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Triacylglycerol structures and the chocolate fat bloom mechanism

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Chapter 3

Structures of mono-unsaturated triacylglycerols

Part II

The β_2 polymorph

based on *Acta Cryst.* (2006) B62, 1131-1138.

3.1 Abstract

An improved crystal-structure model has been established for the β_2 polymorph of the symmetric mono-unsaturated triacylglycerol 1,3-distearoyl-2-oleoylglycerol (SOS) and the equivalent β -V polymorph of Ivory Coast cocoa butter. In addition, the crystal structures of the β_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 β_2 -POS and β_2 -SOA has not been previously reported in 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 ($> 127 \text{ \AA}$). The oleic chains are packed together and sandwiched in between saturated chain layers, forming acyl-chain ‘three-packs’. An analysis of the β_2 polymorphs and β_1 polymorphs (van Mechelen *et al.*, 2006). 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.

3.2 Introduction

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 β_2 phase or better known as β -V. Inevitably, a $\beta_2 \rightarrow \beta_1$ phase transition takes place in cocoa butter, commonly referred to as the β -V \rightarrow β -VI transition that brings forth fat bloom on the 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 (Chapter 2; part I, van Mechelen *et al.*, 2006), we presented the crystal-structures of the β_1 polymorph of several mono-unsaturated TAGs and the (similar) β -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 β_1 polymorphs led us to re-analyze an earlier

reported structure determination of the β_2 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 β_2 crystal-structure model. Using high-resolution powder diffraction data, we have been able to solve the β_2 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 β_2 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 literature. The novel β_2 structural model will be discussed in relation to the model of the β_1 polymorphs and provide an explanation of the mechanism of the phase transition that causes the formation of fat bloom on dark chocolate.

3.3 Experimental Methods

3.3.1 Samples, sample preparation and data collection

Samples of POP, SOS and POS (purity $\sim 97.6\%$) and SOA (purity $\sim 97.7\%$) were obtained from Unilever Research Laboratories (Vlaardingen, The Netherlands) and Unilever R&D Colworth (Sharnbrook, England). Ivory Coast cocoa butter was obtained from ADM (Koog a/d Zaan, The Netherlands). The materials used for this publication (all racemic mixtures) are from the same batches that were used for the β_1 polymorphs presented in Part I (Chapter 2; van Mechelen *et al.*, 2006). Samples of POP, SOS and POS, at delivery in their β_1 polymorphs, were put in glass capillaries and carefully heated up to their melting points. After melting all solid material (established visually), the melt was cooled to 296 K to grow the β_2 polymorph. The Ivory Coast cocoa butter was treated in the same way to obtain a pure β_2 polymorph. The SOA sample was originally delivered as β_1 polymorph. To our surprise, after a couple of years of storage in the laboratory (most of the time in the refrigerator at 283 K), it turned out to be in the β_2 phase. The β_2 -SOA powder was put in a glass capillary for data collection.

β_2 -POS was crystallized with the same treatment used for the growth of other β_2 polymorphs. The original powder, a pure β_1 polymorph, was heated to just above the melting point at 313 K and subsequently cooled down to 296 K, at which temperature the β_2 polymorph readily crystallized. When stored at 295 K the β_2 polymorph converts at least partly to β_1 over several months. To explain the unexpected crystallization of this novel β_2 -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 β_2 -POS sample was measured at the high-resolution powder station of beam line BM01b at the ESRF (Grenoble, France). For β_2 -POP and β -V Ivory Coast data was collected at the high-resolution powder station ID31 at the ESRF (Grenoble, France). The β_2 -SOS and β_2 -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 $K\alpha_1$ X-ray beam. The X'celerator strip detector was used at its maximum active length of $2.17^\circ 2\theta$. Data collection conditions for each of the samples are given in Table 3.1. The capillaries were spun in all cases. The continuous scans were binned with a step size of $0.005^\circ 2\theta$ for the BM01b and ID31 data and a step size of $0.008^\circ 2\theta$ for the X'pert Pro-alpha1 data.

Some of the samples appeared to have a small impurity line from unknown origin between the first and the second reflection. This peak has been excluded from the refinement in all cases (see Table 3.1).

Melting points have been determined with a Linkam DSC600 (Linkam Scientific Instruments Ltd, Tadworth, United Kingdom). Samples were heated at 2 K min^{-1} . The melting points given in Table 3.1 are the temperatures at which the melting peaks are at maximum. The DSC traces showed no evidence for the coexistence of more than one phase.

3.3.2 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 (Chapter 2; Part I, van Mechelen *et al.*, 2006) about the β_1 structures of SatOSat'-type compounds. For the β_2 polymorphs in the present publication we did not start the indexing from scratch, but used the unit cells of the corresponding β_1 structures instead. With help of the program *CheKcell* (Laugier & Bochu, 2001), in all cases good indexing was found by 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 β_1 structure was put into the refined β_2 unit cell and powder patterns were subsequently calculated for various monoclinic space groups with a fourfold general position, and compared with the experimental β_2 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 β_1 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 β_1 structures, including the application of soft distance, angle and planar 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 β_1 models. The

