Triacylglycerol Structures and Cocoa-butter Crystallization.
van Langevelde, A.J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

General introduction
1.1 Introduction

Many people all over the world like chocolate. In The Netherlands 4 – 5 kg of chocolate confectioneries per capita has been consumed in 1997 and the chocolate consumption is still increasing. However, the logistics of chocolate and chocolate products is a serious problem. Badly conditioned transport and storage facilities may result in a greyish-white appearance at the chocolate surface causing the products to appear aged and musty (Fig. 1.1). Since the colour and gloss of food products play an important role in their acceptance by consumers, chocolate manufactures still receive many complaints about white-looking chocolate. Although this so-called “bloom” effect can be ascribed to light scattering on a roughened chocolate surface, many people are suspicious about the product’s freshness. Therefore, a lot of research has been carried out all over the world to reveal the mechanism of bloom formation and to avoid or delay its occurrence.

![Figure 1.1](image-url) Two bars of dark chocolate of which the right one is bloomed.

1.2 Chocolate

1.2.1 History

Originally the cacao trees with their fruits, which contain the cacao beans (see §1.2.2), were found in Central and Middle America by the inhabitants, Toltecs and other peoples living in that region, who ate the pulp of the cacao fruit only, because the beans were too bitter. Later, one of them put the beans in a fire and a marvellous flavour was released. He ground the beans with stones resulting in a creamy mass. This is the beginning of chocolate. The Mayas and Aztecs used cacao beans to prepare a delightful drink called xocotlatl, consisting of cocoa, vanilla, pepper and other spices.

From the very early days of cacao the peoples of Central America, Mayas and Aztecs, used beans also as a form of payment. The use of cacao beans as currency must have become established
before A.D. 1000. On his fourth voyage to America, Columbus landed on 30th July 1502 in Nicaragua and became the first European to discover cacao beans. However, Columbus, who was still searching for the sea route to India, was not interested in cocoa. Hernando Cortez, who conquered parts of Mexico in approximately 1520, was the first (in 1528) to bring cocoa and the utensils necessary for its preparation to Europe, although he did not find the taste of cocoa particularly pleasant.

1.2.2 Cacao beans

Cacao trees having the botanical name ‘Theobroma cacao’ are found in the geographical belt bounded by the Tropics of Cancer and Capricorn, 20° north and south of the equator. This tropical region includes Central and South American, West African and Asian countries. The fruit of the cacao tree is a pod containing 30 – 45 cacao beans. It is shaped like an elongated acorn squash (15 – 30 cm) and comes in a variety of green, yellow, orange, red and brown colours. The beans are white (prior to fermenting) and approximately 2 cm long. Cacao trees produce each about 30 pods during the whole year and deliver 1000 – 1500 kg dry beans per hectare per year. After 5 – 6 months the ripe fruits are harvested and the beans are stored for a few days under banana leaves to ferment. During the fermentation process the flavour and brown colour develop. Before shipping the beans are dried under the sun for two weeks.

A large amount of cacao beans is shipped to the seaport of Amsterdam (The Netherlands) where about 500,000 ton cacao beans, more than 20 % of the world production, are handled annually (Port Management of Amsterdam, 2000), and processed in many cocoa industries, located in the “Zaan” region, a few kilometres to the north of Amsterdam.

It seems that the words cacao and cocoa have become interchangeable. For clarity here the term cacao is used when referring to the trees and raw products and cocoa to indicate an (industrially) processed product (butter, powder etc.).

1.2.3 Cocoa butter

In the cocoa industry the beans are cleaned and dried. After breaking the beans their shells and nibs (= kernels) are separated. The nibs are milled resulting in a fine cocoa mass from which the fat part, cocoa butter (yellowish), and cocoa powder (brown) are obtained by compressing it at high pressure. To enhance the solubility of the cocoa powder in water or milk and to improve its flavour and colour, the nibs are alkalized before milling. This procedure was invented in 1828 by the Dutch chocolate master C.J. van Houten and is still called “Dutching”.

Chocolate contains 25 – 34 % cocoa butter, 5 – 19 % cocoa powder, 42 – 51 % sugar and 0 – 26 % milk powder, depending on the type of chocolate. Cocoa butter is one of the most important ingredients of chocolate, and it largely determines its physical properties. To obtain a constant high level of quality products, the production of cocoa butter should meet high-quality standards. However, the physical properties of cocoa butter depend on many parameters, such as the type of cacao tree, the country of origin and the season of harvesting, but also on human-influenced factors like duration of fermentation. All these parameters determine the exact composition of a cocoa butter resulting in specific melting and solidification trajectories.
Although solid fats, like cocoa butter at room temperature, seem soft and amorphous, they have a rather high degree of crystallinity. Moreover, fats can appear in different crystalline forms each having its specific physical properties. This phenomenon is called polymorphism and plays a major role in many food-processing problems. The total content of solid fat material (SFC) depends on the temperature of the fat. At low temperature a large amount of the fat is solid, whereas the SFC lowers with increasing temperature until the fat is completely molten.

