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

Citation for published version (APA):
Chapter 9

Cocoa-butter long spacings and the memory effect

submitted for publication

Abstract

Depending on the maximum temperature before cooling cocoa butter re-crystallizes in the $\beta(V)$ and/or $\beta(VI)$ phase. In order to obtain structural information of the seed material initiating this re-crystallization process, experiments with cocoa butter were performed at a small-angle X-ray scattering station. Crystallization of cocoa butter at various crystallization temperatures provided long $d$-spacing values ($d > 45 \, \text{Å}$) which can be attributed to the polymorphic phases. From the re-crystallization experiments it is concluded that the seeds initiating rapid-starting re-crystallization have an SOS-dominated triple chain-length packing. Furthermore, the $\beta(VI)$ phase of cocoa butter seems to adopt a similar packing. The seed crystals that initiate the slow-starting re-crystallization and results in the $\beta(V)$ phase are likely to be different from those giving the $\beta(VI)$ phase.
9.1 Introduction

Cocoa butter, a vegetable fat, is widely applied in food products. It is not only appreciated for giving the product a good food structure, but for its melting characteristics as well. Since cocoa butter shows polymorphic behaviour a conditioned crystallization process is required to obtain the desired polymorphic phase and its associated melting range. The phase behaviour of cocoa butter is very complex and is influenced by composition and thermal history. Therefore, the physical behaviour of cocoa butter and related fats has been subject of research for many years (§1.4.3).

In spite of all efforts there is still no consensus about the number and characteristics of cocoa butter phases. The nomenclature used in this paper (γ, α, β' and two β phases β(V) and β(VI)) are taken from Vaeck (1960) and Wille and Lutton (1966). Cocoa-butter phases are identified by their typical X-ray powder diffraction (XRPD) pattern in the 3−6 Å region (Fig. 9.1), the so-called “fingerprint region”, which originates from the different hydrocarbon chain packings. Since a different chain packing corresponds with a different phase, the information provided by the fingerprint region is sufficient to distinguish between the various phases. Moreover, by monitoring this small region (from 15° to 30° 2θ in XRPD with CuKα radiation) it is possible to perform real-time experiments at laboratory scale (Van Malssen et al., 1996a). Recently, also the full XRPD patterns, including peaks at long d-spacing values, were used to characterize the various cocoa-butter phases (Loisel et al., 1998; Van Gelder et al., 1996).

From the phase-transition scheme of cocoa butter crystallizing under static isothermal conditions, it is concluded that the γ phase is very unstable, that α and β’ phases are metastable and that the β phases have the highest stability (Fig. 7.5). All the phases except the stable β phases, can be obtained by crystallization from molten cocoa butter that has been heated to a sufficiently high temperature. Direct crystallization of the β phase from the melt was only observed by solidification of cocoa butter which was heated only a few degrees above its melting end point (MEP) before cooling (Van Malssen et al. 1996b and 1999), the so-called “memory effect” of cocoa butter. Van Malssen et al. (1996b) defined the β-memory point temperature (β-MPT) as the temperature at which cocoa butter in the β phase has to be heated, to prevent re-crystallization into the β phase within 45 min when cooled at 25°C. Furthermore, they found a correlation between β-MPT and the content of 1,3-distearoyl-2-oleoyl-glycerol (SOS) and stearic acid in cocoa butter. Loisel et al. (1998) discussed this correlation and stated, based on long-spacing information that was obtained under different experimental conditions, that the memory effect is correlated with the trisaturated TAG concentration. Recently, we studied the effect of the maximal temperature before re-crystallization (Tmax) and the crystallization temperature (Tcrys) on the re-crystallization behaviour of cocoa butter (Van Langevelde et al., 2000; Chapter 8). Depending on the composition of cocoa butter and on Tmax both a rapid-starting and a slow-starting re-crystallization was observed. It was concluded that the rapid-starting re-crystallization is induced by high-melting SOS-rich seed crystals. In view of this discussion we investigated crystallization and re-crystallization of cocoa butter with small-angle X-ray scattering (SAXS). Sofar, all our results have been based on XRPD studies in the fingerprint region, but the long d-spacing values may also present valuable information about the different polymorphs and their phase transitions. Time-resolved diffraction of long spacings (40 Å < d < 70 Å) is difficult to perform at our laboratory time-resolved XRPD (tr-
Cocoa-butter long spacings and the memory effect

XRPD) equipment because of the relative weak diffraction. These studies may be feasible only with high-resolution XRPD stations or SAXS stations at synchrotron sources. This study describes the long-spacing results of time-resolved diffraction experiments of Bahia cocoa butter performed at the SAXS station of the DUBBLE beamline at the ESRF in Grenoble (BM26a; Bras et al., 1998).