Table 3.1: Experimental and structural details of β_2 -structures

	β_2 -POP	β_2 -SOS	β_2 -POS	β_2 -SOA	β -V Ivory
Chem. form.	$C_{53}H_{100}O_6$	$C_{57}H_{108}O_6$	$C_{55}H_{104}O_6$	$C_{59}H_{112}O_6$	$C_{54.96}H_{104}O_6$
M_r	833.38	889.48	861.43	917.54	861.38
Cell setting	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic
space group	Cc	Cc	Cc	Cc	Cc
$T_{\text{data coll.}}$ (K)	280	298	250	298	280
a, b, c (Å)	5.447 (1), 121.62 (2), 8.220 (1)	5.440 (1), 130.305 (1), 8.221 (1)	5.424 (1), 126.53 (2), 8.121 (2)	5.4385 (1), 135.29 (1), 8.213 (2)	5.442 (1), 127.64 (1), 8.214 (2)
β (°)	88.78 (1)	88.75 (1)	88.51 (2)	88.64 (2)	88.69 (9)
V (Å ³)	5444.1 (2)	5825.8 (4)	5571.5 (6)	6040.1 (11)	5704.0 (4)
Z	4	4	4	4	4
D_x (Mg m ⁻³)	1.017	1.014	1.027	1.015	0.997
T_m (K)	310	317	308	315	305
Radiation type	Synchrotron	Cu $K\alpha_1$	synchrotron	Cu $K\alpha_1$	synchrotron
Specimen form, colour	Cylinder: solid fat, white	Cylinder: solid fat, white	Cylinder: solid fat, white	Cylinder: powder, white	Cylinder: solid fat, yellowish white
Specimen size (mm)	5 × 1 × 1	12 × 1 × 1	20 × 1 × 1	12 × 0.7 × 0.7	5 × 1 × 1
Diffractionmeter	ESRF ID31	X'pertPro- α 1	ESRF BM01b	X'pertPro- α 1	ESRF ID31
2θ range (°)	0.50 - 58.0	0.52 - 60.0	0.136 - 30.48	0.79 - 50.0	0.50 - 57.96
R factors and goodness of fit	$R_p = 0.067$ $R_{wp} = 0.099$ $R_{exp} = 0.024$ $S = 4.37$	$R_p = 0.038$ $R_{wp} = 0.058$ $R_{exp} = 0.016$ $S = 3.86$	$R_p = 0.079$ $R_{wp} = 0.086$ $R_{exp} = 0.018$ $S = 5.07$	$R_p = 0.063$ $R_{wp} = 0.085$ $R_{exp} = 0.032$ $S = 2.70$	$R_p = 0.056$ $R_{wp} = 0.065$ $R_{exp} = 0.021$ $S = 3.15$
λ (Å)	1.24993	1.54059	0.79948	1.54059	1.24993
Excl. region(s)	No	1.56-1.864	0.985-1.075	0.65-0.685	1.36-1.8
No. of param.	505	550	525	561	548

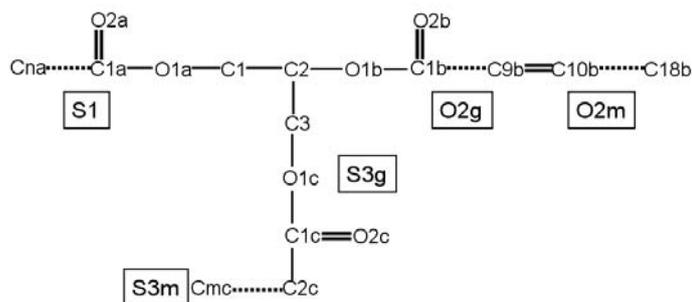


Fig. 3.1: Chemical structure diagram of SatOSat'-type TAGs. The structural subscripts m and n ($=16, 18, 20$) label the acyl chain lengths while the S1, S3g, S3m, O2g, and O2m label the saturated acyl chain and the acyl-chain parts. The subscripts g and m denote glycerol side and methyl side, respectively.

experimental details for all compounds are listed in Table 3.1. A schematic drawing of the TAG molecules with atom and chain labeling is given in Fig. 3.1. Preferred orientation (PO) was corrected for by the March–Dollase function (March, 1932; Dollase, 1986) for β -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 Figs. 3.8 –3.12 (see § 3.9 appendix).

3.4 Results and discussion

3.4.1 Data collection

Fig. 3.2 gives an overview of the experimental diffraction patterns of all samples, with the 2θ -scale being converted to the same scale (2θ , Cu $K\alpha_1$) and ordered from top to bottom with increasing length of the b axis. As for the β_1 structures, the low-angle parts of the diffraction patterns ($2\theta < 10^\circ$, Cu $K\alpha_1$ radiation) are dominated by the $(0k0)$ reflections and are quite similar.

The synchrotron pattern upon which the $P\bar{1}$ β_2 -SOS structural model (Peschar *et al.*, 2004) is based has also been included in Fig. 3.2. In spite of its overall good quality, it has been established that the beam-stop partly shielded off the intensity of the (010) and (020) reflections (for the cell listed in Table 3.1).