1.2.4 Triacylglycerols

Triacylglycerols (TAGs), the main constituents of fats, are esterifications of glycerol with three long-chain fatty acids (Fig. 1.2). Since each chain can differ in length and degree of saturation, many different types of TAGs exists. A three-letter code, based on the nomenclature of the three fatty acids, is used to denote TAGs. The systematic and trivial names of the most important fatty acids are given in Table 1.1. The first letter of the trivial name of the three fatty-acid chains in mutual order form the three-letter code. For example, 1,3-dipalmitoyl-2-stearoyl-glycerol is the trivial name for 1,3-dihexadecanoyl-2-octadecanoyl-glycerol and has the three-letter code PSP. Mono-acid TAGs are named even simpler: trimyristin is the trivial name for 1,2,3-tri-tetradecanoyl-glycerol with three-letter code MMM. In the case that a specific fatty acid does not have a trivial name the number of carbon atoms in the chain is given e.g. C_{13}C_{17}C_{13} is the abbreviation for 1,2,3-tri-tridecanoyl-glycerol. Since the trivial name of different fatty acids can start with the same letter, the notation C_{ab} with ‘a’ the number of carbon atoms and ‘b’ the number of double bounds is also frequently used. For example, the TAG denoted by the three-letter code POS can also be described as C_{16:0}C_{18:1}C_{18:0}.

![Figure 1.2 Chemical structure diagram of a triacylglycerol.](image-url)
**General introduction**

**TABLE 1.1 Fatty-acid nomenclature**

<table>
<thead>
<tr>
<th>chain length : double bonds</th>
<th>systematic name</th>
<th>trivial name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0</td>
<td>methanoic</td>
<td>formic</td>
</tr>
<tr>
<td>2:0</td>
<td>ethanoic</td>
<td>acetic</td>
</tr>
<tr>
<td>3:0</td>
<td>propanoic</td>
<td>propionic</td>
</tr>
<tr>
<td>4:0</td>
<td>butanoic</td>
<td>butyric</td>
</tr>
<tr>
<td>5:0</td>
<td>pentanoic</td>
<td>valeric</td>
</tr>
<tr>
<td>6:0</td>
<td>hexanoic</td>
<td>caproic</td>
</tr>
<tr>
<td>7:0</td>
<td>heptanoic</td>
<td>enanthic</td>
</tr>
<tr>
<td>8:0</td>
<td>octanoic</td>
<td>caprylic</td>
</tr>
<tr>
<td>9:0</td>
<td>nonanoic</td>
<td>pelargonic</td>
</tr>
<tr>
<td>10:0</td>
<td>decanoic</td>
<td>capric</td>
</tr>
<tr>
<td>11:0</td>
<td>undecanoic</td>
<td>–</td>
</tr>
<tr>
<td>12:0</td>
<td>dodecanoic</td>
<td>lauric</td>
</tr>
<tr>
<td>13:0</td>
<td>tridecanoic</td>
<td>–</td>
</tr>
<tr>
<td>14:0</td>
<td>tetradecanoic</td>
<td>myristic</td>
</tr>
<tr>
<td>15:0</td>
<td>pentadecanoic</td>
<td>–</td>
</tr>
<tr>
<td>16:0</td>
<td>hexadecanoic</td>
<td>palmitic</td>
</tr>
<tr>
<td>16:1</td>
<td>hexadec-cis-9-enoic</td>
<td>palmitoleic</td>
</tr>
<tr>
<td>17:0</td>
<td>heptadecanoic</td>
<td>margaric</td>
</tr>
<tr>
<td>18:0</td>
<td>octadecanoic</td>
<td>stearic</td>
</tr>
<tr>
<td>18:1</td>
<td>octadec-cis-6-enoic</td>
<td>petroselenic</td>
</tr>
<tr>
<td>18:1</td>
<td>octadec-cis-9-enoic</td>
<td>oleic</td>
</tr>
<tr>
<td>18:1</td>
<td>octadec-trans-9-enoic</td>
<td>elaidic</td>
</tr>
<tr>
<td>18:2</td>
<td>octadec-cis-9,cis-12-dienoic</td>
<td>linoleic</td>
</tr>
<tr>
<td>18:3</td>
<td>octadec-cis-6,cis-9,cis-12-trienoic</td>
<td>γ-linolenic</td>
</tr>
<tr>
<td>18:3</td>
<td>octadec-cis-9,cis-12,cis-15-trienoic</td>
<td>α-linolenic</td>
</tr>
<tr>
<td>19:0</td>
<td>nonadecanoic</td>
<td>–</td>
</tr>
<tr>
<td>20:0</td>
<td>icosanoic</td>
<td>arachidic</td>
</tr>
<tr>
<td>21:0</td>
<td>henicosanoic</td>
<td>–</td>
</tr>
<tr>
<td>22:0</td>
<td>docosanoic</td>
<td>behenic</td>
</tr>
<tr>
<td>22:1</td>
<td>docosa-cis-13-enoic</td>
<td>erucic</td>
</tr>
<tr>
<td>22:1</td>
<td>docosa-trans-13-enoic</td>
<td>brassidic</td>
</tr>
<tr>
<td>23:0</td>
<td>tricosanoic</td>
<td>–</td>
</tr>
<tr>
<td>24:0</td>
<td>tetracosanoic</td>
<td>lignoceric</td>
</tr>
<tr>
<td>25:0</td>
<td>pentacosanoic</td>
<td>–</td>
</tr>
<tr>
<td>26:0</td>
<td>hexacosanoic</td>
<td>cerotic</td>
</tr>
</tbody>
</table>

It should be noted that TAGs are optically active when the fatty acids at the two outer glycerol positions are different. In products of standard synthetic preparations, as well as in many natural fats, such asymmetric TAGs are present as racemic mixtures. Most work that has been done with chiral TAGs, actually concerns mixtures of enantiomers (De Jong, 1980).
Cocoa butter is a complex mixture of about thirty different types of TAGs though also small amounts of di- and monoacylglycerols and some other minor components are present in cocoa butter. However, compared to other natural fats like butterfat, its composition is relatively simple: the three major TAGs POP, SOS and POS, make up about 14, 23 and 35 %, respectively. The physical properties of TAG mixtures are largely determined by the physical properties of the individual TAGs constituting the mixture. For example, the melting trajectory of cocoa butter is composed by the melting ranges of all the individual crystallites of the cocoa butter. The melting range of an individual crystallite on its turn depends on its TAG composition and crystalline form.

