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Published in:
Acta Crystallographica. Section B-Structural Science

Citation for published version (APA):
Structures of mono-unsaturated triacylglycerols. II. The \(\beta_2\) polymorph

Jan B. van Mechelen, Rene Peschar and Henk Schenk
An improved crystal structure model has been established for the $\beta_2$ polymorph of the symmetric mono-unsaturated triacylglycerol 1,3-distearoyl-2-oleoylglycerol (SOS) and the equivalent $\beta$-V polymorph of Ivory Coast cocoa butter. In addition, the crystal structures of the $\beta_2$ polymorphs are reported for the triacylglycerols 1,3-dipalmitoyl-2-oleoylglycerol (POP) and 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS), which are, together with SOS, the major components of cocoa butter, and that of 1-stearoyl-2-oleoyl-3-arachidoylglycerol (SOA). The existence of $\beta_2$-POS and $\beta_2$-SOA has not been previously reported in the literature. All structures have been solved from high-resolution laboratory or synchrotron powder diffraction data with the direct-space parallel-tempering program FOX and refined with the Rietveld module of GSAS. All compounds crystallize in similar monoclinic unit cells ($Cc$) with very long $b$ axes (> 127 Å). The oleic chains are packed together and sandwiched between saturated chain layers, forming acyl-chain three-packs. An analysis of the $\beta_2$ polymorphs and $\beta_1$ polymorphs [van Mechelen et al. (2006). Acta Cryst. B62, 1121–1130] shows that they contain the same three-packs and differ only in the symmetry relation between the three-packs. The three-pack build-up provides an explanation of the mechanism of the phase transition that causes the formation of fat bloom on dark chocolate.

1. Introduction

The polymorphic phase transitions of fats and their constituent triacylglycerols (TAGs) in consumer products are generally unwanted because they lower the quality. Chocolate, for example, contains cocoa butter that, with the common industrial tempering process, usually crystallizes in the second-highest melting phase, the $\beta_2$ phase, which is better known as $\beta$-V. Inevitably a $\beta_2 \rightarrow \beta_1$ phase transition takes place in cocoa butter, commonly referred to as the $\beta$-V $\rightarrow \beta$-VI transition that brings forth fat bloom on chocolate. It has been hypothesized that this and other polymorphic phase transition processes involve (re-) packing of the long fatty-acid acyl chains and/or layers. To establish the precise mechanism of such phase transition processes, crystal-structure models are indispensable.

In the previous paper (Part I; van Mechelen et al., 2006) we presented the crystal structures of the $\beta_1$ polymorph of several monounsaturated TAGs and the (similar) $\beta$-VI polymorph of cocoa butter. All these models were obtained using direct-space search techniques and high-resolution laboratory and synchrotron powder diffraction data. The experience gained with the structure determination and refinement of the $\beta_1$
polymorphs led us to re-analyze an earlier reported structure determination of the β2 polymorph of SOS and the (similar) β-V polymorph of Ivory Coast cocoa butter (Peschar et al., 2004). In this paper we present a novel β2 crystal-structure model. Using high-resolution powder diffraction data, we have been able to solve the β2 polymorph structures of 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,3-distearoyl-2-oleoylglycerol (SOS) and the β-V polymorph of Ivory Coast cocoa butter. We also solved the β1 polymorph crystal structures of 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS) and 1-stearoyl-2-oleoyl-3-arachidoylglycerol (SOA). To our knowledge, the latter two are novel polymorphs whose existence has not been previously reported in the literature. The novel β2 structural model will be discussed in relation to the model of the β1 polymorphs and provide an explanation of the mechanism of the phase transition that causes the formation of fat bloom on dark chocolate.