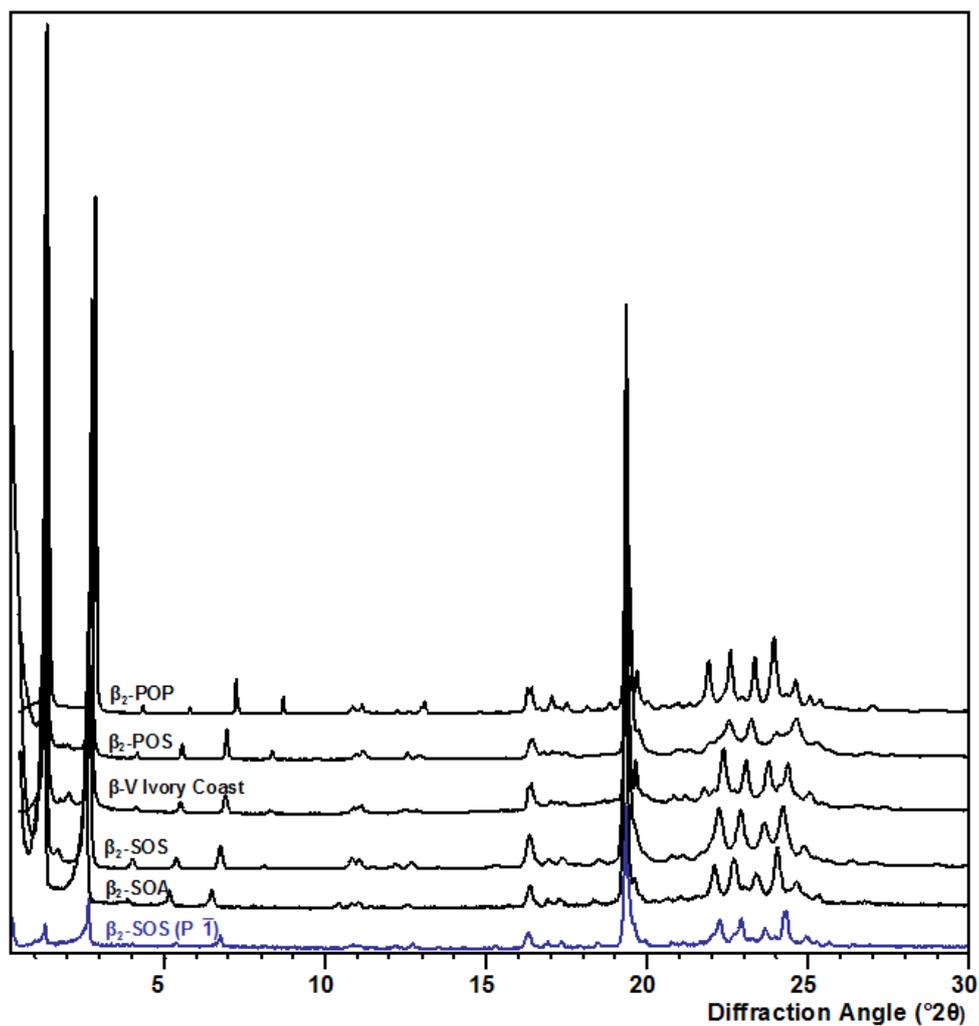


Fig. 3.2: Overview of β_2 diffraction patterns with 2θ -scale converted to Cu $K\alpha_1$ radiation. The bottom pattern was used for the structure determination of the $P\bar{1}$ model (Peschar *et al.*, 2004).

Although at that time this problem was recognized and did not seem to influence the model seriously, our recent experience with solving β_2 (this Chapter) and β_1 polymorphs (Chapter 2; 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. 3.3 zooms in on a part of the fingerprint area of Fig. 3.2 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: with increasing length of the b axis the intensity of the $(\bar{1}, 13, 1)$ 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.

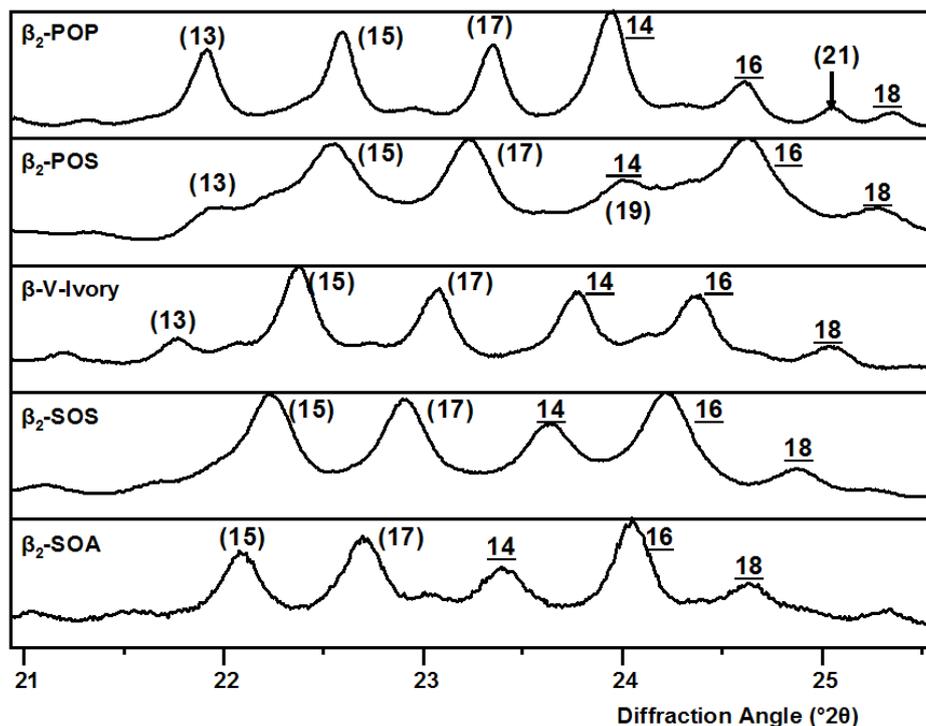


Fig. 3.3: Higher-angle part of Fig. 3.2 showing the differences between the β_2 -patterns. $(1, k, \bar{1})$ peaks are labelled (k) and $(0, k, 2)$ peaks are labelled \underline{k}

3.4.2 Anisotropic cell-parameter contraction.

Besides the room temperature data of β_2 -SOS, a data set collected at 115 K at station BM01b was also available. Unfortunately, this sample appeared to contain too much of the β_1 polymorph to be useful for structure refinement, but it

was still possible to extract the β_2 unit-cell dimensions. A comparison of the unit-cell parameters of both data sets (Table 3.2) 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 β_2 -POS (Table 3.1) 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.