1.3 X-ray diffraction: a brilliant tool

1.3.1 X-rays

The part of the electromagnetic spectrum between ultraviolet light and gamma radiation is called the X-ray region. X-rays, discovered by Röntgen in 1895, have an approximate range of wavelengths of 0.1 to 100 Å and are usually produced by decelerating rapidly moving electrons very quickly and converting their energy of motion into a quantum or radiation. The wavelength $\lambda$ of the emitted radiation depends on the energy of the electrons. The X-rays used in crystallographic analysis are rather soft ($0.5 < \lambda < 2.5 \, \text{Å}$) and have a limited penetration depth. For laboratory practice X-rays are generated by an X-ray tube. The basic parts of an X-ray tube are a source of electrons (cathode) and a metal anode target. In the tube electrons are accelerated by an electric field and directed against the metal target, which slows them rapidly by multiple collisions and a continuum of radiation is formed called white X-ray radiation. The distribution of intensity over the radiation continuum depends primarily on the accelerating voltage and only to a smaller extent on the nature of the target material, the latter resulting in a number of sharp spikes of high intensity. These peaks, characteristic for the element of which the target is made and used to classify the tubes, arise when inner-shell vacancies are refilled by higher-shell electrons. For example, for a Cu anode the characteristic Cu$K\alpha$ wavelength is 1.5418 Å due to the $L \rightarrow K$ shell transition. The monochromatic high-intensity radiation with the characteristic target-metal wavelength is used for X-ray analysis.

Nowadays, synchrotron radiation, which is electromagnetic radiation emitted during the acceleration of charged high-energy particles (electrons or positrons), is also often used for X-ray analysis. It was first generated in the bending magnets of accelerators built for high-energy particle-physics research. Since particle-physics accelerators were inadequate to meet the increasing demand for synchrotron radiation, dedicated storage rings were constructed. Moreover, the brilliance of a source could be tremendously increased by introducing magnetic insertion devices (undulators and wigglers) in the storage rings. The brilliance of a radiation source is defined as the number of photons emitted per second, per unit source size, per unit space angle and for a bandwidth of 1/1000 of the photon energy. Covering the whole spectral range from microwaves to hard X-rays, the radiation produced by these storage rings comes in the form of a fine and very intense beam, similar to that of a laser. Such a third generation source, based essentially on insertion devices, was built in Grenoble (France). For the construction of this European Synchrotron Radiation Facility (ESRF),
General introduction

European co-operation was needed in view of its complexity, cost and experimental potential. The X-ray beams at the ESRF are about a trillion times brighter (i.e. a factor of $10^{12}$) than those produced by conventional X-ray tubes.

1.3.2 Diffraction by crystals

A crystal is defined as a regular repetition of a certain basic group of atoms in three-dimensional space, extending over a distance of millions of molecular dimensions. The basic group of atoms, the structural unit, is the smallest set of atoms from which the crystal can be built by translation only. Since X-rays interact with electrons, a crystal can be regarded as a three-dimensionally periodic electron-density distribution. Points with identical surroundings, each associated with a structural unit, constitute a translation lattice. A translation lattice can be described by three basis vectors $\mathbf{a}$, $\mathbf{b}$ and $\mathbf{c}$. The parallelepiped spanned by the basis vectors is the unit cell of the lattice which is defined by its six cell constants, the three edges $a$, $b$ and $c$, and the three enclosed angles $\alpha$, $\beta$ and $\gamma$. Three points of a translation lattice define a so-called lattice plane. All members of a set of parallel lattice planes are occupied in an identical fashion by the lattice points and the set has a constant interplanar spacing $d$. The orientation of the (lattice) planes in the lattice is characterized by means of three integers $h$, $k$ and $l$ (so-called Miller indices), indicating that the set member closest to a chosen origin (at an arbitrary lattice point) intercepts the $a$, $b$ and $c$ axis at $a/h$, $b/k$ and $c/l$, respectively. To explain the diffraction theory more conveniently, Ewald introduced the reciprocal lattice based on the reciprocal basis vectors $\mathbf{a}^*$, $\mathbf{b}^*$ and $\mathbf{c}^*$. These vectors are defined by Gibbs as follows: $\mathbf{a}^*$ is perpendicular to $\mathbf{b}$ and $\mathbf{c}$, $\mathbf{b}^*$ is perpendicular to $\mathbf{a}$ and $\mathbf{c}$, and $\mathbf{c}^*$ is perpendicular to $\mathbf{a}$ and $\mathbf{b}$. The reciprocal lattice is a collection of lattice points. The virtual line from the origin to a reciprocal lattice point is equal to vector $\mathbf{H}$.

$$\mathbf{H} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^* \quad (1.1)$$

The reciprocal vector $\mathbf{H}$ is perpendicular to the lattice planes $(hkl)$ and its length is equal to $1/d_{\text{hkl}}$.