2. Experimental methods

2.1. Samples, sample preparation and data collection

Samples of POP, SOS and POS (∼97.6%), and SOA (∼97.7%) were obtained from Unilever Research Laboratory (Vlaardingen, The Netherlands) and Unilever R&D Colworth (Sharnbrook, UK). Ivory Coast cocoa butter was obtained from ADM (Koog a/d Zaan, The Netherlands). The materials used for this publication (all racemic mixtures) were from the same batches that were used for the β1 polymorphs presented in Part I (van Mechelen et al., 2006). Samples of POP, SOS and POS, as their β1 polymorphs, were placed into glass capillaries and carefully heated up to their melting points. After melting all the solid material (established by DSC), the melt was cooled to 296 K to grow the β2 polymorph. The Ivory Coast cocoa butter was treated in the same way to obtain a pure β2 polymorph. The SOA sample was originally delivered as the 1β1 polymorph. To our surprise, after a couple of years in storage in the laboratory (most of the time in the refrigerator at 283 K), it turned out to be in the β2 phase. The β2-SOA powder was then placed in a glass capillary for data collection.

β2-POS was crystallized by the same treatment used for the growth of other β2 polymorphs. The crystallized powder, a pure β2 polymorph, was heated to just above the melting point at 313 K and subsequently cooled down to 296 K, at which temperature the β2 polymorph readily crystallized. When stored at 295 K the β2 polymorph converts at least partly to β1 over several months. To explain the unexpected crystallization of this novel β2-POS polymorph additional melt and crystallization experiments were carried out.

All samples were checked for the coexistence of more than one polymorph on the in-house X'pert Pro-alpha1 diffractometer (PANalytical, Almelo, The Netherlands) before carrying out a full data collection. The β2-POS sample was measured at the high-resolution powder station of beamline BM01b at the ESRF (Grenoble, France). For β2-POP and β2-VI-Ivory Coast, data was collected at the high-resolution powder station ID31 at the ESRF (Grenoble, France). The β2-SOS and β2-SOA samples were measured on the in-house X'pert Pro-alpha1 diffractometer. This diffractometer was equipped with a sealed Cu X-ray tube, 0.01 rad primary and secondary Soller slits and a hybrid monochromator that produces a parallel Cu Kα1 X-ray beam. The X'celerator strip detector was used at its maximum active length of 2.17°–20°.

Data collection conditions for each of the samples are given in Table 1. The capillaries were spun in all cases. The continuous scans were binned with a step size of 0.005° 2θ for the BM01b and ID31 data and a step size of 0.008° 2θ for the X'pert Pro-alpha1 data.

Some of the samples appeared to have a small impurity line of unknown origin between the first and the second reflection. This peak has been excluded from the refinement in all cases (see Table 1).

Melting points have been determined with a Linkam DSC600 (Linkam Scientific Instruments Ltd, Tadworth, UK). Samples were heated at 2 K min⁻¹. The melting points given in Table 1 are the temperatures at which the melting peaks are at a maximum. The DSC traces showed no evidence for the coexistence of more than one phase.

2.2. Indexing, model building, structure determination and refinement

The indexing of powder diffraction patterns of mono-unsaturated TAGs is a complicated process, as already explained in our publication (Part I; van Mechelen et al., 2006) about the β1 structures of SatSOat'-type compounds. For the β2 polymorphs in the present publication we did not start the indexing from scratch, but used the unit cells of the corresponding β1 structures instead. With the help of the program Chekcell (Laugier & Bochu, 2001), in all cases good indexing was found by the alternate coupling of the appropriate Miller indices to peak position markers in the observed diffraction pattern and unit-cell refinement. The most probable space group (Cc) was found with the help of the structure solution program FOX (Favre-Nicolin & Černý, 2002) in the following way. The asymmetric unit (Z-matrix description) of a TAG β1 structure was placed in the refined β2 unit cell and powder patterns were subsequently calculated for various monoclinic space groups with a fourfold general position, and compared with the experimental β2 data.

The model has been optimized in Cc with the parallel tempering mode of FOX and a Z-matrix description of the molecule. The same settings of degrees of freedom were used as in the structure solution of the β1 structures, i.e. rotation and translation of the molecule and gradual inclusion of torsion-angle flexibility at the glycerol group.

The Rietveld structure refinement was carried out with the program GSAS (Larson & Von Dreele, 1987) and the refinement strategy was essentially the same as for the β1 structures, including the application of soft distance, angle and planar

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1 Supplementary data for this paper are available from the IUCr electronic archives (Reference: DR5012). Services for accessing these data are described at the back of the journal.
Table 1

Experimental and structural details of $\beta_1$-structures.