Table 3.2: Indexed unit-cell parameters of β_2 -SOS

	Literature [†]	Literature [‡]	Indexed [§]	Indexed [§]	Indexed [§]	Indexed [§]
Station	BM01b	Calculated	BM01b	BM01b	X'pert	BM01b
$T_{\text{data coll.}}$ [K]	273	-	273	273	298	115
a [Å]	5.462	5.462	5.46	5.455	5.45	5.44
b [Å]	65.36	130.62	65.50	130.78	130.35	130.22
c [Å]	8.211	8.211	8.20	8.215	8.225	8.03
α [°]	89.72	89.77	89.75	90	90	90
β [°]	88.83	88.83	88.80	88.75	88.85	88.08
γ [°]	87.78	90.17	87.75	90	90	90
V [Å ³]	2928.4	5856.7	2930.	5859.	5826.	5685.
M_{20}	13		22	20	21	15

[†] Cell parameters of refined model from Peschar *et al.* (2004)

[‡] Transformed from [†] using $a' = a$, $b' = -a + 2b$, $c' = c$

[§] This work, indexed with program LSQDETC (see Part I, van Mechelen *et al.*, 2006)

3.4.3 The novel β_2 -POS and β_2 -SOA polymorphs.

The occurrence of the novel β_2 -POS polymorph is attributed to the presence of small amount of a high-melting crystalline phase in the original POS batch that probably acts as a seeding template for β_2 crystallization. Additional experiments revealed that if the POS sample is heated above 321 K, the β_2 polymorph no longer crystallizes. A possible explanation is that beyond this temperature the crystal lattice of the high-melting seeding phase is destroyed after which it remains dissolved in the POS liquid and does not crystallize as a separate

seeding phase. Fatty-acid methyl ester analyses showed the POS sample to contain 0.1% of a fully saturated C20 chain and HPLC indicated the presence of 0.3% of a C58 compound. In view of this, the most likely candidate is β_1 -AOA (C58) that has a $T_m = 321$ K (Koyano *et al.*, 1990) although this also implies that β_1 seeds can instigate β_2 crystallization. The seeding effect of the addition of a few tenths of a percent of a Sat-O-Sat type TAG to chocolate is well known (Koyano *et al.*, 1990).

The unexpected conversion of the SOA powder from the β_1 polymorph into the β_2 polymorph was a very slow process. The complete conversion took ~ 5 years. Attempts to obtain the β_1 polymorph by tempering the β_2 polymorph failed and crystallization from an acetone solution at room temperature only produced the β_2 polymorph. The surprising β_2 preference of this SOA sample may be related to the lower-melting polymorphs by impurities, as has been reported before (Lovegren *et al.*, 1976; Lovegren & Gray, 1978). More experimental work is needed to verify this hypothesis.

3.4.4 Indexing, structure determination and refinement of the β_2 polymorphs.

Surprisingly, for all β_2 samples discussed in this paper, the powder patterns calculated with the β_2 cells and β_1 coordinates showed a remarkable similarity with the observed β_2 patterns in case of in 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 suggest a close relationship between the β_1 and β_2 structures.

If the triclinic unit cell of the $P\bar{1}$ β_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_2 -SOS (Table 3.2), taking into account that the temperatures at data collection differed 30 K. Before the monoclinic β_1 cells with the double-length b axis had been found, this type of solution had not been considered for the β_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 3.2). It should be noted that the unit-cell parameters of the refined structure (Table 3.1) differ somewhat from the original values obtained in the indexing process (Table 3.2) 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_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 PO direction. Although at 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 $P\bar{1}$ structural model and hid its imperfections.

3.5 The novel β_2 polymorph crystal-structure model

3.5.1 Conformation of TAGs

The conformation of the TAG molecules in the β_2 (and β -V) crystal structures is identical to that of the β_1 polymorphs, and to that in the previous $P\bar{1}$ 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 (Chapter 2; van Mechelen *et al.*, 2006) and in Fig. 3.4, in which Ivory Coast β -V is given as an example. These conformational characteristics are in full agreement with other physical data in literature. From FT-Raman carbonyl

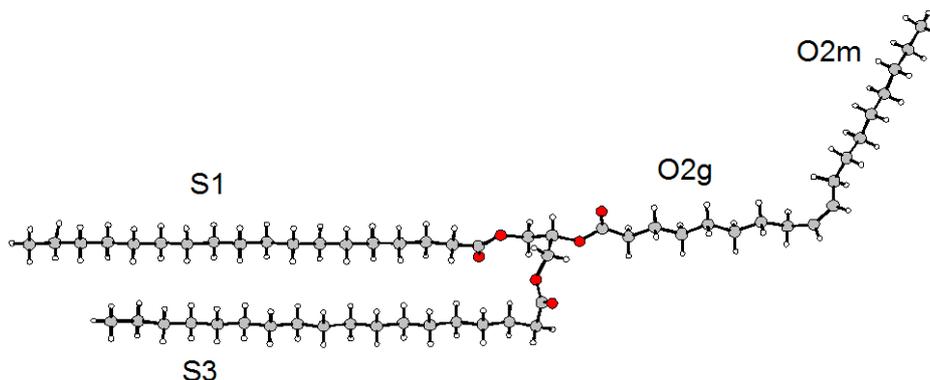


Fig. 3.4: Conformation of the β -V Ivory Coast average molecule.

stretching data, Sprunt *et al.* (2000) inferred that two of the three carbonyl groups in β_1 -SOS as well as β_2 -SOS should be in a *trans* conformation and one in a *gauche* conformation. The carbonyl conformations in our β_1 and β_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 β polymorphs based on FT-IR results. Just like in the β_1 polymorphs there is no significant difference between the β_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.