A beam of X-rays falling on a crystal will be scattered. The effect can be considered as reflection by all lattice planes simultaneously. Constructive interference occurs only when the scattered waves are in phase i.e. when the path difference is an integral multiple ($n$) of $\lambda$. The reflection condition is:

$$n\lambda = 2d_{\text{hkl}} \sin \theta \quad (1.2)$$

This is Bragg's law, which relates the direction $\theta$ of the scattered beam to the interplanar distance $d_{\text{hkl}}$ while $n$ indicates the order of the reflection (Fig. 1.3). For describing the reflection condition of Bragg in the reciprocal space a sphere of radius $1/\lambda$ is constructed around a reciprocal origin. Whenever a reciprocal lattice point coincides with this sphere, Bragg's law is satisfied and reflection occurs. By rotating the lattice about its origin, various reciprocal lattice points can be brought into coincidence with the surface of the sphere of reflection and the corresponding reflection can be observed.
The scattering amplitude of a crystal in a certain direction $\mathbf{H}$ will be the resultant of the scattering amplitudes of all unit cells. Since X-rays are scattered by electrons, the scattering quantity $\rho(r)$, is equal to the time-average of the electron density in the crystal. The amplitude of scattering by the unit cell $F_{hkl}$

$$F_{hkl} = \sum_{j=1}^{N} f_j \exp[2\pi i (hx_j + k y_j + l z_j)]$$

is a function of the atomic positions $(x_j, y_j, z_j)$ and the atomic scattering factors $f_j$. The $F_{hkl}$ are Fourier-transform related to the electron-density distribution within the cell.

$$\rho(x, y, z) = \frac{1}{V} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} F_{hkl} \exp[-2\pi i (hx + ky + lz)]$$

For calculation purposes the electron density $\rho(x,y,z)$ is usually approximated by a collection of spherical atomic distributions $\rho(x_j, y_j, z_j)$ that are Fourier-transform related to the $f_j$.

1.3.3 Structure-analysis techniques

The electron-density distribution of the structural unit i.e. the crystal structure, can be reconstructed via a Fourier synthesis (see (1.4)). The Fourier coefficients $(F_{hkl})$ required consist of the amplitudes of the reflections, derived from the intensities of the beams diffracted by a single
General introduction

crystal in the directions $\mathbf{H}$, and the phases of the reflections. In general, the phases cannot be measured in a diffraction experiment. Therefore, mathematical methods have been developed to derive a set of phases from which an initial structure model can be constructed. The correctness of this structure model is given by the $R$ value, which compares the experimental intensities with the intensities calculated from the model,

$$
R = \frac{\sum_{hkl} \left| F_{hkl}^{\text{obs}} - F_{hkl}^{\text{calc}} \right|}{\sum_{hkl} \left| F_{hkl}^{\text{obs}} \right|} \quad (1.5)
$$

This $R$ value is minimized by refining the structural model using a least-squares method. With the present mathematical methods and the availability of large computers, rather complex crystal structures with more than a hundred atoms in the structural unit can be determined on a routine basis. For small single crystals or large complex structures like proteins, synchrotron radiation is indispensable to perform a successful data collection and structure determination.

However, it is not always possible to grow good-quality single crystals. Often a compound only crystallizes as a conglomerate of a large number of small crystals \textit{i.e.} a polycrystalline powder. The three-dimensional diffraction pattern will be projected onto a one-dimensional intensity pattern as function of the diffraction angle $\theta$. Since single-crystal X-ray diffraction is no longer feasible, special X-ray powder diffraction (XRPD) methods have been developed. XRPD is a common technique to identify an unknown powder sample: the intensities and $d_{\text{at}}$ values of the peaks observed at the XRPD pattern can be compared with the intensities and $d_{\text{at}}$ values of many known powder samples in an electronic database. Crystal-structure determination from powder data, although in principle the same as from single-crystal data, has some specific difficulties. All reflections with (approximately) equal $\theta$ values will diffract simultaneously, and not separately as with a single crystal, so considerable overlap may occur, which can seriously hamper the determination of the individual reflection intensities. In the last decade, many efforts have been put in the development of structure-determination-from-powder-data methods and last year about fifty crystal structures could be determined from powder data world wide. Furthermore, using synchrotron radiation high-resolution XRPD patterns, with significantly reduced overlap of reflections, can be obtained.

With the development of position-sensitive detectors, capable to collect the complete or a part of the $2\theta$ range of an XRPD pattern at once, real-time experiments became available so changes in the crystalline state of a powder sample can be monitored. In general, this type of experiments is most easily carried out with high-intensity synchrotron radiation, but it is also feasible to do these experiments with laboratory equipment if the sample diffracts sufficiently strong. At the Laboratory for Crystallography (Universiteit van Amsterdam, Amsterdam, The Netherlands) a real-time X-ray powder diffractometer has been constructed using a linear diode-array detector (Van Malssen, 1994; Van Malssen \textit{et al.}, 1994). The detector has 1024 channels which were made sensitive to X-rays.
using an Y$_2$O$_3$:Tb scintillation layer. Depending on the sample type and the intensity of the X-ray beam, experiments can be carried out at dynamic processes on a time scale as small as 0.1 s. Since this diffractometer is equipped with a temperature-controlled sample environment, the effect of temperature on the crystalline state can be studied. The temperature of the sample may be varied between 80 – 400 K or 248 – 400 K depending on the coolant used. Disadvantages of this diffractometer are the limitations in the $2\theta$ range which can be monitored at once and the non-optimal resolution of the XRPD patterns due to defocusing at the linear detector.

