidic acids (known also as YN) drew the most attention in the chocolate industry since they were designed to serve as lecithin replacers. YN, also called synthetic lecithin, acts in nearly the same way as lecithin but exhibits a more constant composition and efficiency. YN allows processing the chocolate at lower temperatures and simplifies the handling of chocolate masses, especially in the case of low shear rates during molding, and facilitates the removal of air bubbles (Rector, 2000; Schuster, 1985). Moreover, it becomes possible to create innovative products with very thin coatings or layers and filigree surface designs. The optimization of coating thickness and cocoa butter content also means economizing on the material costs of chocolate products.

Analysts are predicting a significant shortage of non-genetically modified (GM) lecithin in the near future. This indication is forcing chocolate and other food product producers to search for alternatives to lecithin as a rheology modifier. Some of the compounds that were mentioned include monoglyceride citrate, lysolecithin, and polyglycerol polyricinoleate, and others such as sorbitan tristearate and monoglycer-



Fig. 12.14. Chemical structure of glycerol monostearate.

ide stearate. Palsgaard (a Danish producer) recently launched the citric acid ester of fatty acids (monoglycerides citrate) as a lecithin replacer.

In conclusion, the main role of emulsifiers in the manufacture of chocolate is related to the fact that the three major ingredients are immiscible in each other and are dispersed in the molten cocoa butter liquid fraction, imparting strong interactions. The emulsifiers coat the sugar and cocoa solid particles, preventing strong interactions (and aggregations), and allow minimum friction and high mobility of the particles and thus easier manufacturing conditions.

Lecithin performs best as a shear stress reducer and viscosity controller once added at ca. 0.3–0.4 wt% of the total mass. All other alternatives give only partial results.

Polymorphism

Fat molecules can crystallize under controlled conditions in more than one unit cell. Such assemblies in a unit crystalline cell are known as polymorphic structures (and the phenomenon is polymorphism).

Cocoa butter (CB) can crystallize in a number of polymorphic forms as a function of triglyceride composition, with fatty acid composition influencing how liquid fat solidifies (Afoakwa et al., 2007; Awua, 2002), and various physical crystallization conditions. CB has six polymorphic forms (I–VI), the principals being α , β , and β' (Table 12-D) (Afoakwa et al., 2007). Form V (also termed the β form) is generally the most desirable form in well-controlled crystallization conditions (best tempered chocolate), giving a glossy appearance, good snap, contraction, and resistance to bloom (Beckett, 2000).

Table 12-D. Characteristic Powder XRD Wide Angle Reflections (Short Spacings) for the Various Polymorphic Forms of Cocoa Butter.

Polymorphic form	Short spacings (Å)
γ (sub- α)	3.87 (m), 4.17 (s)
α	4.20 (vs)
β_2'	3.87 (vw), 4.20 (vs)
β_1'	3.75 (m), 3.88 (w), 4.13 (s), 4.32 (s)
β_2	3.65 (s), 3.73 (s), 3.87 (w), 3.98 (s), 4.22 (w), 4.58 (vs), 5.13 (w), 5.38 (m)
β_1	3.67 (s), 3.84 (m), 4.01 (w), 4.21 (vw), 4.53 (vs), 5.09 (vw), 5.37 (m)

^a the relative intensity is noted as very strong (vs), strong (s), medium (m), weak (w), or very weak (vw).

Source: Larsson, 1994.

The identification, isolation, and characterization of each polymorph were completed after significant separation and purification efforts and after developing reliable analytical tools to provide consistent information on each polymorph. Many of the old studies dealing with the polymorphism of cocoa butter are not relevant today and do not well characterize the polymorphs. The most important techniques that were used to describe the different polymorphs are XRD, FTIR, solid state NMR, and DSC (Figs. 12.15 and 12.16).

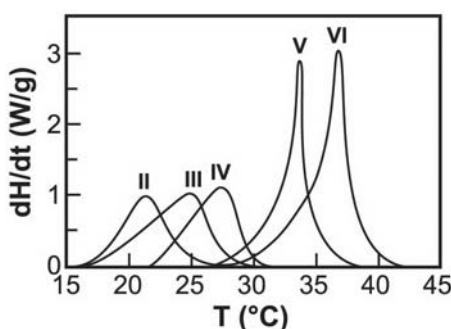


Fig. 12.15. The enthalpy of fusion vs. temperature of the six different polymorphs of cocoa butter. The peak of each endothermic event is the melting point of the specific polymorph.

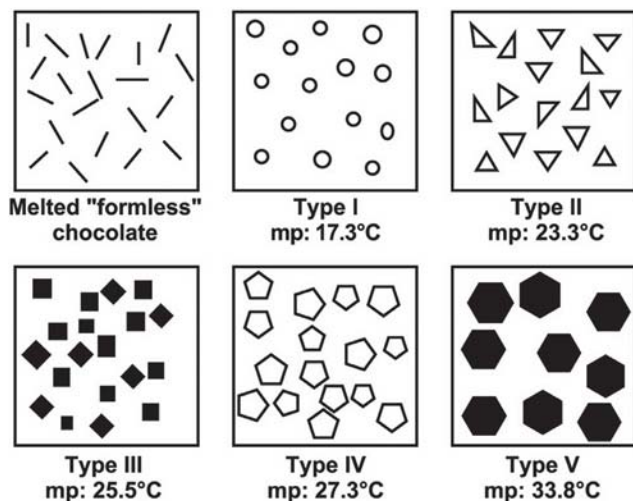


Fig. 12.16. Schematic representation of the crystal size and shape of the six polymorphs of cocoa butter. Source: Art and Science of Homemade Chocolate.

The form I is obtained when the molten cocoa butter (or the chocolate) is cooled rapidly (also called fast quenching). This will result in chocolate that has a melting point of 17°C. The second form, II, is obtained if the molten chocolate is cooled at the rate of 2°C per minute. This will result in a melting point of 23°C. Polymorph III is formed from aged polymorph II. The melting point of polymorph III is 25.5°C. If the chocolate is kept at 20°C, the resulting chocolate will have polymorph IV with a melting point of 27°C. If the process is done under shearing (stirring), polymorph V will be obtained. The chocolate made with this polymorph has a melting point of around 33.8°C. If form V is aged (stored) for a certain period of time, it transforms to polymorph VI. This chocolate has a melting point of 36°C and is hard to melt in our mouth. It tastes disgusting (Art and Science of Homemade Chocolate).

The different polymorphic structures were well documented and their crystalline characteristics were determined by SAXS (Fig. 12.17), DSC, FTIR, etc.

The tempering process of chocolate is done by a high shear seeding process that is beneficial in controlling the kinetics of nucleation and the polymorphic transformations. By applying strongly accelerated shear forces (acting in high shear flow fields) the overall quality of products improves, as fat bloom is reduced (Afoakwa et al., 2007; Windhab et al., 2002). During tempering, the temperatures are precisely controlled and the agitation provided enhances nucleation rates. As the viscosity increases, the chocolate is reheated again in the third stage to prevent runaway solidification. In the fourth stage, crystals are matured. Chocolate might be cooled (tempered) by the use of high pressure (150 bars) (Yasuda & Mochizuki, 1992). This increases the chocolate melting point and causes it to solidify into solid crystals of all polymorphic forms.

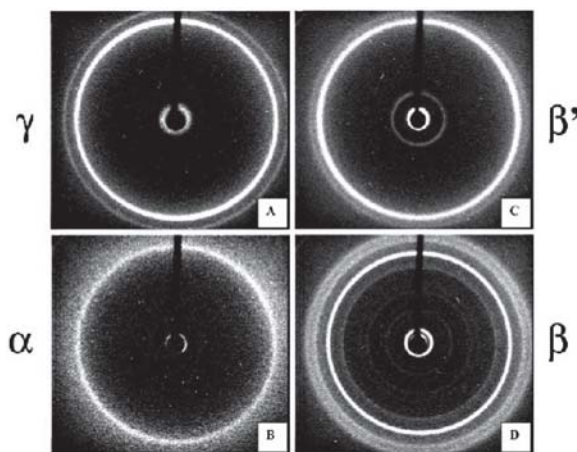


Fig. 12.17. Characteristic XRD patterns of the various polymorphic forms of cocoa butter crystallized at -20°C for 2 min (A), 5°C for 2 min (B), 5°C for 5 days (C), and 22°C for 28 days (D). Source: Marangoni & McGauley, 2003.

When pressure is released, lower polymorphic forms melt leaving behind tempered chocolate. Subsequent batches can be seeded with stable fat crystals.

A well-tempered chocolate will have good shape, color, gloss, contraction from the mold, better weight control, provide a stable product—harder and more heat resistant (fewer finger marks during packaging), and have a longer shelf-life. The tempering regime for milk chocolate differs slightly from that for dark chocolate due to the influence of milk fat molecules on crystal lattice formation (Afoakwa et al., 2007; Haylock & Dodds, 1999). Milk chocolate contains a proportion of butter fat that causes a eutectic effect, which prevents bloom formation and results in a lower melting point, softening of texture, and lowering of temperature to obtain crystal seed for the tempering process (around 29.4°C compared to 34.5°C for plain chocolate).

If chocolate is poorly tempered, the outcome is the β form IV, which rapidly transforms into form V (Table 12-E). This influences color, as reflected light is disoriented by unstable, disorganized crystal growth. Untempered chocolate is soft and not effectively demolded. In cocoa butter, forms V and VI are the most stable forms. Form VI is difficult to generate although it is formed during lengthy storage of tempered chocolate accompanied by fat bloom. In addition, form VI has a high melting temperature (36°C), and crystals that are large and gritty on the tongue. The unstable form I has a melting point of 17°C and is rapidly converted into form II that transforms more slowly into forms III and IV. Polymorphic triglyceride forms differ in distance between fatty acid chains, angle of tilt relative to plane of chain end methyl group, and manner in which triglycerides pack during crystallization (Talbot, 1999).

Polymorphic form is determined by processing conditions. Fatty acids crystallize in a double- or triple-chain form depending on triglyceride composition and positional distribution. Form IV crystallizes in a double-chain form, form V in a triple-chain system that enables closer packing and greater thermodynamic stability. Unstable lower polymorphic forms (II and III) transform into higher melting, more stable forms, with closer packing and lower volume. These changes can be observed in terms of overall contraction of the chocolate, appearance, or undesirable fat bloom

Table 12-E. Melting Point and Chain Packing of the Polymorphic Forms of Cocoa Butter.

Polymorphic forms of cocoa butter		Melting point (°C)	Chain packing
Form I	β_2'	16–18	Double
Form II	α	21–22	Double
Form III	Mixed	25.5	Double
Form IV	β_1	27–29	Double
Form V	β_2	34–35	triple
Form Vi	β_1'	36	Triple

Source: Talbot, 1999.

formation at rates dependent on relative stabilities of the polymorphic forms and temperature (Talbot, 1999). For chocolate to be in an appropriate polymorphic form, tempering is crucial, influencing final quality characteristics such as color, hardness, handling, finish, and shelf-life characteristics.

Tempering involves pre-crystallization of a small proportion of triglycerides, with crystals forming nuclei (1–3% total), for the remaining lipid to set in the correct form. Tempering has four key steps: melting to completion (at 50°C), cooling to point of crystallization (at 32°C), crystallization (at 27°C), and conversion of any unstable crystals (at 29–31°C) (Talbot, 1999) (Figure 12.3 of this chapter). Tempering sequence is a function of recipe, equipment, and the final purpose. Time–temperature combinations are of paramount importance in process design and in continuous tempering; molten chocolate is usually held at 45°C then gently cooled to initiate crystal growth.

Sorbitan Esters

The polymorphic behavior of cocoa butter in the presence of several food emulsifiers serving as crystal structure modifiers was investigated. Emphasis was placed on transitions among the relatively stable forms IV, V, and VI, which are significant for the confectionery industry (Garti et al., 1986).

As known from industry work, within the series of sorbitan esters and ethoxylated sorbitan esters, the solid emulsifiers were the most efficient in retarding transition of form V into the VI modification. Blends of sorbitan monostearate (Span 60), ethoxylated sorbitan monostearate (Tween 60), and Span 60-Tween 65 used in the present study were particularly effective. Surprisingly, it was found that some combinations of emulsifiers accelerate the transition of form IV into form V. Transition of form V into form VI occurs via the solid state, and other transitions are known to take place via the liquid phase. Emulsifier was found to increase the liquid fraction of the fat prior to its transition. Mechanistic considerations concerning these transitions are suggested. Synergism between Span 60 and Tween 60 may suggest that the emulsifiers do not function in a complete molecular dispersion, but are solubilized into each other. A relation between emulsifier structure and the ability to retard $V \rightarrow VI$ transformation has been demonstrated. It may be suggested that the effect on $IV \rightarrow V$ transition is due just to the increase in liquid fraction, but the effect on $V \rightarrow VI$ transition is, in addition, related closely to the emulsifier's physical structure. We may imagine that a high melting point emulsifier will have its carbon chains tightly packaged, producing a rigid structure. Any polymorphic transition through solid phase, associated with slight movement of fat molecules, will be hindered by the rigidity of that structure. Lack of rigidity will allow eventual undulation of the carbon chains, thus permitting the slight movement of the fat molecules (Fig. 12.18).

In order to better understand the effect of emulsifiers on CB, attempts were made to study the effect of emulsifiers on the two major pure fractions of CB, namely SOS and POP.

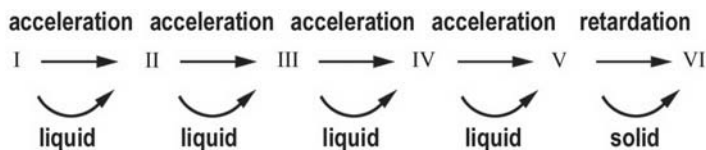


Fig. 12.18. Emulsifier effect on polymorphic transformations of cocoa butter.

The polymorphic behavior of 1,3-distearoyl-2-oleo glycerol (SOS) was investigated in the presence of a selected food emulsifier, sorbitan monostearate. Five polymorphs, named α , γ , pseudo- β' , and β_2 , and β_1 , were crystallized and identified by both x-rays and DSC. Each polymorph was treated using two selected protocols—the “screen cycle” and the “aging cycle”. It has been demonstrated that sorbitan monostearate, when present in the molten fat, will significantly retard the $\alpha \rightarrow \gamma$ transition during both the screen cycle and the aging cycle. A retardation effect was not detected for the γ to pseudo- β' transition (during the screen or the aging cycle). The emulsifier that is inserted into the fat behaves as a conventional impurity or seed, causing significant reduction in the melting temperatures of each polymorph. As a result, the fat tends to melt prior to its transition. The behavior of SOS in the presence of the sorbitan monostearate was found therefore to be different than that of SSS (tristearin), and may explain, in part, the absence of the strong blooming transition to high melting polymorph that occurs when the fat is rich in SOS (Jacome Guth et al., 1989; Garti et al., 1985, 1986, 1989; Schlichter-Aronhime & Garti, 1988; Schlichter-Aronhime et al., 1988a, 1988b).

Effects of three emulsifiers, distilled monoglycerides (DMG), sorbitan monostearate (Span 60), and sorbitan tristearate (Span 65), on the crystallization kinetics of SSS, 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS), and 1,3-distearoyl-2-oleoylglycerol (SOS) were studied by DSC.

The data presented in this paragraph are based on Wei's dissertation abstract (Wei, 2005): “In constant cooling crystallization, all three emulsifiers did not change the nucleation behavior of SSS but slowed down the crystal growth rate and the polymorphic transformations. In contrast all three emulsifiers increased the initial crystallization temperature of POP, POS, and SOS, and had different effects on the melting behaviors and polymorphic transformations. Avrami analysis results showed no change on the n values for SSS, while for POP, POS, SOS, different results were obtained from different emulsifiers. Effects of tripalmitin (PPP) on the crystallization behavior of SSS were also studied. PPP decreased the growth rate of SSS and significantly changed the polymorphic forms of SSS obtained while Span 65 did not. Effects of four emulsifiers on the crystallization kinetics of cocoa butter were studied by DSC and NMR. Acetic acid monoglyceride esters had almost no effect on the nucleation and melting behaviors of cocoa butter while distilled monoglycerides, Span 60 and Span 65 all increased the initial crystallization temperature and changed the melting behaviors of cocoa butter. The Avrami analysis results from both DSC and NMR

indicated that all emulsifiers decreased the n value and increased the k value. At higher isothermal temperatures, higher n values were obtained. In addition, the n values from DSC results were higher than those obtained from NMR results.”

Monoglyceride Lactates

Influence of concentration of emulsifier Lactem P 22 (lactic acid esters of mono- and diglyceride) as a blooming inhibitor on the quality and fat bloom stability of laboratory-made samples of chocolate was investigated. This ingredient was added during the pre-crystallization process of chocolate mass (Jovanovic & Pajin, 2004).

Statistical results processing was performed according to the Response Surface Methodology. Physical characteristics of chocolate-hardness and -lightness were obtained by instrument methods. The results have shown that monoglyceride lactates of fatty acids (mostly stearic acid; Lactem P22) improve fat bloom stability of chocolate samples but decrease sensory quality of chocolate. Better sensory quality of chocolate was achieved with combination of the crystallization promoter—glycerol tristearate (TAG; Dynasan 118). The best sensory quality of chocolate has been obtained with 0.5% TAG and 1% and 2% monoglyceride lactates (Fig. 12.19A and B). Samples of chocolate that were pre-crystallized at 23°C and with 3% Lactem P22, showed the greatest fat bloom stability (Jovanovic & Pajin, 2004). Fig. 12.20 shows

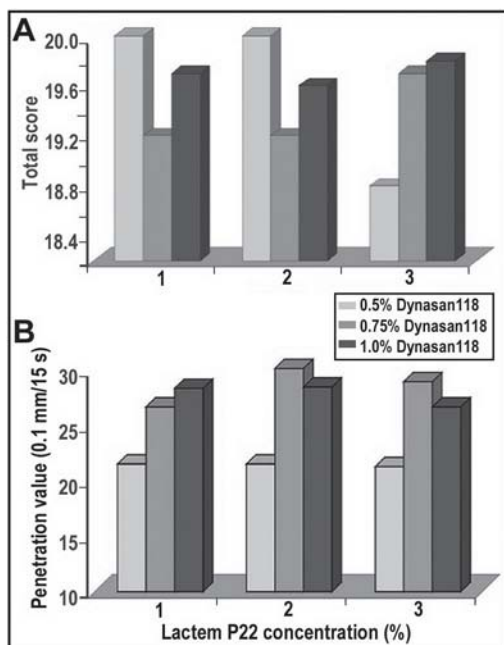


Fig. 12.19. (A) Influence of the concentration of Lactem P22 and concentration of Dynasan 118 on chocolate sensory quality. (B) Influence of the Lactem P22 and Dynasan 118 on the penetration value—hardness of the chocolate samples. Source: Jovanovic & Pajin, 2004.

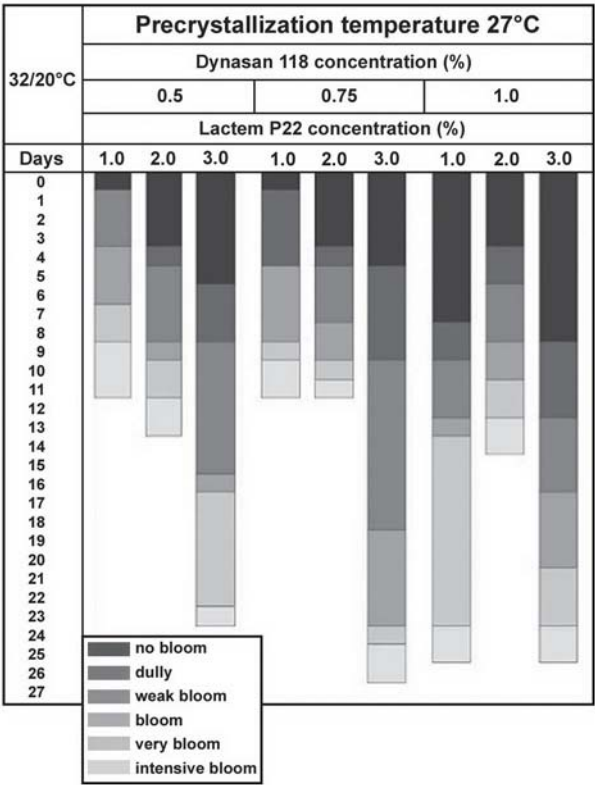


Fig. 12.20. Dynamics of the fat chocolate bloom test at 32/20°C. Source: Jovanovic & Pajin, 2004.

the dynamics of chocolate blooming depending on the concentration of Lactem P22 and Dynasan 118. Results indicate an interaction between Lactem P22 and Dynasan 118, which is reflected in the increase of fat bloom stability with the addition of higher concentrations of these materials. The greatest fat bloom stability was defined as the total number of cycles that at the same time has the largest number of cycles prior to the first sign of blooming (Jovanovic & Pajin, 2004).

Lactic acid monoglyceride and diglyceride are recommended as additives to improve the shine in the production of chocolate-like products (compound coatings). The authors (Jovanovic & Pajin, 2004) showed that this emulsifier could be used in chocolate production as a blooming inhibitor to achieve satisfactory physical, chemical, and sensory attributes. Minor alterations of certain pre-crystallization

parameters proved necessary in order to apply Lactem P22 successfully. The most important changes were a lower temperature of pre-crystallization and the addition of a crystallization promoter (Jovanovic & Pajin, 2004).

Lecithins

A very interesting study on the effect of sugar particles and lecithin was carried out. It was found that at low hold temperature (13°C) crystallization was fast, independent of shear rate, and produced more of the low melting polymorph, whereas at higher hold temperatures (20°C and 23°C) crystallization of a higher melting temperature polymorph was hastened by the action of shear. The reduction in induction times with increased shear rate suggests that shear is important at the nucleation stage. This would tend to rule out interpolymorphic transitions as the key mechanism to tempering in this case (Dhonsi & Stapley, 2006).

The addition of sugar resulted in much shorter induction times, which were only shear dependent at 23°C (i.e., not at 20°C as was the case with plain cocoa butter). Sugar appears to act as a heterogeneous nucleation agent that preferentially promotes the formation of lower polymorphs. The effect of lecithin was to slightly retard induction times compared to the cocoa butter/sugar mixture, which may be caused by lecithin coating the sugar crystals (Dhonsi & Stapley, 2006).

Conclusions

Fifty years of efforts to understand the bloom phenomenon in dark chocolate led scientists to understand that polymorphic transition from form V to form VI is not the cause for bloom but rather a result of poor tempering and a non-optimal tempering and cooling profile.

Moreover, it was recognized that emulsifiers, and mainly lecithin, are essential additives to control the rheology behavior of the chocolate mass during the different stages of crystallization and are not essential in controlling the polymorphic transformations, since even if the polymorphic transitions from form V $\rightarrow$ VI are slowed down or almost prevented, it will not minimize the bloom phenomenon if the tempering is not properly performed.

It was also recognized that lecithin performance is almost ideal and it is very difficult to find lecithin replacers. However, the use of lecithin together with PGPR or synthetic YN will have a synergistic effect on the chocolate mass Casson viscosity and other important properties such as gloss and mechanical strength.

Emulsifiers are crystal structure modifiers and have a strong tendency to adsorb onto the solid particles of sugar and fat; while changing the rheology of the system they also serve as crystallization retarding agents, slowing down the polymorphic transition. Lipophilic surfactants have been experimentally used but they were not essential in most chocolate applications.

References

- Aeschlimann, J.M.; Beckett, S.T. International inter-laboratory trials to determine the factors affecting the measurement of chocolate viscosity. *J. Texture Stud.* 31, 2000, 541–576.
- Afoakwa, E.O.; Paterson, A.; Fowler, M. Effects of particle size distribution and composition on rheological properties of dark chocolate. *Eur. Food Res. Technol.* (published online, April 2007a) doi: 10.1007/s00217-007-0652-6.
- Afoakwa, E.O.; Paterson, A.; Fowler, M. Factors influencing rheological and textural qualities in chocolate—A review. *Trends Food Sci. Technol.* 18, 2007b, 290–298.
- Afoakwa, E.O.; Paterson, A.; Fowler, M. Effects of particle size distribution and composition on rheological properties of dark chocolate. *Eur. Food Res. Technol.* 226, 2008, 1259–1268.
- Afoakwa, E.O.; Paterson, A.; Fowler, M.; Vieira, J. Microstructure and mechanical properties related to particle size distribution and composition in dark chocolate. *Int. J. Food Sci. Technol.* 44, 2009a, 111–119.
- Afoakwa, E.O.; Paterson, A.; Fowler, M.; Vieira, J. Comparison of rheological models for determining dark chocolate viscosity. *Int. J. Food Sci. Technol.* 44, 2009b, 162–167.
- The Art and Science of Homemade Chocolate, Tempering—Deconstruction and Reconstruction & Illustrated Tempering, online website: <http://chocolatealchemy.com/illustrated-tempering/>.
- Awua, P.K. *Cocoa Processing and Chocolate Manufacture in Ghana*. Essex, UK, David Jamieson and Associates Press Inc., 2002.
- Beckett, S.T. *Industrial Chocolate Manufacture and Use*. Oxford, Blackwell Science, 3rd ed., 1999, 153–181, 201–230, 405–428, 460–465.
- Beckett, S.T. *The Science of Chocolate*. London, Royal Society of Chemistry, 2000.
- Bouzas, J.; Brown, B.D. Interactions affecting microstructure, texture, and rheology of chocolate confectionery products. In A.G. Goankar (Ed.), *Ingredient Interactions: Effects on Food Quality*. New York, Marcel Dekker, 1995, 451–527.
- Casson, N. *Rheology of Disperse Systems*, (C.C. Mill, editor), London, Pergamon Press, 1959, chapter 5.
- Ceci, L.N.; Constenla, D.T.; Crapiste, G.H. Oil recovery and lecithin production using water degumming sludge of crude soybean oils. *J. Sci. Food Agric.* 88, 2008, 2460–2466.
- Chevalley, J. Chocolate flow properties. In *Industrial Chocolate Manufacture and Use* (edited by S.T. Beckett). New York, NY, Chapman & Hall, 1999, 182–199.
- Cook, L.R. *Chocolate Production and Use*, Harcourt Brace, New York, 1984.
- Dhonsi, D.; Stapley, A.G.F. The effect of shear rate, temperature, sugar and emulsifier on the tempering of cocoa butter. *J. Food Eng.* 77, 2006, 936–942.
- Garti, N.; Binyamin, H.; Aserin, A. Stabilization of water-in-oil emulsions by submicrocrystalline alpha-form fat particles. *J. Am. Oil Chem.* 75, 1998, 1825–1831.
- Garti, N.; Schlichter-Aronhime, J.; Sarig, S. The role of chain length and an emulsifier on the polymorphism of mixtures of triglycerides. *J. Am. Oil Chem. Soc.* 66, 1989, 1085–1089.
- Garti, N.; Schlichter, J.; Sarig, S. Polymorphic transformations of cocoa butter in the presence of emulsifier, studied by the DSC. *Thermochim. Acta* 85, 1985, 517–520.

- Garti, N.; Schlichter, J.; Sarig, S. Effect of food emulsifiers on polymorphic transitions of cocoa butter. *J. Am. Oil Chem. Soc.* 63, 1986, 230–236.
- Gobley, T.N. Recherches chimiques sur le jaune d'œuf de poule. *C.R. Hebd. Séances Acad. Sci.* 21, 1845a, 988.
- Gobley, T.N. Sur l'existence des acides oléique, margarique et phosphoglycérique dans le jaune d'œuf. Premier Mémoire: Sur la composition chimique du jaune d'œuf. *C.R. Hebd. Séances Acad. Sci.* 21, 1845b, 766.
- Gobley, T.N. Recherches chimiques sur le jaune d'œuf – Examen comparatif du jaune d'œuf et de la matière cérébrale. *J. Pharm. Chim.* 11, 1847, 409, 412.
- Gobley, T.N. Recherches chimiques sur les œufs de carpe. *J. Pharm. Chim.* 17, 1850, 401, and 18, 1850, 107.
- Gobley, T.N. Recherches chimiques sur le cerveau. *J. Pharm. Chim.* 19, 1874a, 346–354.
- Gobley T.N. Recherches chimiques sur le cerveau. *J. Pharm. Chim.* 20, 1874b, 161–166.
- Gobley, T.N. Sur la lécithine et la cérébrine. *J. Pharm. Chim.* 20, 1874c, 98–103.
- Hasenhuettl, G.L.; Hartel, R.W. *Food Emulsifiers and Their Applications*. New York, Chapman & Hall, 1997.
- Haylock, S.J.; Dodds, T.M. Ingredients from milk. In S.T. Beckett (Ed.), *Industrial Chocolate Manufacture and Use* (3rd ed.). Oxford: Blackwell Science, 1999, 137–152.
- Heath, H.B. Emulsifiers and stabilizers in food processing. *Food Flavouring Ingredients Packaging and Processing* 4, 1982, 24–27.
- Höfs, M.; Taschke, H. Emulgatoren: Hilfsmittel mit vielfältigen wirkmechanismen. *Zucker-und Süßwarenwirtschaft* 55, 2002, 6–8.
- Jacome Guth, O.; Aronhime, J.; Garti, N. Polymorphic transitions of mixed triglycerides, SOS, in the presence of sorbitan monostearate. *J. Am. Oil Chem. Soc.* 66, 1989, 1606–1613.
- Jovanovic, O.; Pajin, B. Influence of lactic acid ester on chocolate quality. *Trends Food Sci. Tech.* 15, 2004, 128–136.
- Karupaiah, T.; Sundram, K. Effects of stereospecific positioning of fatty acids in triacylglycerol structures in native and randomized fats: a review of their nutritional implications. *Nutr. Metab. (London)*, 4, 2007, 16.
- Larsson, K. *Lipids-Molecular Organization, Physical Functions and Technical Applications*; Dundee, The Oily Press, 1994, 7–47.
- Lee, S.; Heuberger, M.; Rousset, P.; Spencer, N. D. Chocolate at a sliding interface. *J. Food Sci.* 67, 2002, 2712–2717.
- Marangoni, A.G.; McGauley, S.E. Relationship between crystallization behavior and structure in cocoa butter. *Cryst. Growth Des.* 3, 2003, 95–108.
- Martin, R.A., Jr. Chocolate. *Adv. Food Res.* 31, 1987, 211–342.
- Minifie, B.W. Uses of lecithin in confectionery products and food drinks. *J. Am. Oil Chem.* 57, 1980, A119–A119.
- Nebesny, E.; Źyzelewicz, D. Effect of lecithin concentration on properties of sucrose-free chocolate masses sweetened with isomalt. *Eur. Food Res. Technol.* 220, 2005, 131–135.

- Prawira, M.; Barringer, S.A. Effects of processing and ingredients on milk chocolate quality. *Foods & Food Ingredients Journal of Japan* 213, 2008, 737–749.
- Rector, D. Chocolate—controlling the flow. Benefits of polyglycerol polyricinoleic acid. *The Manufacturing Confectioner* 80, 2000, 63–70.
- Rousset, <http://www.sciencedirect.com/science/article/pii/S0021967302003709-COR1#COR1> Ph.; Sellappan, P.; Daoud, P. Effect of emulsifiers on surface properties of sucrose by inverse gas chromatography. *J. Chromatogr. A* 969, 2002, 97–101.
- Schantz, B.; Linke, L.; Rohm, H. Effects of different emulsifiers on rheological and physical properties of chocolate. 3rd Int. Symp. Food Rheology and Structure 2001, 329–333.
- Schantz, B.; Rohm, H. Influence of lecithin–PGPR blends on the rheological properties of chocolate. *LWT- Food Sci. Technol.* 38, 2005, 41–45.
- Schlichter-Aronhime, J.; Garti, N. Solidification and polymorphism in cocoa butter and the blooming problems. In: *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti and K. Sato, Marcel Dekker, New York, 1988, 363–393.
- Schlichter-Aronhime, J.; Sarig, S.; Garti, N. Dynamic control of polymorphic transformation in triglycerides by surfactants: the button syndrome. *J. Am. Oil Chem. Soc.* 65, 1988a, 144–150.
- Schlichter-Aronhime, J.; Sarig, S.; Garti, N. Reconsideration of polymorphic transformations in cocoa butter using the DSC. *J. Am. Oil Chem. Soc.* 65, 1988b, 1140–1143.
- Schuster, G. *Emulgatoren in Lebensmitteln*. Berlin, Heidelberg, Springer, 1985.
- Segall, S.D.; Artz, W.E.; Raslan, D.S.; Ferraz, V.P.; Takahashi, J.A. Analysis of triacylglycerol isomers in Malaysian cocoa butter using HPLC-mass spectrometry. *Food Res. Int.* 38, 2004, 167–174.
- Seguine, E.S. Casson plastic viscosity and yield value: what they are and what they mean to the confectioner. *The Manufacturing Confectioner* 11, 1988, 57–63.
- Smiles, A.; Kakuda, Y.; MacDonald, B.E. Effect of degumming reagents on the recovery and nature of lecithins from crude canola, soybean and sunflower oils. *J. Am. Oil Chem. Soc.* 65, 1988, 1151–1155.
- Sokmen, A.; Gunes, G. Influence of some bulk sweeteners on rheological properties of chocolate. *LWT—Food Sci. Technol.* 39, 2006, 1053–1058.
- Svanberg, L.; Ahrne, L.; Loren, N.; Windhab, E. Effect of sugar, cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems. *J. Food Eng.* 104, 2011, 70–80.
- Talbot, G. Chocolate temper. In S.T. Beckett (Ed.), *Industrial Chocolate Manufacture and Use* (3rd ed.). Oxford: Blackwell Science, 1999, 218–230.
- Vannieuwenhuyzen, W. The industrial uses of special lecithins – A review. *J. Am. Oil Chem.* 58, 1981, 886–888.
- Vernier, F. C. Influence of emulsifiers on the rheology of chocolate and suspensions of cocoa or sugar particles in oil. PhD Diss., Department of Chemistry, University of Reading, 1998.
- Walter, P.; Cornillon, P. Influence of thermal conditions and presence of additives on fat bloom in chocolate. *J. Am. Oil Chem. Soc.* 78, 2001, 927–932.

- Wei, D. Effect of emulsifiers on the crystallization of kinetics of triglycerides and cocoa butter. Ph.D. Diss., University of California, 2005.
- Wilson, R.; van Schie, B.J.; Howes, D. Overview of the preparation, use and biological studies on polyglycerol polyricinoleate (PGPR). *Food Chem. Toxicol.* 46, 1998, 711–718.
- Windhab, E.J.; Mehrle, Y.; Stierli, F.; Zeng, Y.; Braun, P.; Boller, E. Verbesserung der Fettreifresistenz durch neuartiges Temperieren e Kontinuierliche Impfkristallisation. Schoko-Technik, Köln, Germany, 2002.
- Yasuda, A.; Mochizuki, K. Behaviour of triglycerides under high pressure. In C. Balny, R. Hayashi, K. Heremans, & P. Masson (Eds.), *High Pressure and Biotechnology*. Japan: Meiji Seika Kaisha Ltd., 1992, 255–259.
- Ziegler, G.; Hogg, R. Particle size reduction. In *Industrial Chocolate Manufacture and Use* (S.T. Beckett, ed.). New York, Chapman & Hall, 1999, 182–199.

13

Methods of Studying Cocoa Butter and Bloom

Daniel Kalnin

*KTH Royal Institute of Technology, School of Chemical Science and Engineering,
Division of Surface and Corrosion Science, Stockholm, Sweden*

Introduction

This chapter reviews methods used to analyze cocoa butter and fat bloom structure and help proper manufacturing in an effort to prevent bloom formation. Bloom prevention is a key issue for chocolate producers hence methods for correctly tempering cocoa butter in chocolate are most appropriate for preventing bloom, as are methods to verify the correct temper of a chocolate mass. This theory is very straightforward because in practice every chocolate mass, each production line, and each chocolate product have their own optimal temper. A good temper allows an appropriate chocolate mass, production facility, and geometry to work out well together in a final product. Hence it is sometimes also interesting to follow every step in the process of production. In general there are several types of methods to verify the temper; however, as for the methods for analyzing fat bloom, some analysis might be useful. There are inline (which might be online) measurements as well as offline measurements. Some of the online measurements will in turn change the temper itself, for example, ultrasound. On the other hand caution should be applied for offline measurements since the temper that is measured—using available techniques—is the temper of the “production facility.” This could mean to take a sample of chocolate mass and place it into whatever sample environment is desired.

Then the methods that are related to the different processing steps are summarized. To evaluate the quality of the final product, however, blooming is important to master since it can be evaluated easily by an untrained eye.

It is assumed that sensory aspects are not relevant for mere bloom stability and cocoa butter structure considered in this chapter. Therefore, only physical methods are reviewed in this chapter. All methods are ordered alphabetically however there is a clear distinction of sets of methods and one might be tempted to classify between contact and noncontact methods. One could also speculate between spectroscopic and mechanical methods. However, this classification leads to confusion since methods that in theory can be noncontact methods can influence the measurement due to radiation as well as mechanical or contact measurements.

Hence in the summary an evaluation of the pertinence of those measurements is attempted. Another important issue is the relevance of the measurement itself—meaning the amount of sample to be investigated and the relevance of that amount of sample to the surface of the chocolate to be investigated. In this respect the analyst should undertake two approaches: (1) a broad approach mimicking the eye since, as we all know, one single spot of dust can ruin a clean window as one whitish spot can ruin a chocolate bar. This is due to the sensitivity of the eye alone thanks to scattered light. (2) In-depth analysis of the occurring bloom should be a relevant sample portion at a micro level.

More information on methods will be detailed in alphabetical order in order to allow rapid access

Scanning Probe Microscopy

Scanning probe microscopy, as for example atomic force microscopy (AFM), can provide topographic information of a relatively small surface or cross section, with a resolution on a nanometer scale.

Since AFM is a scanning probe technique, in which an object (could be a fat crystal or a protein particle or a sugar particle) that can just be the sharp tip (a couple of micrometers long and often less than 100 Å in diameter) positioned on a cantilever (100 to 200 μm long) the interaction with the surface of the sample is monitored. Mainly, it is scanned in a raster-pattern along the surface. In an ideal case, forces between the tip and the sample surface cause the cantilever to bend, or deflect. A detector measures the cantilever deflection as the tip is scanned over the sample, or the sample is scanned under the tip. It is possible to record the deflection as function of time (and hence space) and thereby form a 2D image of the surface topography with close to atomic resolution. Deflection and twisting of the tip is measured by a laser spot reflected from the tip to a photodiode and this deflection provides information about the sample surface height. A feedback mechanism can be employed to keep the force between sample and tip constant. Fig. 13.1 shows a photo of an AFM and its principle of function using the deflection of a laser as detection.

Several forces typically contribute to the deflection of an AFM cantilever. The force most commonly associated with AFM is an inter-atomic force called the van der Waals force. The dependence of the van der Waals force upon the distance between the tip and the sample is shown in Fig. 13.2.

Two distance regimes are labeled in Fig. 13.2: (1) the contact regime, and (2) the noncontact regime. In the contact regime, the cantilever is held less than a few Ångströms from the sample surface, and the interatomic force between the cantilever and the sample is repulsive. In the non-contact regime, the cantilever is held on the order of tens to hundreds of Ångströms from the sample surface, and the inter-atomic force between the cantilever and sample is attractive.

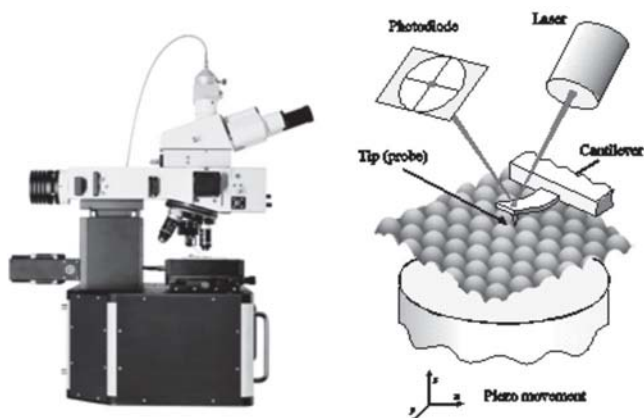


Fig. 13.1. Atomic force microscope and its principle of function using laser deflection from a cantilever to a photodiode.

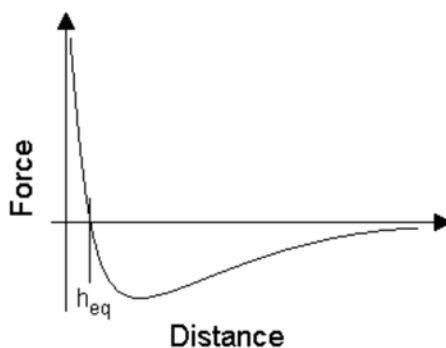


Fig. 13.2. Interatomic forces as a function of distance between the probe and the probed surface.

Hard samples can be investigated in contact mode, where the distance sample-to-tip is maintained constant. In contact mode, also known as repulsive mode, an AFM tip makes soft “physical contact” with the sample. As the scanner gently traces the tip across the sample (or the sample under the tip), the contact force causes the cantilever to bend to accommodate changes in topography.

However, for soft samples such as chocolate, other more gentle modes of AFM have been utilized, e.g. tapping mode where the tip is not “scraped” along the surface as is the case for the just described contact mode, but “tapped” along the surface. In tapping mode the cantilever is oscillated externally at its resonance frequency.

Changes in the oscillation amplitude, phase and resonance frequency provide information about the sample.

Pulsed force mode is a scan technique that minimizes destructive lateral forces that may be induced by standard contact mode, and makes it possible to scan soft samples, e.g., chocolate. The cantilever is oscillated so that it comes in contact with the sample in every cycle, and then enough restoring force is provided by the cantilever spring to detach the tip from the sample. Pulsed force mode employs an oscillating cantilever to probe a sample's surface and produces simultaneous but separate maps of sample topography, stiffness, and adhesion. Stiffness is a primary mechanical characteristic, and gives an indication of the extent to which a material will deform under an applied force. A sample's stiffness is measured by calculating the average rate of probe deflection as the tip is oscillated into contact with the surface with increasing force.

The maximum tensile force that is observed during the retraction phase of the probe oscillation is used to determine the adhesion between the tip and sample. A sample with a strong adhesive interaction will hold the tip in contact as the cantilever attempts to pull it away from the surface. Thus a large force will be required in order to make the tip snap away from the surface. The ability to acquire simultaneous stiffness and adhesion data in conjunction with a topography image allows the user to map physical properties that are frequently independent of the surface morphology.

Besides acquisition of topographical data, AFM can be used to measure the friction between tip and surface. When scanning in contact mode any hindrance in the path of the tip will induce torsion in the cantilever. This torsion can be measured by the reflecting laser beam method and can subsequently be converted into an image. The friction measured by this method clearly does not correspond to the classical macroscopic concept of friction. Macroscopic friction effectively corresponds to a very large number of tips simultaneously interacting with a surface consisting of a very large number of bumps, while this "microscopic" friction-measurement is the most local measurement of friction attainable.

When analyzing chocolate surfaces with AFM, the results mainly used are produced topological images of the surfaces, obtained in tapping mode. Fig. 13.3 shows topography images of a fat crystals laying on a chocolate surface. Here it can be seen that surface of chocolate under different storage conditions changes its surface. A clear tendency can be seen. However the field of view does not allow to judge the overall condition of the chocolate since there is no insurance whether measurements are done on a sugar or cocoa particles underlying fat layer (Smith & Dahlman, 2005). It can also be interesting to combine topological images with an overly of one of these material properties, in order to find information on the relation of material properties to particular topological features. There using the

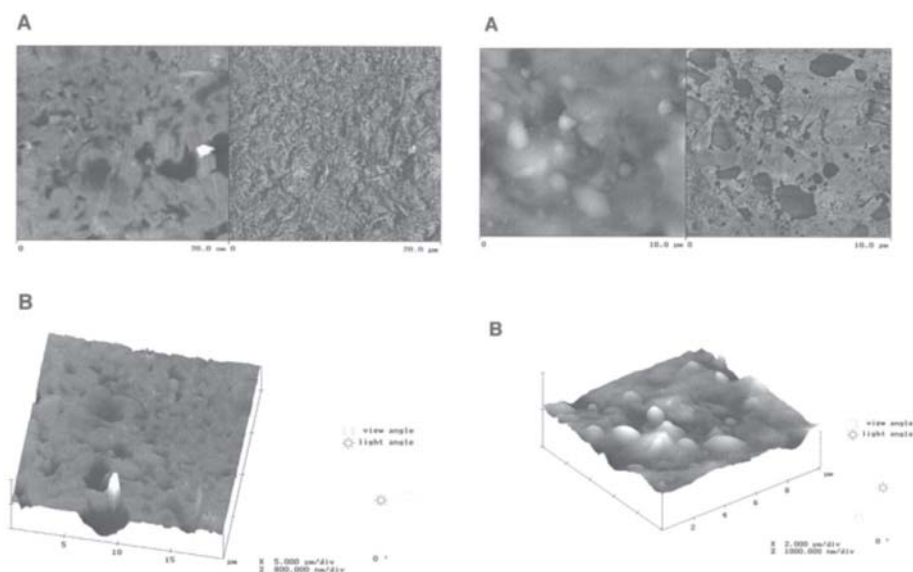
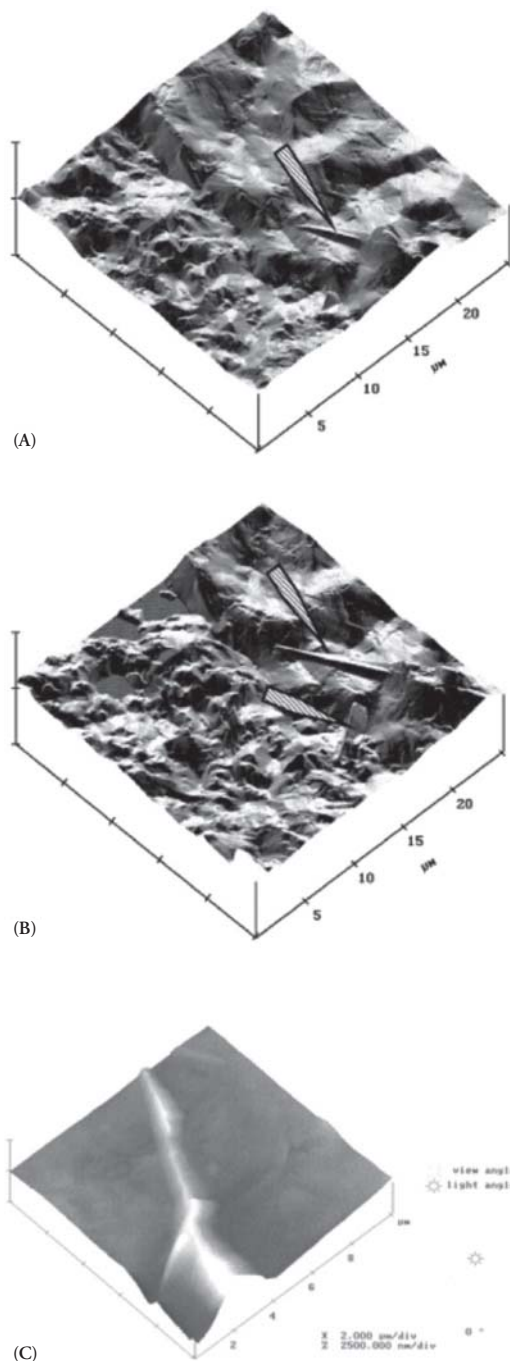


Fig. 13.3. Topography image of a fat crystal on chocolate. Left: Image of a fresh chocolate surface. (A) Topographic and phase images; (B) a 3-D representation of the topographic image ($20 \times 20 \mu\text{m}$; $z = 800 \text{ nm}$ per division). Right: Images of chocolate after 4 wk of storage. (A) Topographic and phase images; (B) a 3-D representation of the topographic image ($10 \times 10 \mu\text{m}$; $z = 1,000 \text{ nm}$ per division). Source: Smith & Dahlman, 2005.

phase image (Fig. 13.3A, right) one can reveal the difference in consistency of the surface.

The AFM technique is particularly useful when very detailed information on small surface features, e.g., crystals, protrusions, etc., are the primary objective of the analysis. A difficulty with the AFM technique in characterizing a surface is the fact that only a very small surface is typically analyzed, e.g. $10 \times 10 \mu\text{m}$. This area is smaller, or in the same range, as the particles in the chocolate. This becomes of particular importance in the cross-sectional analysis, where the material properties, rather than the topology, are analyzed (Hodge & Rousseau, 2002). Thus, in order to obtain statistically relevant data, a very large number of areas need to be analyzed, which often is impractical and too time consuming. Eventually fat crystals can be detected on a micron scale (Fig. 13.4). However combined with other techniques, it can be used as a proof of principle that different crystal states of chocolate surface may generally look the same while being different crystal types inducing different surface roughness, e.g. Fig. 13.5.

Fig. 13.4. Cocoa butter fat crystal growth on the surface of milk chocolate (scale: $25\mu\text{m}/25\mu\text{m}/5\mu\text{m}$). (A) Shows the surface of milk chocolate after 18 cycles. (B) Shows crystals after 54 cycles. Arrows identify crystals that have grown (Rousseau, 2006). (C) A 3-D topographic image of chocolate after 20 wk of storage ($10 \times 10 \mu\text{m}$; $z = 2,500 \text{ nm}$ per division). Source: Smith & Dahlman, 2005)



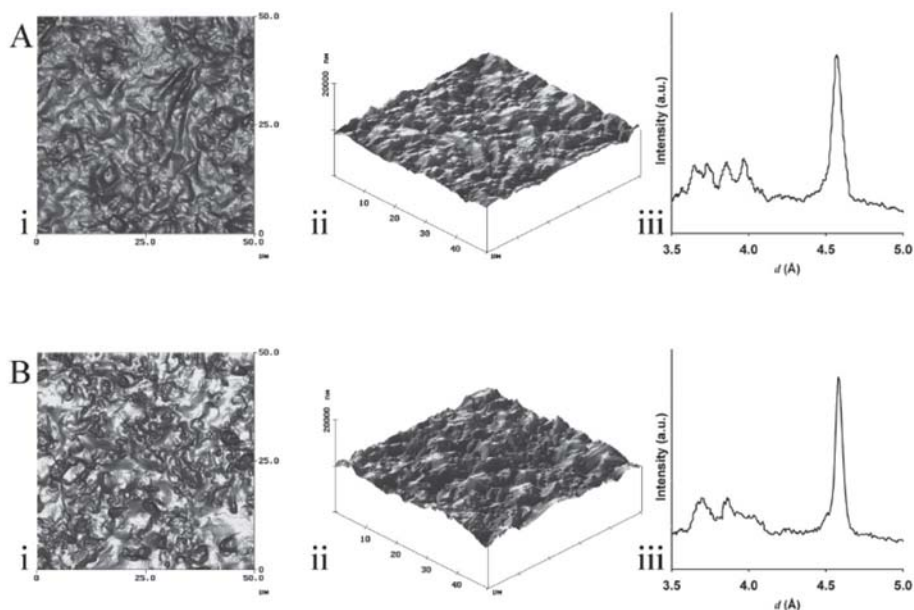


Fig. 13.5. Result of temperature cycling between 20 and 34°C for one and two cycles (A, B). AFM images of chocolate wafer surface and corresponding XRD patterns of washed samples show concurrent evolution of surface roughness and polymorphic transition of form V to form VI. Source: Hodge & Rousseau, 2002.

Colorimetry

Colorimetry is a simple method to determine the color of chocolate samples. By calculating the whiteness index, the whitening of the chocolate surface caused by the formation of fat bloom can be evaluated. This method is particularly useful for large surfaces and hence can be used statistically.

Color is a subjective, complex three-dimensional property. The human eye possesses receptors for only three colors: red, green, and blue, the so-called primary colors. However, all colors are seen as combinations of these primary colors. In principle, a color sensation is described by three attributes: hue, saturation, and lightness. When plotting all visible colors, one obtains a three-dimensional figure.

After a calibration with a white standard plate and a background measurement, the chocolate samples can be measured for their L^* , a^* and b^* values. The following equation defines a whiteness index where L^* is the lightness component, which ranges from 0 to 100, while a^* (green to red) and b^* (blue to yellow) are two chromatic components:

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

This parameter has been the most widely used color parameter in chocolate storage studies for many years. During storage both WI and L^* increase due to the formation of fat bloom on the chocolate surface. However, this method is not as sensitive as visual fat bloom determination. Longchamp and Hartel (2006) noted that the WI of improperly tempered chocolate change significantly despite visual detection of numerous white spots. Furthermore, the method is limited when non-homogeneous color patterns are present on the sample's surface. Commercial colorimeters usually only cover small areas (2–5 cm²) which are chosen by the operator, not taking into account the color of the whole sample. However it was determined that the rate and extent of bloom formation on untempered chocolate is to be dependent on the RH of storage, with bloom occurring more readily at very low RH (Figs. 13.6 and 13.7)

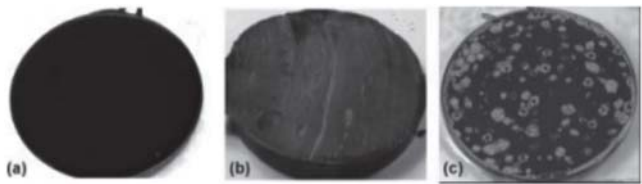


Fig. 13.6. Microphotographs of well-tempered (unbloomed) chocolate (a), surface dulling of over-tempered chocolate (b) and bloom on untempered chocolate (c). All samples were stored for 1 week at room temperature.

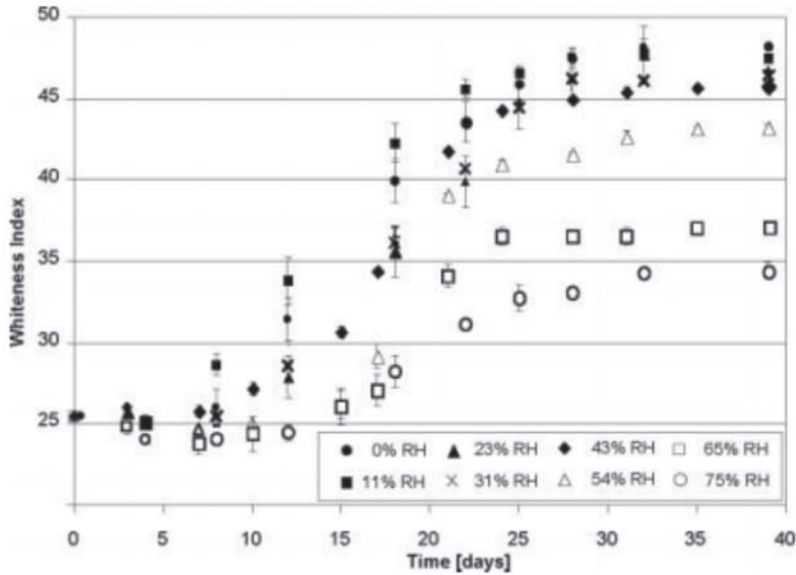


Fig. 13.7. Changes in whiteness index of untempered chocolate stored at different RH and room temperature, as measured by colorimetry.

also difference in the whitening profile due to the use of different sugar types were detected (Bricknell & Hartel, 1998)

Confocal Microscopy

Confocal laser scanning microscopy is used to determine the microstructure of chocolate at the micrometer level. The microstructure can be linked to differences in processing and storage conditions, and ingredients as well as to fat bloom development.

In a confocal laser scanning microscope (CLSM), the image information is obtained from an optical section i.e. one focal plane in the material. The laser beam can penetrate into a bulk sample, and local information about the structure can be determined at different depths. By adding stacks of images from adjacent focal planes, it is possible to construct three-dimensional images of the microstructure. Food components can be specifically labeled with fluorescent dyes and detected separately. This gives us important information about the lateral distribution of components and how they perform in the environment of the food matrix. Having access to special stages allows us to follow structural changes as a function of parameters such as time, temperature, stress and shear, and in a sense we can mimic events in the food process or the breakdown of a food during consumption.

The principle of a confocal microscope and its main parts are illustrated in Fig. 13.8. Following the ray path in an epi-microscope, laser light of different wavelengths is focused a first time in the illumination aperture, passes the beam splitter, goes through the objective and then hits the sample. The laser light that comes into the sample excites fluorescent molecules and the emitted light passes the objective and the beam-splitter and subsequently reaches the pinhole. Light coming from out-of-focus is physically rejected by the pinhole in front of the detector. This means that only light from the focus plane is reaching the detector. This gives a lot of advantages besides the possibilities of fluorescence microscopy such as better resolution and no requirement of sample sectioning.

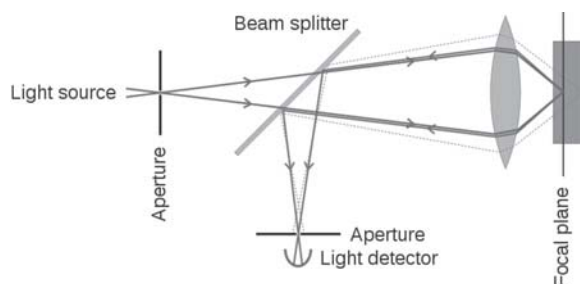


Fig. 13.8. The principle of a confocal microscope and its main parts.

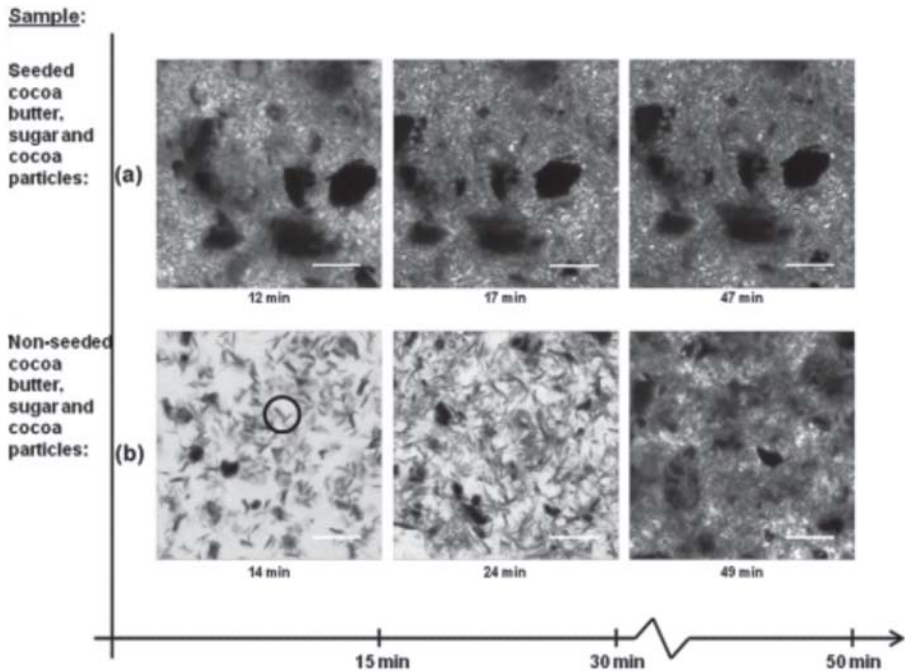


Fig. 13.9. CLSM micrograph of a chocolate model system surface. Here seeded (a) and nonseeded (b) samples containing cocoa butter, sugar, and cocoa particles. The characteristic rod-shaped crystals, highlighted by a circle in (b) and connected to nonseeded samples containing cocoa particles are explicitly detected, scale bars represent 12.5 μm and the time indicated below each image correlates to minutes kept at 14°C. The color is artificial. Source: Svanberg et al., 2011.

The microstructure of chocolate and pralines can be revealed at the micrometer level using CLSM. Since CLSM is a fluorescence microscope, staining of the structures of interest is needed. The staining involves use of fluorescent molecules that have high affinity to certain parts of the microstructure or that dissolves in certain phases such as fat. The microstructure of chocolate mainly consists of cocoa butter, cocoa particles, sugar and lecithin. An example of a cocoa butter microstructure is shown in Fig. 13.9. Lecithin can be made visible by covalent binding of a fluorescent group to the lecithin molecules. Liquid fat or semi-crystalline fat can be made visible in the CLSM by fat soluble fluorescent molecules like BODIPY. Fat crystals are very dense and the staining cannot easily penetrate into the fat crystals. Thus, fat crystals appear as dark areas in the CLSM micrographs there the crystal habitus can be determined.

The CLSM micrographs are evaluated either by visual inspection or image analysis. The CLSM method works well to get an overview of the microstructure, to

get the distribution of fat, cocoa particles, and sugar in the sample. Sample preparation is very important for what kind of information that can be concluded from the micrographs. The fluorescent molecules that stain different parts of the sample can be introduced either during the production of chocolate before the fat crystallization or in already produced chocolate samples. The fat crystal network determines many of the properties of the praline. If the BODIPY is introduced already at the production of the chocolate, it is possible to nicely follow the fat crystallization and the influence of solid particles on fat crystallization (Svanberg et al., 2011). However, if the staining is introduced after production it is more elaborate to get detailed information about the fat crystal network and more research is needed.

CLSM can also be used without staining for the detection of chocolate bloom or surface roughness. Using the height assessment of the confocal can be used to quantifying the surface roughness commercial chocolate samples and following the kinetics of surface changes under fat bloom inducing conditions. This was shown to be possible by Pedreschi (Pedreschi, Aguilera, & Brown, 2002) and Briones (Briones, Brown, & Aguilera, 2006). In that way confocal light microscopy can be used to characterize surface properties of foods as well as changes during storage distribution when these alterations are beyond the visual range (Fig. 13.10).

Raman Microscopy

Confocal Raman microscopy is a useful method to study the distribution of the different chemical components in a chocolate product and the physical state of the components. Confocal Raman microscopy is used in order to analyze the local distribution of different components within a sample. This technique offers the possibility to acquire a Raman spectrum and hence chemical information of a specially defined sample, with a resolution down to the optical diffraction limit. Further, the analysis can be performed at ambient conditions, without requiring any special sample preparation. Confocal Raman microscopy combines two different techniques, namely confocal microscopy and Raman spectrometry. In confocal microscopy a small pinhole, sitting in conjunction to the focus of the objective, allows only the light coming from the focal plane to pass into the detector. This focal plane can be moved several micrometers into the sample, which gives the opportunity of gaining 3D information. Raman spectroscopy uses the effect of inelastic scattering of monochromatic light on molecules, and shows the elastic scattered light energy-shifted peaks, which are characteristic for the scattering molecule and result from the excitation of vibrations in the molecule. Performing Raman microscopy, a Raman spectrum is recorded on every image pixel. Thus, when analyzing dedicated peaks of the spectra, a variety of images can be generated using only a single set of data. Postmeasuring data evaluation makes identification of different components in the sample possible.

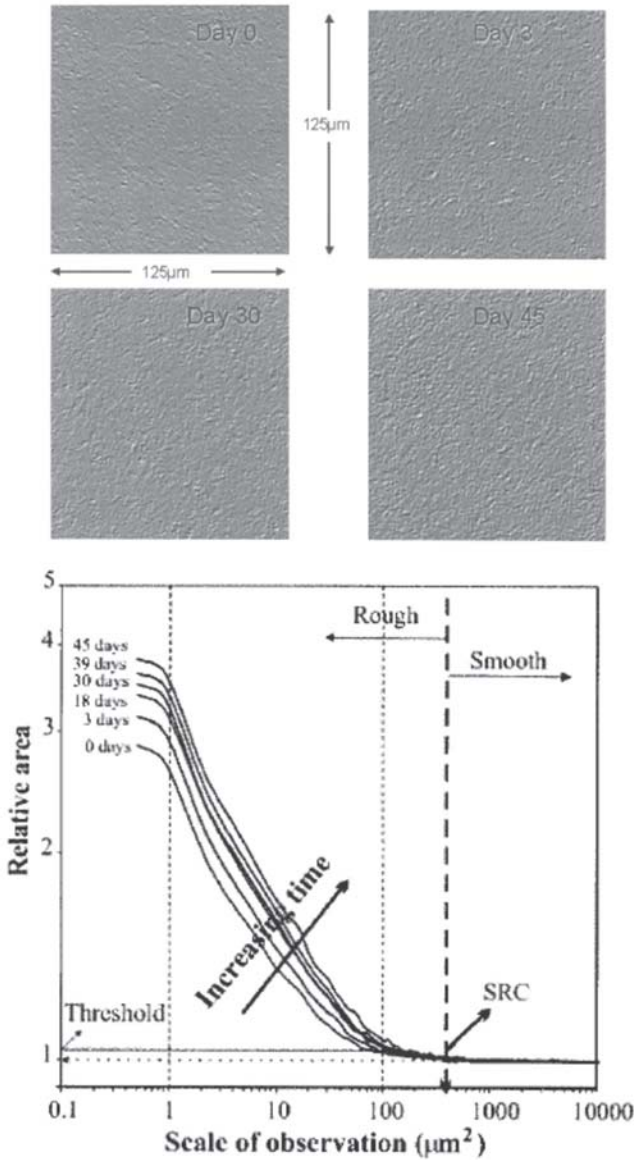


Fig. 13.10. Top: Height maps constructed from four surfaces measured after 0, 3, 30, and 45 d of storage. Bottom: Relative surface area as a function of scale of observation (μm^2) for chocolate bar during selected storage times.

Confocal Raman microscopy measurements can be performed e.g. with a WITec alpha300 system which has a lateral resolution of 300 nm and a vertical resolution of 500 nm, using a 532.18 nm laser for excitation. The integration time per Raman spectra is normally 0.1 s, which gives an advantageous signal-to-noise ratio at the same time as it does not destroy the sample. Further, the microscope can be equipped with a Linkam cold stage set to 3°C, preventing the sample from melting. Due to fluorescence when analyzing milk- and dark chocolate (probably due to cocoa solids), only white chocolate or cocoa butter samples can be analyzed with this technique. The scan range for an image can vary, and a larger scan range leads to a longer scan time, e.g., a scan range of $60 \times 60 \mu\text{m}$ with 140×140 pixels in xy-direction will take a few minutes to perform. In order to be able to determine the distribution of the components, reference spectra have to be recorded for all the components in the sample. No sample preparation is needed for this technique.

The data can be evaluated using the software program WITec project 1.92 (Ulm, Germany), which together with the reference spectra will generate result images showing the distribution of the different components in the sample. Fig. 13.11 shows an example over xy- and xz-result images, where the distribution of fat and sugar at

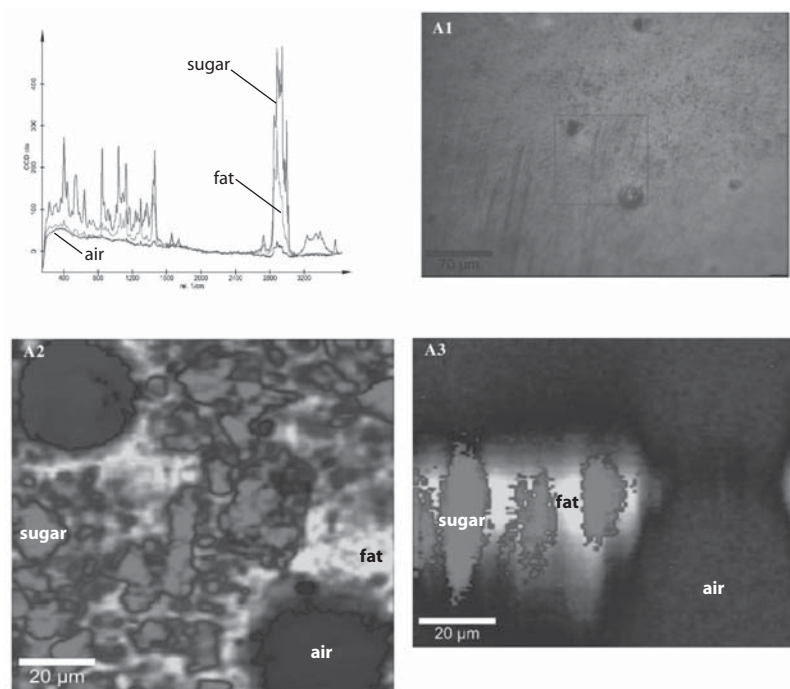


Fig. 13.11. Top left: Average spectra, according to color codes in image and depth scans; (light gray) fat, (medium gray) sugar, and (dark gray) air.

a white chocolate surface has been determined. Those analyses can be performed as a function of time eventually at the same spot. Here the micro distribution of cocoa butter, cocoa particles as well as sugar could be determined. However, the fluorescence of cocoa particles generally prohibits the observation using Raman microscopy unless the fluorescence is absorbed by filters.

Microscope images, image scans (xy), and depth scan (xz) made on filled white chocolate; A2 shows an image scan over the marked area in microscope image A1. A3 shows a depth scan over the marked white line in A2 (Dahlenborg, 2008).

Thermal Analysis

Differential scanning calorimetry (DSC) can be used to investigate the melting behavior and crystallization of fats (e.g. cocoa butter) in chocolate products. Thus, DSC can provide melting points and enthalpies for the correct determination of the polymorphic forms of cocoa butter TAGs.

Calorimetry is a method that measures heat which means that heat is exchanged. Since many chemical reactions and physical transitions are connected to heat, either by consumption or generation of heat, calorimetry is a useful method for investigating these kinds of processes. Especially food materials are very suitable to analyze with calorimeters to get information about the thermal and thermodynamic stability of various phases. FSC is often used to investigate the melting behavior and crystallization of cocoa butter in chocolate products (see also Table 13-B).

DSC measures heat capacity as a function of temperature. The principal with DSC is to measure the difference between the heat flow (J/s or W) of a sample and a reference as a function of temperature or time while exposed to a controlled temperature-time program. During scanning, peaks or inflection points can be observed and these are reflecting the thermally induced transitions of the material. Depending on the direction of the peak, the nature of the transition is either endothermic (heat absorbing) or exothermic (heat releasing) or mixtures of both of them as occurs during phase transitions. For example, solid material that melts display endothermic behavior while crystallization displays exothermic. The sample and the reference are placed in small individual pans or crucibles, 10–100 μl , that are either opened or hermetically sealed before placed in the DSC.

During DSC analysis melting/crystallization curves will be recorded. Fig. 13.12 shows a melting curve of tempered chocolate. The temperature at the different peaks gives the melting temperature of the fat crystals, and thus, the polymorphic forms. By studying the peaks in the recorded melting/crystallization curves and relating this to the melting points of different polymorphs one can determine the polymorphic form/forms of the fat.

Using PCA De Graef et al. (2005) determined that, provided other data like SFC from pNMR and hardness, DSC data could be unnecessary for determining whether a chocolate sample shall or shall not bloom. However it is very likely to be seen from

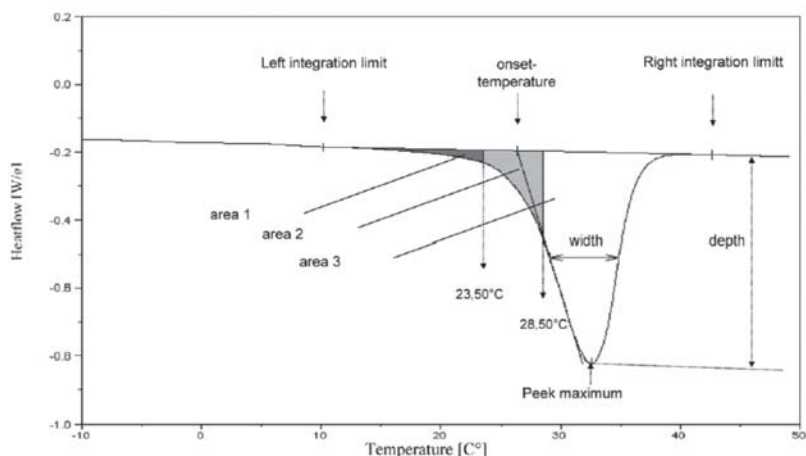


Fig. 13.12. DSC profile of a chocolate sample. There the melting peak is divided into different areas and the onset temperature is correctly determined. Source: De Graef et al., 2005.

DSC alone that phase transitions occurred in chocolate if only one technique can be used. It is to be stated that the time at which the analysis is performed is crucial for determination. It has been shown that initially “well tempered” chocolate samples turn out to transform totally in their polymorph (Kalinin, 2011). Fig. 13.13 indicates that samples of different tempering origin which show initially well tempered behavior do transit in polymorphic form, hence pointing out that the timely analysis is relevant for investigation of bloom in chocolate and prediction thereof is based on the knowledge of the kinetics of phase transitions.

Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy (EDS) is a useful method to achieve migration profiles from a filling into a chocolate shell and to the surface. This method is an analytical technique used for elemental analysis and chemical characterization of a sample. Almost all elements in the periodic table, from Na to Pu, can be measured. Energy dispersive X-ray spectroscopy (EDS) is a powerful micro analytical tool that is compatible with scanning electron microscopes (SEM). EDS can be used to rapidly evaluate the elemental constituents of a sample. It is not only qualitative results that can be obtained with EDS, but also accurate quantitative results can be achieved. The generation of the electron beam is the same for EDS as for SEM. When the electron beam interacts with the sample, a beam electron can interact with a sample atom and ejecting an electron from an inner shell. The atom is then left in an excited state with an electron hole in the inner shell. An electron from an outer shell, with higher energy, then fills the hole and the atom relaxes back to its ground state, low energy state. The energy difference

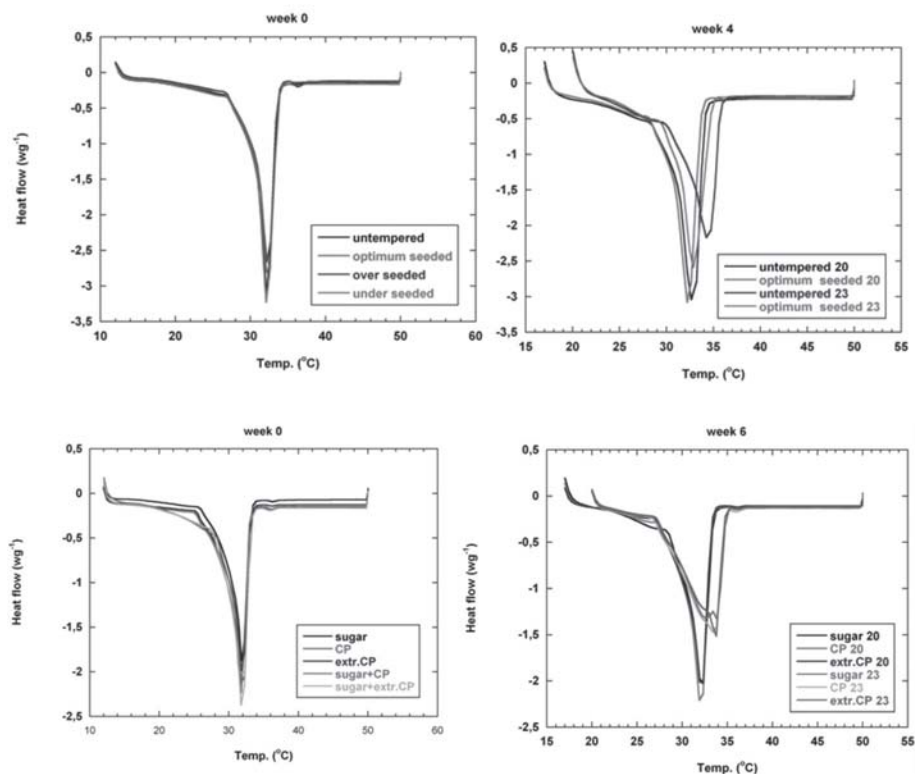


Fig. 13.13. DSC graph for initial melting behavior after 0 weeks and after 4 weeks for samples with different crystallization stored at 20°C and 23°C, respectively.

between the outer high energy shell and the inner shell is emitted as x-ray photons and this energy difference is specific for each element. The different elements in the sample therefore generate x-ray photons with energies specific for only these elements, and by measuring the emitted x-rays it is possible to determine the elemental composition of the specimen. During deceleration the electron loses some energy, which is emitted as a photon and the radiation that occurs is called Bremsstrahlung (“breaking radiation”). This radiation can be seen in the EDS spectrum as the continuum background, and prevents fully quantitative determination of the atomic species.