<table>
<thead>
<tr>
<th>Crystal data</th>
<th>$\beta_1$-POP</th>
<th>$\beta_1$-SOS</th>
<th>$\beta_1$-POS</th>
<th>$\beta_1$-SOA</th>
<th>$\beta_1$-V Ivory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C$<em>{53}$H$</em>{100}$O$_6$</td>
<td>C$<em>{57}$H$</em>{108}$O$_6$</td>
<td>C$<em>{55}$H$</em>{104}$O$_6$</td>
<td>C$<em>{59}$H$</em>{112}$O$_6$</td>
<td>C$<em>{54.96}$H$</em>{108}$O$_6$</td>
</tr>
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<td>Monoclinic, Cc</td>
<td>Monoclinic, Cc</td>
<td>Monoclinic, Cc</td>
<td>Monoclinic, Cc</td>
<td>Monoclinic, Cc</td>
<td>Monoclinic, Cc</td>
</tr>
<tr>
<td>Temperature (K)</td>
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<td>298</td>
<td>250</td>
<td>298</td>
<td>280</td>
</tr>
<tr>
<td>$a$, $b$, $c$ (Å)</td>
<td>5.447 (1), 122.62 (2), 8.220 (1)</td>
<td>5.440 (1), 130.30 (1), 8.221 (1)</td>
<td>5.424 (1), 126.53 (2), 8.121 (2)</td>
<td>5.438 (1), 135.29 (1), 8.213 (2)</td>
<td>5.442 (1), 127.64 (1), 8.214 (2)</td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>88.78 (1)</td>
<td>88.75 (1)</td>
<td>88.51 (2)</td>
<td>1.36–1.8</td>
<td>88.69 (9)</td>
</tr>
<tr>
<td>$V$ (Å$^3$)</td>
<td>5825.8 (4)</td>
<td>5571.5 (4)</td>
<td>6040.1 (1)</td>
<td>5704.0 (4)</td>
<td>7004.0 (4)</td>
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<tr>
<td>Specimen size (mm)</td>
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<td>12 × 1 × 1</td>
<td>20 × 1 × 1</td>
<td>12 × 0.7 × 0.7</td>
<td>5 × 1 × 1</td>
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<tr>
<td>Specimen preparation temperature (K)</td>
<td>280</td>
<td>298</td>
<td>250</td>
<td>298</td>
<td>280</td>
</tr>
<tr>
<td>Radiation type</td>
<td>Synchrotron</td>
<td>Cu $K_\alpha$</td>
<td>Synchrotron</td>
<td>Cu $K_\alpha$</td>
<td>Synchrotron</td>
</tr>
<tr>
<td>Specimen form, colour</td>
<td>Cylinder (particle morphology: solid fat), white</td>
<td>12 × 1 × 1</td>
<td>Cylinder (particle morphology: solid fat), white</td>
<td>12 × 0.7 × 0.7</td>
<td>5 × 1 × 1</td>
</tr>
<tr>
<td>Data collection</td>
<td>Diffractometer</td>
<td>ESF ID21</td>
<td>X’pert-Alpha1</td>
<td>ESF BM01b</td>
<td>X’pert-Alpha1</td>
</tr>
<tr>
<td>$2\theta$ (°)</td>
<td>26$<em>{\min}$ = 0.009, 26$</em>{\max}$ = 58.0</td>
<td>26$<em>{\min}$ = 0.52, 26$</em>{\max}$ = 60.0</td>
<td>26$<em>{\min}$ = 0.136, 26$</em>{\max}$ = 30.48</td>
<td>26$<em>{\min}$ = 0.79, 26$</em>{\max}$ = 50.0</td>
<td>26$<em>{\min}$ = 0.003, 26$</em>{\max}$ = 77.96</td>
</tr>
<tr>
<td>Refinement</td>
<td>$R_p = 0.067$, $R_{wp} = 0.099$, $R_{F0} = 0.024$, $S = 4.37$</td>
<td>$R_p = 0.038$, $R_{wp} = 0.055$, $R_{F0} = 0.016$, $S = 3.86$</td>
<td>$R_p = 0.079$, $R_{wp} = 0.086$, $R_{F0} = 0.018$, $S = 5.07$</td>
<td>$R_p = 0.063$, $R_{wp} = 0.085$, $R_{F0} = 0.032$, $S = 2.70$</td>
<td>$R_p = 0.056$, $R_{wp} = 0.065$, $R_{F0} = 0.021$, $S = 3.15$</td>
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<td>1.54059</td>
<td>0.79948</td>
<td>1.54059</td>
<td>1.24993</td>
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<tr>
<td>Specimen preparation</td>
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<td>Constrained to parent site</td>
<td>Constrained to parent site</td>
<td>Constrained to parent site</td>
<td>Constrained to parent site</td>
</tr>
<tr>
<td>(Δ/$\sigma$)$_{max}$</td>
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<td>0.15</td>
<td>0.54</td>
<td>1.92</td>
<td>0.09</td>
</tr>
<tr>
<td>R factors and goodness-of-fit</td>
<td>$R_p = 0.067$, $R_{wp} = 0.099$, $R_{F0} = 0.024$, $S = 4.37$</td>
<td>$R_p = 0.038$, $R_{wp} = 0.055$, $R_{F0} = 0.016$, $S = 3.86$</td>
<td>$R_p = 0.079$, $R_{wp} = 0.086$, $R_{F0} = 0.018$, $S = 5.07$</td>
<td>$R_p = 0.063$, $R_{wp} = 0.085$, $R_{F0} = 0.032$, $S = 2.70$</td>
<td>$R_p = 0.056$, $R_{wp} = 0.065$, $R_{F0} = 0.021$, $S = 3.15$</td>
</tr>
</tbody>
</table>