![Graph showing X-ray diffraction patterns for different phases of cocoa butter](image)

**FIGURE 9.1** Fingerprint region ($\lambda = 1.5418 \text{ Å}; d$-spacing values 3.0 to 6.0 Å) of the XRPD patterns of the cocoa-butter phases. The characteristic $\gamma$, $\alpha$ and $\beta'$ phases were measured of Cameroon cocoa butter after isothermal crystallization at -10.0, 0.0 and 20.0° C, respectively (Van Malssen et al., 1999). The $\beta(V)$ and $\beta(VI)$ phases were measured of the Bahia Brazil cocoa butter after isothermal crystallization at 22.0° C and from bulk material, respectively. These are the clearest X-ray powder diffraction patterns of the $\beta(V)$ and $\beta(VI)$ phases observed so far (Fig. 8.4a; Van Langevelde et al., 2000).
9.2 Materials and methods

9.2.1 Samples, sample preparation and data collection

Experiments were performed with cocoa butter originating from Bahia Brazil, which was obtained from ADM Cocoa B.V. (Koog aan de Zaan, The Netherlands). The iodine value of the cocoa butter was determined by the Wijs method (IUPAC method 2.205; Paquot and Hautfenne, 1987; Table 9.1), the triglyceride composition was determined with GLC (IUPAC method 2.323) and the fatty-acid composition with GLC via fatty-acid methyl esters (IUPAC methods 2.301 and 2.302).

Samples for temperature-controlled SAXS experiments were prepared by pressing cocoa butter in standard aluminium sample pans for differential scanning calorimetry (DSC; TA Instruments, Leatherhead, UK). To allow X-ray access to the sample, holes were punched in the pan and lid, which were covered with mica windows having a thickness of 0.025 mm (Ryan, 1993). Samples for tr-XRPD were prepared by pressing cocoa butter into a temperature-controlled sample holder resulting in a sample size of 10 x 15 x 1 mm$^3$ (~150 mg) with a flat surface.

<table>
<thead>
<tr>
<th>Table 9.1</th>
<th>Iodine value and, triglyceride and fatty acid content of the Bahia Brazil cocoa butter (Table 8.2; Van Langevelde et al., 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine value</td>
<td>40.3</td>
</tr>
<tr>
<td>Triglyceride</td>
<td></td>
</tr>
<tr>
<td>C48</td>
<td>0.2</td>
</tr>
<tr>
<td>C50</td>
<td>16.7</td>
</tr>
<tr>
<td>C52</td>
<td>45.6</td>
</tr>
<tr>
<td>C54</td>
<td>35.9</td>
</tr>
<tr>
<td>C56</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Synchrotron X-ray diffraction images were made at the SAXS station mounted on beamline BM26a of the DUBBLE CRG (ESRF, Grenoble, France; Bras, 1998). The instrument is equipped with a DSC cell to perform temperature-controlled measurements (Bras et al., 1995). The wavelength was fixed at 1.24 Å and the camera length was chosen such that the minimum observable $d$-spacing value was 40 Å. The detector was calibrated using the $d$ spacings of rat-tail collagen. The 40 Å < $d$ < 70 Å region of the SAXS images were analyzed using the programs READSAXS (Driessen, Laboratory for Crystallography, Amsterdam, The Netherlands), FIT2D (Hammersly, 1997), BSL (Bordas and Mant, CLRC Daresbury Laboratory, Warrington, United
Cocoa-butter long spacings and the memory effect

Kingdom) and XOTOKO (Bordas and Mant, CLRC Daresbury Laboratory, Warrington, United Kingdom). The standard deviation of the d-spacing values in this region is ± 0.2 Å. The data was normalized by dividing the images by the value of the second ionisation chamber. The integrated intensity $I_d(q)$ was determined by integrating the image over a quart-circle sector and multiplying the result with $q^2$. This gives a minimum observed d-spacing value of 45.6 Å for the integrated intensity. The $q^2$ correction (for geometrical considerations) is commonly used for lamellar systems (Russell, 1991). Contour plots of $I_d(q)$ vs. frame number and time were made using the program GNUPLOT v3.7 (Williams and Kelley, 1998).