By using EDS, the migration in chocolate can be followed using an adapted labeled TAG which can be incorporated in the filling. The elements that will appear when analyzing both chocolate and filling are C and O. Therefore a labeled TAG (TAG) should be added to the filling in a suitable concentration. By detecting the element from the labeled TAG we will be able to follow the migration from the filling into and through the chocolate shell.

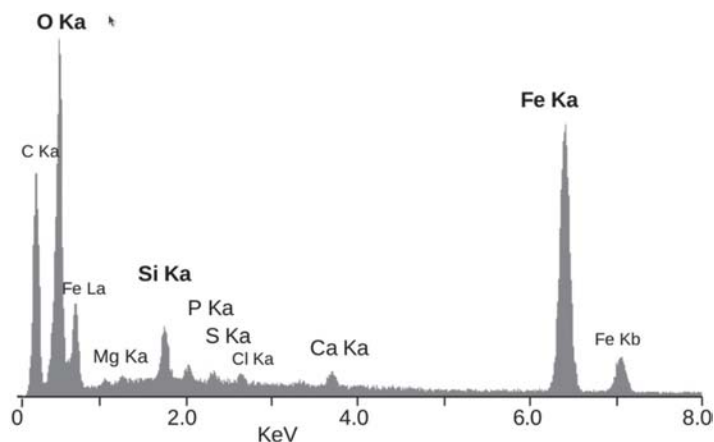


Fig. 13.14. EDS spectrum showing the amount of carbon different atomic species of a mineral enabling detailed determination of atomic composition.

In order to follow the migration of the labeled TAG, the analyzed surface is the surface of an intersection of the sample is analyzed. This intersection is obtained by cutting the samples with a razor blade. The concentration of the labeled TAG is measured in the shell at different spots, over a line. By measuring at different distance from the filling and over a line, one can follow how the labeled TAG migrates through the shell. From each measuring spot, an EDS spectrum is obtained indicating the different elements in the shell. From the spectrum, also the weight percent (Wt%) and the atomic percent (At%) of each element can be obtained (Fig. 13.14).

The EDS analyzes can be performed on the chocolate shell intersections, moving from the filling to the upper surface of the chocolate shell. By plotting the concentration of a labeled TAG as a function of the distance from the filling a migration profile can be achieved as in HPLC.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is used to determine the TAG concentration of pure fats or fat-containing samples. Furthermore, it is a useful method to study the kinetics of oil migration in composed chocolate products by determining the TAG composition of a chocolate in contact with a filling as a function of storage time.

High performance liquid chromatography is used to separate certain components, such as TAGs, on a thin column containing a solid stationary phase. If fats or oils are injected in a chromatographic system, the different TAGs will move with the liquid mobile phase through the system. Depending on their interaction with the stationary

phase, they will move through the system at a different speed. The higher the interaction between the TAG and the stationary phase is, the more the TAG will be retained by the system. For the separation and analysis of TAGs in oils and fats, reversed phase high performance liquid chromatography (RP-HPLC) is generally preferred. "Reversed-phase" indicates that the mobile phase is more polar than the stationary phase, whereas in "normal-phase" the stationary phase is more polar (Rombaut, De Clercq, Foubert, & Dewettinck, 2009)

Several detectors can be used in combination with the chromatographic system. For TAGs in oils and fats, very often an evaporative light scattering detector (ELSD) is used. In such a detector, the column effluent is first nebulized, then the solvent is evaporated and finally the remaining non-volatile components scatter the incident light. The scattered light is directly related to the quantity of the component. Another type of detector that can be used for TAG analysis is a refractive index (RI) detector. In this detector, the difference in refractive index between a reference cell (containing the solvent) and a measuring cell (containing the solvent and the sample's TAGs) is measured. This difference is directly related to the TAG concentration. However, RI detection is not compatible with gradient elution whereas ELSD detection is. More recently, a charged aerosol detector (CAD) detector has been proposed to quantify the TAG concentration of samples. In this detector, also nebulization of the column effluent and evaporation of the solvent takes place, but the components are then charged before detection occurs.

In order to study oil migration using high performance liquid chromatography, a setup in which two components (chocolate and filling) are brought into contact with each other is required. When studying oil migration in filled chocolates (type pralines), the outer chocolate layer can be removed with a scalpel, hereby ensuring that an equally large amount of chocolate is removed from each sample. However, most studies use model systems for filled chocolates. In order to monitor the TAG migration, the chocolate layer can be sliced at regular time intervals using a microtome.

Prior to HPLC analysis, the fat of chocolate samples or other fat-containing samples (e.g. cocoa mass, hazelnut mass, etc.) should be extracted by means of a solvent like petroleum ether or diethylether. When determining the TAG concentration of pure fats or oils (e.g., cocoa butter, hazelnut oil, palm oil, etc.) the samples do not require an extraction step. The (extracted) fat should then be diluted to an appropriate concentration, depending on the chromatography system that is used.

The three main TAGs of chocolate, POP, POS, and SOS, can be easily distinguished using HPLC. At higher elution times peaks appear in the chromatogram. These correspond to the filling-specific TAGs, like triolein (OOO) and LOO. When plotting the relative triolein concentration of each chocolate layer as a function of time, the kinetics of oil migration into the chocolate can be studied; see Fig. 13.15 (Depypere, De Clercq, Segers, Lewille, & Dewettinck, 2009). Here the ratio between different TAGs is used to follow blooming ability of a filled chocolate. This method

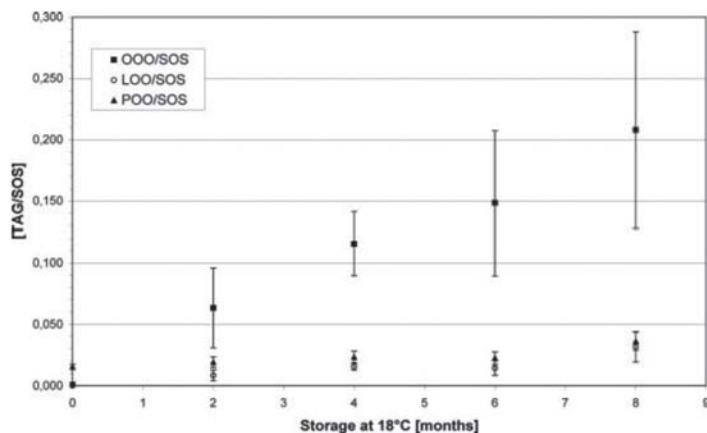


Fig. 13.15. Evolution of [OOO/SOS], [LOO/SOS] and [POO/SOS] in chocolate sampled from the outer surface of filled chocolates during prolonged storage at 18°C. Source: Depypere et al., 2009.

enabled them to propose a heat treatment that retards fat bloom when pralines are stored at -20°C or 4°C .

Low Vacuum Scanning Electron Microscopy

Low Vacuum Scanning Electron Microscopy (LV SEM) is a technique which is suitable for investigation of chocolate surfaces and for characterization of topological changes over time.

A scanning electron microscope (SEM) is an electron microscope that uses a beam of electrons, under high vacuum (10^{-6} Torr), to create a three-dimensional-like image of the sample surface. Topographic images can be obtained in the magnification range 10–10,000 $\times$. The sample area to be examined is irradiated with a finely focused electron beam, generated from the electron gun, and images are formed when the electron beam is swept in a raster across the sample surface. The electron beam can also be static to obtain an analysis at one position. From the interaction of the electron beam with the sample, signals in form of secondary electrons (SE), backscattered electrons (BSE), characteristic x-ray and other photons of various energies are produced. These signals are collected by a detector system that converts the signals into point-by-point intensity changes on a viewing screen and an image is produced. Since SEM utilizes vacuum conditions and electrons to create images, the surface of non-metal samples need to be covered with a thin layer of a highly conductive material to make it more conductive.

Low vacuum scanning electron microscopy (LV SEM) is a type of SEM that can operate while the specimen chamber contains a gas at a pressure of 0.1–20 Torr and

while the electron column is still under high vacuum. This instrument, with a gaseous environment, makes it possible to observe images of specimens with poor conductivity without coating them with a conducting metal layer. LV SEM is therefore appropriate for chocolate samples. Analyzing an uncoated sample leads to a sacrifice in magnification and resolution, since the chocolate surface might melt when irradiated with the electron beam. However, by analyzing the samples without a coating, the real surface of the chocolate can be studied. The LV SEM system almost appears identical to the conventional SEM except that LV SEM has a pressure-limiting aperture that isolates the specimen chamber from the high vacuum region, around the electron gun. Further the specimen chamber is pumped by a separate pumping line. When the electron beam interacts with the specimen, the same signals as in high-vacuum SEM are produced. To collect the SE signals, special detectors known as environmental SE (ESE) detectors or gaseous SE (GSE) detectors are used.

LV SEM is a technique producing high resolution images of a sample surface. Due to the way in which the image is created, LV SEM images have a characteristic three-dimensional appearance and are useful for judging the surface structure of a chocolate sample. The instrument can be equipped with a cold stage set to 5°C, keeping the chocolate samples from melting. Mixed detectors (GSE 75% and BSE 25%), at 15 keV and at low vacuum mode with 0.7–0.9 Torr, which gives a RH of 13% approximately are the parameters used. These parameters are chosen considering that they cause minimal damage of the chocolate surface, and for optimizing the imaging quality. Analyses of the samples are performed without sample preparation, i.e. without coating the sample surface. This leads to a sacrifice in magnification and resolution; however, by analyzing without coating the true surface can be monitored. After each analysis the monitored samples should be sacrificed due to the possible impact from the microscope such as electron beam, temperature, humidity and pressure (Figs. 13.16–18).

A more systematic approach was undertaken by Dahlenborg et al. (2011) where profilometry was coupled systematically with LV SEM. They have shown that early recognition of surface roughness changes monitored by both LV SEM and profilometry could be a powerful tool in the future.

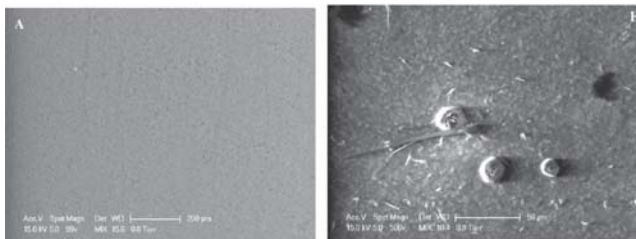


Fig. 13.16. This figure shows result images of fresh and bloomed chocolate. The left result image represents a fresh chocolate surface, and the result image to the right represents a heavily bloomed chocolate sample.

Fig. 13.17. Result images from LV SEM of fresh and bloomed chocolate samples (A) 0 days; (B) 1 day; (C) 2 days; (D) 3 days. Scale bar = 100 mm. Source: Rousseau & Smith, 2008.

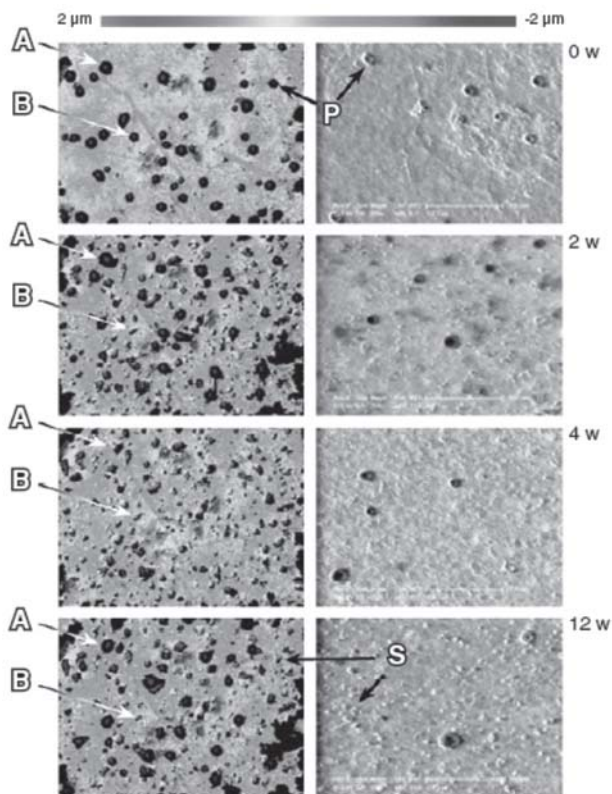
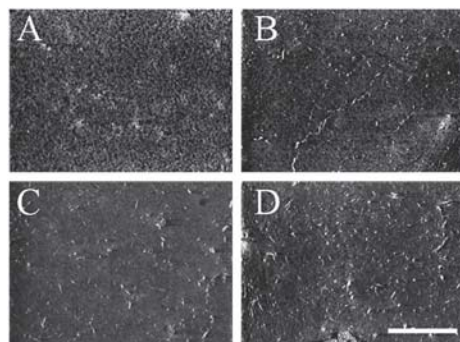


Fig. 13.18. Surface images of white chocolate pralines stored at a constant temperature (18°C). Left: 2D profilometry images (350 * 263 mm), produced from the same spot on the same sample, weeks 0, 2, 4, 12, 36. Some of the protrusions disappear and then reappear (A), while some change their appearance over time (B). Right: LV SEM images, from different samples, weeks 0, 2, 4, 12 including protrusions (P), ridge-like topological features (S) and needle-shaped crystals (C).

Nuclear Magnetic Resonance

By use of the Nuclear Magnetic Resonance (NMR) technique the solid fat content of a pure fat suspension or matrix can be determined. Furthermore the sample does not get destroyed during evaluation and the analysis speed is that high (<10 s), that crystallization kinetics can be determined precisely.

In chocolate mainly the fat matrix determines the product texture. Therefore the knowledge about the solid fat content as a function of temperature is of utmost importance. Since cocoa butter and other vegetable fats are natural products and contain several fatty acids resulting in a number of fatty acid combinations within TAGs their melting points are technically intervals instead. Furthermore the origin or even the year of harvest can result in significant differences in temperature stability. For this reason the knowledge about the solid fat content distribution is mandatory for comparison and is already an indicative parameter for quality consistency. In addition to that the combination of TAGs can lead to polymorphic behavior of crystalline structures resulting in diverse temperature stabilities (see tempering). This has to be kept in mind even by comparison of differently processed or stored fats of the same type.

The analysis principle is the same as for all the NMR techniques on the market. A permanent magnetic field ($B=0.47\text{T}$) influences the mean spin direction of the molecules protons. By applying current pulses a second magnetic field is generated perpendicular towards the permanent field. Thereby the spin direction of protons is temporarily deflected by 90° resulting in a measurable magnetic induced momentum. As soon as the pulse stops (μs) the spin direction relaxes towards its initial status. Solid and liquid fat molecules possess different relaxation times. By following the induced momentum over time the ratio of solid/liquid fractions can be determined. The Solid Fat Content (SFC) describes the fraction of solid fat in percentage of the whole sample.

There exist two principles of final evaluation of the solid fat content. By use of the so-called direct method (AOCS Official Method Cd 16b-93, AOCS, 1999b) the values of solid and liquid fractions are directly compared with each other. Within the indirect method only the values (AOCS Official Method Cd 16-81, AOCS, 1999a) of the liquid fractions are determined and compared with a completely liquid sample.

By following the AOCS description of the method a strict preconditioning for the fat sample has to be fulfilled before a representative evaluation of solid fat content can be performed. The reason for this is to achieve the equilibrium state of the sample. From a crystallographic point of view this guideline is not valid for fats showing complicated polymorphic behavior such as cocoa butter. For achieving a representative result triple measurements on different samples should be performed. During operation it is important to avoid fat sticking outside the tubes since once fat gets in direct contact with the measuring cell, the results are falsified and the cell has to be replaced.

Since the NMR-software interprets directly the relaxation times of solid and liquid fraction of fat into SFC values, the evaluation of the results is straightforward.

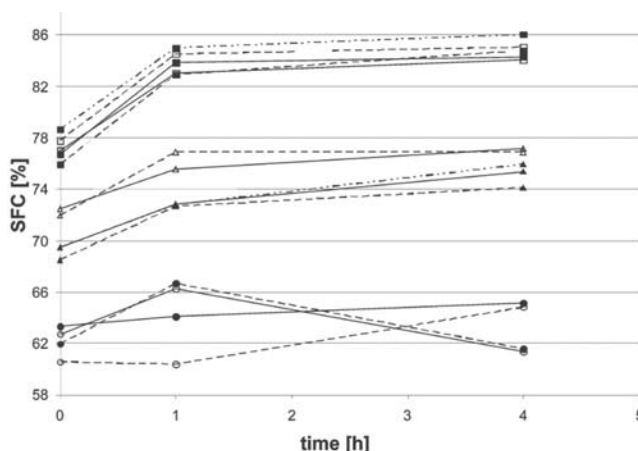


Fig. 13.19. Evolution of the SFC values during storage time. Open symbol: short residence time tempering; closed symbol: long residence time tempering; square: 0% butter oil; triangle: 3% butter oil; circle: 6% butter oil; —: undertempered (T1); - - -: well tempered (T5); - · - · -: overtempered (T9). (Graef et al 2005).

Fig. 13.19 shows the results of SFC distributions of different vegetable fats used in chocolate manufacturing. The direct method was used for determination of the values. The graph points out that cocoa butter is the most stable fat within this comparison over the whole temperature range. It shows a minor crystalline fraction melting from 10–25°C and a major between 25–35°C resulting. The results are in accordance with the characteristics of chocolate where it is generally stable at regular room temperature but melts rapidly in direct sunshine or holding in your hands for more than a few seconds. Palm kernel fat instead shows overall lower values and melts constantly over a range from 10–30°C. This characteristic is one reason why palm kernel fat is often used in fat based fillings of filled chocolate products. Palm mid fraction seems to be even less stable than palm kernel at low temperatures showing a strong melting between 10–25°C but indicates more stable crystals between 25–35°C. As a result of this palm mid fraction finally is even higher stable than palm kernel fat.

With this measuring technique empirically gained knowledge can be precisely quantified and compared with each other. Once set up, the equipment is easy to handle and low intense in terms of maintenance. In the same manner high resolution NMR can be tuned in order to calibrate for different polymorphic forms

In general the relaxation times of different Carbon atoms as well as the difference in chemical shift can therefore be calibrated for further use. This has been demonstrated by Adam-Berett et al. (2007). There, model systems of pure TAGs have been investigated Table 13-A.

Table 13-A. Temperatures of Crystallization Used to Form the A and the B Polymorphs, Melting Temperatures of the Polymorphs, T₁ Relaxation Values at –20°C and 20 Mhz and M₂ Measured at 10°C.

Triglycerides		Temperature of crystallization (°C)	Temperature of formation of β form (°C)	Melting temperature (°C)	T ₁ (ms) at –20 °C and 20 Mhz	M ₂ (s ⁻²) at 10 °C
Tricaprin CCC (C10)	α	–50	25	–15.0	229	Melt
	β			31.5	3828	10 236
Trilaurin LLL (C12)	α	–20	41	15.0	249	7346
	β			46.5	3728	10 744
Trimyristin MMM (C14)	α	2	51	33.0	153	6345
	β			57.0	4797	11 140
Tripalmitin PPP (C16)	α	2	58	44.7	137	6393
	β			66.4	3986	11 224
Tristearin SSS (C18)	α	2	68	54.9	126	6583
	β			73.1	4340	11 330

Profilometry

Profilometry is a method that can be used to directly follow the development and kinetics of a surface and hence of surface topology on a chocolate product.

Profilometry is a microscope based method used to measure surface profiles, in order to quantify its roughness. The results are displayed on a color display as solid images and plots, and there is also access to several roughness parameters. Vertical resolution is usually in the nanometer level.

A noncontact profilometer is an advantageous method when analyzing chocolate samples since no surface damage will occur. Further, this technique provides the possibility to return to the same sample area on each analysis occasion. This gives an opportunity to follow and compare surface changes in that specific area of interest. As a result of this, noise (normally observed due to the use of different spots for data collection) can be reduced. Non-contact profilometry is based on white light interferometry where the sample surface is scanned by using a light beam. Movement of the stage, supporting the sample, along two axes (X,Y) and measurement of the movement of the light beam (Z) allows 3-D measurement of the topography. Light from the microscope divides within the interferometric objective; one portion reflects from the sample surface and another portion reflects from an internal, high quality reference surface in the objective. Both portions are then directed onto a solid-state camera (CCD). Interference between the two light wave fronts result in an image of light and dark bands, called fringes, which indicate the surface structure of the sample. The sample is scanned by vertically moving the objective with a piezoelectric transducer. As the objective scans, a video system captures intensities at each camera pixel. These intensities are converted into images by a software application. In addition, the images are displayed on a video monitor, which magnifies the camera image 26 times.

Profilometry provides result images and quantitative data over surface roughness and surface waviness, which can be associated with fat bloom development at the surface. However, there are some limitations. The sample reflectivity must be high



Fig. 13.20. Chocolate sample fixed to a microscope slide which fits onto the profilometry stage.

enough (better than 0.1%), the roughness (peak-to-valley) should be $<100\text{ }\mu\text{m}$ and the local inclination of the topological features should not be too steep. The dimension of this inclination depends on the objective and magnification used. The number of points displays the number of valid data points in the data set, i.e. the number of camera pixels used in the analysis. Thus, due to topological surface features with high inclinations, some profilometry data might not be interpretable and will then be illustrated with black color in the result images.

A chocolate sample can be fixed with glue to a microscope slide which fits onto the profilometry stage (Fig. 13.20), and will thereby keep the same position at each analysis occasion. The different analysis points have known x- and y-coordinates, and by means of these coordinates the different analysis points can be retrieved, and therefore the same area ($\pm 20\text{ }\mu\text{m}$) can be analyzed at each analysis occasion. The stage has to be tilted in order to level the sample surface. This is done by minimizing the number of fringes on the instrument screen. The fringes can be located by focusing the sample surface. After adjustment of the light level and determination of the working distance (the distance from the end of the objective to the sample surface when focused) the measurement can be performed. Depending on the working distance the scan takes 10 to 60 seconds. In order to achieve a result image of $350 \times 263\text{ }\mu\text{m}$, with a theoretical lateral resolution of $0.88\text{ }\mu\text{m}$, a $20\times$ interference objective with $1\times$ zoom can be used. Further, there is access to several objective options and magnifications in order to obtain different result image areas.

The data can be analyzed using different applications in the analysis and control software. In order to quantify the surface topology, there is access to several surface topological parameters. Roughness (R_q), waviness (W_q), peak number, peak height and peak area are result parameters that can be useful for the analysis of chocolate surfaces. Peak number (peaks per observation area) is the peak count or the number of peaks included in the analysis. A peak is defined as a data point whose height is above an operator-selected bandwidth. The band is centered on the mean plane of the data, where the upper edge of the band is the reference value for finding peaks. Thus, it is

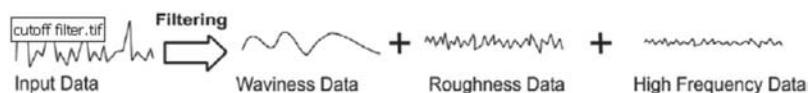


Fig. 13.21. The input data can be separated into waviness, roughness and high frequency components.

of importance that the choice of reference values is well considered. Peak height (μm) is the height for each peak included in the peak analysis and peak area (μm^2) is the area of each peak included in the peak analysis. The roughness parameter, R_q (μm), is the root-mean-square (rms) roughness and the waviness parameter, W_q (μm), is the root-mean-square (rms) waviness. Thus, they are defined as the average of the measured height deviations taken within the evaluation length or area and measured from the mean linear surface:

$$\text{rms} = \sqrt{\frac{1}{L} \int_0^L z^2(x) dx} \quad \mu\text{m}$$

$Z(x)$ represents the height elements along the profile and L is the number of discrete elements. By digital filtering (cut-off filter), the input data for the sample can be separated into waviness, roughness and high frequency components (Fig. 13.21). This filter includes a high filter wavelength which defines the noise threshold; all shorter spatial wavelengths are considered noise. It also includes a low filter wavelength which defines the form threshold where all longer spatial wavelengths are considered form (waviness). Everything between these two wavelengths is assigned to roughness.

This technique allowed Dahlenborg et al. (2011) to closely follow surface morphology as a function of storage time even on white pralines (Fig. 13.22).

X-ray Diffraction

X-ray diffraction is used to characterize the structure and hence polymorphism of fats and oils in food products, such as chocolate. Cocoa butter exhibits a complex polymorphism. Six different polymorphic forms are generally recognized. They are indicated by a Greek letter, a Roman number or both. Additionally the lateral chain arrangement should be noted (Kalnin et al., 2004) since it is important for the distinction of TAG crystal forms in general (Fig. 13.23).

The most common nomenclature is that proposed by Wille and Lutton (1966), who described polymorphic forms I to VI. However, Van Malssen et al. (1999) suggested that the forms III and IV are not really distinct. The polymorphic forms $\gamma(\text{I})$, $\alpha(\text{II})$ and β' (III and IV) are very unstable and are rapidly transformed into the more stable forms β_1 and β_2 (Table 13-B). It has not yet been denied that lipids exhibit a polymorphism of monotropic type. Therefore polymorphic transition from a less

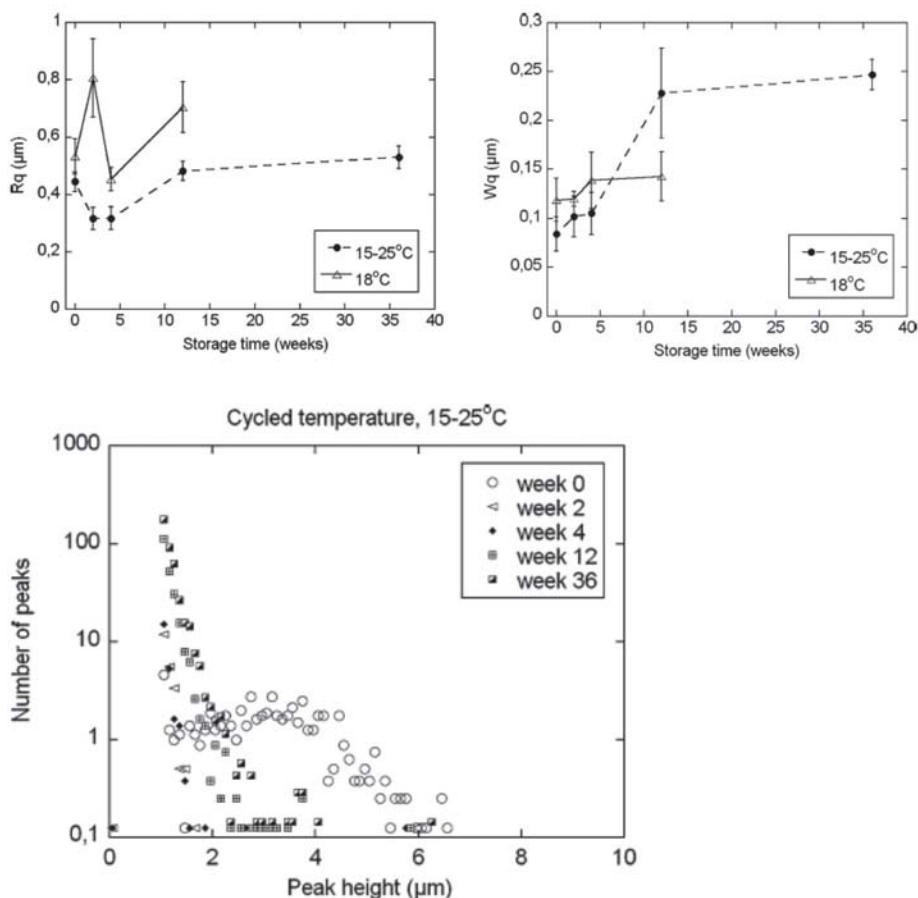


Fig. 13.22. Top left: Rq as a function of time for samples stored at 18°C (open triangles) and at 15–25°C (filled circles), respectively, including standard error of mean. Top right: Wq as a function of time for samples stored at 18°C (open triangles) and at 15–25°C (filled circles), respectively, including standard error of mean. Bottom: Number of peaks per observation area (350 9 263 lm) as a function of peak height for samples stored at a cycled temperature (15–25°C). The number is calculated as a mean of $n = 9$. The standard error of mean is ± 1.0 .

stable to a more stable polymorph is irreversible. This means, that in practice only the polymorphic forms β'_1 to β_1 are important: well-tempered chocolate appears in the β_2 form, the β'_1 form is found in untempered chocolate and the β_1 form is characteristic for bloomed chocolate or chocolate stored for a long time. The polymorphic form β_2 gives chocolate the desired properties such as a high gloss, a good snap, a good hardness, maximal contraction and resistance towards oil migration and fat bloom. Fat

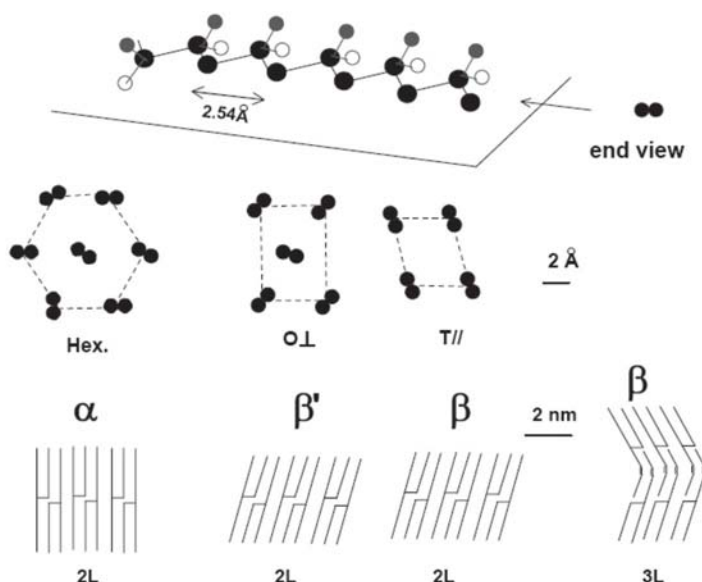


Fig. 13.23. General overview on TAG structures in the solid state.

Table 13-B. Melting Points of Different Polymorphic Forms of Cocoa Butter (Additional Variability Is Found for Cocoa Butter of Different Origin).

Melting Point (°C) of the Different Polymorphic Forms of Cocoa Butter (literature values)

References						
14	10	15	17	18	19	20
18 (γ)	17.3 (I)		14.9–16.1 (I)	13 (VI)	16–18 (γ)	13.1 (I)
23.5 (α)	23.3 (II)		17–23.2 (II)	20 (V)	20.7–24.2 (α)	17.7 (II)
	25.5 (III)	20.7 (III)	22.8–27.1 (III)	23 (IV)		22.4 (III)
28 (β ^{''})	27.5 (IV)	25.6 (IV)	25.1–27.4 (IV)	25 (III)	26–28 (β')	26.4 (IV)
33 (β')	33.87 (V)	30.8 (V)	31.3–33.2 (V)	30 (II)	33.7–34.9 (β)	30.7 (V)
34 (β)	36.3 (VI)	32.3 (VI)	33.8–36 (VI)	33.5 (I)		33.8 (VI)

bloom crystals appear in the β_1 polymorph. Fat bloom is thus always accompanied with a polymorphic transition from β_2 to β_1 (Lonchamp & Hartel, 2004).

The polymorphism of cocoa butter can be characterized using differential scanning calorimetry (see DSC) or X-ray diffraction (XRD). The application of XRD to study crystal polymorphism in real chocolate has been described in a limited number of publications. The main difficulty herein lays in the fact that the WAXS-pattern of sugar partially overlaps with the signal from the cocoa butter so that XRD cannot be applied directly to chocolate when only poor resolution WAXS is available. Several ways to overcome this problem have been suggested in literature. These include

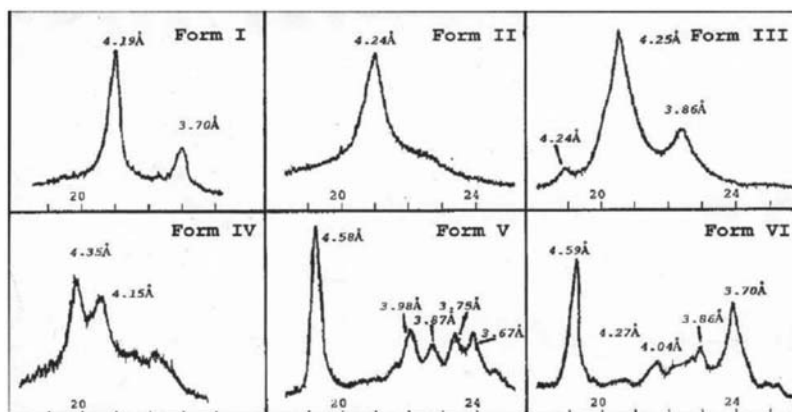


Fig. 13.24. Characteristic WAXS-pattern of the different polymorphic forms of West African cocoa butter. Source: Timms, 2003.

classification of the sugar and removal of the sugar by a solid-liquid extraction (Le Révérend et al., 2009).

For chocolate samples, the sugar should be removed prior to XRD analysis. Usually the sugar is removed by rinsing the chocolate in cold water. Therefore, ground chocolate is rinsed in water at 4–6°C for several hours and the water should be changed regularly. After drying, the chocolate is then inserted into a sample holder, thereby assuring that the surface is very flat. Guthrie et al. (2005) demonstrated that for certain dark chocolate samples, the removal of sugar is not required.

Fig. 13.24 shows the WAXS pattern of the different polymorphic forms of a West African cocoa butter (Timms, 2003). From the 2θ angle, the d spacing can be calculated according to Bragg's law:

$$n\lambda = 2d \sin \theta$$

where n is a positive whole number, λ is the wavelength of the X-ray source, d is the space between crystal planes and θ is the angle between the incident and the diffracted wave.

The unstable polymorphs (γ , α , and β') show typical peaks around 4.20 Å, whereas the stable polymorphs (β_2 and β_1) are characterized by a peak around 4.58 Å, as can be seen in Fig. 13.24 and Table 13-C (Loisel et al., 1998). However, the β_2 polymorph is characterized by four distinct d spacings, whereas the β_1 polymorph displays only three lines in the WAXS pattern where the mid spacing line at 3.75 Å is totally lacking.

In conclusion, for proper investigation of cocoa butter, a thermal method at the end of the production facility where the proper temper should be achieved is recommended. Additionally a spectroscopic method for determining the structure for the

Table 13-C. Characteristic Peaks in the XRD Pattern for the Different Cocoa Butter Polymorphs (Loisel 1998; Simplified Version: Le Révérend, 2009).

Long- and Short-Spacings (Å) of the Different Polymorphic Forms of Cocoa Butter (literature values) ^a						
Reference	I (2L) ^b β'-sub(α)	II (2L) ^b α	III (2L) ^b β' ₂	IV (2L) ^b β' ₁	V (3L) ^b β ₂	VI (3L) ^b β ₁
10	4.19 (vs)	49 (vs) ^c	49 (vs) ^c	45 (vs) ^c	63.1 (m) ^c	63.1 (vs) ^c
	3.70 (s)	16.3 (s) ^c	4.25 (vs)	14.87 (s) ^c	32.2 (vs) ^c	32 (?) ^c
		4.24 (vs)	3.86 (s)	4.35 (vs)	12.8 (s) ^c	12.76 (s) ^c
				4.15 (vs)	4.58 (vs)	4.59 (vs)
					3.98 (vs)	3.70 (s)
15					3.67 (w)	
	54 (s) ^c	51 (vs) ^c	51 (vs) ^c	49 (vs) ^c	66 (s) ^c	63 (s) ^c
	4.17 (s)	4.20 (vs)	4.20 (vs)	14.8 (w) ^c	33 (s) ^c	31 (s) ^c
	3.87 (m)		3.87 (w)	4.32 (s)	12.8 (m) ^c	12.7 (mw) ^c
				4.13 (s)	4.58 (vs)	4.53 (vs)
16					3.98 (ms)	3.84 (m)
					3.65 (ms)	3.67 (s)
				45 ^c	64 ^c	63.8 ^c
				4.35	4.58	4.59
				4.15	3.98	3.7

distinct chocolate to be analyzed should undermine the correct temper at least once. Special care has to be taken for observing and maintaining “one” thermal history. Temperature control is crucial not only for the production process but also for the analytical process.

For the investigation of bloom, an optical method like profilometry is efficiently used. For methods like LV SEM or use of laser light, great care should be taken for temperature control since one can easily burn bloom crystals away. A combination of both a technique with high and low magnification should be wisely used since bloom is not a punctual occurrence but a global behavior. Provided an equal temper in a chocolate one can still observe fat bloom as time passes by. The underlying reason for the time delay is still not understood completely . . . the cause for fat bloom is thermodynamics. Therefore there is no straightforward predictive measurement for fat bloom, yet.

References

- Bricknell, J.; Hartel, R.W. Relation of fat bloom in chocolate to polymorphic transition of cocoa butter. *J. Am. Oil Chem. Soc.* 1998, 75(11), 1609–1615.
- Briones, V.; Brown, C.; Aguilera, J. Scale-sensitive fractal analysis of the surface roughness of bloomed chocolate. *J. Am. Oil Chem. Soc.* 2006, 83(3), 193–199. doi: 10.1007/s11746-006-1193-z.
- Dahlenborg, H. Prediction of chocolate bloom. Master Thesis in Surface Chemistry, KTH, Stockholm, 2008.
- De Graef, V.; Foubert, I.; Agache, E.; Bernaert, H.; Landuyt, A.; Vanrolleghem, P.A.; Dewettinck,

- K. Prediction of migration fat bloom on chocolate. [10.1002/ejlt.200401059]. *Eur. J. Lipid Sci. Technol.* 2005, 107(5), 297–306.
- Depypere, F.; De Clercq, N.; Segers, M.; Lewille, B.; Dewettinck, K. Triacylglycerol migration and bloom in filled chocolates: Effects of low-temperature storage. *Eur. J. Lipid Sci. Technol.* 2009, 111(3), 280–289. [10.1002/ejlt.200800179].
- Hodge, S.; Rousseau, D. Fat bloom formation and characterization in milk chocolate observed by atomic force microscopy. *J. Am. Oil Chem. Soc.* 2002, 79(11), 1115–1121. doi: 10.1007/s11746-002-0613-4.
- Hodge, S.M.; Rousseau, D. Fat bloom formation and characterization in milk chocolate observed by atomic force microscopy. *J. Am. Oil Chem. Soc.* 2002, 79(11), 1115–1121.
- Kalnin, D. Effect of chocolate shell microstructure and storage on fat bloom, In *Structure and Processing for High Quality Pralines*; Ahrné, L., Ed., Report on Deliverable 2.4, 2011.
- Lonchampt, P.; Hartel, R.W. Surface bloom on improperly tempered chocolate. *Eur. J. Lipid Sci. Technol.* 2006, 108(2), 159–168. doi: 10.1002/ejlt.200500260
- Pedreschi, F.; Aguilera, J.M.; Brown, C.A. Characterization of the surface properties of chocolate using scale-sensitive fractal analysis. *Int. J. Food Prop.* 2002, 5(3), 523–535. doi: 10.1081/jfp-120015489
- Rombaut, R.; De Clercq, N.; Foubert, I.; Dewettinck, K. Triacylglycerol analysis of fats and oils by evaporative light scattering detection. *J. Am. Oil Chem. Soc.* 2009, 86(1), 19–25. doi: 10.1007/s11746-008-1316-9
- Rousseau, D. On the porous mesostructure of milk chocolate viewed with atomic force microscopy. *LWT-Food Science and Technology* 2006, 39(8), 852–860. doi: 10.1016/j.lwt.2005.06.009
- Smith, P.R.; Dahlman, A. The use of atomic force microscopy to measure the formation and development of chocolate bloom in pralines. *J. Am. Oil Chem. Soc.* 2005, 82(3), 165–168. doi: 10.1007/s11746-005-5167-3
- Svanberg, L.; Ahrné, L.; Lorén, N.; Windhab, E. Effect of sugar, cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems. *J. Food Eng.* 2011, 104(1), 70–80. doi: 10.1016/j.jfoodeng.2010.09.023

14

New Method to Study Molecular Interactions in Fats—Synchrotron Radiation Microbeam X-ray Diffraction

Satoru Ueno*Hiroshima University*

Introduction

Lipids are a major component of foods, cosmetics, and pharmaceuticals. They are generally included as multi-component systems for real applications. Therefore, complicated physical properties are presented by reflecting two types of interaction (Sato & Ueno, 2005): (i) among different intermolecular types of lipid molecules and (ii) among intra-molecular fatty acid moieties in a lipid. Hence, physical properties should be studied both in single-component systems and in multi-component systems. In particular, binary mixture studies have provided much knowledge about molecular interactions among different lipid molecules (Timms, 1984; Small, 1988; Sato & Ueno, 2001; Larsson et al., 2006).

Many physical chemical studies have focused on triacylglycerols (TAGs), some of the most useful lipids for industry. Diversified molecular interactions among TAGs occur in polymorphism and mixing behavior. Many types of polymorphs appear in each TAG and in mixed TAGs. Binary mixture systems typically exhibit three phases (Rossell, 1967): solid solution, eutectic, and molecular compound formation. According to physical chemical research, these phenomena are critically influenced by three factors (Zhang, 2007; Larsson et al., 2006): fatty acid compositions of TAGs, glycerol backbone conformation (chair or tuning fork), and methyl end packing interaction. Consequently, physical chemical research is the key to clarifying the complicated phenomena in natural fats and oils.

Physical chemical studies have used various methods, including differential scanning calorimetry (DSC), polarized optical microscopy, transmission and scanning electron microscopy (TEM and SEM), atomic force microscopy (AFM), X-ray diffraction (XRD), and Fourier transformed infrared spectroscopy (FT-IR). FT-IR is a powerful method to determine fatty-acyl-chain conformation in a TAG molecule (Kobayashi, 1988). AFM is useful for visible observation of the surface at the molecular level. Among the above methods, XRD is the most powerful method of obtaining structural information (Small, 1988; Kaneko, 2001). However, measuring the local

area in a sample (e.g., the local structure of a crystal in margarine, part of an emulsion droplet such as the oil-water interface, the air-water interface of an air bubble in ice cream, the local area of chocolate such as the critical area between bloom and non-bloom) is difficult when using XRD because XRD data is integral and the average of all the irradiated area, which is usually millimeter (mm) size.

Synchrotron radiation microbeam XRD (SR- μ -XRD) is a new and powerful method for measuring a local area in a sample. Because the X-ray beam is micrometers (μm) in size, we are able to measure a critical area (e.g., oil-water or water-air interface between bloom and non-bloom areas in the local area of spherulite or a small crystal).

Here I will highlight SR- μ -XRD measurement. First, the experiment set-up is introduced. The experiment results of spherulite (Ueno et al., 2008; Bayes-Garcia et al., 2011), oil-water interface on oil-in-water (O/W) emulsion (Shinohara et al., 2008; Arima et al., 2009), and granular crystal analysis in water-in-oil (W/O) emulsion (Tanaka et al., 2009) are then presented.

Synchrotron Radiation Microbeam X-ray Diffraction

Microbeam X-ray Diffraction

A microbeam is a micrometer-sized X-ray beam that can be produced by several optics (e.g., Fresnel zone plate, K-B mirror, and micrometer-sized pinhole). SR is suitable for producing the microbeam because of its high directionality. Although conventional XRD involves easier sample preparation and time-resolved measurement than μ -XRD, it can provide only averaged structural information from the crystals in the millimeter-size beam. However, SR- μ -XRD, having a diameter of less than 10 μm , enables time-resolved and spatial analysis of fat structures.

While SR- μ -XRD analyses have been applied to many of biological materials, e.g., amylopectin in starch (Buleon et al., 1997; Lemke et al., 2004; Chanzy et al., 2006), human hair (Kajiura et al., 2006), biological tissues (Riekell & Vollrath, 2001; Seidel et al., 2008; Kuckzumow et al., 2000; Nakano et al., 2002; Paris & Muller, 2003; Drakopoulos et al., 2005; Fratzl & Weinkamer, 2007; Yagi et al., 2009) and blended polymers (Müller et al., 2000; Nozue et al., 2003; Nozue et al., 2004a; Nozue, 2004b; Riekell & Davies, 2005; Kikuzuki et al., 2010; Kajioka et al., 2010)), very little research has been performed on fat structures. Since the diameter of the SR-microbeam is of micro-meter dimensions, the local structures of a fat crystal network can be measured at different scales of microanalysis [Fig. 14.1 (Acevedo & Marangoni, 2010)]. Also, the fat crystals in complicated colloidal systems (e.g., emulsion, whipped systems, and gels) can be analyzed with microbeam techniques.

The difficulties of μ -XRD experiments include optics, aiming the microbeam, fixing samples, preparing samples preparation, avoiding target overlapping along the m-beam, irradiation damage, thermal damage, and reaction damage (Fig. 14.2a).

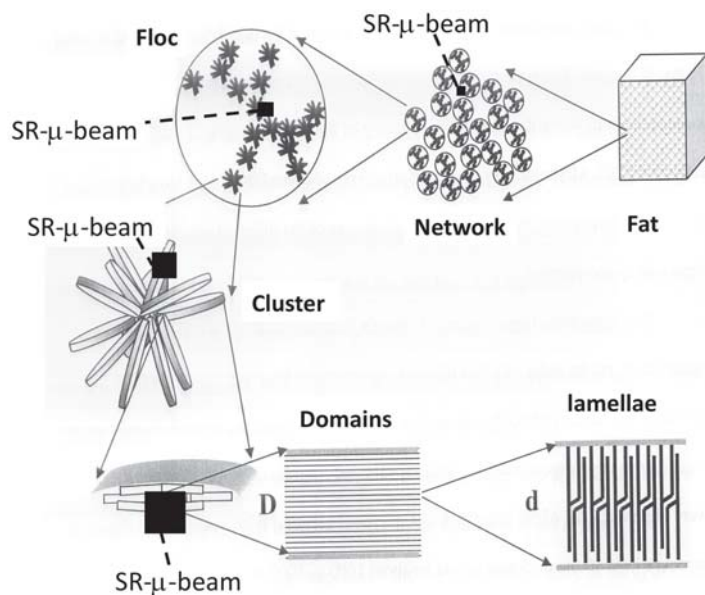


Fig. 14.1. Spatial analysis of fat structures: the capability of SR- μ -XRD analysis. Partly alteration of Fig. 7 in the reference. Source: Acevedo & Marangoni, 2010.

(a)

- ◆ Optics
- ◆ Targeting of μ -beam
- ◆ Fixation of samples
- ◆ Sample preparation
- ◆ Avoiding target-overlapping along μ -beam
- ◆ Irradiation damages
- ◆ Thermal damages
- ◆ Reaction damages

(b)

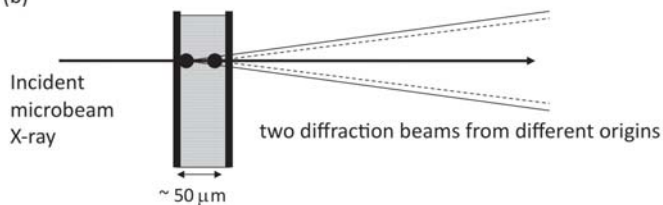


Fig. 14.2. (a) Difficulties of μ -XRD experiment, (b) Schematic explanation for overlapping a few or more diffractions from different origins.

XRD measurement always requires considerable optics. For the μ -XRD experiment, we need a two-dimensional (2D) detector (e.g., a CCD camera or an imaging plate) in order to measure the anisotropy of the sample structure. It is very difficult to aim the micrometer-size beam. We must know where the microbeam is in order to penetrate the right position in the sample, especially for scanning (or mapping) measurement, so the sample must be fixed. Even if the sample moves just $1\mu\text{m}$, the data must be revised from before the movement. Prepared samples must be thin for the diffraction beam to penetrate. Usually, an optical microscope is used for to check whether or not the microbeam is at the right position in the sample; thus, light from the light source penetrates the sample (Fig. 14.3), and the sample must be thin enough for light to penetrate. Avoiding target-overlapping along the microbeam is closely related to sample preparation. If the sample is thick, there will be diffractions from different positions of the same diffraction sources on the irradiation beam in the sample; thus, the diffraction peak will be broader because of the overlapping diffractions from different origins whose distances are on a micrometer level (Fig. 14.2b). In a real system, this overlapping diffraction peak is problematic for analysis if the limit of resolution is less than the full width at half maximum of the overlapping diffraction peak. Therefore, a thinner sample is better for analysis. However, a thicker sample is better for the experiment because it facilitates obtaining higher intensity of the diffraction peak. Irradiation damage, thermal damage, and reaction damage may occur after sample irradiation. The incident SR beam is usually concentrated by a K-B mirror and results in a condensed microbeam. When the microbeam penetrates the sample, the sample may be irradiated and damaged by the intense, condensed beam, and the sample temperature may increase. Reaction damage may occur after irradiation or a temperature increase of the sample.

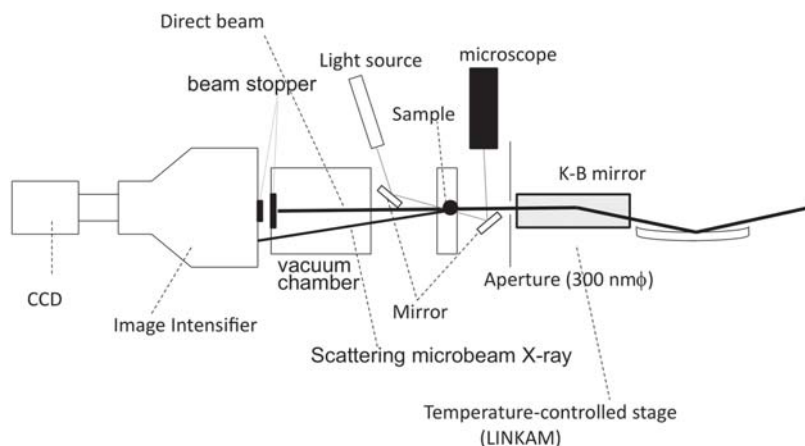


Fig. 14.3. Schematic diagram of SR- μ -XRD beam line of BL-4A, Photon Factory, KEK, Japan.

Fig. 14.3 presents a schematic diagram of the small-angle μ -XRD at BL-4A in the synchrotron radiation source, Photon Factory, KEK National Laboratory, Japan (Iida & Noma, 1993). The microbeam X-ray penetrates the sample. The microbeam position in the sample can be controlled by changing the sample position by an X (left-right) axis – Y (up-down) axis stepping motor. The position of the microbeam in the sample is always traced by optical microscopy. Scattered X-ray beams from the sample are introduced to a vacuum chamber and detected by a 2D image intensifier CCD camera system. According to this optics, only small-angle X-ray diffraction (SAXD) (i.e., a chain-length structure) is detected.

Data Analysis of μ -XRD

The μ -XRD experiment involves two typical data analyses: long-spacing analysis by diffraction angle (2θ) extension and azimuthal angle (χ) extension. Both analyses are usually applied to 2D data.

Long-spacing Analysis

The distances between all diffraction spots and the center are converted into diffraction angles (2θ) (i.e., 2θ extensions). All values are then calculated using long spacing for SAXD and short spacing for WAXD. Long spacing corresponds to the chain-length structure and short spacing corresponds to the subcell structure, so the polymorphic form can be determined. If only SAXD is measured, the polymorph can at least be deduced. Using 2D detectors, the orientation of the fat crystal structure is also determined by looking at anisotropy or isotropy diffraction patterns, especially in the small-angle region. If the diffraction data is anisotropic with two arc peaks, the fat crystals are well-oriented (Fig. 14.4a). In contrast, if the diffraction patterns are quite isotropic, the fat crystals are randomly oriented (Fig. 14.4b).

Scanning Analysis

With an extremely small microbeam (e.g., 5×5 mm square [Fig. 14.5a (Arima et al., 2009)]), a scanning study (see next paragraph) can be performed in order to determine the structure information of the polymorphism and the orientation at different local positions inside a sample. The diffraction intensity along azimuthal angles χ at the fixed 2θ angle is extended to compare the relative degree of ordering of lamellar planes of the fat crystals (Figs. 14.5b and 14.5c). In the analysis of the azimuthal angle extension, lamellar planes were better oriented when a narrower peak was observed.

The scanning study involved the following method. (i) The scanning sample position was determined for each several or several tens of micro-meters. (ii) The μ -XRD measurement was performed at each position for the same irradiation time. (iii) The data were sequenced one-by-one in order. We could determine the anisotropy or isotropy at each local position using this scanning study.

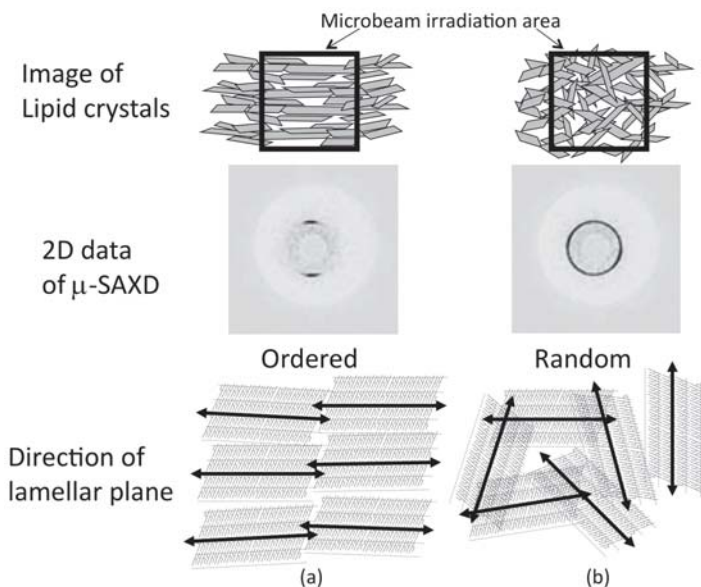
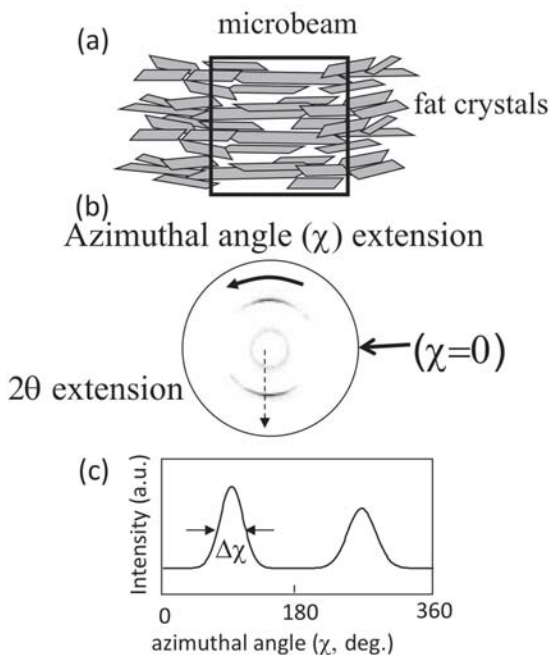


Fig. 14.4. μ-SAXD 2D diffraction patterns. (a) ordered, (b) random.

Fig. 14.5. Azimuthal angle extension. (a) Microbeam $5 \times 5 \mu\text{m}$ square, (b) Azimuthal angle (χ) and 2θ , (c) Azimuthal angle (χ) extension. Adapted from Arima et al. 2009, Fig. 1.



Examples of Microbeam XRD Experiments

Spherulite

Trilaurin

Microstructures and molecular arrangements during solid-state $\beta' \rightarrow \beta$ polymorphic transformation in 2D spherulites of trilaurin (LLL) grown within thin spaces between PET films were investigated (Ueno et al., 2008). The 2D spherulites of β' (Fig. 14.6a) were composed of nanometer-sized crystals. Fig. 14.7a presents the result of μ -XRD scanning measurement. A scanning study was performed for each 20 μm step. The center position exhibited a ring diffraction pattern, indicating that the nanometer-sized crystals around the center assembled with random orientation. However, in all the data on off-center positions, the lamellar planes were oriented parallel to the radial direction of the spherulites in the nanometer-sized crystals (Fig. 14.7b). Therefore, when liquid LLL crystallizes to β' form, crystallization should occur in the following three processes: (i) (nucleation) poly-nuclei with random orientation at the center of spherulite, (ii) (crystal growth) progressing along the direction of fatty acid chain packing (Fig. 14.8), and (iii) (lamellar plane) formation of the radial direction from the center position of the spherulite. For polymorphic transformation, it was verified that the orientations of the long-chain axes of the β form remained unchanged with respect to those of the β' form following solid-state transformation from β' to β . Fig. 14.9 presents the result of μ -XRD scanning measurement after transformation from β' form (Fig. 14.6a) to β form (Fig. 14.6b). All local diffraction data presented in Fig. 14.7a and Fig. 14.9 correspond to the same position. Both figures indicate the same kind of diffraction pattern, suggesting that the molecular arrangement of LLL during the $\beta' \rightarrow \beta$ polymorphic transformation occurred through template effects of the lamellar structures of the mother phase of β' .

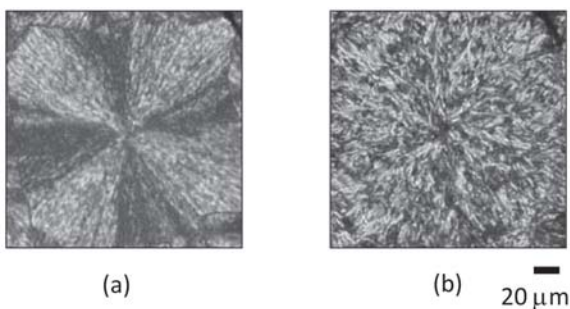


Fig. 14.6. Polarized microscopic images of LLL ; (a) the two-dimensional spherulite of trilaurin β' form, and (b) it's transformed β polymorph. Adapted from Ueno et al., 2008, Fig. 1.

Fig. 14.7. (a) μ -XRD scanning patterns of the spherulites of LLL β' form shown by a 20 μm step. The black bold type box means center position. Adapted from Ueno et al., 2008, Fig. 4(a).

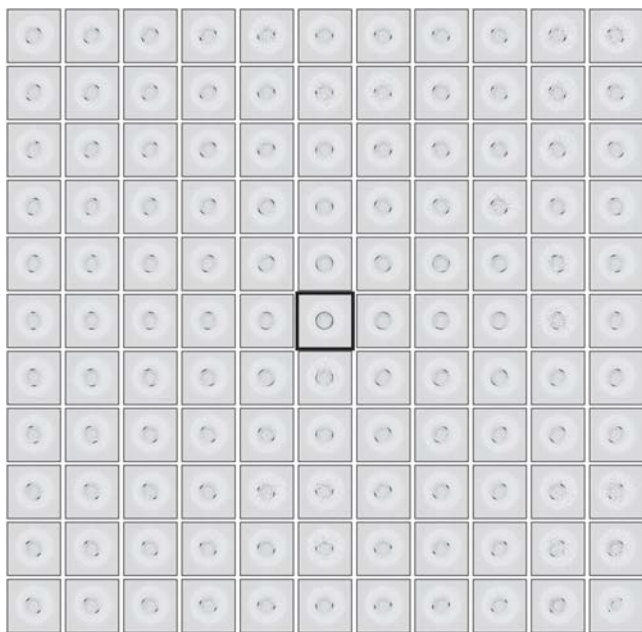
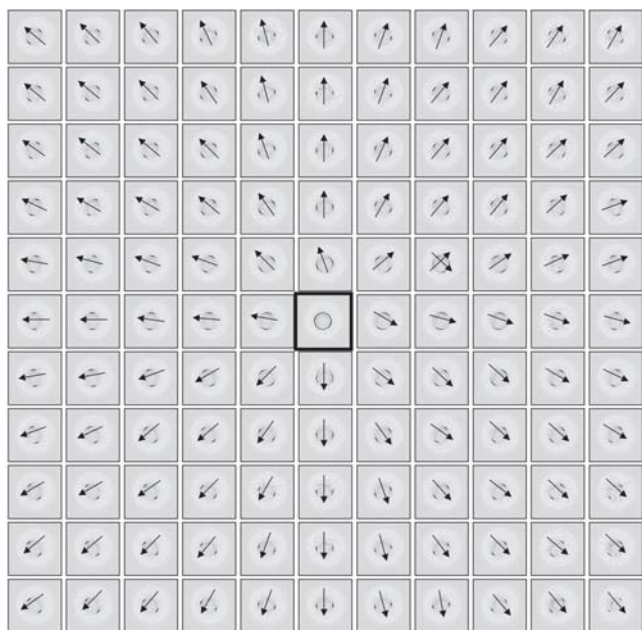


Fig. 14.7. (b) μ -XRD scanning patterns of the spherulites of LLL β' form with arrows in Fig. 14.7(a). The black bold type box means center position. Arrows mean the direction of lamellar planes.



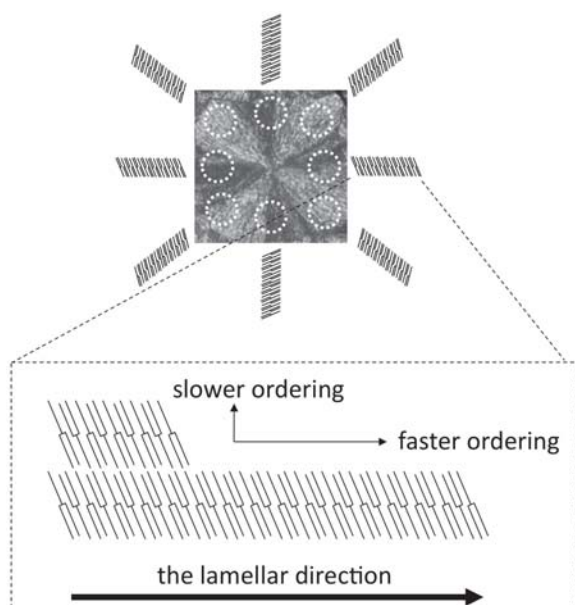


Fig. 14.8. Crystallization process of spherulite of trilaurine (LLL).

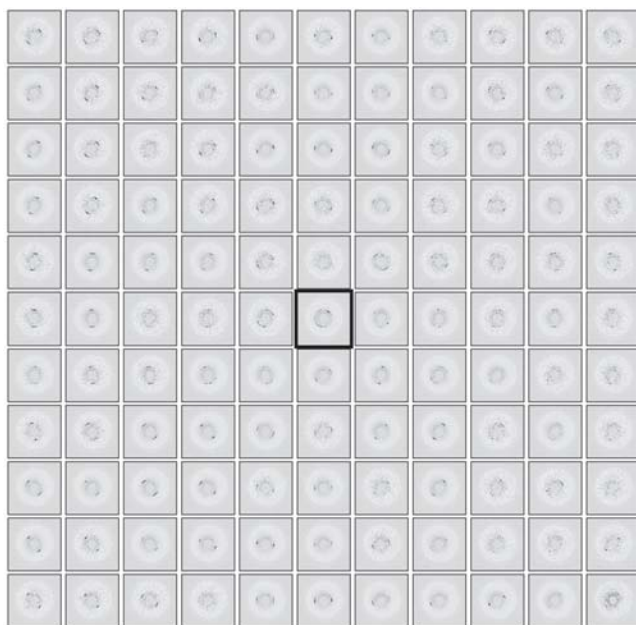


Fig. 14.9. μ -XRD scanning patterns of the spherulites of LLL β form shown by a 20 μ m step. The black bold type box means center position. Adapted from Ueno et al., 2008, Fig. 4(b).

POP

No μ -XRD measurement has been performed for cocoa butter. However, POP was measured by μ -XRD scanning (unpublished). Fig. 14.10 depicts the isothermal crystallization of the spherulite of 1,3-dipalmitoyl-2-oleoyl-sn-glycerol (POP) measured by polarized optical microscopy at 20°C after cooling from 60°C. The polymorph of this spherulite was β' form with a long spacing of 4.2 nm (Fig. 14.11). The spherulite filled the field of vision of the microscope 20 min after reaching 20°C. Fig. 14.11 presents the result of μ -XRD scanning measurement shown by a 10 μ m step. The same

60°C $\rightarrow$ 20°C (isothermal)

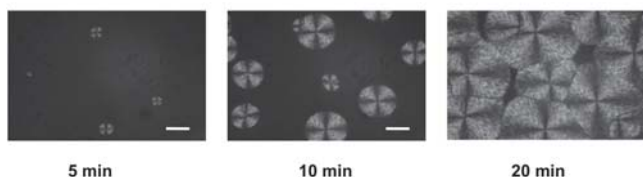


Fig. 14.10. Polarized optical microscope Images of crystallization process of spherulite of POP after cooling from 60 to 20°C.

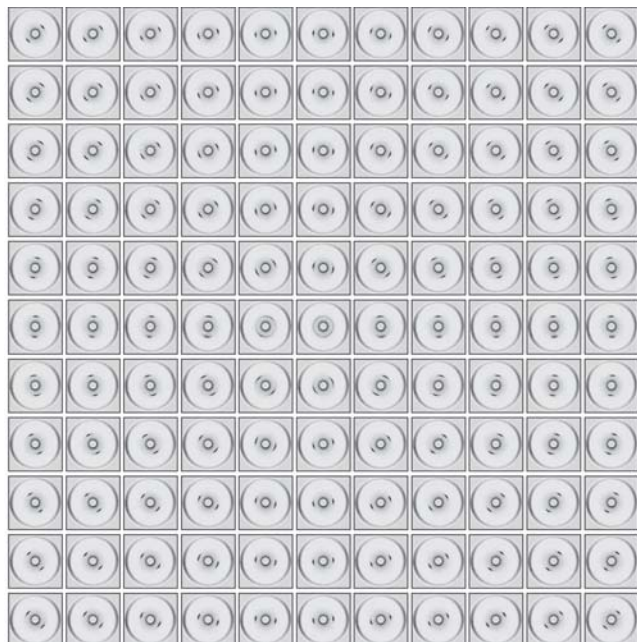


Fig. 14.11. Scanning μ -XRD diffraction patterns of spherulite of POP β' form shown by a 10 μ m step at 20°C after quenching from 60°.

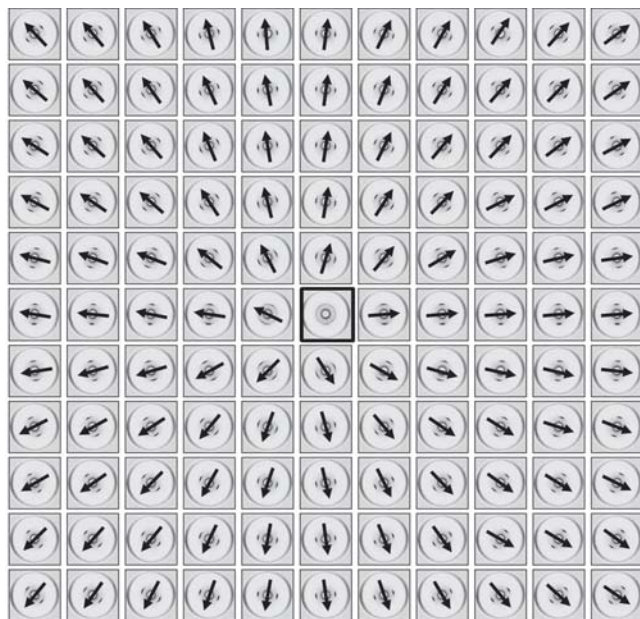


Fig. 14.12. μ -XRD scanning patterns of the spherulites of POP β' form with arrows shown by a 10 μm step. The black bold type box means center position. Arrows mean the direction of lamellar planes.

kind of arc patterns appeared in all XRD data at local positions, except for the center of the spherulite. After drawing arrows to indicate the direction of the lamellar plane in each local area in Fig. 14.12, the same arrow pattern as in Fig. 14.7b appeared. Therefore, the spherulite of POP β' crystallized along the lamellar dimension from liquid and resulted in the plate-like structure (Fig. 14.8).

Binary Mixtures of POP/OPO

The microstructure of spherulite made of two TAGs (POP and 1,3-dioleoyl-2-palmitoyl-sn-glycerol (OPO)) was studied recently (Bayes-Garcia et al., 2011). According to this previous study, POP and OPO make a molecular compound (MC) formation at an equal ratio grown from their binary mixture of neat liquid (Minato et al., 1997) and even in *n*-dodecane solution of 50% and 20% concentration (Ikeda et al., 2010). What is the microstructure when an MC and a pure component exist at the same time? At a concentration ratio of POP:OPO = 75:25, POP and MC are formed at an equal ratio. In turn, at a concentration ratio of POP:OPO = 25:75, MC and OPO are formed at an equal ratio. The molecular structures of POP, OPO, and MC are quite different, as seen in the triple-chain-length structures of POP and OPO, and the double-chain-length

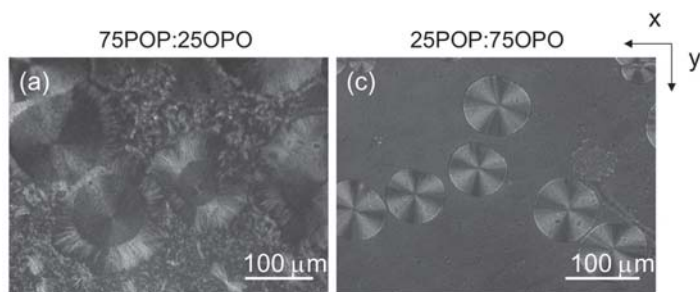


Fig. 14.13. Polarized microscopic images of spherulites of POP:OPO binary mixtures crystallized from neat liquid. (a) 75POP:25OPO grown at 16°C, (b) 25POP:75OPO grown at 6°C. X and Y directions in the spherulites are shown. Adapted from Bayes-Garcia et al., 2011, Fig. 2.

structure of MC. Thus, microstructure measurement of the spherulite of the binary mixtures of POP and OPO may provide information about the crystallization of MC, POP, and OPO by using μ -XRD scanning measurement.

Using the results of μ -XRD scanning measurement, POP:OPO = 75:25 and POP:OPO = 25:75 were examined in a neat liquid and with 50% *n*-dodecane solution. At POP:OPO = 75:25 crystallized from neat liquid, a double-chain-length MC β form (β_{MC} -2) was located in the inner part of a spherulite, and a triple-chain-length POP β form (β_{POP} -3) appeared near the outer part. However, at POP:OPO = 25:75 crystallized from neat liquid, a triple-chain-length OPO β form (β_{OPO} -3) predominated over β_{MC} -2 at the crystallization temperatures examined. These heterogeneous distributions were due to the different crystallization rates of MC, and component POP and OPO crystals. These results were confirmed by polarized optical microscopy. The morphology of the spherulites of POP:OPO = 75:25 grown from 50% *n*-dodecane and POP:OPO = 25:75 grown from neat liquid and 50% *n*-dodecane was homogeneous, whereas that of POP:OPO = 75:25 grown from neat liquid was heterogeneous (Fig. 14.13).

For clarification, Figs. 14.14–14.16 present one result. Fig. 14.14 presents the μ -XRD scanning patterns of the spherulites of POP/OPO = 75/25 grown from neat liquid. Scanning measurement was performed at a distance of 5 μ m. Fig. 14.15 presents the precise analysis of positions 1, 2, and 3 drawn in the previous figure. For position 1 (Fig. 14.15a), a single diffraction peak at $2\theta = 2.0^\circ$, which corresponds to the 002 reflection of 6.4 nm, appeared (i.e., the long spacing of the triple-chain-length POP β form (β_{POP} -3)). The broken line drawn in the XRD data at position 1 (Fig. 14.15a), was aligned along the arc pattern (i.e., the lamellar plane of fat crystals). In position 2 (Fig. 14.15b), three diffraction peaks at $2\theta = 1.3^\circ$, 2.0° , and 2.7° appeared. Peaks at $2\theta = 1.3^\circ$ and 2.7° correspond to the 001 and 002 reflections of the 4.6 nm of the double-chain-length MC β form (β_{MC} -2). The diffraction peak

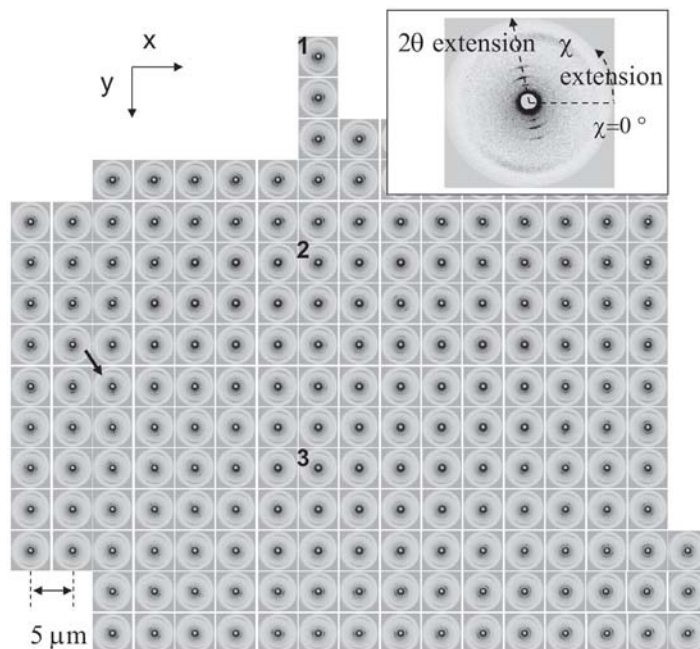


Fig. 14.14. Scanning SR- μ -XRD patterns taken at the all positions of a spherulite of the mixture of 75POP:25OPO grown from neat liquid, and enlarged 2D pattern of the position noted by an arrow (inserted). Adapted from Bayes-Garcia et al., 2011, Fig. 3.

at $2\theta = 2.0^\circ$ corresponds to the 002 reflection of the 6.4nm of $\beta_{\text{POP}}-3$. For position 3 (Fig. 14.15c), a ring diffraction pattern in the 2D data and two diffraction peaks ($2\theta = 1.3^\circ$ and 2.7°) in the 2θ extension appeared. Therefore, only $\beta_{\text{MC}}-2$ crystals existed, and they had a randomly oriented lamellar arrangement. The directions of the lamellar planes of all local XRD data in Fig. 14.14 are indicated in Fig. 14.16. According to Fig. 14.16, random orientation of the lamellar planes as depicted in position 3 in Fig. 14.15c appeared in the center area of the spherulite. However, the crystals directed parallel to the radial direction appeared in the peripheral region in Fig. 14.16.