restraints, and rigid bodies. In the model for the Ivory Coast cocoa butter the occupancy of the last two C atoms of the saturated chains was fixed at 0.5, in view of the experience with occupancy refinement of the cocoa butter $\beta_1$ models. The experimental details for all compounds are listed in Table 1. A schematic drawing of the TAG molecules with atom and chain labeling is given in Fig. 1. The preferred orientation (PO) was corrected for by the March–Dollase function (March, 1932; Dollase, 1986) for $\beta_1$-V Ivory Coast (axis: [010] ratio = 1.04 correction range: min = 0.87, max = 1.07). For the other samples no significant PO was found. The final results of the refinements are shown in the figures given in the supplementary material.

3. Results and discussion

3.1. Data collection

Fig. 2 gives an overview of the experimental diffraction patterns of all the samples, with the $2\theta$ scale being converted to the same scale ($2\theta$, Cu $K\alpha$) and ordered from top to bottom with increasing length of the $b$ axis. As for the $\beta_1$ structures, the low-angle parts of the diffraction patterns ($2\theta < 10^\circ$, Cu $K\alpha$ radiation) are dominated by the (0k0) reflections and are quite similar.

The synchrotron pattern upon which the $\beta_1$-$\beta_1$-SOS structural model (Peschar $et$ $al.$, 2004) is based has also been included in Fig. 2. In spite of its overall good quality, it has

![Figure 1](image1.png)

Figure 1

Chemical structure diagram of SatOSat'-type TAGs. The structural subscripts $m$ and $n$ (= 16, 18, 20) label the atom numbers while S1, S3g, S3m, O2g and O2m label the saturated acyl chain and the acyl chain parts g and m denote the glycerol and methyl sides, respectively.

been established that the beam-stop partly obstructed the intensity of the (010) and (020) reflections (for the cell listed in Table 1). Although this problem was recognized at that time and did not seem to influence the model seriously, our recent experience with solving \( \beta_2 \) (this paper) and \( \beta_1 \) polymorphs (Part I; van Mechelen et al., 2006) has led to the opinion that correct low-angle intensities are essential to obtain the most realistic structural model.

Fig. 3 zooms in on part of the fingerprint area of Fig. 2 and the reflections have been marked with their Miller indices. The reflections shift to lower angles with increasing length of the \( b \) axis and also change in relative intensity. This effect is most clearly visible for the two outermost reflections: on increasing the length of the \( b \) axis the intensity of the (1,13,1) reflection gradually disappears, while that of the (0,16,2) reflection increases. This effect can be attributed to a change in orientation of the chain layers relative to the crystal planes as a function of the length of the \( b \) axis.