Long-range ordering of molecules (in crystals) will result in diffraction intensity in the long $d$-spacing region ($d > 25 \, \text{Å}$). Since the diffraction intensity in the long $d$-spacing region is often moderate or weak, it is difficult to measure it accurately with standard XRPD, especially for real-time experiments. For monitoring the long $d$-spacing region at a small time scale, small-angle X-ray scattering at synchrotron facilities can be used (Russell, 1991). The high flux and small divergence of the synchrotron radiation beam result in a reduced signal-to-noise ratio through which weak intensity peaks can be evaluated. With the introduction of two-dimensional detectors time-resolved measurements of the long $d$-spacing region can be performed. At the ESRF (Grenoble, France) a SAXS station is mounted at beam line BM26a of the DUBBLE CRG (Bras, 1998). Since this SAXS station is equipped with a temperature-controlled sample environment, temperature-dependent processes can be studied.

1.4 X-ray diffraction applied to TAGs and cocoa butter

1.4.1 Polymorphism

Long-chain organic compounds, such as alkanes, fatty acids and TAGs, show polymorphism: their hydrocarbon chains easily pack in different ways resulting in several different crystalline forms. The various crystalline forms or polymorphs can be identified by XRPD ($\S$ 1.3.3) via short $d$-spacings values ($d \approx 3 – 6 \, \text{Å}$), related to the lateral chain packing, and long $d$-spacings values ($d > 25 \, \text{Å}$). The latter result from the layer thickness which is directly associated with the length of the hydrocarbon chains and with the angle of tilt ($\tau$) between the chain axes direction and the basal plane.

The short $d$-spacings originate from the dominant lateral packing of the hydrocarbon zigzag planes. Since the lateral packing of one zigzag is repetitive in the direction of the chain axes, a subcell representing only the lateral chain packing can be derived. Three major subcell types are encountered in practice: the $T_m$, $O_\perp$ and $H$ subcell. In the triclinic $T_m$ subcell all the zigzag chains are parallel packed, in the orthorhombic $O_\perp$ subcell the zigzag chains are orthogonal packed (Fig. 1.5), whereas in the hexagonal $H$ subcell the zigzag chain orientation is randomly distributed as a result of statistical disorder (Table 1.2).

The parallel packing of hydrocarbon chains in the triclinic subcell is the most stable lateral packing. The lateral packing in the hexagonal subcell is unstable and the orthogonal lateral chain packing in the orthorhombic subcell is metastable.
In case of TAGs and their mixtures, the \( \alpha \) phase corresponds to the unstable H subcell packing, the \( \beta' \) phase to the orthogonal lateral chain packing and the \( \beta \) phase to the stable triclinic subcell packing. This is the case for cocoa butter and its main constituent TAGs. Nevertheless, TAGs and fats that are \( \beta' \) stable do exist, for example milkfat. Generally, phase transitions of TAGs from the unstable towards the more stable phases are irreversible.

For TAGs, two types of chain-length packing can be distinguished: a double and triple chain-length packing (Fig. 1.4).

**TABLE 1.2 Characteristics of TAG polymorphs (Abrahamsson et al., 1978; Hoerr and Paulicka, 1968)**

<table>
<thead>
<tr>
<th>Polymorph</th>
<th>( d )-spacing value</th>
<th>subcell lattice</th>
<th>chain orientation</th>
<th>symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>4.15</td>
<td>hexagonal</td>
<td>random</td>
<td>H</td>
</tr>
<tr>
<td>( \beta' )</td>
<td>3.80, 4.20</td>
<td>orthorhombic</td>
<td>orthogonal</td>
<td>( O_\parallel )</td>
</tr>
<tr>
<td>( \beta )</td>
<td>3.65, 3.85, 4.57</td>
<td>triclinic</td>
<td>parallel</td>
<td>( T_{\parallel} )</td>
</tr>
</tbody>
</table>

**FIGURE 1.5** The triclinic \( T_{\parallel} (a) \) and orthorhombic \( O_\parallel (b) \) subcell of lateral hydrocarbon chain packing.

11
1.4.2 Crystal structures determined by others

Although many different types of TAGs exist, crystal structures of TAGs are very scarce and until now only a few crystal structures of long-chain TAGs have been published, all of them in the \( \beta \) phase. Jensen and Mabis (1963) presented a full single-crystal structure determination of \( \beta \)-CCC from an incomplete three-dimensional data set, obtained through equi-inclination Weissenberg photographs collected with FeK\( \alpha \) radiation. The crystal structure refined poorly (\( R = 0.179 \)), but later a further refinement based on photometric data resulted in a final \( R \) value of 0.051 (Jensen and Mabis, 1966). Larsson (1965) presented the crystal structure of \( \beta \)-LLL, refined to an \( R \) value of 0.20. L\( ^{\text{br}} \)L\( ^{\text{br}} \)L\( ^{\text{br}} \), an isomorphously crystallizing heavy-atom analogue of LLL, was used to derive the initial model. Gibon et al. (1984) further refined the structure of \( \beta \)-LLL to a final \( R \) value of 0.06.