Oil-in-Water Emulsion

Previous studies reviewed by Sato (Larsson et al., 2006) suggested that crystallization in the oil droplet of oil-in-water (O/W) emulsion might occur both from liquid oil in the droplet and on the oil-water interface in the droplet. In particular, if a hydrophobic emulsifier whose melting point is somewhat higher than that in the oil phase is placed in the oil droplet as an additive, surface heterogeneous crystallization should

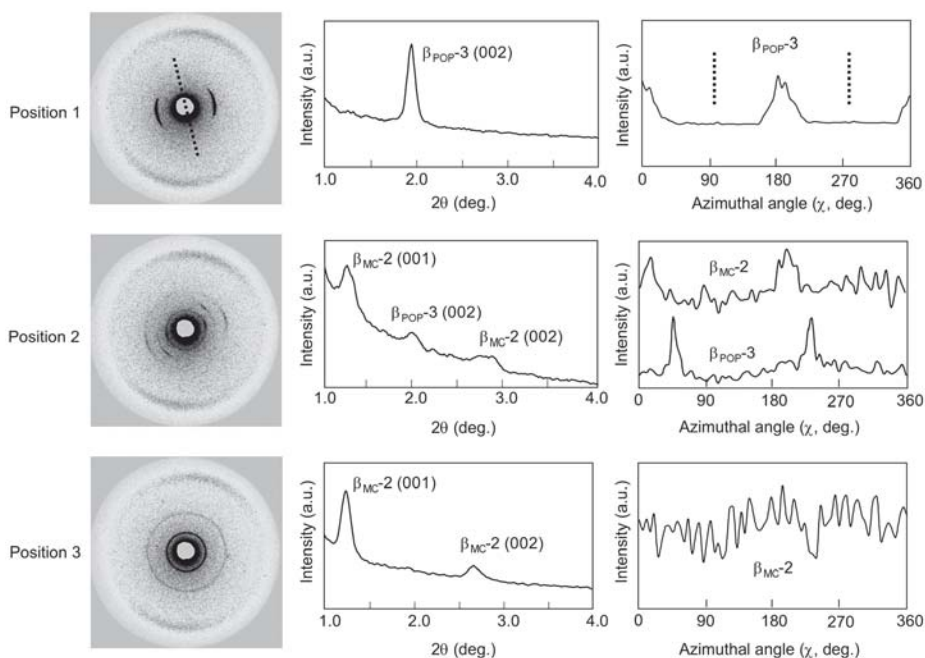
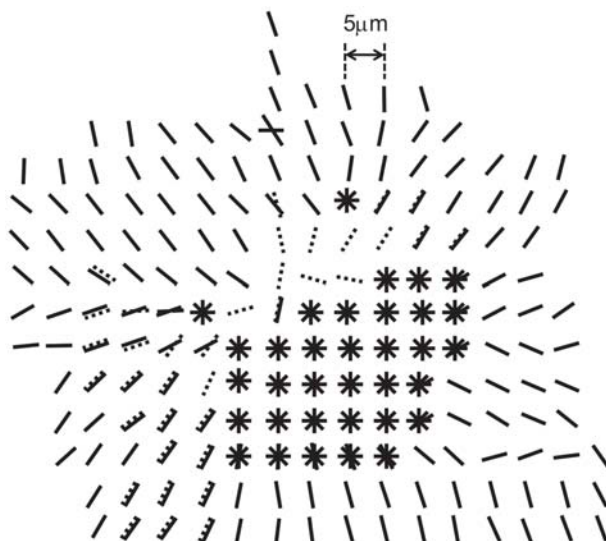


Fig. 14.15. Enlarged patterns taken at three positions noted by 1, 2 and 3 in Fig. 14.14. 2θ diffractions patterns (left), 2θ extension (middle) and χ extension (right). Adapted from Bayes-Garcia et al., 2011, Fig. 4.

Fig. 14.16. Lamellar plane directions of the crystals at the all positions examined of the spherulite of 75POP:25OPO shown in Fig. 14. Solid and dotted lines correspond to $\beta_{\text{POP-3}}$ and $\beta_{\text{MC-2}}$, respectively. Asterisk means random orientation of the lamellar plane. Adapted from Bayes-Garcia et al., 2011, Fig. 5.



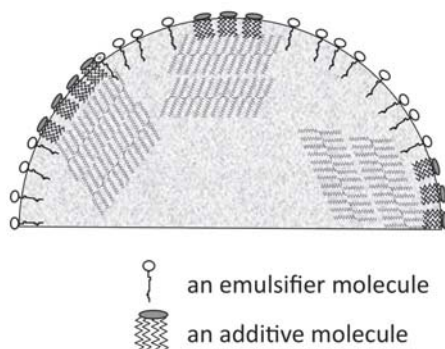


Fig. 14.17. Model of interfacial heterogeneous nucleation of fat crystals in an emulsion droplets assisted by additives. Adapted from Arima et al. 2009, Fig. 8.

occur on the oil-water interface of the oil droplet (Fig. 14.17). The additive must be on the surface of the oil droplet and crystallize in the cooling temperature of the O/W emulsion. After the additive crystallizes, it should work as a template for crystallization of an oil droplet. Therefore, crystallization of an oil droplet in O/W emulsion should occur on the oil-water interface. This phenomenon has been observed by polarized optical microscopy. In addition, μ -XRD measurement has been employed to observe crystallization on the oil-water interface of an oil droplet. Here, two examples of μ -XRD studies are introduced: (i) crystallization of *n*-alkane-in-water emulsion (Shinohara et al., 2008) and (ii) crystallization of palm mid fraction (PMF)-in-water emulsion (Arima et al., 2009).

n-Alkane-in-Water Emulsion

Fig. 14.18 depicts the μ -XRD patterns of three positions of a crystallized 50 μm droplet in *n*-hexadecane-in-water emulsion that was prepared using polyoxyethylene sorbitan mono-laurate (Tween 20) as an emulsifier (Shinohara et al., 2008).

While no additive was used in this system, evidence of surface heterogeneous crystallization was observed. XRD data taken at the upper side, center, and lateral side of the droplet were measured at the shorter camera length, at a distance between the sample and the detector (20cm), to detect SAXD and WAXD simultaneously. The black circle in the figure corresponds to the diffraction pattern from mylar film used as a window of the sample. Diffraction inside the circle corresponds to SAXD, and that outside the circle corresponds to WAXD. In Fig. 14.18b, irradiated at the center of an oil droplet, diffraction peaks corresponding to SAXD and WAXD appear with no orientation (an asterisk in Fig.14.8b). However, in Fig. 14.18a, irradiated at the upper side oil-water interface of the droplet, the 001, 002, and 003 reflections corresponding to the lamellar distance are distributed along the meridional direction of

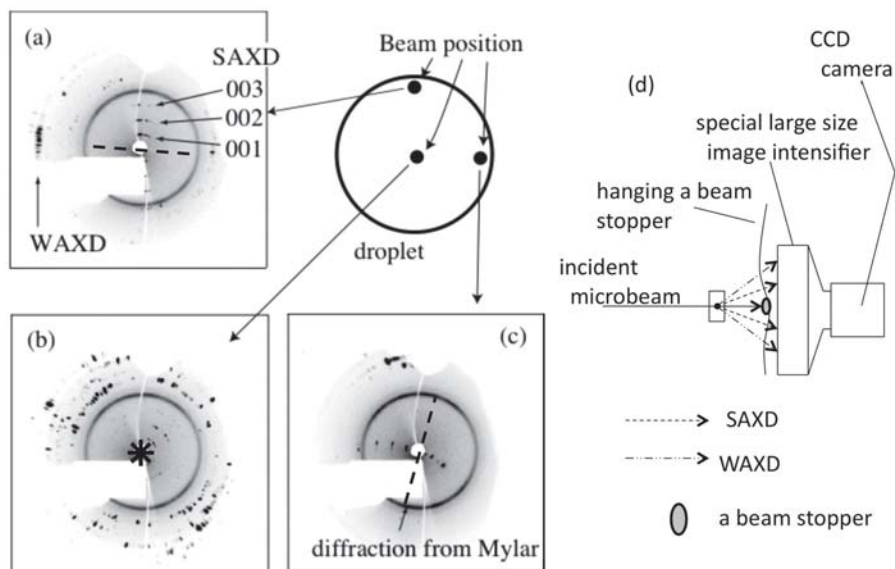


Fig. 14.18. μ -XRD patterns of three positions of a crystallized droplet in *n*-hexadecane-in-water emulsion measured by a special experimental system drawn in (d). (a) upper side interface, (b) center, (c) lateral side interface, in a droplet. (d) schematic diagram of this experiment. Adapted from Shinohara et al., 2009, Fig. 2.

the figure (a broken line in the Fig. 14.18a, and WAXD patterns corresponding to the subcell structure are distributed along the equatorward direction. In addition, the distribution directions of the SAXD (a broken line in Fig. 14.18c and WAXD taken from the lateral side interface in Fig. 14.18c are rotated almost 90° from those in Fig. 14.18a. These results indicate that the crystallized *n*-hexadecane molecules align their molecular axes normal to the interface, along which the long chains of the hydrophobic moiety of the surfactant are arranged, because the SAXD peak is observed only when the angle between the long axis and incident X-rays becomes nearly perpendicular.

Palm Mid Fraction-in-Water Emulsion

The μ -SAXD experiment has been examined in order to observe the crystallization of PMF-in-water emulsion (Arima et al., 2009). Two samples were employed: (i) hydrophilic polyoxyethylene sorbitan mono-oleate (Tween 80) alone used as an emulsifier (Tween 80 emulsion), and (ii) Tween 80 for the emulsifier and hydrophobic sucrose palmitic acid oligoester (P-170) for the additive (Tween 80+P-170 emulsion). The μ -SAXD study had the following three results. (i) The lamella planes of PMF crys-

tals near the oil-water interface of the droplet in the Tween 80+P-170 emulsion were mostly parallel to the interface, whereas the lamella planes of PMF crystals were not always aligned with the oil-water interface in the Tween 80 emulsion droplet. (ii) The degree of orientation of the lamellar planes of PMF crystals, which was evaluated from the full width at half maximum of 2D μ -SAXD patterns with respect to the azimuthal angle extension, was remarkably higher in the Tween 80+P-170 emulsion than in the Tween 80 emulsion. (iii) Polymorphic transformation of PMF from α to β' in the Tween 80+P-170 emulsion was retarded, compared to that in the Tween 80 emulsion.

As an example, Fig. 14.19 presents the results of μ -SAXD scanning measurement for a droplet in Tween80 (Fig. 14.19a) and Tween 80+P-170 (Fig. 14.19b) emulsions. The scanning step is 5 μm . The diameter of the droplet is 45 μm . The oil-water interface is denoted as a dotted line. Comparison of the result presented in Fig. 14.19b with that presented in Fig. 14.19a suggests that the ratio of the local XRD data indicating sharp arc peaks increased. This tendency is more clearly observed in the local XRD data near the oil-water interface. Therefore, we compare the azimuthal angle (χ) extension patterns of the 2D μ -SAXD data acquired at the positions that are rotated each 45° and noted as positions 1 to 8 in Figs. 14.19a and b. Fig. 14.20 depicts the χ extension patterns for positions 1 to 8 in Fig. 14.19. Fig. 14.20a indicates the χ extensions of these eight positions for a droplet in Tween80. The relationships among the χ values at the diffraction peaks and those of neighbor positions in the figure are irregular; in other words, the diffraction peaks appeared in an irregular manner. In Fig. 14.20b, however, the relationships among the χ values at the diffraction peaks and those of neighboring positions in the figure are in order. If a diffraction peak appeared at any χ value, the corresponding diffraction peak appeared at $\chi \pm 45^\circ$ in the next position. If this diffraction peak continued for several positions, the χ value shifted every 45°. The diffraction peak (arrows in Fig. 14.20b) shifted continuously. This is evidence that surface heterogeneous nucleation occurred at the oil-water interface. We obtained this evidence using μ -SAXD measurement.

Water-in-Oil Emulsion

Hydrogenated fats are high-melting fat materials including *trans*-fatty acid moiety in their molecules, which has been reported to decrease HDL cholesterol, increase LDL cholesterol (Mensink & Katan, 1990; Judd et al., 1994), and result in a high risk of arterial sclerosis (Tanaka et al., 2009). Therefore, *trans*-fat alternatives have been studied, and palm oil is one of the most appropriate choices. However, when palm oil was added in the production of margarine or fat spread, granular crystals often appeared in the products during or after production, degrading the quality of the products (Smith, 2001; Miskandar et al., 2002). Many studies have been conducted to clarify the formation mechanism of granular crystals in palm-oil-based margarine (Chong et al., 2007; Che Man et al., 1999; Ishikawa, 1980; Watanabe et al., 1992; Miura & Konishi, 2001; Tanaka et al., 2007; Garbolino et al., 2005).

Fig. 14.19. (a) μ -SAXD patterns of PMF crystals taken at different positions in an emulsion droplet emulsified with Tween 80. A dotted line indicates an oil-water interface of the droplet. Adapted from Arima et al., 2009, Fig. 3.

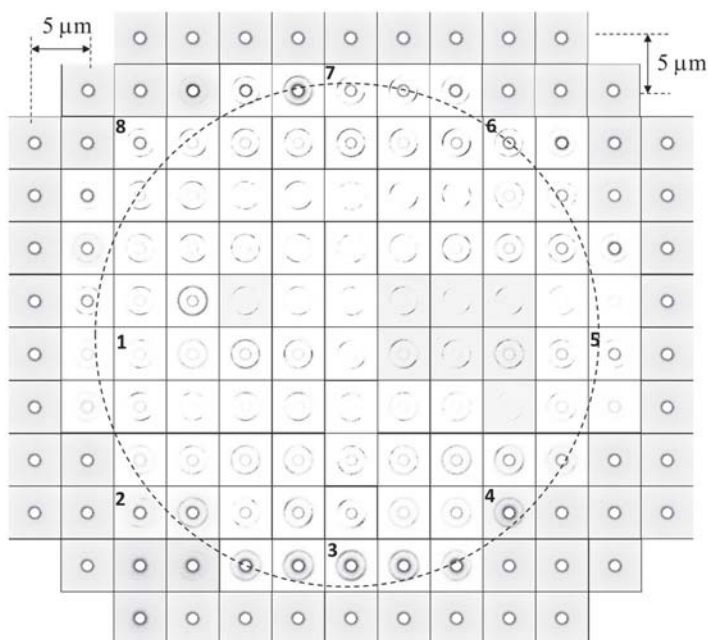
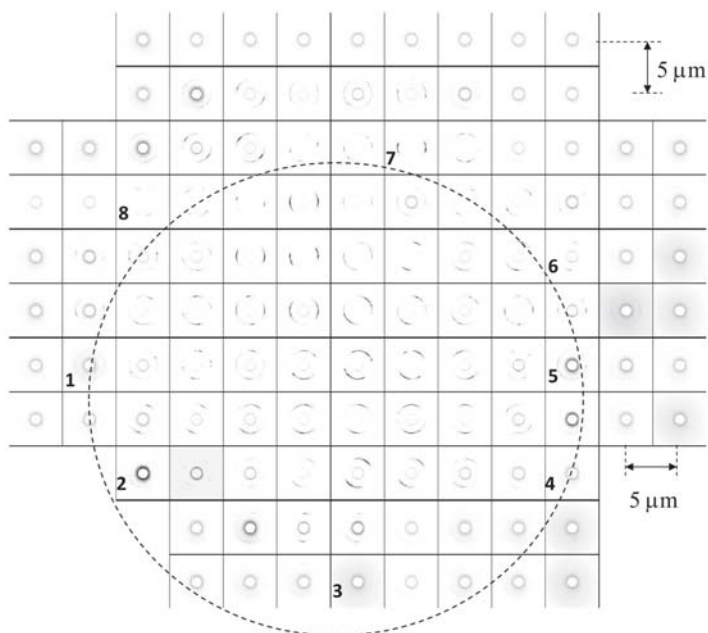


Fig. 14.19. (b) μ -SAXD patterns of PMF crystals taken at different positions in a Tween 80+P-170 emulsion droplet. A dotted line indicates an oil-water interface of the droplet. Adapted from Arima et al., 2009, Fig. 5.



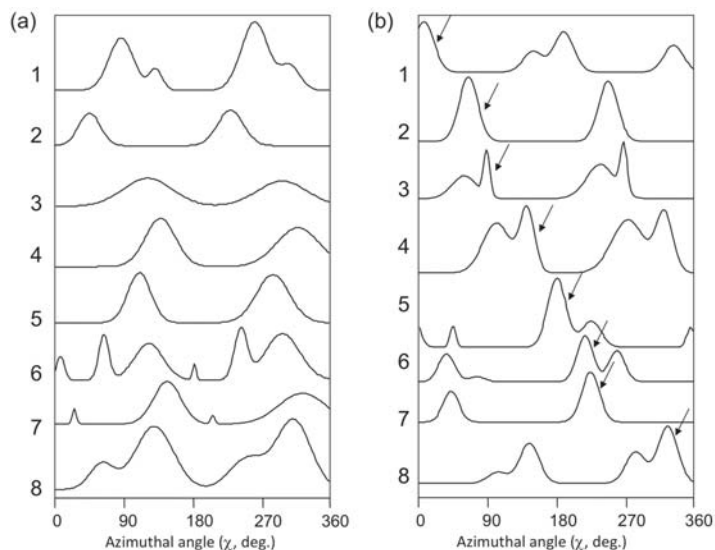


Fig. 14.20. Azimuthal angle extension of μ -SAXD patterns of PMF crystals in emulsion droplets. (a) eight positions of Tween 80 emulsion droplet shown in Fig. 19(a), (b) eight positions of Tween 80+P-170 emulsion droplet shown in Fig. 14.19(b). Adapted from Arima et al., 2009, Fig. 6.

We used DSC, XRD, SEM, polarized optical microscopy, and μ -XRD to clarify the structure and growth mechanism of granular crystals. Here, we present the μ -XRD results of the structure of a granular crystal produced from fat spread. Fig. 14.21 depicts the sample preparation for the μ -XRD scanning measurement. A 2 mm-diameter granular crystal was removed from the fat spread which was keeping at 7°C and 15°C alternately changed condition for more than 1 year. After the granular crystal was removed, the central part was sliced out (Fig. 14.21) and put in a sample cell. The cell was set in the temperature control holder (LINKAM) and maintained at 5°C. Fig. 14.22 presents the μ -SAXD scanning data for the sliced sample of a granular crystal. The scanning distance was 100 μ m. Three different XRD patterns appeared, representing three regions in the granular crystal: the exterior region (region I), which is outside the solid line; the peripheral region (region II), which is between the broken line and the solid line; and the core region (region III), which is inside the broken line. Region I is actually normal fat spread around the granular crystal. To investigate regions II and III in more detail, the typical μ -SAXD patterns at positions 1 and 2 were chosen for both regions and analyzed. Fig. 14.23a presents typical examples in position 1, and Fig. 14.23b presents typical examples in position 2. In position 1, two strong diffraction peaks appeared at 6.3 and 3.1 nm, corresponding to the first and second diffraction peaks of a

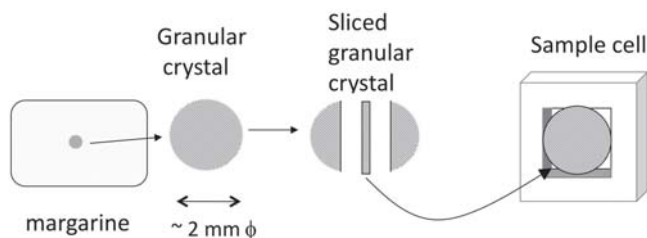


Fig. 14.21. Schematic diagram of sample preparation for the μ -XRD scanning measurement. Adapted from Tanaka et al. 2009, Fig. 1.

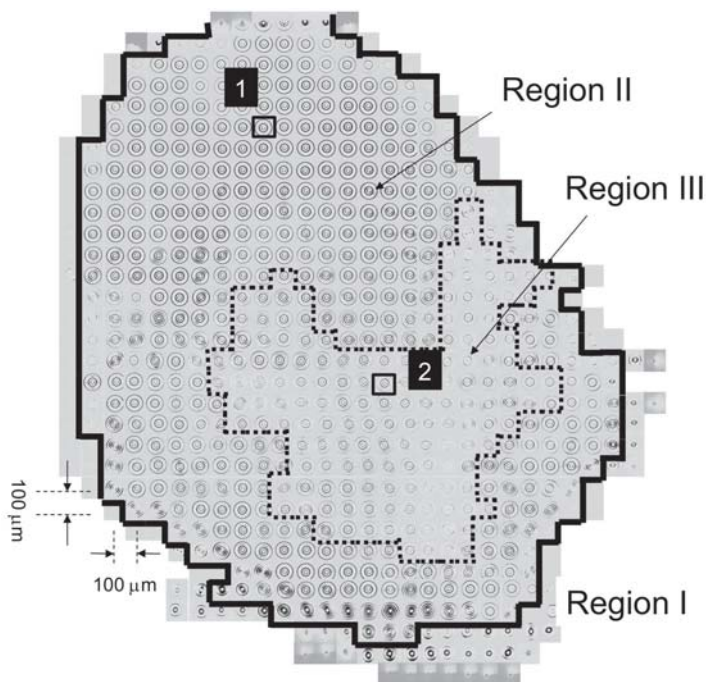


Fig. 14.22. μ -SAXD patterns of the sliced sample of a granular crystal. The scanning distance was 100 μm . Three regions, I – III, presented in the granular crystal. Adapted from Tanaka et al. 2009, Fig. 7a.

triple-chain-length structure. A very weak diffraction peak appeared at 4.2 nm, corresponding to a double-chain-length structure. A triple-chain-length structure is dominant in position 1. In contrast, for position 2, three weak peaks appeared at 6.2, 4.2, and 3.1 nm. They correspond to the first diffraction peak at 6.2 nm and the second diffraction peak at 3.1 nm for a triple-chain-length structure, and the

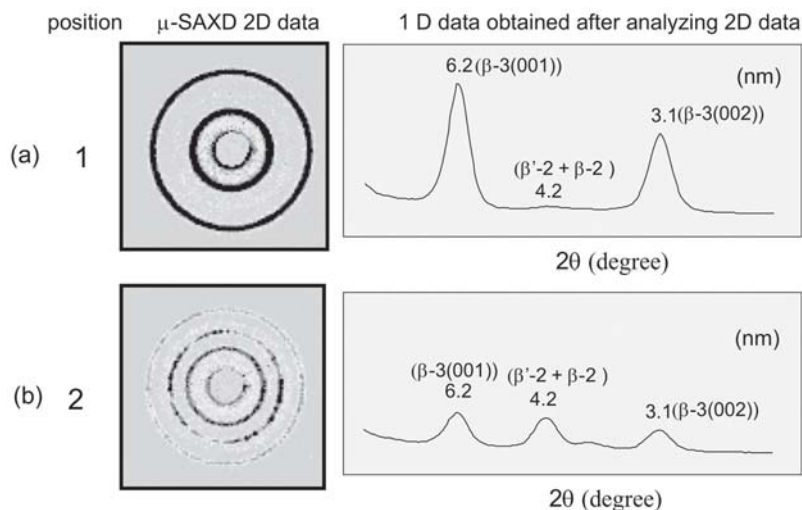


Fig. 14.23. Magnified XRD patterns and those 2 θ extensions in position 1 and 2 shown in Fig. 14.22. (a) for position 1 and (b) for position 2. Adapted from Tanaka et al. 2009, Fig. 7b.

first diffraction peak at 4.2 nm for a double-chain-length structure. Considering the composition of the high-melting fats of the sample, POP β form is a unique composition exhibiting a triple-chain-length structure. Therefore, the triple-chain-length structure with the first diffraction peak at 6.2 nm and the second diffraction peak at 3.1 nm is the β form of POP ($\beta_{\text{POP-3}}$). Thus, the composition of region II, including position 1, is dominated by $\beta_{\text{POP-3}}$. In the core region (region III), double- and triple-chain-length structures are mixed. For double chain length structures, β and β' of tripalmitin (PPP), tristearine (SSS) and the molecular compound of POP and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol (PPO), and β' of POP are origins (i.e., $\beta'-2$ and $\beta-2$ ($\beta_{\text{PPP-2}}$, $\beta'_{\text{PPP-2}}$, $\beta_{\text{SSS-2}}$, $\beta'_{\text{SSS-2}}$, $\beta_{\text{POP+PPO-2}}$, $\beta'_{\text{POP+PPO-2}}$, $\beta'_{\text{POP-2}}$). $\beta_{\text{POP-3}}$ should be present in region III.

In conclusion, the structure of a granular crystal consists of two layers, the core area and the peripheral area. The core area contains mixed double- and triple-chain-length structures, and the peripheral region is dominated by $\beta_{\text{POP-3}}$.

Conclusions

I have described the synchrotron radiation μ -XRD method and its application to the study of spherulite O/W and W/O emulsion. μ -XRD is a powerful method for clarifying the microstructure and mesostructure of fats (Fig. 14.1). No μ -XRD experiment has been performed to study chocolate and cocoa butter. However, this method

may be useful for studying oil migration and the blooming phenomenon. Ganache chocolate (O/W emulsion chocolate) should be a good sample for μ -XRD. The use of μ -XRD for chocolate or cocoa butter may be reported in the near future.

Acknowledgments

I thank Emeritus Professor K. Sato (Hiroshima University) for encouraging me in the writing this chapter and for providing unpublished data of POP study. I gratefully acknowledge the help of Professor A. Iida, the station manager of BL-4A at the Photon Factory, KEK National Institute, Japan. I also appreciate the experiment and analysis support provided by Dr. Y. Shinohara and Professor Y. Amemiya (University of Tokyo).

The μ -XRD studies described in this chapter have been supported by the Ministry of Education, Culture, Sports, Science, and Technology (Grant-in-Aid, 20540397) of Japan and the Food Nanotechnology Project, the Ministry of Agriculture, Forestry and Fisheries of Japan. The experiments were performed with the approval of the Photon Factory Program Advisory Committee (Proposal No. 2006G303, 2008G201, 2010G114, and 2010G115).

References

- Acevedo N.C.; Marangoni A.G. Characterization of the Nanoscale in Triacylglycerol Crystal Networks. *Cryst. Growth Des.* 2010, 10, 3327–3333.
- Arima, S.; Ueno, S.; Ogawa, A.; Sato, K. Scanning Microbeam Small-Angle X-ray Diffraction Study of Interfacial Heterogeneous Crystallization of Fat Crystals in Oil-in-Water Emulsion Droplets. *Langmuir*, 2009, 25 (17), 9777–9784.
- Bayes-Garcia, L.; Calvet, T.; Cuevas-Diarte, M.A.; Ueno, S.; Sato, K. Heterogeneous Microstructures of Spherulites of Lipid Mixtures Characterized with Synchrotron Radiation Microbeam X-ray Diffraction. *Cryst. Eng. Comm.* 2011, 13, in press.
- Buleon, A.; Pontoire, B.; Riekkel, C.; Chanzy, H.; Helbert, W.; Vuong, R. Crystalline Ultrastructure of Starch Granules Revealed by Synchrotron Radiation Microdiffraction Mapping Macromolecules 1997, 30, 3952–3954.
- Chanzy, H.; Putaux, J. L.; Dupeyre, D.; Davies, R.; Burghammer, M.; Montanari, S.; Riekkel, C. Morphological and structural aspects of the giant starch granules from *Phajus grandifolius*. *J. Struct. Biol.* 2006, 154, 100–110.
- Che Man, Y.B.; Haryati, T.; Ghazali, H.M.; Asbi, B.A. Composition and thermal profile of crude palm oil and its products. *J. Am. Oil Chem. Soc.* 1999, 76, 237–242.
- Chong, C.L.; Kamarudin, Z.; Lusieur, P.; Marangoni, A.; Bourgoaux, C.; Ollivon, M. Thermal and structural behaviour of crude palm oil: crystallisation at very slow cooling rate. *Eur. J. Lipid Sci. Technol.* 2007, 109, 410–421.
- Drakopoulos, M.; Sergienko, P.M.; Snigireva, I.; Snigirev, A.; Vazina, A.A. Application of microbeam technique for the small-angle diffraction studies of a living tissue. *Nucl. Instrum. Meth. A*, 2005, 543, 161–165.

- Fratzl, P.; Weinkamer, R. Nature's hierarchical materials. *Prog. Mater. Sci.*, 2007, 52, 1263–1334.
- Garbolino, C.; Bartoccini, M.; Floter, E. The influence of emulsifiers on the crystallization behavior of a palm oil-based blend. *Eur. J. Lipid Sci. Technol.* 2005, 107, 616–626.
- Iida, A.; Noma, T. Beam Interactions with Materials and Atoms, *Nucl. Instrum. Methods Phys. Res. B* 1993, 82, 129–138.
- Ikedo, E.; Ueno, S.; Miyamoto, R.; Sato, K. Phase Behavior of a Binary Mixture of 1,3-Dipalmitoyl-2-oleoyl-sn-glycerol and 1,3-Dioleoyl-2-palmitoyl-sn-glycerol in n-Dodecane Solution. *J. Phys. Chem. B* 2010, 114, 10961–10969.
- Ishikawa, H.; Mizuguchi, T.; Kondo, S. Studies on granular crystals growing in palm oil (in Japanese). *J. Jpn. Oil Chem. Soc.* 1980, 29, 235–242.
- Judd, J.T.; Clevidence, B.A.; Muesing, R.A.; Wittes, J.; Sunkin, M.E.; Podczasy, J.J. Dietary trans fatty acid: effect of plasma lipids and lipoproteins of healthy men and women. *Am. J. Clin. Nutr.* 1994, 59, 861–868.
- Kajioka, H.; Yoshimoto, S.; Gosh, R.C.; Taguchi, K.; Tanaka S.: A. Toda. Microbeam X-ray diffraction of non-banded polymer spherulites of it-polystyrene and it-poly(butene-1). *Polymer* 2010, 51, 1837–1844.
- Kajiura, Y.; Watanabe, S.; Itou, T.; Nakamura, K.; Iida, A.; Inoue, K.; Yagi, N.; Shinohara Y.; Amemiya, Y. Structural analysis of human hair single fibres by scanning microbeam SAXS, *J. Struct. Biol.*, 2006, 155, 438–444.
- Kaneko, F. Polymorphism and Phase Transitions of Fatty Acids and Acylglycerols. *Crystallization Processes in Fats and Lipid Systems*, Gardi N., Sato K., Eds.; Marcel Dekker Inc.: New York, 2001; pp.53–97.
- Kikuzuki, T.; Shinohara, Y.; Nozue, Y.; Kazuki I.; Amemiya, Y. Determination of lamellar twisting manner in a banded spherulite with scanning microbeam X-ray scattering. *Polymer* 2010, 51, 1632–1638.
- Kobayashi, M. Vibrational Spectroscopic Aspects of Polymorphism and Phase Transition of Fats and Fatty Acids, *Crystallization and Polymorphism of Fats and Fatty Acids*, Gardi, N. and Sato, K. Eds; Surfactant Science Series Volume 31; Marcel Dekker INC.: New York, 1988; pp. 139–187.
- Kuckzumow, A.; Chevallier, P.; Dillmann, P.; Wajnberg P.; Rudas, M. Investigation of petrified wood by synchrotron X-ray fluorescence and diffraction methods, *Spectrochim. Acta B*, 2000, 55, 1623–1633.
- Larsson, K; Quinn, P; Sato, K; Tiberg, F. Solid-state behavior of polymorphic fats and fatty acids. *Lipids: Structure, Physical Properties and Functionality*. 1st Edition; The Oily Press Lipid Library; The Oily Press: Bridgewater, 2006; Vol.19, pp 9–71.
- Lemke, H.; Burghammer, M.; Flot, D.; Rosse, M.; Riekel, C. Structural Processes during Starch Granule Hydration by Synchrotron Radiation Microdiffraction. *Biomacromolecules* 2004, 5, 1316–1324.
- Mensink, R.B.; Katan, M.B. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N. Eng. J. Med.* 1990, 323, 439–445.
- Minato A.; Ueno S.; Smith K.; Amemiya Y.; Sato K. Thermodynamic and Kinetic Study on Phase Behavior of Binary Mixtures of POP and PPO Foming Molecular Compound Systems. *J. Phys. Chem. B* 1997, 101, 3498–3505.

- Miskandar, M.S.; Che Man, Y.B.; Yusoff, M.S.A.; Rahman, R.A. Effect of scraped-surface tube cooler temperatures on the physical properties of palm oil margarine. *J. Am. Oil Chem. Soc.* 2002, 79, 931–936.
- Miura, S.; Konishi, H. Crystallization behavior of 1, 3-dipalmitoyl-2-oleoil-glycerol and 1-palmitoyl-2, 3-dioleoil-glycerol. *Eur. J. Lipid Sci. Technol.* 2001, 103, 804–809.
- Müller, M.; Riekel, C.; Vuong, R.; Chanzy, H. Skin/core micro-structure in viscose rayon fibres analysed by X-ray microbeam and electron diffraction mapping. *Polymer*, 2000, 41, 2627–2632.
- Nakano, T.; Kaibara, K.; Tabata, Y.; Nagata, N.; Enomoto, S.; Marukawa K.; Umakoshi, Y. Unique alignment and texture of biological apatite crystallites in typical calcified tissues analyzed by microbeam x-ray diffractometer system. *Bone*, 2002, 31 (4), 479–487.
- Nozue, Y.; Kurita, R.; Hirano, S.; Kawasaki, N.; Ueno, S.; Iida, A.; Nishi T.; Amemiya, Y. Spatial distribution of lamella structure in PCL/PVB band spherulite investigated with microbeam small- and wide-angle X-ray scattering. *Polymer*, 2003, 44, 6397–6405.
- Nozue Y.; Hirano S.; Kurita R.; Kawasaki N.; Ueno S.; Iida A.; Nishi T.; Amemiya Y. Co-existing handednesses of lamella twisting in one spherulite observed with scanning microbeam wide-angle X-ray scattering. *Polymer*, 2004a, 45, 8299–8302.
- Nozue Y.; Hirano S.; Kawasaki N.; Ueno S.; Yagi N.; Nishi T.; Amemiya Y. Penetration of PBSU spherulite into PVDC-VC spherulite observed with microbeam- and macrobeam-SAXS/WAXS measurements, *Polymer*, 2004b, 45, 8593–8601.
- Paris, O.; Muller, M. Scanning X-ray microdiffraction of complex materials: Diffraction geometry considerations. *Nucl. Instrum. Meth. B*, 2003, 200, 390–396.
- Rossell, J.B. Phase Diagrams of Triacylglyceride Systems. *Adv. Lipid Res.* 1967, 5, 353–408.
- Riekel, C.; Vollrath, F. Spider silk fibre extrusion: combined wideand small-angle X-ray microdiffraction experiments. *Biol. Macromolec.* 2001, 29, 203–210.
- Riekel, C.; Davies, R.J. Applications of synchrotron radiation micro-focus techniques to the study of polymer and biopolymer fibers. *Curr. Opin. Colloid Int.* 2005, 9, 396–403.
- Sato, K.; Ueno S. Molecular Interactions and Phase Behavior of Polymorphic Fats. *Crystallization Processes in Fats and Lipid Systems*, Garti, N., Sato, K., Eds.; Marcel Dekker Inc.: New York, 2001; pp.177–210.
- Sato, K.; Ueno, S. Polymorphism in Fats and Oils. *Bailey's Industrial Oil and Fat Products*, 6th edition; Shahidi, F., Ed.; John Wiley & Sons: Hoboken, New Jersey, 2005; Vol. 1, pp 77–120.
- Seidel, B.; Gourrier, A.; Burghammer, M.; Riekel, C.; Jeronimidis, G.; Paris, O. Mapping fibre orientation in complex-shaped biological systems with micrometre resolution by scanning X-ray microdiffraction. *Micron* 2008, 39, 198–205.
- Shinohara, Y.; Takamizawa, T.; Ueno, S.; Sato, K.; Kobayashi, I.; Nakajima, M.; Amemiya, Y. Microbeam X-ray Diffraction Analysis of Interfacial Heterogeneous Nucleation of n-Hexadecane inside Oil-in-Water Emulsion Droplets. *Cryst. Growth Des.*, 2008, 8, 3123–3126.
- Small, D. M. *Glycerides. The Physical Chemistry of Lipids*; 2nd Edition; Handbook of Lipid Research; Plenum: New York, 1988; Vol. 4, pp. 345–394.
- Smith, K.W. Crystallization of palm oil and its fractions. *Crystallization Processes in Fats and Lipid Systems*, Garti, N., Sato, K. Eds.; Marcel Dekker: New York, 2001, pp. 357–380.

- Tanaka, L.; Miura, S.; Yoshioka, T. Formation of granular crystals in margarine with excess amount of palm oil. *J. Am. Oil Chem. Soc.* 2007, 84, 421–426.
- Tanaka, L.; Tanaka, K.; Yamato, S.; Ueno S.; Sato, K. Microbeam X-ray Diffraction Study of Granular Crystals Formed in Water-in-Oil Emulsion. *Food Biophys.* 2009, 4, 331–339.
- Timms, R.E. Phase Behavior of Fats and Their Mixtures. *Prog. Lipid Res.* 1984, 23, 1–38.
- Ueno, S.; Nishida, T.; Sato, K. Synchrotron Radiation Microbeam X-ray Analysis of Microstructures and the Polymorphic Transformation of Spherulite Crystals of Trilaur. *Cryst. Growth Des.* 2008, 8 (3), 751–754..
- Watanabe, A.; Tashima, I.; Matsuzaki, N.; Kurashige, J.; Sato, K. On the formation of granular crystals in fat blends containing palm oil. *J. Am. Oil Chem. Soc.* 1992, 69, 1077–1080.
- Yagi, N.; Ohta, N.; Matsuo, T. Structure of amyloid fibrils of hen egg white lysozyme studied by microbeam X-ray diffraction. *Int. J. Biol. Macromol.*, 2009, 45, 86–90.
- Zhang, L.; Ueno, S.; Miura, S.; Sato, K. Binary Phase Behavior of 1,3-Dipalmitoyl-2-oleoyl-sn-glycerol and 1,2-Dioleoyl-3-palmitoyl-rac-glycerol. *Jam. Oil Chem. Soc.* 2007, 84, 219–227.

15

Milk Fat and Cocoa Butter

Serpil Metin¹ and Richard W. Hartel²

¹Cargill Incorporated, Wayzata, MN; and ²University of Wisconsin, Madison, WI

Introduction

Milk chocolate provides one of the most popular flavor combinations—the special appeal of chocolate and the creaminess of milk fat. This combination makes milk chocolate the favorite of many consumers. As delicious as milk chocolate is, the mixture of milk fat and cocoa butter provides challenges for controlling lipid interactions to give consistent product quality. From phase behavior to crystallization kinetics, understanding the interactions between milk fat and cocoa butter is critical to controlling the quality characteristics and shelf life of milk chocolate.

Milk Fat and Milk Fat Fractions

Both milk fat and milk fat fractions have been used in chocolate, although intact milk fat is by far the most common form in commercial chocolates. Despite the potential benefits of using milk fat fractions in chocolate, the economic realities of milk fat fractionation have prevented their widespread commercial application to date.

Milk Fat

Milk fat in milk is found in finely divided globules surrounded by a natural globule membrane made of various polar lipids. Although its primary role is to provide nutrition for the young, milk fat finds widespread application in a range of food products, including chocolate. Milk fat is a primary ingredient in milk chocolate, providing both flavor and texture modification, but it is also often used in dark chocolate to moderate texture and physical properties through its phase interactions with cocoa butter.

Milk Fat Composition

Milk fat is arguably one of the most complex fats found in nature and varies widely from animal to animal. For the most part, milk fat used in chocolate comes from the

cow; thus, the discussion here relates exclusively to bovine milk fat. Milk fat contains over 400 different fatty acids (Jensen and Clark, 1988), including saturated with a wide range of carbon chain length, unsaturated, *trans*, branched, and even cyclic fatty acids. The levels of the main fatty acids in milk fat are given in Table 15-A (O'Donnell-Megaro et al., 2011), although there can be substantial variability in milk fat composition due to feeding and genetic differences. One of the unique aspects of milk fat is the presence of a substantial content of short-chain fatty acids (particularly C4:0 and C6:0). These are preferentially located on the *sn*-3 carbon of the glycerol

Table 15-A. Fatty Acid Composition of Milk Fat (g/100 g fatty acids).

Fatty acid	Mean
C4:0	4.15
C6:0	2.13
C8:0	1.19
C10:0	2.59
C12:0	2.87
C14:0	9.53
C14:1	0.82
C15:0	0.89
C16:0	28.08
C16:1	1.48
C17:0	0.52
C18:0	11.68
C18:1, <i>trans</i> -6	0.32
C18:1, <i>trans</i> -9	0.29
C18:1, <i>trans</i> -10	0.55
C18:1, <i>trans</i> -11	1.48
C18:1, <i>trans</i> -12	0.54
C18:1, <i>cis</i> -9	23.58
C18:2, <i>cis</i> -9, <i>cis</i> -12	3.19
C18:2, <i>cis</i> -9, <i>trans</i> -11	0.55
C18:2, <i>trans</i> -10, <i>cis</i> -12	-
C18:3	0.38
C20:0	0.09
Other	3.09

Source: O'Donnell-Megaro et al., 2011.

molecule. Both the amount and location of these shorter-chain fatty acids play a major role in milk fat properties, including melting properties and flavor. Another unique compositional characteristic of milk fat is the concentration of conjugated linoleic acid (C18:2) or CLA, widely known to have unique health benefits.

According to Jensen (2002), if milk fat contained only 400 different fatty acids and they were randomized on the glycerol molecule, milk fat would contain 64,000,000 different triacylglycerol (TAG) species. Since the distribution of fatty acids in milk fat is not randomized, there are many fewer TAG species present, but still Jensen (2002) states that several thousand different TAG may be present in milk fat. Because of this complexity, a complete analysis of the TAG profile in milk fat is not feasible. In one of the most comprehensive studies of the TAG composition, Gresti et al. (1993) identified over 250 different TAG in milk fat from HPLC analysis. Interestingly, no single TAG species was present at greater than 4.2% (molar basis). A listing of the ten most prevalent TAG found in milk fat, as determined by Gresti et al. (1993), is given in Table 15-B.

Based on the differences in short chain, long chain and unsaturated fatty acids in these TAG, it should come as no surprise that milk fat crystallization is extremely complex, with crystals composed of multitudes of different TAG. When milk fat is cooled, TAG with similar melting points come together to form mixed crystals (crystals containing a wide range of different TAG), with composition depending greatly on the conditions of crystallization (rate of cooling, agitation, etc.). Further, because of the complex mixed crystals formed, milk fat generally crystallizes only in the β' polymorph.

Table 15-B. Concentration (mole %) of the Ten Most Prevalent Triacylglycerols (TAG) Identified in Milk Fat.

TAG ¹	Concentration
C4:0; C16:0; C18:1	4.2
C4:0; C16:0; C16:0	3.2
C4:0; C14:0; C16:0	3.1
C14:0; C16:0; C18:1	2.8
C16:0; C18:1; C18:1	2.5
C4:0; C16:0; C18:0	2.5
C16:0; C16:0; C18:1	2.3
C16:0; C18:0; C18:1	2.2
C6:0; C16:0; C18:1	2.0
C4:0; C14:0; C18:1	1.8

¹not in positional order

Data from Gresti et al., 1993.

Although TAG are the major component of milk fat (97 to 98%), there are numerous minor lipid classes in milk fat that at times may influence crystallization behavior. Diacylglycerol (DAG) levels vary from 0.28 to 0.59wt%, monoacylglycerol (MAG) levels from 0.16 to 0.38wt%, and free fatty acids (FFA) from 0.1 to 0.44wt% (Jensen, 2002). Cholesterol is also found in milk fat at about 0.4 to 0.45%. Phospholipid levels vary from 0.2 to 1.0wt%, with the primary contributors being phosphatidylcholine (34.5 mol%) and phosphatidylethanolamine (31.8 mol%), with sphingomyelin (25.2 mol%) also generally being counted in the phospholipid category (Jensen, 2002). The components and levels of these minor lipids depend greatly on the method of processing milk fat.

Physical Properties

The most important physical properties of milk fat pertinent to mixtures with cocoa butter are melting point, melting profile, and crystallization and polymorphic behavior. Due to the diverse molecular composition, the crystallization behavior of milk fat is quite complex. Crystallization of milk fat is affected by degree of supercooling, inter-solubility between the components, method of crystallization, polymorphism, and controlled time and temperature treatment (tempering). All of these factors should be taken into account to understand milk fat crystallization (Mulder & Walstra, 1974).

The wide range of molecular species in milk fat results in a melting temperature range from -40 to 40°C (Mulder & Walstra, 1974). This melting temperature range is not the composite of the melting point of the component triacylglycerols since higher melting components dissolve in the liquid fat (Walstra, 1987). At any intermediate temperature, milk fat is a mixture of liquid and solid, defined by the phase interactions of the multitude of different TAG present.

The melting point temperature, or clear point, generally refers to the temperature where the solid fat content goes to zero. Clear point is typically measured visually as the temperature at which the highest-melting, most stable crystal finally disappears. For milk fat, this temperature is typically around 35 to 37°C , but may be as high as 40°C depending on the source (animal, feeding regimen, season, etc.). More commonly, a softening point is given as an indicator of the melting point, often with the Mettler melting point apparatus. Because approximately 2.5% solid fat content (SFC) remains at the Mettler softening point, this measure generally is several degrees lower than the final melting point.

As noted above, milk fat exhibits a melting range from -40 to $+40^{\circ}\text{C}$, with SFC varying across that temperature range as well. However, the exact nature of the SFC melting profile depends to some extent on the source of milk fat since feeding strategies can influence composition of milk fat. Although traditional seasonal differences in milk fat are often mitigated by feeding strategies, it is common that winter milk fat is slightly harder than summer milk fat at all temperatures, with a slightly higher final melting point as well.

Based on its molecular complexity, milk fat does not exhibit the complex polymorphism of simpler fats (e.g., cocoa butter). Milk fat is often thought to crystallize in a single form, the β' polymorph, but recent studies have shown that milk fat's polymorphic behavior is actually much more complex. The γ and α forms were observed, dependent on temperature and cooling rate, in addition to the stable β' form (ten Grotenhuis et al., 1999). The β polymorph was not observed. In contrast, Lopez et al. (2007) found six different types of crystals in milk fat, including a β polymorph. Shear forces were shown to enhance the polymorphic transition from α to β' forms (Mazzanti et al., 2009).

Compared to cocoa butter, milk fat crystallizes slightly faster when cooled under the same conditions (Metin, 1997). Given the large difference in TAG molecular diversity, the fact that milk fat still crystallizes more readily is an interesting result. This is not an effect of apparent thermodynamic driving force (or subcooling) since both cocoa butter and milk fat have about the same final melting point. Apparently, the ability of the different milk fat TAG to form into mixed crystal, with a loose crystal lattice structure, is greater than that of the much more similar TAG in cocoa butter.

In bulk milk fat, nucleation is always heterogeneous, where crystallization starts at the surface of extraneous particles (catalytic impurities) (Mulder & Walstra, 1974). Higher supercoolings or lower temperatures increase the possibility that a solid particle can catalyze nucleation. Hence, the nucleation rate is higher at deeper (lower) supercoolings. Fat crystals formed during cooling accompany nucleation of other crystals of slightly different composition since they serve as catalytic impurities. Even monoglycerides and free fatty acids can act as catalytic impurities in crystallization of milk fat (Walstra, 1987).

Crystal growth is relatively slow in milk fat. Generally, growth rate of crystals depends on the super saturation, diffusion rate of molecules to the crystal surface and time necessary for a molecule to fit on the crystal lattice. Diffusion is not important in growth of milk fat crystals since there are many triacylglycerols similar to those forming the crystal lattice. These molecules can fit easily into the crystal lattice, but also can occupy a vacant site on the crystal surface for a while before diffusing away. A period of time is required until a suitable triacylglycerol arrives at the vacant site for incorporation into the crystal. This competition delays crystal growth (Mulder & Walstra, 1974). Growth of milk fat crystals under isothermal conditions may take up to 24 hours to be complete. For example, at 11°C, only half of the crystallization was complete after 1 hour (Walstra, 1987).

Grall and Hartel (1992) investigated effects of crystallization temperature, agitator velocity, cooling rate and scale of operation on crystallization kinetics of milk fat in a stirred suspension crystallizer. Due to the heterogeneity milk fat, crystallization temperature significantly affected crystal structure and kinetic rates. The rate of formation and growth of crystals was dependent on impeller shear, and heat and mass transfer conditions, which varied with agitator speed and scale of operation. As cooling rate increased, nucleation rate increased, but crystal growth rate decreased.

As discussed previously, crystallization of milk fat is significantly influenced by intersolubility, which was defined as co-crystallization and formation of mixed crystals (Deffense, 1993). Due to large variety of triacylglycerols in milk fat, mixed crystals containing two or more different components are quite likely to form. Triacylglycerols can be easily arranged in a mixed crystal having α -modification since it has the least dense chain-packing structure. Most mixed crystals are not stable and rearrange into purer crystals, often accompanied by polymorphic transformation. However, unstable polymorphs of mixed crystals may persist longer in milk fat (Tverdokhlebov & Vergelesov, 1970; Mulder & Walstra, 1974). Milk fat consists of mainly three major groups of glycerides (low-, middle- and high-melting), which melt and crystallize separately, and they behave as independent solid solutions (Timms, 1980). The formation of solid solutions between glycerides greatly influences the rheological properties of milk fat. The thermal history of the milk fat influences the groups of mixed crystals and polymorphic behavior during crystallization (Mulder & Walstra, 1974).

The crystallization temperature influences the formation of different polymorphic forms, the size of the crystals and the composition of solid and liquid milk fat (Kaylegian et al., 1993). When milk fat is held at a constant temperature, there may be additional crystallization of liquid triacylglycerols, which have not been crystallized upon cooling, and polymorphic transitions and recrystallization of impure and mixed crystals may occur. This additional crystallization increases solid fat content. Polymorphic transitions may result in volume and energy changes, and may affect solid fat content. Recrystallization may cause a minor increase in solid fat content (van Beresteyn, 1972). Upon cooling of molten milk fat, small crystals form first with needle, or platelet, shape. As crystals grow, the needles aggregate into spherulites, consisting of radial needles, which may be at most 50 μ m in diameter (Mulder & Walstra, 1974). If crystallization is very slow, large crystals form which result in a soft fat because liquid oil can move more freely between crystals. Rapid cooling to a low temperature gives rise to numerous small crystals. Tempering causes an increase in crystal size of existing crystals until the system equilibrates at a given temperature (Kaylegian et al., 1993).

The work of Marangoni and Hartel (1998) illustrated that milk fat crystals are formed in spherulites with wispy extensions emanating from a central nucleus. These spherulites extended radially outwards and interacted with other spherulites in the crystalline network. Crystalline network was modified when minor lipids were removed from the milk fat. The newly formed crystalline network no longer contained spherulitic crystals, but portrayed a random wispy microstructure. Minor changes in chemical composition of milk fat resulted in very different microstructures in the final product.

Consequently, the crystallization of milk fat is very complex due to the large number of molecular species involved. Although the effects of the various factors on crystallization of milk fat are understood qualitatively, we are still far from a satisfactory quantitative description of milk fat crystallization.

Milk Fat Fractions

Because of the numerous triglycerides present in milk fat and their wide range of melting points, milk fat has been a prime target for fractionation. By separating milk fat triglycerides according to their melting point, a variety of fractional components with widely different characteristics can be produced. Some of these fractions have been found to be beneficial in chocolate, particularly for the prevention of bloom. Some notable references on milk fat fractionation and the properties of the various fractions include Deffense (1993), Kaylegian and Lindsay (1995), and Hartel and Kaylegian (2001).

Fractionation Technologies

Numerous methods have been used to fractionate milk fat, including solvent fractionation, dry or melt fractionation, and solid layer fractionation (Hartel & Kaylegian, 2001). Dry fractionation is generally considered the method of choice to separate milk fat into components with different melting properties since it is relatively efficient and retains the natural flavors of milk fat.

Dry fractionation, so called because no solvent is used to enhance the separation, involves cooling the molten fat to a specific temperature with controlled agitation to produce a crystalline slurry that is then filtered under pressure to squeeze liquid oil from the crystal component. The solid phase, often called a stearin fraction, is made up preferentially of high-melting TAG, while the liquid phase passing through the filter, called the olein fraction, contains TAG with lower melting point. The crystallization temperature determines the amount of solid phase yield and which TAG are separated, with higher temperatures giving lower yield of a fraction with higher melting point. Due to the high viscosity of the crystal slurry, a substantial amount, up to 50%, of the liquid phase remains in the solid fraction. This limits the sharpness of the separation of TAG to a large extent. High pressure filters are used to squeeze as much liquid from the crystals as possible, but even high pressure filters leave behind 20 to 30% liquid phase. Despite this lack of efficiency in separation, dry fractionation is the commercial method of choice for milk fat because it provides a natural separation with retention of all flavors.

To produce a range of milk fat fractions with different melting points, multiple fractionation steps are required (Kaylegian & Lindsay, 1995). For example, the first fractionation step may occur at 25°C to yield a high-melting fraction with a melting point over 40°C. The liquid that passes through the filter is then sent to a second crystallizer, where temperature is lowered further. A second crystallization and filtration step produces another set of stearin and olein fractions, with lower melting points. Sequential crystallization and filtration allows milk fat fractions with melting points as low as about 10°C to be produced.

Solvent fractionation separates the components of milk fat based on the solubility of the milk fat TAG in a solvent like acetone or hexane (Kaylegian & Lindsay, 1995).

The melted fat is added to the solvent and cooled to a set temperature with appropriate agitation to form individual crystals. The crystallization temperature determines which of the TAG crystallize, with higher temperatures giving TAG with higher melting points. The presence of solvent allows relatively sharp separation of TAG, in part because of the reduced viscosity of the slurry in filtration. Vacuum separation is then used to remove the acetone from the milk fat fractions, leading to a loss of volatiles.

Layer fractionation involves passing the molten milk fat across a cold tube to allow high melting TAG to crystallize. Although great success is seen with this technology in a variety of separations, the relatively slow growth rate of milk fat crystals makes this separation method untenable for fractionating milk fat (Peters et al., 1999). When the solid layer is grown too fast, too many low-melting TAG are incorporated into the solid layer to make this process attractive.

Composition of Milk Fat Fractions

In general, milk fat is comprised of three different components with high, medium and low-melting points. Table 15-C describes the characteristic TAG and melting properties associated with each component. However, a wide range of milk fat fractions can be produced through multiple fractionation steps by controlling temperature at each step. Composition (fatty acids, TAG, minor lipids) also depends on processing parameters that include cooling rate, agitation rate, efficiency of filtration (pressure or vacuum), and the starting material for fractionation (anhydrous milk fat or a previously fractionated starting material).

Fatty acid and acyl carbon profiles of typical milk fat fractions are given in Tables 15-D and 15-E, respectively (Timms, 1980; Hartel & Kaylegian, 2001). Since dry fractionation is based on differences in melting point, it is not surprising that low-melting milk fat fractions are richer in short chain and unsaturated fatty acids than high-melting fractions and contain preferentially TAG with higher acyl carbon number. The minor lipids also partition to some extent during milk fat fractionation. Fatty acids and, to some extent, 1,3-diacylglycerols preferentially partition into the high-melting fractions, whereas 1,2-diacylglycerols preferentially partition into the low-melting fractions (Tietz, 1998).

Table 15-C. Classes of Triacylglycerol (TAG) in Milk Fat.

Class	TAG composition	Yield (%)	Melting temperature (°C)
High-melting	Long-chain saturated fatty acids	5	>50
Middle-melting	2 long-chain saturated fatty acids and 1 short-chain or unsaturated fatty acid	25	35–40
Low-melting	1 long-chain saturated fatty acid and 2 short-chain or unsaturated fatty acids	70	<15

Source: Timms, 1980.

Table 15-D. Fatty Acid Distribution (wt%) in Typical Milk Fat Fractions.

Fatty acid	Milk fat	High-melting fraction	Medium-melting fraction	Low-melting fraction
C4:0	4.0	—	2.1	4.9
C6:0	2.8	0.1	1.5	3.0
C8:0	1.3	0.1	0.8	1.5
C10:0	2.6	0.6	2.0	3.1
C12:0	3.0	1.6	2.5	3.4
C14:0	10.4	10.5	10.2	10.5
C16:0	26.1	40.2	35.1	22.7
C18:0	13.3	31.6	22.0	9.2
C18:1	24.4	8.9	14.9	28.0
C18:2	1.7	0.6	0.9	2.1

Source: Timms, 1980.

Table 15-E. Triacylglycerol (TAG, in terms of acyl carbon number) Distribution in Typical Milk Fat Fractions.

TAG	Milk fat	High-melting fraction	Medium-melting fraction	Low-melting fraction
24	0.3	—	—	0.4
26	0.3	—	—	0.4
28	0.5	—	—	0.5
30	0.9	—	—	1.4
32	2.2	—	0.1	2.9
34	5.4	0.1	1.1	7.0
36	10.6	0.2	6.2	12.9
38	12.9	0.3	10.3	15.0
40	10.0	0.6	7.6	11.7
42	6.2	1.9	7.0	6.2
44	5.7	5.2	8.5	4.8
46	6.6	12.4	9.9	5.3
48	9.0	20.7	12.2	6.7
50	12.3	28.3	17.5	9.2
52	11.3	22.4	14.9	9.4
54	5.5	7.3	4.6	6.1
56	0.3	0.5	0.1	—

Source: Timms, 1980.

Monoacylglycerols were found at such low levels that it was not clear if there was a preferential partitioning or not.

Physical Properties of Milk Fat Fractions

The melting profiles of milk fat fractions are determined principally by the fatty acid and TAG profiles. Melting temperatures of milk fat fractions vary from as low as about 8°C for a super-olein to about 50°C for a super-stearin through control of fractionation conditions (Kaylegian & Lindsay, 1995). Solid fat curves for different milk fat fractions are shown in Fig. 15.1. As expected, milk fat fractions with higher melting point have higher SFC throughout the range of melting temperatures.

Crystallization rates of different milk fat fractions are also dependent on fatty acid and TAG profiles, but may also be influenced by the specific minor lipids present. For example, crystallization rates at 15°C of various milk fat fractions were different than the initial anhydrous milk fat from which they were made (Metin, 1997). Crystallization rates generally increased with increasing melting point, although certain middle-melting fractions did not follow this trend, indicating perhaps the effects of specific TAG or minor lipid impurity on crystallization rate (Metin, 1997; Tietz, 1998).

The polymorphic behavior of milk fat fractions undoubtedly depends on the molecular composition of the specific fraction. Although details are generally lacking,

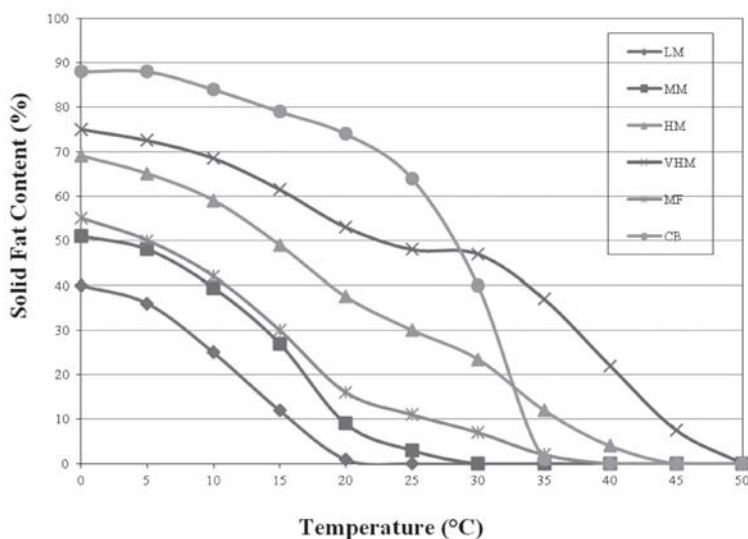


Fig. 15.1. SFC curves for a range of milk fat fractions. CB – cocoa butter, MF – milk fat, VHM – very high melting fraction, HM – high melting fraction, MM – middle melting fraction, LM – low melting fraction. Source: Tietz, 1998.

some studies have been done on polymorphic behavior of milk fat fractions. Lopez et al. (2006) and Lopez and Ollivon (2009) characterized the polymorphic behavior of a stearin and olein fraction. During crystallization, different polymorphic forms were observed in the stearin and olein fractions, and these forms were both different from the native milk fat used to make the fractions. Under the study conditions, both the α and β' polymorphs were found in the high-melting stearin fraction, whereas only the α polymorph was observed in the low-melting olein fraction. Upon warming, recrystallization into the β' form occurred in both fractions, although the packing arrangements of TAG molecules into the crystal lattice were slightly different between the two fractions.

Phase Behavior of Cocoa Butter-milk Fat Blends

Co-crystallization of cocoa butter with milk fat or milk fat fractions affects both phase behavior and the solid fat content of these binary blends. It has been showed that the addition of over 2% milk fat to cocoa butter results in softening of the blend along with changing its crystallization kinetics (Timms, 1980; Lohman & Hartel, 1994).

Timms (1980b) constructed phase and isosolid diagrams for binary blends of cocoa butter with i) milk fat, ii) middle- melting milk fraction and iii) low-melting milk fat fraction in order to investigate eutectic interactions causing the softening of cocoa butter by milk fat. Up to 50% milk fat addition, a single β -3 (cocoa butter-like) solid solution was observed. These findings may suggest that chocolate containing up to 50% milk fats may be produced; however, this would only be possible if problems such as bloom formation, poor gloss and inadequate contraction could be avoided in these blends.

According to Timms (1980b), the middle-melting fraction of milk fat formed a eutectic with cocoa butter. The acylglycerols in this fraction were responsible for eutectic interaction and limited solubility in the solid phase of cocoa butter and milk fat. The low-melting milk fat fraction was found to be mainly responsible for the softening effect of milk fat on cocoa butter because this fraction caused dilution interactions in the blend. Additionally, if the mixture contained more than 30% milk fat, the middle-melting milk fat fraction caused softening of cocoa butter.

An isosolids diagram between cocoa butter and milk fat, showing eutectic behavior, is shown in Fig. 15.2 (from Metin, 1997). At low milk fat addition levels (10–20%), there was a substantial decrease in solid fat content low temperatures (high solid fat). This caused a significant softening of the cocoa butter at room temperature without a significant effect on the melting point (1–5% solid fat). At higher addition levels (30–50%), there was a eutectic effect at higher temperatures, with a slight decrease in melting point. Fig. 15.2 shows the complex interactions that occur between the diverse TAG found in milk fat and cocoa butter.

Since low-melting glycerides of milk fat are believed to be responsible, at least in part, for the softening of chocolate when milk fat is added, the low-melting glycerides

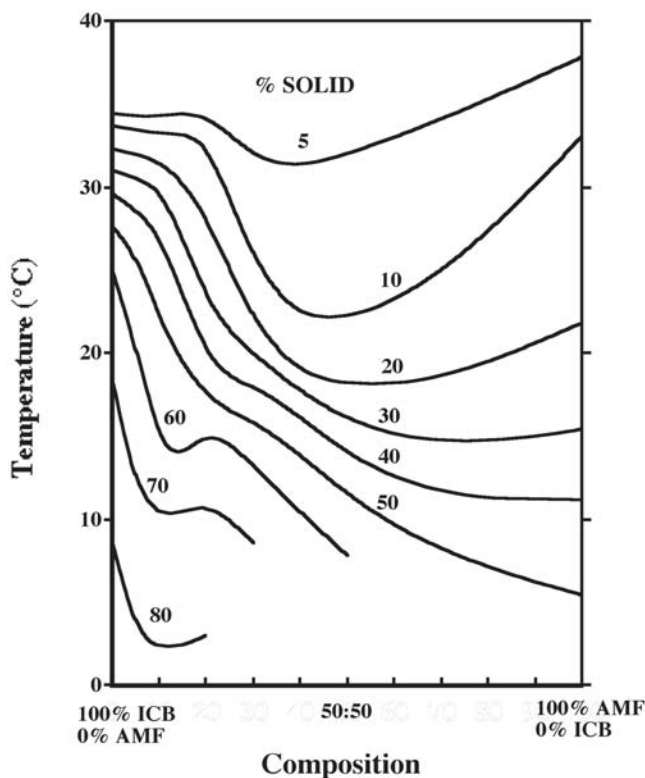


Fig. 15.2. Isosolids diagram for a mixture of milk fat and cocoa butter. Source: Metin, 1997.

can be removed by fractionation. Separation of the low-melting glycerides from the intact milk fat concentrates middle- and high-melting glycerides in the hard fraction. However, Timms and Parekh (1980) found that such a hard fraction also resulted in softer blends with cocoa butter than unmodified fat, showing a eutectic softening effect. Transformation of unsaturated low-melting glycerides to middle-melting glycerides by hydrogenation may be a solution to prevent softening of cocoa butter by dilutional softening, but this gives rise to eutectic interactions between middle-melting glycerides and cocoa butter, and this also causes softening (Jordan, 1986; Timms & Parekh, 1980). However, Hendrickx et al. (1971) reported that up to 6% hydrogenated butterfat could successfully replace cocoa butter. Above this limit, chocolate lost its brittle properties and the snap was too soft. Interestingly, replacing a portion of the cocoa butter in chocolate with hydrogenated butterfat exhibited a favorable retarding effect on fat bloom, even at room temperature for 2 years, so this may be an advantage in chocolate for tropical countries.

Lambelet and Raemy (1983) reported the isosolid diagram of a high-melting (41°C melting point) milk fat fraction and cocoa butter mixture stored at 15°C for one month. Up to a level of 30% milk fat fraction in the mixture, the isosolid lines were very close to each other indicating a sharp melting fat mixture (nonplastic fat). In this composition range, physical properties of the fat blends were very similar to cocoa butter, with a slight softening at higher solid fat content. Above 30–40% milk fat fraction in the fat blend, isosolid lines separated from each other indicating the dominance of milk fat on the physical properties of the fat blend (plastic fat). The isosolid lines also showed the existence of significant eutectic formation at higher addition levels of this milk fat fraction to cocoa butter revealing significant softening of the mixture, especially at lower temperatures.

The incorporation of native and interesterified milk fat fractions (obtained by either multiple step dry fractionation or supercritical fluid extraction) into chocolate was examined by Bystrom and Hartel (1994). Similar to other studies, the incorporation of anhydrous milk fat to cocoa butter caused a softening effect at about 40% level. Addition of interesterified anhydrous milk fat still softened cocoa butter, but the effect was slightly less significant. The high-melting fraction showed more compatibility with cocoa butter than the intact milk fat, particularly after interesterification. Timms and Parekh (1980) also showed that interesterified milk fat was slightly more compatible with cocoa butter than unmodified milk fat. However, they reported that interesterification resulted in loss of flavor in milk fat.

Crystallization and Microstructure of Milk Fat and Cocoa Butter Blends

Cocoa butter and milk fat, being the main fats in chocolate, determine its final properties. Cocoa butter contributes 30–40% (by weight) to finished chocolate and determines texture, gloss, snap and mouth-feel of the chocolate products. Up to 30% milk fat (on a fat basis) is added to milk chocolate, and it is often added at lower levels, about 5%, to dark chocolate to control hardness. The addition of milk fat to cocoa butter is limited by its crystallization behavior and its functionality. Rates of nucleation, growth and polymorphic transformations of blends of these two fats are important in final chocolate product quality and storage conditions (Metin & Hartel, 1998).

Crystallization and Microstructure

Controlling the physical behavior of chocolate or any food system requires knowledge of its microstructure. Understanding microstructure is important in establishing the relationship between composition, processing, and final functionality of chocolate. Crystalline microstructure can provide insight on the size and shape of the crystals and the mechanism of crystallization (Dickinson, 1995). Studying nucleation and its

relation to microstructure in chocolate may provide information on how to control formation of a desired fat microstructure, hence its final texture and other important parameters.

The macroscopic rheological properties of polycrystalline networks of lipids are important in foods containing high fat food, and their sensory attributes (spreadability, sensory, mouthfeel, texture, etc.) are dependent on the mechanical strength of the network of fat crystals (Marangoni & Narine, 2005). The mechanical properties of a fat crystal network are affected by solid fat content and the nature of the crystalline microstructure (Liang et al., 2008). Since milk fat alters the rate of cocoa butter crystallization, its presence undoubtedly changes the nature of the crystalline microstructure on chocolate products.

In fats, nucleation rate depends on the nucleation of a particular polymorphic form. Different polymorphic forms differ in solubility and stability. The least stable α -form has the lowest surface tension and heat of crystallization. A small difference in surface tension can result in a large difference in nucleation rate between less-stable and more-stable polymorphic forms (Timms, 1995). The α -form crystallizes when the temperature of fat is decreased below its melting point. The more stable polymorphic forms can also be obtained directly from the melted fat, but the nucleation rate is usually very low. For example, the rate of nucleation of more stable forms of tripalmitin (β' and β) directly from the melt was found to be 3 to 10 times slower than the transformation of α -form to more stable forms, β' and β (Larsson & Dejmek, 1990).

The rate of nucleation plays a significant role in the microstructure of a product since the number of crystals and their initial structure affect the final crystal morphology (Hartel, 2001). Formation of many nuclei leads to the presence of many small and uniform crystals in the final product. Formation of few nuclei, in contrast, results in the presence of large fat crystals, which in general, are irregular in shape.

After nucleation, crystal growth continues until phase equilibrium is reached (Hartel, 2001). Crystal growth is also affected by the same parameters that affect nucleation. These include cooling rate, agitation, and impurities. Rapid cooling leads to the growth of uniform small crystals, whereas slow cooling results in fewer but larger crystals. Rapid agitation may break the crystals into smaller crystals (a form of secondary nucleation). Low agitation rate may result in lower crystal growth rates as both heat and mass transfer are limited. Impurities can either decrease or increase crystal growth. Incorporation of impurities into crystalline matrix can inhibit crystal growth and may lead to irregular shaped crystals. On the other hand, impurities can co-crystallize with TAG and can incorporate liquid TAG into the crystalline network (Sato and Garti, 1998). In general, impurities affect crystal growth by changing solution properties and affecting solid-liquid interface. These will lead to selective adsorption of impurities on various crystal faces resulting in differences in growth of crystal faces.

Crystallization of Cocoa Butter

Crystallization of cocoa butter is important in determining final physical and textural parameters of chocolate. Typically, a long induction period is observed prior to initiation of nucleation of cocoa butter due the difficulty in producing stable nuclei (Ziegleder, 1988). A few molecules are involved in the formation of a single nucleus in cocoa butter, and less than 1% of the total fat available solidifies in nucleation of cocoa butter (Davis & Dimick, 1986). During crystal growth, following nucleation, the crystals are surrounded by the liquid cocoa butter mass. The TAGs in cocoa butter easily crystallize as they reach the surface of the nuclei. The solidification time required for the molten cocoa butter depends on number of nuclei, crystal growth rate and crystallization temperature (Davis, 1988). Crystal growth continues until fat crystals touch each other and phase equilibrium is attained at crystallization temperature. As mentioned previously, the crystal size depends on both number of nuclei and growth rate. At the end of crystal growth, cocoa butter has approximately 50 to 70% solid fat depending on crystallization temperature and its origin (Davis, 1988). The last stage of cocoa butter solidification is sometimes referred to as crusting-over (Davis & Dimick, 1986). In this stage, individual fat crystals may form bridges from one to another, and the rate of solidification is drastically lower than that of the growth stage. At the end of the crusting-over period, cocoa butter may be solidified to 80% solid fat. Polymorphic transitions, recrystallization and/or phase changes are postulated to occur during the crusting-over stage of crystallization (Davis, 1988). The type of seed crystals is important in formation of stable polymorphs. Rate of nucleation and crystal growth also have an impact on the crystalline network and final solidification of cocoa butter. Cocoa butters with different chemical compositions show different crystallization rates (Foubert et al., 2004). Since cocoa butter is composed mainly of three fatty acids resulting in predominantly SOS, POP and POS triacylglycerols, they show similar packing behavior in crystal formation. As a result of this, cocoa butter has a very sharp melting profile.

Because of the industrial importance of cocoa butter, a considerable amount of work on crystallization kinetics of cocoa butter has been done to improve the tempering process (Windhab, 2009). For example, Svanberg et al. (2011) investigated the effects of the primary components of chocolate (sugar, cocoa particles, and lecithin) on kinetics of cocoa butter crystallization and chocolate microstructure using seeded and unseeded precrystallization techniques. Seeded samples formed multiple nucleation sites, inducing rapid crystal growth. The unseeded samples showed development of large spherical crystals in some domains, while a more heterogeneous microstructure was observed in other domains. Lecithin significantly impacted crystallization kinetics in both seeded and unseeded samples. In the seeded samples, lecithin significantly reduced the induction time in the presence of sugar and cocoa particles. In unseeded samples, lecithin acted as the nucleation site for cocoa butter crystals.

The ability of seed crystals in chocolate to crystallize the remaining cocoa butter during chocolate solidification was studied by Kinta and Hartel (2010). A minimum amount of stable seed crystals was required to properly crystallize the chocolate. If too few stable seeds were present, nucleation of the unstable β' crystals dominated crystallization and led to bloom formation. When the number of stable seeds was greater than the critical number, their growth overwhelmed nucleation of the unstable polymorph and bloom was prevented.

Hachiya et al. (1989a) studied the effects of type of seed crystals on the solidification kinetics of cocoa butter. The crystallization rate was greatly enhanced by the addition of seed crystals, with crystallization rate increasing in the order of addition of less stable to more stable forms. On the other hand, the polymorphic form of the seed crystals did not have any effect on the final polymorphic form obtained (β -polymorph). Polymorphism of the bulk crystal was primarily influenced by the degree of supercooling. The seed crystals served only as an accelerator of crystallization, not a modifier for polymorphic form.

Hachiya et al. (1989b) also evaluated the effects of chemical composition and polymorphic form of seed crystals on the kinetics of solidification of cocoa butter. Where molecular and thermodynamic properties of seed crystals were similar to those of cocoa butter, solidification rate of cocoa butter was enhanced. For seed crystals of cocoa butter (either β polymorph), the more stable polymorph was found to be most effective in enhancing crystallization rate of cocoa butter. They also studied seed crystals according to their melting point. Tristearin seed crystals (SSS), having the highest melting point, did not significantly enhance the crystallization rate. Therefore, the melting point of the seed crystals was not an effective parameter, but rather the similarity in crystal structure between cocoa butter and seed crystals was the most effective factor for acceleration of the solidification of cocoa butter.

Influence of Minor Components on Cocoa Butter Crystallization

In general, minor components of the fats, such as free fatty acids, mono- and diacylglycerols (MAG and DAG, respectively) and phospholipids, have been shown to influence crystallization of TAGs (Chaiseri & Dimick, 1995).

Wahnelt et al. (1991) showed that the influence of DAG on the crystallization of cocoa butter is dependent on the position of the hydroxyl group on the molecule. The addition of 10% 1,2- or 1,3-dipalmitin resulted in an increase in crystallization time, with 1,2-dipalmitin showing a greater inhibitory effect. The addition of DAG did not affect the final polymorphic form of cocoa butter; however, addition of DAGs to cocoa butter extended the life time of the α -form by inhibiting the polymorphic transformation.

Savage and Dimick (1995) investigated the effect of phospholipids on cocoa butter crystallization. Even though only a small amount of phospholipids are present in cocoa butter, initial crystals formed at 26.5°C contained higher concentrations

(4–5%) of phospholipids. No correlation was found between the nucleation times and the total phospholipids content of initial crystals. However, good correlation was determined between particular phospholipid species and crystallization rate. They postulated that the crystallization of cocoa butter was influenced by mesomorphic behavior of phospholipids (having the ability to form liquid crystals during solid-liquid transition) and by the configurational structure of phospholipids (normal hexagonal and inverse hexagonal mesophases).

Crystallization of Cocoa Butter with Milk Fat or Milk Fat Fractions

Milk fat is often added in chocolate as a texture modifier, bloom inhibitor and cocoa butter replacer. The physical, textural, sensory and shelf life properties of chocolate products depend on the microstructure formed during the crystallization of cocoa butter and milk fat below their respective melting temperatures, and are affected by the processing and storage conditions. Therefore, it is important to understand the crystallization behavior of these two chemically different fats when blended. Effective replacement of cocoa butter with milk fat or milk fat fractions is limited since crystallization behavior and kinetics of cocoa butter are affected by the interactions between the fats and processing parameters. For example, it is generally accepted that addition of milk fat or milk fat fractions to cocoa butter results in undesirable softening of chocolate and alteration of crystallization kinetics of cocoa butter (Timms, 1980; Hartel, 1996).

Polymorphism

The polymorphic behavior of mixtures of cocoa butter with up to 30% milk fat in the total fat content do not change the polymorphic behavior of cocoa butter (Jewell & Bradford, 1981). However, the melting points of polymorphic forms of the cocoa butter in the blends were lower than for pure cocoa butter, as shown in Table 15.F. In addition, the rate of transformation of each cocoa butter polymorph to the next higher melting one decreases in the presence of milk fat. The transformation rate of Form V to Form VI slows down, which may account for the retardation of fat bloom in cocoa butter by addition of milk fat (Sonwai and Rousseau, 2010). Timms (1980b) reported that addition of up to 50% milk fat to cocoa butter did not change the polymorphic form of the fat phase, since the fat phase behaved as a single (cocoa butter-like) solid solution.

Crystallization Kinetics and Microstructure

It is well known that milk fat slows the rate of crystallization of cocoa butter, with a greater effect with higher levels of milk fat (Hogenbirk, 1990). This effect was more pronounced with soft cocoa butters than hard ones, since softer cocoa butters

Table 15-F. Polymorph Melting Points.