Real-time XRPD patterns were taken using the tr-XRPD equipment as used in earlier studies, with the same instrumental setting (Van Malssen et al., 1996a). The use of CuKα radiation and a diffraction range of 14.6 - 29.6° 2θ resulted in an observed d range of 3.0 - 6.1 Å.

9.2.2 MEP determination and crystallization from the melt

In order to determine the melting end point (MEP) of Bahia cocoa butter, a sample was kept 20 min at each of the temperatures given in Table 9.2. The first 5 min of each of these 20 min periods was used for acclimatization and in the successive 15 min a diffraction image was collected. Each next temperature in the range was reached from the preceding one by heating 20° C min⁻¹.

Crystallization from the melt and phase transitions of cocoa butter at various pre-set solidification temperatures $T_p$ were investigated. Samples were heated to 60° C and kept at that temperature for several minutes and then cooled to 40° C at 5° C min⁻¹. Next the measurements were started whilst cooling the sample at 5° C min⁻¹ to $T_p$ and, subsequently, keeping it at that temperature for about 60 min. During cooling and the holding period images were made with a life time of 30 s and a dead time of 0.001 s.

In order to attribute the observed peaks correctly to the various cocoa-butter phases, the SAXS measurements are supported with laboratory tr-XRPD experiments that have been carried out using identical temperature profiles and an exposure time of 3 x 10 s per pattern.

9.2.3 Melting and re-crystallization experiments near the MEP

The memory effect of cocoa butter was investigated by performing re-crystallization experiments and comparing the results with the crystallization and phase transitions of the memory-free crystallization experiments. Each re-crystallization experiment was started with a freshly prepared cocoa-butter sample. Samples were taken from the bulk of which the temperature had not exceeded its MEP during the last few years. After being at 20° C for 5 min, samples were heated to a maximum temperature ($T_{max}$) with a heating rate of 5° C min⁻¹. At $T_{max}$ it was kept for 5 min, before cooling the sample at 5° C min⁻¹ to a crystallization temperature $T_{cryst}$, at which it was kept for 60 min. During the whole temperature profile measurements were made with a life time of 30 s and a dead time of 0.001 s.
9.3 Results

9.3.1 Melting end point

The first temperature $T_f$ encountered without significant diffraction intensity is considered as the MEP. At the starting temperature $T_f = 20^\circ C$ two diffraction peaks were observed ($d = 64.8$ and $47 \, \text{Å}$). The intensity of the strongest peak ($d = 64.8 \, \text{Å}$) was determined at the various $T_f$ values by subtracting the background from intensity $I$ at the top of the diffraction peak (Table 9.2). From the resulting temperature $T_f$ vs. intensity $I$ curve the MEP was determined as $35.0 \pm 0.5^\circ C$ (Fig. 9.2).

### Table 9.2 Temperature $T_f$ vs. intensity $I$ for the diffraction peak at 64.8 Å during melting of β-cocoa butter

<table>
<thead>
<tr>
<th>$T_f$ (°C)</th>
<th>$I$ (a.u.)</th>
<th>$T_f$ (°C)</th>
<th>$I$ (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>2147</td>
<td>32.0</td>
<td>556</td>
</tr>
<tr>
<td>25.0</td>
<td>2071</td>
<td>32.5</td>
<td>585</td>
</tr>
<tr>
<td>27.5</td>
<td>1885</td>
<td>33.0</td>
<td>415</td>
</tr>
<tr>
<td>28.0</td>
<td>1817</td>
<td>33.5</td>
<td>73</td>
</tr>
<tr>
<td>28.5</td>
<td>1726</td>
<td>34.0</td>
<td>145</td>
</tr>
<tr>
<td>29.0</td>
<td>1727</td>
<td>35.0</td>
<td>0</td>
</tr>
<tr>
<td>29.5</td>
<td>1597</td>
<td>35.5</td>
<td>0</td>
</tr>
<tr>
<td>30.0</td>
<td>1339</td>
<td>38.0</td>
<td>0</td>
</tr>
<tr>
<td>30.5</td>
<td>995</td>
<td>40.0</td>
<td>0</td>
</tr>
<tr>
<td>31.0</td>
<td>830</td>
<td>50.0</td>
<td>0</td>
</tr>
<tr>
<td>31.5</td>
<td>586</td>
<td>60.0</td>
<td>0</td>
</tr>
</tbody>
</table>

9.3.2 Crystallization from the melt

For the crystallization experiments the time $t$ (min) is relative to $t_0$, which is the time that the cocoa butter reaches $T_p$. Contour plots of $I_c(q)$ vs. frame number and time are shown for most crystallization experiments (Fig. 9.3).