### 3.2. Anisotropic cell-parameter contraction

Besides the room-temperature data of \( \beta_2 \)-SOS, a data set collected at 115 K at the BM01b station was also available. Unfortunately, this sample appeared to contain too much of the \( \beta_1 \) polymorph to use for structure refinement, but it was still possible to extract the \( \beta_2 \) unit-cell dimensions. A comparison of the unit-cell parameters of both data sets (Table 2) reveals an anisotropic contraction as the temperature is lowered with the second shortest (\( c \)) axis being influenced the most. This anisotropic behaviour is also the reason for the relatively small \( c \) axis of \( \beta_2 \)-POS, as these data were collected at a temperature which was 40 K lower than that of the other four samples. This difference in temperature hampers the comparison of the cell dimensions within the series.

### 3.3. The novel \( \beta_2 \)-POS and \( \beta_2 \)-SOA polymorphs

The occurrence of the novel \( \beta_2 \)-POS polymorph is attributed to the presence of a small amount of a high-melting crystalline phase in the original POS batch that probably acts as a seeding template for \( \beta_2 \) crystallization. Additional experi-
Table 2
Indexed unit-cell parameters of β\textsubscript{2}-SOS.

<table>
<thead>
<tr>
<th>Station</th>
<th>Literature†</th>
<th>Calculated</th>
<th>Literature‡</th>
<th>Indexed§</th>
<th>Indexed¶</th>
<th>Indexed∥</th>
<th>Indexed‡‡</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>BM01b</td>
<td>BM01b</td>
<td>BM01b</td>
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<tr>
<td>T (K)</td>
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<td>273</td>
<td>273</td>
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<tr>
<td>α (Å)</td>
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<td>8.211</td>
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<td>V (Å\textsuperscript{3})</td>
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<tr>
<td>M\textsubscript{20}</td>
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<td>22</td>
<td>20</td>
<td>21</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Cell parameters of refined model from Peschar et al. (2004) ‡ Transformed from cell parameters of refined model from Peschar et al. (2004) using $a' = a - a + b + c = c$ †‡ This work, indexed with the program LSQDETC (see Part I; van Mechelen et al., 2006).

Figure 4
Conformation of the β-V Ivory Coast average molecule.

3.4. Indexing, structure determination and refinement of the β\textsubscript{2} polymorphs

Surprisingly, for all β\textsubscript{2} samples discussed in this paper, the powder patterns calculated with the β\textsubscript{2} cells and β\textsubscript{1} coordinates showed a remarkable similarity with the β\textsubscript{2} patterns observed in the space group Cc, but not in other monoclinic space groups with $Z = 4$ and a single molecule in the asymmetric unit. Since the complete intensity patterns were well covered by the calculated reflection positions and complied with the space-group extinctions of Cc, this space group was taken as the most likely candidate. The similarity of the unit cells and the single molecule in the asymmetric unit in both cases suggests a close relationship between the β\textsubscript{1} and β\textsubscript{2} structures.

If the triclinic unit cell of the $P\bar{1}$ β\textsubscript{2}-SOS structure is transformed to a cell that is twice as large with $b' = -a + 2b$, an almost monoclinic cell is obtained with dimensions that are very close to those of the refined monoclinic cell of β\textsubscript{2}-SOS (Table 2), taking into account that the temperatures at data collection differed by 303 K. Before the monoclinic β\textsubscript{1} cells with the double-length $b$ axis had been found, this type of solution had not been considered for the β\textsubscript{2}-SOS and β-V cocoa butter polymorphs, in particular because the indexing figure of merit, $M_{20}$, is better for the $P\bar{1}$ solution (Table 2). It should be noted that the unit-cell parameters of the refined structure (Table 1) differ somewhat from the original values obtained in the indexing process (Table 2) and the $M_{20}$ indexing figure of merit is also lower for cell parameters of the refined structure. The reason is that in the current version of GSAS the asymmetry of the peaks can be fitted satisfactorily only if changes in the unit-cell parameters are allowed for.