The crystal structure of a TAG with unequal fatty-acid chain lengths was published by Doyne and Gordon (1968). They determined the crystal structure of 2-(11-bromoundecanoyl)-1,3-di-decanoyl-glycerol (CL\( ^{\text{br}} \)C), a heavy-atom analogue of CLC, which is a C\( _n \)C\( _{n+2} \)C\( _n \)-type (\( n \) = even) TAG. The crystal structure of a TAG having one short acyl chain, 1,2-di-hexadecanoyl-3-ethanoyl-sn-glycerol (PP2) was published by Goto et al. (1992). Only recently, the crystal structure of a TAG with a double bond in each chain, 1,2,3-tri-octadec-trans-9-enoyl-glycerol (EEE) was published by Culot et al. (2000). Furthermore, unit-cell parameters and space groups were determined for some TAGs crystallized in the \( \beta \) phase.

Unit-cell parameters and space group of \( \beta' \)-LLL, \( \beta' \)-PSP, \( \beta' \)-C\( _{11} \)C\( _{11} \)C\( _{11} \) and \( \beta' \)-LML were determined from single crystals (Birker et al., 1991; Hernqvist, 1988; Hernqvist and Larsson, 1982;
General introduction

Larsson, 1965b), but efforts to determine the crystal structure of TAGs in the β′ phase were not successful.

1.4.3 Cocoa-butter crystallization

Major parts of the research on the polymorphic behaviour of cocoa butter have been carried out using differential scanning calorimetry (DSC; Cebula and Smith, 1991; Manning and Dimick, 1983; Ziegleder, 1990). In a DSC experiment the heat flow necessary to change the temperature in both the sample and a reference is compared. Since it is not possible to establish the crystalline forms of cocoa butter unambiguously from DSC data, the interpretation of the experimental data easily leads to false conclusions. Furthermore, using DSC for determination of the crystalline form destroys the sample.

With XRPD the main crystalline forms of cocoa butter (γ, α, β′ and β) can be identified unambiguously, because the various packing modes of the hydrocarbon chains of the constituting TAGs result in characteristic peaks in the 3 – 6 Å region of the XRPD pattern (see §1.4.1). However, the polymorphic behaviour of cocoa butter is more complex than that of their pure TAGs. The diversity of experimental results, often obtained under different experimental conditions or using non-specified samples, has led to contradictions and confusion about the number of solid cocoa-butter phases, especially the existence of sub-forms in the β′ and β classes, and their physical nature in particular the melting points/ranges. The different nomenclatures, to name the cocoa-butter phases, used in literature are based on the nomenclatures by Vaecck (1960) and by Lutton and Wille (1966). The names of the cocoa-butter phases occurring in this thesis, γ, α, β′ and two β phases: β(V) and β(VI), are based on these two principal nomenclatures and real-time experiments (Van Malsse et al., 1999). An overview of the different nomenclatures is given in Table 1.3, which is an extension of a Table by Dimick and Davis (1986).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>γ 18.0</td>
<td>γ 17.3 I</td>
<td>α 23.5 II</td>
<td>β 25.5 III</td>
<td>β ′ 27.3 IV</td>
<td>β ′ 25.6 III</td>
<td>β ′ 25.0 IV</td>
<td>β ′ 22.4 β′ 20-27</td>
</tr>
<tr>
<td>α 23.5</td>
<td>α 23.3 II</td>
<td>β 33.0 V</td>
<td>β 33.8 V</td>
<td>β 30.8 II</td>
<td>β 30.0 V</td>
<td>β 30.7 β(V) β(VI)</td>
<td></td>
</tr>
<tr>
<td>β 34.5</td>
<td>β 36.3 VI</td>
<td>β 32.2 I</td>
<td>β 33.5 VI</td>
<td>29-34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Names given and melting points (°C) of cocoa butter phases found by various authors.
From the phase-transition scheme of mechanically-static cocoa butter it can be deduced that the $\gamma$ phase is very unstable, that $\alpha$ and $\beta'$ phases are metastable and that the $\beta$ phases have the highest stability (Fig. 1.6). The phase transitions from less stable to more stable phases are generally irreversible and depend on temperature and time (Van Malssen et al., 1999). All cocoa-butter phases may crystallize from totally molten cocoa butter except for the two $\beta$ phases.

**FIGURE 1.6** Cocoa butter phase-transition scheme (Van Malssen et al., 1999). Both isothermal and non-isothermal phase transitions. *This non-isothermal transition has been reported in the literature (Wille and Lutton, 1966). **This non-isothermal transition has been reported in literature (Wille and Lutton, 1966) and has been observed in our experiments (Van Langevelde, A.J. and Van Malssen, K.F., unpublished data).