- $T_p = 25^\circ C$  
  Within the experiment time no diffraction peaks have been observed after cooling liquefied cocoa butter to $25^\circ C$, neither in SAXS nor in XRPD.

- $T_p = 20^\circ C$  
  (Fig. 9.3a)  
  At this $T_p$ value a small diffraction peak with a $d$-spacing value of $49.6 \, \text{Å}$ was observed after 7 min. The intensity of this peak enhanced slowly until the end of the experiment time had been reached. No significant diffraction peaks were observed with XRPD.

- $T_p = 15^\circ C$  
  (Fig. 9.3b)  
  A sharp and rather intense diffraction peak at $54.9 \, \text{Å}$ was observed between $t = 1$ and $9$ min. A broad diffraction peak with a $d$-spacing value of $49.6 \, \text{Å}$ started at $t = 2$ min and increased slowly. From $t = 27$ min, its intensity decreased slowly, until it was
Cocoa-butter long spacings and the memory effect

completely gone at $t = 58$ min. Between $t = 28$ min and the end of the experiment also a broad vague band of diffraction peaks was observed around $d = 44.8$ Å. At the XRPD patterns a peak with a $d$-spacing value of about 4.1 Å was observed from $t = 1$ min. From $t = 28$ min till the end of the experiment two diffraction peaks were observed at 3.8 and $\sim 4.2$ Å, respectively. Since the 4.1 and 4.2 Å peaks were very close to each other, it was not clear whether the peak at 4.1 Å slowly disappeared while at 4.2 Å a peak appeared or whether the 4.1 Å peak slowly shifted to 4.2 Å.

$T_p = 10^\circ$ C
(Fig. 9.3c)

A sharp diffraction peak at 54.7 Å was observed between $t = -1$ and 27 min and in the same period a peak at $\sim 4.1$ Å. From $t = 3$ min a broad peak with a $d$-spacing value of 48.7 Å was observed, which became sharper near the end of the experiment. At the end of the experiment, a clear shoulder at 46 Å was observed as well. At the XRPD patterns a peak at 4.2 Å was observed from $t = 3$ min and remained till the end of the experiment. It was not clear from the XRPD patterns whether the 4.1 Å peak shifted to 4.2 Å or slowly disappeared. From $t = 31$ min till the end of the experiment also a diffraction peak at 3.8 Å was observed.

$T_p = 5^\circ$ C
(Fig. 9.3d)

At this $T_p$ value a diffraction peak at 54.8 Å was observed from $t = -1$ min, which shifted within 3 min to a $d$-spacing value of 53.8 Å and returned to its original $d$-spacing value within 12 min, at which it remained until it disappeared at $t = 38$ min. A diffraction peak with a $d$-spacing value of 51.0 Å was observed from $t = 3$ min, which broadened and shifted to a broad band of diffraction peaks with a $d$-spacing range of 45-50 Å remaining till the end of the experiment. In XRPD a peak at $\sim 4.1$ Å was observed from $t = -1$ min. Later, this peak was gone and a peak at 4.2 Å was observed remaining till the end of the experiment. From $t = 32$ min till the end of the experiment a peak at 3.8 Å was observed as well.

**FIGURE 9.2** Melting curve of β-cocoa butter from Bahia Brazil determined from the diffraction peak with a $d$-spacing value of 64.8 Å (see Table 9.2).
Figure 9.3abc Contour plots $I(q)$ vs. frame number and time of memory-free cocoa-butter crystallization. The contour levels are at 10 a.u. intervals starting at 10 a.u. The time $t$ (min) is relative to $t_0$, which is the time that cocoa butter reaches $T_p$. Cocoa butter was heated 5 min at 60°C and subsequently it was kept at 40°C before cooling at 5°C min$^{-1}$ to $T_p$ values of a) 20°C, b) 15°C and c) 10°C, respectively.
Cocoa-butter long spacings and the memory effect