3.5.2 Built-up and stacking of 'three packs'

As in the β_1 polymorphs (space group $P2_1/n$), in space group Cc of the β_2 -polymorphs the same two symmetry operators (x, y, z) and $(x + \frac{1}{2}, \bar{y} + \frac{1}{2}, z + \frac{1}{2})$ define a 'three-pack' that consists of an unsaturated zone which is sandwiched by two saturated ones (Fig. 3.5). Within the resolution of the data, the β_1 and the β_2 polymorphs have an identical molecular conformation and 'three-pack' built-up. The sole difference between the β_1 and the β_2 crystal-structural models resides in the different stacking of neighbouring 'three-packs': in β_1 they are related by inversion centers while in the β_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 β_2 polymorphs are inclined towards each other and form a terrace-type methyl end-plane (Fig. 3.5a). The flat molecules are packed in layers and the layers of molecules of adjacent 'three-packs' are shifted relative to each other (Fig. 3.5c). De Jong *et al.* (1991) already speculated about the existence of a simple layer-stacking difference between β_1 and β_2 , although they believed that only single-crystal diffraction could give an answer.

3.5.3 Subcell

When the 'three-packs' are cut in half at the C9b=C10b bond, a view parallel to the chain direction (Fig. 3.6) shows a good alignment of the S and O chains that together form a M_{\parallel} -subcell. This subcell differs from subcells proposed for the S and O chains in literature (Sato *et al.*, 1989). Yano *et al.* (1993) judged the FT-IR data of β_2 -SOS to be inconclusive regarding the subcell structure and attributed this to an incomplete degree of polarization of the β_2 -SOS crystal. Inhomogeneity of β_2 , also mentioned by Yano *et al.* (1993) but assumed by them to

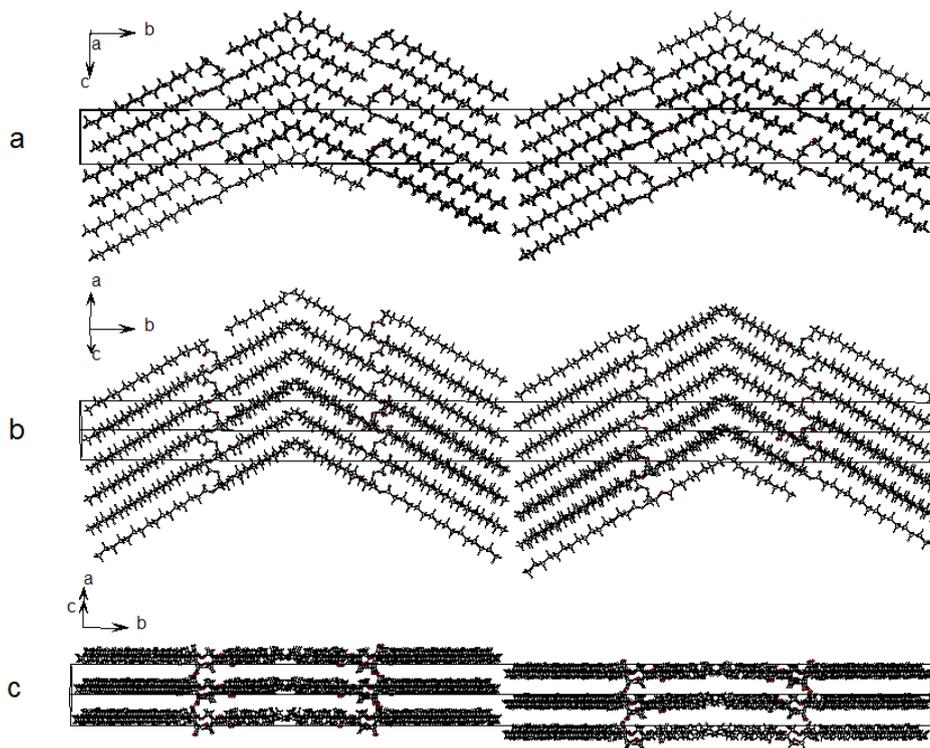


Fig. 3.5: Packing of β_2 -POS. (a) view down the a axis showing the (0 16 2) layers, (b) view parallel to $(\bar{1}01)$ showing the $(\bar{1}171)$ layers and (c) view parallel to (101) showing the shifted molecule layers.

be less plausible, seems to be a more likely explanation than incomplete polarization. The reflections in the fingerprint area of the β_2 TAGs are always broader than those of the β_1 , thus complying with an enhanced crystallinity of the latter.

3.5.4 Fingerprint area interpretation

The directions of the main crystal planes in the view parallel to the chain direction (Fig. 3.6) have been marked with their Miller indices and correspond to reflections in the enlarged fingerprint area (Fig. 3.5), except the (101) . The position of this latter plane (Fig. 3.6) is in between the layers of molecules and coincides

with the b axis in Fig. 3.5. The (111) plane that causes the β characteristically dominant diffraction maximum in the fingerprint area (Fig. 3.2, $19.5^\circ 2\theta$) 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 the $(\bar{1}01)$ (Figs. 3.5a and b) show the layered packing of the individual acyl chains along the same directions as in marked Fig. 3.6. The fingerprint area contains two classes of reflections, the $(0k2)$ with $k = 14 - 20$ and the $(\bar{1}k1)$ with an odd k ranging from 13 to 21 (Fig. 3.3). 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.