A second reason why the new β\textsubscript{2} model is more plausible is that the preferred orientation in the $P\bar{1}$ model was assumed to
be present at the stage of model finding with FOX with [111] as the PO direction. Although on other occasions such an assumption has been successful, it seems that this assumed PO compensated for the too low intensity of the (111) peak in the P1 structural model and hid its imperfections.

4. The novel \( \beta_2 \) polymorph crystal-structure model

4.1. Conformation of TAGs

The conformation of the TAG molecules in the \( \beta_2 \) (and \( \beta \)-V) crystal structures is identical to that of the \( \beta_1 \) polymorphs, and to that in the previous P1 model: a flat conformation, parallel S1 and S3 chains with a gauche bend in S3 and a skew cis skew geometry at the double bond in the O2 chain. More details can be found in Part I (van Mechelen et al., 2006) and in Fig. 4, \( \beta \)-V, in which Ivory Coast is given as an example. These conformational characteristics are in full agreement with other physical data in the literature. From FT-Raman carbonyl stretching data, Sprunt et al. (2000) inferred that two of the three carbonyl groups in \( \beta_1 \)-SOS as well as \( \beta_2 \)-SOS should be in a trans conformation and one in a gauche conformation. The carbonyl conformations in our \( \beta_1 \) and \( \beta_2 \) structures are in line with the findings of Sprunt et al. (2000). The skew cis skew' conformation at the C=C bond has also been suggested by Yano et al. (1993) for both \( \beta \) polymorphs based on FT-IR results. As with the \( \beta_1 \) polymorphs, there is no significant difference between the \( \beta_2 \) conformations of symmetric and asymmetric TAGs.

In view of the resolution of the data, a detailed analysis of the glycerol torsion angles has not been carried out.

4.2. Build-up and stacking of ‘three-packs’

As in the \( \beta_1 \) polymorphs (space group \( P2_1/n \)), in the \( Cc \) space group of the \( \beta_2 \) polymorphs the same two symmetry operators \((x, y, z)\) and \((x + \frac{1}{2}, -y + \frac{1}{2}, z + \frac{1}{2})\) define a three-pack that consists of an unsaturated zone which is sandwiched by two saturated ones (Fig. 5). Within the resolution of the data, the \( \beta_1 \) and the \( \beta_2 \) polymorphs have an identical molecular conformation and three-pack build-up. The sole difference between the \( \beta_1 \) and the \( \beta_2 \) crystal-structure models resides in the different stacking of neighbouring three-packs: in \( \beta_1 \) they are related by inversion centers, while in the \( \beta_2 \) polymorph they are related by the \( Cc \) lattice-centering operator \((\frac{1}{2}, \frac{1}{2}, 0)\). The saturated chains of adjacent three-packs in the \( \beta_2 \) polymorphs are inclined towards each other and form a terrace-type methyl end-plane (Fig. 5a). The flat molecules are packed in layers and the layers of molecules of adjacent three-packs are shifted relative to each other (Fig. 5c). De Jong et al. (1991) already speculated about the existence of a simple layer-stacking difference between \( \beta_1 \) and \( \beta_2 \), although they believed that only single-crystal diffraction could give an answer.

4.3. Subcell

When the three-packs are cut in half at the C9b-C10b bond, a view parallel to the chain direction (Fig. 6) shows a good alignment of the S and O chains that together form an \( M_1 \) subcell. This subcell differs from subcells proposed for the S and O chains in the literature (Sato et al., 1989). Yano et al. (1993) judged the FT-IR data of \( \beta_2 \)-SOS to be inconclusive regarding the subcell structure and attributed this to an incomplete polarization of the \( \beta_2 \)-SOS structure. The inhomogeneity of \( \beta_2 \), also mentioned by Yano et al. (1993) although assumed to be less plausible, seems to be a more likely explanation than incomplete polarization. The reflections in the fingerprint area of the \( \beta_2 \) TAGs are always broader than those of the \( \beta_1 \), thus complying with an enhanced crystallinity of the latter.