Nowadays, research at cocoa-butter crystallization is mainly focused on parameters influencing the polymorphic behaviour of cocoa butter. This knowledge is used in manufacturing processes to direct the crystallization behaviour by controlling these parameters. One of these parameters is the crystallization temperature, which may be varied during cocoa-butter crystallization (Loisel et al., 1998a; Van Malssen et al., 1996abc). These temperature variations, called tempering, are used to crystallize cocoa butter in one of the most stable phases. Since this is not straightforward, various tempering methods are still considered (Bomba, 1993; Loisel et al., 1997; Seguine, 1991). To crystallize a stable phase of cocoa butter also seeding methods have been
investigated (Adenier et al., 1978; Hachiya et al., 1989abc; Schlichter-Aronhime and Garti, 1988). In the case of seeding, cocoa-butter crystallization is not initiated by primary nucleation, as is the case for tempering, but the seed material starts the crystallization process. The crystallized phase depends on both the seed material and crystallization temperature. Cocoa-butter crystallization is also influenced by the addition of other natural fats and oils like milkfat (Campbell et al., 1969; Cebula and Ziegleder, 1993; Hartel, 1996; Lohman and Hartel, 1994). Furthermore, emulsifiers are often added to cocoa butter to change its behaviour (Garti et al., 1986; Loisel et al., 1998b).

1.5 The blooming problem

There are two types of bloom at chocolate: sugar bloom and fat bloom (Fig. 1.7). Sugar bloom arises from changes in crystal size and distribution of sugar particles due to moisture (Bomba, 1993), whereas fat bloom originates from physical changes of the fat in chocolate (Seguine, 1990; Pczczola, 1997). Recently, Lohman and Hartel (1994) summarized the reasons for fat-bloom formation in chocolate. Most of these concern the crystallization conditions of chocolate. In our opinion the underlying physical principle can be regarded as being related to either Ostwald ripening or to phase transitions of cocoa butter (Paulicka, 1973), or a combination of these two processes. Ostwald ripening is the formation of large crystals at the expense of the smaller ones, shifting the crystal-size distribution to larger crystal sizes (Boistelle, 1988). The critical radius $r^*$ of a crystal increases at decreasing concentration of the solution ($C_s$). Crystals with $r < r^*$ dissolve and crystals with $r > r^*$ grow until a thermodynamic equilibrium is reached. Cocoa butter and chocolate contain a liquid fat fraction, which mainly consists of low-melting unsaturated TAGs. These low-melting TAGs can be regarded as the solvent. The concentration of high-melting TAGs in the solvent $C_s$ decreases due to the growth of fat crystals with $r > r^*$. Migration of low-melting unsaturated TAGs also decreases $C_s$ and, consequently, enhances Ostwald ripening at the chocolate surface (Ziegleder and Schwingshandl, 1998). The formation of large cocoa-butter crystals in chocolate creates small crevices at the surface that are perceived as bloom. Fat bloom arising from phase transitions of cocoa butter can be divided in bloom originating from a phase transition of i) $\beta'$ $\rightarrow$ $\beta$ and ii) $\beta(V)$ $\rightarrow$ $\beta(VI)$ (Fig. 1.7). In the first case the cocoa butter in chocolate was crystallized in the $\beta'$ phase and transforms rather rapid to the $\beta$ phase. In the last case the cocoa butter was crystallized in the $\beta(V)$ phase and transforms slowly to the $\beta(VI)$ phase (Wille and Lutton, 1966). Both these re-crystallization processes may result in the appearance of bloom and may appear simultaneously with Ostwald ripening.

Since fat bloom at chocolate is an undesired phenomenon, manufacturing methods have been developed to exclude or delay its occurrence. One should be aware of the difference between exclusion and delay in the further consideration. Delay means that a certain process still takes place but at a lower rate, while exclusion means that the (blooming) process does not take place at all. To exclude bloom formation originating from a $\beta'$ $\rightarrow$ $\beta$ phase transition, chocolate's cocoa butter should be crystallized in the $\beta$ phase, from which it is impossible to have a $\beta'$ $\rightarrow$ $\beta$ phase transition. However, crystallization of completely molten cocoa butter directly in the $\beta$ phase was never observed by Van Malssen et al. (1996ac, 1999) despite their exhaustive efforts. To crystallize chocolate in the $\beta$ phase, at the start of the crystallization a sufficient amount of nuclei in the $\beta$
phase has to be present in the liquid. When the nuclei are homogeneously spread through the chocolate liquor, a uniformly crystallized product in the $\beta$ phase will be obtained. The widely used tempering and seeding methods are based on this concept.

![Diagram of Bloom Formation](image)

**Figure 1.7** Scheme of bloom formation on chocolate.

At this moment, no method is described in literature to exclude bloom, which originates from a $\beta(V) \rightarrow \beta(VI)$ phase transition completely. A definite solution would be to crystallize the cocoa butter in chocolate directly in the $\beta(VI)$ phase, so avoiding a $\beta(V) \rightarrow \beta(VI)$ phase transition. However, it is extremely difficult to crystallize the chocolate product in the $\beta(VI)$ phase and hitherto only methods to delay bloom formation were developed, but even this approach is not straightforward. The $\beta(V)$ phase has to be stabilized and/or the $\beta(VI)$ destabilized in order to slow down the $\beta(V) \rightarrow \beta(VI)$ phase-transition rate. Besides lowering temperature no other physical treatment is readily used in practice to achieve this lower phase-transition rate except for the addition of special components to the chocolate products. Milk fat and emulsifiers are frequently used to stabilize the $\beta(V)$ phase resulting in delayed fat-bloom formation (§ 1.4.3).