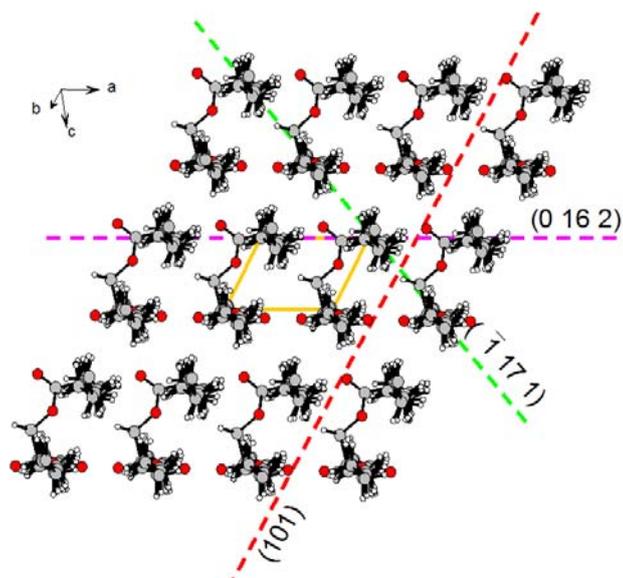


Fig. 3.6: View parallel to the chain direction of half a POS ‘three-pack’ layer cut at the double bond zone. The O and S chains are well aligned and form a M_{\parallel} subcell.

3.5.5 Methyl end-plane packing and melting points

The methyl end-planes in the β_1 and β_2 structures are essentially the same, though the interaction at the end-plane between the adjacent ‘three-packs’ is different owing to the parallel (β_1) and inclined (β_2) orientation of the S chains, respectively. This difference complies with polarized-microprobe FT-IR results of β_1 and β_2 SOS (Yano *et al.*,1993). In β_1 -SOS the ν_{as} (CH_3) stretching showed a strong and sharp polarization-dependent peak whereas in β_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 β_1 and β_2 polymorphs, the lower melting points of the β_2 polymorph can only be explained by stacking differences and/or reduced van der Waals interactions at the methyl end-plane. For example, in 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 relative larger occupiable volume (= volume large enough to fit an atomic probe) (van Langevelde *et al.*, 2001; Helmholtz *et al.*, 2002). However, calculation of the occupiable volume at the methyl end-plane with the program *Cerius²* (Molecular Simulations Inc., 2000) using a probe of 1.6 Å and atomic van der Waals radii for the β_1 polymorphs (Chapter 2; van Mechelen *et al.*, 2006) and β_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 β_2 polymorphs discussed in this paper versus the β_1 polymorphs from Part I (van Mechelen *et al.*, 2006) and
- III. the asymmetric monounsaturated β_1 TAGs versus the symmetric ones (both see Part I).

It seems likely that the enhancement of the longest d spacing corresponds to a reduced interaction at the methyl end-plane, although this can not be traced to specific enhanced contact distances. The reason is the limited resolution of the data that, just like in the β_1 models, causes a poorly defined parallelism of the zigzag acyl-chain planes S1, S3 and O2g as well as a poorly defined total length of the chains.

3.5.6 The β_2 to β_1 phase transition

To transform a β_2 structure into a β_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 β_2 has to change into the parallel interface found in β_1 , which is energetically favourable. This ‘flip over’ does not seem easily realized in the solid state but it is imaginable that liquidized top layers of β_2 crystallites can undergo such a transition after which they may serve as β_1 seeds. In this way, it is comprehensible that cycles of slightly heating

and cooling promotes the β_2 to β_1 transition. This mechanism also complies with the results of re-crystallization experiments with cocoa butter (van Langevelde *et al.*, 2001): when β -VI is melted just a few degrees above its melting point and subsequently cooled, it re-crystallizes (fast) as β -VI. If the melting temperature is increased a few degrees more, upon cooling β -V re-crystallizes, though much slower than the β -VI. Melting beyond the so-called memory-point temperature does not yield any β crystallization upon cooling but only β' 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 β -V to β -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