4.4. Fingerprint area interpretation

The directions of the main crystal planes in a view parallel to the chain direction (Fig. 6) have been marked with their Miller indices and correspond to reflections in the enlarged fingerprint area (Fig. 5), except (101). The position of this latter plane (Fig. 6) is between the layers of molecules and coincides with the \( b \) axis in Fig. 5. The (111) plane that causes the \( \beta \) characteristically dominant diffraction maximum in the fingerprint area (Fig. 2, 19.5° 20) is slightly tilted relative to (101) and cuts the shifted molecular layers of two adjacent three-packs. Views along the \( a \) axis and parallel to (101) (Figs. 5a and b) show the layered packing of the individual acyl chains along the same directions as marked in Fig. 6. The fingerprint area contains two classes of reflections: \((0k2)\) with \( k = 14-20 \) and \((1k1)\) with an odd \( k \) ranging from 13 to 21. Both types of lattice planes are slightly inclined towards each other and their intensity fades away when the lattice plane direction deviates more from the chain direction.
4.5. Methyl end-plane packing and melting points

The methyl end-planes in the $\beta_1$ and $\beta_2$ structures are essentially the same, although the interaction at the end-plane between the adjacent three-packs is different owing to the parallel ($\beta_1$) and inclined ($\beta_2$) orientation of the S chains, respectively. This difference complies with the polarized-microprobe FT-IR results of $\beta_1$- and $\beta_2$-SOS (Yano et al., 1993). In $\beta_2$-SOS the $\nu_{as}(\text{CH}_3)$ stretching showed a strong and sharp polarization-dependent peak, whereas in $\beta_2$-SOS this peak was broader and less polarization-dependent.

In view of the identical molecular TAG conformation and three-pack built-up in the $\beta_1$ and $\beta_2$ polymorphs, the lower melting points of the $\beta_2$ polymorph can only be explained by stacking differences and/or reduced van der Waals interactions at the methyl end-plane. For example, in the case of the monoacid saturated TAGs, the lower melting points of the odd-numbered series members could be explained by a less dense packing at the methyl end-plane, as exemplified by their relatively larger occupiable volume (= volume large enough to fit an atomic probe; Van Langevelde, Van Malssen et al., 2001; Helmholdt et al., 2002). However, calculation of the occupiable volume at the methyl end-plane with the program Cerius$^2$ (Molecular Simulations Inc., 2000) using a probe of 1.6 Å and atomic van der Waals radii for the $\beta_1$ polymorphs (paper I) and $\beta_2$ polymorphs (this paper) of POP, SOS, POS and SOA did not result in significantly different volumes, although the lack of atomic resolution prevents us from drawing further conclusions from this observation.

An analysis of the results shows that a reduction of the melting point is accompanied by an enlargement of the longest (observable) d spacing. This observation holds for:

(i) the odd-numbered monoacid saturated TAGs versus the even-numbered ones,

(ii) the monounsaturated $\beta_2$ polymorphs discussed in this paper versus the $\beta_1$ polymorphs from Part I (van Mechelen et al., 2006) and

(iii) the asymmetric monounsaturated $\beta_1$ TAGs versus the symmetric ones (both see Part I).

It seems likely that the enlargement of the longest d spacing corresponds to a reduced interaction at the methyl end-plane, although this cannot be traced to specific enhanced contact distances. The reason is the limited resolution of the data that, as in the $\beta_1$ models, causes a poorly defined parallelism of the zigzag acyl-chain planes S1, S3 and O2g, as well as the total length of the chains.

4.6. The $\beta_2$ to $\beta_1$ phase transition

To transform a $\beta_2$ structure into a $\beta_1$ structure half of the three-packs have to be ‘flipped over’ along a line parallel to the b axis and in this transformation the inclined acyl-chain interface between the three-packs in $\beta_2$ has to change into the parallel interface found in $\beta_1$, which is energetically favourable. This ‘flip over’ does not seem easily realised in the solid state, but it is imaginable that liquidized top layers of $\beta_2$ crystals can undergo such a transition, after which they may serve as $\beta_1$ seeds. In this way, it is comprehensible that slight cyclic heating and cooling promotes the $\beta_2$ to $\beta_1$ transition. This mechanism also complies with the results of re-crystallization experiments with cocoa butter (Van Langenvelde, Peschar & Schenk, 2001): when $\beta$-VI is melted to just a few degrees above its melting point and subsequently cooled, it recrystallizes (fast) as $\beta$-VI. If the melting temperature is increased a few degrees more, upon cooling $\beta$-V recrystallizes, although much more slowly than $\beta$-VI. Melting beyond the so-called memory-point temperature does not yield any $\beta$ crystalization upon cooling but only $\beta'$ or lower melting polymorphs, depending on the crystallization temperature. Apparently, up to the memory point, the three-pack structure remains intact (dispersed) in the liquid state, but beyond this point the three-pack structures disintegrate. This mechanism is also consistent with seeding the liquid with a few tenths of a percent of a solid Sat-O-Sat’-type TAG (containing three-packs) at a temperature below the memory disappearance point, upon which the seeds ‘restore’ the original phase (Koyano et al., 1990).