FIGURE 9.3def Contour plots $I_d(q)$ vs. frame number and time of memory-free cocoa-butter crystallization. The contour levels are at 10 a.u. intervals starting at 10 a.u. The time $t$ (min) is relative to $t_0$, which is the time that cocoa butter reaches $T_p$. Cocoa butter was heated 5 min at 60°C and subsequently it was kept at 40°C before cooling at 5°C min$^{-1}$ to $T_p$ values of $d)$ 5°C, $e)$ 0°C and $f)$ -5°C, respectively.
A sharp diffraction peak with \(d\)-spacing value of 54.6 Å, observed from 5 min after start of the cooling, shifted to 53.8 Å within 3 min. At \(t = 11\) min the peak split up in two with \(d\)-spacing values of 52.6 and 55.0 Å. The intensity of the diffraction peak at 55.0 Å decreased slowly until this peak was disappeared at \(t = 54\) min. The other one broadened and remained till the end of the experiment. In XRPD a diffraction peak at \(\sim 4.1\) Å was observed in the same period as the diffraction peak at 54.6 Å. From \(t = 0\) min till the end of the experiment also diffraction peaks were observed at 4.2 and 3.8 Å.

At \(T_p = -5^\circ\) C (Fig. 9.3f) and \(T_p = -10^\circ\) C the observed (changes in the) diffraction patterns are quite similar to those at \(T_p = 0^\circ\) C, except for the peak at \(d = 55.6\) Å that was still clearly present at \(T_p = -5^\circ\) C, but that was almost disappeared at \(T_p = -10^\circ\) C.

### 9.3.3 Melting and re-crystallization experiments near the MEP

For the re-crystallization experiments the time \(t\) (min) is relative to \(t_0\), which is the time that the cocoa butter reaches \(T_{cryt}\). Before the start of each experiment a diffraction peak with a \(d\)-spacing value of 64.8 Å was observed. In Fig. 9.4 relevant contour plots of \(I_f(q)\) vs. frame number and time are shown for most re-crystallization experiments.

**\(T_{max} = 34^\circ\) C and \(T_{cryt} = 15^\circ\) C**

The intensity of the peak at 64.8 Å decreased but remained observable during heating at \(T_{max}\). Upon subsequent cooling to \(T_{cryt}\) this peak became more intense again after \(t = 1\) min.

**\(T_{max} = 34.5^\circ\) C and \(T_{cryt} = 15^\circ\) C**

Also during heating at this \(T_{max}\) value the peak with a \(d\)-spacing value of 64.8 Å did not fully disappear. This peak became more intense again 3 min after cooling to \(15^\circ\) C.

**\(T_{max} = 34.5^\circ\) C and \(T_{cryt} = 10^\circ\) C (Fig. 9.4a)**

The diffraction peak at 64.8 Å became slightly more intense between \(t = -2\) and 1 min and remained till the end of the experiment, whilst from \(t = -2\) min a diffraction peak at 49.2 Å was observed. This peak shifted and broadened within 3 min to a \(d\)-spacing value of 45.5 Å, while its intensity slowly decreased until the end of the experiment was reached. Between \(t = 0\) and 4 min also a diffraction peak with \(d\)-spacing value of 54.7 Å was observed.

**\(T_{max} = 35^\circ\) C and \(T_{cryt} = 15^\circ\) C (Fig. 9.4b)**

During heating at \(T_{max} \geq 35.0^\circ\) C no diffraction peaks were observed at all, even the peak at 64.8 Å fully disappeared at these temperatures. After heating at this \(T_{max}\) value and subsequent cooling to \(15.0^\circ\) C a diffraction peak at 49.4 Å was observed from \(t = -1\) min. This peak disappeared at \(t = 28\) min. A very broad and vague diffraction peak with a \(d\)-spacing value of 66.0 Å was observed from \(t = 4\) min and remained till the end of the experiment.
Cocoa-butter long spacings and the memory effect

$T_{\text{max}} = 35.5^\circ \text{C}$ and $T_{\text{crys}} = 15^\circ \text{C}$ (Fig. 9.4c)

Heating cocoa butter at this $T_{\text{max}}$ value and afterwards cooling to 15.0$^\circ \text{C}$ resulted in a diffraction peak observed at 49.2 Å between $t = 0$ and 51 min, a peak observed at 54.7 Å between $t = 0$ and 3 min, and a broad peak observed at 66.0 Å from $t = 16$ min till the end of the experiment.