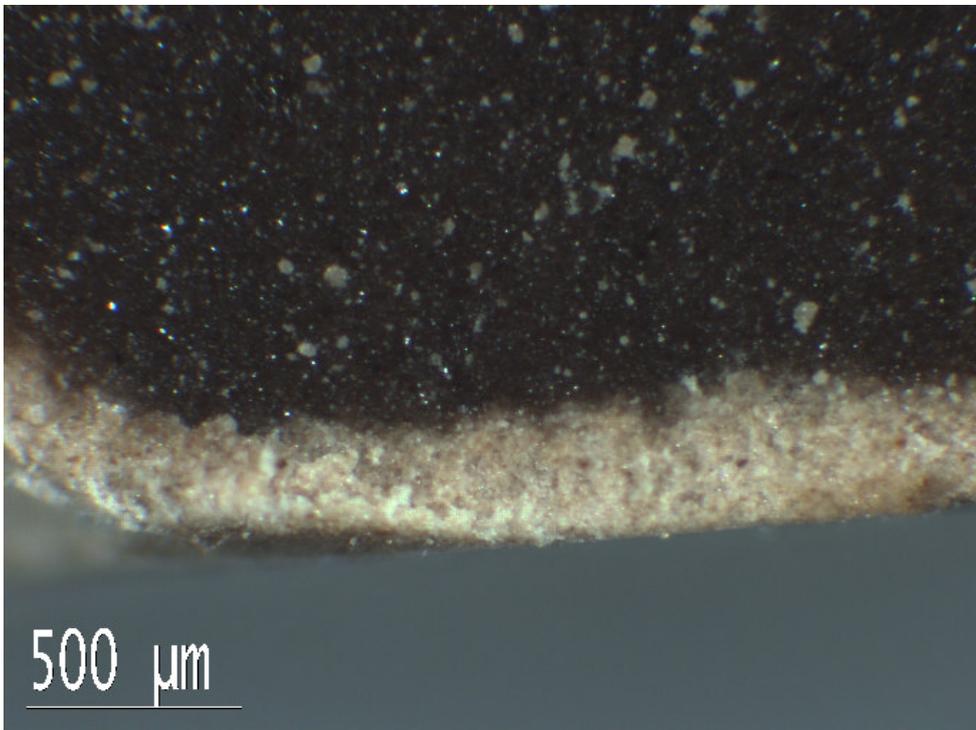


Fig. 3.7: Light microscope image of the rupture surface of bloomed dark chocolate.

chocolate, fat bloom is visible as a thin white layer. Using a light microscope the fat bloom is visible as a layer that locks the dark particles that color the bulk material (Fig. 3.7). A possible explanation is that the cocoa butter ‘three-packs’ migrate to the surface and (re-)crystallize as β -VI while the colouring 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 (Lonchamp & Hartel, 2004, and references therein), the principal components of the ‘three-packs’.

3.6 Conclusions

The structure determination results of the β_2 polymorphs and the previously solved β_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 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 β_2 -SOS and β -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 β_1 polymorphs, which was not possible without human interference.

The ‘three-pack’ built-up of both the β_2 and β_1 polymorphs provides a simple explanation for the β_2 to β_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 β_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 templating 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 β_2 and β_1 patterns, it could be established which reflections are responsible for the β -characteristic diffraction maxima in the fingerprint area. As the unit-cell parameters change anisotropically as 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.

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3.9 Appendix

Rietveld refinement results of β_2 structures

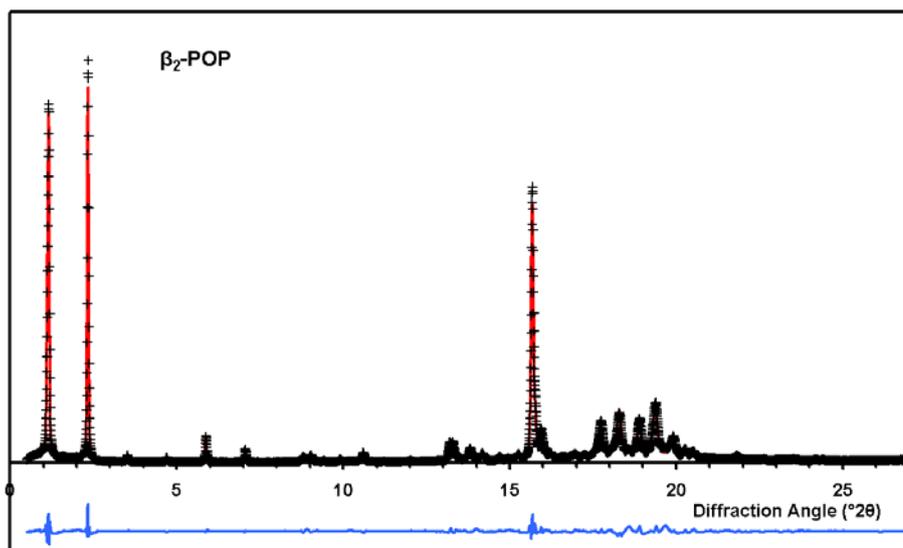


Fig.

3.8: Rietveld refinement results: Measured (+), calculated (solid) diffraction traces and the difference trace (lower curve) of the refined β_2 -structure of POP.

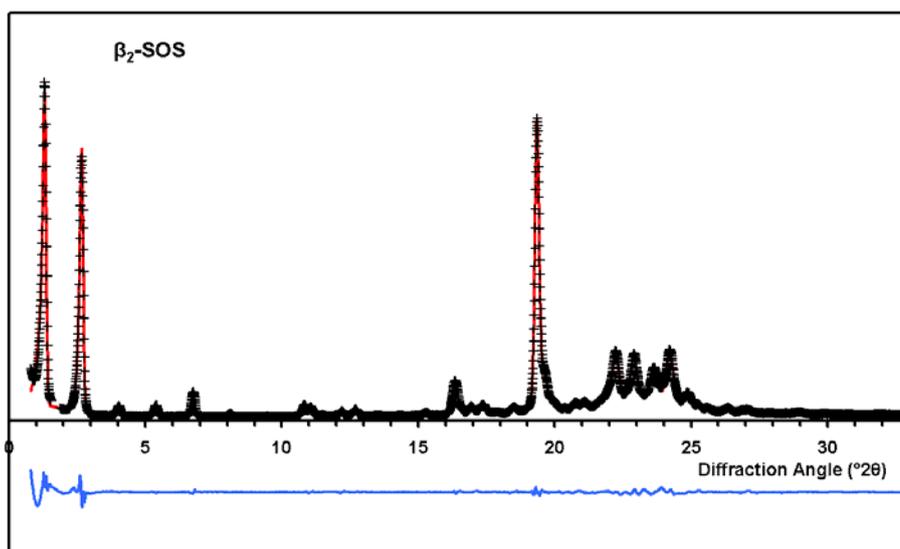


Fig. 3.9: Rietveld refinement results: Measured (+), calculated (solid) diffraction traces and the difference trace (lower curve) of the refined β_2 -structure of SOS.