Form ^a	Melting Point (°C)		X-ray Characterization ^b
	CB	CB/MF (70/30)	
I	17.5	Not determined	β_2^1
II	23.5	15	α
III	25.5	19	$\alpha + \beta^1$
IV	27.5	25	β_1^1
V	33.8	31	β_2
VI	38.4	Not detected	β_3

^aAfter Wille and Lutton (1966).

^bNomenclature after Larsson (1966).

Source: Jewell & Bradford, 1981.

originally crystallize slower than hard butters. The effect is also seen whether crystallization occurs statically or under shear (Maleky & Marangoni, 2008). Despite a minor decrease in melting point (about 1°C), both a delay in onset of crystallization and a decrease in the total solid fat content were observed with addition of 10% milk fat to cocoa butter, whether crystallized statically or dynamically.

The desired polymorphic form, β -V, is not obtained by cooling only, it also requires uniform application of shear. Several recent studies have observed the influence of shear on cocoa butter crystallization kinetics and polymorphic transformations (Windhab, 1999; Bolliger et al., 1998; Mazzanti et al., 2003). Shear effects on crystallization can occur through either acceleration of solid state phase transformation or crystal orientation (Maleky & Marangoni, 2008). Maleky and Marangoni (2008) discussed the importance of critical shear rate required to form proper seed crystals. As the shear rate increased, the polymorphic transformation rate also increased. However, the inhibitory effect of milk fat on the cocoa butter polymorphic transformation was also observed under shear conditions. Shear also accelerates the crystal orientation, with alignment of triglyceride molecules in the shear field resulting in parallel orientation that helps them to move and pass other.

Marangoni and Narine (2005) focused on determination of effects of solid fat content, polymorphism and network microstructure on the elasticity of the blends of milk fat fractions and their blends with cocoa butter (CB) under static crystallization process at 22°C. Their main goal was to identify key indicators of mechanical strength in these blends. In the blend of high-melting milkfat fraction (HMF) with CB, the storage modulus (G') increased as the proportion of HMF in the blends increased. The phase diagram of the blends showed the presence of monotectic solution behavior indicating ideal solution behavior between the two fats and formation of mixed crystals in the solid state. The structure of the solid state of the β form of CB was

affected by addition of HMF, at 2, 5, and 10% levels, and it is reverted to the characteristics of β -3 pattern of CB. Mixtures containing 20% and above levels of HMF, fat crystals were in a β' -2 polymorphic form. Microstructure of the fat crystal network clearly changed from 100% HMF to 100% CB. The spatial distribution of mass in fat crystal networks characterized by their mass fractal dimension, also changed for different mixtures. Changes in polymorphism were mirrored by the changes in the geometry of networks. They suggested that under the conditions of the study, the geometry of the network would be strongly influenced by the polymorphism of the solid state. However, no direct correlation could be made to mechanical response (G') by these two factors. They have shown that mechanical properties of the blends of milk fat fractions with cocoa butter are mainly controlled by phase behavior, not by the microstructure or polymorphism of the fat crystal network. The amount of solids directly affected the elastic behavior of the material. The knowledge of molecular composition of the solid state was sufficient for the prediction of mechanical response in the systems studied.

The Avrami model has been used to describe isothermal fat crystallization (Metin, 1997). Several authors have reported the values of the exponent in Avrami's equation corresponding to the kinetics and shape of the crystals. Modifications of Avrami's equation have also been discussed (Liu & Swant, 2002, Ng, 1975, Marangoni, 1998). Further, Foubert et al. (2002 and 2003) proposed a mathematical model to describe fat crystallization. Metin and Hartel (1998) applied the Avrami equation to the isothermal crystallization at 15°C of binary mixtures of cocoa butter with milk fat or milk fat fractions. Avrami analysis indicated an n value of 4 for cocoa butter crystallization suggesting heterogeneous nucleation with spherulitic growth from sporadic nuclei for crystallization mechanism. For milk fat, the n value was 3, suggesting that the crystallization mechanism was instantaneous heterogeneous nucleation with spherulitic growth. For milk fat fractions, the obtained n value was 2, which suggested that the mechanism was high nucleation rate at the beginning of crystallization decreasing with time, and plate-like growth. Although strict application of Avrami's exponents to specific lipid crystallization mechanisms is not correct, these results strongly suggest that the nature of cocoa butter crystallization is changed with the addition of milk fat and milk fat fractions.

Isothermal kinetics of pre-crystallized cocoa butter and milk chocolate were experimentally investigated by Padar and coworkers (2008). Two pre-crystallization techniques were utilized, seed with cocoa butter crystal suspension and conventional chocolate tempering. Unseeded cocoa butter was used as a control. The change in solid fat content was measured under quiescent conditions at different temperatures, and microscopic information of shape and rate of crystal growth was also collected. They demonstrated that only one of the three theoretical equations of Avrami model fits well the experimental single component crystallization kinetics. Although Avrami's approximate equation fits the experimental data well, it does not enable us to know the exact type of crystal growth. Since fractionation of TAGs was observed

during crystallization of multicomponent cocoa fat systems, they concluded that the Avrami model cannot be used for multicomponent crystallization processes observed in fat systems. Therefore, understanding of both thermodynamic factors and kinetic aspects of crystallization is necessary for improvement of the currently used models.

Minor lipid components, such as diacylglycerols (DAG), monoacylglycerols (MAG), cholesterol and phospholipids, play a key role in crystallization and microstructure of milk fat-cocoa butter blends. Tietz and Hartel (2000) showed that the level of milk fat minor lipids impacted the crystallization behavior of the blends and physical properties of chocolate. Normal levels of milk fat minor lipids resulted in the shortest onset nucleation time and inhibited bloom formation the best. Both removing the milk fat minor lipids and doubling the level of milk fat minor lipids resulted in a longer onset nucleation time, slower crystallization rate and more rapid bloom development in chocolate. In addition, the crystalline microstructure of blends without minor components contained irregular primary and secondary crystals with inclusions of liquid fat, whereas, the microstructure of blends made with normal levels of minor lipids contained spherical and more uniform crystals. Milk fat minor lipids, even though small in concentration, affected the crystallization of milk fat-cocoa butter blends and also had an impact on the chocolate microstructure and bloom development in chocolate.

Milk Fat in Chocolate

Milk fat is found quite extensively in chocolate. Milk chocolate of course contains a substantial amount of milk ingredient, including fat, but even dark chocolate often contains a portion of milk fat. Milk fat moderates the hardness of cocoa butter through its effect on phase behavior and helps to prevent fat bloom in storage. The inhibitory effect of milk fat on cocoa butter crystallization must be offset by changes to the tempering process. Tempering must be adjusted to account for both the amount of milk fat addition and the nature of the fat phase added. That is, addition of milk fat and its fractions may require substantial modification to tempering protocols to obtain well-tempered chocolate.

Milk Fat (or Fractions) Addition to Chocolate

There are several ways by which milk fat can be added into milk chocolate. Dried whole milk powder, whether spray or roller-dried, is a common method of adding milk ingredients to chocolate. Alternatively, dried skim milk powder can be added along with anhydrous milk fat to obtain the desired composition levels. Another method is to make milk crumb, a cooked and dried mixture of milk, sugar, and sometimes, chocolate liquor. Recently, Franke and Heinzelmann (2008) developed an extrusion process to enhance the use of whole milk powder in chocolate. Each of these methods produces slightly different results in terms of flavor and textural properties

(Attaie et al., 2003; Keogh et al., 2003; Liang & Hartel, 2004), in part because of the nature of the interaction between milk fat and cocoa butter.

One of the main effects of milk ingredient addition to chocolate is due to the extent of milk fat that is actually capable of mixing with and influencing the cocoa butter. On one extreme is spray-dried whole milk powder, which contains about 29 to 30% milk fat. Because of the drying process, the milk fat in dried whole-milk powder is effectively encapsulated within the powder particles and only a small amount (1 or 2%) is actually free to mix with cocoa butter. Roller-dried whole milk powder contains more free fat (about 25%) so more milk fat is free to interact with the cocoa butter in the chocolate. At the other extreme is the combination of dried skim powder plus anhydrous milk fat. In this case, the entire amount of milk fat is free to mix with the cocoa butter, significantly influencing crystallization, physical properties and shelf stability. Note that these free milk fat values are estimated based on the nature of the milk powder. Since the milk powder goes through the refining and conching stages of chocolate manufacture, it is likely that additional free fat is released from the particle fragments. Still, there are significant differences in the amount of milk fat available to mix with the cocoa butter for different milk products.

The free milk fat level has numerous effects on chocolate properties. For one, free milk fat in milk chocolate correlates directly with the viscosity of the melted chocolate mass (Attaie et al., 2003; Keogh et al., 2003; Liang & Hartel, 2004). Total fat content of chocolate is based on the sum of milk fat and cocoa butter, regardless of where the milk fat is located. When there is more free milk fat capable of mixing with cocoa butter (and not entrapped within a powder matrix), the effective liquid fat available to disperse the particulate matter in chocolate (sugar, cocoa solids, milk powder) is greater. Thus, viscosity is decreased as the particles of chocolate are further dispersed.

In general, higher levels of milk fat addition in chocolate results in softer chocolate (Full et al., 1996), according to the eutectic mixing behavior seen in Section 3. In the same way, the free milk fat content of the milk ingredient used to manufacture the chocolate also influences hardness. As free milk fat content increased, hardness of the chocolate decreased (Liang & Hartel, 2004).

The use of milk fat fractions in chocolate has a significant effect on chocolate properties. Full et al. (1996) showed that increasing addition levels of all milk fat fractions, from low-melting to high-melting, cause softening of chocolate (both analytical and sensory). However, little significant difference was observed for different milk fat fractions as compared to anhydrous milk fat. These results matched those of Barna et al. (1992) and Bystrom (1993), where all milk fat fractions, including the high-melting fraction, caused a significant decrease in hardness over the cocoa butter control. In contrast, Tietz (1998) found that the melting point of the milk fat fraction did indeed significantly influence hardness of milk chocolate made with 10% milk fat component. The hardness of the chocolate decreased proportionally with the melting point of the milk fat fraction, with a very high-melting fraction (clear point of 47.4°C) giving significantly harder chocolate than the equivalent anhydrous milk fat addition.

Tempering

The free milk fat content in milk chocolate also influences cocoa butter crystallization, thereby influencing tempering conditions. The inhibitory effects of milk fat on cocoa butter crystallization indicate that tempering of milk chocolate will be different from that of dark chocolate (with no milk fat added). In general, chocolate makers know that tempering milk chocolate requires lower temperatures than generally used for dark chocolate. The higher crystallization rate at the lower tempering temperature offsets the inhibition effect of milk fat on cocoa butter crystallization. However, the extent of deviation due to milk fat addition is generally a matter of trial and error since it depends on the type of cocoa butter, the nature of the milk fat added (particularly milk fat fractions), and the free fat level.

The free milk fat content in a milk chocolate significantly affects cocoa butter crystallization (Liang & Hartel, 2004). The more free fat milk fat available to mix with the cocoa butter, the greater inhibition and the lower the temperatures required to provide good temper. The aim of tempering of chocolate is to pre-crystallize the liquid mass with a large number of cocoa butter seed crystals in the stable polymorphic form. The lower temperature increases crystallization rate, offsetting the inhibitory effects of milk fat on cocoa butter crystallization, allowing the desired seed population to be developed. The specific tempering conditions for different combinations of milk fat and cocoa butter must be determined, to a large extent, by trial and error.

The use of milk fat fractions adds another level of complexity to controlling cocoa butter crystallization since each fraction has a different phase behavior and inhibitory effect with cocoa butter. Yella Reddy et al. (1996) determined the tempering conditions needed to produce chocolates with high levels of milk fat fractions. Because of the inhibition effect on cocoa butter crystallization, both a decrease in tempering temperature and an increased time at tempering temperature were required to ensure adequate tempering as determined by chocolate properties after subsequent cooling. Different maximum addition levels were found for the different milk fat fractions. In particular, the high-melting fraction was impossible to temper at levels above 35% (fat basis) because of the increased viscosity. The high melting point of this fraction resulted in excessive milk fat crystallization at temperatures where cocoa butter crystallization was insufficient to seed the mass.

Chocolate Bloom in the Presence of Milk Fat

Milk fat is widely recognized as a fat bloom inhibitor in chocolate although the exact mechanism for this behavior is not completely understood. Since fat bloom in chocolate is covered in chapters 7–8 and 13, only the specific effects of milk fat and milk fractions will be addressed here.

Since milk fat is an excellent bloom inhibitor, bloom resistance is also affected by the free milk fat content of the milk ingredient added to milk chocolate. Liang and

Hartel (2004) showed that the free fat content of the milk ingredient correlated with bloom stability, as expected. The more milk fat available to mix with the cocoa butter, the greater the bloom inhibition.

One hypothesis for the inhibitory effect of milk fat on bloom in chocolates is that it slows the polymorphic transition of cocoa butter. Note that several studies (Adenier et al., 1993; Bricknell & Hartel, 1998) have shown that the transition from the reasonably stable β V polymorph to the most stable β VI polymorph is not the cause of fat bloom during chocolate storage since chocolates were made that showed no evidence of visual bloom despite the presence of the β VI polymorph. Still, every study that has characterized the polymorphic form of bloom has shown it to be the β VI polymorph. That is, the polymorphic transition appears to be a necessary, but not sufficient, step in bloom formation. For visual bloom, the recrystallized cocoa butter must also have the proper size and shape at the chocolate surface to reflect light in such a way that there is a visual change in appearance.

Bricknell and Hartel (1998) also showed that milk fat and milk fat fractions, even at an addition level of only 2%, did indeed have an effect on both visual bloom in milk chocolate and the polymorphic transition. Using a chocolate made with amorphous sugar to allow x-ray analysis, it was shown that anhydrous milk fat delayed the onset of the polymorphic transition compared to a similar chocolate with no milk fat. The expected bloom inhibition was demonstrated on a similar chocolate made with crystalline sucrose since no visual bloom was observed on the chocolate made with amorphous sugar. The high-melting milk fat fraction has the largest effect, inhibiting both the polymorphic transformation and visual bloom to a greater extent than anhydrous milk fat. On the other hand, the low-melting milk fat fraction was seen to promote both the polymorphic transformation and visual bloom formation.

Atomic force microscopy has been used to probe the surface of chocolate to better understand the effects of milk fat on bloom formation (Hodge & Rousseau, 2002; Rousseau, 2006). This technique allows observation of structural developments on the chocolate surface during storage. The surface of milk chocolate shows irregular structures at the $<3 \mu\text{m}$ scale, with evidence of numerous microscopic pores. During temperature cycling, larger crystals begin to form, often around the opening of a pore. Subsequent studies (Sonwai & Rousseau, 2008; Sonwai & Rousseau, 2010) have shown that larger-scale structures develop on the surface of milk chocolate over time during temperature cycling. Liquid fat appears to well up out of the surface and crystallize over time, showing evidence of the β V to β VI polymorphic transition. The addition of milk fat to chocolate reduces the initial roughness of the chocolate surface, decreases the extent of liquid welling into cone structures, delays the solidification rate of the cones, and delays the onset of the polymorphic transformation. This leads to a decrease in development of surface whiteness (bloom).

Summary

The addition of milk fat to chocolate creates a complex mixture of TAGs whose crystallization behavior is quite complex. There are several notable changes in chocolate due to the effects of milk fat addition.

The softening effect from adding milk fat to cocoa butter is easily explained through phase behavior. This explains the softening of milk chocolate, although milk fat is also used by chocolate manufacturers to control the hardness of dark chocolate. Milk fat also has an inhibitory effect on cocoa butter crystallization, which means that chocolate containing milk fat must be tempered at different conditions. Specifically, milk chocolate is tempered at slightly lower temperatures than dark chocolate to offset the inhibitory effects and allow more rapid cocoa butter crystallization. Finally, milk fat is known as a bloom inhibitor, most likely through its effect on slowing the polymorphic transformation of cocoa butter to the most stable polymorph, β -VI. However, the exact process for this inhibition is still the topic of research.

References

- Adenier, H.; Chaveron, H.; Ollivon, M. Mechanism of fat-bloom development on chocolate. In *Developments in Food Science*; Charalambous, G., Ed.; Elsevier Science Publishers B.V.: New York, Vol. 33, 1993; p. 353.
- Attaie, H.; Breitschuh, B.; Braun, P.; Windhab, E.J. The functionality of milk powder and its relationship to chocolate mass processing, in particular the effect of milk powder manufacturing and composition on the physical properties of chocolate masses. *Int. J. Food Sci. Technol.* 2003, 38, 325–335.
- Barna, C.M.; Hartel, R.W.; Metin, S. Incorporating milk fat fractions into milk chocolate. *Manufact. Confect.* 1992, 72(6), 107.
- Bigalli, G. Practical aspects of the eutectic effect on confectionery fats and their mixtures. *Manufact. Confect.* 1988, 68(5), 65–80.
- Bolliger, S.; Breitschuh, B.; Stranzinger, M.; Wagner, T.; Windhab, E.J. Comparison of precrystallization of chocolate. *J. Food Eng.* 1998, 35(3), 281–297.
- Bricknell, J.; Hartel, R.W. Relation of fat bloom in chocolate to polymorphic transition of cocoa butter. *J. Amer. Oil Chem. Soc.* 1998, 75(11), 1609.
- Bystrom, C.E.; Hartel, R.W. Evaluation of milk fat fractionation and modification techniques for creating cocoa butter replacers. *Lebensmittel-Wissenschaft u. Technologie* 1994, 27(2), 142–150.
- Chaiseri, S.; Dimick, P. Dynamic crystallization of cocoa butter. I. Characterization of simple lipids in rapid- and slow-nucleating cocoa butters and their seed crystals. *J. Amer. Oil Chem. Soc.* 1995, 72, 1491–1496.
- Davis, T.R.; Dimick, P.S. The process of cocoa butter solidification. *Manufact. Confect.* 1986, 66, 123–128.
- Davis, T.R. Thermal and compositional properties of Ivory Coast cocoa butter seed crystals. Ph.D. Thesis. The Pennsylvania State University, 1988.

- Deffense, E. Milk fat fractionation today: a review. *J. Amer. Oil Chem. Soc.* 1993, 70(12), 1193–1201.
- Dickinson, E. *New Physico-Chemical Techniques for the Characterization of Complex Food Systems*; Blackie Academic & Professional: London, Glasgow, 1995; pp. 54–68.
- Foubert, I.P.A.; Vanrolleghem, B.; Vanhoutte, B.; Dewettinck, K. Dynamic mathematical model of the crystallization kinetics of fats. *Food Res. Intern.* 2002, 35(10), 945–956.
- Foubert, I.; Dewettinck, K.; Vanrolleghem, P. Modelling of the crystallization kinetics of fats. *Trends Food Sci. Technol.* 2003, 14(3), 79–92.
- Foubert, I.; Vanrolleghem, P.A.; Thas, O.; Dewettinck, K. Influence of chemical composition on the isothermal cocoa butter crystallization. *J. Food Sci.* 69(9), E478–E487.
- Franke, K.; Heinzlmann, K. Structure improvement of milk powder for chocolate processing. *Int. Dairy J.* 2008, 18, 928–931.
- Full, N.A.; Yella Reddy, S.; Dimick, P.S.; Ziegler, G.R. Physical and sensory properties of milk chocolate formulated with anhydrous milk fat fractions. *J. Food Sci.* 2006, 61(5), 1068–1072, 1084.
- Grall, D.S. Kinetics of butterfat crystallization from the melt. MS Thesis. University of Wisconsin–Madison, 1991.
- Grall, D.S.; Hartel, R.W. Kinetics of butterfat crystallization. *J. Amer. Oil Chem. Soc.* 1992, 69(8), 741.
- Gresti, J.; Bugaut, M.; Maniogui, G.; Bezard, J. Composition of molecular species of triacylglycerols in bovine milk fat. *J. Dairy Sci.* 1993, 76, 1850–1869.
- Hachiya, I.; Koyano, T.; Sato, K. Seeding effects on crystallization behavior of cocoa butter. *Agric. Bio. Chem.* 1989b, 53(2), 327–332.
- Hachiya, I.; Koyano, T.; Sato, K. Seeding effects on crystallization of cocoa butter and dark chocolate. I. Kinetics of solidification. *J. Amer. Oil Chem. Soc.*, 1989b, 66(12), 1757–1762.
- Hartel, R.W. Application of milk fat fractions in confectionery. *J. Amer. Oil Chem. Soc.* 1996, 73(8), 945–953.
- Hartel, R.W. *Crystallization in Foods*; Aspen Publ.: New York, 2001.
- Hartel, R.W.; Kaylegian, K.E. Advances in milk fat fractionation: technology and applications. In *Crystallization Processes in Fats and Lipid Systems*; Garti, N., Sato, K., Eds.; Marcel Dekker, New York, 2001.
- Hendrickx, H.; DeMoor, H.; Huyghebaert, A.; Janssen, G. Manufacture of chocolate containing hydrogenated butterfat. *Rev. Inter. Chocolate* 1971, 26, 190–193.
- Hodge, S.M.; Rousseau, D. Fat bloom formation and characterization in milk chocolate observed by atomic force microscopy. *J. Amer. Oil Chem. Soc.* 2002, 79(11), 1115–1121.
- Hogenbirk, G. Compatibility of specialty fats with cocoa butter. *Manufact. Confect.* 1984, 64(6), 59–63.
- Jensen, R.G. The composition of bovine milk fat. *J. Dairy Sci.* 2002, 85, 295–350.
- Jensen, R.G.; Clark, R.M. Lipid composition and properties. In *Fundamentals of Dairy Chemistry*, 3rd ed.; Wong, N., Ed.; Van Nostrand Reinhold Company: New York, 1988; pp. 171–213.

- Jewell, G.G.; Bradford, L. Considerations in chocolate formulation. *Manufact. Confect.* 1981, 61(1), 26–30.
- Kaylegian, K.E.; Lindsay, R.C. *Handbook of Milkfat Fractionation Technology and Applications*; Amer. Oil Chem. Soc. Press: Champaign, IL, 1995.
- Kaylegian, K.E.; Hartel, R.W.; Lindsay, R.C. Applications of modified milk fat in food products. *J. Dairy Sci.* 1993, 76, 1782–1796.
- Keogh, M.K.; Murray, C.A.; O’Kennedy, B.T. Effects of selected properties of ultrafiltered spray-dried milk powder on some properties of chocolate. *Int. Dairy J.* 2003, 13, 719–726.
- Kinta, Y.; Hartel, R.W. Bloom formation on poorly-tempered chocolate and effects of seed addition. *J. Amer. Oil Chem. Soc.* 2010, 87, 19–27.
- Lambele, P.; Raemy, A. Iso-solid diagrams from thermal analysis data. *J. Amer. Oil Chem. Soc.* 1983, 60(4), 845–847.
- Larsson, K. Classification of glycerides crystal forms. *Acta. Chem. Scand.* 1966, 20 (8), 2255–2560.
- Larsson, K. Molecular arrangements in glycerides. *Fette Seifen Anstrich.* 1972, 74, 136–145.
- Larsson, K.; Djemek, P. Crystal and liquid crystal structures of lipids. In *Food Emulsions*; Larsson, K., Ed.; Marcel Dekker, Inc.: New York, 1990; pp. 97–125.
- Liang, B.; Hartel, R.W. Effects of milk powders in milk chocolate. *J. Dairy Sci.* 2004, 87(1), 20–31.
- Liang, B.; Shi, Y.; Hartel, R.W. Correlation of rheological and microstructural properties in a model lipid system. *J. Amer. Oil Chem. Soc.* 2008, 85, 397–404.
- Liu, X.Y.; Sawant, P.D. Mechanism of the formation of self organized microstructures in soft functional materials. *Advanced Materials* 2002, 14(6), 421–426.
- Lohman, M.H.; Hartel, R.W. Effect of milk fat fractions on fat bloom in dark chocolates. *J. Amer. Oil Chem. Soc.* 1994, 71(3), 267–276.
- Lopez, C.; Bourgaux, C.; Lesieur, P.; Ollivon, M. Coupling of time-resolved synchrotron X-ray diffraction and DSC to elucidate the crystallisation properties and polymorphism of triglycerides in milk fat globules. *Lait* 2007, 87, 459–480.
- Lopez, C.; Bourgaux, C.; Lesieur, P.; Riaublanc, A.; Ollivon, M. Milk fat and primary fractions obtained by dry fractionation. 1. Chemical composition and crystallization properties. *Chem. Phys. Lipids* 2006, 144, 17–33.
- Lopez, C.; Ollivon, M. Triglycerides obtained by dry fractionation of milk fat. 2. Thermal properties and polymorphic evolutions on heating. *Chem. Phys. Lipids* 2009, 159, 1–12.
- Lovegren, N.V.; Gray, M.S.; Feuge, R.O. Effect of liquid fat on melting point and polymorphic behavior of cocoa butter and a cocoa butter fraction. *J. Amer. Oil Chem. Soc.* 1976, 53(3), 108–112.
- Maleky, F.; Marangoni, A.G. Process development for continuous crystallization of fat under laminar shear. *J. Food Eng.* 2008, 89, 399–407.
- Manning, D.M.; Dimick, P.S. Crystal morphology of cocoa butter. *Food Microstructure* 1985, 4, 249–265.

- Marangoni, A. G. On the use and misuse of the Avrami equation in characterization of the kinetics of fat crystallization. *J. Amer. Oil Chem. Soc.* 1998, 75(10), 1465–1467.
- Marangoni, A.G.; Hartel, R.W. Visualization and structural analysis of fat crystal networks, *Food Technol.* 1998, 52(9), 46–51.
- Mazzanti, G.; Guthrie, S.E.; Sirota, E.B.; Marangoni, A.G.; Idziak, S.H.J., Orientation and phase transitions of fat crystals under shear. *Crystal Growth Design* 2003, 3 (5), 721–725.
- Mazzanti, G.; Marangoni, A.G.; Idziak, S.H.J. Synchrotron study on crystallization kinetics of milk fat under shear flow, *Food Res. Int.* 2009, 42, 682–694.
- Metin, S. Crystallization behavior and kinetics of blends of cocoa butter and milk fat or milk fat fractions, PhD Dissertation, University of Wisconsin, Madison, 1997.
- Metin, S.; Hartel, R.W. Thermal analysis of Isothermal crystallization kinetics in blends of cocoa butter with milk fat or milk fat fractions. *J. Amer. Oil Chem. Soc.* 1998, 75(11), 1617–1624.
- Mulder, H.; Walstra, P. The Milk Fat Globule. Emulsion science as applied to milk products and comparable foods. Commonwealth Agricultural Bureau, Farnham Royal, Bucks, England, 1974.
- Mullin, J.W. Crystallization, 4th ed.; Butterworth Heinemann Limited: London, 1995.
- Ng, W.L. Thermal decomposition in the solid state. *Australian J. Chem.* 1975, 28(6), 1169–1178.
- O'Donnell-Megaro, A.M.; Barbano, D.M.; Bauman, D.E. Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. *J. Dairy Sci.* 2011, 94, 59–65.
- Padar, S.; Heelani, S.A.K.; Windhab, E.J. Crystallization kinetics of cocoa fat systems: experiments and modeling, *J. Amer. Oil Chem. Soc.* 2008, 85, 1115–1126.
- Peters, S.; Ulrich, J.; Tiedtke, M.; Hartel, R.W. Milk fat fractionation by solid layer melt crystallization, *J. Amer. Oil Chem. Soc.* 1999, 76(5), 579–584.
- Rousseau, D. On the porous mesostructure of milk chocolate viewed with atomic force microscopy. *Lebensmittel, Wissenschaft Tech.* 2006, 39, 852–860.
- Sato, K.; Garti, N. Crystallization and polymorphic transformations: an introduction. In *Crystallization and Polymorphism of Fats and Fatty Acids*. Vol. 31; Garti, N., and Sato, K., Eds.; Marcel Dekker, Inc.: New York, 1998.
- Savage, C.M.; Dimick, P.S. Influence of phospholipids during crystallization of hard and soft cocoa butters. *Manufact. Confect.* 1995, 75(5), 127–132.
- Sonwai, S.; Rousseau, D. Fat crystal growth and microstructural evolution in industrial milk chocolate. *Crystal Growth & Design* 2008, 8(9), 3165–3174.
- Sonwai, S.; Rousseau, D. Controlling fat bloom formation in chocolate — impact of microstructure and fat phase crystallisation. *Food Chem.* 2010, 119, 286–297.
- Svanberg, L.; Ahrne, L.; Loren, N.; Windhab, E. Effects of sugar, cocoa particles and lecithin on cocoa butter crystallization in seeded and non-seeded chocolate model systems. *J. Food Eng.* 2011, 104(1), 70–80.
- ten Grotenhuis, E.; van Aken, G.A.; van Malssen, K.F.; Schenk, H. Polymorphism of milk fat studied by differential scanning calorimetry and real-time powder X-ray diffraction. *J. Am. Oil Chem. Soc.* 1999, 76(9), 1031–1039.

- Tietz, R.A. The effects of milk fat minor lipids on the crystallization of milk fat-cocoa butter blends and bloom formation in chocolate. MS Thesis, University of Wisconsin-Madison, 1998.
- Tietz, R.A.; Hartel, R.W. Crystallization and microstructure of milk fat cocoa butter blends related to bloom formation in chocolate. *J. Amer. Oil Chem. Soc.* 2000, 77(7), 763–772.
- Timms, R.E. The physical properties of milk fat and blends of milk fat with other oils and fats. In *Proceedings of Milk Fat Symposium*, Victoria, Australia. 1979.
- Timms, R.E. The phase behaviour and polymorphism of milk fat, milk fat fractions and fully hardened milk fat. *Austral. J. Dairy Technol.* 1980a, 35(2), 47–53.
- Timms, R. E. The phase behaviour of mixtures of cocoa butter and milk fat. *Lebens. Wissen. Technol.* 1980b, 13, 61–65.
- Timms, R.E. Crystallization of fats. In *Developments in Oils and Fats*; Hamilton, R.J., Ed.; Chapman & Hall: Glasgow, 1995; pp. 204–223.
- Timms, R.E.; Parekh, J.V. The possibilities for using hydrogenated, fractionated or interesterified milk fat in chocolate. *Lebens. Wissen. Technol.* 1980, 13, 177–181.
- Tverdokhle, G.V.; Vergelesov, V.M. Factors controlling crystallization of milk fat. XVIII International Dairy Congress, Vol. 1E, Sydney, 1970, 211–212.
- van Beresteijn, C.H. Polymorphism in milk fat in relation to the solid/liquid ratio. *Nether. Milk Dairy J.* 1972, 26, 117–130.
- Wahnelt, S.; Meusel, D.; Tulsner, M. Der Einfluß Isomerer Diglycerides auf Phasenumwandlungen von Kakaobutter-Untersuchungen mittels Isothermer DSC. *Fat Sci. Technol.* 1991, 93(5) 174–178.
- Walstra, P. Fat crystallization. In *Food Structure and Behaviour*; Blandshard, J.M., Lillford, P., Eds.; Academic Press, Inc.: Orlando, Florida, 1987.
- Wille, R.L.; Lutton, E.S. Polymorphism of cocoa butter. *J. Am. Oil. Chem. Soc.* 1966, 43, 491–496.
- Windhab, E.J. New developments in crystallization process. *J. Thermal Anal. Calorimetry* 1999, 57, 171–180.
- Windhab, E.J. Tempering. In *Industrial Chocolate Manufacture and Use*, 4th ed.; Beckett, S.T., Ed.; Wiley-Blackwell: Oxford, UK, 2009; pp. 276–319.
- Ziegleder, V.G. Crystallization of chocolate masses. Part 1. *Zucker-und-Suss. Wirtschaft*, 1988, 41, 165–168.

16

Molecular Interactions of Triacylglycerides in Blends of Cocoa Butter with *trans*-free Vegetable Oils

Jorge F. Toro-Vazquez, Míriam A. Charó-Alonso, Juan A. Morales-Rueda, and J. David Pérez-Martínez

Facultad de Ciencias Químicas, Centro de Investigación y Estudios de Posgrado, Universidad Autónoma de San Luis Potosí, México

Introduction

In the native state cocoa butter (CB) is a semisolid fat that exhibits brittleness below 20°C, a soft texture between 28°C and 32°C, and melts completely right below the human body temperature. Therefore, the use of cocoa butter as the main lipid phase in chocolate formulation provides unique characteristics of texture, mouthfeel, and flavor release to chocolate and other confectionery products. Most of the cocoa butter's functional properties are associated with its triacylglyceride (TAG) composition predominantly constituted by mono-unsaturated symmetrical TAG with oleic acid in the sn-2 position, i.e., POP, POSt, and StOSt (where P = palmitic acid, O = oleic acid, and St = stearic acid). These three triacylglycerides (TAGS) make up approximately 75% to 82% of its components (Lipp et al., 2001; Segall et al., 2005). Other minor components of cocoa butter are mono-glycerides (MAGS), diacyl-glycerides (DAGS), phospholipids, glycolipids, sterols, free fatty acids, and fat-soluble vitamins (Calliauw et al., 2008; Dimick, 1999; Foubert et al., 2004). Differences in the proportion between the main TAG and the concentration of minor components (i.e., MAGS, DAGS, and phospholipids) influence the physical properties of cocoa butter. Within this context, Foubert et al. (2004) using DSC, principal component analysis, and multiple linear regression described the effect of the natural variation in composition on crystallization kinetics of 20 different cocoa butter crystallized under isothermal and static conditions. The authors concluded that the ratio of saturated to unsaturated fatty acids and the ratio of monounsaturated to diunsaturated triacylglycerols have an important positive effect on the maximum extent of crystallization (as measured from the exotherm of crystallization) and reduced the induction time of the α to β' polymorphic transition. In the same way, the more the concentration of unsaturated fatty acids and diunsaturated TAGS, the lower the maximum extent of crystallization and the higher the induction time of the α to β' polymorphic transition. Overall, the concentration of DAGS showed the most deleterious effect on the crystallization rate constant (Foubert et al., 2004).

Because of the homogeneity in TAG composition and conformation, polymorphism in cocoa butter is complex. Willie & Lutton originally identified six polymorphs (1966), and named them with the Roman numerals I to VI in increasing order of melting temperature. More recent studies using X-ray diffraction (Guthrie et al., 2005; Mazzanti et al., 2007; Loisel et al., 1998; van Malssen et al., 1999) and differential scanning calorimetry (Fessas et al., 2005; Stapley et al., 1999) use the most common Greek letters' nomenclature for polymorphs. The correspondence between this nomenclature and the ones used by Willie & Lutton (1966), the characteristic diffraction peaks and melting temperature of the cocoa butter polymorphs have been summarized by Le Révérend et al. (2010) as follows:

- Form I (γ) is the most unstable polymorph with a melting temperature around 14°C and a very strong diffraction peak at 4.18 Å.
- Form II (α) has a melting temperature around 20°C and shows a very strong peak at 4.20 Å.
- Form III (β') with a melting temperature around 22°C and a very strong diffraction peak at 4.20 Å.
- Form IV (β') has a melting temperature around 24°C and two strong X-ray diffraction peaks at 4.13 Å and 4.32 Å. According to van Malssen et al. (1999) forms III and IV exist as a range of β' polymorphic phases rather than as two separate polymorphic phases.
- Form V (β') melts at around 30°C and shows a very strong diffraction peak at 4.58 Å.
- Form VI (β) has a melting temperature around 32°C and a strong X-ray diffraction peak at 4.59 Å and differs from polymorph V only by the lack of a mid intensity peak at 3.75 Å.

Form V is considered the optimal polymorph by the confectionery industry, since it provides the appropriate melting temperature interval to allow chocolate storage at room temperature, and provides a smooth melting when chocolate is heated in the mouth. Additionally, polymorph V provides chocolate products with the snap (ability to break apart easily), good demolding properties (contraction), good quality finish in terms of color and gloss, and resistance to fat bloom. Fat bloom is a physical defect that appears during chocolate storage that results in loss of gloss or to the appearance of undesirable white spots or a streaky grey-white finish on the chocolate surface (Kinta & Hartel, 2010; Lonchampt & Hartel, 2006) derived from the polymorphic transition of TAG crystals of cocoa butter (i.e., POP) from form V (β') into form VI (β) (Sato & Koyano, 2001). Unfortunately, polymorph V is not readily obtained in the bulk of chocolate by simple cooling, but rather by using a tempering protocol (i.e., time-temperature program) applied

under the action of shear to develop sufficient cocoa butter crystal seeds of the form V to nucleate the remaining TAGS into the appropriate polymorph (Marty & Marangoni, 2009). Thus, shearing and temperature history are vital for the tempering process.

Although cocoa butter is used as the main lipid phase in chocolate manufacture, due to cost or technical limitations cocoa butter is also used in combination with other vegetable oils, such as hydrogenated or partially hydrogenated soybean oil and palm oil (i.e., cocoa butter extenders or cocoa butter replacers) in the elaboration of compound coatings used in confectionery products. Vegetable fats might be even designed to resemble the functional properties of cocoa butter (i.e., sharp melting profile) and then used as 100% of the fat portion for the production of confectionary fats (i.e., cocoa butter substitutes). In other cases, fully refined vegetable oils and fats might be blended with cocoa butter, particularly in the development of low-*trans* or *trans*-free chocolate fillings and coatings. In all these systems, as in cocoa butter, the molecular interactions occurring among TAG families determine the thermodynamic conditions (i.e., supercooling and supersaturation) that drive the formation of a solid (i.e., crystal) in a liquid phase and the phase behavior of the solid phase. The resulting three-dimensional TAG crystal network and the phase behavior of TAGS are major factors determining the physical and functional properties (i.e., rheology, liquid phase entrapment, mouthfeel) of the crystallized systems such as the confectionary coatings (Pérez-Martínez et al., 2007). Additionally, processing conditions such as cooling rate, thermal history (i.e., crystallization temperature and time-temperature conditions), and stirring conditions have additional significant effects on the crystallization kinetics and physical properties of the crystallized systems.

In any case, we must control three major physical processes when processing cocoa butter or cocoa butter blended with other vegetable fats or oils, namely, (i) crystallization of TAGS, including in this kinetics, size, shape, and polymorphic state of the crystals; (ii) development of the crystal network; and (iii) rheology of the chocolate mass or of the confectionary blend (Toro-Vazquez et al., 2004). In this chapter we discuss the molecular interactions occurring among TAGS of cocoa butter with other TAGS, particularly TAGS present in *trans*-free vegetable oils, and the effect of these interactions on cocoa butter crystallization kinetics, polymorphism, and crystal network microstructure. Furthermore, we address the effect of such molecular interactions on supercooling, the thermodynamic variable that drives TAG crystallization. Major TAGS in *trans*-free vegetable oils have a higher degree of unsaturation than the symmetrical saturated-unsaturated-saturated TAG and the saturated TAG present in cocoa butter. Therefore, in cocoa butter-vegetable oil blends the molecular compatibility among the native TAGS in cocoa butter is modified affecting crystallization kinetics and, subsequently, the physical and functional properties of the crystallized system (i.e., of *trans*-free compound coatings and fillings) are different from the ones observed in cocoa butter.

Crystallization of Blends of Cocoa Butter with *trans*-free Vegetable Oils

In the development of low-*trans* or *trans*-free chocolate fillings and coatings, fully refined vegetable oils and fats are blended with cocoa butter. the nature of interaction between cocoa butter and other vegetable oils and fats, such as palm mid-fraction, palm kernel stearin, hydrogenated palm kernel stearin, Illipe, sal stearin, cottonseed oil and others has been studied using a macroscopic perspective (Hogenbirk, 1984; Lovegren et al., 1976; Alia et al., 1991; Alia & Oh. Flingoh, 1993; Paulicka, 1970, 1973; Smith et al., 2004). Blending of vegetable oils with fats such as cocoa butter, provides flexibility to achieve particular functional properties to crystallized confectionary systems. However, if certain limit of cocoa butter is exceeded, the blend becomes prone to phase separation (i.e., eutectic behavior) with the subsequent oil/fat migration. Thus, when cocoa butter-vegetable oil blends are used as coating, TAG crystallization might occur on the surface because of fat blooming. From the above, the complex molecular interactions that occur among TAGS as a function of the crystallization conditions must be first understood to achieve particular physical and functional properties through cocoa butter blending with vegetable oils.

Phase behavior of cocoa butter-vegetable oil blends is mainly studied using solid fat content (SFC) diagrams, particularly iso-solid diagrams. Iso-solid diagrams define curves indicating the temperatures at which constant solid to liquid ratios (i.e., constant SFC) can be found as a function of the mixture composition. The SFC values used in the iso-solid diagrams ought to be determined under equilibrium conditions so the corresponding equilibrium polymorphic form is attained. However, in most cases near equilibrium conditions are assumed in the determination of iso-solid diagrams. The SFC of mixed lipid system at a given temperature is the result of the molecular interactions among the TAGS present in the oils and/or fats present in the blends. Thus, the iso-solid diagrams show the different behavior describing the compatibility between TAGS present in the blends, i.e., monotectic solid solution behavior (full compatibility between the TAGS present in the blend), monotectic partial solution behavior (partial compatibility between the TAGS present in blend), and eutectic behavior (incompatibility between the TAGS present in the blend) (Braipson-Danthine & Deroanne, 2006; Marangoni & Lencki, 1996). Since the SFC determines in great extent the mechanical behavior of lipid systems, the iso-solid diagrams are used as a guideline to establish whether the vegetable oil blends are suitable for specific applications. However, crystallized vegetable oil blends with similar SFC might have developed different polymorphs with different three-dimensional organization and, therefore, each system observing distinctly rheological properties. This is the main reason why the rheological behavior of cocoa butter (Brunello et al., 2003) and different kind of TAGS blends such as butterfat-vegetable oil blends (Marangoni & Rousseau, 1996), and cocoa butter-vegetable oil blends (Pérez-Martínez et al.,

2005 and 2007) is not strongly related just to SFC, but rather to a combination of factors including crystal polymorphism and the microstructure (i.e., fractal dimension) of the crystal network.

Liquid Structural Organization of TAG Molecules in Cocoa Butter and Cocoa Butter-Vegetable Oils Blends

The development and attainment of critical number of TAG molecules structured in a liquid state or mesophase are requirements to achieve TAG nucleation. All the works associated with proposing molecular arrangements for TAG melts agrees on this (Cebula et al., 1992; Corkery et al., 2007; Hernqvist, 1984; Larsson, 1972, 1992; Minato et al., 1997; Ueno et al., 1999). Nevertheless, the actual structural liquid crystalline-like state organization of TAGS [i.e., nematic (Cebula et al., 1992), smectic (Larsson, 1972), or discotic (Corkery et al., 2007)] has not been established. Our group has studied the liquid structural organization of TAG molecules in blends of palm stearin in sesame oil (Toro-Vazquez et al., 2000) and blends of cocoa butter in soybean and canola oil (Pérez-Martínez et al., 2005) using the equilibrium melting temperature (T_M°). Although Hoffman & Weeks (1962) developed the T_M° parameter for polymers, this principle also applies for the true melting temperature of nonpolymeric pure compounds (i.e., nonionic molecules, ionic or metallic crystals). In this section we introduce some concepts about T_M° to support its application to investigate the molecular compatibility between TAGS in cocoa butter-vegetable oil blends.

T_M° is the hypothetical melting temperature of an assembly of molecules (i.e., TAGS) of infinite size, so that the surface effect on the melting temperature is negligible (Hoffman & Weeks, 1962). An additional assumption is that at T_M° the structural organization of such assembly of molecules (i.e., TAG liquid crystals) is in equilibrium with the molecules in the melt. Therefore, aggregations of molecules without the correct 3-D arrangement to develop a stable crystal, and therefore small crystals, will melt below T_M° (Hoffman & Weeks, 1962). In correspondence, for a particular mixture of TAG families (i.e., cocoa butter-vegetable oil blend) T_M° may occur at high temperatures if the molecules in the melt are oriented to an appreciable extent. Consequently, T_M° is a constant that describes the structural organization of the molecules in the melt (i.e., TAGS) and is a parameter specific for each particular system and polymorph (Pérez-Martínez et al., 2005).

Within this framework, following a slight modification of the methodology proposed by Hoffman & Weeks (1962), Pérez-Martínez et al. (2005) determined that T_M° for the β' polymorph of cocoa butter in a 30% cocoa butter-soybean oil blend was 65.8°C ($\pm 3.0^\circ\text{C}$) and 33.7°C ($\pm 4.9^\circ\text{C}$) in a 30% cocoa butter-canola oil blend. the authors noted that at the 30% cocoa butter concentration used in the blends no mixed crystals were developed between tags of cocoa butter and TAGS of canola or soybean oil (i.e., just TAGS from cocoa butter crystallized in the blends).

Table 16-A. Triacylglyceride (TAGS) Concentration in Cocoa Butter, Canola, and Soybean Oils.

TAGS ^a	Cocoa Butter ^b	Canola ^b	Soybean ^b
LLnL	n.d. ^c	n.d.	3.18 ± 0.17
LLL	n.d.	0.78 ± 0.10	21.39 ± 0.45
LLnP	n.d.	n.d.	1.16 ± 0.16
LLPo	n.d.	4.48 ± 0.68	n.d.
LLO	n.d.	7.35 ± 0.47	21.18 ± 0.87
LLnEo	n.d.	8.90 ± 0.44	n.d.
LLP	n.d.	n.d.	14.49 ± 0.64
LOO	n.d.	26.14 ± 0.01	9.88 ± 0.12
LLSt	n.d.	n.d.	3.84 ± 0.23
PLO	0.16 ± 0.03	n.d.	10.42 ± 0.03
POL	n.d.	3.34 ± 0.12	n.d.
PLP	0.55 ± 0.38	n.d.	1.70 ± 0.39
OOO	0.48 ± 0.59	39.41 ± 1.38	3.67 ± 0.03
StLO	0.26 ± 0.21	n.d.	2.93 ± 0.04
POO	1.86 ± 0.05	4.28 ± 0.18	2.72 ± 0.26
PLSt	1.99 ± 0.02	n.d.	1.36 ± 0.35
PPO	n.d.	0.97 ± 0.20	n.d.
POP	14.80 ± 0.04	n.d.	0.39 ± 0.18
StOO	3.76 ± 0.31	1.86 ± 0.10	0.84 ± 0.43
StLSt	1.20 ± 0.05	n.d.	0.32 ± 0.19
POSt	43.81 ± 0.64	n.d.	0.50 ± 0.02
PPSt	0.32 ± 0.04	n.d.	n.d.
StOSt	29.33 ± 0.05	n.d.	n.d.
PStSt	0.21 ± 0.08	n.d.	n.d.
StOA	1.00 ± 0.03	n.d.	n.d.
StStSt	0.20 ± 0.02	n.d.	n.d.
Unidentified	n.d.	2.46 ± 0.60	n.d.

^a P, palmitic; Po, palmitoleic; St, stearic; O, oleic; L, linoleic; Ln, linolenic; A, arachidic; Eo, gadoleic.

^b % w/w, mean and standard deviation of 2 independent determinations (n = 2).

^c Not detected.

Source: Pérez-Martínez et al., 2007.

We proposed that given the high extent of unsaturation of major TAGS present in soybean oil (e.g., 21.4% LLL, 21.1% LLO, and 14.5% LLP; Table 16-A) these TAGS have a lower extent of molecular compatibility with the symmetrical TAGS from cocoa butter (i.e., POP, POSt, StOSt) than the major TAGS present in canola oil (e.g., 39.4% OOO and 26.1% LOO; Table 16-A). This observation was latter corroborated by Campos et al. (2010) in blends of cocoa butter with 1% and 5% of 1,2,3-trilinoleoyl-glycerol. Thus, in comparison with TAGS of canola oil, TAGS from soybean oil modified at a lower extent the native liquid structural organization developed by TAGS of cocoa butter (i.e., the original liquid structure of TAGS from cocoa butter was preserved in the 30% cocoa butter-soybean oil blend). However, since major TAGS of canola oil have a higher structural compatibility with the symmetrical TAGS of cocoa butter, the native liquid structural organization developed by TAGS of cocoa butter was altered in the 30% cocoa butter-canola oil blend. Therefore, in comparison with the 30% cocoa butter-soybean oil blend a lower temperature (i.e., lower T_M°) was required in the 30% cocoa butter-canola oil blend to achieve the correct three-dimensional arrangement of TAG molecules to develop a stable nucleus.

From the previous discussion, the difference in T_M° values between the cocoa butter-soybean oil blend and the cocoa butter-canola oil blend (Pérez-Martínez et al., 2005) was mainly associated to the different molecular compatibility between the symmetrical TAGS from cocoa butter and TAGS from soybean oil and canola oil. However, additional molecular interactions between TAGS and TAGS with other lipid components have to be considered. Thus, in a parallel study our group investigated the crystallization process of TAGS in native cocoa butter and cocoa butter without polar lipids (CB-WPL), using a mechanical spectrometer to measure the phase shift angle (δ) during the cooling process ($1^\circ\text{C}/\text{min}$) from 80°C until achieving a particular crystallization temperatures (i.e., 18.5°C , 19.0°C , 19.5°C , and 20°C) (Toro-Vazquez et al., 2005). δ is defined as $\tan^{-1}(G''/G')$, and is a sensitive parameter that evaluates the viscoelastic changes of complex systems. In particular, in a vegetable oil or completely melted cocoa butter $\delta = 90^\circ$. However, once TAGS achieve the lamellar organizations that develop the nucleus as a result of a temperature decrease, a drastic decrease in δ must follow the phase change from liquid-crystalline structure to solid formation (i.e., TAGS nucleation). As cooling proceeds nucleation and crystal grow δ decreases and eventually become zero when the system achieves full crystallization. Within this framework, the δ rheograms for cocoa butter and CB-WPL showed that the onset for TAGS nucleation, independent of T_C , occurred during the cooling stage at 59.8°C ($\pm 3.7^\circ\text{C}$) in cocoa butter and at 39.5°C ($\pm 1.5^\circ\text{C}$) in CB-WPL (Fig. 16.1) (Toro-Vazquez et al., 2005). We associated the difference in the onset of nucleation between CB-WPL and cocoa butter to the nucleating role of phosphatidylcholine and phosphatidylethanolamine proposed by Dimick (1994, 1999). Accordingly, the less polar phospholipids (i.e., phosphatidylcholine and phosphatidylethanolamine) act as a nucleating agent for TAGS through the development of an inverse hexagonal mesophase (H_{II}), with trace amounts of water acting as the core.

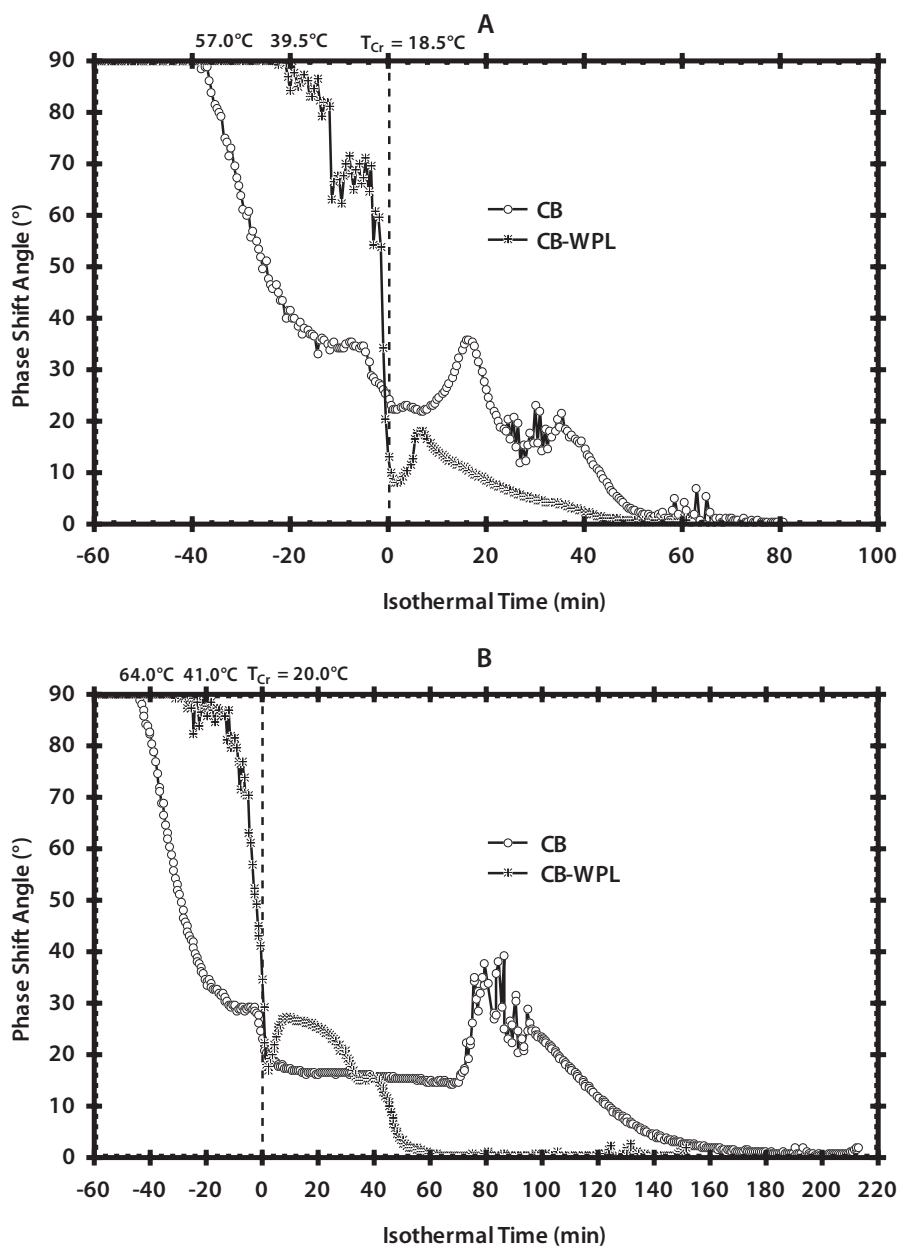


Fig. 16.1. Phase shift angle rheograms for the crystallization of CB and CB-WPL at T_{Cr} of 18.5°C (A) and 20.0°C (B). Data represent the means of two independent determinations. The onset of crystallization under non-isothermal conditions (i.e., before the dotted line) for CB and CB-WPL is shown. Source: Toro-Vazquez et al., 2005.

Therefore, without the presence of this type of phospholipids (i.e., CB-WPL), no inverse hexagonal mesophase would be developed in the cocoa butter melt, resulting in a requirement for higher thermodynamic drive (i.e., higher supercooling and therefore a lower temperature) to achieve TAG nucleation (Toro-Vazquez et al., 2005).

Considering that T_M° is the hypothetical melting temperature of an assembly of TAGS in equilibrium with TAG molecules in the melt, while the δ rheograms determine the temperature at which TAGS develop a lamellar organization that result in nucleation, both methodologies ought to provide similar nucleation temperatures. Thus, the T_M° for the 30% cocoa butter-soybean oil blend ($65.8^\circ\text{C} \pm 3.0^\circ\text{C}$) is in close agreement with the onset of nucleation for cocoa butter ($59.8^\circ\text{C} \pm 3.7^\circ\text{C}$) determined by rheometry. In the same way, the T_M° for the 30% cocoa butter-canola oil blend ($33.7^\circ\text{C} \pm 4.9^\circ\text{C}$) is of similar magnitude order that the nucleation temperature for CB-WPL ($39.5^\circ\text{C} \pm 1.5^\circ\text{C}$) determined with the δ rheograms. Although refined soybean and canola oil were used by Pérez-Martínez et al. (2005) in their study, it is well known that refined soybean oil has higher phospholipid concentration than canola oil. Particularly, the kind of phospholipids mainly involved as TAG nucleating agent for cocoa butter (i.e., phosphatidylethanolamine and phosphatidylcholine) (Arruda & Dimick, 1991). Within this context, canola oil might produce a higher diluting effect of the native phospholipids present in cocoa butter than soybean oil, and from here the similarity between the T_M° for 30% cocoa butter-canola oil blend and the nucleation temperature for CB-WPL. In contrast, the higher concentration of phospholipids in soybean oil resulted in a lower diluting effect of native phospholipids of cocoa butter, and thus the similar values of T_M° for 30% cocoa butter-soybean oil blend and the nucleation temperature for cocoa butter. incidentally, under similar crystallization conditions, DSC measurements provided an onset of crystallization of 15.0°C ($\pm 0.0^\circ\text{C}$) in CB-WPL and of 15.5°C ($\pm 0.1^\circ\text{C}$) in cocoa butter. Evidently, the δ rheograms are more sensitive to detect the nucleating role of phospholipids than DSC (Toro-Vazquez et al., 2005). Nevertheless, the comparison between results must be done with caution since differences in weight or volume of samples used, equipment design and its impact on the thermodynamics of the system, and heat and mass transfer conditions existing in each measurement device affect crystallization to a different extent. Such factors must be kept in mind when comparing crystallization data obtained by rheometry and DSC thermograms.

On the other hand, in collaboration with Dr. Marangoni's group we recently investigated the pre-nucleation behavior of blends of 25% tripalmitin (TP) or 25% tristearin (TS) in triolein, high oleic safflower oil, or soybean oil using anisotropy, viscosity measurements, molecular mechanics simulations, and calculation of the activation free energy to develop a stable nucleus (ΔG_c°) (Dibildox-Alvarado et al., 2010a, 2010b). These studies showed that the presence of TAG molecules containing at least one chain of palmitic acid (i.e., POO, LLP, PLO) is a requisite to induce a molecular interaction through van der Waals forces with TP or TS, developing pre-nucleation structuring of TAGS. According to TAG composition (Dibildox-Alvarado et al., 2010a), no palmitic acid was present in triolein, while palmitic acid was present in high oleic safflower

oil and in soybean oil as LLP, PLO/POL, and POO. The results indicated that the molecular interactions between TP and TAGS with at least one palmitic acid from soybean and high oleic safflower oil occurred in the liquid phase during isothermal conditions before nucleation takes place. For TS blends, the molecular interactions occurred during the cooling stage well before attaining supercooling conditions required for TAGS nucleation. This as evaluated by the increase in anisotropy (i.e., liquid structure) observed before detecting crystal nucleation by DSC in the TP and TS blends with soybean and high oleic safflower oil (Dibildox-Alvarado et al., 2010b). This behavior was directly associated to the development of mixed TAGS lamellar liquid structures between TP or TS with LLP, PLO/POL, and POO, with the corresponding increase in the microviscosity of the blends. Interesting, the TP-triolein and TS-triolein blends did not show an increase in anisotropy before TP and TS nucleation (Dibildox-Alvarado et al., 2010b). The increase in anisotropy observed in the blends of TP and TS with soybean and high oleic safflower oil was larger as the concentration of LLP, POO, and PLO/POL increased in the blends, and thus was higher in the soybean oil blends, and lower in the high oleic safflower blends. Since triolein had a purity higher than 99% and therefore no content of TAGS molecules with palmitic acid, the corresponding TS and TP blends in triolein did not show any increase in the anisotropy value (Dibildox-Alvarado et al., 2010b). Within this framework, we observed that the higher T_M° showed by the 30% cocoa butter-soybean oil blend might be associated with the higher concentration of TAGS with at least one palmitic acid present in soybean oil (i.e., 32.74%; Table 16-A). This, in contrast with the lower concentration of TAGS with palmitic acid (i.e., 8.59%; Table 16-A) present in canola oil with the corresponding lower T_M° showed by the 30% cocoa butter-canola oil blend. These results suggest that the major symmetrical TAGS from cocoa butter (i.e., POP, POSt, StOSt) might interact with TAGS with palmitic acid present in the vegetable oils, resulting in the development of a mixed TAGS liquid structure. Since TAGS with at least one palmitic acid were present in higher concentration in soybean oil (i.e., 32.74%) than in canola oil (i.e., 8.59%), T_M° was higher in the 30% cocoa butter-soybean oil blend than in the 30% cocoa butter-canola oil blend. This pointed out that TAG molecules might developed a liquid structural organization at a higher temperature (i.e., higher T_M°) in the 30% cocoa butter-soybean oil blend than in the 30% cocoa butter-canola oil blend.

Supercooling, the difference between the temperature of crystallization (T_{Cr}) and the melting temperature (i.e., T_M°), is the thermodynamic drive for crystallization. The results reported in the literature using simple and complex blends of TAGS indicate that the molecular relationships occurring in the liquid phase among the symmetrical TAGS from cocoa butter and the unsaturated TAG from the vegetable oils, the phospholipid type and concentration present in both cocoa butter and the vegetable oil, and also the concentration of TAGS with at least one palmitic acid in the vegetable oil are factors that determine the liquid structural organization of TAGS, and from here the thermodynamic condition (i.e., $T_M^\circ - T_{Cr}$) driving TAGS crystallization kinetics. The resulting physical properties of crystallized cocoa butter-vegetable oil blends, in

turn determine the functionality (i.e., rheology, liquid phase entrapment, mouthfeel) of products such as *trans*-free fillings and coatings.

Supercooling and Cooling Rate Effect on Crystallization of Cocoa Butter-Vegetable Oil Blends

Functional properties of fat-based products (i.e., *trans*-free fillings and coatings based on cocoa butter-vegetable oil blends) are mainly determined by the SFC and the crystal habit of TAGS, including the polymorphic phase, crystal size and shape, and the three-dimensional TAG crystal organization that physically entraps the liquid phase. Crystal habit is determined by the thermodynamic condition, heat, mass, and momentum transfer conditions prevalent during crystallization. Within this framework, Campos et al. (2010) studied the presence of small concentrations (i.e., 1% and 5%) of 1, 2, 3-tristearoyl-glycerol (SSS) or 1, 2, 3-trilinoleoyl-glycerol (LLL) on crystal habit, crystallization behavior, microstructure, and textural properties of cocoa butter. Previous to this study, using a more complex system our group investigated the effect of T_{Cr} (i.e., 9.5°C to 13.5°C) and cooling rate (i.e., 1°C/min and 5°C/min) on the SFC, the crystallization kinetics (i.e., the crystallization rate constant, z), and the three-dimensional organization (i.e., fractal dimension, D) developed by TAGS in blends of cocoa butter with canola or soybean oil (Pérez-Martínez et al., 2007). Several authors have investigated the crystallization kinetics of multicomponent TAGS systems under different crystallization conditions, including cooling rate (Campos et al., 2002; deMan, 1964; Herrera & Hartel, 2000a, 2000b, 2000c; Humphrey & Narine, 2007; Litwinenko et al., 2002). However, Pérez-Martínez et al. (2007) and Campos et al. (2010) studied the crystallization kinetics of cocoa butter using model systems where different extent of molecular interactions and compatibility occurred between the major TAGS present in the crystallizing phase (i.e., cocoa butter) and other TAGS intentionally added to the system as a solvent phase (i.e., unsaturated TAGS from canola or soybean oil) or in minor quantities (i.e., SSS or LLL).

In the study of Pérez-Martínez et al. (2007), although similar T_{Cr} values were used to investigate the crystallization behavior of cocoa butter blended with vegetable oils, given the different extent of molecular compatibility between the symmetrical TAGS of cocoa butter and the major TAGS in soybean oil (i.e., LLL, LLO and LLP) or canola oil (i.e., OOO and LOO) (Table 16-A), each type of cocoa butter blend had a particular T_M° . The overall result is that supercooling (i.e., $T_{Cr} - T_M^\circ$) was higher in the 30% cocoa butter-soybean oil than in the 30% cocoa butter-canola oil. The results of this investigation (Pérez-Martínez et al., 2007) showed that, at the different T_{Cr} and cooling rates investigated, cocoa butter crystallized and melted in the 30% cocoa butter-vegetable oil blends (Figs. 16.2 and 16.3) following the same crystallization and melting behavior as the one observed by pure cocoa butter (Toro-Vazquez et al., 2004).

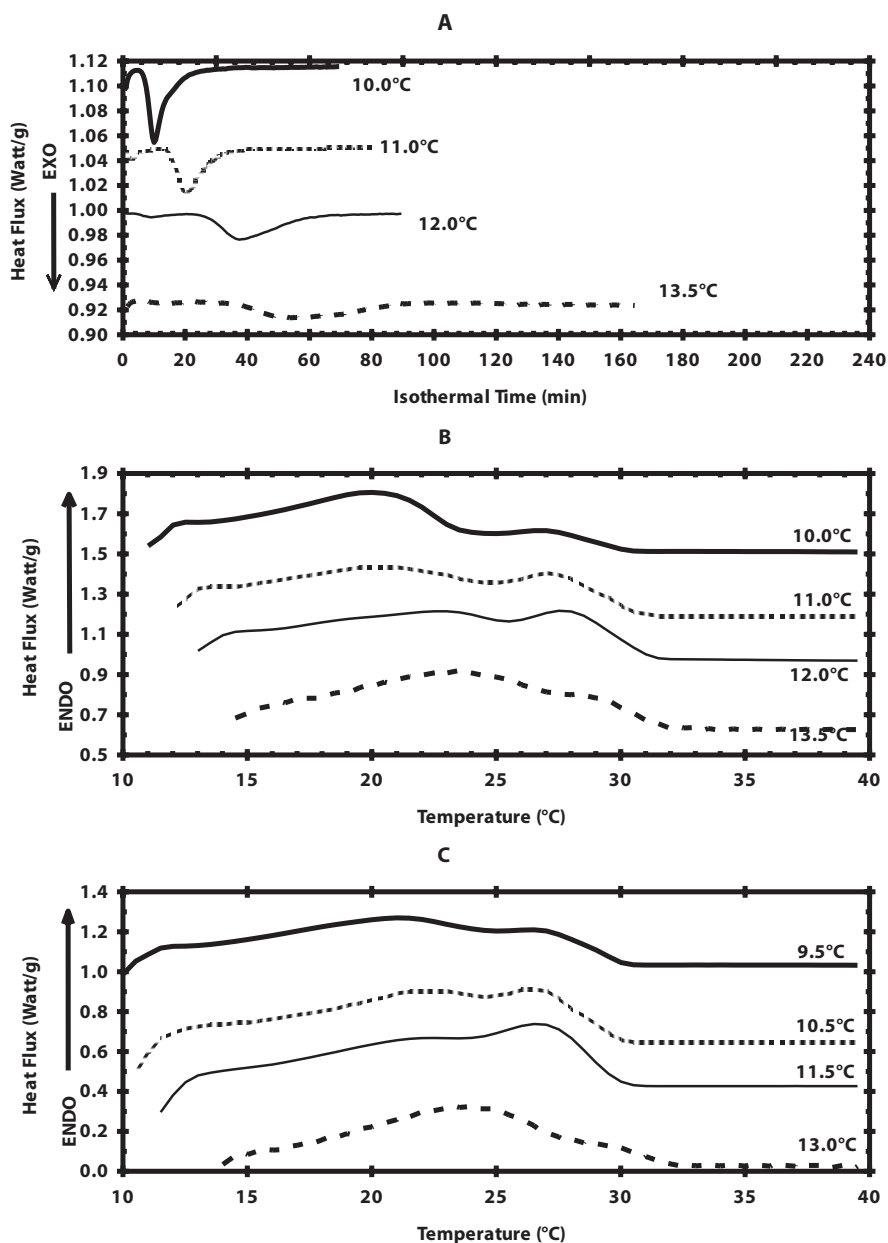


Fig. 16.2. Isothermal crystallization thermograms for the 30% CB-soybean oil blend at different T_{Cr} using a cooling rate of $1^{\circ}\text{C}/\text{min}$ (A). Melting of the 30% CB-soybean oil blend (B) and the 30% CB-canola oil blend (C) after crystallization at the corresponding T_{Cr} using a cooling rate of $1^{\circ}\text{C}/\text{min}$. Modified from Pérez-Martínez *et al.*, 2007.

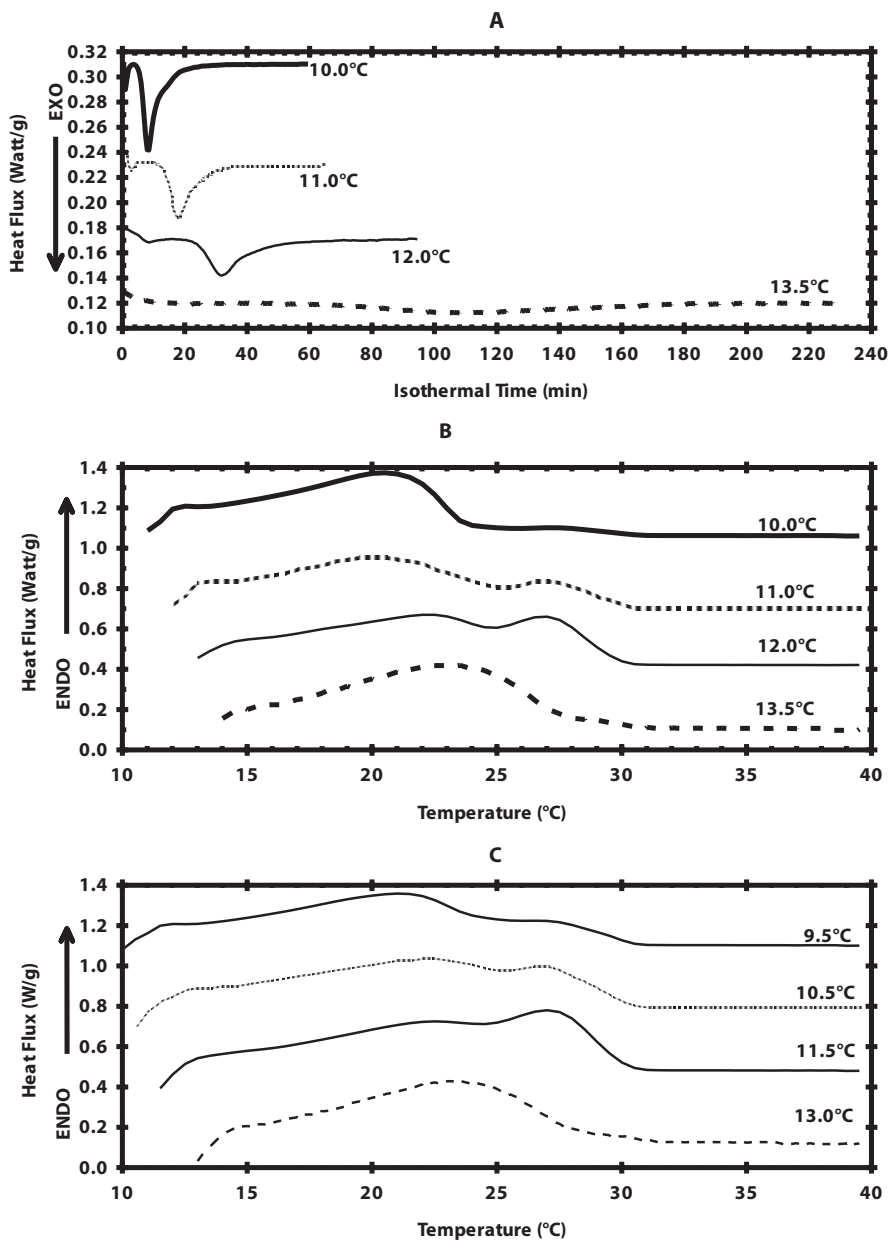


Fig. 16.3. Isothermal crystallization thermograms for the 30% CB-soybean oil blend at different T_c using a cooling rate of 5°C/min (A). Melting of the 30% CB-soybean oil blend (B) and the 30% CB-canola oil blend (C) after crystallization at the corresponding T_c using a cooling rate of 5°C/min. Modified from Pérez-Martínez et al., 2007.

This showed that highly unsaturated TAGS do not co-crystallize with TAGS from cocoa butter, observation later confirmed by Campos et al. (2010) using X-ray analysis in blends of LLL with cocoa butter. thus, as with pure cocoa butter the first exotherm was assigned to the α polymorph and the second exotherm to the β' polymorph (Figs. 16.2 and 16.3). In correspondence, independent of the cooling rate used for crystallization, the first endotherm observed in the melting thermograms was assigned to the β' melting and the second endotherm to β polymorph melting (Figs. 16.2B and 16.2C; Figs. 16.3B and 16.3C) (Pérez-Martínez et al., 2007). Given the lower viscosity prevalent in the cocoa butter-vegetable oil blends in comparison with cocoa butter melt, molecular mobility is enhanced accelerating polymorphic transformations in comparison with the ones occurring in the cocoa butter melt. Campos et al. (2010) later corroborated this observation comparing the rate for the β' to β transition in cocoa butter and in cocoa butter with 1% and 5% of LLL (Fig. 16.4). Therefore, in the cocoa butter-vegetable oil blends the β polymorph was easily developed mainly

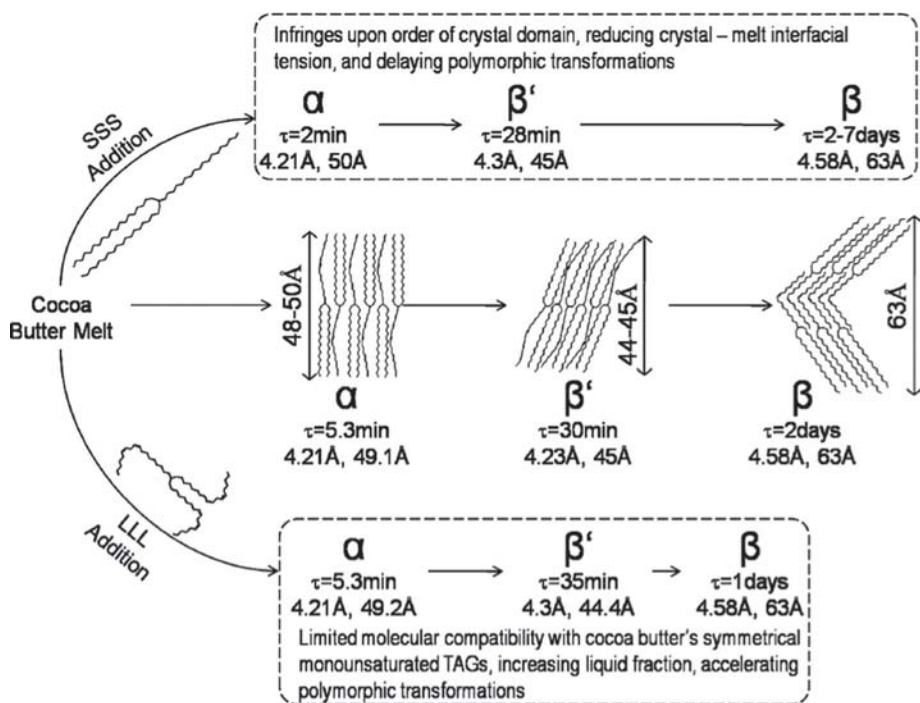


Fig. 16.4. Schematic representation of CB's crystallization, in its native state and upon addition of either saturated tristearin (SSS) or unsaturated trilinoleate (LLL) at 20°C. Indicated are times (τ) in which each polymorphic form was observed with X-ray diffraction, as well as their short and long spacings. Source: Campos et al., 2010.

through the $\alpha \rightarrow \beta' \rightarrow \beta$ polymorphic transition. As in cocoa butter, the presence of the α polymorph was necessary during crystallization of cocoa butter-vegetable oil blends for the formation of β crystals via the $\alpha \rightarrow \beta' \rightarrow \beta$ polymorphic transition since TAGS from cocoa butter do not nucleate directly from the melt into the β phase (Marangoni & McGauley, 2002).