$T_{\text{max}} = 36^\circ \text{C}$ and $T_{\text{crys}} = 20^\circ \text{C}$ (Fig. 9.4d)

A vague diffraction peak at 49.8 Å was observed from $t = 1$ min. Although the intensity of this peak was decreasing, it remained till the end of the experiment. A diffraction peak at 65.7 Å, which was observed from $t = 5$ min, remained till the end of the experiment as well, but the intensity of this peak was increasing.

$T_{\text{max}} = 36^\circ \text{C}$ and $T_{\text{crys}} = 25^\circ \text{C}$ (Fig. 9.4e)

Only one peak was observed in the re-crystallization experiment with $T_{\text{max}} = 36.0^\circ \text{C}$ and $T_{\text{crys}} = 25.0^\circ \text{C}$. This peak with a $d$-spacing value of 65.5 Å was observed from $t = 6$ min till the end of the experiment.

$T_{\text{max}} = 37^\circ \text{C}$ and $T_{\text{crys}} = 15^\circ \text{C}$ (Fig. 9.4f)

Heating at 37.0$^\circ \text{C}$ and subsequent cooling to 15.0$^\circ \text{C}$ revealed an intense diffraction peak observed at a $d$-spacing value of 54.6 Å between $t = 0$ and 8 min, a slowly increasing peak with a $d$-spacing value of 49.2 Å observable between $t = 1$ and 46 min, and a peak with a $d$-spacing value of 45.2 Å observed from $t = 15$ min till the end of the experiment.

9.4 Discussion

9.4.1 Crystallization from the melt

The starting phase of the Bahia Brazil cocoa butter used in our experiments was β(VI) (Chapter 8; Van Langevelde et al., 2000) with long spacings at 64.8 and 47 Å. The phase-transition scheme (Fig. 7.5) and XRPD patterns (compare with Fig. 9.1) support the attribution of the other diffraction peaks, which were observed in the crystallization experiments, to various cocoa-butter phases.

The diffraction peak at 53.8 Å observed for $T_p$ values of 5.0, 0.0, -5.0 and -10.0$^\circ \text{C}$ is attributed to the γ phase of cocoa butter. This phase has also diffraction peaks at 3.8 and 4.2 Å and is formed at low crystallization temperatures ($T_p < 5.0^\circ \text{C}$), as is known from mechanically-static isothermal phase-transition studies (Van Malssen et al., 1999). Remarkably, the diffraction peak at 53.8 Å was splitting up into two peaks with $d$-spacing values of ~52.7 and ~55.6 Å for $T_p$ values of 0.0, -5.0 and -10.0$^\circ \text{C}$ (Fig. 9.3def), whereas under the same conditions no changes were observed at the XRPD patterns. These observations can be explained by assuming lipid segregation to occur in the γ phase. TAG molecules with similar chain length and conformational likeliness aggregate to crystalline compounds, but without changing the lateral γ-chain packing of the TAG molecules. Lipid segregation has also been observed by others using different techniques (Dimick and
FIGURE 9.4abc Contour plots $I_d(q)$ vs. frame number and time of cocoa-butter re-crystallization. The contour levels are at 10 a.u. intervals starting at 10 a.u. The time $t$ (min) is relative to $t_0$, which is the time that cocoa butter reaches $T_{\text{crys}}$. Cocoa butter was heated 5 min at $T_{\text{max}}$ before cooling at 5° C min$^{-1}$ to $T_{\text{crys}}$. a) $T_{\text{max}} = 34.5°\ C$ and $T_{\text{crys}} = 10°\ C$, b) $T_{\text{max}} = 35°\ C$ and $T_{\text{crys}} = 15°\ C$ and c) $T_{\text{max}} = 35.5°\ C$ and $T_{\text{crys}} = 15°\ C$. 

160
Cocoa-butter long spacings and the memory effect

**FIGURE 9.4** Contour plots $I_c(q)$ vs. frame number and time of cocoa butter re-crystallization. The contour levels are at 10 a.u. intervals starting at 10 a.u. The time $t$ (min) is relative to $t_0$, which is the time that cocoa butter reaches $T_{cryl}$. Cocoa butter was heated 5 min at $T_{max}$ before cooling at 5° C min⁻¹ to $T_{cryl}$. d) $T_{max} = 36° C$ and $T_{cryl} = 20° C$, e) $T_{max} = 36° C$ and $T_{cryl} = 25° C$ and f) $T_{max} = 37° C$ and $T_{cryl} = 15° C$, respectively.
Manning, 1987; Chaiser and Dimick, 1995). In line with the observed rapid-starting re-crystallization of cocoa butter and the proposed existence of high-melting SOS-rich crystals in this Bahia Brazil cocoa butter (§8.3.3; Van Langevelde et al., 2000), one of the diffraction peaks possibly may originate from a SOS-dominated crystalline lattice.