### 1.6 Scope of this thesis

#### 1.6.1 Aim

Fat-bloom formation at chocolate is still a serious problem for chocolate manufactures and ideally a process should be developed to make quality chocolate which is free of bloom formation, but without requiring additives. Therefore, the cocoa butter of chocolate should crystallize directly in the $\beta(VI)$ phase (see §1.5). To achieve this, it is essential to get a better understanding of the irreversible $\beta' \rightarrow \beta$ and $\beta(V) \rightarrow \beta(VI)$ phase transitions of cocoa butter at the molecular level and of the experimental conditions at which the phase transitions occur. Since cocoa butter is a conglomerate of crystallites, each having its own TAG composition, phase-transition mechanisms
of cocoa butter will be described by theoretical models only. Nevertheless, to construct these models, crystal-structure information and insight in the phase-transition mechanisms of individual TAGs at the atomic scale are necessary.

1.6.2 Approach

To describe the phase-transition mechanisms of TAGs at the molecular level, accurate three-dimensional atomic models of different types of pure TAGs in various phases are necessary. However, these are very scarce and all known crystal structures of TAGs are crystallized in the β phase (see §1.4.2). Therefore, a major research effort has been put into crystal-structure determination of TAGs and, in particular, of TAGs crystallized in the β' phase. Since single-crystals for crystal-structure determination were not available for most of the TAGs, crystal structures have been determined from powder data instead. Since cocoa butter is a complex mixture of different TAGs, molecular models of the various cocoa-butter phases and their transitions can not be constructed yet. Therefore, the second part of the research was focused on the crystallization conditions of the various phases, the experimental conditions of their transitions to occur and, in particular, on the influence of time and temperature on the re-crystallization behaviour of cocoa butter. Therefore, the temperature of cocoa-butter samples of different origins were varied conform many different temperature profiles, while the XRPD pattern was monitored at the same time.

In Chapter 2 the crystal structure of β-1,2,3-tri-hexadecanoyl-glycerol (β-PPP) from single-crystal X-ray diffraction data is presented. By assuming appropriate transformations it is shown that this structure and the two known crystal structures of this β-C₆C₆C₆-type (n = even) TAGs series form a homologous series. As a result an overlap model is build from which the structure of another series member is predicted. The implications of this approach is that with one known crystal structure of a homologous series, the structure of the other series members may be determined from high-resolution XRPD data. The effectiveness of this approach is illustrated in Chapter 3 where the crystal-structure determination of β-1,2,3-tri-tetradecanoyl-glycerol (β-MMM) and β-1,2,3-tri-octadecanoyl-glycerol (β-SSS) from high-resolution synchrotron XRPD data is described.

In Chapter 4 the crystal structure of β-1,2,3-tri-tridecanoyl-glycerol (β-C₁₃C₁₃C₁₃), an odd-numbered member of the C₆C₆C₆-type TAG series, is presented. The crystal structure has been determined from high-resolution synchrotron XRPD data and is the first known crystal structure of an odd-numbered TAG. On basis of these crystal structures the melting-point alternation of odd and even-numbered β-C₆C₆C₆-type series members is discussed.

For some series members of the β'-stable C₆C₆₂C₆-type (n = even) TAG series unit-cell parameters and space groups have been determined. The implications of these results for the crystal packing, assuming straight TAG molecules, are discussed in Chapter 5. The crystal structures of two members of the β'-C₆C₆₂C₆ series are described in Chapter 6, being the first known crystal structures of a TAG in the β' phase. One of them has been determined from single-crystal synchrotron data and the other from high-resolution synchrotron XRPD data. On basis of these crystal structures the differences between the β' and β phase are discussed.

In the Chapters 7 – 9 the time and temperature-dependent crystallization behaviour of cocoa butter is described.
A complete isothermal phase-transition scheme of cocoa butter under mechanically static conditions is presented in Chapter 7. The observed phase behaviour of cocoa butter is explained on basis of the concept of individual crystallite phase behaviour of cocoa butter. During this extensive study no direct crystallization of cocoa butter from the melt in the most stable β phase has been observed. However, to avoid the unwanted β' → β phase transformation of cocoa butter, direct crystallization of cocoa butter in the β phase is essential. This can be achieved by re-crystallization of incompletely molten cocoa butter, where remaining crystalline material initiates the crystallization. The influence of maximum and crystallization temperatures on the re-crystallization behaviour of cocoa butter is described in Chapter 8. The results are discussed in respect to the composition of the cocoa butters used.

In Chapter 7 – 8, only short d-spacing values were monitored during the cocoa butter (re-)crystallization experiments, while long d-spacing values may provide information about the chain-length packing of crystallized cocoa butter. To characterize the cocoa-butter phases in more detail, the long d-spacing region (40 Å < d < 70 Å) of cocoa butter crystallizing at various crystallization temperatures has been monitored at the SAXS station of DUBBLE (ESRF, Grenoble, France). Moreover, to obtain structural information at the long d-spacing level of the crystalline material responsible for cocoa-butter re-crystallization, experiments of re-crystallizing cocoa butter has been performed at various maximum and crystallization temperatures. These experiments and their results are discussed in Chapter 9.

The knowledge of the polymorphic behaviour of cocoa butter obtained in this study has been utilized to develop a new chocolate manufacturing process. Since this newly developed process is still in a patenting procedure, it is not described in this thesis.

Bibliography


Callebau, (1999). All about chocolate ...


Encarta® 98. Encyclopaedia Winkler Prins Editie.


**General introduction**


**References**


General introduction


Port Management of Amsterdam (2000). Personal communication.