From the above, it is evident that Pérez-Martínez et al. (2007) did not observe relevant differences in the crystallization and melting behavior between the 30% cocoa butter-soybean oil and the 30% cocoa butter-canola oil blends as evaluated by DSC. In the same way, the microscopic observation of the crystallized blends showed similar behavior between the blends. Figs. 16.5 and 16.6 show polarized light microphotographs of crystallized 30% cocoa butter-soybean oil and 30% cocoa butter-canola oil blends obtained at crystallization times where an equilibrium SFC value was achieved at 1°C/min and 5°C/min (i.e., 30 min after the SFC measurements achieved a sustained plateau). Thus, in both type of blends and independent of cooling rate, as T_{Cr} decreased and SFC increased the overall TAG crystal size decreased (Figs. 16.5 and 16.6). However, under certain supercooling conditions (i.e., T_{Cr} between 11.5°C and 13.5°C) TAG crystals with two different sizes, identified as small β' and large β crystals, were present in the crystallized blends (Figs. 16.5C to 16.5F; Figs 16.6C to 16.6F). Additionally, the authors observed in the cocoa butter-vegetable oil blends that decreasing T_{Cr} (i.e., increasing supercooling) at both cooling rates produced a decrease in β' crystal size and the large β crystals became less evident until, at the highest supercooling conditions (i.e., T_{Cr} of 9.5°C and 10°C) just aggregates of very small β' crystals were observed (Figs. 16.5A and 16.5B; Figs. 16.6A and 16.6B). In contrast, as T_{Cr} increased above 10°C β crystals became more evident and dramatically showed an increase in size (Pérez-Martínez et al., 2007) (Figs. 16.5C to 16.5F; Figs 16.6C to 16.6F). It is important to consider that the common crystal morphology observed with pure cocoa butter was not observed in the cocoa butter-vegetable oil blends, mainly because the lower viscosity present in the blends reduced the higher limitation of mass transfer present during crystallization of cocoa butter.

In spite the similarities in the crystallizing behavior between the 30% cocoa butter-soybean oil and the 30% cocoa butter-canola oil blend, a more detailed analysis of the results obtained by Pérez-Martínez et al. (2007) showed that T_{Cr} and cooling rate had a differential effect on SFC, the crystallization kinetics (i.e., z) (Table 16-B and Table 16-C), and the relationship between SFC and the rheological properties of the crystallized blends. The overall crystallization rate constant (z) and the Avrami index (n) included in Table 16-B and Table 16-C, were determined by the authors (Pérez-Martínez et al., 2007) by fitting the SFC values as a function of time to the Avrami equation. z is a parameter that includes the nucleation and crystal growth rate, while n is function of the time dependence of crystal nucleation (i.e., sporadic or instantaneous) and the dimensionality of the crystal growth process. Thus, the n values showed that, independent of the T_{Cr} TAGS of cocoa butter followed a spherulitic crystal growth mechanism in both blends.

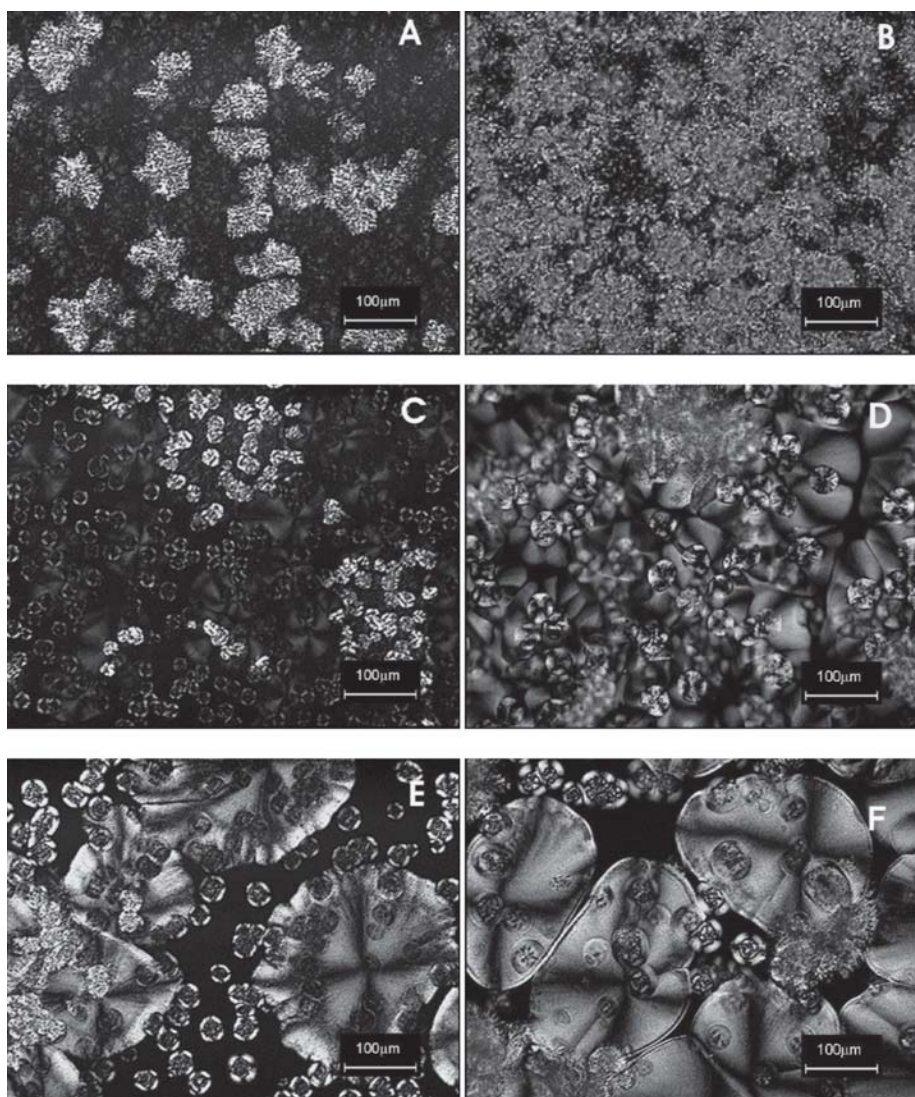


Fig. 16.5. Polarized light microphotographs for the 30% CB-canola oil blend crystallized using a cooling rate of 1°C/min (A, C, E) and 5°C/min (B, D, F) at 9.5°C [after 90 min(A) or 120 min (B)], 11.5°C [after 190 min (C) or 240 min (D)], and 13°C [after 450 min (E) or 680 min (F)]. The minutes indicate the crystallization time under isothermal conditions. Source: Pérez-Martínez et al., 2007).

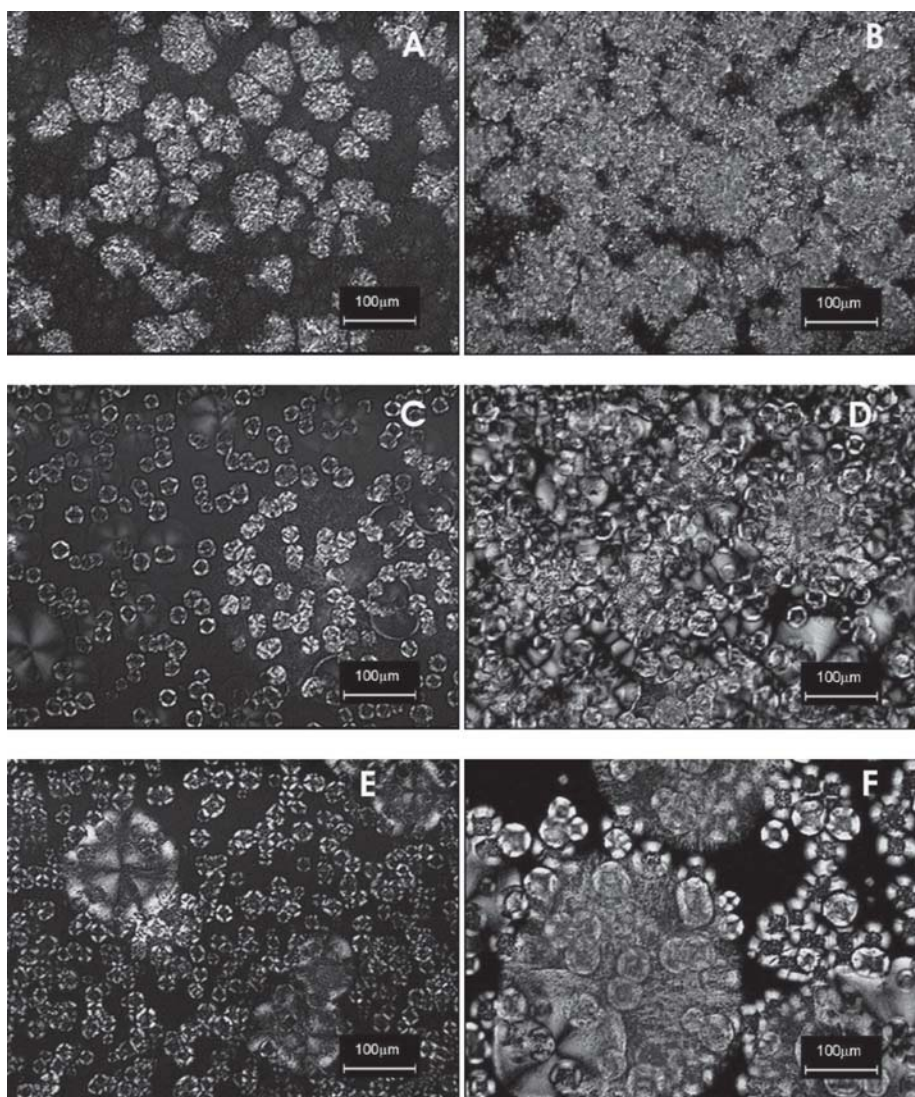


Fig. 16.6. Polarized light microphotographs for the 30% CB-soybean oil blend crystallized using a cooling rate of 1°C/min (A, C, E) and 5°C/min (B, D, F) at 10°C [after 140 min (A) or 120 min (B)], 12°C [after 220 min (C) or 240 min (D)], and 13.5°C [after 420 min (E and F)]. The minutes indicate the crystallization time under isothermal conditions. Source: Pérez-Martínez et al., 2007.

Table 16-B. Solid Fat Content (SFC), Crystallization Rate Constant (z), Avrami Index (n), and Fractal Measurement (D) for the 30% CB/Soybean Oil Blend as a Function of Crystallization Temperatures (T_{cr}) Achieved at the Cooling Rates of 1°C/min and 5°C/min.

T_{cr} (°C)	SFC (%)	$z \times 10^{-7}$ (min ⁻ⁿ)	n	D
<i>1 °C/min</i>				
10	26.3 (1.0) ^b	102.1 (12.4) ^b	3.34 (0.26) ^b	2.79 (0.02) ^b
11	25.2 (0.9) ^b	19.41 (1.07) ^b	3.25 (0.20) ^b	2.77 (0.02) ^b
12	24.2 (0.9) ^b	31.20 (4.39) ^b	3.42 (0.81) ^b	2.78 (0.01) ^b
13	22.1 (0.4) ^b	0.024 (0.001) ^b	4.16 (0.25) ^b	2.76 (0.01) ^b
<i>5 °C/min</i>				
10	25.4 (1.1) ^b	645.9 (75.4) ^c	3.07 (0.45) ^b	2.86 (0.01) ^c
11	24.5 (1.4) ^b	8.91 (2.84) ^c	3.66 (0.11) ^b	2.82 (0.00) ^c
12	22.6 (0.8) ^b	0.01 (0.00) ^c	4.33 (0.01) ^b	2.80 (0.01) ^b
13	21.8 (1.2) ^b	0.01 (0.00) ^b	4.02 (0.07) ^b	2.78 (0.01) ^b

^aFor all measurements we show the mean and standard deviation of at least two independent determinations.

^{b,c}Within each column and same T_{cr} values with the same letter are statistically the same at the two cooling rates. A different letter indicates statistical difference ($P < 0.05$).

Adapted from Pérez-Martínez et al. 2007.

Table 16-C. Solid Fat Content (SFC), Crystallization Rate Constant (Z), Avrami Index (N), and Fractal Measurement (D) for the 30% CB/Canola Oil Blend as a Function of Crystallization Temperatures (T_{cr}) Achieved at the Cooling Rates of 1°C/min and 5°C/min.

T_{cr} (°C)	SFC (%)	$z \times 10^{-7}$ (min ⁻ⁿ)	n	D
<i>1 °C/min</i>				
9.5	24.1 (0.2) ^b	352.7 (15.5) ^b	2.82 (0.39) ^b	2.84 (0.00) ^b
11	23.5 (0.0) ^b	4.76 (0.273) ^b	3.44 (0.11) ^b	2.80 (0.05) ^b
12	22.4 (0.0) ^b	1.42 (0.195) ^b	3.59 (0.44) ^b	2.45 (0.04) ^b
13	20.5 (0.0) ^b	0.030 (0.002) ^b	3.60 (0.04) ^b	1.66 (0.37) ^b
<i>5 °C/min</i>				
9.5	24.5 (0.6) ^b	5375.8 (17.4) ^c	2.44 (0.05) ^b	2.85 (0.02) ^b
11	23.5 (0.9) ^b	1.67 (0.22) ^c	4.23 (0.32) ^c	2.83 (0.01) ^b
12	22.6 (0.5) ^b	0.01 (0.001) ^c	4.40 (0.10) ^c	2.62 (0.04) ^b
13	20.9 (1.2) ^b	0.01 (0.007) ^b	3.50 (0.13) ^b	1.72 (0.24) ^b

^aFor all measurements we show the mean and standard deviation of at least two independent determinations.

^{b,c}Within each column and same T_{cr} values with the same letter are statistically the same at the two cooling rates. A different letter indicates statistical difference ($P < 0.05$).

Adapted from Pérez-Martínez et al., 2007.

Additionally, the fractional values of n (Table 16-B and Table 16-C) observed in both type of blends seemed to be in line with the simultaneous development of TAG crystals with two different sizes (i.e., small β' and large β crystals; Figs. 16.5C to 16.5F; Figs 16.6C to 16.6F). In the same way, for both cocoa butter-vegetable oil blends the authors did not find a significant effect of cooling rate on the SFC (Table 16-B and Table 16-C). Nevertheless, although slightly higher (i.e., 0.5°C higher) T_{Cr} 's were used with the 30% cocoa butter-soybean oil blend, independent of the cooling rate used higher SFC were achieved with this blend than with the 30% cocoa butter-canola oil blend. additionally, the behavior of z showed that crystallization occurred at the fastest rate at the lower T_{Cr} , particularly at the cooling rate of 5°C/min and with the 30% cocoa butter-canola oil blend (Table 16-B and Table 16-C). However, as T_{Cr} increased and cooling rate decreased higher z values were obtained with the 30% cocoa butter-soybean oil blend (Table 16-B). These results confirmed that higher supercooling conditions were prevalent in the cocoa butter-soybean oil blend than in the cocoa butter-canola oil blend (Pérez-Martínez et al., 2005, 2007). The different supercooling conditions were the result of the different extent of molecular compatibility between the major symmetrical TAG families of cocoa butter and the major unsaturated TAG families present in each vegetable oil, as affected by T_{Cr} and cooling rate.

The main difference observed by Pérez-Martínez et al. (2007) between the cocoa butter-vegetable oil blends was associated with the relationship between the rheological properties (i.e., viscoelasticity, G') and the SFC. Thus, as observed by other authors (Herrera & Hartel, 2000a), a positive significant linear relationship between $\text{Log}(G')$ and $\text{log}(\text{SFC})$ was observed at both cooling rates but just with the 30% cocoa butter-soybean oil blend. With the 30% cocoa butter-canola oil blend, a peculiar behavior was observed since as T_{Cr} increased (i.e., supercooling decreased) and SFC decreased G' showed an increment. Under such low supercooling conditions (i.e., T_{Cr} of 13°C) achieved at both cooling rates, the crystallized 30% cocoa butter-canola oil blend was formed by a mixture of small β' and large β crystals (Figs. 16.5E and 16.5F) providing high viscoelasticity (i.e., G') and resistance to flow (i.e., yield stress, σ^*) at low SFC (i.e., 20.5–20.9%) and D (i.e., 1.66–1.72) values. The 30% cocoa butter-soybean oil blend achieved similar G' and σ^* values independent of cooling rate only at high supercooling conditions (i.e., T_{Cr} of 10°C). In this case, the crystal network was formed mainly by small β' crystals (Figs. 16.6A and 16.6B) with SFC (i.e., 25.4–26.3%) and D (i.e., 2.86–2.79) values higher than in the 30% cocoa butter-canola oil blend at low supercooling (Pérez-Martínez et al., 2007). The authors concluded that the rheological behavior observed by the cocoa butter-vegetable oil blends was the result of a cooperative structural effect derived from the right proportion between β' and β crystals and the right β' to β crystal size ratio. Thus, cocoa butter-vegetable oil blends might provide high G' and σ^* values even with low SFC and D values (Pérez-Martínez et al., 2007). These results agree with the ones obtained with cocoa butter by Brunello et al. (2003). Based on a model developed to estimate G' , these authors concluded that the mechanical properties of cocoa butter are not

only governed by the amount of SFC, but also by the three-dimensional organization of the crystal network (i.e., D) as affected by polymorphism (Brunello et al., 2003).

On the other hand, the results obtained by Campos et al. (2010) showed that the addition of small proportion of specific TAGS (i.e., SSS or LLL) to cocoa butter modified the native saturation conditions of the cocoa butter melt, affecting its crystallization kinetics and crystal habit. Thus, the results of this investigation showed that the high melting saturated SSS added to cocoa butter decreased the supercooling conditions required for crystallization, reducing the temperature and time for cocoa butter crystal nucleation. Additionally, Campos et al. (2010) concluded that given the straighter spatial structure of SSS relative to cocoa butter's unsaturated symmetric TAGS (i.e., which have bends in the unsaturated oleic acid), its inclusion in the crystal structure modifies the crystal domain, reducing the crystal-melt interfacial tension. The overall result is a delay in the polymorphic transition from metastable forms into the stable β form as determined by wide and small angle X-ray diffraction done at 20°C and 24°C (Campos et al., 2010). Thus, despite the lower induction times for crystallization observed by blends of cocoa butter with 1% or 5% of SSS, the presence of this fully saturated TAG delayed polymorphic transformations in cocoa butter. In contrast and in agreement with the results obtained by Pérez-Martínez et al. (2007), in cocoa butter added with 1% or 5% of LLL, the limited molecular compatibility between the major TAGS of cocoa butter and the fully unsaturated LLL avoided the co-crystallization process. This resulted in an increase in the liquid fraction that enhanced TAGS molecular mobility and, therefore, the β' to β polymorphic transition occurred at a faster rate and larger crystals were observed in comparison with the crystals developed by cocoa butter in the presence of SSS (Campos et al., 2010). A schematic representation of these events in native cocoa butter and cocoa butter blended with SSS or LLL is shown Fig. 16.4.

Conclusions

In the elaboration of compound coatings used in confectionery products, cocoa butter is used in combination with other vegetable oils such as hydrogenated or partially hydrogenated soybean oil and palm oil. In other cases, fully refined vegetable oils and fats might be blended with cocoa butter in the development of low-*trans* or *trans*-free chocolate fillings and coatings. The results obtained in simple and complex model systems, showed that in cocoa butter-vegetable oil blends the molecular interactions occurring among TAG families determine the thermodynamic conditions (i.e., supercooling) that drive the formation of a solid (i.e., crystal) in a liquid phase and the phase behavior of the solid phase. In particular, the prevalent supercooling condition in cocoa butter-vegetable oil blends is the result of the different extent of molecular compatibility between the major symmetrical TAG families of cocoa butter and the major unsaturated TAG families present in each vegetable oil. Overall, the higher the structural compatibility between the major TAGS of the vegetable oil with the

symmetrical TAGS of cocoa butter, the more the native liquid structural organization developed by TAGS of cocoa butter is altered. Thus, blends of canola oil with cocoa butter have lower equilibrium melting temperature (i.e., T_M°) and, therefore, lower supercooling (i.e., $T_M^\circ - T_{Cr}$) than cocoa butter-soybean oil blends. Additional molecular interactions involved in the establishment of the liquid structural organization of TAGS in cocoa butter-vegetable oil blends, and therefore in the thermodynamic condition (i.e., $T_M^\circ - T_{Cr}$) driving TAGS crystallization kinetics include, the phospholipids' type and concentration present in both cocoa butter and the vegetable oil, and the concentration of TAGS with at least one palmitic acid in the vegetable oil.

The results discussed indicate that the molecular interactions occurring between TAGS in cocoa butter-vegetable oil blends determine crystal habit, crystallization kinetics, rate of polymorphic transitions, microstructure, and the mechanical properties that in turn determine the functionality (i.e., liquid phase entrapment, mouthfeel) of *trans*-free fillings and coatings. However, in industrial crystallizers (i.e., scraped surface crystallizers) crystallization of cocoa butter-vegetable oil blends would take place under a different thermodynamic scenario involving sinusoidal temperature fluctuations, secondary nucleation, and crystal attrition promoted by shearing.

References

- Alia, A.R.; Embonga, M.S.; Omare, H.; Oh Flingohb, C.H. Effect of Oleo-Disaturated Triacylglycerol Content on Properties of Palm Mid Fraction, Sal Stearin and Borneo Tallow Blends, *J. Am. Oil Chem. Soc.* 1991, 68, 328–331. DOI: 10.1007/BF02657687
- Alia, A.R.; Oh Flingoh, C.H. Effect of Cocoa Butter on Compatibility of Specialty Fats. *Elaeis* 1993, 5, 86–91.
- Arruda, D.H.; Dimick, P.S. Phospholipid Composition of Lipid Seed Crystal Isolates from Ivory Coast Cocoa Butter. *J. Am. Oil Chem. Soc.* 1991, 68, 385–390. DOI: 10.1007/BF02663754
- Braipson-Danthine, S.; Deroanne, C. Determination of Solid Fat Content (SFC) of Binary Fat Blends and Use of These Data to Predict SFC of Selected Ternary Fat Blends Containing Low-Erucic Rapeseed Oil. *J. Am. Oil Chem. Soc.* 2006, 83, 571–581. DOI: 10.1007/s11746-006-1242-7
- Brunello, N.; McGauley, S.E.; Marangoni, A.G. Mechanical Properties of Cocoa Butter in Relation to its Crystallization Behavior and Microstructure. *Lebensm. Wiss. Technol.* 2003, 36, 525–532. DOI:10.1016/S0023-6438(03)00053-7
- Calliau, G.; Ayala, J. V.; Gibon, V.; Wouters, J.; De Greyt, W.; Foubert, I.; Dewettinck, K.. Models for FFA-removal and Changes in Phase Behavior of Cocoa Butter by Packed Column Steam Refining. *J. Food Eng.* 2008, 89, 274–284.
- Campos, R.; Narine, S. S.; Marangoni, A. G. Effect of Cooling Rate on the Structure and Mechanical Properties of Milk Fat and Lard. *Food Res. Int.* 2002, 35, 971–981. PII: S0963–9969(02)00159-X
- Campos, R.; Ollivon, M.; Marangoni, A.G. Molecular Composition Dynamics and Structure of Cocoa Butter. *Cryst. Growth Des.* 2010, 10, 205–217. DOI: 10.1021/cg900853e

- Cebula, D.J.; McClements, D.J.; Povey, M.J.W.; Smith, P.R. Neutron Diffraction Studies of Liquid and Crystalline Trilaurin. *J. Am. Oil. Chem. Soc.* 1992, 69, 130–136. DOI:10.1007/BF02540562
- Corkery, R.W.; Rousseau, D.; Smith, P.; Pink, D.A.; Hanna, C.B.; A Case for Discotic Liquid Crystals in Molten Triglycerides. *Langmuir* 2007, 23, 7241–7246. PMID: 17511482
- deMan, J. M.. Effect of Cooling Procedure on Consistency, Crystal Structure and Solid Fat Content of Milk Fat. *Dairy Industr.* 1964, 29, 244–246.
- Dibildox-Alvarado, E.; Laredo, T.; Toro-Vazquez J.F.; Marangoni A. Pre-Nucleation Structuring of TAG Melts Revealed by Fluorescence Polarization Spectroscopy and Molecular Mechanics. *J. Am. Oil Chem. Soc.* 2010a, 87, 1115–1125. DOI 10.1007/s11746-010-1596-8.
- Dibildox-Alvarado, E.; Marangoni, A. G.; Toro-Vazquez, J. F. Pre-nucleation Structuring of Triacylglycerides and Its Effect on the Activation Energy of Nucleation. *Food Biophys.* 2010b 5, 218–226. DOI 10.1007/s11483-010-9163-2
- Dimick, P. S. Influence de la Composition sur la Cristallisation du Beurre de Cacao. *La Cristallization*, 3ième Colluge 1994, 33–46.
- Dimick, P. S. Compositional Effect on Crystallization of Cocoa Butter. *Physical Properties of Fats, Oils and Emulsifiers*. Widlak, N. Ed.; AOCS Press: Champaign, IL, USA, 1999; 140–163.
- Fessas, D.; Signorelli, M.; Schiraldi, A. Polymorphous Transitions in Cocoa Butter. A Quantitative DSC Study. *J Therm Anal Calorim.* 2005, 82:691–702. DOI: 10.1007/s10973-005-0952-7
- Foubert, I.; Vanrolleghem, P.A.; Thas, O.; Dewettinck, K.; Influence of Chemical Composition on the Isothermal Cocoa Butter Crystallization, *J. Food Sci.* 2004, 69, E478–E487. DOI: 10.1111/j.1365-2621.2004.tb09933.x
- Guthrie, S.E.; Mazzanti, G.; Idziak, S.H.J. X-ray Phase Identification of Chocolate is Possible without the Removal of Sugar. *Eur. J. Lipid Sci. Technol.* 2005, 107:656–659. DOI:10.1002/ejlt.200501178
- Hernqvist, L. On the Structure of Triglycerides in the Liquid State and Fat Crystallization. *Fette, Seifen, Anstrichmittel* 1984, 86, 297–300. DOI: 10.1002/lipi.19840860802
- Herrera, M. L.; Hartel, R. W. Effect of Processing Conditions on the Crystallization Kinetics of Milk Fat Model Systems. *J. Am. Oil Chem. Soc.* 2000a, 77, 1177–1187. DOI: 10.1007/s11746-000-0184-4.
- Herrera, M. L.; Hartel, R. W. Effect of Processing Conditions on the Crystallization Kinetics of Milk Fat Model Systems: Rheology. *J. Am. Oil Chem. Soc.* 2000b, 77, 1189–1195. DOI: 10.1007/s11746-000-0185-3
- Herrera, M. L.; Hartel, R. W. Effect of Processing Conditions on the Crystallization Kinetics of Milk Fat Model Systems: Microstructure. *J. Am. Oil Chem. Soc.* 2000c, 77, 1197–1204. DOI: 10.1007/s11746-000-0186-2
- Hoffman, J.D.; Weeks, J.J. Melting Process and the Equilibrium Melting Temperature of Polychlorotrifluoroethylene, *J. Res. Natl. Bur. Std.* 1962, 66, 13–28.
- Hogenbirk, G. Compatibility of Specialty Fats with Cocoa Butter. *Manuf. Confect.* 1984, 64:59–63.

- Humphrey, K. L.; Narine, S. S. Diminishing Marginal Utility of Cooling Rate Increase on the Crystallization Behavior and Physical Properties of a Lipid Sample. *J. Am. Oil Chem. Soc.* 2007, 84, 709–716. DOI 10.1007/s11746-007-1092-y
- Kinta, Y.; Hartel, R. W. Bloom Formation on Poorly-Tempered Chocolate and Effects of Seed Addition. *J. Am. Oil Chem. Soc.* 2010, 87, 19–27. DOI: 10.1007/s11746-009-1473-5
- Larsson, K. Molecular Arrangement in Glycerides. *Fette, Seifen, Anstrichmittel* 1972, 74, 136. DOI:10.1002/lipi.19720740302
- Larsson, K. On the Structure of the Liquid State of Triglycerides. *J. Am. Oil Chem. Soc.* 1992, 69, 835–836. DOI:10.1007/BF02635928
- Le Révérend B. J. D.; Fryer P.J.; Coles S.; Bakalis S. A Method to Qualify and Quantify the Crystalline State of Cocoa Butter in Industrial Chocolate. *J. Am. Oil Chem. Soc.* 2010, 87, 239–246. DOI: 10.1007/s11746-009-1498-9
- Lipp, M.; Simoneau, C.; Ulberth, F.; Anklam, E.; Crews, C.; Brereton, P.; de Greyt, W.; Schwack, W.; Wiedmaier, C. Composition of Genuine Cocoa Butter and Cocoa Butter Equivalents. *J. Food Compos. Anal.* 2001, 14, 399–408. DOI:10.1006/jfca.2000.0984
- Litwinenko, J.W.; Rojas, A.M.; Gerschenson, L.N.; Marangoni, A.G. Relationship Between Crystallization Behavior, Microstructure, and Mechanical Properties in a Palm Oil-Based Shortening. *J. Am. Oil Chem. Soc.* 2002, 79, 647–654. DOI: 10.1007/s11746-002-0538-y
- Loisel, C.; Keller, G.; Lecq, G.; Bourgaux, C.; Ollivon, M. Phase Transitions and Polymorphism of Cocoa Butter. *J. Am. Oil Chem. Soc.* 1998, 75, 425–439. DOI: 10.1007/s11746-998-0245-y
- Lonchampt, P.; Hartel, R. W. Surface Bloom on Improperly Tempered Chocolate. *Eur. J. Lipid Sci. Tech.* 2006, 108, 159–168. DOI:10.1002/ejlt.200500260
- Lovegren, N.V.; Gray, M.S.; Feuge, R.O. Polymorphic Changes in Mixtures of Confectionery Fats. *J. Am. Oil Chem. Soc.* 1976, 53, 83–88. DOI: 10.1007/BF02637399
- Marangoni, A. G.; McGauley, S. E. Relationship Between Crystallization Behavior and Structure in Cocoa Butter. *Cryst. Growth Des.* 2002, 3, 95–108. DOI: 10.1021/cg025580l
- Marangoni, A. G.; Rousseau, D. Is Plastic Fat Rheology Governed by the Fractal Nature of the Fat Crystal Network? *J. Am. Oil Chem. Soc.* 1996, 73, 991–994. DOI: 10.1007/BF02523406
- Marangoni, A.G.; Lencki, R.W. Ternary Phase Behavior of Milk Fat Fractions. *J. Agric. Food Chem.* 1998, 46, 3879–3884. DOI: 10.1021/jf9801668
- Marty, S.; Marangoni, A. G. Effects of Cocoa Butter Origin, Tempering Procedure, and Structure on Oil Migration Kinetics. *Cryst. Growth Des.* 2009, 9, 4415–4423. DOI: 10.1021/cg9004505
- Mazzanti, G.; Guthrie, S.E.; Marangoni, A.G.; Idziak, S.H.J. A Conceptual Model for Shear-Induced Phase Behavior in Crystallizing Cocoa Butter. *Cryst. Growth Des.* 2007, 7, 1230–1241. DOI: 10.1021/cg050467r
- Minato, A.; Ueno, S.; Smith, K.; Amemiya, Y.; Sato, K. Thermodynamic and Kinetic Study on Phase Behavior of Binary Mixtures of POP and PPO Forming Molecular Compound Systems. *J. Phys. Chem. B*, 1997, 101, 3498–3505. DOI: 10.1021/jp962956v

- Paulicka, F.R. Phase Behavior of Fats in Confectionery Coatings, Proceedings of the Annual Production Conference, 1970, 24, 43–46.
- Paulicka, F.R., Phase Behavior of Cocoa Butter Extender, *Chem. Ind.* 1973, 17, 835–839.
- Pérez-Martínez, D.; Álvarez-Salas, C.; Charó-Alonso, M.; Dibildox-Alvarado, E.; Toro-Vazquez, J.F. The Cooling Rate Effect on the Microstructure and Rheological Properties of Blends of Cocoa Butter with Vegetable Oils. *Food Res. Int.* 2007, 40, 47–62. DOI:10.1016/j.foodres.2006.07.016
- Pérez-Martínez, D.; Alvarez-Salas, C.; Morales-Rueda, J.A.; Toro-Vazquez, J. F.; Charó-Alonso, M.A.; Dibildox-Alvarado, E. The Effect of Supercooling on Crystallization of Cocoa Butter-Vegetable Oil Blends. *J. Am. Oil Chem. Soc.* 2005, 82, 471–479. DOI: 10.1007/s11746-005-1096-z
- Sato, K.; Koyano, T. Crystallization Properties of Cocoa Butter. *Crystallization Processes in Fats and Lipid Systems*. Garti, N.; Sato, K. Eds.; Marcel Dekker: New York, 2001; 429–456.
- Segall, S.D.; Artz, W.E.; Raslan, D.S.; Ferraz V.P.; Takahashi, J.A. Analysis of Triacylglycerol Isomers in Malaysian Cocoa Cutter using HPLC–Mass Spectrometry. *Food Res. Int.* 2005, 38, 167–174. DOI:10.1016/j.foodres.2004.09.008
- Smith, K. W.; Cain, F. W.; Talbot, G. Nature and Composition of Fat Bloom from Palm Kernel Stearin and Hydrogenated Palm Kernel Stearin Compound Chocolates, *J. Agric. Food Chem.* 2004, 52, 5539–5544. DOI: 10.1021/jf049401e
- Stapley, A.G.F.; Tewkesbury, H.; Fryer, P.J. The Effects of Shear and Temperature History on the Crystallization of Chocolate. *J. Am. Oil Chem. Soc.* 1999, 76, 677–685. DOI: 10.1007/s11746-999-0159-3
- Toro-Vazquez, J.F.; Briceño-Montelongo, M.; Dibildox-Alvarado, E.; Charó-Alonso, M.A.; Reyes-Hernández, J. Crystallization Kinetics of Palm Stearin in Blends with Sesame Seed Oil. *J. Am. Oil Chem. Soc.* 2000, 77, 297–310. DOI: 10.1007/s11746-000-0049-x
- Toro-Vazquez, J.F.; Pérez-Martínez, D.; Dibildox-Alvarado, E.; Charó-Alonsom M.; Reyes-Hernández, J. Rheometry and Polymorphism of Cocoa Butter Crystallization Under Static and Stirring Conditions. *J. Am. Oil Chem. Soc.* 2004, 81, 195–202. DOI: 10.1007/s11746-004-0881-z
- Toro-Vazquez, J.F.; Rangel-Vargas, E.; Dibildox-Alvarado, E.; Charó-Alonso, M.A. Crystallization of Cocoa Butter with and without Polar Lipids Evaluated by Rheometry, Calorimetry and Polarized Light Microscopy. *Eur. J. Lipid Sci. Tech.* 2005, 107, 641–655. DOI: 10.1002/ejlt.200501163
- Ueno, S.; Minato, A.; Yano, J.; Sato, K. Synchrotron Radiation X-ray Diffraction Study of Polymorphic Crystallization of SOS from Liquid Phase. *J. Cryst. Growth* 1999, 198, 1326–1329. DOI: 10.1016/S0022-0248(98)01018-5
- van Malssen, K.; van Langevelde, A.; Peschar, R.; Schenk, H. Phase Behavior and Extended Phase Scheme of Static Cocoa Butter Investigated with Real-Time X-ray Powder Diffraction, *J. Am. Oil Chem. Soc.* 1999, 76, 669–676. DOI: 10.1007/s11746-999-0158-4
- Wille, R.; Lutton, E. Polymorphism of Cocoa Butter. *J. Am. Oil Chem. Soc.* 1966, 43, 491–496. DOI: 10.1007

17

Methods and Technologies Related to Shea Butter Chemophysical Properties and to the Delivery of Bioactives in Chocolate and Related Products

Orit Segman, Zeev Wiesman, and Leonid Yarmolinsky

Phyto-Lipid Biotechnology Lab (PLBL), Department of Biotechnology Engineering, Faculty of Engineering Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

Introduction

The importance of natural vegetable oils and fats in nutrition and commerce have increased, because they are considered healthier sources of dietary energy and antioxidants and also supply bio-fuels and raw materials for the manufacture of industrial products (Okullo et al., 2010; Jatto et al., 2010). They are used in food, cosmetic, pharmaceutical and chemical industries (Badifu, 1993; Eke et al., 2004). Although the major raw materials for production of vegetable oil in the world are sunflower, palm, soya bean, and cotton seeds, the usefulness of indigenous and semi-domesticated plants and trees as vegetable oil source, is starting to percolate into the awareness and knowledge of agro-foresters, entrepreneurs, and the public (Leakey, 1999; Teklehaimanot, 2004; Dianda et al., 2009; Kante et al., 2009).

The shea butter tree [family Sapotaceae, *Vitellaria paradoxa* C.F. Gaertner, synonym *Butyrospermum parkii* (G. Don) Hepper], is indigenous to the wild and dry savannah belt of West Africa from Senegal in the west to Sudan in the east, and grows onto the foothills of the Ethiopian highlands. It occurs in 19 countries across the African continent (viz.: Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Ethiopia, Ghana, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo, Uganda, Democratic Republic of the Congo, and Guinea), in which it was probably subjected to an unconscious domestication processes (Lovett & Haq, 2000; Maranz & Wiesman, 2003; USAID, 2004).

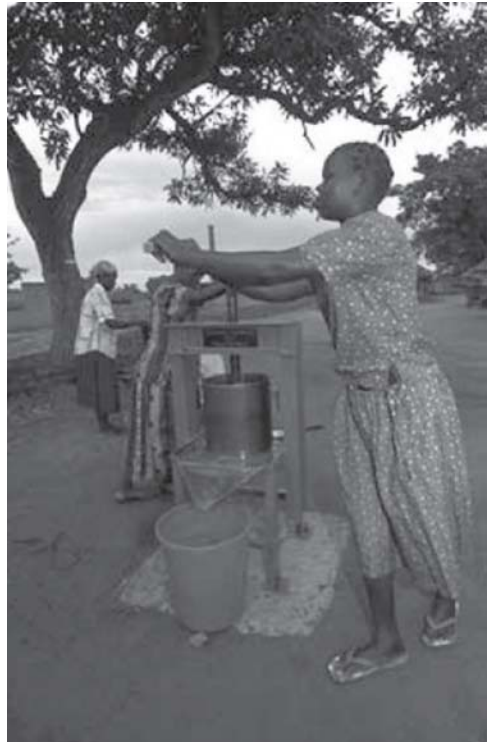
It is categorized as a vulnerable species by the International Union for Conservation of Nature (IUCN), meaning that it is likely to become endangered, unless the circumstances threatening its survival and reproduction improve (Forestry working group, 2004, Ugehe et al., 2008, Moore, 2008, IUCN, 2010, Byakagaba et al., 2011).

The tree is widely referred to by the common name, Karité, which is preferred by French, Portuguese, Spanish, and Italian speakers. Shea butter, which is the fat extracted from the seeds of the shea tree fruits is becoming increasingly popular as a component of cosmetic formulations, in addition to its longstanding use as a cocoa butter substitute in the chocolate industry (Maranz, 2004; Elias, 2006).

The pulp of the ripe fallen fruit (also described as the famine food) is consumed by the people and the large seed (the shea nut) is saved enclosed for butter extraction (Maranz, 2004). The nuts are parboiled, sun dried and then lightly crushed with pestle and mortar to remove the outer shell, in order to expose the oil bearing kernels. The kernels are processed to obtain shea oil which solidifies to form a solid fat, butter-like substance, called shea butter (Olajide et al., 2000). Among some ethnic groups, the oil is used as a cooking fat and the shea tree flowers are made into fritters. Besides the dietary contribution of shea fruit and butter, the species plays a great role medicinally; traditionally processed shea butter is traded in Africa outside of the *Vitellaria* zone mainly for use as a skin cream and as a medicinal ointment (Bail et al., 2009).

For centuries, the collection and processing of nuts into butter in Africa has been carried out by women. It is estimated that over 2 million women in 13 African coun-

Fig. 17.1. Shea nut grinding and pressing procedure. Source: USAID, 2002.



tries produce shea butter both for cash and food. Trade in shea tree products has been reported to improve the incomes and living standards of rural farm families and the economies of exporting countries (USAID, 2002; Vermilye, 2004; Kante, 2007).

Variation in oil content, physical and chemical properties of shea butter, both within and between populations, is the result of genetic, geographical, and climatic differences across West Africa and other Sub-Saharan regions (Maranz et al., 2004; Maranz & Wiesman, 2004; Di Vincenzo et al., 2004; Jatto et al., 2010). The lack of sufficient plantations of shea trees and the fact that shea butter is traditionally produced in poor conditions and distinct manufacturing processes are other factors which contribute to this variation (Figs. 17.1–17.4). The latter may also lead to the yield of poor quality products that do not conform to the Codex Alimentarius standards (Megnanou et al., 2007).

Henceforth, the characterization of the triglycerides profile, phenolic compounds, tocopherol, and phospholipid contents in raw materials, which is essential for the



Fig. 17.2. Shea fruit. Source: Masters, Yidana & Lovett, 2004.

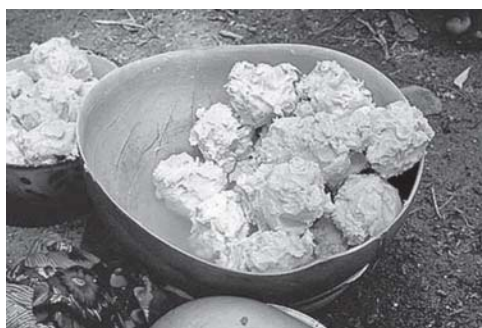


Fig. 17.3. Shea butter. Source: Masters et al., 2004.



Fig. 17.4. From left to right: shea kernels and nuts. Source: Olajide et al., 2000.

production of high quality and nonfraudulent engineered aliments will be needed. In addition, the increased demand for low-cost equivalents as ingredients in the chocolate industry (especially in Europe, Japan, and North America) has prompted investigators to search for new methods and tools to further investigate the physicochemical characteristics of shea butter and similar edible vegetable oils.

The objective of this review chapter is to gather the available information regarding methods and technologies related to shea butter chemophysical properties and to the delivery of bioactives in chocolate and related products.

Shea Butter Chemical Composition

Saponifiable Fraction

The average fat content in shea kernels varies between 45–53% and depends on the origin of the samples (fat content of beans grown in East Africa have a greater oil content than beans from West Africa) and the extraction methods used (Fig. 17.5).

Among 18 saturated and unsaturated five fatty acids characterized in the shea kernel fat, the following five predominate: palmitic, stearic, oleic, linoleic, and arachidonic with mean percentage of 4, 41.5, 46.4, 6.6, and 1.3, respectively. The fatty acid composition is dominated by stearic and oleic acids, which together account for 85–90% of the total fatty acids (Maranz et al., 2004, Akihisa et al., 2010). Cis-vaccenic and linolenic acids were found to be present in relatively low levels (0.41% and 0.31% respectively) in shea butter (Davrieux et al., 2010).

A broad research of the shea butter lipid composition, conducted by Maranz and Wiesman (2004) from the Ben Gurion University of the Negev in Israel, demonstrated that the principle triglycerides of karité butter were found to be stearic-oleic-stearic (SOS, 13–45%) and stearic-oleic-oleic (SOO, 28–30%). The major triglycerides measured in samples from Uganda were SOO (33.39%), OOO (19.07%), and SOS (19.87%), whereas those from Mali, Burkina Faso, and Nigerian shea butters were 40% SOS, 26% SOO, and 10% OOO. The levels of the triglycerides: Palmitic-Oleic-Stearic (POS) were relatively low (3.6–7.6%) (see data in Table 17-A).

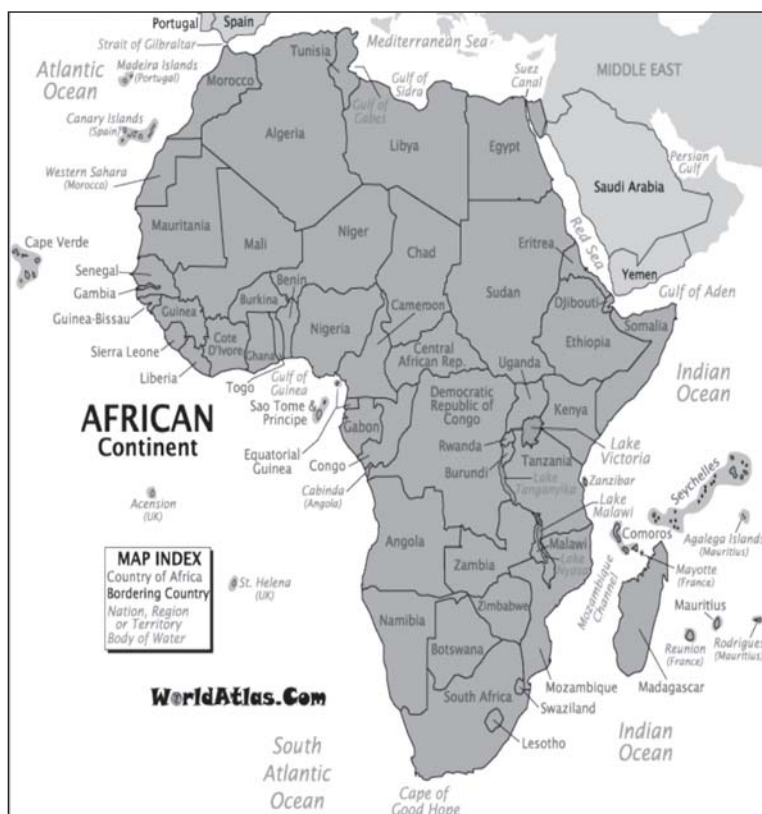


Fig. 17.5. Map of Africa. Source: <http://www.worldatlas.com/webimage/countrys/africa/africa.gif>.

The relative proportions of the two fatty acids: stearic and oleic, produces differences in shea butter consistency. The high stearic acid content gives the shea butter its solid consistency, while higher percentage of oleic acid determines how soft and liquid-like it would be (Maranz et al., 2004; Di Vincenzo et al., 2005). These findings are very important and relevant to the chocolate industry, since the latter is interested only in the shea hard fraction that can be delivered by the West African zones.

Unsaponifiable Fraction

Shea butter is popular in skin care and cosmetic product formulations in part due to the unusually high level (5–15%) of nonsaponifiable lipid (NSL) constituents in the fat, which is a potentially rich source of vitamin E (a natural antioxidant).

Table 17-A. Summary of Triglyceride Data—Site Means and Stearic Acid Means.

Summary of Triglyceride data - Site Means and Stearic acid means

Pescara and Aarhus combined datasets

Country	Site	Total PolyUnsat	Total DiUnsat	Total MonoUnsat	Total TriSat	% SOS	% 18:0	N
Burkina	Sapone	19.71	29.16	51.13	0.00	45.1	43.7	8
Mali	Niamana	18.52	30.84	50.62	0.02	44.7	42.6	9
Burkina	Lan	17.74	32.28	50.05	0.00	43.9	30.9	8
Mali	Badougou	19.00	31.74	49.27	0.07	43.2	27.2	9
Burkina	Tambissini	19.75	31.66	48.59	0.00	42.8	45.3	8
Mali	Koumantou	18.03	34.40	47.53	0.06	41.9	29.5	9
Burkina	Souly	18.69	34.01	47.31	0.00	40.3	45.2	9
Mali	Massala	20.84	32.24	46.92	0.02	40.5	41.8	9
Burkina	Siena	20.97	32.45	46.58	0.00	41.5	44.2	7
Nigeria	Kontagora	20.40	33.16	46.21	0.20	39.8	28.9	11
Burkina	Yasso	20.14	34.09	45.77	0.00	40.4	45.8	7
Burkina	Touroum	21.09	33.54	45.35	0.04	39.8	45.8	5
Burkina	Peni	19.82	35.08	45.11	0.00	40.0	43.6	8
Mali	Gouani	23.36	31.61	44.98	0.09	39.2	28.1	9
Mali	Sebekoro	22.68	32.22	44.91	0.20	38.3	43.8	9
Nigeria	Mokwa	25.20	30.07	44.54	0.19	38.4	42.1	10
Mali	Fourou	21.38	34.62	44.01	0.00	38.9	27.4	11
Mali	M'Peresso	24.02	33.78	42.16	0.06	36.8	42.5	8
Mali	Sirakorola	23.77	36.43	39.79	0.04	34.2	44.4	9
Uganda	Okwang	30.45	40.70	28.85	0.00	23.6	42.8	5
Uganda	Lapono	29.04	44.56	26.40	0.00	21.2	30.9	1
Uganda	Acowa	33.58	40.28	26.14	0.00	21.9	23.2	3
Uganda	Lira-Palwo	32.37	42.57	24.99	0.08	20.0	40.8	4
Uganda	Olilim	30.28	44.85	24.87	0.00	20.3	43.3	2
Uganda	Kuju	34.43	41.02	24.56	0.00	19.6	30.5	1
Uganda	Orum	35.99	40.88	23.13	0.00	19.1	43.4	1
Uganda	Abim	35.73	42.87	21.38	0.03	16.5	22.4	4
Uganda	Patongo	36.09	43.53	20.38	0.00	16.4	43.5	1
Uganda	Asamuk	45.72	37.82	16.46	0.00	13.1	27.0	1
Uganda	not given	51.65	35.95	12.40	0.00	10.1	42.6	1
Uganda	All Grps	22.55	33.94	43.48	0.05			187

Source: <http://www.prokarite.org/vitellaria-dbase-EN/index-EN.html>.

About two-thirds of the vitamin E found in shea butter occurs in the form of *Alpha-tocopherol*, a component that has the highest antioxidant activity among the tocopherols in most of the cases; followed by δ (15%), γ (14%), and β (7%) tocopherols (Maranz & Wiesman, 2004).

Shea butter NSL also contains relatively high total levels (up to 6%) of phytosterols (generally regarded as cholesterol reducing nutritional factors): *campesterol*, *stigmasterol*, *α -sitosterol*, and *α -spinosterol*, triterpenes alcohols in the form of 4,4'-dimethylsterols: *cinnamic acid esters (oryzanols)*, *α -amyrin*, *beta-amyrin*, *parkeol*, *butyrospermol*, and *lupeol*; hydrocarbons such as *karitene* and phenolic compounds (Peers, 1977; Itoh et al., 1980; Crews et al., 1997; Di Vincenzo et al., 2005; Krist et al., 2006).

The phenolic profile of shea butter is composed of catechin family compounds similar to those found in green tea, which has gained wide attention recently as an antioxidant-rich health beverage (Maranz et al., 2003). The most prominent component in shea kernels was gallic acid (27% of the mean total content of the nine catechin family compounds cited here) followed by: Gallocatechin (23%), epigallocatechin (16%), epigallocatechin gallate (13%), gallocatechin gallate (7%), and catechin, epicatechin, and epicatechin gallate (3% each). Quercetin was present only in trace amounts, averaging 0.1% of the total phenolic compounds (Maranz & Wiesman, 2004). However, the authors revealed that considerable loss (90-98%) of these components occurs, after the extraction of the butter.

Henceforth, by the application of shea butter as cocoa butter equivalent and improver, the chocolate industries can enhance the bioactive components such as natural antioxidants and vitamins in the final product. As a consequence, increased stability and longer shelf life can be attributed to the latter.

Regional Variation

Maranz et al. (2004) discovered that Ugandan shea populations produce consistently high oleic acid butter (51.2–62.1%), that is liquid at warm ambient temperatures in comparison to West African shea butter (Senegal to Nigeria), which is more variable in its oleic acid content (ranging from 37.1–54.7%). These results are consistent with those obtained by Davrieux et al. (2010) in other study in which it was confirmed that East African Shea butters were richer in oleic acid (56.64%) than West African butters (45.91%). Conversely, the relative stearic acid percentage was encountered higher in West Africa (40.91%) than in East Africa (29.72%) in both studies.

In general, shea butter from the east (Ethiopia, Southern Sudan, Uganda, and Democratic Republic of Congo), comes from the subspecies *nilotica* that has a higher oleic content compared with the subspecies *paradoxica* distributed in West and Central Africa (Davrieux et al., 2010; Ugese et al., 2010).

Likewise, considerable variation was observed in the NSL content within countries and between different regions. Shea samples from western area of the shea belt

(Cote d'Ivoire, Ghana, Nigeria, and Camaroon), differed from the eastern area (Sudan and Uganda); the former exhibited higher NSL content than the latter. Similarly, the proportion of triterpene alcohols varied within a country and between western and eastern regions. Samples from the western area contained more triterpene alcohols in the NSL than those from eastern zone, with intermediate levels in the central part (Akihisa et al., 2010). According to Di Vincenzo et al. (2005), West African provenances had significantly higher levels of both acetyl and cinnamyl triterpenes than did provenances of East Africa.

Finally, Maranz and Wiesman (2004) observed that shea trees in the hottest part of the Sahel zone may produce shea butter containing almost 10 times as much vitamin E and 15 times as much alpha-tocopherol as *Vitellaria* populations in cool highland areas. They concluded that the amount of Alpha-tocopherol in shea butter increased as both total tocopherols and the percentage of Alpha-tocopherol augmented with temperature.

The regional variation resumed here may play a key role in the assessment of food authenticity and traceability for commercial reasons and for the assurance of public health. Conjointly, it may assist in the categorization of the opposed types of shea butter according to the compatibility these raw materials will possess to certain designated functionalities.

Shea Butter Physical Properties

Melting Point

The melting point (complete fusion) recorded for shea butter originated in Ghana was 38.5–39.5°C, which is higher than that registered for cocoa butter (34.34°C). The slip point was also higher than that observed for cocoa butter (36.7–37.4°C and 33.3–33.5°C respectively) (Adomako, 1977). The melting point is mostly determined by the ratio of saturated-unsaturated-saturated triglycerides (e.g.: SOS, SLiS, POS) to saturated-unsaturated-unsaturated (SOO, SLiO, POO, etc.) (Beckett, 2000). This ratio in the karité butter samples analyzed by Maranz et al. (2004) varied from 0.5 (Uganda) to 2.3 (Lan, Burkina Faso).

Viscosity

The viscosity of shea butter is high below 40°C, but decreases when it becomes quite fluid over 60°C. This parameter is to be taken into consideration for the sake of oil extraction efficiency, the latter is inversely related to the former (FAO, 2006). Additionally, it is a variable that must adapted to the machinery of the chocolate industry.

The viscosity values obtained for shea oil samples from the shea districts of Uganda are reported to be in the range of 2.4 to 2.8 cP at 34–35°C (Okullo et al., 2010).

Oxidative Stability

Many components like the tocopherols and phenolic compounds that constitute the unsaponifiable fraction, have been reported to possess antioxidant properties of utmost importance for the oxidative stability of vegetable oils (Enweremadu & Alamu, 2010). Acid value, peroxide value, saponification value and iodine value are indicators of the suitability of the vegetable oils as raw materials and ingredients in aliments, since the increase in these values can be associated with rancidity of oils due to oxidation. These values exhibited by shea butter, were lower or equal to those measured for most edible vegetable oils (Obi & Oyinlola, 1996; Okullo et al., 2010); henceforth, its incorporation may confer minor rancidity and in consequence a longer shelf life of the final products.

Industrial Chocolate Application

Cocoa butter (CB) is the natural fat extracted from the seeds of the *Theobroma cacao* tree and its color is slightly yellowish. Cocoa butter is an important ingredient in the chocolate and related confectionery industries. It is responsible for the different favorable characteristics such as hardness at room temperature, brightness and fast and complete melting when placed in the mouth. It also improves the process of dispersion of other chocolate ingredients (Spangenberg & Dionisi, 2001).

In view of the fact that cocoa butter is the most expensive chocolate component whose price and availability often change, there have been long term efforts to replace it, fully or in part, with other vegetable fats, so-called cocoa butter alternatives (CBA), which would be cheaper but would not influence the resulting sensorial properties of chocolate (Kaphueakngam et al., 2009). In certain cases, the addition of other vegetable fats might have favorable effects on the chocolate quality, e.g. its resistance to bloom, increased milk tolerance and improvement on the function of some cocoa butters of lower quality (Maheshwari & Yella Reddy, 2005).

The continued research in the field of confection science resulted in the development of fats resembling the characteristics of cocoa butter. These fats have become known by the term: hard butters (Lovegren et al., 1976; Adomako, 1977; Shukla, 2005). The latter were developed using palm kernel, coconut, palm, and other exotic oils such as sal, shea, mango kernel, and illipe as raw materials (Van Pee et al., 1981). The processes involved in producing such fats included hydrogenation, interesterification, solvent or dry fractionation, and blending. The most elementary hard butters are manufactured by combining the processes of hydrogenation and fractionation (Pinyaphong & Phutrakul, 2009).

The most important CBA are the cocoa butter equivalents (CBE): non-lauric plant fats, which are similar in their physical and chemical properties to cocoa butter and miscible with it in any amount without altering the properties of cocoa butter (fully compatible with cocoa butter). To be equivalent to cocoa butter a CBE needs to have a

concentration of total SOS triglycerides similar to that found in cocoa butter. In total, cocoa butter typically contains about 80% of these triglycerides. Therefore, the vegetable fats usually have to undergo some form of fractionation process to concentrate the SOS type of triglyceride necessary for equivalence to cocoa butter (Talbot et al., 2006).

According to the Codex Alimentarius (FAO/WHO, 2001) and the 2003 EU regulations (Directive 2000/36/EC), only seven vegetable oils/fats of tropical origin (alone or in mixture) can be used in EU chocolate and shea kernel fat is one of them. In order to prevent adulteries and to ensure the delivery of a standardized and suitable chocolate for sale, an addition of CBE at levels up to only 5% based on the finished products, is allowed. Moreover, these regulations specify that adequate methods should be developed for the detection of CBA.

Cocoa Butter Improvers (CBIs) and Equivalents (CBEs)

In the chocolate industry, shea butter owes its importance to its similarity to cocoa butter. Shea fats with melting points equal to or less than hard cocoa butter are designated CBEs, while those with a higher melting point are qualified as CBI that can be defined as similar to CBEs, but with higher content in solid triglycerides. The latter are used for improving soft cocoa butters (Lipp & Anklam, 1998). On the other hand, the low content of POS in karité butter compared to that in cocoa butter, makes it attractive as a bloom suppressant when blended into chocolate (Beckett, 2000) Table 17-B.

Before its incorporation as CBE, Shea butter requires fractionation, because the content of SOS is too low for its effective use as a raw material for CBE formulation. By fractionation, the SOS levels can be increased to levels similar to those found in

Table 17-B. Fatty Acid Profiles of Shea Butter versus the Competitors.

Oil type	Lauric (12:0)	Myristic (14:0)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linoletic (18:3)
Cocoa butter			25	35	30–40	2–4	
Olive			12	2	72	8	1
Palm oil (husk)			42	4	38	9	
Coconut	44	16	8	3	5	2	
Shea butter							
Literature	0–0.5	0–1.6	3–9	30–50	41–50	4–11	0–7.5
Uganda			6.5	26.4	59.3	6.2	0.2
Nigeria			3.2	38.9	47.5	6.5	0.2
Burkina Faso			12.1	42.5	39.3	4.5	0.2
Mali			19	31.1	42.6	5.7	0.2

Source: Ferris et al., 2001.

cocoa butter; solvent fractionation gives a useful stearin that augments the melting point of cocoa butter (Pease, 198; Buchgraber, 2004). The process is highly specialized and conducted by only a few companies in Europe and Japan.

All shea nuts and butter comes from West Africa, principally from Ghana, Burkina Faso, Mali, and Cote D'Ivoire. Given that CBE manufacturers are interested in the shea hard fraction, the industry has paid no interest in shea nuts produced in Uganda, Sudan, and Kenya (Ferris et al., 2001).

Shea butter CBEs have to compete with CBEs produced from palm oil mid-fraction, illipe oil (from the nuts of the mahuwa tree, found mainly in Indonesia and Malaysia), and sal fat (from the sal tree in India). Of these competing fats and oils, palm oil mid-fraction is dominant, sal fat is generally regarded as inferior, and Illipe oil is hard to obtain. However, shea butter CBE manufacturers are unlikely to change their processes to suit other fats and oils, because they have invested heavily in highly specialized processing technology for transforming shea butter into CBEs. That places shea nuts and butter in a quite strong competitive position (Ferris et al., 2001; USAID, 2004).

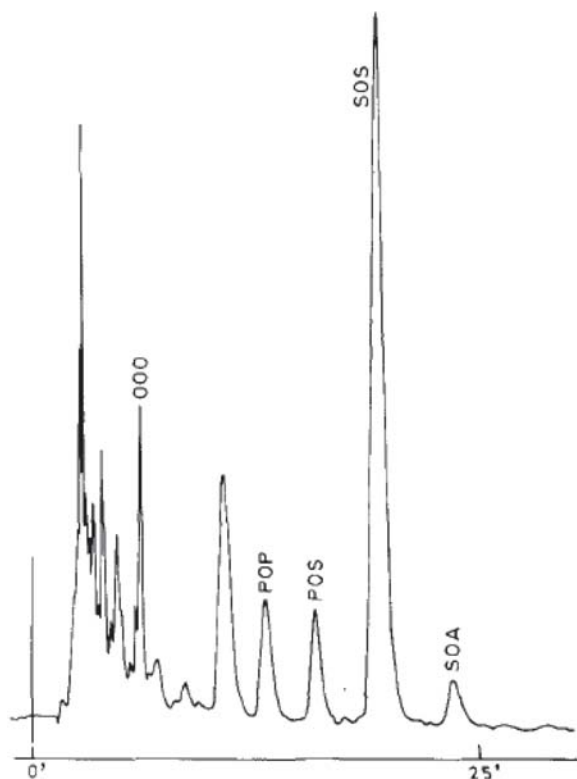
Analytical Tools and Methodologies

Chromatography—HPLC, GLC

The Directive 2000/36/EC of the European Parliament and the Council has specified a number of vegetable fats other than cocoa butter (CB) that may be added to chocolate products to a maximum of 5% of the total weight of the finished product (meaning that fat extracted from chocolates could contain about 15% CBE) (Talbot & Slager, 2008). The mandatory labeling of the addition of CBE and labeling to indicate that those fats have not been added is allowed. However and unlike the case of olive oil that has a legal definition backed by officially sanctioned methods of analysis, the analysis of cocoa butter is not governed by legal definitions in order to protect consumers from fraudulent malpractice (Dionisi et al., 2004). Therefore, a perceived need exists within official control laboratories for the availability of useful and precise methods for the quantification of such vegetable fats in chocolate.

For identity control purposes of fats and oils, in particular of CBEs in cocoa butter, the knowledge of the triglyceride (TG) composition has become an indispensable tool (Neri et al., 1998). In general, CBEs contained substantially more POP and less POS than cocoa butter, but similar amounts of SOS. Since POS is neither contained in substantial amounts in palm oil nor in shea, sal, or kokum fat, and unless illipé fat or illipé-containing CBEs are added, commercial CBEs contain normally less POS than genuine cocoa butter. This fact provides the basis for commonly used analytical techniques to determine the presence and quantity of CBEs in cocoa butter (Lipp et al., 2001).

Reversed phase high performance liquid chromatography (RP-HPLC) has become the most popular separation and analysis method for TAG species in oils and fats.



Figs. 17.6. Brominated shea-stearine.

Geeraert and De Schepper (1983) have developed a simple and rapid RP-HPLC technique for the identification and quantitative determination of cocoa butter equivalents (CBE) in cocoa butter (CB) and chocolate products. Before the RP-HPLC analysis, the triglycerides are brominated (addition of bromine to the double bounds), in order to separate between the symmetrical mono-unsaturated triglycerides from the di- and polyunsaturated triglycerides, and to increase the UV absorption of unsaturated and monounsaturated triglycerides at 215 nm. They obtained excellent separation with the eluents: acetonitrile:isooctane:isopropanol (5:2:3) on R:Sil C18 HL 5 μm (Figs. 17. 6 and 17.7).

Nowadays, the use of an *evaporative light scattering detector (ELSD)* for the HPLC analysis of oils and fats is generally preferred. It is a highly sensitive detector with a uniform response resulting in a better separation and in a shorter analysis time. However, one should take into account that for oils or fats with an asymmetrical TG profile, a serious underestimation of minor TG species can occur, hence, results should be interpreted with care; when they injected a certificated cocoa butter stan-

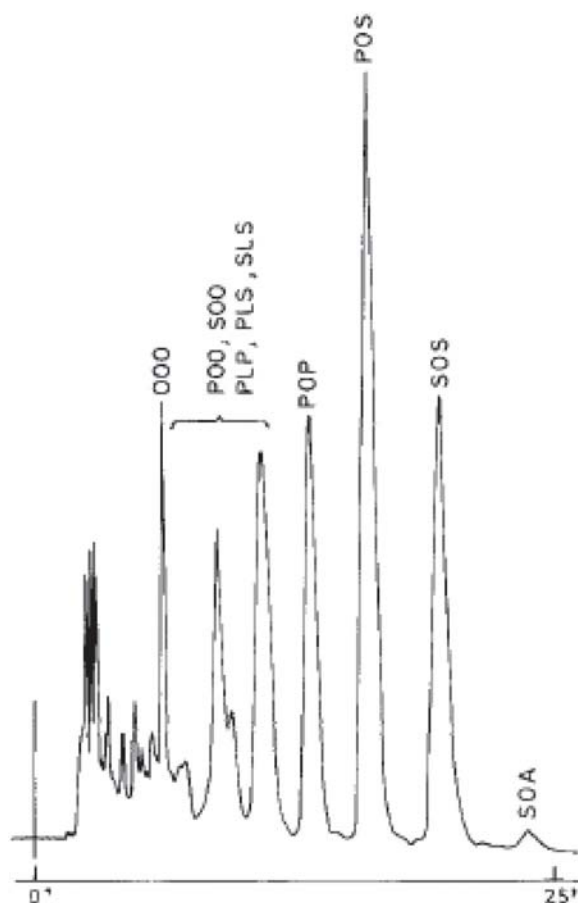


Fig. 17.7. Brominated cocoa butter.

dard, Rombaut et al. (2009) observed that at low concentrations, the minor species POO and PPP/SOO were underestimated and the main components POS/SLS were overestimated. In the same proposed method, the main component POS seemed to be underestimated at higher concentrations. It is an extremely critical issue since the three TGs (POP, PLS, and POS) and the two TG ratios (POS/PLP and POP/PLS) were found as particularly significant to discriminate between cocoa butter and CBE (Dionisi et al., 2004).

The *gas chromatographic methods* based on the determination of the main triglycerides, lead mostly to good results (Hites, 1970). They enable the TG separation according to the carbon number, also providing a separation based on the degree

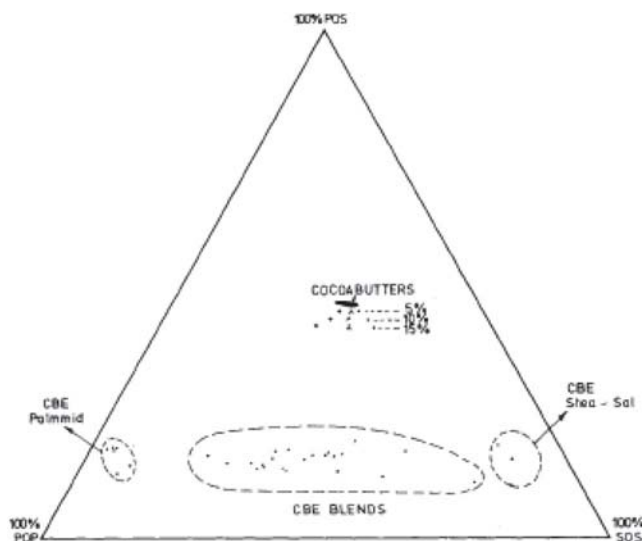


Fig. 17.8. Ternary diagram of normalized brominated POP, POS, SOS content from cocoa butters, cocoa butter equivalents, and mixtures of both.

of fatty acid unsaturation. Using *high-temperature capillary Gas Chromatography* (HT-GC), Geeraert and Sandra (1986) reported that by comparing POP:POS:SOS ratio in natural cocoa butters, additions of 5% CBE can be easily detected by placing the relative POP:POS:SOS percentages in a ternary diagram; hence the addition of a shea stearin CBE (high SOS content) could be easily detected and quantified due to the disturbance of the specific POP:POS:SOS ratio of pure cocoa butter (Fig. 17.8).

Yet, although these methods have been found quite sensitive for the quantification of some CBE (Kurvinen et al., 2002), Problems can occur especially when the chemical and physical behavior of CBE is very similar to cocoa butter. Additionally, these methods are interfered by the large variation of the composition of cocoa butter due to climatologic and geographical differences (Anklam et al., 1997).

To answer those difficulties, other methods such as *Pyrolysis-mass spectrometry*, have been developed. Pyrolysis-mass spectrometry (Py-MS), is an analytical fingerprinting technique applied for a fast profiling of food and food ingredients. In this method, the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry. It gave good results when optimized with a supervised training of a neural network for the desired net response, by adjusting samples into identifiable groups. Training of the network is performed by exposing each sample of the calibration set to the network and computing the weights

accordingly. Shea butter for example, could not be assigned to one group, because the characteristics of one sample resembled those in the group of palm mid fraction and the other belonged with sal fat (Anklam et al., 1996; Anklam et al., 1997).

Another methodology that may provide excellent compositional information is when GC–MS analysis is preceded by HPLC isolation of each TG present in the sample, further fatty acid hydrolysis and methylation. Unfortunately and in addition to other disadvantages such as the co-elution of different TG and the use of toxic chemicals as mobile phase in the HPLC method, it is very time consuming (Buchgraber et al., 2000; Segall et al., 2005).

A European collaborative study has been conducted in order to validate a *high resolution gas chromatography (HR-GC)* method for the detection of CBE in CB and plain chocolate. The fat is separated into TG fractions according to their acyl-C numbers and within a given number also according to unsaturation. The presence of CBE is detected by linear regression analysis applied to individual TG fractions of the fat analyzed. The method performed well with a detection limit of least 2% of CBE admixture to CB corresponding to 0.4% in chocolate (assumed 20% fat content of chocolate) (Buchgraber & Anklam, 2002).

Prompted by the same European project, an inter-comparison study between several laboratories in Europe have demonstrated that *high-temperature capillary Gas Chromatography (HT-GC)* is a robust and reliable technique for the characterization of cocoa butter, by determining its triglyceride pattern (Buchgraber et al., 2003). The participants were asked to apply an in-house validated analytical procedure of their own choice for the analysis of the TG profile of CB and prepare a mixture consisting of POP, POS, POO, SOS, SOO present in proportions as normally found in CB for calibration purposes. These TG reference substances with a purity of at least 99% were obtained by the collaborators from *Sigma-Aldrich* (various countries) and *Larodan* (Malmö, Sweden). Moreover, all had to achieve a separation of the critical pairs POS/POO and SOS/SOO of the same training CB sample, with chromatographic resolution of at least 1.0.

Negative ion CI-MS (tandem MS method) enabled a fast and reliable determination of regio-isomeric structure and M.W. of TG species in CB. It requires only minimal sample preparation which may be considered an advantage if a fast and simple method is desired for a large number of samples. Nevertheless, it was observed that the differences caused by the addition of CBE were minor, leading to the conclusion that MS cannot be used alone to quantify added CBE, but in combination with other analytical methods and multivariate statistical data analysis programs (Kurvinen et al., 2002).

NMR

Accurate determination of the amount of solid fat in edible fats and oils is an essential requirement in the food industry. The measured values are used for process control

(e.g. during hydrogenation, interesterification and blending), because close control of the *solid fat content (SFC)* is often required for the development of new products and for quality-control purposes (Gribnau, 1992). Moreover, on the basis of measurements of the solid fat level at different temperatures, a number of physical and organoleptic properties, such as hardness, physical appearance, spreadability, heat resistance, mastication quality, mouth-feel and flavor release, can be predicted (Torbica et al., 2006). Thus, the knowledge of SFC simplifies the usage of fats in the technological process of chocolate manufacturing.

The *melting and crystallization behavior* of vegetable oils and fats is very important for functionality in many prepared food products. These thermal properties depend on the triacylglycerol (TG) profile in vegetable oils and fats and can therefore be used in qualitative and quantitative ways for identification of vegetable oils and fats (Santana et al., 2008).

Over the past three decades, pulsed nuclear magnetic resonance (NMR) has become a dominant and routine measuring technique for the determination of SFC in fats (Lipp & Anklam, 1998). In this technique, *Fat hardness* can be expressed as the percentage of solid fat content at a given temperature (Gilabert-Escriva et al., 2002). Additionally, it is considered the standard procedure for determining melting behavior and for monitoring the oxidation of vegetable oils and fats (Knothe, 2003).

Although gas chromatography (GC) is usually the method of choice for determining the *fatty acid profile*, the common unsaturated fatty acids (oleic, linoleic, linolenic) in an oil or fat can be quantified using ^1H -NMR. This method utilizes the area per proton (determined by integration) and gives equations for determining the amounts of the unsaturated fatty acids. The results agree well with GC conducted as a control, although reportedly ^{13}C -NMR results correlate even better with GC (Knothe, 2003).

In later studies, the possibility to estimate the polymorphic structure of the crystals of fat and its modifications by analysis of the spin–lattice relaxation time, has been demonstrated (Lucas et al., 2005). This measurement is important since X-ray diffraction and microscopic analyses of binary blends of studied oils, have demonstrated that changes in the hardness are controlled not only by the SFC, but also by polymorphism and the materials microstructure (Braipson-Danthine & Deroanne, 2004). It will also aid in studying the chocolate bloom phenomenon as a function of thermal history and the different ingredients (i.e. CBE, CBIs) added to the cocoa butter (Walter & Cornillon, 2001; Janssen & MacGibbon, 2007).

Petersson, Anjou, and Sandstrom (1985) have demonstrated that the SFC in pure fat can be controlled by the tempering temperature in the pre-treatment procedure before NMR analysis. They developed a general pretreatment method acceptable for CB and CBE (a mix including fractions of shea butter fractions and of other vegetable fats used commonly), that gave stable and reliable SFC measurements in a short time.

In a continuous study conducted later (Petersson, 1986), it was concluded that very different SFC results can be obtained both in pure fat and chocolate with various tempering temperatures and storage temperatures, respectively. For example, the way of tempering the fats used in dark chocolates will not reproduce the same situation when applied to the normally produced and stored dark chocolates with the mentioned fats incorporated.

The same author also reveals the fact that milk fat acts as a diluter of SFC and that the tolerance of cocoa butter and CBE to milk fat must be measured. The latter is a crucial issue, because it determines the crystallization time and microstructure uniformity, thus affecting the organoleptic properties of the chocolate (Petersson, 1986).

Analysis of Minor Lipid Components

A combination of approaches rather than a single technique will probably be required in order to detect vegetable fats other than cocoa butter in chocolate products. And though the determination of the TG profile is a key parameter both for authenticity assessment of cocoa butter and for the estimation of the CBE content in chocolate in most of them, the analysis of minor lipid constituents as an optional tool for the same purpose, should be considered.

Recent investigations show that the patterns of steryl esters (the esterified form of sterols) remain unchanged after butter deodorization (Gordon & Griffith, 1992; Gordon & Miller, 1997). That is in addition to the advantageous facts that cocoa butter belongs to a low content steryl ester group and that slight variations in the absolute amounts of steryl esters between samples of cocoa butter from distinct geographical origin, were observed (Kamm et al., 2001).

The 4,4'-dimethylsterol (triterpene alcohol) fraction have also been proved to remain stable and thus are of special interest for discriminative purposes (especially in the case of shea butter that contains considerable amount of these compounds). Cycloartenol and 24-methylene cycloartanol are the major triterpenic alcohols in cocoa butter, while CBE raw materials contain in addition considerable amounts of α -amyrin, butyrospermol and lupeol (shea) and β -amyrin and ψ -taraxasterol in sal and illipé. Henceforth, the presence of sal, shea, and illipé in cocoa butter and confectionery can be detected by determination of triterpenoids such as α -amyrin and ψ -taraxasterol (Ulberth & Buchgraber, 2000; Ulberth & Buchgraber, 2003). Gas chromatographic and NMR afford a good evidence for the presence of certain types of triterpenes (Itoh et al., 1975; Saleem, 2009) (Fig. 17.9)

Another interesting approach is to detect the steradiens resulting from the dehydration of major plant sterol: beta-sitosterol, campesterol, stigmasterol, in the fractionation process of CBEs. Crews et al. (1997, 1999) have demonstrated that steradiene analysis using GC-MS, can indicate the presence of refined fats from mango, palm, illipé, kokum, sal, and shea in chocolate products (Fig. 17.10).