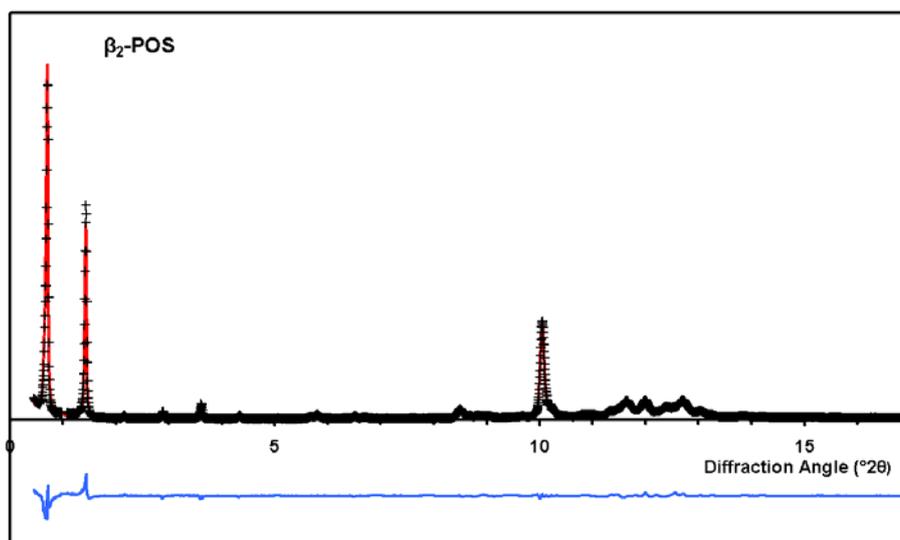


Fig. 3.10: Rietveld refinement results: Measured (+), calculated (solid) diffraction traces and the difference trace (lower curve) of the refined β_2 -structure of POS.

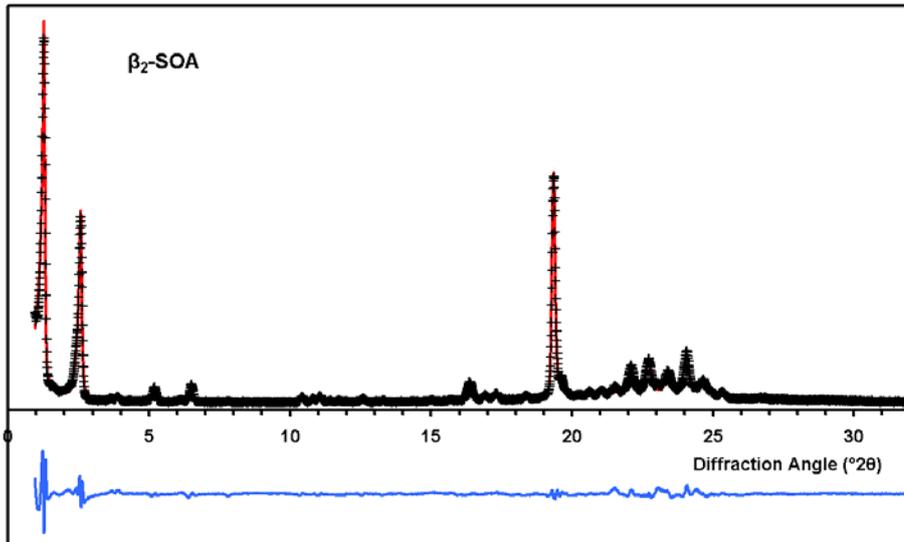


Fig. 3.11: Rietveld refinement results. Measured (+), calculated (solid) diffraction traces and the difference trace (lower curve) of the refined β_2 -structure of SOA.

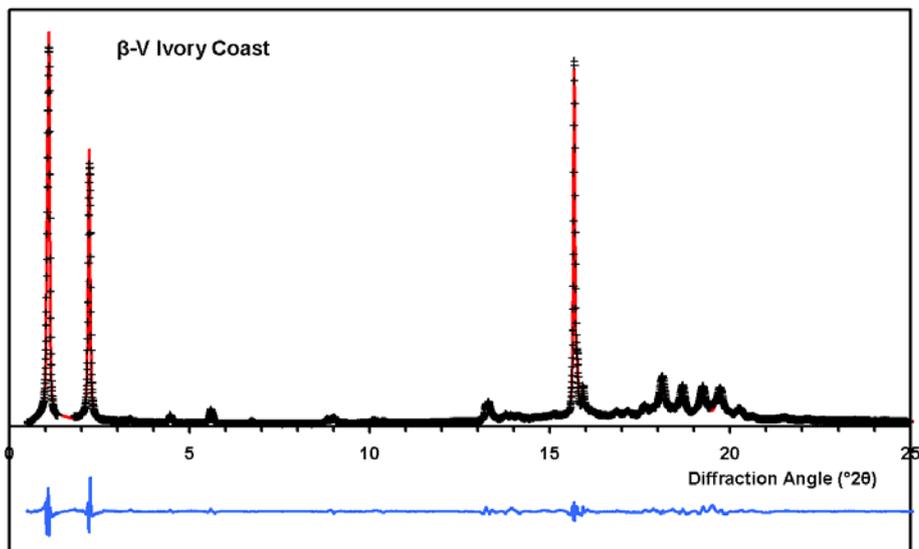


Fig. 3.12: Rietveld refinement results: Measured (+), calculated (solid) diffraction traces and the difference trace (lower curve) of the refined β -V structure of Ivory Coast cocoa butter.