The occurrence of fat bloom on dark chocolate that accompanies the $\beta$-V to $\beta$-VI phase transition can now be understood in terms of three-pack structure migration and crystallization. At the rupture surface of a bloomed piece of dark chocolate, fat bloom is visible as a thin white layer. Using a light microscope the fat bloom layer locks the dark particles that color the bulk material (see supplementary material). A possible explanation is that the cocoa butter three-packs migrate to the surface and (re-)crystallize as $\beta$-VI while the coloring particles do not migrate. A migration that is enabled by the liquid state and is stimulated by a tempering process (cyclic heating and cooling) is also in line with analyses of fat bloom on dark non-bloomed chocolate that showed similar to slightly enhanced levels of POP, POS and SOS (Lonchampt & Hartel, 2004, and references therein), the principal components of the three-packs.

5. Conclusions

The structure determination results of the $\beta_2$ polymorphs and the previously solved $\beta_1$ polymorphs point out that structural imperfections are easily masked when data lack atomic resolution or when incorrect assumptions are made with respect to physical parameters. The option to model the presence of the preferred orientation can be useful but, apparently, has to be applied very carefully. In retrospect, even if the monoclinic cells would have been available at the time for $\beta_2$-SOS and $\beta$-V cocoa butter, it is unlikely that the current model would have been found. Essentially the same problems would have been encountered as during the structure determination of the $\beta_1$ polymorphs, which was not possible without human intervention.

The three-pack built-up of both the $\beta_2$ and $\beta_1$ polymorphs provides a simple explanation for the $\beta_2$ to $\beta_1$ phase transition and (re-)crystallization experiments of cocoa butter. Also, the occurrence of fat bloom can be understood as resulting from three-pack migration to the surface. In this respect, the discovery of the novel $\beta_2$ polymorphs of POS and SOA is of
interest since their crystallization seems attributable to the presence of three-packs of higher-melting TAGs that act as template seeds.

The enlargement of the longest axis seems related to a reduction of the melting point. A reduced interaction at the methyl end-plane seems likely, but could not be attributed unequivocally to specific increased interatomic contact distances because of the resolution of the data.

Based on the indexed \(\beta_2\) and \(\beta_1\) patterns, it could be established which reflections are responsible for the \(\beta\)-characteristic diffraction maxima in the fingerprint area. As the unit-cell parameters change anisotropically as a function of temperature, with the middle axis changing the most, and the peak positions also dependent on the chain length, the fingerprint area can only be interpreted completely if a reliable indexing is available.

The authors thank ADM Cocoa NL for providing the cocoa butter sample and Unilever Research Vlaardingen and Unilever Research Colworth for the pure triacylglycerols. The authors acknowledge the ESRF (Grenoble, France) for providing the facilities to perform the synchrotron diffraction experiments. The authors thank W. van Beek (Swiss–Norwegian CRG beamline BM01b) and E. Sonneveld (UvA) for their help in the collection of the \(\beta_2\)-POS, and I. Margiolaki (ID31), K. Goubitz (UvA) and R. B. Helmholdt (UvA) for their help in collecting data for \(\beta\)-V Ivory Coast. Dr V. Favre-Nicolin is gratefully acknowledged for proving several updated \(\beta\) versions of FOX with improved functionality. The investigations have been supported by the Netherlands Foundation for Chemical Research (NWO/CW) with financial aid from the Netherlands Technology Foundation (STW: project 790.350.405). The members of the User Committee of this project are thanked for stimulating discussions and continued interest.

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