The α phase was crystallized at $T_p$ values of 15.0, 10.0 and 5.0°C, having diffraction peaks at $d$-spacing values of 49.6, 48.7 and 51.0 Å, respectively (Fig. 9.3b-d). Therefore, it seems that the exact $d$-spacing values of the diffraction peaks of the α phase depend on the crystallization temperature. In contrast, the diffraction peak at short $d$ spacing of the α phase has a constant value of 4.2 Å. These observations may be explained by considering cocoa butter to be conglomerate of crystallites (§7.4.4). The TAG distribution of the crystallites formed at the various $T_{crys}$ values is expected to be slightly different and may result in slightly different long $d$-spacing values, but similar lateral chain packing.

The broad diffraction peak at 45 Å, its shoulder at 46 Å and the broad range of peaks between 45-50 Å which were observed at the $T_p$ values of 15.0, 10.0 and 5.0°C, respectively (Fig. 9.3b-d), are attributed to the β' phase, because under the same conditions diffraction peaks at 3.8 and ~4.2 Å were observed. Similar to earlier observations in the short $d$-spacing region (Van Malssen et al., 1999), the diffraction peaks of β' were observed at slightly different $d$-spacing values for cocoa butter crystallizing at different temperatures (§7.4.2). Therefore, these peaks support the existence of a β' phase range.

Cooling of totally-molten cocoa butter at $T_p \leq 15$°C with a cooling rate of 5°C min⁻¹ resulted in all cases in the initial formation of crystalline material with diffraction peaks at ~54.7 and 4.1 Å (Fig. 9.3b-f). Although one may be tempted to interpret this to be the α phase, it is not likely to be so because the α phase has a diffraction peak at a short $d$-spacing value of 4.2 Å and a peak at a long $d$-spacing value of about 50 Å (the exact $d$-spacing value depends on $T_p$). Upon cooling of totally-molten cocoa butter, apparently nuclei are formed with a chain-length packing of ~54.7 Å and a lateral chain packing similar to the packing of the α phase. Since these nuclei transform rapidly to the well-known cocoa-butter phases, they are only for a short time present. Although real-time X-ray diffraction was applied in our earlier studies of cocoa-butter crystallization (Chapter 7; Van Malssen et al., 1999), formation of such nuclei was never observed before. The cooling rate and composition of the cocoa butter are the only two parameters which differ from the previous experiments. Since both will influence the formation of initial seed crystals, further research is necessary to figure out what’s happening during primary nucleation of cocoa butter.

### 9.4.2 Melting and re-crystallization experiments near the MEP

It is likely that in the re-crystallization experiments with low $T_{max}$ values (34.0 and 34.5°C), cocoa-butter crystals of the original β(VI) phase of cocoa butter remained present during heating to $T_{max}$ (Fig. 9.4a), because the diffraction peak at 64.8 Å did not fully disappear and upon subsequent cooling to 15°C the cocoa butter re-crystallized rapidly in its original β(VI) phase. On the other hand, when heating cocoa butter to $35.0 \leq T_{max} < 37.0$°C the diffraction peaks of the β(VI) phase disappeared completely (Fig. 9.4b-e). Upon subsequent cooling to $15 \leq T_{crys} \leq 25$°C a diffraction peak at ~66 Å was observed. This peak, that is significantly different from the 64.8 Å peak of the
Cocoa-butter long spacings and the memory effect

β(VI) phase, is attributed to the β(V) phase. Moreover, re-crystallization in the β(V) phase started a few minutes later compared to re-crystallization of the β(VI) phase. This confirms earlier observations that the phase in which cocoa butter re-crystallizes depends on the maximum temperature before re-crystallization (Van Langevelde et al., 2000). Since the β phase was not observed at the memory-free cocoa-butter crystallization experiments (see §9.4.1), it is concluded that re-crystallization via the memory effect largely accelerates the formation of β-cocoa butter.