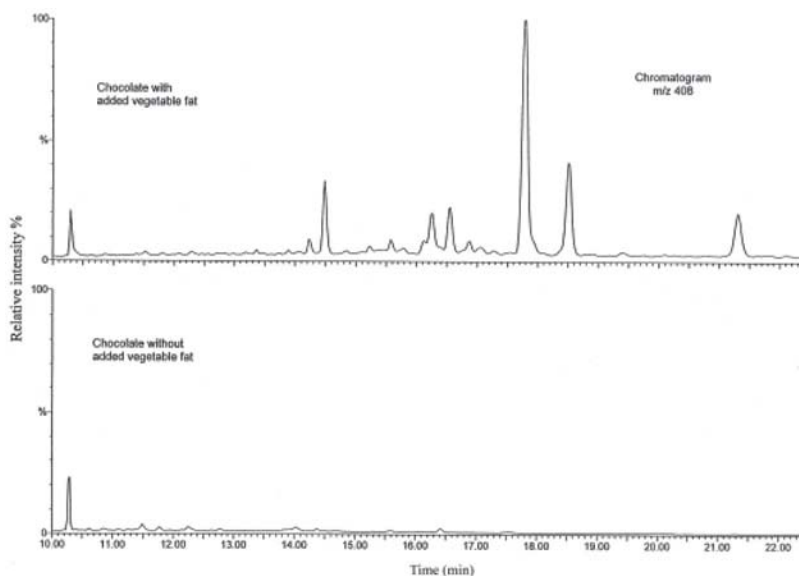


Fig. 17.9. Selected ion chromatogram of the triterpene sterene for extract of chocolates with and without added vegetable fat. Source: Crews et al., 1999.

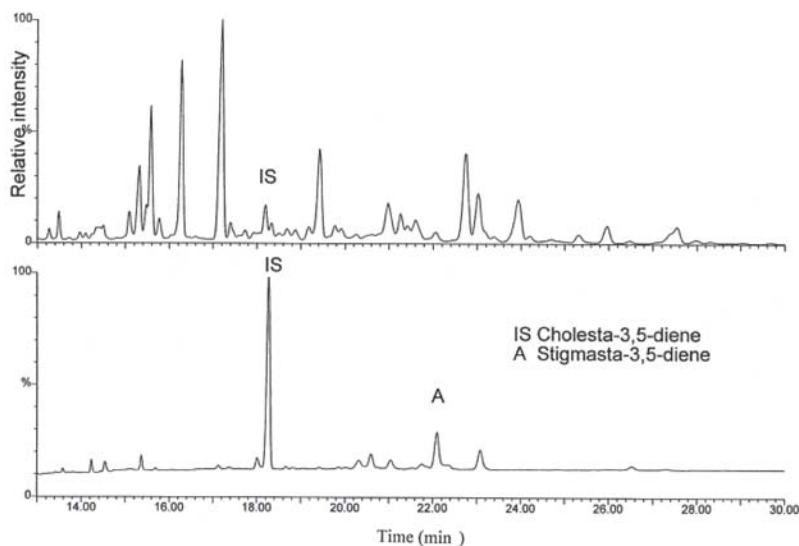


Fig. 17.10. Total ion current chromatogram of steradiens in refined shea butter (top) and in shea stearin fraction (bottom). Source: Crews et al., 1999.

Conclusions

The predominated fatty acids in shea butter are oleic and stearic acid, however, only the hard fraction is useful to the chocolate industry. For that reason it is important to remember that shea butter obtained in the West African zone is richer in stearic acid than the one obtained in the eastern areas of this continent. Due to its chemical and physical properties, shea butter is an excellent cocoa butter equivalent and improver, which helps in achieving the desirable organoleptic characteristics of the chocolate. Moreover, the high levels of antioxidants in shea butter may impede rancidity and confer more stability to confectionary products.

The detection of shea butter hard fractions, as well as that of other CBE, can be accomplished by the combination of various methods. The examination of the fatty acid profile is the main useful target, nevertheless, the identification and quantification of characteristic minor components is also a powerful tool.

References

- Abidemi, T.A.; Adebayo, O.J.; Idowu, O.; Agbotoba, M.O. Short Communication: Nutrient content and anti-nutritional factors in shea butter (*Butyrospermum parkii*) leaves. *African Journal of Biotechnology* 2009, 8(21), 2 November, 5888–5890.
- Adomako, D. Fatty acid composition and characteristics of *Pentadesma butyracea* fat extracted from Ghana seeds. *J. Sci. Fd. Agric.* 1977, 28, 384–386.
- Akihisa T., Kojima N., Katoh N., Ichimura Y., Suzuki H., Fukatsu M., Maranz S., and Masters E.T.: Triterpene alcohol and fatty acid composition of shea nuts from seven African countries. *Journal of oleo science*, 2010, 59, 7, 351–360.
- Anklam E., Bassani M.R., Eiberger T., Kriebel S., Lipp M., Matissek R. Characterization of cocoa butters and other vegetable fats by pyrolysis-mass spectrometry. *Fresenius J. Anal. Chem.* 1997, 357: 981–984.
- Apgar J.L., Shively C.A., and Tarka S.M. Digestibility of cocoa butter and corn oil and their influence on fatty acid distribution in rats. *American Institute of Nutrition. The Journal of Nutrition*, jn.nutrition.org.
- Badifu G.I.O. Lipid composition of Nigerian *Butyrospermum paradoxum* kernel. *Journal of Food Composition and Analysis* 1989, 2, 238–244.
- Badifu G.I.O: Food potentials of some unconventional oilseeds grown in Nigeria—A brief review. *Plant Foods for Human Nutrition* 1993, 43: 211–224.
- Bail S., Krist S., Masters E., Unterweger H., Buchbauer G.: Volatile compounds of shea butter samples made under different production conditions in western, central and eastern Africa. *Journal of Food Composition and Analysis* 2009, 22, 738–744.
- Beckett S. 2000. *The Science of Chocolate*. The Royal Society of Chemistry: Cambridge, 175 pp.
- Braipson-Danthine S., and Deroanne C. Influence of SFC, microstructure and polymorphism on texture (hardness) of binary blends of fats involved in the preparation of industrial shortenings. *Food Research International* 2004, 37, 941–948.

- Buchgraber M., Ulberth F., and Anklam E. Cluster analysis for the systematic grouping of genuine cocoa butter and cocoa butter equivalent samples based on triglyceride patterns. *J. Agric. Food Chem.* 2004, 52, 3855–3860.
- Buchgraber M., Ulberth F., Anklam E. Interlaboratory evaluation of injection techniques for triglyceride analysis of cocoa butter by capillary gas chromatography. *Journal of Chromatography A* 2004, 1036, 197–203.
- Buchgraber M. and Anklam E. Validation of a method for the detection of cocoa butter equivalents in cocoa butter and plain chocolate. European Commission, Joint Research Center, Institute for Reference Materials and Measurements, Food Safety and Quality Unit, 2003.
- Buchgraber M., Ulberth F. and Anklam E. Comparison of HPLC and GLC techniques for the determination of the triglyceride profile of cocoa butter. *J. Agric. Food Chem.* 2000, 48, 3359–3363.
- Buchgraber M., Ulberth F. and Anklam E. Capillary GLC: a robust method to characterize the triglyceride profile of cocoa butter—results of an inter-comparison study. *Eur. J. Lipid Sci. Technol.* 2003, 105, 754–760.
- Bohačenko I., Kopicová Z. and Pinkrová J. Chocolate authenticity control concerning compliance with the conditions for adding cocoa butter equivalents as laid down by Directive 2000/36 EC. *Czech J. Food Sci.* 23(1), 27–35.
- Byakagaba P., Eilu G., Okullo J.B.L., Tumwebaze S.B. and Mwavu E.N. Population structure and regeneration status of *Vitellaria paradoxa* (C.F.Gaertn) under different land management regimes in Uganda. *Agricultural Journal* 2011, 6(1), 14–22.
- Crews C., Calvet-Sarrett R. and P. Brereton: The analysis of sterol degradation products to detect vegetable fats in chocolate. *JAOCs* 1997, 74(10), 1273–1280.
- Crews C., Calvet-Sarrett R. and P. Brereton. Identification of steroidal hydrocarbon in refined confectionery fats by gas chromatography–mass spectrometry. *Journal of Chromatography A* 1999, 847, 179–185.
- Damiani P., Cossignani L., Simonetti M. S., Blasi F., Petrosino T. and Neri A. Identification of cocoa butter equivalents added to cocoa butter. III. Stereospecific analysis of triacylglycerol fraction and some its subfraction. *Eur Food Res Technol* 2006, 223: 645–648.
- Davrieux F., Alla F.-O., Piombo G., Kelly B., Okullo J.B., Thiam M., Diallo O.B.R., and Bouvet J.-M. Near infrared spectroscopy for high-throughput characterization of shea tree (*Vitellaria paradoxa*) nut fat profiles. *J. Agric. Food Chem.* 2010, 58, 7811–7819.
- Di Vincenzo D., Maranz S., Serraiocco A., Vito R., Wiesman Z., and Bianchi G. regional variation in shea butter lipid and triterpene composition in four African countries. *J. Agri. Food. Chem.* 2005, 53, 7473–7479.
- Dianda M., Bayala J., Diop T., Ouédraogo S.J. Improving growth of shea butter tree (*Vitellaria paradoxa* C.F.Gaertn.) seedlings using mineral N, P and arbuscular mycorrhizal (AM) fungi. *Biotechnol. Agron. Soc. Environ.* 2009 13(1), 93–102.
- Dionisi F., Golay P.-A., Hug B., Baumgartner M., Callier P., and Destailats F.R. Triacylglycerol analysis for the quantification of cocoa butterequivalents (CBE) in chocolate: feasibility study and validation. *J. Agric. Food Chem.* 2004, 52, 1835–1841.

- Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption. Official Journal, L 197, 03/08/2003, 19–25.
- Eke, U.B., Dosumu, O.O., Oladipo, E. and Agunbiade, F.O. Analysis of locally produced soap using sheabutter oil (SBO) blended with palm-kernel oil (PKO). *Nigerian Journal of Science* 2004, 38, 19–24.
- Elias M., Bayala J. and Dianda M. Impediments and innovations in knowledge sharing: the case of the African shea sector. *KM4D Journal* 2(1): 52–67.
- Eze J.I. and Ejilah I.R. Tested performance parameters of diesel fuel and transesterified sheanut oil blends in compression ignition engine. *Global Journal of Researchers in Engineering*, April 2010, 10(1) (Ver 1.0), 84–92.
- Farines M., Soulier R. and Soulier J. Analysis of the triglycerides of some vegetable oils. *Journal of Chemical Education*, May 1988, 65(5), 464–466.
- Food and Agricultural Organization (FAO). Technology for agriculture: Replacing the need for labour intensive traditional methods with a mechanical press for making Shea butter in northern Ghana. <http://www.fao.org/teca/content/replacing-need-labour-intensive-traditional-methods-mechanical-press-making-shea-butter-nort>. 2006.
- Food and Agricultural Organization/World Health Organization (FAO/WHO). Food Standards Programme: Report of the Nineteenth Session of the Codex Committee on Cocoa Products and Chocolate. Fribourg, Switzerland, 3–5 October, 2001.
- Ferris R.S.B, Collinson C., Wanda K., Jagwe J. and Wright P. Evaluating the marketing opportunities for Shea nut and Shea nut processed products in Uganda. Natural Resources Institute, USAID, 1–76, October 2001.
- Forestry Working Group: Shea Forest Stands Mapping and Inventory in Yei County, South Sudan. May 2004.
- Geeraert E. and De Schepper D. Structure elucidation of triglycerides by chromatographic techniques: Part 2: RP HPLC of triglycerides and brominated triglycerides. *Journal of High Resolution Chromatography & Chromatography Communications*, March 1983, 6, 123–132.
- Geeraert E. and Sandra P. Capillary GC of triglycerides in fats and oils using a high temperature phenylmethylsilicone stationary phase. Part II. The analysis of chocolate fats. *JAOCS*, January 1987, 64(1), 100–105.
- Gilbert-Esciva, M.V., Goncalves, Silva C.R. and A. Figueira. Fatty acid and triacylglycerol composition and thermal behaviour of fats from seeds of Brazilian Amazonian *Theobroma* species. *Journal of Science, Food and Agriculture*, 82, 1425–1431.
- Gordon M.H. and Griffith R.E. Steryl ester analysis as an aid to the identification of oils in blends. *Food Chemistry* 43 (1992) 71–78.
- Gordon M.H. and Miller L.A.D. Development of steryl ester analysis for the detection of admixtures of vegetable oils. *JAOCS* 1997, 74(5).
- Gribnau M.C.M. Determination of solid/liquid ratios of fats and oils by low-resolution pulsed NMR. *Trends in Food Science & Technology*, August/September 1992, Vol. 3, 186–190.

- Hites R.A. Quantitative analysis of triglyceride mixtures by mass spectrometry. *Analytical Chemistry*, Vol. 42, No. 14, 1736–1740, December 1970.
- Itoh T., Uetsuki T., Tamura T. and Matsumoto T. Characterization of triterpene alcohols of seed oils from some species of Theaceae, Pholaccaceae and Sapotaceae. *Lipids* 1980, 15(6), 407–411.
- Itoh T., Tamura T. and Matsumoto T. 24-methylenedammarenol: a new triterpene alcohol from shea butter. *Lipids* 1975, 10(12), 808–813.
- IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. www.iucnredlist.org.
- Janssen P.W.M. and MacGibbon A.K.H. Non-isothermal crystallization of bovine milk fat. *J Am Oil Chem Soc* 2007, 84, 871–875.
- Jatto W. O., Yuanfa L., Shan L., Wang X. and Aworh O. C. Liquid-gas chromatographic analysis of fatty acid content of south-western Nigerian shea butter (*Vitellera paradoxum*). *EJEAFCh* 2010, 9(2), 358–363.
- Kamm W., Dionisi F., Fay L.-B., Hischenhuber C., Schmarr H.-G., Engel K.-H. Analysis of steryl esters in cocoa butter by on-line liquid chromatography–gas chromatography. *Journal of Chromatography A* 2001, 918, 341–349.
- Kante A. Rural women's perceptions of the diffusion of technological innovations that increase quality shea butter production in Mali. Thesis of master, Montana state university, 2007.
- Kante A., Igo C.G., Frick M.J. Enhancing effectiveness of extension efforts: A case study of Malian shea butter producers. *Journal of International Agricultural and Extension Education* 2009, 16(1).
- Kaphueakngam P., Flood A. and Sonwai S. Production of cocoa butter equivalent from mango seed almond fat and palm oil mid-fraction. *As. J. Food Ag-Ind.* 2009, 2(04), 441–447.
- Knothe G. Quantitative analysis of mixtures of fatty compounds by ¹H-NMR. *Lipid Technology*, September 2003, 111–114.
- Knothe G. ¹H-NMR Spectroscopy of fatty acids and their derivatives. www.lipidlibrary.co.uk.
- Krist S., Bail S., Unterweger H., Ngassoum M. B., Mohagir A. M., Buchbauer G. Volatile compounds of original African black and white shea butter from Tchad and Cameroon. *Eur. J. Lipid Sci. Technol.* 2006, 108, 583–588.
- Kurvinen J.-P., Sjövall O., Tahvonen R., Anklam E. and Kallio H. Rapid MS method for analysis of cocoa butter TAG. *JAOCs* 2002, 79(7), 621–626.
- Leakey R.R.B. Potential for novel food products from agroforestry trees: A review. *Food Chemistry* 1999, 66, 1–14.
- Lehrian D.W., Keeney P.G. Butler D.R. Triglyceride characteristics of cocoa butter from cacao fruit matured in a microclimate of elevated temperature. *JAOCs*, February 1980, 66–69.
- Lipp M. and Anklam E. Review of cocoa butter and alternative fats for use in chocolate—Part A. Compositional data. *Food Chemistry* 1998, 62(1), 73–97.
- Lipp M. and Anklam E. Review of cocoa butter and alternative fats for use in chocolate—Part B. Analytical approaches for identification and determination. *Food Chemistry* 1998, 62(1), 99–108.

- Lipp M., Simoneau C., Ulberth F., Anklam E., Crews C., Brereton P., De Greyt W., Schwack A. W. and Wiedmaier C. Composition of genuine cocoa butter and cocoa butter equivalents. *Journal of Food Composition and Analysis* 2001, 14, 399–408.
- Lovegren N.V., Gray M.S. and Feuge R.O. Effect of liquid fat on melting point and polymorphic behavior of cocoa butter and a cocoa butter fraction. *Journal of the American Oil Chemists' Society*, March 1976, 53, 108–112.
- Lovett P. N. and Haq N. Evidence for anthropic selection of the Sheanut tree (*Vitellaria paradoxa*). *Agroforestry Systems* 2000, 48: 273–288.
- Lucas T., Le Ray D., Barey P., Mariette F. NMR assessment of ice cream: Effect of formulation on liquid and solid fat. *International Dairy Journal* 2005, 15, 1225–1233.
- Maranz S. and Wiesman Z. Evidence for indigenous selection and distribution of the shea tree, *Vitellaria paradoxa*, and its potential significance to prevailing parkland savanna tree patterns in sub-Saharan Africa north of the equator. *Journal of Biogeography* 2003, 30, 1505–1516.
- Maranz S., Wiesman Z. and Garti N. Phenolic constituents of shea (*Vitellaria paradoxa*) kernels. *J. Agric. Food Chem.* 2003, 51, 6268–6273.
- Maranz S. and Wiesman Z. Influence of climate on the tocopherol content of shea butter. *J. Agric. Food Chem.* 2004, 52, 2934–2937.
- Maranz S., Wiesman Z., Bisgaard J. and Bianchi G. Germplasm resources of *Vitellaria paradoxa* based on variations in fat composition across the species distribution range. *Agroforestry Systems* 2004, 60: 71–76.
- Maranz S. Characterization of shea butter tree (*Vitellaria paradoxa* Gaertner) populations across Africa by seed fat content and composition. Thesis of doctorate, The Ben-Gurion university of the Negev, 2004.
- Masters E.T., Yidana J.A. and Lovett P.N. El fortalecimiento de la buena gestión por medio del comercio: los productos del árbol de karité en África. *Unasylva* 2004, 55(4), 46–53.
- Megnanou R.-M., Niamke S. and Diopoh J. Physicochemical and microbiological characteristics of optimized and traditional shea butters from Côte d'Ivoire. *African Journal of Biochemistry Research*, September 2007, 1(4), 41–47.
- Maheshwari B. and Yella Reddy S. Application of kokum (*Garcinia indica*) fat as cocoa butter improver in chocolate. *J Sci Food Agric* 2005, 85:135–140.
- Moore S. The role of *Vitellaria paradoxa* in poverty reduction and food security in the Upper East region of Ghana. *Earth & Environment* 2008, 3, 209–245.
- Neri A., Simonetti M. S., Cossignani L., Damiani P. Identification of cocoa butter equivalents added to cocoa butter. I. An approach by fatty acid composition of the triacylglycerol sub-fractions separated by Ag+-HPLC. *Z Lebensm Unters Forsch A* 1998, 206, 387–392.
- Okullo J.B.L, Omujal F., Agea J.G., Vuzi P., Namutebi A., Okullo J.B.A. and Nyanzi S.A. Physico-chemical characteristics of shea butter (*Vitellaria paradoxa* C.F. Gaertn.) oil from the shea districts of Uganda. *AJFAND* 2010, 10(1).
- Olajide J.O., Ade-Omowaye B.I.O., Otunola E.T. Some physical properties of shea kernel. *J. Agric. Engng Res.* 2000, 76, 419–421.

- Padley F.B. and Timms R.E. The determination of cocoa butter equivalents in chocolate. *JAOCS* September 1980, 286–292.
- Pease J.J. Confectionery fats from palm oil and lauric oil. *JAOCS*, February 1985, 62(2), 426–430.
- Peers K. E.: The non-glyceride saponifiables of shea butter. *J. Sci Fd Agric.* 1977, 28, 1000–1009.
- Petersson B., Anjou K. and Sandstrom L. Pulsed NMR method for solid fat content determination in tempering fats. Part I: cocoa butters and equivalents. *Fette, Seifen, Anstrichmittel* 1985, 87(6), 222–230.
- Petersson B. Pulsed NMR method for solid fat content determination in tempering fats, part ii: cocoa butters and equivalents in blends with milk fat. *Fette, Seifen, Anstrichmittel* 1986, 88(4), 128–136.
- Pinyaphong P. and Phutrakul S. Modification of palm oil structure to cocoa butter equivalent by *Carica papaya* lipase-catalyzed interesterification. *World Academy of Science, Engineering and Technology* 2009, 54, 536–540.
- Pocklington W.D. and Hautfenne A. Determination of triglycerides in fats and oils, results of a collaborative study and the standardised method. *Pure & Appl. Chem.* 1985, 57(10), 1515–1522.
- Rombaut R., De Clercq N., Foubert I. and Dewettinck K. Triacylglycerol analysis of fats and oils by evaporative light scattering detection. *J Am Oil Chem Soc* 2009, 86, 19–25.
- Saleem M. Lupeol, a novel anti-inflammatory and anti-cancer dietarytriterpene. *Cancer Lett.* 2009 November 28, 285(2), 109–115.
- Santana A., Fernandez X., Larrayoz M.A., Recasens F. Vegetable fat hydrogenation in supercritical-fluid solvents: Melting behavior analysis by DSC and NMR. *J. of Supercritical Fluids* 2008, 46 322–328.
- Segall S.D., Artz W.E., Raslan D.S., Ferraz V.P., Takahashi J.A. Analysis of triacylglycerol isomers in Malaysian cocoa butter using HPLC–mass spectrometry. *Food Research International* 2005, 38, 167–174.
- Shukla V.K.S. Confectionery lipids. *Bailey's Industrial Oil and Fat Products*, Sixth Edition, 2005, 159–173.
- Spangenberg J.E. and Dionisi F. Characterization of cocoa butter and cocoa butter equivalents by bulk and molecular carbon isotope analyses: implications for vegetable fat quantification in chocolate. *J. Agric. Food Chem.* 2001, 49, 4271–4277.
- Talbot G. and Slager H. Cocoa butter equivalents and improvers: Their use in chocolate and chocolate-coated confectionery. *Agrofood Industry Hi-tech*, May/June 2008, 19(3), 28–29.
- Talbot G., Smith K. and Zand I. Effects of trisaturated triglycerides on chocolate rheology. *Euro Fed Lipid Congress*, Madrid, October 2006.
- Teklehaimanot Z. Exploiting the potential of indigenous agroforestry trees: *Parkia biglobosa* and *Vitellaria paradoxa* in sub-Saharan Africa. *Agroforestry Systems* 2004, Volume 61–62, Numbers 1–3, 207–220.
- Timms R.E. Cocoa butter, a unique vegetable fat. *Lipid Technology Newsletter*, October 1999, pp. 101–107. <http://www.britanniafood.com/english/tc07.htm>.

- Torbica A., Jovanovic O., Pajin B. The advantages of solid fat content determination in cocoa butter and cocoa butter equivalents by the Karlshamns method. *Eur Food Res Technol* 2006, 222: 385–391.
- Ugese F.D., Baiyeri K.P. and Mbah B.N. Leaf area determination of shea butter tree (*Vitellaria paradoxa* C.F. Gaertn.). *Int. Agrophysics* 2008, 22, 167–170.
- Ugese F.D., Baiyeri K.P. and Mbah B.N.: Determination of growth stages and seedling structures associated with slow emergence of shea butter tree (*Vitellaria paradoxa* C. F. Gaertn.) seedlings. *Journal of Animal & Plant Sciences* 2010, 8(2), 993–998.
- Ugese F.D., Baiyeri K.P. and Mbah B.N. Agroecological variation in the fruits and nuts of shea butter tree (*Vitellaria paradoxa* C. F. Gaertn.) in Nigeria. *Agroforest Syst* 2010, 79, 201–211.
- Ulberth F. and Buchgraber M. Analytical platforms to assess the authenticity of cocoa butter. *Eur. J. Lipid Sci. Technol.* 2003, 105, 32–42.
- Ulberth F. and Buchgraber M. Authenticity of fats and oils. *Eur. J. Lipid Sci. Technol.* 2000, 102, 687–694.
- United States agency for international development (USAID): A marketing manual for west Africa: Buying and selling shea butter. October, 2002.
- Van Pee W.M., Boni L.E., Foma M.N. and Hendriks A. Fatty acid composition and characteristics of the kernel fat of different mango (*Mangifera indica*) varieties. *J. Sci. Food Agric.* 1981, 32, 485–488.
- Vermilye K.L. *Vitellaria paradoxa* and the feasibility of a shea butter project in the north of Cameroon. Thesis of Master, The University of Montana, 2004.
- Walter P. and Cornillon P. Influence of thermal conditions and presence of additives on fat bloom in chocolate. *JAOCS* 2001, 78(9).

18

Enzymatic and Other Modification Techniques to Produce Cocoa Butter Alternatives

Stefanie Verstringe, Nathalie De Clercq, Tuyet Mai Nguyen, Sheida Kadivar, and Koen Dewettinck

Laboratory of Food Technology and Engineering, Department of Food Safety and Food Quality, Ghent University, Ghent, Belgium

Introduction

Cocoa butter (CB) is an essential ingredient in chocolate as it forms the continuous phase of chocolate. It is responsible for the gloss, texture, and typical melting behavior of chocolate. An increasing demand for chocolate and chocolate-type products increases the demand for cocoa from year to year (Shukla, 2005). Although cocoa butter is the ideal ingredient, the varying supply and increasing price depending on fluctuating cocoa bean prices, forced manufacturers to seek alternatives. Cocoa butter can be replaced by other vegetable fats, collected under the name cocoa butter alternatives (CBAs). Attempts were made as early as 1930 by confectioners to use fats other than cocoa butter but they failed due to incompatibility of the fat blends. The continued research in the field of confection science resulted in the development of fats resembling the characteristics of cocoa butter (Shukla, 2005). The CBAs are divided into three main categories according to their functionality and similarity to cocoa butter (Lipp and Anklam, 1998a): the cocoa butter equivalents (CBEs), the cocoa butter replacers (CBRs) and the cocoa butter substitutes (CBSs). Table 18-A lists the different CBAs with their main properties.

The CBEs are nonlauric fats with similar physicochemical characteristics as cocoa butter and are therefore compatible with cocoa butter in every amount without altering the properties of cocoa butter. A CBE should allow processing of chocolate products in an identical manner to that of cocoa butter based products. The CBEs are subdivided into two groups. The first group, the cocoa butter extenders (CBEXs), are defined by Timms (2003) as fats that extend or dilute cocoa butter to make it more economical feasible. The second group, the cocoa butter improvers (CBIs), are characterized by a high content of StOSt. StOSt increases the solid fat content and as such the melting point and the hardness of chocolate. Chocolates with CBIs have better resistance to softness and fat bloom at higher ambient temperatures, such as in summer or in tropical climates (Lipp & Anklam, 1998a; Timms, 2003). Illipe butter, shea fraction, and kokum fat have a higher content of StOSt and have been reported to

Table 18-A. Classification of Cocoa Butter Alternatives (Norberg, 2006; Stewart and Timms, 2002; Talbot, 2009; Timms, 2003).

	CBE	CBR	CBS
Origin	Illipe butter Palm oil Sal fat Shea butter Kokum butter Mango kernel fat	Palm oil Soybean oil Rapeseed oil Cottonseed oil	Palm kernel oil Coconut oil
Processing	Hydrogenation Fractionation Interesterification	Hydrogenation Fractionation	Hydrogenation Fractionation
TAG composition	Similar to CB	Different from CB	Different from CB
Lauric acid	Non lauric	Non lauric	Lauric (45–55% lauric acid)
Compatibility to CB	Compatible	Compatible in small ratios	Incompatible
Crystallization	Tempering to obtain stable polymorphic form	Crystallize directly from the melt in the stable polymorphic form	Crystallize directly from the melt in the stable polymorphic form
Application	*5% replacement on total product *Compound	Compound	Compound

impart these qualities and can therefore be used to harden cocoa butter and chocolate products (Maheshwari & Reddy, 2005).

The nonlauric CBRs consist of fractions of hydrogenated oils: palm, soybean, cotton, corn, peanut, safflower and sunflower oil. They are hydrogenated either alone or as a blend under selective, *trans*-promoting (elaidic acid) conditions to give relatively steep SFC melting curves (Timms, 2003). They possess good flavour, odour and colour properties and do not need tempering (Shukla, 2005). As a result of the similarity in the chain length and the molecular weight, products of this type can tolerate up to 25% cocoa butter on a fat basis when used in confectionary coating. Outside these boundaries eutectics occur due to incompatibility. This gives rise to softer products and provokes loss of gloss and fat bloom formation (Talbot, 2009). The high level of *trans* fatty acids in these products is a significant disadvantage as the consumption of *trans* fatty acids may be seen as a risk factor in cardiovascular disease (Stewart, 2005). To address the issue of *trans* fatty acids in CBRs, manufacturers are trying to produce low *trans* or even no-*trans* alternatives (Talbot, 2009).

Unlike the CBEs and CBRs, the CBSs fats contain a large amount (45–55%) of lauric acid (C12:0) and a considerable amount (15–20%) of myristic acid (C14:0)

(Stewart & Timms, 2002). They can be produced from palm kernel and/or coconut oil by means of fractionation and/or hydrogenation (Talbot, 2009). Palm kernel oil is more useful than coconut oil, because it has a higher content of oleic acid and a lower content of short-chain (C6:0 to C10:0) fatty acids (Timms, 2003). Due to the presence of lauric and myristic acid, CBSs have a sharp melting profile. This melting profile gives a good mouth feel to the compound chocolates. Due to their triacylglycerol composition there is a considerable degree of incompatibility between lauric CBSs and cocoa butter. CBSs and cocoa butter form strong eutectics. CBSs are suitable to substitute cocoa butter completely or an addition of cocoa butter up to 5% is acceptable (Lipp & Anklam, 1998a; Talbot, 2009). If more than 5% cocoa butter is present in the fat phase, excessive softening and fat bloom occurs. Therefore compound chocolates with CBSs have to be made with low-fat cocoa powders instead of cocoa mass. This makes it difficult to achieve a compound with a strong cocoa flavour (Stewart & Timms, 2002).

CBRs and CBSs have a different crystallization behaviour compared to cocoa butter. They crystallize in a stable form directly from the melt, so no tempering is needed. If these fats are cooled very quickly (shock cooling), small crystals are formed with uniform sized crystals. Small crystals give excellent gloss and have a good bloom stability (Norberg, 2006).

In the European Union, the use of vegetable fats is regulated by the EU Directive 2000/36/EC relating to cocoa and chocolate products intended for human consumption. They are defined as nonlauric vegetable fats, which are rich in symmetrical monounsaturated triacylglycerols (TAG) of the type POP, POSt, and StOSt. They are miscible in any proportion with cocoa butter, and are compatible with its physical properties (melting point and crystallization temperature, melting rate, need for tempering phase). They are obtained only by the processes of refining and/or fractionation, which excludes enzymatic modification of the triacylglycerol structure. This description corresponds with the definition of a CBE. Prior to 2003 any vegetable fat could be used, provided it met the properties mentioned above. But since 3rd of August 2003 an amendment on the EU Directive 2000/36/EC stated that only six vegetable fats, i.e. illipe, palm oil, sal, shea, kokum gurgi and mango kernel can be used in chocolate. The addition of these fats cannot exceed 5% of the finished product after deduction of the total weight of any other edible matter used, without reducing the minimum content of cocoa butter or total dry cocoa solids. Coconut oil is an exception as it may be used in chocolate for the manufacturing of ice cream and similar frozen products. Chocolate products which contain vegetable fats other than cocoa butter following the EU Directive, may be marketed as chocolate, provided that their labeling is supplemented by a clearly legible statement: 'contains vegetable fats in addition to cocoa butter'. This statement must be in the same field of vision as the list of ingredients, in lettering at least as large and in bold with the sales name nearby (Talbot, 2009).

Countries outside the EU have their own national regulations. For example the United States do not permit the use of vegetable fat other than cocoa butter in

chocolate, but do allow its use in 'chocolate and vegetable fat coatings'. Also the Codex Alimentarius standard STAN 87-1981 was revised in 2003 (FAO/WHO, 2003) to permit the use of vegetable fats in chocolate up to a level of 5% of the finished product. The standards published by Codex Alimentarius are used by many countries as the basis for determining the acceptability of foods. The standard has many similarities with the EU Directive (Martinez-Inchausti, 2009). Most countries permit higher levels of CBEs in chocolate, but these products cannot be labelled 'chocolate'. However, as noticed by Talbot (2009), legislation can change so it is desirable to check the legislation of the specific country before applying a CBA in a chocolate product.

Modification Techniques for the Production of CBAs

The specific fatty acid profile and their distribution on the glycerol backbone in natural oils and fats affect their functional properties like crystallization and melting behavior. Generally, oils and fats have a limited use in their native form. Therefore, fat modification technologies were developed driven by the need to modify the physical and chemical properties of natural oils and fats in order to extend their applicability. The best known processes applied in edible oil industry are hydrogenation, interesterification and fractionation. They can be used alone or in combination to engineer a wide variety of fats and oils into products having different consistencies, melting points and textures or, in this case, to meet the requirements of a cocoa butter alternative. Generally, the use of only one technique is not sufficient and several techniques need to be combined together to produce a cocoa butter alternative fat.

Enzymatic Interesterification for the Production of CBAs

Interesterification is an important modification technique resulting in the redistribution of the fatty acids along the glycerol backbone, which results in a change of the physicochemical properties of the fat. It refers to the reaction of triacylglycerols within or between the molecules or with other acyl donors or alcohol moieties, producing new esters by an interchange of fatty acid groups. Interesterification therefore collects a group of reactions i.e. an ester with a fatty acid (acidolysis), an alcohol (alcoholysis) or another ester (ester-ester exchange). It can be conducted via the catalysis of a chemical or an enzyme. By this way, it is divided into chemical and enzymatic interesterification (Ahmadi et al., 2009; Xu et al., 2006). Chemical interesterification, which leads to a random distribution of the fatty acids on the glycerol backbone, has long been used to modify oils and fats into functional products (Marangoni & Rouseau, 1998). Today, enzymatic interesterification (EIE) can be conducted in specific or nonspecific conditions depending on the regioselectivity of the used enzyme (Kim & Yoon, 2003). Compared to chemical interesterification, it is carried out under milder conditions leading to fewer side reactions, less post-treatment and in consequence less oil losses (Zhang et al., 2004). Nonspecific EIE (ester-ester exchange)

is the most applied technique for the production of commodity fats to modify the overall melting properties, to increase compatibility within the solid phase and to enhance the plasticity of the final product (Costales-Rodriguez et al., 2009). Specific EIE (acidolysis or ester-ester exchange) can be used for the production of tailor-made products like cocoa butter equivalents, anti-bloom agents, breast milk substitutes and low calorie fats (Costales-Rodriguez et al., 2009; Huey et al., 2009; Xu, 2000).

Different varieties of lipases are used nowadays in industries due to their regiospecificity and stereospecificity in production of specific structures. Among all types of lipases, microbial lipases are most interesting because of their different specifications and thermostability without the need of a co-lipase. Many different microbial lipases are used in lipid modification although only a few of them are commercially available. Among them, Lipozyme IM, in which *Rhizomucor miehei* lipase is immobilized on ion exchange resin, is widely used in the production of structured lipids. It is the most favorable lipase in terms of reactivity and stability.

Due to their specificity, lipase-catalyzed reactions are the ideal tool to produce tailor-made fat products. In this way, they are predominantly used to produce cocoa butter equivalents with a very similar triacylglycerol composition compared to genuine cocoa butter. This is possible because vegetable oils often contain oleic acid in the sn-2 position. The regiospecificity of a 1,3-specific lipase can then be employed to incorporate the proper saturated fatty acids, i.e. palmitic and stearic acid, in the 1- and 3-positions of a low-cost oil until a similar triacylglycerol composition of cocoa butter is obtained. In exceptional cases, lipases are applied to modify the physical properties of an abundant and low-cost starting material in order to be able to use the modified product as a cocoa butter-like fat. To the best of our knowledge, enzymatic modification of lauric fats in order to make them suitable as cocoa butter substitutes has not been used.

Suitable Substrates for the Enzyme-catalyzed Production of CBAs

Xu (2000) gives an overview of sources of oils and fats that contain abundant oleic acid at their sn-2 position (Table 18-B). Palm oil midfraction and Chinese vegetable

Table 18-B. Sources of Oils and Fats that Contain Abundant Oleic Acid at Their sn-2 position, POP or StOSt (Xu, 2000).

Oleic acid at the sn-2 position	POP	StOSt
High oleic sunflower oil	Palm oil midfraction	Sal fats
Tea seed oil	Chinese vegetable tallow	Mango fat
Olive oil		Illipe fat
High oleic canola oil		Kokum fat
Palm oil midfraction		Shea oil
Cocoa butter		
Chinese vegetable tallow		

tallow are rich in POP while a limited number of natural oils and fats are rich in StOSt (Table 18-B). The latter five oils and fats, i.e. sal, mango, illipe, kokum, and shea fat, together with palm oil, form the six base oils which are allowed to be used in CBEs in the European Union. Only illipe and kokum contain about an equal amount of SOS type (S, saturated) triacylglycerols as cocoa butter. However, illipe is rarely used for reasons of availability and quality. Shea, sal and mango fat are subjected to a single-stage fractionation to separate the higher melting SOS triacylglycerols from the lower melting diunsaturated and triunsaturated triacylglycerols. Palm oil undergoes a double-stage fractionation to separate the SOS triacylglycerols. After the fractionation, the amount of SOS triacylglycerols has raised to about 80%. However, in none of the six cases, the POP:POSt:StOSt ratio is similar to that of cocoa butter. Therefore, in practice, blending of the components is needed to obtain a suitable cocoa butter equivalent.

The seeds from the *Sapium sebiferum* tree yield two different lipids: Chinese vegetable tallow, from the outer seed coating, and stillingia oil, from the kernel. Stillingia oil is nutritionally unacceptable due to the presence of estolide-type acylglycerols. Therefore, the possible contamination of Chinese vegetable oil with stillingia oil is the major problem of its use in edible products (Jeffrey & Padley, 1991). This is probably the reason why, so far, no scientific research has been reported using Chinese vegetable tallow as substrate oil for the production of cocoa butter equivalents.

Acyl Donors Used for the Enzyme-catalyzed Production of CBAs

Next to the substrate oil or fat, an acyl donor is necessary to produce the desired triacylglycerol composition. Three types of acyl donors can be used for the production of cocoa butter alternatives: triacylglycerols, fatty acid esters and free fatty acids. The choice depends on product purity, reactivity, purification technology and price. Triacylglycerols as acyl donors are at a disadvantage because similar triacylglycerol species are produced than those produced by randomization (Mohamed et al., 1993). In general, triacylglycerols as acyl donors have a lower reactivity while free fatty acids have a higher reactivity. The reactivity of free fatty acids and their ethyl esters does not differ clearly. Fatty acid esters are more volatile than free fatty acids and so they offer the advantage to be more easily separated by distillation. Opposite results were observed related to the diacylglycerol formation. Bloomer et al. (1991) noted a higher diacylglycerol formation when ethyl esters were used instead of free fatty acids, whereas Gitlesen et al. (1995) saw the reverse. Diacylglycerol formation is disadvantageous as it may lead to acyl migration and the formation of unwanted trisaturated triacylglycerols. Overall, free fatty acids are the most often used acyl donors due to their easy availability, low price and high reactivity (Xu, 2000).

Fatty Acids and Fatty Acid Esters as Acyl Donors. When using free fatty acids or fatty acid esters as acyl donors in a 1,3-specific lipase-catalyzed interesterification reac-

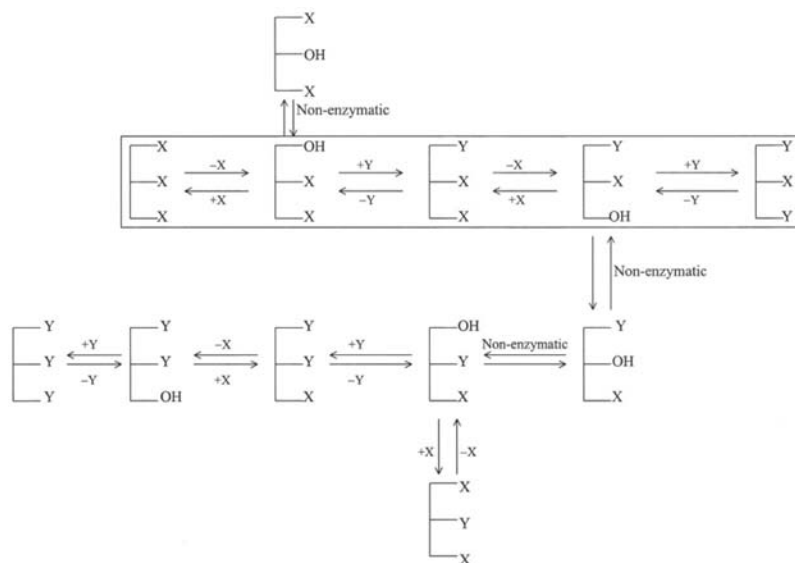


Fig. 18.1. Reaction scheme of a 1,3-specific lipase-catalyzed interesterification between triacylglycerols (XXX) and free fatty acids or fatty acid esters (Y). The main reactions are enclosed in the rectangle. X and Y are different fatty acids (Xu, 2000).

tion, three main triacylglycerol species are formed: XXX, sn-YXX/XXY, and sn-YXY (Fig. 18.1).

No other isomers will be formed theoretically as the used lipase is 1,3-specific. However, as shown in Fig. 18.1, side reactions can occur due to the formation of diacylglycerols. These intermediates of the main reactions can cause acyl migration which leads to the formation of by-products. Factors that are affecting acyl migration are temperature, reaction time, water content, water activity, enzyme content and reactor type. Overall, the amount of by-products is usually small (Xu, 2000). Ciftci et al. (2008) used a model enzymatic acidolysis system to study the effects of different reaction parameters on the production of the three major triacylglycerols in cocoa butter. Palmitic and stearic acid were therefore incorporated into triolein under the catalysis of Lipozyme. Concerning the substrate mole ratio, the best results were obtained at 1:3:3 (OOO:palmitic acid:stearic acid) mole ratio. At higher ratios, the excess of palmitic and stearic acid causes a decrease in the activity of the enzyme. The same amounts of palmitic and stearic acid were incorporated so it could be concluded that Lipozyme IM does not have higher reactivity and/or selectivity for palmitic over stearic acid or vice versa.

An overview of studies using free fatty acids and/or fatty acid esters as acyl donors is listed in Table 18-C.

Table 18-C. Authors Using Free Fatty Acids and/or Fatty Acid Esters as Acyl Donors for the Enzyme-catalyzed Synthesis of Cocoa Butter Alternatives.

Target CBA	Substrate ¹	Acyl donor ¹	Enzyme used	Optimized conditions	Reference
CBE	Oils and fats with high 2-oleyl TAG content	Alcohol ester of stearic acid and/or palmitic acid	A 1,3-specific lipase	Enzyme load: 0.1-10% Water: 0.01-0.18%	Matsuo et al. (1981a)
CBE	Palm oil midfraction	Stearic acid	Aspergillus niger lipase	Solvent: petroleum ether T: 40°C Time: 16h	Macrae (1984)
CBE	A triacylglycerol	Free fatty acids	Lipase from Aspergillus niger, Mucor species or Rhizopus species	T: 0-60°C Time: <2h	Macrae and How (1989)
CBE	A glyceride oil	Palmitic or stearic acid	Rhizopus delemar lipase	Not mentioned	Tanaka et al. (1989)
CBE	Palm oil midfraction	Ethyl stearate	Twelve commercially available lipases	Solvent: heptane T: 40°C Time: 24h	Bloomer et al. (1990)
CBE	Mahau, kokum, dhupa, sal and mango fat	Palmitate and stearate methyl ester	Lipozyme	Solvent: hexane Enzyme load: 6% T: 60°C Time: 4h	Sridhar et al. (1991)
CBE	Palm olein	Stearic acid	Lipozyme IM20	Solvent free Enzyme load: 10% Water: 1% T: 60°C Time: 20h	Chong et al. (1992)
CBE	Palm oil midfraction	Stearic acid	Rhizopus arrhizus lipase	Solvent: hexane	Mojovic et al. (1993)
CBE	High-oleic-acid rapeseed oil	Stearic acid and ethyl stearate	Rhizopus arrhizus lipase	Solvent free T: 50°C Time: 24h	Gitlesen et al. (1995)
CBE	Palm oil midfraction	Stearic acid	Rhizopus arrhizus lipase	Solvent: hexane Enzyme loading: 10.33 U/mg celite Aw: 0.432	Ju et al. (1996)
CBE	Palm oil midfraction	Stearic acid	Rhizopus arrhizus lipase	Solvent: hexane	Huang and Ju (1997)

CBE	Piqui (Caryocar brasiliense Camb) oil	Stearic acid	Lipozyme	Enzyme load: 10% T: 70°C Time: 4h	Facioli and Goncalves (1998)
CBE	Palm oil	Stearic acid	Lipozyme	Solvent: SC-CO ₂ at 200 bar T: 44.2°C	Liang et al. (1998)
CBE	Palm oil midfraction	Stearic acid	Lipozyme	T: 65-70°C	Undurraga et al. (2001)
CBE	Strychnos madagascariensis oil, Trichelia emetica oil and Ximenia caffra oil	Palmitic and stearic acid	Lipozyme	Solvent: 2,2,4-trimethylpentane Aw: 0.4 T: 60°C Time: 4h	Khumalo et al. (2002)
CBE	Tea seed oil	Methyl palmitate and methyl stearate	Porcine pancreas lipase	Solvent: hexane Enzyme load: 15% Water: 10mg/g T: 35°C Time: 60h	Wang et al. (2006)
CBE	Triolein	Palmitic and stearic acid	Lipozyme IM	Solvent: hexane Enzyme load: 20% Water: 5% T: 45°C Time: 10h	Ciftci et al. (2008)
CBE	Pentadesma butyacea butter	Ethyl palmitate	Lipozyme TL IM	Nonpolar solvent Enzyme load: 8% T: 55°C	Tchobo et al. (2009)
CBE	Palm oil	Methyl stearate	Carica papaya lipase	Solvent free Enzyme load: 18% Aw: 0.11 T: 45°C Time: 4h	Pinyaphong and Phutrakul (2009)
CBE	Triolein or high oleic sunflower oil	Stearic acid	Rhizopus oryzae lipase	T: 65-80°C	Favre et al. (2010)
Hard butter with high StOSt content	Different substrate oils	A fatty acid or fatty acid ester	Lipozyme TL IM	Water: <0.02% T: 40-50°C	Kang et al. (2010)
CB-like fat	Beef tallow	Stearic acid	Lipozyme IM60	Solvent: hexane T: 58°C Time: 24h	Osborn and Akoh (2002)
CB-like fat	Refined olive pomace oil	Palmitic and stearic acid	Lipozyme IM	Solvent free Enzyme load: 40% T: 45°C Time: 3h	Ciftci et al. (2009)

¹In case of optimization, the most suitable one is reported.

At the end of the seventies, Unilever was the first to patent a process for the enzymatic modification of oils and fats (Coleman & Macrae, 1977). A few years later, Fuji Oils patented a process for the production of a cocoa butter equivalent. Fats and oils containing triacylglycerols rich in oleic acid at the sn-2 position were to be transesterified with an alcohol ester of stearic acid and/or palmitic acid in the presence of a 1,3-specific lipase (Matsuo et al., 1981a). At the end of the eighties, Unilever patented the production of a cocoa butter equivalent by interesterifying a fatty acid ester reactant, preferably a triacylglycerol and optionally including free fatty acids, by a lipase from *Aspergillus niger*, *Mucor* species or *Rhizopus* species (Macrae & How, 1989). At the same time, the Kao Corporation from Japan filed a patent concerning the enzymatic treatment of a glyceride oil with palmitic or stearic acid in the presence of *Rhizopus delemar* lipase in order to produce a cocoa butter equivalent (Tanaka et al., 1989). A more recent patent describes the production of a hard butter with high StOSt content with Lipozyme TL IM using different substrate oils and a fatty acid or fatty acid ester (Kang et al., 2010). Another process that has been patented recently, is the interesterification of triolein or high oleic sunflower oil with stearic acid in the presence of a lipase from *Rhizopus oryzae*. After fractionation of the interesterified product and blending with palm midfraction, the mixture can be used as a cocoa butter equivalent (Favre et al., 2010).

Common Substrates

A lot of research focuses on the use of palm oil and its fractions for the production of cocoa butter alternatives. Palm oil is a low cost fat and is often readily available. The main triacylglycerol component of palm oil is POP, which can be converted to POSt and StOSt by selective incorporation of stearic acid in the sn-1- and 3-position by using a 1,3-specific lipase and a stearic acid-rich acyl donor. Pinyaphong and Phutrakul (2009) used *Carica papaya* lipase to modify palm oil to a cocoa butter equivalent. *C. papaya* lipase was prepared from the unripe fruit of Thai papaya tree. Different reaction parameters were tested, among which the acyl donor. Three different types, methyl stearate, ethyl stearate and stearic acid, were used and it was found that, for this reaction system, methyl stearate was the most appropriate stearyl donor to yield a product with a similar composition as cocoa butter. The cocoa butter equivalent produced under the optimized reaction conditions had similar chemical and physical properties, such as iodine value, saponification value and melting point, as natural cocoa butter. Liang et al. (1998) studied the interesterification of palm oil and stearic acid in the presence of Lipozyme and supercritical carbon dioxide as solvent in a continuous flow system. When the stearic acid content was abundant, Lipozyme was found to predominantly catalyze the interesterification of POP and stearic acid to POSt and POO and stearic acid to StOO.

Palm oil midfraction is obtained by double fractionation of palm oil and is often used as a raw material to produce cocoa butter equivalents. Already in 1985, Macrae

described the enzymatic conversion of a mixture of palm mid fraction and stearic acid into a cocoa butter equivalent fat by using an *Aspergillus niger* lipase. However, the kieselguhr-immobilized enzyme was difficult to use in practice (Holm & Cowan, 2008). A few years later, Bloomer et al. (1990) screened twelve commercially available lipase preparations in their ability to catalyze the interesterification of palm mid fraction and ethyl stearate to form a cocoa butter equivalent. Five fungal lipase preparations were found to be suitable to incorporate enough stearate: Lipozyme, Enzymatix F3 (*Rhizopus* sp.), Enzymatix F4 (*Rhizopus* sp.), Gist-brocades *R. arrhizus* lipase and Röhm 2212E (fungal origin). In later studies, *Rhizopus arrhizus* lipase was used to convert a mixture of palm mid fraction and stearic acid into a cocoa butter equivalent (Huang & Ju, 1997; Ju et al., 1996; Mojovic et al., 1993). Undurraga et al. (2001) used Lipozyme to convert a mixture of palm mid fraction and stearic acid into a cocoa butter equivalent. Palm oil midfraction was chosen in terms of cost, availability and composition while stearic acid was preferred due to its availability and low cost. Because the application concerns a food process, a solvent free system was used. The interesterified product was distilled through which free fatty acids were totally eliminated, but diacylglycerols formed during the reaction were not removed. Due to the presence of diacylglycerols and a small fraction of trisaturated triacylglycerols formed during the reaction, the melting profile of the interesterified product was similar to cocoa butter but showed several distinct peaks. This does not warrant the use of the interesterified product as a good quality cocoa butter equivalent. The authors note that further purification of the product is technically feasible but this could make the overall process uneconomical.

The research of Chong et al. (1992) was based on palm olein as substrate oil. The incorporation of stearic acid gave rise to the formation of about 40% of the three main cocoa butter triacylglycerols. After removal of the free fatty acids, these triacylglycerols were selectively fractionated with hexane and/or acetone. In this way, 78–89% of cocoa butter-like triacylglycerols could be isolated. The triacylglycerol composition and melting profile of the end product were comparable to those of cocoa butter but the overall process yielded only 25% of the original palm olein.

Sridhar et al. (1991) modified five Indian vegetable fats (sal, kokum, mahua, dhupa and mango fat) by Lipozyme-catalyzed ester interchange with methyl palmitate and/or stearate. The aim was to increase the level of SOS triacylglycerols in order to be able to use the modified products as a cocoa butter alternative. As the indigenous vegetable fats contain major proportions of oleic and stearic acids, palmitic acid had to be incorporated through lipase-catalyzed interesterification. Addition of a stearate source was necessary to restore the depletion of stearate during ester interchange. The authors' preliminary observed that the ester interchange method was better than acidolysis and that methyl esters were slightly better incorporated than ethyl esters. In conclusion, they found that interesterified dhupa, kokum and sal fats were similar to cocoa butter in fatty acid composition, the sn-2 position of the triacylglycerols, as well as glyceride composition. Of these, interesterified kokum possessed physical and chemical properties closest to that of cocoa butter.

Alternative Substrates

A wide range of other vegetable oils have been explored to serve as starting material for the production of a cocoa butter equivalent. These include refined olive pomace oil, *Pentadesma butyracea* butter, tea seed oil, *Strychnos madagascariensis* oil, *Trichelia emetica* oil and *Ximenia caffra* oil, Piqui oil, and high-oleic-acid rapeseed oil. However, none of these alternative sources has found general applicability.

Olive pomace oil is extracted from the olive pomace which is left after mechanical pressing of olives. It is considered a low-quality oil because of long storage times and high temperature drying. However, its chemical composition after refining does not differ from refined olive oil. Olive pomace oil has high sn-2 position oleic acid content and a low cost. It is therefore an ideal substrate for the production of a fat enriched in the three major triacylglycerols present in cocoa butter. Recently, Ciftci et al. (2009) studied the production of a cocoa butter like fat from olive pomace oil and palmitic and stearic acid by enzymatic acidolysis in the presence of Lipozyme. Concerning the substrate mole ratio, a higher amount of stearic acid was needed as it was observed that stearic acid is a poor acyl donor compared to palmitic acid. This is in contrast with a previous research of the authors where it was found that, using triolein as substrate and in the presence of Lipozyme, equal amounts of palmitic and stearic acid were incorporated. The modified product was characterized in terms of triacylglycerol content, thermal characteristics and microstructure. It had a melting and SFC profile comparable to that of cocoa butter. Moreover, polarized light microscope images showed no drastic changes in the microstructure between the modified olive pomace oil and cocoa butter. In a next step, binary blends of cocoa butter and the cocoa butter-like fat were prepared in different proportions and their physicochemical characteristics (triacylglycerol content, melting profile, solid fat content and microstructure) were analyzed to determine the compatibility limit (Ciftci et al., 2010). It appeared that the addition of cocoa butter-like fat to cocoa butter should be limited to 30% in order to save the physical and chemical properties of the product. Use of enzymatically modified olive pomace oil can reduce costs and creates value to a by-product.

The African butter from *Pentadesma butyracea* contains a high amount of oleic acid at the sn-2 position with StOO and StOSt as the main triacylglycerol components. Tchobo et al. (2009) used the butter as starting material for the synthesis of a cocoa butter equivalent. Enzymatic incorporation of ethyl palmitate gave rise to an increase of the amount of POSt and a strong reduction in StOSt content. StOSt was converted to POSt and POP while StOO was converted to POO. Therefore, fractionation of the butter was necessary to eliminate StOO in order to prevent POO formation. The melting profile of the modified solid fraction of *Pentadesma butyracea* butter was similar to cocoa butter. Only minor differences were observed, particularly due to the presence of trisaturated triacylglycerols.

Tea seed is considered as a by-product of tea-processing although its oil content is around 30–32% and it resembles olive oil. Wang et al. (2006) claimed that tea seed oil is an ideal starting material for the synthesis of cocoa butter equivalents as oleic

acid constitutes about 74–87% of the triacylglycerols at the sn-2 position. They prepared a cocoa butter equivalent by interesterifying the tea seed oil with methyl palmitate and methyl stearate using porcine pancreas lipase immobilized on a macroporous resin. The prepared products contained the same amount of palmitic and stearic and a slightly higher amount of oleic acid compared to cocoa butter and were composed mainly of SUS triacylglycerols. Due to the slightly higher oleoyl content, the product had a lower melting point compared to cocoa butter. The dilatation curves of the CBE and cocoa butter were similar but the melting range of the CBE was slightly wider.

Khumalo et al. (2002) investigated the potential of three abundant and underutilised vegetable oils to be used as starting materials for the enzymatic production of cocoa butter equivalents. Oils were extracted from the inner skin of *Strychnos madagascariensis* fruit shell, *Trichelia emetica* seed and *Ximenia caffra* seed. *S. madagascariensis* and *X. caffra* oil contain a high amount of oleic acid while *T. emetica* contains a low amount. In the *X. caffra* and *T. emetica* oil, oleic acid was more concentrated in the sn-2 position of triacylglycerols while in the case of *S. madagascariensis* oleic acid is evenly distributed among the three positions. Based on triacylglycerol analysis of the reaction products, it was concluded that the products derived from *S. madagascariensis* and *X. caffra* oil compared best with natural cocoa butter and were therefore the two most suitable substrates for the production of cocoa butter equivalents.

Caryocar brasiliense is known as piqui throughout Brazil. This indigenous word means “thorny covering,” referring to the characteristic endocarp that is covered with numerous slender thorns. The species is economically exploited for the oil contained in the mesocarp which is used as butter substitute and the oil contained in the fruit kernel which is used as cooking oil (Maia et al., 2008). Facioli and Gonçalves (1998) transformed piqui oil into a cocoa butter equivalent fat through an enzymatic interesterification reaction using Lipozyme to incorporate stearic acid. Through optimization of the reaction parameters they were able to produce a fat with a comparable triacylglycerol composition as cocoa butter.

Gitlesen et al. (1995) showed in their study that rapeseed oil with a high content of oleic acid is a useful starting material for the production of a confectionary fat. When using ethyl stearate instead of stearic acid, the amount of diacylglycerols and consequently acyl migration and unwanted saturated fatty acids in the 2-position could be reduced. This is in contrast with the observation of Bloomer et al. (1991), who noted a higher diacylglycerol formation when ethyl palmitate was used instead of palmitic acid. When using immobilized *Rhizopus arrhizus* lipase instead of Lipozyme, the highest triacylglycerol content was observed due to reduced hydrolysis. The main components in the enzymatically modified fat were SOS and SOO, which are useful components in confectionary fats. By increasing the ratio of acyl donor to triacylglycerol, the equilibrium can be shifted towards higher stearic acid content in the 1- and 3-positions. As the palmitic acid content of the high-oleic-acid rapeseed oil is only 3.2%, a palmitic acid donor should most likely be added.

Osborn and Akoh (2002) tried to utilize an animal by-product, beef tallow, as cocoa butter-like fat by modifying it enzymatically in the presence of stearic acid and

Lipozyme IM60. The modified beef tallow was applied in dark chocolate samples to fully replace cocoa butter. Beef tallow is a readily available and inexpensive fat but has an elevated saturated fatty acid content which is nutritionally disadvantageous. Modifying the beef tallow gave rise to a slightly better fatty acid profile. However, the melting profile and melting behaviour of the modified product differed from cocoa butter and softer chocolate samples without snap were obtained. The advantage of using modified beef tallow is that it reduces fat bloom development. In conclusion, enzymatically modified beef tallow may be useful to replace cocoa butter in chocolate coatings or in confectionery fillings where snap is not a desirable characteristic. However, the health concerns currently associated with beef tallow are not completely removed by the enzymatic treatment and must be kept in mind.

Triacylglycerols as Acyl Donors. The reaction scheme of an interesterification of two triacylglycerols by means of a chemical catalyst and a 1,3-specific lipase is presented in Fig. 18.2. They both provide the same triacylglycerol species but the proportions will differ. Only in some special cases, the undesired triacylglycerol species can be separated from the desired ones by means of fractionation. However, this is only possible when a difference in melting point exists between the two groups. In general, the use of triacylglycerols as acyl donors will yield mixtures similar to randomized products (Xu, 2000). Due to this drawback, triacylglycerols, and by extension oils and fats, are not often used as acyl donors. An overview of existing literature is given in Table 18.D

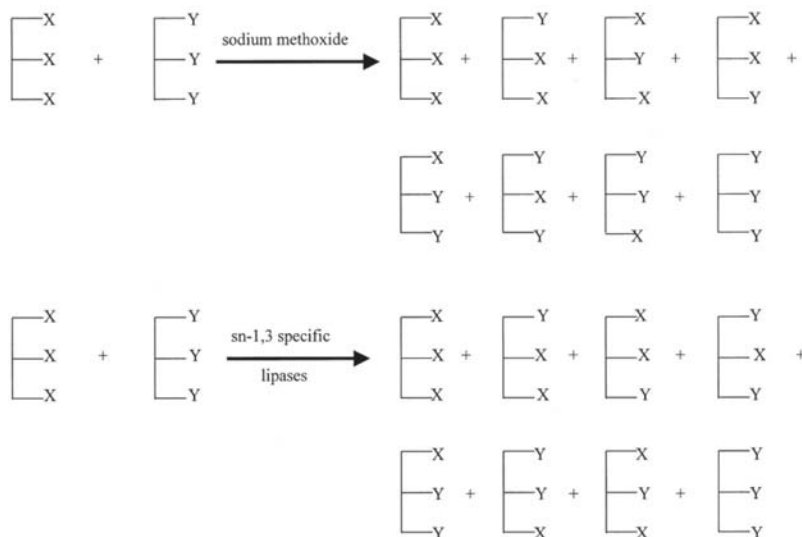


Fig. 18.2 Reaction scheme of an interesterification of two triacylglycerols by means of a chemical catalyst and a 1,3-specific lipase. X and Y are different fatty acids (Xu, 2000).

Table 18-D. Authors Using Triacylglycerols as Acyl Donors for the Enzyme-Catalyzed Synthesis of Cocoa Butter Equivalents and Cocoa Butter-like Fats.

Target CBA	Substrate ¹	Enzyme used	Optimized conditions	Reference
CBE	Olive oil and hydrogenated cottonseed oil	Lipozyme	Solvent free Enzyme load: 10% T: 70°C Time: 4h	Chang et al. (1990)
CBE	Palm oil and tristearin	Lipozyme IM-20	Solvent: SC-CO ₂ at 1500 psi, hexane and t-butyl alcohol Water: 5% pH: 7 T: 50°C Time: 3h	Liu et al. (1997)
CBE	Refined, bleached and deodorized palm oil and fully hydrogenated soybean oil	Lipozyme IM	Solvent free Enzyme load: 10% T: 70°C Time: 4h	Abigor et al. (2003)
CBE	Lard and tristearin	Lipozyme IM-20	Solvent: SC-CO ₂ at 17 Mpa Aw: 0.33 pH: 9 T: 50°C Time: 3h	Liu et al. (2007)
CBE	Camel hump fat and tristearin	Lipozyme TL IM	Solvent: SC-CO ₂ at 10 Mpa Enzyme load: 10% Water: 10% T: 40°C Time: 3h	Shekarchizadeh et al. (2009)
CB-like fat	Anhydrous milk fat and fractions thereof	Immobilized lipase	CO ₂ at atmospheric pressure or at supercritical pressure T: 50°C Time: 24h	Bystrom and Hartel (1994)
CB-like fat	Fully hydrogenated vegetable oil rich in stearic acid and a triacylglycerol rich in caproic, caprylic and capric acid	Immobilized lipase	Solvent free	Bertoli et al. (1995)
CB-like fat	Beef tallow	Candida antarctica (SP 435) lipase	Solvent: hexane T: 58°C Time: 24h	Osborn and Akoh (2002)
CB-like fat	Hydrogenated tea seed oil and solid fraction of fractionated tea seed oil	Lipozyme TL IM	Solvent free Enzyme load: 10% T: 60°C Time: 8h	Zarringhalami et al. (2010)

¹In case of optimization, the most suitable one is reported.

Palm oil, olive oil, lard, and camel hump fat have been enzymatically modified in the presence of a stearic acid donor like tristearin or a fully hydrogenated vegetable oil, in order to produce a cocoa butter equivalent. Abigor et al. (2003) used palm oil as POP source, fully hydrogenated soybean oil as stearic acid donor and Lipozyme IM as 1,3-specific lipase. They isolated the cocoa butter-like fats by fractional crystallization from acetone and after removing the mono- and diacylglycerols by silica chromatography, they obtained a product with a triacylglycerol composition closely resembling that of cocoa butter. Moreover, the melting point of the interesterification product was comparable to that of cocoa butter.

Liu et al. (1997) determined the effect of lipase, acyl donor, and type of oil on the synthesis of cocoa butter equivalent. Five different lipases were tested and palm oil, olive oil and peanut oil were examined as substrate oils. Stearic acid, stearic acid ethyl ester, stearic anhydride and tristearin were tested as possible acyl donors. The authors found that cocoa butter equivalent could be most efficiently and specifically synthesized by interesterification of palm oil and tristearin in the presence of Lipozyme IM-20 as catalyst. The melting point of the produced cocoa butter equivalent after removal of fatty acids and fractionation was 34.3°C.

In a later research, Liu et al. (2007) compared three different substrate oils (palm oil, lard and beef tallow) and their possibility to produce a cocoa butter analog using tristearin as acyl donor. They found that the amount of POST and POO was similar to that of cocoa butter when the analog was prepared with lard.

Camel hump fat predominantly contains palmitic, stearic and oleic acid and is therefore a possible substrate for cocoa butter alternative production. Shekarchizadeh et al. (2009) optimized the process conditions to achieve a maximum yield of cocoa butter analog produced by enzymatic interesterification of camel hump fat and tristearin using immobilized *Thermomyces lanuginosus* lipase under SC-CO₂. However, the authors did not provide further information concerning the chemical composition and physical properties of the produced cocoa butter analog.

In the research of Chang et al. (1990), olive oil was used in combination with hydrogenated cottonseed oil as palmitic and stearic acid donor. The cocoa butter-like fat was isolated from the reaction mixture by fractional crystallization from acetone at two different temperatures. The produced fat matched well with cocoa butter but contained slightly more diacylglycerols and had a higher melting point which may cause a waxy mouthfeel upon application in a chocolate formulation.

Several studies have been performed on the enzymatic modification of a substrate oil or fat in order to make a cocoa butter-like fat, i.e. a fat which can be used in chocolate formulations but has a different triacylglycerol composition than cocoa butter. All studies are characterized by the fact that they tested the applicability of the cocoa butter-like fat in a chocolate formulation. An extensive research has been conducted by Zarringhalami et al. (2010) on the use and applicability of tea seed oil in the production of a cocoa butter-like fat. As tea seed oil has only limited commercial applications in its original form, a hydrogenated tea seed oil and the solid

fraction of tea seed oil were enzymatically interesterified to produce a structured lipid. Hydrogenation was performed because tea seed oil has a very low amount of stearic acid and the solid fraction instead of the liquid fraction was used because of its higher stearic acid content. No POST and POP and a negligible amount of StOSt is found in tea seed oil. After enzymatic interesterification, the amounts of POST, POP and StOSt have increased but not to the level present in cocoa butter. The produced cocoa butter-like fat was used in dark chocolate formulations to replace cocoa butter for 5, 10, 15, and 20%. Hardness, bloom development, melting behavior, polymorphic structure and sensory tests revealed that the cocoa butter-like fat produced from tea seed oil could be used up to 10% replacement of cocoa butter without adverse effects.

Osborn and Akoh (2002) modified an animal by-product, beef tallow, by using *Candida antarctica* lipase. The modified beef tallow was then applied in dark chocolate samples to completely replace cocoa butter. Randomization of the beef tallow did not improve the fatty acid profile from a nutritional point of view. The melting behaviour of the randomized beef tallow differed from cocoa butter but the addition did not lead to a significant softening of the chocolate samples. It also reduced the fat bloom development. The authors stated that the modified beef tallow could be useful to replace cocoa butter in chocolate coatings to reduce costs by using an animal by-product but health concerns must be beared in mind.

Bystrom and Hartel (1994) fractionated milk fat utilizing two different techniques: multiple step melt crystallization and supercritical CO₂ extraction. The fractions were further modified enzymatically under conditions of CO₂ at atmospheric pressure or CO₂ at supercritical pressures. A lipase from *Candida rugosa* immobilized by adsorption to silica gel was used for the enzymatic interesterification, after which free fatty acids, mono- and diacylglycerols were removed. The products were then tested for use in chocolate by the production of milk chocolates with 10 or 20% cocoa butter replacement. It was found that the chocolates with 10 or 20% cocoa butter replacement were softer than the control chocolates. The modified milk fat fractions were thus not suitable for use in milk chocolate formulations. The authors stated that the replacement of cocoa butter in a dark chocolate confection could be a more suitable application.

Bertoli et al. (1995) filed a patent describing the production of a cocoa butter-like fat. A fully hydrogenated vegetable oil rich in stearic acid is interesterified with a triacylglycerol rich in caproic, caprylic and capric acid such that the amount of tristearin becomes lower than 5%. The interesterified mixture is bleached and deodorized and can be used as a cocoa butter-like fat.

Comparison of Fatty Acids/Fatty Acid Esters and Triacylglycerols as Acyl Donors. When formulating a cocoa butter equivalent by interesterifying two oils or fats, for example olive oil and hydrogenated cottonseed oil, fractionation is applied to isolate the cocoa butter-like fat (Abigor et al., 2003; Chang et al., 1990). The studies using free fatty

acids or fatty acid esters as acyl donor, only applied fractionation to remove unwanted triacylglycerols from the substrate (Tchobo et al., 2009) or in case the amount of desired triacylglycerols formed, i.e. POP, POSt, and StOSt, is too low and can be elevated by fractionation (Chong et al., 1992).

Several studies used different acyl donors to determine the most suitable one for the specific reaction system. Liu et al. (1997) and Liu et al. (2007) were the only ones who included a triacylglycerol in the selection set, i.e. tristearin, and eventually selected it as a better acyl donor than stearic acid, ethyl stearate and stearic anhydride for the interesterification catalyzed by Lipozyme. Two studies, both using a different lipase, found that methyl esters are a better acyl donor than ethyl esters. Sridhar et al. (1991) used Lipozyme and compared methyl palmitate/stearate with ethyl palmitate/stearate. Pinyaphong and Phutrakul (2009) found that methyl stearate performed better than ethyl stearate and stearic acid for the *Carica papaya* lipase-catalyzed reaction. Two researches using palmitic and stearic acid as acyl donors and Lipozyme as catalyst came to different conclusions regarding the selectivity of the enzyme. When using triolein as substrate, the enzyme was found to have the same reactivity for palmitic acid and stearic acid (Ciftci et al., 2008), whereas, when olive pomace oil was used as substrate, stearic acid was found to be a poor acyl donor compared to palmitic acid (Ciftci et al., 2009). Gitlesen et al. (1995) preferred ethyl stearate above stearic acid as the ethyl ester reduced acyl migration and thus the formation of saturated fatty acids in the 2-position. This is in contrast with the observations of Bloomer et al. (1991) who noted a higher diacylglycerol formation when ethyl esters were used instead of free fatty acids. In conclusion, it can be stated that different parameters are influencing the operation of the enzyme-catalyzed reaction and that optimization of the reaction parameters is a prerequisite for an optimal production process.

Supercritical Carbon Dioxide in the Enzyme-catalyzed Production of CBAs

A number of studies used supercritical carbon dioxide as a solvent for the enzymatic interesterification reaction (Bystrom & Hartel, 1994; Liang et al., 1998; Liu et al., 2007; Liu et al., 1997; Shekarchizadeh et al., 2009). A fluid is in its supercritical state at temperatures and pressures higher than its critical values. At the critical temperature and pressure and above, the distinction between the gaseous and liquid phases disappears. A supercritical fluid has a density, and thus a solvating power, approaching that of a liquid, while the low viscosity, high diffusivity and very low surface tension result in a faster mass transport capacity. For food applications, carbon dioxide is always chosen as it is a low cost, non-toxic, nonflammable and environmentally friendly solvent. Moreover, it has a moderate critical temperature (31°C) and pressure (7.4 Mpa) and it can be easily separated from the reaction medium by post-reactional depressurization (Temelli & Guclu-Ustundag, 2005). As supercritical fluids can also be used for extraction and fractionation processes, a coupling of these processes with

the enzymatic interesterification is possible. Liang et al. (1998) attempted to fractionate a mixture of palm oil and stearic acid in supercritical CO₂ before the enzymatical interesterification but did not succeed. The use of cosolvents to improve the selectivity was suggested as a possible solution. Bystrom and Hartel (1994) used supercritical CO₂ both as a fractionation medium to separate anhydrous milk fat into high-, middle- and low-melting fractions and as a solvent for the enzymatic interesterification of the high- and middle-melting fractions.

Other Modification Techniques for the Production of CBAs

Next to enzymatic interesterification, a number of other techniques are employed to produce cocoa butter alternatives or an ingredient of it. These are blending, hydrogenation, fractionation and single cell oil production. An overview of modification techniques other than enzymatic interesterification used in the production of CBAs is given in Tables 18.E and 18.F.

Blending

Blending is a physical technique to produce CBAs or its ingredients by combining natural and/or modified oils and fats under certain conditions. For efficient blending, some rules need to be taken into account. Firstly, all the initial components should be liquid. The agitation should be conducted in an effective way, meaning that it should be strong enough to create a homogeneous mixture, however, it should not be too strong to avoid too much air coming into the oil. Secondly, sufficient time is also required for the blending (Smith, 2001). Having been discussed before, the main triacylglycerols in cocoa butter are POP, POSt and StOSt. Other types of fats also contain POP, POSt and StOSt but in different ratios compared to cocoa butter. According to Timms (2003), no natural fat contains as much POSt as cocoa butter. From this point of view, it is not possible to produce a CBE that completely resembles genuine cocoa butter only by blending natural fats (Smith, 2001).

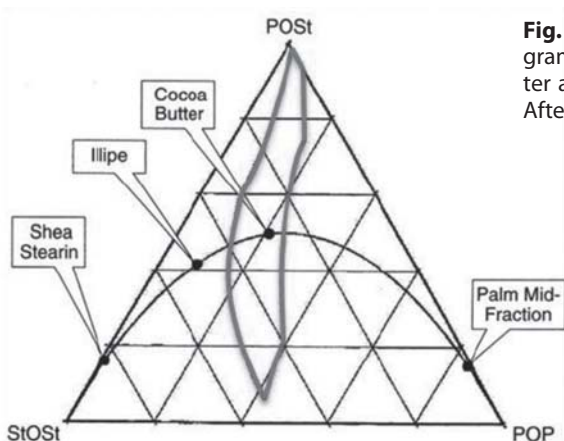
Fig. 18.3 presents a ternary phase diagram of POP, POSt and StOSt. The area within the red line represents all possible ratios of POP, POSt and StOSt which have the same tempering characteristics as cocoa butter (Padley et al., 1981; Timms, 2003). From this diagram, it is clear that the triacylglycerol composition of a CBE does not have to be exactly the same as cocoa butter. This gives different sources of oils and fats the opportunity to be used in the production of CBEs. However, it is not possible to mix POP, POSt and StOSt in any ratio as eutectic phenomena can occur between POP-StOSt and POP-POSt. This eutectic behaviour does not occur in POSt-StOSt mixtures (Fig. 18.4) (Timms, 2003). During blending, this remark should be taken into consideration in order to avoid mixed crystals which have different melting and crystallization behavior.

Table 18-E. Authors Using Modification Techniques Other than Enzymatic Interesterification to Produce Cocoa Butter Alternatives.

Target CBA	Starting material	Technique	Reference
CBE	Oleic acid from pecan oil, stearic acid and palmitic acid Olive oil and hydrogenated cottonseed oil	Esterification or interesterification and fractionation	Feuge et al. (1958)
CB-like fat	Hydrogenated cottonseed oil and triolein	Chemical interesterification and fractionation	Decossas et al. (1962)
CB-like fat	Cottonseed oil stearine	Hydrogenation and solvent fractionation	Feuge et al. (1973); Lovegren et al. (1973)
CB-like fat	1-palmitoyl-3-stearoyl glycerol, 1,3-distearoyl glycerol, 1,3-dipalmitoyl glycerol and oleic anhydride	Acylation	O'Connor and Wyness (1976)
CB-like fat	1-palmitoyl-3-stearoyl glycerol and oleic anhydride	Acylation	Yetter (1976)
CBE	Mango fat	Acetone fractionation	Baliga and Shitole (1981)
CBE	Shea butter, mango kernel fat, Borneo tallow, sal, kokum, mahua and palm oil	Blending and fractionation	Pairaud et al. (1982)
CBE	Sal fat and palm midfraction	Fractionation and blending	Jasko and Domek (1984)
CB-like fat	Tallow	Fractionation	Craig Jr. et al. (1987)
CB-like fat	Phulwara butter	Fractionation	Reddy and Prabhakar (1994b)
CBE	Kokum fat and Phulwara butter	Dry fractionation and blending	Reddy and Prabhakar (1994a)
CB-like fat	Glycerin compositions containing acyl groups derived from C12-C24 saturated linear fatty acids	Esterification and propoxylation	Cooper (1995)
CB-like fat	Joboba oil and hydrogenated joboba wax esters	Transesterification	Sessa (1996)
CBE	Palm oil and sal fat	Co-fractionation (blending followed by acetone fractionation)	Ali (1996)
CBE	Mahua and Kokum fat	Blending and fractionation	Jeyarani and Reddy (1999)
CB-like fat	Palm kernel oil	SC-CO2 fractional extraction	Hassan et al. (2000)
CB-like fat	Pili nut oil	Fractionation	Kakuda et al. (2000)
CBE	Simarouba glauca fat	Solvent or dry fractionation	Jeyarani and Reddy (2001)
CBS	Palm kernel oil, hydrogenated palm kernel oil, palm kernel stearin and hydrogenated palm kernel stearin	Blending	Nalur (2001)
CB-like fat	High stearic soybean oil	Fractionation	Knowlton (2001)
CB-like fat	Palm kernel oil	SC-CO2 fractional extraction	Norulaini et al. (2004)
CBS	Palm kernel oil	Dry fractionation	Calliauw et al. (2005)
CB-like fat	Palm kernel oil	SC-CO2 fractional extraction	Zaidul et al. (2006)
CBE	Mango seed almond fat and palm midfraction	Blending	Kaphueakngam et al. (2009)
CBE	High stearic – high oleic sunflower oil	Solvent fractionation (hexane)	Salas et al. (2011)
CB-like fat	High oleic – high stearic sunflower oil	Dry fractionation	Bootello et al. (2011)

Table 18-F. Authors Using Microorganisms to Produce Cocoa Butter Alternatives.

Target CBA	Substrate	Microorganism	Reference
CBE	Mixed carbon source	A microorganism capable of producing fats and oils rich in 1,3-disaturated-2-unsaturated triacylglycerols	Matsuo et al. (1981b)
CBE	Cyclopropene fatty acid compounds	A suitable lipid accumulating microorganism	Moreton and Clode (1988)
CBE	N-limited medium	<i>Apiotrichum curvatum</i>	Hassan et al. (1993)
CBE	Glucose	<i>Mucor</i> species	Roux et al. (1994)
CBE	Nitrogen limited medium with acetic acid as carbon source	<i>Mucor circinelloides</i>	Roux et al. (1995)
CBE	Prickly-pear juice	<i>Cryptococcus curvatus</i>	Hassan et al. (1995)
CBE	Industrial fats containing stearic, oleic, linoleic and palmitic acid	<i>Yarrowia lipolytica</i>	Papanikolaou et al. (2001)
CBE	Saturated free fatty acids, technical glycerol and glucose	<i>Yarrowia lipolytica</i>	Papanikolaou et al. (2003)
CBE	N-acetylglucosamine medium	<i>Cryptococcus curvatus</i>	Wu et al. (2010)
CBE	Yeast Extract Peptone Dextrose (YEPD) medium under sulfate-limited conditions	<i>Rhodospiridium toruloides</i> Y4	Wu et al. (2011)

**Fig. 18.3.** POP/POST/StOSt ternary diagram showing positions of cocoa butter and some StOSt-type raw materials. After Padley et al. (1981); Smith (2001).

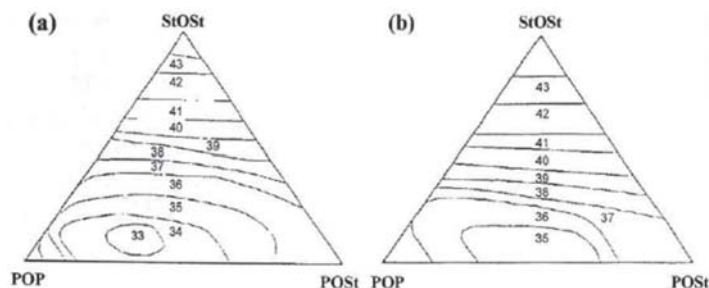


Fig. 18.4. Ternary isosolid diagrams for mixtures of POP, POST and StOSt: (a) 25% isosolid lines, indicating temperatures (°C) at which SFC = 25%; (b) 0% isosolid lines. Source: Timms (2003) as given by Wesdorp (1990).

Hydrogenation

Hydrogenation is an old technique in the oils and fats industry but its use has been reduced recently as partial hydrogenation can create *trans* fatty acids. Nevertheless, hydrogenation is still used, especially in the production of fats rich in saturated fatty acids, which can be used in the formulation of CBAs, or to create a seeding effect during fat crystallization. Some hydrogenated oils and fats used in making CBAs are fully hydrogenated soybean oil (Abigor et al., 2003), hydrogenated cottonseed oil (Chang et al., 1990; Decossas et al., 1962; Feuge et al., 1958), hydrogenated tea seed oil (Zar-ringhalami et al., 2010), a hydrogenated derivative from animal fat (Papanikolaou et al., 2003), hydrogenated palm kernel oil (Nalur, 2001), hydrogenated coconut oil (Nalur, 2001), and a fully hydrogenated vegetable oil (Bertoli et al., 1995).

Fractionation

Fractionation is used in the production of CBAs to obtain fractions with properties compatible with cocoa butter or to obtain fractions that can be used for the formulation of CBAs. The technique involves a fractional crystallization followed by a separation between the liquid olein fraction and the crystallized stearin fraction (Calliauw et al., 2005; Deffense, 2000; Timms, 1985). Basically, there are three main strategies for this technology: dry fractionation, solvent fractionation, and detergent fractionation (Deffense, 2000). The two first techniques have been employed a lot in the production of CBAs. For solvent fractionation, mostly hexane and acetone are utilized. Timms (1985) reported that acetone fractionation offers several benefits in CBE preparation as compared to dry fractionation. With the addition of acetone, the viscosity is reduced and the isolated solid fat can be washed. This helps reducing the amount of entrapped liquid oil. Multiple washings can be executed to remove the olein fraction thoroughly out of the stearin fraction. In addition, the concentration of more polar lipids such as mono- and diacylglycerols can be decreased as they go into the olein part. However, the operation cost for solvent fractionation may not be economical.

A lot of studies use the fractionation technique to separate the desired composition from the fat obtained by another modification technique (Abigor et al., 2003; Ali, 1996; Chang et al., 1990; Chong et al., 1992; Decossas et al., 1962; Feuge et al., 1973; Feuge et al., 1958; Jeyarani & Reddy, 1999; Lovegren et al., 1973; Pairaud et al., 1982). On the other hand, fractionation on itself is also used as only technique to obtain a cocoa butter-like fat. Substrates that have been fractionated include mango fat (Baliga & Shitole, 1981), tallow (Craig Jr. et al., 1987), Pili nut oil (Kakuda et al., 2000), *Simarouba glauca* fat (Jeyarani & Reddy, 2001), high stearic soybean oil (Knowlton, 2001), Phulwara butter (Reddy & Prabhakar, 1994b), palm kernel oil (Calliauw et al., 2005) and high stearic-high oleic sunflower oil (Bootello et al., 2011; Salas et al., 2011). In some cases, the obtained fractions are blended to yield a suitable end product (Jasko & Domek, 1984; Reddy & Prabhakar, 1994a).

Supercritical Carbon Dioxide Extraction/Fractionation. Next to the use of supercritical CO₂ as a solvent for conducting enzymatic interesterification reactions, it can also be used for extraction and fractionation processes. Recently, the supercritical CO₂ extraction process has attracted considerable attention as a promising alternative to conventional solvent extraction or mechanical pressing of oilseeds (Herrero et al., 2006; Norulaini et al., 2004; Zaidul et al., 2006). Supercritical CO₂ is very well suited for this application because its low viscosity enables good penetration into solids and lipid materials are highly soluble in it. Supercritical fractionation of extracts can be achieved by fractional extraction or fractional separation. During fractional extraction, different fractions are obtained throughout the extraction process due to differences in solubility behavior or a different location of the solutes in the solid sample matrix. On the other hand, fractional separation is performed after the extraction step using a series of separators operated at different conditions to adjust the solvent power/density. Separation of the supercritical solvent from the extract is achieved by decreasing the pressure or by elevating the temperature or both (Temelli & Guclu-Ustundag, 2005). A good review about supercritical CO₂ in lipid extraction can be found in Sahena et al. (2009).

Supercritical CO₂ was used to extract palm kernel oil from the palm kernel, a by-product of the palm oil industry. Palm kernel oil is rich in lauric acid, myristic acid and oleic acid and has a solid consistency at ambient temperatures but melts below 30°C. Because triacylglycerols with short chains are more soluble in supercritical CO₂, the composition of the oil extracted varied with time. Earlier fractions were rich in short-chain triacylglycerols while later fractions were rich in longer chain triacylglycerols and unsaturated triacylglycerols. Fractionation of the palm kernel oil could thus be achieved by collecting the extracted oil at different time or volume intervals (Bharath et al., 1992; Hassan et al., 2000; Norulaini et al., 2004; Zaidul et al., 2006). According to the authors, the fractions with decreased short-chain and increased longer chain triacylglycerols can possibly be used for blending to formulate a cocoa butter alternative (Zaidul et al., 2006).

Microbial Lipids

Lipid biotechnology has expanded continuously in the last years, as concerns about health, nutritional labeling and environment are increasing. A lot of research concerning the production of lipids derived from microbial sources [the so-called “single-cell oils (SCO)”] has been carried out in recent years. Despite the fact that the production cost of microbial lipids is higher than that of conventional vegetable oils, the production of microbial lipids with compositions similar to high valued fatty materials such as cocoa butter has been economically considered, especially when lower cost raw materials are utilized. The principle of single cell oil lipids is based on the capacity of various microorganism to synthesize lipids with specific structure and composition. The oleaginous microorganisms (yeasts, molds, algae) are reported to be capable of accumulating significant quantities of lipid, even up to more than 60% of their dry biomass weight (Hassan et al., 1994). This phenomenon usually happens under the deficiency of a certain nutrient component in the culture medium (Papanikolaou & Aggelis, 2011; Wu et al., 2010).

At the end of the eighties, yeast lipids with a composition similar to cocoa butter have been produced on industrial scale (Kyle and Ratledge, 1992). In Hassan et al. (1994), it is shown that the oleaginous yeast *Cryptococcus curvatus* ATCC 20509 is able to produce a lipid product with a composition comparable to cocoa butter. The same yeast was shown of being able to use N-acetylglucosamine as carbon and nitrogen source to synthesize a lipid that could be explored as a cocoa butter equivalent (Wu et al., 2010). Another oleaginous yeast *Yarrowia lipolytica* LGAM S(7)1 showed the potential of producing a cocoa butter substitute from mixtures of industrial fats containing stearic, oleic, linoleic and palmitic acid (Papanikolaou et al., 2001). In another study on *Yarrowia lipolytica* ACA-DC 50109, single-cell oil having a composition similar to cocoa butter was produced from mixtures of saturated free fatty acids, technical glycerol and glucose (Papanikolaou et al., 2003). The most recent study on *Rhodospiridium toruloides* Y4 under sulfate-limited conditions also presented a promising result for cocoa butter-like fat. Some other researches on microbial lipids are listed in Table 18-E. The biochemistry of single cell oil production and its potential applications can be found in the review of Papanikolaou and Aggelis (2011).

Detection Techniques

According to the EU Directive 2000/36/EC, only 5% (on product base) other vegetable fats can be added to chocolate. Therefore it is important to detect and quantify the addition of vegetable fats, other than cocoa butter, in chocolate.

Various components of cocoa butter have been targeted as indicator for the presence of other vegetable fats. Crews et al. (1997) analysed the sterol degradation products. Besides sterols, 4-methylsterols, triterpene alcohols (Gegiou & Staphylakis, 1985; Lipp & Anklam, 1998b; Soulier et al., 1990) have been used for the detection of foreign fats in chocolate (Simoneau et al., 1999; Ulberth & Buchgraber, 2003).

Because triacylglycerols are the most important component in cocoa butter and CBEs, their analysis has been investigated intensively. The most common methods are gas chromatography (Barcarolo & Anklam, 2001; Fincke, 1980; Guyon et al., 2003; Minim & Cecchi, 1999; Padley & Timms, 1980; Simoneau et al., 1999; Young, 1984) and high performance liquid chromatography (Buchgraber et al., 2004; Damiani et al., 2006; Dionisi et al., 2004). Ratios of the three main triacylglycerols (POP, POST and StOSt) are typical for cocoa butter. As such, the content of foreign fats in cocoa butter can be quantified (Lipp and Anklam, 1998b).

In 2009, the AOCS (American Oil Chemists' Society) reapproved the AOCS Official Method Ce 11-05; Determination of Cocoa Butter Equivalents in Cocoa Butter and Plain Chocolate. The quantification is performed with high-resolution capillary gas chromatography (HR-GC) of triacylglycerols. Afterwards, the data are evaluated by either linear regression analysis or partial least squares regression analysis. When using the first method, the relative proportions of the three main triacylglycerol fractions are analyzed and the presence of other fats can be determined down to a level of 2%. When analyzed with partial least squares regression, the relative proportions of the five main triacylglycerols (POP, POST, POO, StOSt, and StOO) are quantified at a level of 5% (Firestone, 2009). The principle of the method is based on the fact that for cocoa butter, POST content is constant for wide variations of POP and StOSt. All CB/CBE mixtures will cause the triacylglycerol analysis to deviate from the CB-line to the extent that their POST value differs from the POST value of cocoa butter (Firestone, 2009). Two types of standardised analytical toolboxes, CoCal1 and CoCal2, have been developed to facilitate the detection and quantification of CBEs in dark chocolate and milk chocolate respectively (Buchgraber & Androni, 2008).

References

- Abigor, R.D., Marmer, W.N., Foglia, T.A., Jones, K.C., DiCiccio, R.J., Ashby, R., Uadia, P.O., (2003). Production of cocoa butter-like fats by the lipase-catalyzed interesterification of palm oil and hydrogenated soybean oil. *Journal of the American Oil Chemists Society* 80(12), 1193–1196.
- Ahmadi, L., Wright, A.J., Marangoni, A.G., (2009). Structural and Mechanical Behavior of Tristearin/Triolein-rich Mixtures and the Modification Achieved by Interesterification. *Food Biophysics* 4(2), 64–76.
- Ali, A.R.M., (1996). Effect of co-fractionation technique in the preparation of palm oil and sal fat based cocoa butter equivalent. *International Journal of Food Sciences and Nutrition* 47(1), 15–22.
- Baliga, B.P., Shitole, A.D., (1981). Cocoa butter substitutes from mango fat. *Journal of the American Oil Chemists Society* 58(2), 110–114.
- Barcarolo, R., Anklam, E., (2001). Development of a rapid method for the detection of cocoa butter equivalents in mixtures with cocoa butter. *Journal of Aoac International* 84(5), 1485–1489.
- Bertoli, C., Hosszu-Sackett, K., Melachouris, N., Traitler, H., (1995). Preparation of butterfat and vegetable butter substitutes, US Patent.

- Bharath, R., Inomata, H., Adschiri, T., Arai, K., (1992). Phase-equilibrium study for the separation and fractionation of fatty oil components using supercritical carbon-dioxide. *Fluid Phase Equilibria* 81(1–2), 307–320.
- Bloomer, S., Adlercreutz, P., Mattiasson, B., (1990). Triglyceride interesterification by lipases. 1. Cocoa butter equivalents from a fraction of palm oil. *Journal of the American Oil Chemists Society* 67(8), 519–524.
- Bloomer, S., Adlercreutz, P., Mattiasson, B., (1991). Triglyceride interesterification by lipases. 2. Reaction parameters for the reduction of trisaturate impurities and diglycerides in batch reactions. *Biocatalysis* 5, 145–162.
- Bootello, M.A., Garces, R., Martinez-Force, E., Salas, J.J., (2011). Dry fractionation and crystallization kinetics of high-oleic high-stearic sunflower oil. *Journal of the American Oil Chemists Society* 88(10), 1511–1519.
- Buchgraber, M., Androni, S., (2008). Determination of milk fat and cocoa butter equivalents in milk chocolate. *Agro Food Industry Hi-Tech* 19(3), 25–27.
- Buchgraber, M., Ullberth, F., Emons, H., Anklam, E., (2004). Triacylglycerol profiling by using chromatographic techniques. *European Journal of Lipid Science and Technology* 106(9), 621–648.
- Bystrom, C.E., Hartel, R.W., (1994). Evaluation of milk-fat fractionation and modification techniques for creating cocoa butter replacers. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* 27(2), 142–150.
- Calliauw, G., Foubert, I., De Greyt, W., Dijckmans, P., Kellens, M., Dewettinck, K., (2005). Production of cocoa butter substitutes via two-stage static fractionation of palm kernel oil. *Journal of the American Oil Chemists Society* 82(11), 783–789.
- Chang, M.K., Abraham, G., John, V.T., (1990). Production of cocoa butter-like fat from interesterification of vegetable-oils. *Journal of the American Oil Chemists Society* 67(11), 832–834.
- Chong, C.N., Hoh, Y.M., Wang, C.W., (1992). Fractionation procedures for obtaining cocoa butter-like fat from enzymatically interesterified palm olein. *Journal of the American Oil Chemists Society* 69(2), 137–140.
- Ciftci, O.N., Fadiloglu, S., Gogus, F., (2009). Utilization of Olive-Pomace Oil for Enzymatic Production of Cocoa Butter-like Fat. *Journal of the American Oil Chemists Society* 86(2), 119–125.
- Ciftci, O.N., Fadiloglu, S., Kowalski, B., Gogus, F., (2008). Synthesis of cocoa butter triacylglycerols using a model acidolysis system. *Grasas Y Aceites* 59(4), 316–320.
- Ciftci, O.N., Gogus, F., Fadiloglu, S., (2010). Performance of a Cocoa Butter-Like Fat Enzymatically Produced from Olive Pomace Oil as a Partial Cocoa Butter Replacer. *Journal of the American Oil Chemists Society* 87(9), 1013–1018.
- Coleman, M.H., Macrae, A.R., (1977). Verfahren zur umlagerung von fettsäureresten in fettreaktionsteilnehmern, German Patent.
- Cooper, C.F., (1995). Reduced calorie cocoa butter substitutes, US Patent. .
- Costales-Rodriguez, R., Gibon, V., Verhe, R., De Greyt, W., (2009). Chemical and Enzymatic Interesterification of a Blend of Palm Stearin: Soybean Oil for Low trans-Margarine Formulation. *Journal of the American Oil Chemists Society* 86(7), 681–697.

- Craig Jr., J.C., Kozempel, M.F., Elias, S., (1987). Continuous fractionation of tallow and production of a cocoa butter-like plastic fat, US Patent.
- Crews, C., CalvetSarrett, R., Brereton, P., (1997). The analysis of sterol degradation products to detect vegetable fats in chocolate. *Journal of the American Oil Chemists Society* 74(10), 1273–1280.
- Damiani, P., Cossignani, L., Simonetti, M.S., Blasi, F., Petrosino, T., Neri, A., (2006). Identification of cocoa butter equivalents added to cocoa butter. III. Stereospecific analysis of triacylglycerol fraction and some its subfraction. *European Food Research and Technology* 223(5), 645–648.
- Decossas, K.M., Feuge, R.O., Patton, E.L., Pollard, E.F., Spadaro, J.J., Koltun, S.P., (1962). Cocoa butter-like fat from cottonseed oil—preliminary cost study. *Journal of the American Oil Chemists Society* 39(3), 146–150.
- Deffense, E., (2000). Dry fractionation technology in 2000. *European Journal of Lipid Science and Technology* 102(3), 234–236.
- Dionisi, F., Golay, P.A., Hug, B., Baumgartner, M., Callier, P., Destailats, F., (2004). Triacylglycerol analysis for the quantification of cocoa butter equivalents (CBE) in chocolate: Feasibility study and validation. *Journal of Agricultural and Food Chemistry* 52(7), 1835–1841.
- Facioli, N.L., Goncalves, L.A.G., (1998). Piqui (*Caryocar brasiliense* Camb) oil triglyceride composition modification by enzymatic way. *Quimica Nova* 21(1), 16–19.
- Favre, T.L.F., Kos, H., Bhaggan, K., Fages, S.J., (2010). Process for making a triglyceride composition, WIPO PCT Patent.
- Feuge, R.O., Gajee, B.B., Lovegren, N.V., (1973). Cocoa butter-like fats from fractionated cottonseed oil. 1. Preparation. *Journal of the American Oil Chemists Society* 50(2), 50–52.
- Feuge, R.O., Lovegren, N.V., Cosler, H.B., (1958). Cocoa butter-like fats from domestic oils. *Journal of the American Oil Chemists Society* 35(5), 194–199.
- Fincke, A., (1980). Possibilities and limitations of simple gas chromatographical triglyceride analyses to detect alien fats in cocoa butter and chocolate fats. *Deutsche Lebensmittel-Rundschau* 76(5), 162–167.
- Firestone, D., (2009). Official methods and recommended practices of the AOCS, 6th edition. AOCS Press, Campaign, Illinois, USA.
- Gegiou, D., Staphylakis, K., (1985). Detection of cocoa butter equivalents in chocolate. *Journal of the American Oil Chemists Society* 62(6), 1047–1051.
- Gitlesen, T., Svensson, I., Adlercreutz, P., Mattiasson, B., Nilsson, J., (1995). High-oleic-acid rapeseed oil as starting material for the production of confectionary fats via lipase-catalyzed transesterification. *Industrial Crops and Products* 4(3), 167–171.
- Guyon, F., Absalon, C., Eloy, A., Salagoity, M.H., Esclapez, M., Medina, B., (2003). Comparative study of matrix-assisted laser desorption/ionization and gas chromatography for quantitative determination of cocoa butter and cocoa butter equivalent triacylglycerol composition. *Rapid Communications in Mass Spectrometry* 17(20), 2317–2322.
- Hassan, M., Blanc, P.J., Granger, L.M., Pareilleux, A., Goma, G., (1993). Lipid production by an unsaturated fatty-acid auxotroph of the oleaginous yeast *Apiotrichum-curvatum* grown in single-stage continuous-culture. *Applied Microbiology and Biotechnology* 40(4), 483–488.

- Hassan, M., Blanc, P.J., Pareilleux, A., Goma, G., (1994). Selection of fatty-acid auxotrophs from the oleaginous yeast *Cryptococcus-curvatus* and production of cocoa butter equivalents in batch culture. *Biotechnology Letters* 16(8), 819–824.
- Hassan, M., Blanc, P.J., Pareilleux, A., Goma, G., (1995). Production of cocoa butter equivalents from prickly-pear juice fermentation by an unsaturated fatty-acid auxotroph of *Cryptococcus-curvatus* grown in batch culture. *Process Biochemistry* 30(7), 629–634.
- Hassan, M.N., Rahman, N.N.A., Ibrahim, M.H., Omar, A.K.M., (2000). Simple fractionation through the supercritical carbon dioxide extraction of palm kernel oil. *Separation and Purification Technology* 19(1–2), 113–120.
- Herrero, M., Cifuentes, A., Ibañez, E., (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. *Food Chemistry* 98(1), 136–148.
- Holm, H.C., Cowan, D., (2008). The evolution of enzymatic interesterification in the oils and fats industry. *European Journal of Lipid Science and Technology* 110(8), 679–691.
- Huang, F.C., Ju, Y.H., (1997). Interesterification of palm midfraction and stearic acid with *Rhizopus arrhizus* lipase immobilized on polypropylene. *Journal of the Chinese Institute of Chemical Engineers* 28(2), 73–78.
- Huey, S.M., Hock, C.C., Lin, S.W., (2009). Characterization of Low Saturation Palm Oil Products after Continuous Enzymatic Interesterification and Dry Fractionation. *Journal of Food Science* 74(4), E177–E183.
- Jasko, J.J., Domek, S.M., (1984). Hard butter and process for making same, US Patent.
- Jeffrey, B.S.J., Padley, F.B., (1991). Chinese vegetable tallow—characterization and contamination by stillingia oil. *Journal of the American Oil Chemists Society* 68(2), 123–127.
- Jeyarani, T., Reddy, S.Y., (1999). Heat-resistant cocoa butter extenders from mahua (*Madhuca latifolia*) and kokum (*Garcinia indica*) fats. *Journal of the American Oil Chemists Society* 76(12), 1431–1436.
- Jeyarani, T., Reddy, S.Y., (2001). Cocoa butter extender from Simarouba glauca fat. *Journal of the American Oil Chemists Society* 78(3), 271–276.
- Ju, Y.H., Chiu, C.K., Huang, F.C., (1996). Interesterification of palm midfraction and stearic acid with *Rhizopus arrhizus* lipase under controlled water activity. *Journal of the Chinese Institute of Chemical Engineers* 27(2), 65–70.
- Kakuda, Y., Jahaniaval, F., Marcone, M.F., Montevirgen, L., Montevirgen, Q., Umali, O., (2000). Characterization of pili nut (*Canarium ovatum*) oil: Fatty acid and triacylglycerol composition and physicochemical properties. *Journal of the American Oil Chemists Society* 77(9), 991–996.
- Kang, J.-H., Lee, S.-B., Song, S.-H., Lee, K.-T., (2010). Cocoa butter equivalents produced by the enzymatic interesterification process and method for preparing the same, WIPO PCT Patent.
- Kaphueakngam, K., Flood, A., Sonwai, S., (2009). Production of cocoa butter equivalent from mango seed almond fat and palm oil mid-fraction. *Asian Journal of Food and Agro-Industry* 2(4), 441–447.

- Khumalo, L.W., Majoko, L., Read, J.S., Ncube, I., (2002). Characterisation of some underutilised vegetable oils and their evaluation as starting materials for lipase-catalysed production of cocoa butter equivalents. *Industrial Crops and Products* 16(3), 237–244.
- Kim, E.J., Yoon, S.H., (2003). Recent progress in enzymatic production of structured lipids. *Food Science and Biotechnology* 12(6), 721–726.
- Knowlton, S., (2001). Fat products from high stearic soybean oil and a method for the production thereof, US Patent.
- Kyle, D.J., Ratledge, C., (1992). *Industrial Applications of Single Cell Oils*. AOCS Press, Champaign (USA).
- Liang, M.T., Chen, C.H., Liang, R.C., (1998). Interesterification of edible palm oil by stearic acid in supercritical carbon dioxide. *Journal of Supercritical Fluids* 13(1–3), 211–216.
- Lipp, M., Anklam, E., (1998a). Review of cocoa butter and alternative fats for use in chocolate—Part A. Compositional data. *Food Chemistry* 62(1), 73–97.
- Lipp, M., Anklam, E., (1998b). Review of cocoa butter and alternative fats for use in chocolate—Part B. Analytical approaches for identification and determination. *Food Chemistry* 62(1), 99–108.
- Liu, K.-J., Chang, H.-M., Liu, K.-M., (2007). Enzymatic synthesis of cocoa butter analog through interesterification of lard and tristearin in supercritical carbon dioxide by lipase. *Food Chemistry* 100(4), 1303–1311.
- Liu, K.J., Cheng, H.M., Chang, R.C., Shaw, J.F., (1997). Synthesis of cocoa butter equivalent by lipase-catalyzed interesterification in supercritical carbon dioxide. *Journal of the American Oil Chemists Society* 74(11), 1477–1482.
- Lovegren, N.V., Gajee, B.B., Gray, M.S., Feuge, R.O., (1973). Cocoa butter-like fats from fractionated cottonseed oil. 2. Properties. *Journal of the American Oil Chemists Society* 50(2), 53–57.
- Macrae, A.R., (1984). Microbial lipases as catalysts for the interesterification of oils and fats, in: Ratledge, C., Dawson, P., Rattray, J. (Eds.), *Biotechnology for the Oils and Fats Industry*. AOCS press, Champaign, IL (USA), pp. 189–198.
- Macrae, A.R., How, P., (1989). Rearrangement process, US Patent.
- Maheshwari, B., Reddy, S.Y., (2005). Application of kokum (*Garcinia indica*) fat as cocoa butter improver in chocolate. *Journal of the Science of Food and Agriculture* 85(1), 135–140.
- Maia, J.G.S., Andrade, E.H.A., da Silva, M.H.L., (2008). Aroma volatiles of pequi fruit (*Caryocar brasiliense* Camb.). *Journal of Food Composition and Analysis* 21(7), 574–576.
- Marangoni, A.G., Rousseau, D., (1998). The influence of chemical interesterification on physicochemical properties of complex fat systems 1. Melting and crystallization. *Journal of the American Oil Chemists Society* 75(10), 1265–1271.
- Martinez-Inchausti, A., (2009). Legal aspects of chocolate manufacture, in: Beckett, S.T. (Ed.), *Industrial chocolate manufacture and use*. Wiley-Blackwell, West-Sussex, pp. 576–594.
- Matsuo, T., Sawamura, N., Hashimoto, Y., Hashida, W., (1981a). Method for producing cacao butter substitute, US Patent.

- Matsuo, T., Terashima, M., Hasimoto, Y., Hasida, W., (1981b). Method for producing cacao butter substitute, US Patent.
- Minim, V.P.R., Cecchi, H.M., (1999). Methodology for the determination of cocoa butter replacements in chocolate by triacylglycerol GC analysis. *Hrc-Journal of High Resolution Chromatography* 22(5), 305–307.
- Mohamed, H.M.A., Bloomer, S., Hammadi, K., (1993). Modification of fats by lipase interesterification. 1. Changes in glyceride structure. *Fett Wissenschaft Technologie—Fat Science Technology* 95(11), 428–431.
- Mojovic, L., Silermarinkovic, S., Kukic, G., Vunjaknovakovic, G., (1993). *Rhizopus-arrhizus* lipase-catalyzed interesterification of the midfraction of palm oil to a cocoa butter equivalent fat. *Enzyme and Microbial Technology* 15(5), 438–443.
- Moreton, R.S., Clode, D.M., (1988). Microbial desaturase enzyme inhibitors and their use in a method of producing lipids, US Patent.
- Nalur, S.C., (2001). Oil blend compositions, US Patent.
- Norberg, S., (2006). Chocolate and confectionery fats, in: Gunstone, F.D. (Ed.), *Modifying lipids for use in food*, part III. Woodhead Publishing, Cambridge, pp. 487–516.
- Norulaini, N.A.N., Zaidul, I.S.M., Anuar, O., Omar, A.K.M., (2004). Supercritical enhancement for separation of lauric acid and oleic acid in palm kernel oil (PKO). *Separation and Purification Technology* 39(3), 133–138.
- O'Connor, D.E., Wyness, G.R., (1976). Process for synthesizing specific complete mixed polyol esters, US Patent.
- Osborn, H.T., Akoh, C.C., (2002). Enzymatically modified beef tallow as a substitute for cocoa butter. *Journal of Food Science* 67(7), 2480–2485.
- Padley, F.B., Paulussen, C.N., Soeters, C., Tresser, D., (1981). Chocolate having defined hard fat, US Patent.
- Padley, F.B., Timms, R.E., (1980). The determination of cocoa butter equivalents in chocolate. *Journal of the American Oil Chemists Society* 57(9), 286–293.
- Pairaud, D.J., Musso, S., Bouvron Nee Ferrenbach, C., Pages-Xatart Pares, X.J.M., (1982). Method of preparing a cocoa butter substitute, US Patent.
- Papanikolaou, S., Aggelis, G., (2011). Lipids of oleaginous yeasts. Part I: Biochemistry of single cell oil production. *European Journal of Lipid Science and Technology* 113(8), 1031–1051.
- Papanikolaou, S., Chevalot, I., Komaitis, M., Aggelis, G., Marc, I., (2001). Kinetic profile of the cellular lipid composition in an oleaginous *Yarrowia lipolytica* capable of producing a cocoa-butter substitute from industrial fats. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 80(3–4), 215–224.
- Papanikolaou, S., Muniglia, L., Chevalot, I., Aggelis, G., Marc, I., (2003). Accumulation of a cocoa-butter-like lipid by *Yarrowia lipolytica* cultivated on agro-industrial residues. *Current Microbiology* 46(2), 124–130.
- Pinyaphong, P., Phutrakul, S., (2009). Synthesis of Cocoa Butter Equivalent from Palm Oil by Carica papaya Lipase-Catalyzed Interesterification. *Chiang Mai Journal of Science* 36(3), 359–368.

- Reddy, S.Y., Prabhakar, J.V., (1994a). Cocoa butter extenders from Kokum (*Garcinia-indica*) and Phulwara (*Madhuca-butyracea*) butter. *Journal of the American Oil Chemists Society* 71(2), 217–219.
- Reddy, S.Y., Prabhakar, J.V., (1994b). Confectionery fat from Phulwara (*Madhuca-butyracea*) butter. *Fett Wissenschaft Technologie-Fat Science Technology* 96(10), 387–390.
- Roux, M.P., Kock, J.L.F., Botha, A., Dupreez, J.C., Wells, G.V., Botes, P.J., (1994). Mucor—a source of cocoa butter and gamma-linolenic acid. *World Journal of Microbiology & Biotechnology* 10(4), 417–422.
- Roux, M.P., Kock, J.L.F., DuPreez, J.C., Botha, A., (1995). The influence of dissolved oxygen tension on the production of cocoa butter equivalents and gamma-linolenic acid by *Mucor circinelloides*. *Systematic and Applied Microbiology* 18(3), 329–334.
- Sahena, F., Zaidul, I.S.M., Jinap, S., Karim, A.A., Abbas, K.A., Norulaini, N.A.N., Omar, A.K.M., (2009). Application of supercritical CO₂ in lipid extraction—A review. *Journal of Food Engineering* 95(2), 240–253.
- Salas, J.J., Bootello, M.A., Martinez-Force, E., Garces, R., (2011). Production of stearate-rich butters by solvent fractionation of high stearic-high oleic sunflower oil. *Food Chemistry* 124(2), 450–458.
- Sessa, D.J., (1996). Derivation of a cocoa butter equivalent from jojoba transesterified ester via a differential scanning calorimetry index. *Journal of the Science of Food and Agriculture* 72(3), 295–298.
- Shekarchizadeh, H., Kadivar, M., Ghaziaskar, H.S., Rezayat, M., (2009). Optimization of enzymatic synthesis of cocoa butter analog from camel hump fat in supercritical carbon dioxide by response surface method (RSM). *Journal of Supercritical Fluids* 49(2), 209–215.
- Shukla, V.K.S., (2005). Confectionery lipids, in: Shahidi, F. (Ed.), *Bailey's Industrial Oil and Fat Products*. John Wiley & Sons, Inc., New Jersey, pp. 159–166.
- Simoneau, C., Hannaert, P., Anklam, E., (1999). Detection and quantification of cocoa butter equivalents in chocolate model systems: analysis of triglyceride profiles by high resolution GC. *Food Chemistry* 65(1), 111–116.
- Smith, K.W., (2001). Cocoa butter and cocoa butter equivalents, in: Gunstone, F. (Ed.), *Structured and modified lipids*. Marcel Dekker, New York (USA), pp. 401–422.
- Soulier, P., Farines, M., Soulier, J., (1990). Triterpene alcohols, 4-methylsterols and 4-desmethylsterols of sal and illipe butters. *Journal of the American Oil Chemists Society* 67(6), 388–393.
- Sridhar, R., Lakshminarayana, G., Kaimal, T.N.B., (1991). Modification of selected Indian vegetable fats into cocoa butter substitutes by lipase-catalyzed ester interchange. *Journal of the American Oil Chemists Society* 68(10), 726–730.
- Stewart, I.M., Timms, R.E., (2002). Fats for chocolate and sugar confectionery, in: Rajah, K.K. (Ed.), *Fats in Food Technology*. Sheffield Academy Press, Sheffield, pp. 159–191.
- Talbot, G., (2009). Vegetable fats, in: Beckett, S.T. (Ed.), *Industrial Chocolate Manufacture and Use*, 1 ed. Wiley-Blackwell, West-Sussex, UK, pp. 415–433.
- Tanaka, Y., Omura, H., Irinatsu, Y., Kobayashi, T., Noguchi, A., (1989). Cacao butter substitute composition, US Patent.

- Tchobo, F.P., Piombo, G., Pina, M., Soumanou, M.M., Villeneuve, P., Sohounhloue, D.C.K., (2009). Enzymatic synthesis of cocoa butter equivalent through transesterification of *Pentadesma butyracea* butter. *Journal of Food Lipids* 16(4), 605–617.
- Temelli, F., Guclu-Ustundag, O., (2005). Supercritical technologies for further processing of edible oils, in: Shahidi, F. (Ed.), *Bailey's Industrial Oil and Fat Products*. John Wiley & Sons, Inc., New Jersey, pp. 397–432.
- Timms, R.E., (1985). Physical-properties of oils and mixtures of oils. *Journal of the American Oil Chemists Society* 62(2), 241–249.
- Timms, R.E., (2003). *Confectionery Fats Handbook*. The Oily Press, Bridgwater, England.
- Ulberth, F., Buchgraber, M., (2003). Analytical platforms to assess the authenticity of cocoa butter. *European Journal of Lipid Science and Technology* 105(1), 32–42.
- Undurraga, D., Markovits, A., Erazo, S., (2001). Cocoa butter equivalent through enzymic interesterification of palm oil midfraction. *Process Biochemistry* 36(10), 933–939.
- Wang, H.X., Wu, H., Ho, C.T., Weng, X.C., (2006). Cocoa butter equivalent from enzymatic interesterification of tea seed oil and fatty acid methyl esters. *Food Chemistry* 97(4), 661–665.
- Wesdorp, L.H., (1990). Liquid-multiple solid phase equilibria in fats – theory and experiments. PhD thesis, Technical University of Delft, The Netherlands.
- Wu, S., Hu, C., Zhao, X., Zhao, Z.K., (2010). Production of lipid from N-acetylglucosamine by *Cryptococcus curvatus*. *European Journal of Lipid Science and Technology* 112(7), 727–733.
- Wu, S., Zhao, X., Shen, H., Wang, Q., Zhao, Z.K., (2011). Microbial lipid production by *Rhodospiridium toruloides* under sulfate-limited conditions. *Bioresource Technology* 102(2), 1803–1807.
- Xu, X., Guo, Z., Zhang, H., Vikbjerg, A.F., Damstrup, M.L., (2006). Chemical and enzymatic interesterification of lipids for use in food, in: Gunstone, F.D. (Ed.), *Modifying lipids for use in food*. Woodhead Publishing Limited, Cambridge, pp. 234–272.
- Xu, X.B., (2000). Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. *European Journal of Lipid Science and Technology* 102(4), 287–303.
- Yetter, J.J., (1976). Process for synthesizing specific complete mixed polyol esters, US Patent.
- Young, C.C., (1984). The interpretation of GLC triglyceride data for the determination of cocoa butter equivalents in chocolate—a new approach. *Journal of the American Oil Chemists Society* 61(3), 576–581.
- Zaidul, I.S.M., Norulaini, N.A.N., Omar, A.K.M., Smith, R.L., (2006). Supercritical carbon dioxide (SC-CO₂) extraction and fractionation of palm kernel oil from palm kernel as cocoa butter replacers blend. *Journal of Food Engineering* 73(3), 210–216.
- Zarringhalami, S., Sahari, M.A., Barzegar, M., Hamidi-Esfehani, Z., (2010). Enzymatically modified tea seed oil as cocoa butter replacer in dark chocolate. *International Journal of Food Science and Technology* 45(3), 540–545.
- Zhang, H., Smith, P., Adler-Nissen, J., (2004). Effects of Degree of Enzymatic Interesterification on the Physical Properties of Margarine Fats: Solid Fat Content, Crystallization Behavior, Crystal Morphology, and Crystal Network. *Journal of Agricultural and Food Chemistry* 52(14), 4423–4431.

19

Confectionery Fats

Kevin W. Smith

Fat Science Consulting Limited, Bedford, U.K.

This chapter will cover the principal classes of confectionery fats. The formulation of the blends will be described, as well as their triacylglycerol composition and polymorphism. The important issue of compatibility with cocoa butter will be discussed. The way in which product processing differs for the various fat types will be considered.

Introduction

Fats are used in confectionery in various applications: bars, shells, enrobings, fillings, toffees (including caramel), and ice cream. This being a book about *Cocoa Butter and Related Fats*, the focus in this chapter will exclude toffee fats and ice cream fats.

Cocoa butter is a unique fat, as described in other chapters in this book. However, over the years, various factors have led to the development of other confectionery fats to extend the use of cocoa butter or to replace it entirely in a product type, be it due to shortage of supply, cost reduction, product improvement, new product characteristics or production convenience. These cocoa butter alternatives (CBA) fall into several categories: cocoa butter equivalents (CBE), cocoa butter replacers (CBR) and cocoa butter substitutes (CBS). Naturally, they have been designed to have very similar melting behavior to cocoa butter (Fig. 19.1) but can have a wide range of properties within each class.

Other fats have been specifically developed to counter bloom formation in chocolate. A short summary of the interaction of these fats with cocoa butter will be given at the end of the chapter.

Principal Sources of Confectionery Fats and Their Components

There are relatively few oils and fats that are widely used in confectionery, although many others are used in specialist applications or small sectors of the market. The

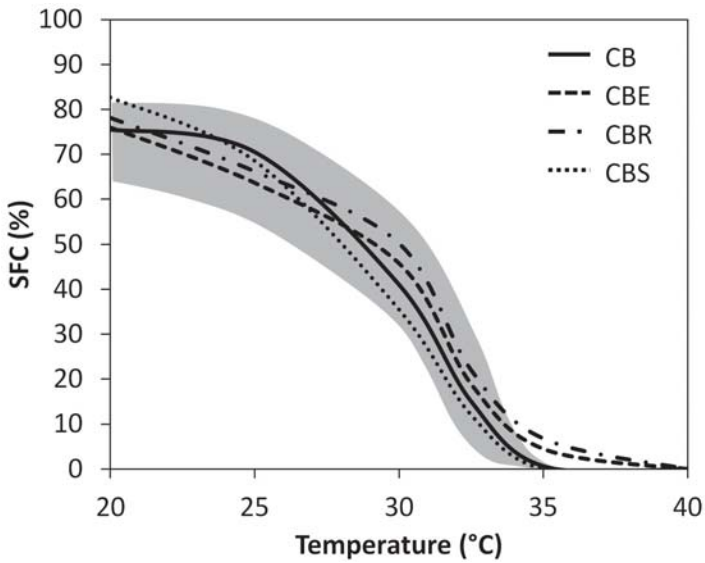


Fig. 19.1. Melting profiles of cocoa butter and typical cocoa butter alternative fats. Shaded area indicates range observed for cocoa butters, while solid line shows one specific cocoa butter.

Table 19-A. Principal Fats Used in Confectionery.

Cocoa butter	
Palm oil	Usually fractionated
Palm kernel oil	Usually fractionated
Shea oil	Usually fractionated
Illipé	
Kokum	
Sal	Usually fractionated
Mango kernel oil	Usually fractionated
Soybean oil	Usually hydrogenated or interesterified
Cottonseed oil	Usually fractionated or hydrogenated
Milk fat	
Various nut oils (e.g. hazelnut)	

principal fats present in confectionery are given in Table 19-A. Oils are generally extracted by crushing seeds, nuts or fruits and expelling the oil by pressing. In some cases the oil is extracted using solvents. In confectionery, many of these oils may be applied directly after refining, without further processing. However, some

may undergo modification to obtain specific properties in the oil. The most widely used modification process is that of fractional crystallization (or fractionation), but hydrogenation, interesterification and simple blending with other oils also have their place.

Fractionation

In concept, fractionation is quite simple. All fats are composed of a range of triacylglycerols (TAG). These TAG vary in melting point and the mix of these TAG gives rise to the particular melting profile of a fat. Table 19-B shows the principal TAG of which palm oil and palm kernel oil are composed, along with the melting points. Since the TAG have different melting points, they can be separated by crystallizing the oil at a temperature at which certain TAG, but not others of lower melting point, will crystallize. In the main, the TAG broadly separate according to the number of saturated acyl groups in the TAG. Table 19-C shows the TAG composition of palm oil and two fractions. It is clear that the fully saturated TAG are present almost in the stearin alone. Following crystallization, the solid fat can be separated from the liquid oil by a filtration technique. Fig. 19.2 shows an overview of the fractionation process and a good description has been given recently by Timms (2005).

Table 19-B. Melting Points of Some Triacylglycerols Present in Confectionery Fats.

Triacylglycerol ¹	Melting Point (°C) ²
StStSt	72.5
PPP	65.9
POP	37.2
POSt	37.1
StOSt	44*
POO	18.5
PLnP	27.1
PEP	54.0
PEE	44.1
StEE	49.7
EEE	42.2
LaLaLa	45.7
OOO	4.8

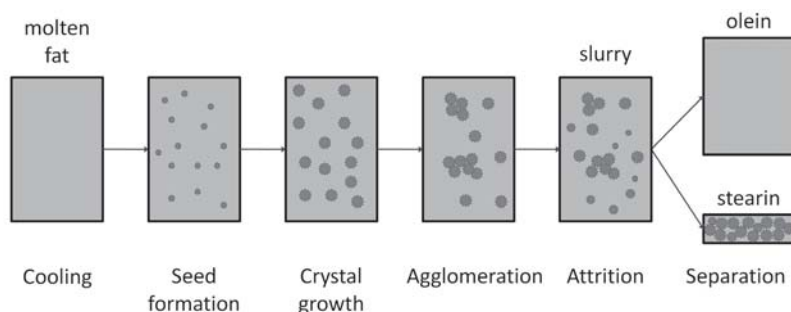
¹St = stearic, P = palmitic, La = lauric, E = elaidic, Ln = linoleic, O = oleic

²most stable polymorph

Table 19-C. Triacylglycerol Composition of Typical Palm Oil, Palm Stearin, and Palm Olein.

Triacylglycerol ¹	Palm Oil	Palm Stearin	Palm Olein
SSS	6.8	33.8	0.1
SOS	32.8	27.8	31.5
SSO	4.7	6	5.1
SLnS	8.7	8.1	9.6
S00	28.8	14.2	33.5
3+ DB	18.1	10	20.2
I.V.	52.8	32	57.2

1. S = saturated, O = oleic, Ln = linoleic, 3+ DB = 3 or more double bonds

**Fig. 19.2.** Schematic overview of fractionation.

Fractionation methods vary but they can be divided into a number of categories. The first classification concerns whether the oil is crystallized from the melt or from solution in an organic solvent. These are termed 'dry' and 'wet' fractionation respectively (although the latter involves no water). The second classification, applying principally to dry fractionation, relates to whether the oil is crystallized under shear (stirring) or quiescently (static). Similarly, the separation of solid and liquid is carried out in various ways: membrane presses, centrifuges, vacuum filter belts.

In dry fractionation, the oil is heated until it is completely liquid. It is cooled, in a controlled manner, until sufficient crystallization has taken place. During this time, agitation or shear may be applied, but need not be. Where the slurry is agitated, the crystals can break up, to a greater or lesser degree. Attrition of the crystals can make separation difficult. Efficiency of the separation of the slurry into solid and liquid phases depends on several factors. It is almost impossible completely to separate the solid (stearin) from the liquid (olein), and some liquid is entrained in

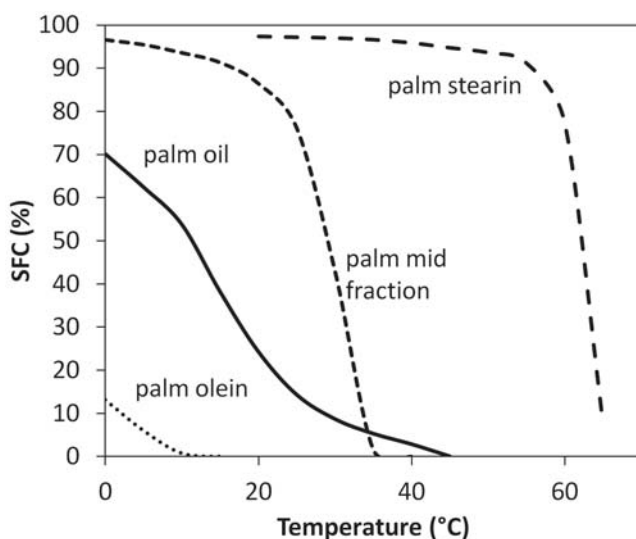


Fig. 19.3. Melting profiles of palm oil and some fractions.

the stearin. High-pressure membrane presses can achieve a good separation between olein and stearin, removing much of the liquid from between the crystals but liquid trapped within crystals is difficult to squeeze out. Thus, for the highest quality stearins, wet fractionation is preferred. Palm kernel oil is routinely fractionated via the dry method, and usually statically crystallized. On the other hand palm oil is usually stirred.

In many cases, a single fractionation step is sufficient (e.g. with palm kernel oil). Other oils (e.g. palm oil) benefit from two or more steps (Kellens et al., 2007) to obtain fractions with specific properties (Fig. 19.3). Palm mid fraction, produced by a two-step (at least) fractionation has a high level of 1,3-dipalmitoyl-2-oleoyl glycerol (POP), which is one of the principal TAG in cocoa butter.

In wet fractionation, the oil is dissolved in an organic solvent, such as acetone, hexane or 2-nitro-propane. As with dry fractionation, it is cooled in a controlled way to a temperature at which crystallization will occur. Unlike dry fractionation, solvent fractionation is never static, but is stirred. Once crystallization has occurred, the solid is separated, frequently using a belt filter, and washed with chilled solvent to remove the liquid oil. Solvent is evaporated from the solid to yield the stearin.

The choice of dry or wet fractionation usually depends on the quality of the desired stearin. In many situations, the olein quality from both methods is very similar. However, wet fractionation yields stearins with a lower amount of entrained olein. In large part, this is due to the fact that the entrained liquid in the crystals is a solution of oil, rather than the oil itself. For this reason, wet fractionation is the preferred method

for the production of the highest quality palm mid fractions and the stearins of oils, such as shea and sal, used in confectionery fats. Nevertheless, fractions of very good quality are possible via dry fractionation if very high pressure presses are used to expel the olein.

Hydrogenation

Hydrogenation involves the addition of hydrogen to unsaturated acyl groups on the TAG, either reducing the number of double bonds, isomerizing them, or removing them entirely (Fig. 19.4). The reaction is carried out with the aid of a catalyst, such as nickel, which has been treated in a certain way (usually with sulfur) in order to control the rate and type of reaction taking place. In the past, especially, certain types of catalyst were developed to prevent complete hydrogenation and to isomerize the *cis* double bonds to *trans*. As seen in Table 19-B, the fewer the double bonds in a TAG, the higher the melting point, and *trans* double bonds yield a higher melting point than *cis*: *rac*-1-palmitoyl-2,3-dioleoyl glycerol (POO) has a melting point of about

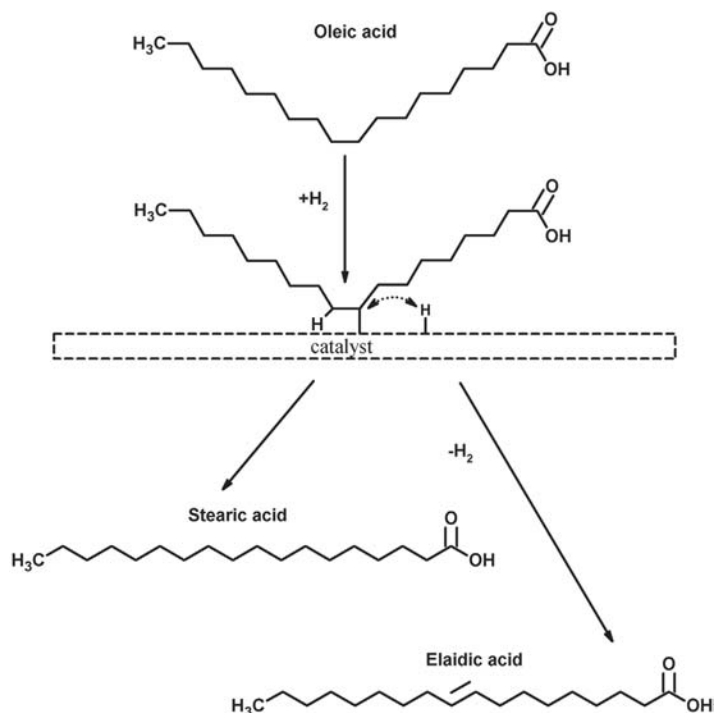


Fig. 19.4. Hydrogenation of *cis* double bonds can lead to saturated or *trans*-unsaturated acyl chains.

18°C but *rac*-1-palmitoyl-2,3-diacylglycerol (PEE) has a melting point of around 44°C. Thus, otherwise liquid oils can be given a range of melting points from semi-solid at room temperature up to 70°C (if fully hydrogenated). This increase in melting point is also referred to as hardening. The amount of unsaturation (double bonds) in a fat is often quantified by the iodine value (IV), which is a figure determined by the amount of iodine that complexes with the double bonds present. Hydrogenation almost always involves a reduction in the IV, even though *trans* double bonds yield the same figure as *cis*. This is because polyunsaturated acyl groups are more susceptible to full hydrogenation than monounsaturated groups. Thus, linoleoyl and linolenoyl groups in an oil are the first to be hydrogenated. The reduction in *cis* unsaturation leads to an increase in the slip melting point of the fat.

Palm kernel oil and palm kernel stearin are frequently fully hydrogenated (i.e. all unsaturates are converted to saturates, and *trans* content is minimal) for use in confectionery, giving rise to a hard but sharp-melting fat (Fig. 19.5). Nonlauric fats, which contain longer chains (C16-C18) are usually partially hydrogenated to a slip melting point of around 37–40°C. These may be used as they are or, since they can have a high-melting ‘tail’, may be fractionated to yield a fat having a sharper melting point. Over the last few years, such fats have been produced in decreasing quantities due to the negative health aspects of *trans* fatty acids (Mensink et al., 2003). Instead, products have been developed that are low or zero *trans*, including those that use fully hydrogenated oils (e.g. soybean) as a component for a blend with unhydrogenated oil that is then interesterified.

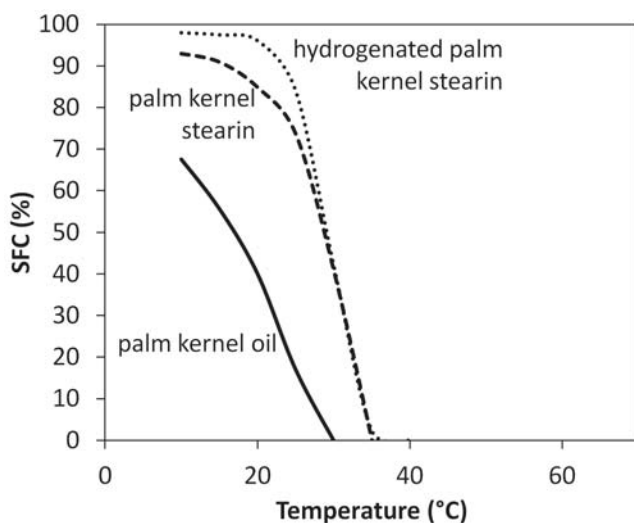


Fig. 19.5. Melting profiles of palm kernel oil and derived fats.

Interesterification

The interesterification process rearranges the acyl groups on the glycerol backbone of the TAG (Fig. 19.6). Traditionally, this has been achieved by using a catalyst such as sodium methoxide under anhydrous conditions and at high temperature. The effect of interesterification on an oil depends on the original TAG present (and the acyl groups or fatty acids that they contain). Interesterifying palm kernel oil, reduces the solid fat contents at most temperatures, leading to a slightly softer fat. With palm oil, it increases the solid fat content at most temperatures, leading to a slightly harder fat (Fig. 19.7). Generally, the process leads to a broader TAG composition. As well as interesterifying a single fat, blends of fats can be interesterified. In this way, the

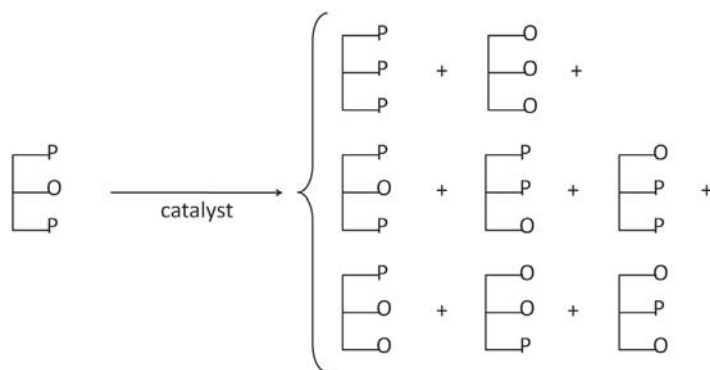


Fig. 19.6. Effect of chemical (or random) interesterification of a mixed triacylglycerol (TAG) on TAG composition.

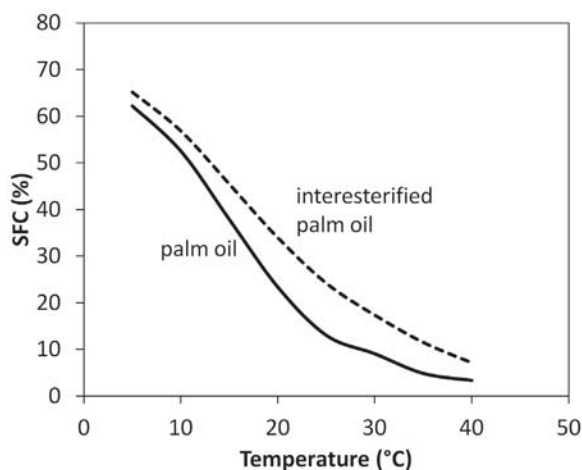


Fig. 19.7. Influence of interesterification on palm oil melting profile.

TAG composition can be broadened even further, with the final composition being dictated by the fatty acid composition of the blend.

For confectionery fats, in particular, monounsaturated TAG are desirable, since they have melting points in the right range. Fats containing such TAG can be produced via by interesterifying a fully hydrogenated oil (e.g. soybean oil) with a non-hydrogenated liquid oil (Petrauskaite et al., 1998, Ribeiro et al., 2009). The product of this reaction can be processed further by fractionation to yield a fat rich in mono-unsaturated TAG.

As an alternative to 'chemical' catalysts, the reaction can be carried out using enzymes, specifically lipases. Unilever, and Novozymes pioneered the use of microbial lipases as catalysts for fat interesterification. It has the advantage that the reaction can be carried out at much lower temperatures, with less risk of damaging the oil in any way. It may also be perceived as more 'natural'. When the process was originally developed, the cost was much more than the 'chemical' route, but enzyme costs have reduced markedly over the last decade. A further advantage over chemical interesterification is that lipases with certain specificities are available; they may operate on certain glycerol positions within the TAG, or may even operate on certain specific fatty acids. The former functionality is made of good use when it comes to creating confectionery fats (Fig. 19.8)

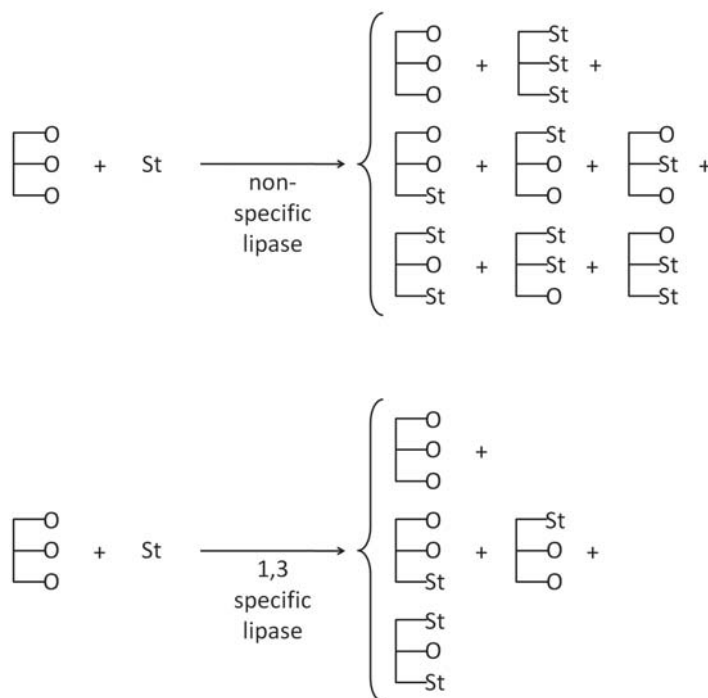


Fig. 19.8. Acidolysis of OOO with St using a non-specific (random) lipase (Top) or 1,3, specific lipase (Bottom).

Cocoa butter contains mainly symmetrical disaturated, mono-unsaturated TAG (SOS), the principal one being *rac*-1(3)-palmitoyl-3(1)-stearoyl-2-oleoyl glycerol (POSt). Since many vegetable fats already have oleic acid in the 2 position of the TAG, a 1,3 specific lipase can be used to place saturated acids on the 1 and 3 positions, yielding cocoa butter-like TAG. In this way, oils such as palm oil (or olein) can be reacted with stearic acid to yield a composition like that of cocoa butter (Macrae, 1984, Undurraga et al., 2001, Abigor et al., 2003). Similarly, a normally liquid oil, high oleic sunflower oil, can be converted into a cocoa butter-like fat by reacting with stearic acid and then fractionating, giving a fat rich in 1,3-distearoyl-2-oleoyl glycerol (StOSt), another of the main TAG in cocoa butter.

Cocoa Butter Equivalents

Composition and Polymorphism

Cocoa butter equivalents (CBE) are very similar to cocoa butter in composition and properties. They are not completely identical in composition to cocoa butter but are based on fats, or blends of fats, which contain high levels of the three principal TAG in cocoa butter: POSt, StOSt, and POP. Typically, CBE component fats have greater than 70%, usually greater than 80% SOS TAG and less than 5% SSS TAG. Cocoa butter exhibits six polymorphs: sub-alpha (I), alpha (II), beta' (III), beta' (IV), beta (V), and beta (IV). Like cocoa butter, all CBE component fats are stable in the beta-3 polymorph.

Component Fats

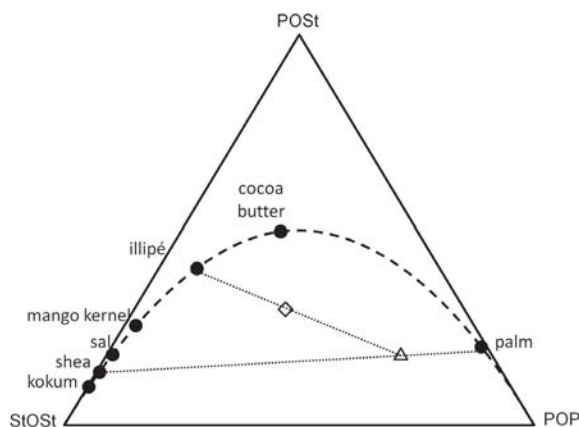
Many fats have been used, developed or proposed as CBE components. However, a few fats dominate. Indeed, within the European Union, use in chocolate is restricted to six specific fat sources: palm (*Elaeis guineensis*, *Elaeis olifera*), shea (*Butyrospermum parkii*), illipé (Borneo tallow, *Shorea stenoptera*), kokum (*Garcinia indica*), sal (*Shorea robusta*), and mango kernel (*Mangifera indica*). The compositions of these six fats are given in Table 19-D. It is clear that certain of these fats can be used directly, without further processing, since their content of SOS type TAG is high and SSS is low (e.g. illipé, kokum). The other fats need to be fractionated to concentrate the SOS TAG. It is equally clear from Table 19-D that palm oil is a key fat, since it is the only one of the group of six that contains a significant proportion of POP. It is also the most abundant of all the CBE component fats. The other five fats contribute mainly StOSt, except illipé, which also contributes POSt. There are many other fats that have high SOS contents, or can be fractionated to yield a composition rich in SOS (including allanblackia, pentadesma, mowrah, phulwara, aceituno, and Chinese vegetable tallow).

In order to increase the level of SOS, palm oil is fractionated, as described above, to yield palm mid fraction, which is rich in POP. Similarly, shea, sal, and mango ker-

Table 19-D. Triacylglycerol Composition of Cocoa Butter and the Six Fats Permitted by the EU for Use in Chocolate.

Triacylglycerol	Cocoa Butter	Palm	Shea	Illipé	Kokum	Sal	Mango Kernel
SSS	1.9	7.7	1.4	1.3	0.8	1.1	1.5
SUS/SSU	86.8	47	56.3	91.8	81.6	75.3	62.7
SUU/USU	10.9	35.4	30.6	6.9	15.2	21.5	30.4
UUU	0.4	9.9	11.7	0	2.4	2.1	5.4
Relative proportion of main cocoa butter TAG							
POP	20.6	80.3	3.0	7.3	0.4	0.7	2.1
POSt	49.6	18.6	10.5	39.9	6.7	16.6	22.2
StOSt	29.8	1.1	86.4	52.8	92.9	82.8	75.7

1. S = saturated, U = unsaturated, P = palmitic, St = stearic, O = oleic

**Fig. 19.9.** Ternary diagram of main cocoa butter TAG, showing the curve of vegetable fat compositions and the position of cocoa butter equivalent components.

nel oil are fractionated to yield stearins that are rich in StOSt, while kokum does not need to be fractionated since it already has a high content of StOSt. Blending these two types of fat (POP-rich and StOSt-rich) together brings the composition close to that of cocoa butter. Incorporation of illipé (both POST-rich and StOSt-rich) into the blend brings it even closer in composition terms to that of cocoa butter.

Although high SOS fats can be blended together to yield a composition similar to that of cocoa butter, it is not feasible to match the composition completely. Fig. 19.9 shows a ternary diagram of the three principal TAG of cocoa butter. Due to the manner in which the TAG are produced in plants, all natural fats lie on the curved line

shown. Fractions of natural fats also lie on the line. The composition of any two component blend will lie on a straight line drawn between the two fats. Clearly, no blend of fats can precisely duplicate the composition of cocoa butter since it lies at the top of the curve. For example, if StOSt-rich shea stearin is blended with POP-rich palm mid fraction, the composition of the blend lies on the dotted line in Fig. 19.9, which evidently runs beneath cocoa butter. If POSt-rich illipé is now added to the shea stearin/palm mid fraction blend (triangle in Fig. 19.9), the composition is shifted towards cocoa butter (diamond in Fig. 19.9). Thus, blends can come close to the composition of cocoa butter, and be comparable in behavior.

POP/POSt/StOSt Ternary Phase Behavior

Fig. 19.10 shows the melting points of mixtures of pure cocoa butter TAG. It is obvious that melting point is higher at high levels of StOSt. It is also evident that there is eutectic softening between POP and StOSt. This means that addition of low levels of shea or sal stearin, for example, to palm mid fraction leads to a reduction in melting point initially, although higher levels increase the melting point beyond that of palm mid fraction (Ali et al., 1992). In order to have a CBE that makes no difference at all to the melting point of cocoa butter, the composition should lie on the same isothermal (temperature) contour as cocoa butter itself. In fact, this was the idea behind one of the early patents covering CBE type fats (Padley et al., 1981).

Comparing Fig. 19.9 and Fig. 19.10, it is obvious that blends of SOS fats can have quite a range of properties, with widely differing melting points. Cocoa butter falls roughly midway in melting point terms. Consequently, the particular use to which a CBE will be put depends on its composition. Traditional CBEs contain a mixture of

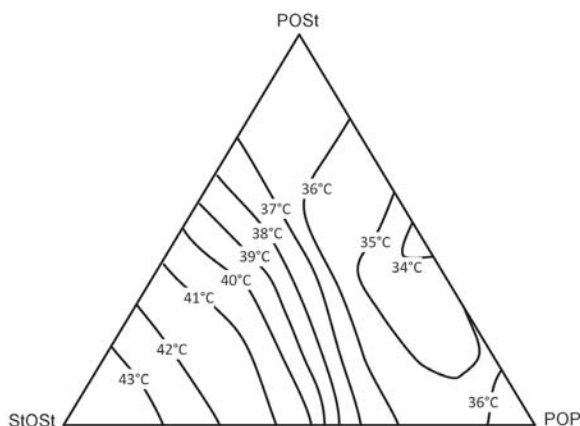


Fig. 19.10. Ternary diagram showing the melting points for mixtures of the main cocoa butter TAG.

POP- and StOSt-rich fats (and sometimes POSt-rich, i.e. illipé) that are very similar to cocoa butter in properties. However, if higher amounts of POP fat are used in the blend, it will be a softer CBE (see Fig. 19.10). At low levels of addition to the fat phase of a product, it can extend the cocoa butter but, at high levels, it will soften the product. On the other hand, if high amounts of StOSt-rich fats are incorporated, the CBE will have a higher melting point and can increase the hardness of cocoa butter, perhaps conferring more heat resistance. Such fats are considered cocoa butter improvers (CBI).

Although Fig. 19.1 shows a CBE with similar SFC to cocoa butter, it should be kept in mind that what matters is not the SFC of the CBE but rather the SFC of the mixture with cocoa butter. Thus, a CBE may have lower SFC than cocoa butter, but produce a completely acceptable melting profile when mixed with cocoa butter. This is because of the presence of the eutectic line in the phase ternary phase diagram, and its use at a relatively low level. For this very good reason, CBE are often compared in blends with cocoa butter at the appropriate level for their application.

Compatibility with Cocoa Butter

Compatibility between TAG is mainly dependent on the similarity of their properties: acyl chains involved (molecular size), melting points, polymorphism (or crystal structure). As would be anticipated, since CBE are so close in composition to cocoa butter, they are extremely compatible. If the solid fat content (SFC) temperature profile is determined, pseudo-phase diagrams (iso-solid) can be interpolated for a number of blends of CBE with cocoa butter (Fig. 19.11, derived from Gordon et al., 1979), and can be supplemented by measuring the polymorphic form by x-ray diffraction. The iso-solid lines between cocoa butter (left of the figure) and CBE (right of the figure) are

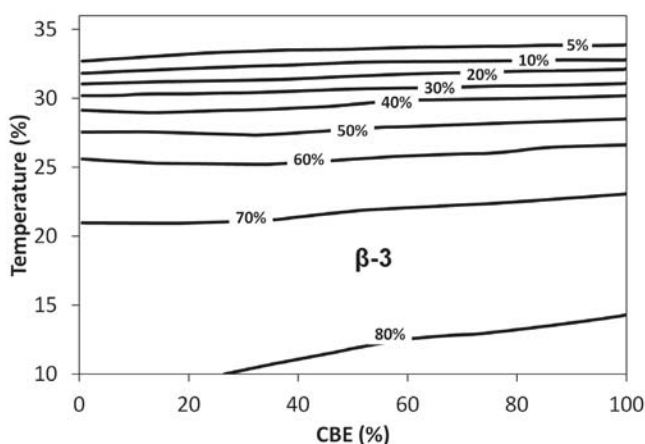


Fig. 19.11. Iso-solid diagram showing compatibility between cocoa butter and CBE. Source: Gordon et al., 1979 © Copyright Wiley-VCH, redrawn with permission.

more or less straight and parallel, indicating that CBE is completely compatible with cocoa butter at all levels. Consequently, CBE can be used at whatever level is required, provided it meets with local regulations (regarding formulation and/or labeling).

Processing Differences Compared with Cocoa Butter

CBE can be used in any formulation that includes cocoa butter, principally chocolate, but also in center fillings like pralines. Due to the intentional similarity of CBE with cocoa butter, little difference is necessary when processing chocolate. Since they are stable in the beta polymorph, they must be tempered. Naturally, much depends on the precise composition of the CBE and the amount present (as a proportion of the fat phase). If the CBE is towards the POP end of the range (soft CBE), it can require slightly lower tempering temperatures compared to cocoa butter. Conversely, a CBE rich in StOSt (CBI) would require higher tempering temperatures. The magnitude of the difference is determined by the proportion of CBE in the fat phase. Within the EU, CBE can be present at up to 5% on chocolate, which translates into around 15–17% of the fat phase. However, it is also possible to formulate a chocolate in which the majority of the fat phase is the CBE. In many countries, this could not be labeled chocolate, but may be considered a ‘compound’ or ‘supercoating’. In this case, the differences in processing conditions are more marked and depend on how far the composition differs from cocoa butter, and in which direction.

Cocoa Butter Replacers

Composition and Polymorphism

In the past, high *trans* cocoa butter replacers (CBR) were widely used in confectionery, but now that *trans* fatty acids have been identified as having a negative effect on health (Mensink et al., 2003), they are used less and less. They replace the majority of the cocoa butter in the fat phase. Recipes often make use of cocoa powder rather than cocoa mass, meaning that cocoa flavor can be less intense than for chocolate. Such fats are prepared by the partial hydrogenation of soft or liquid oils, including palm olein, soybean oil, cottonseed oil and rapeseed oil (or a mixture of such oils). They have a very high *trans* fatty acid content, usually greater than 25%, even up to 55% in coating fats. In order to obtain a sharper melting fat, fractionation is applied to yield a mid fraction with a melting profile similar to cocoa butter. Lower levels of *trans* and softer *trans* fats have been used in toffees and fillings, while high *trans*, sharper melting fats have been used in chocolate compounds.

High *trans* cocoa butter replacer (CBR) fats are mainly composed of PEE (E = elaidic, C18 *trans* mono-unsaturated), StEE, and EEE. CBR are stable in the beta'-2 polymorph, unlike cocoa butter's beta-3. This is probably due to the complexity of the TAG composition, especially the unsaturated acyl chains. During hydrogenation (Fig. 19.4), not only can a *cis* double bond isomerize to *trans*, but the double bond

can migrate along the acyl chain, away from its initial position. This gives rise to many double bond positional isomers, in addition to being *cis* or *trans*, giving a highly complex composition.

Low *trans* CBR

Due to the negative health aspects of *trans* fats, CBR have been developed with very low levels of *trans*, or even zero *trans* (or virtually zero, since simply heating a fat to high temperatures—perhaps during refining—can generate a small quantity, <1%, of *trans* fatty acids). Initial progress in reducing *trans* fatty acids resulted in the development of fats with 5–15% *trans*. However, these still had to be labeled as hydrogenated in many countries. In the last five years, virtually *trans* free CBR have been launched (e.g. Slager et al., 2007). Such CBR may be based on fats similar to CBE components, but having higher asymmetric disaturated, mono-unsaturated TAG (SSO) and other beta' TAG (Cain et al., 1999), or containing a polyglycerol fatty acid ester (Okada et al., 2001). Higher PPO can be achieved in palm fractions by careful control of the fractionation conditions. As with high *trans* CBR, these fats are stable in the β' -2 polymorph.

Compatibility with Cocoa Butter

High *trans* CBR, having mainly C16 and C18 acyl groups, are similar in molecular size to cocoa butter TAG and so can be expected to have some compatibility with cocoa butter. However, they are stable in the β' -2 form, while cocoa butter is stable in the β -3 form, thus they can be expected to have a good deal of incompatibility. Fig. 19.12 shows a pseudo phase diagram using iso-solids to show the interaction

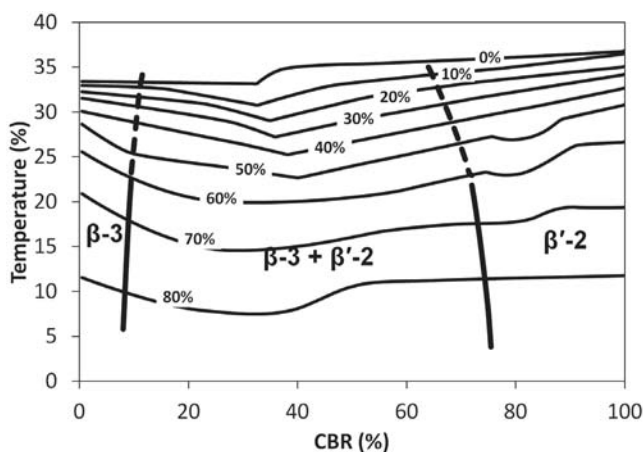


Fig. 19.12. Iso-solid diagram showing incompatibility between cocoa butter and CBR. Source: Gordon et al., 1979 © Copyright Wiley-VCH, redrawn with permission.