For some \( T_{\text{max}}-T_{\text{cryst}} \) experiments also the α phase crystallized, but only for \( T_{\text{cryst}} \leq 15^\circ \text{C} \) crystallization of the α phase started almost simultaneously with the formation of crystalline material via primary nucleation (diffraction peak at ~54.8 Å; see §9.4.1). Compared to memory-free cocoa-butter crystallization at \( T_p \leq 15^\circ \text{C} \), the formation of the α phase started at almost the same time. Apparently, the cocoa-butter crystalline material still present has to compete with the primary-nucleation process at lower \( T_{\text{max}} \) values. Only for \( T_{\text{cryst}} = 10^\circ \text{C} \) the diffraction peak at 45.5 Å of the β' phase was observed.

During heating at \( T_{\text{max}} = 37^\circ \text{C} \) all diffraction peaks disappeared (Fig. 9.4f). Subsequent cooling to 15°C resulted in the formation of an α and β' phase via primary nucleation. In this experiment \( (t < 62 \text{ min}) \) no β formation was observed. This \( T_{\text{max}}-T_{\text{cryst}} \) experiment is comparable to crystallization of memory-free cocoa butter at \( T_p = 15^\circ \text{C} \). Therefore, it is concluded that not enough crystalline material remained present for (re-)crystallization of cocoa butter in the β phase.

9.4.3 Nature of cocoa butter's memory

The results obtained from diffraction at long \( d \)-spacing values confirm that the re-crystallization behaviour of cocoa butter depends on the maximum temperature before re-crystallization (\( T_{\text{max}} \)). Since cocoa butter is considered to be a conglomerate of crystallites having individual triglyceride compositions, it is expected that the amount of remaining crystallites decreases and their composition changes with increasing \( T_{\text{max}} \) values. Our results provide information about the remaining crystallites at various \( T_{\text{max}} \) values, which are responsible for initiation of the re-crystallization process.

For the \( T_{\text{max}} \) values a few degrees Celsius above the MEP the intensity of the peak at 64.8 Å decreased only during heating and upon subsequent cooling the intensity of this peak increased rapidly (Fig. 9.4a). This peak at 64.8 Å, corresponding to the β(VI) phase of cocoa butter, originates from a crystalline lattice in which the TAG molecules are crystallized in a triple chain-length packing (Fig. 9.5). SOS molecules crystallized in the β1 phase adopt a similar packing with a long-spacing reflection of 65 Å. Therefore, these results are in agreement with our earlier interpretation that rapid-starting re-crystallization of the β(VI) phase of cocoa butter is initiated by high-melting SOS-rich crystalline material (§8.3.3; Van Langevelde et al., 2000). The crystalline lattice of the remaining seed crystals is also similar to the β(VI) phase of cocoa butter and therefore it is assumed that the packing of the β(VI) phase is dominated by the triple chain-length packing of SOS-rich crystallites.

During heating of the cocoa butter at \( 35.0 \leq T_{\text{max}} < 37.0^\circ \text{C} \) no significant diffraction peaks were observed in our experiments. It is concluded that almost all SOS-rich cocoa-butter crystallites with triple chain-length packing were molten. Since the cocoa butter has still good re-crystallization
properties crystalline seed material must be present. Since no diffraction peak at \( d = 64.8 \, \text{Å} \) was observed in our experiments, it is not likely that the slow-starting re-crystallization is initiated by seed crystals with the same compositional distribution as those present at \( T_{\text{max}} < 35.0^\circ \text{C} \). Upon cooling the cocoa butter re-crystallizes in the \( \beta(V) \) phase, which does have triple chain-length packing. However, this triple chain-length packing seems to be different from the triple chain-length packing of the \( \beta(VI) \) phase or SOS-dominated crystalline lattice, because the diffraction peak at the long \( d \)-spacing value of the \( \beta(V) \) phase (66 Å) is significantly different from the 64.8 Å peak of the \( \beta(VI) \) phase. Furthermore, also differences within the 3 – 6 Å region of the XRPD pattern were observed (Chapter 8; Van Langevelde et al., 2000).

![FIGURE 9.5](image)

**FIGURE 9.5**  
*a) Triple chain-length packing of TAG molecules. b) Double chain-length packing of TAG molecules.*

These investigations were supported by the Netherlands Foundation for Chemical Research (NWO/CW) with financial aid from the Netherlands Technology Foundation (STW). The authors thank the ESRF (Grenoble, France) and the team of Dr. Wim Bras of the DUBBLE CRG in particular for the opportunity to perform the temperature-controlled SAXS experiments at BM26. They also thank the members of the User Committee for stimulating discussions and continuous interest.

**References**


Cocoa-butter long spacings and the memory effect