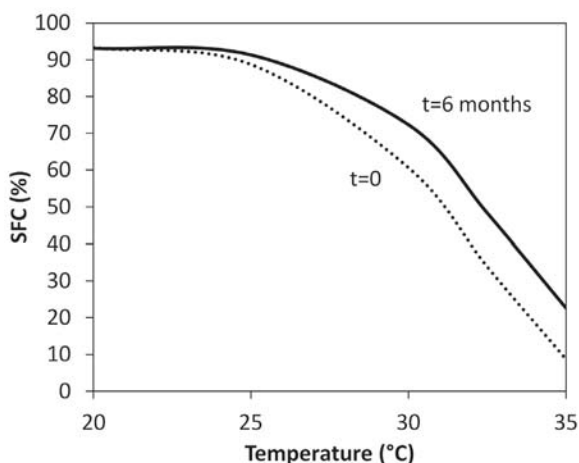


Fig. 19.13. Post-hardening of high *trans* CBR during storage at 20°C.

between CBR and cocoa butter. Bearing in mind that CBR are intended to replace the majority of the cocoa butter (right side of the chart in Fig. 19.12), it is clear that they could tolerate only up to about 20% of cocoa butter (on the fat phase). Any more than this will lead to separation of a β -3 phase from the β '-2 phase, and result in fat bloom formation in a product (or at least loss of gloss). The iso-solid lines are not straight and parallel (as with CBE) but shown an interaction of the CBR with the cocoa butter.

Non-*trans* CBR have similar fatty acids (and molecular size) to cocoa butter, but are stable in the β '-2 form. However, since cocoa butter TAG are similar to a major part of the non-*trans* CBR they are more compatible and may be tolerated at levels above 20% (Fig. 19.13).

Processing Differences Compared with Cocoa Butter

Both high and low *trans* CBR are stable in the β '-2 polymorph, and so do not require tempering, meaning that processing is much easier than for chocolate made with cocoa butter, and they are relatively tolerant of a range of cooling conditions (high *trans* CBR, especially). In general, high *trans* CBR crystallize rapidly but they can have a tendency to post-harden, or increase in melting point and solid fat content at higher temperatures during storage (Padley, 1997). Emulsifiers such as sorbitan tristearate can be added to slow down this process. Low *trans* CBR tend to crystallize less rapidly than high *trans* fats.

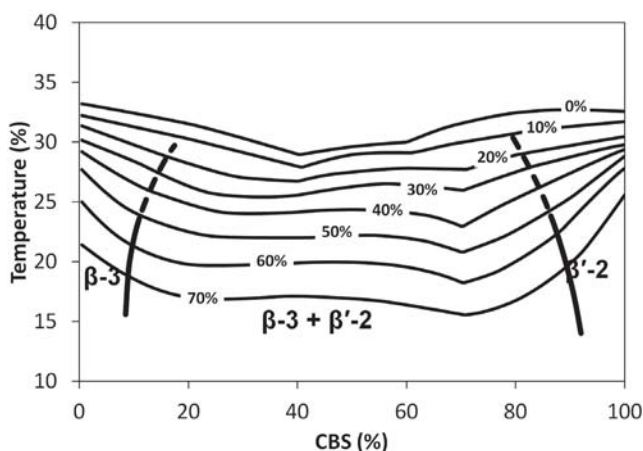


Fig. 19.14. Stabilized (tempered) iso-solid phase diagram of cocoa butter and cocoa butter substitute. Source: Gordon et al., 1979 © Copyright Wiley-VCH, redrawn with permission.

Cocoa Butter Substitutes

Composition and Polymorphism

Lauric cocoa butter substitute (CBS) fats are derived mainly from palm kernel oil and coconut oil and have high levels of lauric acid (>40%). For some applications the fats may be unmodified but, as described earlier, they are likely to be fractionated, hydrogenated or both. Coconut oil has significant amounts of shorter chain fatty acids (C8:0 and C10:0), which mean that it is a relatively soft fat, even when fully hydrogenated (melting point of 34°C). Palm kernel oil has more oleic acid (C18:1) and so yields a harder fat when fully hydrogenated. For the sharpest melting fat, palm kernel oil is fractionated to produce a stearin, which may then be hydrogenated (Fig. 19.5). CBS contain significant levels of TAG such as tri-lauroyl glycerol (LaLaLa) and dimyristoyl-monolauroyl glycerol (MyMyLa). Like CBR, CBS are stable in the β' -2 form.

Compatibility with Cocoa Butter

As for CBR, CBS are used to replace almost all of the cocoa butter in a product. CBS have both a shorter acyl chain length compared to cocoa butter (C12 as opposed to C16 and C18) and a different stable polymorph (β' -2 instead of β -3). As anticipated, CBS fats are almost fully incompatible with cocoa butter. Fig. 19.14 shows significant interaction between CBS and cocoa butter, and indicates that only

a small amount of cocoa butter can be tolerated in the CBS, in practice a maximum of 5% (right hand end of the chart in Fig. 19.14). Beyond this limit, the fat will separate out into two phases, one of which is lauric rich and the other cocoa butter rich. In a compound chocolate, this will lead to fat bloom. Occasionally, emulsifiers like sorbitan tristearate may be added to delay bloom formation (Rossell, 1985). It is also clear from the figure that there is a eutectic between CBS and cocoa butter, with each softening the other. Products containing CBS are frequently made using low fat, or defatted, cocoa powder due to the high incompatibility with cocoa butter. Unfortunately, this leads to much less intense cocoa flavor.

Processing Differences Compared with Cocoa Butter

Since CBS are stable in the β' -2 polymorph, they do not require tempering but can be crystallized directly from the melt. The crystallization rate of CBS fats is often accelerated by the addition fully hydrogenated nonlauric fat, with a melting point typically of around 60°C or more. Such seeding agents are added at levels of only a few percent. Without seeding, CBS needs very deep cooling in order to crystallize at a reasonable rate.

Bloom Retarding Fats

Confectionery bars and coatings can exhibit fat bloom. There are various types of fat bloom and a good review has been published by Lonchampt et al. (2004). This section will cover only fat bloom in cocoa butter chocolate. Bloom in CBR and CBS products is controlled by ensuring that cocoa butter levels are below 20% and 5% of the fat phase, respectively, and addition of certain emulsifiers, like sorbitan tristearate.

Strategies for Bloom Inhibition

There are a number of reasons why fat bloom appears on chocolate products:

- incorrect tempering
- incorrect cooling
- over-heating and melting, followed by cooling
- storage at elevated temperatures (>25°C).

The first two situations will not be considered here, since it is assumed that these factors are already addressed. The third case is typified by the bar of chocolate left in a car on a hot sunny day, which then cools overnight. In essence, bloom results because melting has destroyed the tempered state of the chocolate and subsequent cooling crystallizes the unstable β' polymorph, which then undergoes transformation into

the beta form. This situation can be helped by including a high melting seeding material to induce crystallization into the stable beta form.

The fourth case involves the slow transformation from the beta (V) form into the most stable beta (VI) form. This can be delayed by blocking the crystallization of the most stable form.

Composition and Polymorphism

In the case of uncontrolled melting and recrystallization, 1,3-dibehenyl-2-oleoyl glycerol (BOB) can be used (Hachiya et al., 1989a). Behenic acid is a saturated fatty acid having 22 carbon atoms. Thus, BOB is an SOS TAG and is analogous to POP, POSt, and StOSt. However, it has a melting point of 56°C and can act as a seed for the crystallization of the cocoa butter TAG into the beta (V) form. With such a high melting point, BOB crystals survive melting of the chocolate and provide seeds for subsequent crystallization into the stable form.

In the case of the transition from beta (V) into beta (VI), there are a number of possibilities. Sorbitan tristearate (used to inhibit bloom in CBR and CBS systems as well) and similar emulsifiers reportedly slow the polymorphic transformation (Garti et al., 1986). If the desire is to avoid unnecessary items on the label, TAG solutions exist. Milk fat is well known for its bloom inhibiting effect; dark chocolate often has a small amount of milk fat added for this reason. More effective are bloom retarding fats that incorporate saturated TAG having mixed long (C16, C18) and medium (C10-C14) chain fatty acids (Cain et al., 1995). Thus, they are a specific type of lauric fat. They are stable in the beta' polymorph.

Compatibility with Cocoa Butter

Lauric bloom retarding fats have limited compatibility with cocoa butter. However, in use, only a few percent are required to delay the polymorphic transition (higher levels would lead to softening of the cocoa butter). They have little effect on the tempering process, or the crystallization of chocolate, but act as 'crystal poisons' to block the growth of beta (VI) crystals (Smith et al., 2011). Since migration of oil from the center of a filled product is a leading cause of bloom, bloom retarding fats can be added to the center (to migrate with the oil and slow polymorphic transformation) or used as a 'barrier' layer between the coating and the center (which is carried through into the chocolate by the migrating oil from the center). Care must be taken to formulate so as to have sufficient bloom retarding fat to delay the formation of the beta (VI) polymorph, yet not so much that lauric bloom is promoted.

Filling Fats, Filled Products, and Compatibility

Fillings can fall into the same general classes as the CBA above. They are usually softer versions of CBE, CBR or CBS, with greater amount of unsaturated fatty acids.

Often, nut oils are present in the center, as with pralines, with harder fats supplying the necessary firmness in the center. One of the most important factors to consider is the compatibility between the coating fat and the filling fat. In any system where two fat phases are in contact (as in filled confectionery products), fat will migrate between filling and coating. As already discussed above, fat bloom can result when incompatible fats exist in the same fat phase. Hence, in formulating a product, the type of fat in the center should match the type of fat in the coating.

Factors in Selection of Fat Type

In selecting the fat type to use in a particular product, consideration must be given as to the requirements. If strong chocolate flavor is desired, then a cocoa butter based recipe, possibly with CBE, is the route to take. However, that demands the possession of tempering equipment. In the absence of tempering equipment, non-temper fats need to be used. High *trans* CBR are definitely out of favor because of the health risk, which leaves low (no) *trans* CBR and CBS. Using CBR allows a little more cocoa butter to be present than does CBS, which will bring extra flavor with it. If good oxidative stability is wanted, then CBS have low levels of unsaturated fatty acid and so are the most stable. On the other hand, if there is any chance of live enzyme being present from other ingredients, lauric CBS should be avoided, since hydrolysis of the fat can give rise to a soapy off-taste. Other considerations include the sharpness of melt, the hardness and the crystallization speed. Finally, as discussed in the previous section, the coating fat should match the filling fat.

References

- Abigor, R.D.; Marmer, W.N.; Foglia, T.A.; Jones, K.C.; Diccio, R.J.; Ashby, R.; Uadia, P.O., Production of cocoa butter-like fats by the lipase-catalyzed interesterification of palm oil and hydrogenated soybean oil. *Journal of the American Oil Chemists Society*, 2003, 80(12), 1193–1196
- Ali, A.R.Md.; Embong, M.S.; Flongoh Oh, C.H., Interaction of cocoa butter equivalent component fats in ternary blends. *Elaeis*, 1992, 4(1), 21–26
- Dever Brothers Company, Padley, F.B.; Paulussen, C.N.; Soeters, C.; Tresser, D., Chocolate having defined hard fat. 1981, US Patent 4176322
- Fuji Oil Company, Limited, Okada, T.; Yamada, K.; Nago, A., Hard butter composition and its production. 2001, US Patent 6258398B1
- Garti, N.; Schlichter, J.; Sarig, S., Effect of food emulsifiers on polymorphic transitions of cocoa butter. *Journal of the American Oil Chemists Society*, 1986, 63(2), 230–236
- Gordon, M.H.; Padley, F.B.; Timms, R.E., Factors influencing the use of vegetable fats in chocolate. *Fette Seifen Anstrichmittel*, 1979, 81(3), 116–121
- Hachiya, L.; Koyano, T.; Sato, K., Seeding effects on solidification behavior of cocoa butter and dark chocolate. II. Physical properties of dark chocolate. *Journal of the American Oil Chemists Society*, 1989, 66(12), 1763–1770

- Kellens, M.; Gibon, V.; Hendrix, M.; Greyt, W.D., Palm oil fractionation. *European Journal of Lipid Science and Technology*, 2007, 109, 336–349
- Lonchampt, P.; Hartel, R.W., Fat bloom in chocolate and compound coatings. *European Journal of Lipid Science and Technology*, 2004, 106(), 241–274
- Macrae, A.R., Microbial lipases as catalysts for the interesterification of oils and fats. In *Biotechnology for the Oils and Fats Industry*, Ratledge, C.; Dawson, P.S.S.; Rattray, J., Eds. AOCS Press, Champaign, IL, 1984, 189–198
- Mensink, R.P.; Zock, P.L.; Kester, A.D.; Katan, M.B., Effects of dietary fatty acids and carbohydrates on the ratio of total to LDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition*, 2003, 77(), 1146–1155
- Padley, F.B., Chocolate and confectionery fats. In *Lipid technologies and application*, Gunstone, F.D.; Padley, F.B., Eds. Marcel Dekker, Inc., New York, NY, USA, 1997, 391–432
- Petrauskaite, V.; Greyt, W.D.; Kellens, M.; Huyghebaert, A., Physical and chemical properties of trans-free fats produced by chemical interesterification of vegetable oil blends. *Journal of the American Oil Chemists Society*, 1998, 75(4), 489–493
- Ribeiro, A.P.B.; Grimaldi, R.; Gioielli, L.A.; Gonçalves, L.A.G., Zero trans fats from soybean oil and fully hydrogenated soybean oil: physico-chemical properties and food applications. *Food Research International*, 2009, 42, 401–410
- Rossell, J.B., Fractionation of lauric oils. *Journal of the American Oil Chemists Society*, 1985, 62(2), 385–390
- Slager, H.; Favre, L.; Talbot, G., The ultimate confectionery coating. *Food Marketing and Technology*, 2007, December, 12–14
- Smith, K.W.; Bhagga, K.; Talbot, G.; Van Malssen, K.F., Crystallization of fats: influence of minor components and additives. *Journal of the American Oil Chemists Society*, 2011, 88(8), 1085–1101
- Timms, R.E., Fractional crystallisation – the fat modification process for the 21st century. *European Journal of Lipid Science and Technology*, 2005, 107, 48–57
- Undurraga, D.; Markovits, A.; Erazo, S., Cocoa butter equivalent through enzymic interesterification of palm oil midfraction. *Process Biochemistry*, 2001, 36, 933–939
- Unilever N.V., Cain, F.W.; Hughes, A.D.; Talbot, G., Improved chocolate compositions. 1995, European Patent 0530864B1
- Unilever PLC, Cain, F.W.; Hughes, A.D.; Schmidl, B.; Cebula, D.J., Non-temper confectionery fats. 1999, European Patent 0536842B2

20

Future of Cocoa Butter Research

Nissim Garti¹ and Neil R. Widlak²

¹Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel, and ²ADM Cocoa Division, Archer Daniels Midland Company, Milwaukee, Wisconsin, USA

Introduction

Commercial Perspective

Chocolate and confection are the largest commercial applications for cocoa butter. Expansion of global markets of both cocoa butter and compound coatings will have a strong influence on the future research of cocoa butter and other confectionery fats.

Chocolate made with cocoa butter, established the “gold standard” for delivering what consumers desire from cacao. Government standards of identity for chocolates (dark, milk, white....) established in mature market countries regulate the composition of chocolate products to maintain a minimum standard of quality.

While the market opportunities in mature confectionery markets (Europe and North America) may differ from market opportunities in developing confectionery markets (China, India, Korea, Russia, South America, as examples), both markets share similar technical challenges. Overcoming barriers to market growth due to technical gaps, and not the gaps themselves, will differentiate research priorities between the two markets.

Research Perspective

Cocoa butter is a key ingredient in chocolate. Its chemical and physical structure, along with its crystallization behavior during manufacturing, in the presence of a complex mixture of the other ingredients, sensitive and exact narrow ranges for optimum cooling and storage, provide a great number of opportunities that impact product quality and conditions that produce substandard products that fail to meet minimum acceptable quality standards.

Cocoa butter is a complex mixture of triglycerides that precipitate together in a very harmonic way into as many as six polymorphic structures, depending on the temperatures and physical conditions that are applied. Almost any possible parameter

and variation in the process have been examined extensively by hundreds of laboratories around the world for generations.

Research efforts were made to clarify and to better understand the characteristics of the suspension of the solids (sugars, cocoa solids, and cocoa butter particles) in the molten liquid continuous phase during the process. Hundreds of samples have been analyzed from every possible stage of the crystallization process and within the solid matrix upon cooling (supersaturation, nucleation, growth, and post-crystallization and aging). Many techniques were applied including melting behavior, microscopic observations, solid fat content (SFC), Infrared molecular vibrations, NMR, etc .

In spite of all these efforts, many open questions remain unsolved and many are still in their infancy stage. The controversy on the total mechanism of the fat and chocolate behavior remains part of the scientists' work, there are still quite obscure issues that need additional extensive research work.

In this short chapter, we will stress some of the major issues and technical gaps to be filled through future research.

In many of the studies, scientists deal with confectionary blends of fats together with cocoa butter—or even without it—and these mixtures behave quite differently. Therefore, it is often difficult to deduce much from these studies on the behavior of cocoa butter in pure chocolate. In addition, milk chocolate is quite different in composition from dark chocolate, and the existence of milk solids and milk fat greatly influence the behavior of cocoa butter during the crystallization process. Moreover, today's compositions of chocolate enriched with cocoa solids dictate quite different cocoa butter behavior in the suspension and in the process of its crystallization. Any additional component in the chocolate filling has strong influence on the crystallization process and even more effect during the storage and aging stages. All these complex connections are still quite unclear. Additionally, the push for more healthy food products with reduced sugar levels and minimal saturated fatty acid levels concern most of the population in the modern society. Efforts to replace those ingredients at least in part by other ingredients (e.g., sweeteners, oils) greatly affect cocoa butter crystal behavior. Thus, any generalization and prediction of the behavior of cocoa butter in these complex environments is challenging. Yet a better understanding of the basics on simple chocolate will be very helpful in guiding development of technologies that will facilitate selecting the proper blend of compositions and processes conditions.

Our specific recommendations for future research are concentrated on the behavior of cocoa butter in dark chocolate.

Future Work Recommendations

Chocolate is delightful food that imparts joy and pleasure. To maintain chocolate's delightful properties, it is essential to optimize its texture, mouth feel, mechanical

properties, organoleptic properties, etc. The product must be perfectly understood, balanced, and controlled through every stage of production and distribution.

Chocolate and 'chocolate-like' products (coatings, fillings, etc) exhibit several major problems during shelf life, such as, fat and/or sugar bloom, textural changes, graininess associated with moisture intake, loss of gloss, oxidative instability, and more.

Bloom is the most important phenomenon that needs better understanding and control. In the past, it was concluded fat bloom is related to solid-solid mediated polymorphic transitions of form V to form VI cocoa butter. Efforts were made to identify, characterize, and control this complex polymorphism. Various ways were suggested to crystallize the proper polymorph as a means to prevent, or retard, cocoa butter's transformation to the most stable but undesirable form VI. Form VI was believed to be the primary contributor to graininess and bloom. Several physical controls and many emulsifiers were suggested and shown to achieve this aim. After years of extensive studies, and using new advance analytical and physical methods, it is now clear that the polymorphic transitions are the consequence of the bloom phenomena and not the cause for chocolate bloom.

It seems clear that fat composition (internal composition of SOS, POP vs. SSO and PPO), types of ingredients (sugar, lecithin, etc), and size and shape of the particles, play a significant role in the nucleation and growth stage of the cocoa butter crystals during the cooling. Lack of understanding and control of these variables and the impact of cooling parameters can lead to suboptimal crystallization of the cocoa butter. Uncontrolled nucleation (too slow) and nonuniform crystal growth will lead to nonhomogeneous network structures with an increase of voids that help facilitate migration of liquid oil fractions to the surface of the chocolate. These fractions recrystallize forming an unappealing gray (or white) grainy layer of solid fat (bloom) on the surface of the chocolate. It seems the bloom can occur on chocolate in the preferred form V. Although formation of form V cocoa butter is generally an indication chocolate was produced under ideal conditions (optimum number of crystals, shape, melt profile, etc), it is not a guarantee the proper microstructure or homogeneous network structures were achieved.

It is now postulated that chocolate with no (or minimum) bloom can be manufactured even with polymorph VI cocoa butter provided temperatures during conching and tempering are tightly controlled. Much work remains to prove this new hypothesis and working model. It is our hope that with the use of new and advanced techniques it will be possible, in the future, to verify these concepts and to make chocolate without any bloom (or with minimum bloom).

The controversy between those that relate chocolate to polymorphism transformations and those that claim that it is the outcome of uncontrolled crystallization protocol still must be solved. Bloom occurs in many forms and many causes. The number of possible mechanisms are limited only by our imagination.

Another very important future task is to control the texture of the chocolate to optimize the properties of cocoa butter blends in fillings and coatings composed of cocoa butter and other compounded fats. Texture and organoleptics are strongly dictated by the growth stage of the fat crystals. Crystallization rules and parameters must be applied to improve crystals network formation exhibited in texture of the final products. This is very important for small manufacturers that utilize a significant portion of rework in their compounded chocolates, coatings, and fillings. It is important to be able to change the crystallization conditions that can tolerate additional melting and resolidification. Maintaining consistent flow properties after melting and resolidification will require, in most cases, addition of small amounts of additives.

Many of the "chocolate-like" products for confectionary applications are based on complex mixtures of cocoa butter and other fats such as milk fat, CBS, CBE, and/or palm kernel and hydrogenated palm kernel fats. These binary, tertiary (and greater) blends can form at certain compositions (ratios), under specific conditions, eutectic systems with unique properties (lower melting and slower crystallization behavior), and different physical characteristics (mechanical properties). And some blends may be totally incompatible, forming separated phases with unpredictable and inconsistent crystallization behaviors and subsequent physical properties. It is essential to work on understanding the structure correlations of fat mixtures to the eutectic formation and to distinguish them from diluted solid mixtures. Based on these properties it will be easier to design fats to meet specific properties.

It is well documented that lecithin and other emulsifiers play a very significant role in determining the rheology properties of molten chocolate and in controlling other aspects such as nucleation rates. Lecithin is known to control and reduce plastic Cason viscosity and yield stress by coating sugar particles. The correlations between crystal sugar particles size, amount of sugar, and cocoa butter and the ratio between them is documented, but other properties related to blends of fats and internal fat compositions remain to be illustrated.

It is essential to design more sophisticated experiments to correlate the structure of lecithin to its activity and to better understand why excess lecithin is increasing yield stress.

Synergism between lecithin and other emulsifiers is also important in controlling physical properties of the chocolate. Very little significant work was carried out in understanding the difference in the activity (yields stress and plastic viscosity) of PGPR, Sorbian esters, YN, and lecithin. Very little is known on the uniqueness of each emulsifier in relation to the nucleation and growth rates (crystals shape and size). Even less is known on possible synergism or antagonism of these emulsifiers. New sources of lecithin from sunflower, canola, and corn have been recently introduced to replace soy lecithin. It is important to study and understand the prerequisites of the headgroups and tails of the lecithins to their activity in adsorption on sugar particles,

cocoa powder particles, and in the continuous bulk. So these new sources of lecithin can be optimized for commercial purposes.

The search for other additives (monoglyceride lactate and monoglyceride citrate) that provide gloss, mechanical properties, and other rheological properties are in progress and will have to be further studied.

It seems that better quality and healthier chocolate can be manufactured by incorporation of nutritional additives into the chocolate matrix of the cocoa butter. Vitamins, nutraceuticals, etc., can be now introduced into the chocolate without causing any severe taste and organoleptic alternations. The use of novel natural carriers will facilitate such improvements.

And finally, chocolate is very sensitive to moisture, oxidation reactions, flavor loss, etc. Work must be done to guide manufactures on ways to minimize these phenomena.

In conclusion, although much is known about cocoa butter and chocolate we have still long way to go until this complex yet simple product will be fully understood and optimized.

Contributors

Abraham Aserin

Dr. Aserin is a researcher at the Casali Institute of Applied Chemistry, The Institute of Chemistry, at The Hebrew University of Jerusalem. His research is focused on many practical facets of surfactant chemistry such as emulsions, microemulsions, lyotropic liquid crystals-preparation and characterization, crystallization phenomena in liquid crystalline mesophases and microemulsions, pharmaceutical formulations and advanced drug delivery vehicles dispersions, and improvement of drug bioavailability. He received a B.Sc., M.Sc., and Ph.D. degrees from The Hebrew University of Jerusalem, Israel, in 1978, 1981 and 1987 respectively. Dr. Aserin is the author of more than 160 peer-reviewed scientific articles, 40 reviews, and 2 edited books, and he holds 12 patents. He has educated and tutored 50 M.Sc. and 15 Ph.D. students. He is the co-founder of NutraLease Ltd. Aserin was awarded the *Lester Aronberg Prize* outstanding post-doctoral achievements (1988), the *Velacci Pikowski Prize* (1989) from the Faculty of Science, and the *1st Prize Kaye* of The Hebrew University of Jerusalem on inventions and innovations, "Development of Self-Assembled Nanosized Liquids (NSSL) for Solubilization of Nutraceuticals," 2003.



Rodrigo Campos

Dr. Rodrigo Campos received his Bachelor's degree in Industrial Food Engineering at Monterrey's Tech-Campus Queretaro (Mexico) before relocating to Canada to continue his studies. At the University of Guelph, his graduate research focused on the crystallization and physical properties of edible fats under the supervision of A. G.



Marangoni. He completed his Ph.D. in 2006 on the effects of processing conditions on the crystallization of cocoa butter. While at the University of Guelph, Dr. Campos was a teaching assistant and lecturer for the undergraduate food chemistry curricula. He began his career with Mars Chocolate North America in 2004, accepting a post-doctoral research position to work on the structure-function relationships of confectionary fats. In the ensuing years, he has continued to cultivate his role within Mars and held a variety of research scientist positions. These positions have enabled him to provide analytical support to product development and conduct research on raw materials with a focus on physical chemistry of fats and oils. Currently, Campos is working on cocoa technology, conducting research in the United States and at origin countries around the world.



Miriam Charó-Alonso

Miriam Charó-Alonso holds a B.S. in food engineering from the Autonomous University of San Luis Potosi (UASLP), and a M.Sc. in quality engineering and productivity from the Technological Institute of Superior Studies of Monterrey (ITESM, 2006). She is currently working as an associated professor in the Department of Chemistry at UASLP, with part time assignment at the Food Physicochemistry laboratory, where she is involved in consulting projects for the food industry and provides support in experimental design and statistical analysis. Charó-Alonso is a member of the American Oil Chemists' Society, and the Institute of Food Technology. She is the coauthor of several papers and book chapters involving triacylglyceride crystallization.



Nathalie De Clercq

Nathalie De Clercq graduated as a bio-engineer chemistry at Ghent University in 2004. In the same year, she started her Ph.D. entitled "Changing the functionality of cocoa butter" at the Laboratory of Food Technology and Engineering in collaboration with Barry Callebaut. After the Ph.D. defense in September 2011 she became a postdoctoral assistant and she's now responsible for the chocolate research at UGent Cacaolab.

Koen Dewettinck

Prof. Dr. Ir. Koen Dewettinck is head of the laboratory of Food Technology and Engineering at Ghent University. Together with his research team he tries to gain insight into the microstructural development of mainly fat-rich foods. This has led to four major research pillars: dairy products, oils and fats, chocolate and confectionery products and the innovative design of microstructures. From 2009 on, the chocolate-related research was extended with the UGent Cacaolab. Professor Dewettinck participates in various international organizations concerning microstructural research and organizes different conferences in the food technology area.



Nissim Garti

Nissim Garti is Professor of Chemistry at The Hebrew University of Jerusalem. One of the founders of Adumim Chemicals Ltd., NutraLease Ltd.—a company focused on a nano-encapsulation technology for nutraceuticals, and LDS (Lyotropic Delivery Systems). He received B.Sc., M.Sc. and Ph.D. degrees from The Hebrew University of Jerusalem, Israel, in 1969, 1971 and 1974 respectively. Garti was awarded: Life Time Achievement of the Israeli Association for Food Research and Technology, Tel-Aviv, 2009; The Chung Scientific Award of the AOCS for Outstanding Scientific and Technology Achievements, Orlando, 2009; The Most Innovative Israeli Nanotechnology Award Winner of the CMNC Society, “Food Goes Nano-Liquid Nano Vehicles for Nutraceuticals solubilization and delivery,” Chicago, USA, 2005; The Japanese Award for the Promotion of Senior Foreign Scientists, Hiroshima University, Hiroshima, Japan, 2003; The Best Invention and Innovation of The Hebrew University of Jerusalem 1997; The Most Innovative Food Ingredient Award in Europe (FIE), London, 1997; and The Japan Oil Chemical Society Forum Award for Outstanding Achievement, “Polymorphism in Fats,” Nara, Japan, 1997. Professor Garti is the author of more than 400 publications and holds over 70 patents.



Richard W. Hartel

Richard W. Hartel, Ph.D. has been a Professor of Food Engineering with the Department of Food Science at the University of Wisconsin-Madison since 1986. After receiving a Ph.D. degree in Agricultural and Chemical Engineering at Colorado State University, Dr. Hartel spent



four years as a Research Chemical Engineer at Eastman Kodak Co. before joining the faculty at UW-Madison. His current research interests revolve around various phase transitions in foods. Specific research projects include structure development during manufacture and storage of frozen desserts, the physical chemistry of lipid mixtures, lipid crystallization in foods, bloom formation in chocolates/coatings, sugar crystallization, and drying of whey products.

Dr. Hartel teaches courses in career preparation, food manufacturing, food preservation, food functionality, and candy science.



Sheida Kadivar

Sheida Kadivar obtained a Master's degree in Food Science and Technology at the Tehran Azad University of Research and Science in 2009. In 2010, she started her PhD research entitled Enzymatic production of cocoa butter equivalent at the Laboratory of Food Technology and Engineering.



Daniel Kalnin

Daniel Kalnin studied chemistry at the University of Ulm, and food chemistry at the Technical University of Dresden. He then enrolled in a Ph.D. program with Dr. Michel Ollivon at UMR CNRS 8612 in Chateaufort, France. There he treated lipid crystallization of vegetable and animal fats, mostly using DSC and X-ray diffraction and their coupling for the sake of elucidating thermal and structural behavior of fats in ice-cream. While his Ph.D. involved Nestle, he collaborated with Unilever during his first Post Doc in the Food Physics Group in Wageningen. There he worked in the field of margarine structuring without TAGs. After a year, Dr. Kalnin returned to UMR CNRS 8612 for a French national project dealing with unsaturated fatty acid enriched milk. He then spent three years as a project manager of lipid structures at YKI, the institute for surface chemistry in Stockholm, Sweden, where he consulted for projects involving physical chemistry of colloids or lipid structures in the field of food and pharmaceuticals.

Dr. Kalnin's project portfolio includes dealing with projects of Kraft, PepsiCo, and other global enterprises, as well as the scientific leadership of a work package in the EU project Propalaine, which dealt with migration bloom elimination in filled con-

fectionary. Since September 2011, Dr. Kalnin works at the division for surface and corrosion science at KTH Royal Institute of Technology as invited scientist involved in scattering projects. Dr Kalnin has written more than twelve peer-reviewed journal articles in the field of lipid structures and has authored two book chapters.

Catherine Kwik-Uribe

Catherine Kwik-Uribe is the Director of Research and Development for Mars Botanical, a scientific division of Mars, Incorporated. She received her Ph.D. in the field of nutrition from the University of California, Davis, and spent several years as a post-doctoral fellow at the University of California, Santa Cruz in the Department of Toxicology.

In 2002, Dr. Kwik-Uribe joined Mars, Inc. Since then, Dr. Kwik-Uribe has managed various aspects of a research program which focuses on the development of analytical methods that support the characterization of the phytonutrient composition of cocoa, specifically a class of components known as Cocoa Flavanols, as well as the examination of role of these compounds in supporting human health. Much of the health research focus in recent years has been the potential role of cocoa flavanols in maintaining and improving cardiovascular health. Dr. Kwik-Uribe has co-authored numerous papers focusing on the analysis and benefits of cocoa flavanols. In Mars Botanical, she leads a team that focuses on continuing to examine the chemical and health-promoting properties of cocoa flavanols, and translating this knowledge into the development of new products that can help support human health.

Adam Lechter

Adam Lechter is Product Services and Development Manager for ADM Cocoa, helping customers in North America with new product development and technical services related to chocolate, confectionery coatings, and cocoa powder. He is an active member in the following industry groups: AACT, NCA's scientific and regulatory committees, AOCS, and RCI.

Adam received his Associate's of Occupational Studies from The Culinary Institute of America in Hyde Park, New York, where he was awarded the L'Académie Brillat-Savarin Young Professional Medal of Merit. Adam received his Bachelor's degree from the University of Wisconsin-Madison, majoring in Food Science. He is currently pursuing a Master's Degree in Food Science from the University of Wisconsin-Madison.





Alejandro Marangoni

Alejandro Marangoni is a professor and Canada Research Chair in Food and Soft Materials Science in the department of Food Science at the University of Guelph. His work concentrates on the materials science of foods, particularly fat crystallization and structure, as well as the relationship between food structure and physiological response in humans. His work continues to focus on establishing quantitative relationships between macroscopic functionality and the appropriate length scale within a material.

His current research focuses on food nanostructure, better understanding the nature of van der Waals' forces at the nanoscale, and polymer gelation of anhydrous aliphatic oils.



Stéphanie Marty-Terrade

Stéphanie Marty-Terrade obtained her M.Sc. in Food Science and Technology from the University of Bordeaux (France) in 2003, with a specialization in fats and oils at ITERG. She then pursued her studies at the University of Guelph (Ontario, Canada), within Pr. Alejandro G. Marangoni's laboratory, obtaining her Ph.D. in Food Science in 2009. Dr. Marty-Terrade has been a member of the AOCS since 2004, and was the recipient of the AOCS Honored Student Award in 2006.

From 2007 to 2010, Dr. Marty-Terrade worked within Nestlé at the Product Technology Center in Singen (Germany) as Project Manager and Lipid Specialist. In that role, her fundamental and practical expertise was crucial in developing specialty oils for the culinary business.

She is now Quality and Sustainable Development Manager for a grain company based in Poitiers (France) which produces, among other traditional cereal grains, oil seeds with specific technological and nutritional properties. In her new role, she is notably responsible for developing projects for international food companies as well as projects to implement best practice in social responsibility.

Juan Angel Morales-Rueda

Juan Angel Morales-Rueda has a Ph.D. in Food Science from PROPAC-Autonomous University of Queretaro (2007). He is currently holding a postdoctoral position at the Food Physicochemistry laboratory, Department of Chemistry at the Autonomous University of San Luis Potosí, Mexico. His research interests include the development of organogels using vegetable oil as the liquid phase, and assessment of thermo-mechanical properties of lipid-based edible systems.



Tuyet Mai Nguyen

Tuyet Mai Nguyen obtained her bachelor degree from Ho Chi Minh University of Technology in 2005. In 2009, she obtained her master degree at Ghent University. In 2010, she started working in the laboratory of Food Technology and Engineering, currently on a project entitled “Controlling the Flow Behavior of Chocolate Using Natural Ingredients.”



J. David Perez-Martinez

J. David Perez-Martinez received his Ph.D. in Food Science from PROPAC-Autonomous University of Queretaro in 2007, and is currently working as an associated professor in the Department of Chemistry at the Autonomous University of San Luis Potosi, Mexico. His scientific interests include the relationship between lipid structure-functionality and how processing conditions affect this relationship. He is the coauthor of several papers investigating the effect of supercooling, cooling rate and shearing on the microstructure and physical properties of crystallized lipid systems, mainly cacao butter and its blends with vegetable oil. His current research is associated with the development of *trans*-free multi-phase systems based on organogelation.



Kiyotaka Sato

Kyo Sato retired in 2010 from Laboratory of Food Biophysics, Hiroshima University, where he published over 220 research articles and reviews, and 41 book chapters in the area of lipid crystallography and crystallization. He graduated from Graduate School of Engineering, Nagoya University in 1974, and joined Hiroshima University. Kyo



has been an AOCS member since 1985 and received the Stephen S. Chang Award and Alton E. Bailey Award, along with many other awards from various scientific organizations. He is still active in scientific research, having worked on consultancy in the technical areas of lipid crystallization.

Orit Segman

Orit Segman is a veterinarian specializing in food security and public health. She also holds a B.Sc. Agr., specializing in animal science and M.Sc. in food science and technology, with emphasis on public health and bioactive components. Currently she is collaborating with Professor Zeev Wiesman on various research projects in the phytolipid biotechnology laboratory in the E.D. Bergman institutes for applied research of the Ben Gurion University of the Negev, Israel.

Serpil Metin



Serpil Metin currently leads the Food Material Science group within the Global Food Research of Cargill Incorporated. The group investigates structure/function understanding of food ingredients, including effects of processing steps, and shelf life on desired food structure. Her research interests include food material science and physical chemistry food products, especially in the areas of fat and sugar crystallization. She received her B.Sc. and M.Sc. degrees in Food Engineering, Hacettepe University, Turkey, and Ph.D. degree in Food Science, the University of Wisconsin-Madison. She worked at the Research

and Development Group of M&M/Mars prior to joining Cargill Incorporated. She has published several papers on fat crystallization, and coauthored chapters in four books; *Fat Crystallization in Bailey's Oils and Fat Handbook Volume VI*, *Nutritional Edible Oil Processing*, *Healthful Lipids*, and this current one, *Cocoa Butter and Related Compounds*.



Kevin W. Smith

Kevin Smith spent more than 30 years researching oils and fats with Unilever Research Colworth before starting Fat Science Consulting. He joined Unilever in 1980, from Bath University, beginning work in the Speciality Fats section, investigating the fractionation of palm oil to produce cocoa butter equivalents. This expanded to studying the phase behavior of pure triacylglycerols and to the investigation of DSC as a quality control tool. Other areas included studies

on partially hydrogenated fats, lauric fats, low calorie fat systems, fat migration, bloom retarding fats, non-temper filling fats and lipid-based moisture barriers using techniques such as pulse NMR, Differential Scanning Calorimetry, microscopy (cold-stage and temperature gradient) and x-ray diffraction. He has been involved in projects on chocolate, edible oils, ice cream, baked goods and spreads. He is the Immediate Past Chairman of the Lipids Group of the Society of Chemical Industry and a past board member of the European Federation for Lipid Science and Technology. He is an associate editor of the SCI's *Journal of the Science of Food & Agriculture*.

Lina Svanberg

Lina Svanberg has a M.Sc. in bioengineering at Chalmers University of Technology, Gothenburg, Sweden. She is currently conducting a joint Ph.D. at SIK—the Swedish Institute for Food and Biotechnology and ETH—Swiss Federal Institute of Technology, and will present her thesis in March 2012. Through her research on the process-structure relationship in chocolate confectioneries she has published work on the structure formation and development during storage of cocoa butter as a function of pre-crystallization process and solid particles addition. In addition, she has also developed new methodologies to measure mechanical properties in chocolate and measure the impact of fat or moisture migration.



Geoff Talbot

Geoff Talbot has worked directly or indirectly in the oils and fats industry for over 40 years, much of his time in either Unilever Research or Loders Croklaan. He has been responsible for research and development of speciality fats for use in a wide range of food products, notably fats to replace cocoa butter in coatings and fillings, fats to prevent bloom formation in chocolate, and fats to inhibit moisture migration in composite food systems.

In 2003 Geoff started his own consultancy group, The Fat Consultant. In this capacity he is able to help, advise, and train in all aspects of fats and oils technology and their application in food. He focuses mainly on the applications of oils and fats in food, with special emphasis on getting the functionality of the fat phase correct to achieve particular attributes such as structure and texture, improved shelf-life, and improved nutritional characteristics.

He has worked extensively with clients to define ways of replacing hydrogenated vegetable oils in products to reduce the level of *trans* fatty acids. More recently he



has helped clients reduce the level of saturated fats in their products. He authored a report on possible ways of reducing saturated fats in food, commissioned by the U.K. Food Standards Agency and helped them to define the current position on the level of inclusion of *trans* fatty acids in U.K. food products. He is the author of many articles, papers and book chapters, as well as of the book *Application of Fats in Confectionery*. He has edited two other books—*Science and Technology of enrobed and filled chocolate, confectionery and bakery products*, published in 2010, and one on *Reducing Saturated Fats in Foods*, published in 2011. Since May 2009 he has been a Visiting Research Fellow in the School of Life Sciences at Oxford Brookes University, U.K.



Jorge Fernando Toro-Vazquez

Jorge Fernando Toro-Vazquez, Ph.D. in Food Chemistry from Cornell University, Department of Food Science (1989), is currently working as a Full Professor in the Department of Chemistry, Laboratory of Physical and Chemical Properties of Food at the Autonomous University of San Luis Potosi, UASLP, San Luis Potosi, Mexico.

He has published more than eighty peer-reviewed papers and book chapters, mainly in chemical and physical properties/behavior of food using simple and complex model

systems. His current research projects are associated with phase transitions and structural organization of lipids, their application in vegetable-oil processing and use for developing novel lipid-based trans-free food products. Dr. Toro-Vazquez is an associated editor of the Journal of the American Oil Chemist's Society, reviewer of several scientific journals, and a member of the Mexican Academy of Sciences, the American Oil Chemists Society, and the Institute of Food Technology. The National Council for Science and Technology has twice awarded Dr. Toro-Vazquez with the National Award in Food Science and Technology of Mexico (CONACYT) and is recognized with the highest level (SNI-III) as a National Researcher by CONACYT and the Mexican Ministry of Education.



César Vega

César Vega, a native of Mexico, earned his university degree in Food Engineering (1998) from the Monterrey Institute of Technology in Mexico, his M.Sc. in Food Science (2003) from the University of Guelph in Canada, and his Ph.D. in Food Science (2006) from the University College Cork in Ireland. His areas of expertise are dairy emulsion systems, such as ice cream spray-dried milk products, and lately the physical chemistry of cocoa and

cocoa products, particularly cocoa flavanols. He has published over 20 manuscripts in peer-reviewed journals.

His professional experience includes Procter & Gamble, and Dippin' Dots, Inc. Today he is a research manager at Mars Botanical, a scientific division of Mars, Inc., where he leads the team in charge of incorporating cocoa flavanols into foods, as well as of elucidating the molecular mechanisms that drive the interactions of these compounds within food systems. He also provides guidance to Mars, Inc. in aspects related to food material science.

Dr. Vega is also a passionate cook. He obtained culinary training from Le Cordon Bleu Culinary Arts Institute (2001) and has since been deeply involved in all aspects related to the science of cooking. He was one the expert reviewers in Myrhvold's *Modernist Cuisine*. He is member of the editorial board of the *International Journal of Gastronomy and Food Science* (Elsevier) and he is editor-in-chief and contributor to *The Kitchen as Laboratory: Reflections on the Science of Food & Cooking* (Columbia University Press, 2012).

Stefanie Verstringe

Stefanie Verstringe graduated as a bio-engineer food technology at Ghent University in 2010. In the same year, she started her Ph.D. as a research assistant of the Fund for Scientific Research–Flanders (F.W.O. – Vlaanderen) entitled Influence of partial glycerides on the microstructural development and breakdown of crystallized fats at the laboratory of Food Technology and Engineering.



Zeev Wiesman

Zeev Wiesman is an Associate Professor in the Department of Biotechnology Engineering, Head of Energy Engineering Unit, Faculty of Engineering Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel. He received his university degrees from The Hebrew University of Jerusalem, Faculty of Agriculture, Department of Horticulture (1979–1989). His postdoctoral position was in Plant Molecular Biology Laboratory USDA, Beltsville, Maryland, USA (1990–1992). Professor Wiesman is an expert in plant lipid biotechnologies with emphasis to biofuels, bioenergy and food industries. He was a working package leader for EU project, “Shea butter oil analyses.” The working group includes Oil Technology Institute, Pescara, Italy and Aarhus Oilefabrik A/S, Aarhus, Denmark (1999–2003). Professor Wiesman published a book entitled *Desert Olive Oil Cultivation: Advanced Biotechnologies* (Elsevier, 2009), more than 100 scientific manuscripts, chapters in books and patents.





Neil R. Widlak

Neil is currently the Director of Product Service and Development for ADM Cocoa in North America. Neil started his exploration into the wonderful world of fat crystallization after receiving his B.S. in Food Science from the University of Illinois at Champaign-Urbana, and working for Kraft Foods as a scientist in Kraft's shortening and margarine research labs. Neil earned a Master's in Food Science from the University of Illinois and Masters in Business Administration from Lake Forest Graduate School of Management, while continuing to work at Kraft. During

this time Neil became active in the AOCS, serving as a board member for the North Central Section, Edible Applications Division and as a Member at Large, AOCS Governing Board. Neil chaired numerous AOCS symposia on crystallization of lipids for annual meetings, in addition to chairing three AOCS international conferences on the physical properties of fats and oils in 1997, 2000 and 2009. Neil received the AOCS Fellow Award in 2011.

Leonid Yarmolinsky

Leonid Yarmolinsky is a chemist whose work emphasizes organic chemistry and physical chemistry of complex heterogenic systems. He holds an M.Sc. in organic chemistry awarded in 1985 from the former Soviet Union and also an M.Sc. in desert researches. His Ph.D. is in progress in Ben Gurion University of the Negev, Beer Sheva, Israel. Leonid Yarmolinsky dealt with synthetic fuels (1985–1988) while in the U.S.S.R. He researched changes of chemical properties and characteristics of soils and plants under technogenic stress conditions in the Biology Institute of Dniepropetrovsk State University, Ukraine. Leonid Yarmolinsky worked as a researcher in MS Teck and Catalyteck and as a scientific consultant in the ZET project in Israel. Currently he is collaborating with Professor Zeev Wiesman on various research projects.

INDEX

<u>Index Terms</u>	<u>Links</u>
A	
Acyl donors, cocoa butter alternatives	448
Africa	
map	421
shea butter	417
shea butter regional variation	423
Alkalization	
cocoa	41
cocoa beans by origin	252
Alkaloids, cocoa	47
<i>Alpha</i> -tocopherol, vitamin E	423
Ammonium phosphatide	8
Analytical tools	
evaporative light scattering detector	
(ELSD)	428
gas chromatography (GC)	429
high resolution gas chromatography	
(HR-GC)	431
high-temperature capillary gas	
chromatography (HT-GC)	431
negative ion CI-MS (tandem MS)	431
nuclear magnetic resonance (NMR)	431
pyrolysis-mass spectrometry (Py-MS)	430

Index Terms

Links

Analytical tools (*Cont.*)

reverse phase high performance liquid
chromatography (RP-HPLC) 427

Antioxidant function, flavanols and
procyanidins 56

Aspergillus niger lipase 452 453

Atomic force microscopy (AFM) 38

chocolate bloom 387

chocolate wafer surface 313

cocoa butter fat crystal growth 312

B

Baking chocolate, composition 45

Beef tallow 455 459

Blending, cocoa butter alternatives 461 462

Blending and supply tanks, bloom 186

Blood pressure, flavanols/procyanidins 55

Bloom. *See also* Chocolate fat bloom

blending and supply tanks 186

causes 181

chocolate fat phase 29

composition 182

compositional analysis 179

definition and characterization 174

depositing 188

distribution and storage 190

fats retarding 492

future work 499

incompatible fats 183

Index Terms

Links

Bloom (*Cont.*)

ingredients	185	86
inhibition strategies	492	
oil migration	192	
packaging	190	
processing	186	
static cooling/solidification	189	
storage and cycling conditions	178	
temperature	190	
tempering/dynamic cooling	187	88

Bloom retarding fats

compatibility with cocoa butter	493
composition and polymorphism	493
inhibition strategies	492

Borneo tallow 19

Bragg-Williams approximation, mixing 79

Brazil (Bahia), cocoa butters	214	226	260	266
	268			

C

Cacao and cocoa 36

fermentation 38

harvesting 38

Caffeine, alkaloid 48

Calorimetry. *See* Differential scanning

calorimetry (DSC)

Camel hump fat 458

Candida antarctica lipase 459

Candida rugosa lipase 459

<u>Index Terms</u>	<u>Links</u>			
Canola oil				
cocoa butter-vegetable oil blends	403	408	410	
triacylglyceride concentration	398			
Cardiovascular health, flavanols and				
procyanidins	52			
<i>Carica papaya</i> lipase	452	460		
<i>Caryocar brasiliense</i>	455			
Casson viscosity, lecithin concentration	286			
Catechins	64			
Chain length mismatch (CLM)				
comparing symmetrical/asymmetrical				
binary systems	94			
CSC-CCS with CLM =8	91	92		
LSL-LLS with CLM = 6	89	91		
MSM-MMS with CLM = 4	88			
PSP-PPS with CLM =2	86			
triacylglycerols (TAGS)	74	77		
Chain length packing	22			
Chemical composition				
cocoa	45			
shea butter	420			
China, cocoa butter	260	262	266	268
Chocolate	275			
bloom	29			
cocoa processing	3			
composition and structure	1	25		
cooling profile	277			
fat phase composition	25			
future work recommendations	498			
hardness	27			

Index Terms

Links

Chocolate (*Cont.*)

industrial application	425	
legislative constraints	11	
manufacturing process	277	
milk fat in	384	
production	5	276
recipes	80	
rheology	25	278
sensory properties	29	
tempering	25	296
texture	500	
vegetable oils for low- <i>trans</i> or <i>trans</i> -free	395	

Chocolate fat bloom

characteristics by morphology	210	
confectionery industry	195	
dynamics of test	300	
milk fat	386	
type 1	196	
type 2	200	
type 2-A	203	
type 2-B	204	
type 3	206	

Chocolate industry, tempering	6	269

Chocolate liquor bloom	175	

CoCal1	467	

CoCal2	467	

Cocoa

chemical composition and health	44	
cleaning, breaking and winnowing	41	

Index Terms

Links

Cocoa (*Cont.*)

cocoa butter	44	
cocoa cake	43	
cocoa powder	43	44
industrial processing	41	
nib grinding	43	
pressing	43	
roasting	42	
sterilization and alkalization	41	

Cocoa beans

composition	4	5
fermentation	38	
global production	37	
variation by origin	251	

Cocoa butter

bloom	185		
chocolate composition and structure	25		
cocoa butter equivalents vs.	18		
commercial perspective	497		
composition	12	247	276
composition and physical properties	20		
country of origin	251		
crystallization schematic	145		
effect of environment and agronomic			
practices on composition	247		
extraction and refining	253		
fatty acid and triglyceride variation	13	248	
free fatty acids	18		
future work recommendations	498		
industrial chocolate application	425		

Index Terms

Links

Cocoa butter (*Cont.*)

minor components	16	393
minor triglycerides	16	
oil migration and properties	269	
partial glycerides	17	
phospholipids	18	
polymorphs	394	
processing	44	249 395
research	497	
sorbitan esters	297	
structure and polymorphism	22	
tryacylglyceride concentration	398	
unsaponifiable matter	18	

Cocoa butter alternatives (CBAs)

	433	475
acyl donors	448	
alternative substrates	454	
blending	461	462
classification	444	
comparing fatty acids/fatty acid esters and TAGs	459	
detection techniques	466	
enzymatic interesterification	446	52
enzyme-catalyzed synthesis,	450	
fatty acids and fatty acid esters	448	
fractionation	464	5
hydrogenation	464	
melting profiles	476	
microbial lipids	466	

<u>Index Terms</u>	<u>Links</u>			
Cocoa butter alternatives (CBAs) (<i>Cont.</i>)				
modification techniques for production	461			
production	446			
substrates for enzyme-catalyzed				
production	447	450	451	452
supercritical carbon dioxide in				
production	460			
TAGs as acyl donors	456			
Cocoa butter crystallization	255	379		
fatty acid and TAG and effect on				
properties	259	261		
isolated crystalline phase	122			
kinetics by origin	258	260		
lecithin	237			
microscopy techniques	236	237		
milk fat	381			
milk fat and cocoa butter blends	377			
milk powder	239			
minor components	380			
network formation	255			
noncocoa ingredients	233	241		
origin of cocoa butter	255	258	260	
process	255			
schematic of cocoa butter	145			
sugar with or without emulsifier	234			
temperatures for polymorphs	330			
Cocoa butter equivalents (CBEs)	18	425	426	443
compatibility with cocoa butter	487			
component fats	484			
composition and polymorphism	484			

This page has been reformatted by Knovel to provide easier navigation.

Index Terms

Links

Cocoa butter equivalents (CBEs) (*Cont.*)

POP/POST/StOSt ternary phase

behavior 486

processing differences 488

ternary diagram 485

triacylglycerol composition 485

triacylglycerols as acyl donors 457

Cocoa butter extenders (CBEXs) 443

Cocoa butter improvers (CBIs) 426 443

Cocoa butter replacers (CBRs)

compatibility with cocoa butter 489

composition and polymorphism 488

low-trans 489

processing differences 490

Cocoa butter substitutes (CBSs) 443

bloom 185

compatibility with cocoa butter 491

composition and polymorphism 491

processing differences 492

Cocoa butter-vegetable oil blends

Avrami index 410 411

crystallization rate constant 410 411

fractal measurement 410 411

isothermal crystallization thermograms 404 405

liquid structural organization of TAG

in 397

phase behavior 396

phase shift angle rheograms 400

polarized light microphotographs 408 409

schematic of crystallization 406

This page has been reformatted by Knovel to provide easier navigation.

Index Terms

Links

Cocoa butter-vegetable oil blends (*Cont.*)

solid fat content (SFC)	410	411	
supercooling and cooling rate effect	403	406	411

Cocoa cake

43

Cocoa pods

harvesting	38
maturation time	250
processing	3

Cocoa powder

chocolate	2	
processing	43	44

Cocoa processing

3

Code of Federal Regulations (CFR)

11

Codex Alimentarius

chocolate legislation	12	13
standard for fat	4	
vegetable oil/fats	426	446

Colorimetry

313

Conching

chocolate processing	6
cocoa beans by origin	252

Confectionery fats

475

bloom retarding fats	492	
cocoa butter equivalents (CBEs)	475	484
cocoa butter replacers (CBRs)	475	488
cocoa butter substitutes (CBSs)	475	491
factors in selection	494	

filling fats, filled products and

compatibility	493

fractionation

477

Index Terms

Links

Confectionery fats (<i>Cont.</i>)				
hydrogenation	480			
interesterification	482			
melting points ofTAGs in	477			
principal fats	475	476		
Confections. <i>See also</i> Bloom				
attributes of confectionery fats	180			
wafers with bloom	174			
Confocal laser scanning microscopy (CLSM)				
cocoa butter	228	229	236	237
	238			
microstructure of chocolate	315	318		
Confocal Raman microscopy	317	319		
Cooling, chocolate processing	7			
Cooling rate, cocoa butter-vegetable oil blends	403	406	411	
Cotyledons	39	40		
Country of origin, cocoa butter	251			
Criollo cocoa	36			
<i>Cryptococcus curvatus</i> ATCC 20509	466			
Crystal forms, cocoa butter	23	24		
Crystallization kinetics. <i>See also</i> Cocoa butter crystallization				
cocoa butter origin	258	260		
cocoa butter with milk fat	381			
differential scanning calorimetry (DSC)	108	322		
diglycerides	221	229		
free fatty acids in cocoa butter	215			

Index Terms

Links

Crystallization kinetics (*Cont.*)

phospholipids in cocoa butter	224	
shearwork	108	111
sterol and sterol esters in cocoa butter	226	
total minor lipids in cocoa butter	227	229
triacylglycerols (TAGS)	74	

Crystal sizdshape

cocoa butter polymorphs	294
confectionary fats	180

Crystal structure, cocoa butter

CSC-CCS binary mixtures	83	85	91	92

D

Deodorization

cocoa butter	253	254
free fatty acids	215	

Depositing, bloom

Diacyl-glycerides (DAGs)

Dietary Guidelines Advisory Committee

(2010)	46

Differential scanning calorimetry (DSC)

cocoa butter crystallization	218	223		
cocoa butter polymorphs	164	165	167	168
	294			
crystallization	322			
crystallization kinetics	108	322		
profile of chocolate	321			
thermal properties of cocoa butters	109			

Diglycerides, crystallization

<u>Index Terms</u>	<u>Links</u>			
Diphenylpropane structures	49			
Distribution, bloom	190			
Dry fractionation, milk fat	371			
E				
Economics, confectionary fats	181			
Ecuador, cocoa butter	214	260	262	266
	268			
Elastic bending modulus (E_B)	110	116	139	141
Emulsifiers				
ammonium salts of phosphatidic acids				
(YN)	292			
chocolate recipes	8			
cocoa butter crystallization	234			
glycerol monostearate	292			
lecithins	282			
monoglyceride lactates	299			
polyglycerol polyricinoleate (PGPR)	8	288		
polymorphism	293			
sorbitan esters	297			
synergism of lecithin and	500			
Emulsion				
oil-in-water	351	353	356	357
water-in-oil	355	357		
Endothelial function, flavanols/				
procyanidins	53			
Energy dispersive X-ray spectroscopy				
(EDS)	321			
Enrobing, chocolate processing	7			

<u>Index Terms</u>	<u>Links</u>			
Enzymatic interesterification (EIE), cocoa butter alternatives (CBAs)	446			
Epicatechins	64			
Epidemiological studies	50			
EU Directive 2000/36/EC	11	426	427	445
	466			
European Prospective Incidence into Cancer and Nutrition (EPIC) trial	51			
European Union (EU)				
chocolate legislation	11			
regulations	445			
Evaporative light scattering detector (ELSD)	324			
Extraction, cocoa butter	253			
F				
Family milk chocolate	9			
Fat. <i>See also</i> Confectionery fats				
bloom retarding	492			
chocolate	2	500		
factors in selection	494			
hardness by NMR	432			
microbeam X-ray diffraction (μ -XRD)	340	341		
Fat bloom	195			
<i>See also</i> Bloom				
causes	181			
colorimetry	313			
formation	173			
Fat crystal growth	311	312		

Index Terms

Links

Fat phase composition

bloom resistance	
and formation	29
hardness	27
tempering and rheology	25

Fatty acids

acyl donors for cocoa butter	
alternatives	448
cocoa butter	13
composition of milk fat	366
milk fat fractions	373
positional distribution	276
profiles	106
shea butter vs. competitors	426
triacylglycerols vs.,	
as acyl donors	459
whole cocoa butter and seed	
crystals	17

Fermentation

cocoa beans	38
cocoa beans by origin	252

Fiber, cocoa	46	47

Filled products	493

Filling fats	493

Flavanols	64

antioxidant function	56
blood pressure	55
cardiovascular health	52
endothelial function	53
platelet function	52

Index Terms

Links

Flavonoids	4	64
cocoa	48	
structure and numbering	49	
Food of the Gods	1	57
Forastero cocoa	36	
Formulations,		
chocolate recipes	8	
Fractionation		
cocoa butter alternatives	464	
confectionery fats	477	
milk fat	371	
schematic	478	
supercritical carbon dioxide	465	
Free fatty acids	18	248
Fully hydrogenated		
palm kernel (FHPK)	177	

G

Gas chromatography (GC)			
fatty acid profile	432		
methods	429		
minor lipids	433	434	
Glycerides, partial	17		
Glycerol monostearate (GMS)	292		
Glycosaminoglycans (GAGS)	65	67	68
Glycosidases	39		
Goble, T. N., lecithin	282		
Graph Pad Software Inc.	131		

Index Terms

Links

H

Hardness

chocolate	27
cocoa butter by origin	250

Harvesting,

cocoa pods	38

Health

antioxidant function	56
blood pressure	55
cardiovascular	52
cocoa, chocolate and	50
endothelial function	53
platelet function	52

High-melting seed crystals	17

High performance liquid

chromatography (HPLC)	323

High resolution gas

chromatography (HR-GC)	431	467

High-temperature capillary gas

chromatography (HT-GC)	431

Hildebrand equation,

thermodynamic model	79

Hill's Criteria of

Causality	54

Hydrogenation

cocoa butter	
alternatives	464
confectionery fats	480

Index Terms

Links

I

Illipe	19	20	485
Incompatible fats, bloom	183		
Industrial processing, cocoa	41		
Infrared spectra			
bloom	200	204	
cocoa butter polymorphs	294	295	
Interesterification			
confectionery fats	482		
enzymatic, of cocoa			
butter alternatives	446		
International Confectionery			
Association (ICA)	281		
International Union for			
Conservation of			
Nature (IUCN)	417		
Isolated crystalline phase	118		
chemical makeup	118		
crystallization	122		
TAG composition	120	121	
Ivory Coast			
cocoa butter	262	267	268
cocoa butters	214	226	229
			260

K

Kokum gurgi	19	20	485
Kuna Indians	55		

Index Terms

Links

L

Larodan	431			
Layer fractionation				
milk fat	372			
Lecithins	8	9	301	
Casson viscosity	286			
Casson yield value	287			
chemical structure	283			
chocolate property control	500			
cocoa butter crystallization	237			
fatty acid composition	284			
rheology modifier	282			
Legislative constraints				
Codex Alimentarius	12	13		
European Union (EU)	11	445		
United States	11			
Lipids				
analysis of minor, components	433	434		
cocoa	45			
cocoa butter crystallization	227	229		
microbial	466			
phospholipids in cocoa butter				
crystallization	224			
Liquid structural organization, cocoa				
butter-vegetable oil blends	397			
Low vacuum scanning electron				
microscopy (LV SEM)	325			
LSL-LLS binary mixtures	82	84	89	91

Index Terms

Links

M

Malaysia

cocoa butter	262	267	268

cocoa butters	214	260	

Mango kernel	19	20	485

Marketing, confectionary fats	181		

Mass spectrometry,

negative ion CI-MS	431		

Melting behavior

cocoa butter polymorphs	296	382	394

cocoa butters	24	115	

confectionary fats	180		

profiles of cocoa butter and

alternatives	476		

shea butter	424		

symmetrical/asymmetrical

TAG binary systems	80	84	

Methylxanthines	4		

Microbeam X-ray diffraction. *See*

Synchrotron radiation microbeam

X-ray diffraction (SR- μ -XRD)

Microbial lipids	466		

Microorganisms, cocoa butter

alternatives	463		

Microstructure

cocoa butter blends	109	138	139	140

free fatty acids of cocoa butter	220			

milk fat and cocoa butter blends	377	381		

Index Terms

Links

Milk chocolate				
defined	11			
formulations	9			
Milk fat				
chocolate	384			
chocolate bloom	386			
composition	365			
composition of fractions	372	373	374	
crystallization of cocoa butter with	377			
fatty acid composition	366			
fractionation technologies	371			
fractions	371			
microstructure of cocoa butter blends	377			
phase behavior of cocoa butter blends	375			
physical properties	368			
physical properties of, fractions	374			
Milk powder				
chocolate	2			
cocoa butter crystallization	239			
Minerals, cocoa	46			
Minor lipids, cocoa butter crystallization	227	229		
Mixing phase behavior				
molecular interactions and	157			
triacylglycerols (TAGS)	155			
Modulus of elasticity	142			
Molding, chocolate processing	7			
Molecular composition of cocoa butter	103			
crystallization	111			
crystallization kinetics	108	125		
crystal structure	110	128	144	145

Index Terms

Links

Molecular composition of				
cocoa butter (<i>Cont.</i>)				
fatty acid profiles	106			
isolated crystalline phase	118			
materials and methods	104			
mechanical properties	110	116	139	141
microstructure	109	114	115	138
	139	140		
processing conditions	105	106		
seed crystal isolation	106			
shear work	111			
TAG profiles	107			
thermal behavior	115			
thermal properties	109			
Monoglyceride lactates	299			
Mono-glycerides (MAGS)	393			
Morphology				
chocolate fat bloom	210			
type 1 bloom	196			
type 2-A bloom	200			
type 2-B bloom	204			
type 3 bloom	206			
MSM-MMS binary mixture	81	83	88	
<i>Mucor</i> lipases	452			
N				
National Heart, Lung and Blood Institute				
Family Heart Study	51			
Negative ion CI-MS	431			

<u>Index Terms</u>	<u>Links</u>			
Nib grinding, cocoa	43			
Nigeria, cocoa butter	260	262	267	268
Nomenclature, cocoa butter	24			
Non-contact profilometry	330			
Nuclear magnetic resonance (NMR)	108	328	431	

O

Oil-in-water emulsion	340			
microbeam X-ray diffraction (μ -XRD)	351	353		
<i>n</i> -alkane-in-water	353	354		
palm mid fraction-in-water (PMF)	354	356	357	
Oil migration				
cocoa butter physicochemical				
properties	269			
storage and bloom	192			
Oleic acid, triacylglycerols with	76			
Olive oil	454	458		
Olmec civilization, chocolate	173			
Optical microscopy, bloom	206	208		
Oxidative stability, shea butter	425			

P

Packaging, bloom	190			
Packing, chocolate processing	8			
Palm oil	19	20	458	
hydrogenation	481			
interesterification	482	484		
melting profiles	479	481		
substrate for cocoa butter alternatives	452			

Index Terms

Links

Palm oil (*Cont.*)

triacylglycerol composition 478 485

Partial glycerides 17

Pentadesma butyracea 454

PGPR (polyglycerol polyricinoleate) 8 233 236

emulsifier 288

flow properties of dark chocolate mass

with 289

preparation 286

yield stress and equilibrium viscosity 290 291

Phase behavior

cocoa butter-milk fat blends 375

cocoa butter-vegetable oil blends 396

cocoa butter without polar lipids 399 400 401

POP/POSt/StOSt ternary 486

triacylglycerols (TAGs) 76

Phase diagrams

symmetrical/asymmetrical TAG binary

systems 84

ternary, of POP, POSt and StOSt 461 463 464

thermodynamic analysis of boundaries 79

triacylglycerol mixtures 155

Phospholipids 18

Physical properties

cocoa butter 20

milk fat 368

milk fat fractions 374

triacylglycerols (TAGs) 74

Piqui oil 455

Plain chocolate, formulation 9

Index Terms

Links

Platelet function, cocoa flavanols/ procyanidins	52			
PMF crystals	354	356	357	
Polarized light microscopy (PLM)				
cocoa butter blends	109	113	138	139
	140			
cocoa butter by origin	266	267		
cocoa butter crystallization	221	222	236	
cocoa butter-vegetable oil blends	407	408	409	
POP-OPO binary mixtures	350			
Polymorphism				
bloom retarding fats	493			
cocoa butter	22	26	151	
cocoa butter and component TAGs	160			
cocoa butter equivalents (CBEs)	484			
cocoa butter polymorphs	293	394		
cocoa butter replacers (CBRs)	488			
cocoa butter with milk fat	381	382		
diglycerides	221	229		
free fatty acids (FFA)	215			
microstructure and FFA	220			
minor components of cocoa butter	213			
phospholipids	224			
sterol and sterol esters	226			
total minor lipids	227	229		
triacylglycerols (TAGs)	73	76	152	
X-ray diffraction (XRD)	332			
Polyphenol oxidase	39			
Polyphenols				
activity assessment	65			

<u>Index Terms</u>	<u>Links</u>			
Polyphenols (<i>Cont.</i>)				
boosting effect	69			
classification	63			
skin structure experiment	64			
POP-OPO binary mixtures, microbeam				
X-ray diffraction (μ -XRD)	349			
POP spherulite, microbeam X-ray				
diffraction (μ -XRD)	348			
Pressing, cocoa	43			
Prism 4 software	131			
Proanthocyanidins (PACs)	49	64		
Processing				
bloom	186			
chocolate	5			
cocoa	3			
cocoa butter equivalents	488			
cocoa butter replacers	490			
cocoa butter substitutes	492			
shear work on crystallization	105	106		
Procyanidins	49	64		
antioxidant function	56			
blood pressure	55			
cardiovascular health	52			
endothelial function	53			
platelet function	52			
Prodelphinidins	49			
Profilometry	330			
Proteases	39			
PSP-PPS binary mixtures	80	81	82	86

Index Terms

Links

R

Raman microscopy	317	319
Rapeseed oil	454	455
Recipes, chocolate	8	
Refined cocoa butter	4	
Refining		
chocolate processing	5	
cocoa butter	253	
Response Surface Methodology	299	
Reversed phase high performance liquid		
chromatography (RP-HPLC)	324	427
Rheology	278	
chocolate	280	
chocolate and fat phase	25	
lecithins as, modifier	282	
<i>Rhizomucor miehei</i> lipase	447	
<i>Rhizopus</i> lipase	452	
<i>Rhizopus arrhizus</i> lipase	453	455
<i>Rhizopus delemar</i> lipase	452	
<i>Rhodospiridium toruloides</i> Y4	466	
Roasting		
cocoa beans by origin	252	
cocoa processing	4	42

S

Sal	19	20	485
<i>Sapium sebiferum</i> tree	448		
Saponifiable fraction, shea butter	420		

Index Terms

Links

Scanning electron microscopy			
(SEM)	321		
bloom	175	197	
low vacuum	325		
Scanning probe microscopy	308		
Seed crystals	17	106	
Shea	19	20	485
Shea butter			
analysis of minor lipid components	433	434	
characterization	419		
chemical composition	420		
cocoa butter improvers (CBIs) and			
equivalents (CBEs)	426		
fatty acid profile	426		
grinding and processing nuts	418		
melting point	424		
oxidative stability	425		
photograph	419		
physical properties	424		
regional variation	423		
saponifiable fraction	420		
tree	417		
triglyceride data	422		
unsaponifiable fraction	421	423	
viscosity	424		
Sigma-Aldrich	431		
Single-cell oils (SCO)	466		
Skin structure experiment			
activity of cocoa polyphenols	65		

Index Terms

Links

Skin structure experiment (*Cont.*)

comparing commercially available

product 68

effective dose 66

enhancing effect 69

polyphenols 64

Solid fat content (SFC) 105 108 216 217

261

cocoa butter-vegetable oil blends 396 410 411

evolution during storage 328 329

milk fat fractions 374

nuclear magnetic resonance (NMR) 431

Solidification

bloom 189

cocoa butter blend 184

confectionary fats 180

Solids level, confectionary fats 180

Solvent fractionation 4 371

Sorbitan ester, cocoa butter polymorphs 297

SOS/POP mixture 163

SOS/POP/POP mixture 166

SOS/POS mixture 163

Soybean oil

cocoa butter-vegetable oil blends 403 404 405 407

409 410

tryacylglyceride concentration 398

Spherulites of trilaurin, microbeam

X-ray diffraction (μ -XRD) 345 346 347

Stability, confectionary fats 181

<u>Index Terms</u>	<u>Links</u>			
Static cooling, bloom	189			
Stereomicroscope, bloom	201	205		
Sterilization, cocoa	41			
Sterol and sterol esters, cocoa butter				
crystallization	226			
Storage, bloom	190			
<i>Strychnos madagascariensis</i> oil	454	455		
Sugar				
chocolate	2			
cocoa butter crystallization	234			
Sugar bloom	195			
Supercooling, cocoa butter-vegetable				
oil blends	402	403	407	41
	1			
Supercritical carbon dioxide				
extraction/fractionation	465			
production of cocoa butter alternatives	460			
Surface analysis				
atomic force microscopy (AFM)	308	312	313	
profilometry	330			
Symmetrical monounsaturated				
triglycerides (SOS)	19			
Symmetry, triacylglycerols (TAGS)	74			
Synchrotron radiation microbeam X-ray				
diffraction (SR- μ -XRD)	340			
binary mixtures of POP/OPO	349	352		
data analysis of μ -XRD	343	344		
oil-in-water emulsion	351	353		
palm mid fraction-in-water (PMF)				
emulsion	354	356	357	

<u>Index Terms</u>	<u>Links</u>			
POP	348			
trilaurin	345	346	347	
water-in-oil emulsion	355	357		
Synchrotron radiation X-ray diffraction (SR-XRD), cocoa butter polymorphs	163	165	167	168
T				
Tea seed oil	454	458		
Temperature				
chocolate wafer surface	311	313		
cocoa butter composition	249			
cocoa butter-vegetable oil blends	397			
storage and bloom	190			
Tempering				
bloom	187			
chocolate and fat phase	25			
chocolate processing	6	269	296	
milk fat in chocolate	386			
Tengkawang	19			
Texture, chocolate	500			
<i>Theobroma cacao</i>				
cacao and cocoa	36			
cocoa tree	1	3	35	36
Thermal analysis	320			
<i>Thermomyces lanuginosus</i> lipase	458			
Triacylglycerols (TAGS)	73	151		
acyl donors for cocoa butter alternatives	456			

Index Terms

Links

Triacylglycerols (TAGS) (*Cont.*)

binary systems	77	
chain length mismatch (CLM)	74	77
classes of, in milk fat	372	
cocoa butter by origin	248	
concentration in milk fat	367	
differential scanning calorimetry (DSC)	78	
distribution in milk fat fractions	373	
fatty acidfatty acid esters vs., as acyl donors	459	
high performance liquid chromatography (HPLC)	324	325
materials and methods	77	
melting behavior of symmetrical/ asymmetrical binary systems	80	84
mixing behavior of POP, POS,and SOS	162	
mixing phase behavior	155	
phase diagrams of symmetrical/ asymmetrical binary systems	84	
physical properties	74	
polymorphism	152	
profiles	107	
structure	276	
symmetrical and asymmetrical mixed- acid	76	
symmetry	74	
thermal processing	78	

Index Terms

Links

Triacylglycerols (TAGS) (*Cont.*)

thermodynamic analysis of boundaries			
in phase diagrams	79		
<i>Trichelia emetica</i> oil	454	455	
Triglycerides			
composition in cocoa butter	13		
crystal packing	23	24	
minor	16		
shea butter	422		
Trilaurin spherulites, microbeam X-ray			
diffraction	345	346	347

U

United States, chocolate legislation	11		
Unsaponifiable fraction, shea butter	421	423	
Unsaponifiable matter	18		
USDA Flavonoid Database	50		
USDA Nutrient Database	47		

V

Vegetable fat

chocolate recipes	10t		
cocoa butter processing with	395		
detection techniques	466		
term	2		

Vegetable oils. *See also* Cocoa butter-

vegetable oil blends			
crystallization of cocoa butter (CB)			
and, blends	396		

Index Terms

Links

Vegetable oils (*Cont.*)

melting and crystallization	
behavior	432
substrates for cocoa butter	
alternatives	454
supercooling and cooling rate effect	
on crystallization of CB- blends	403
TAGS in <i>trans</i> -free	395
tryacylglyceride	
concentration	398
Viscosity, shea butter	424
Vitamin E, shea butter	423
<i>Vitellaria paradoxa</i>	417

W

Water-in-oil emulsion	340	355	357
White chocolate			
formulation	10		
surface images	327		
Whiteness index	314		
Whole cocoa butter	17		
Wide-angle X-ray			
diffraction (WAXD),			
cocoa butter polymorphs	160		
Wide-angle X-ray			
scattering (WAXS)	334		
WITec project	319		
Women's Health Study	51		
Woskresensky, Alexander	47		

<u>Index Terms</u>	<u>Links</u>		
X			
<i>Ximenia caffra</i> oil	454	455	
X-ray diffraction (XRD)	332	339	
<i>See also</i> Synchrotron radiation microbeam X-ray diffraction (SR-μ-XRD)			
binary blends of oils	432		
cocoa butter polymorphs	293	294	295
crystal structure of cocoa butters	110	128	
Y			
<i>Yarrowia lipolytica</i> ACA-DC 50109	466		
<i>Yarrowia lipolytica</i> LGAM S(7)	466		
Z			
Zutphen Elderly Study	51		