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The crystal structures of the β_1 and β_2 polymorphs of mono-unsaturated triacylglycerols and cocoa butter determined from high resolution powder diffraction data

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Abstract. Using high-resolution laboratory and synchrotron powder diffraction data and direct-space search techniques, crystal structures have been solved of several pure monounsaturated triacylglycerols, and mixtures thereof, in their highest melting β_1 (= β-VI) and their highest-but-one melting β_2 (= β-V) polymorphs [1-2]. The pure triacylglycerols include two novel polymorphs whose existence has not been reported before in literature. Two of the mixtures are the commercially important β-V and β-VI polymorphs of cocoa butter, a main ingredient of chocolate. The packing of polymorphs indicates a simple mechanism for the β-V to β-VI phase transition that brings forth fat bloom on the chocolate.

Introduction

Triacylglycerols (TAGs) play a dominant role in the properties of consumer products that contain fats and oils. Both TAGs and mixtures of TAGs show polymorphism that depends on the crystallization temperature and the immediate thermal history of the material. With an increase of temperature, or much slower in course of time under isothermal conditions, phase transition processes take place in which the less stable phases melt and higher-melting phases crystallize. In this way, all lower melting phases (γ, α and β') change into the metastable β_2. The latter, however, in its turn eventually changes into the highest melting (β_1) phase [3]. Polymorphic phase transitions are generally undesirable in consumer products. Chocolate, for example, contains cocoa butter that with the common industrial tempering process usually is crystallized in the β_2 phase, better known as β-V. The inevitable $\beta_2 \rightarrow \beta_1$ transition in cocoa butter, commonly referred to as the β-V → β-VI transition, is a well-known, though not appreciated, phenomenon to the manufacturers and consumers as this transition brings forth fat bloom on the chocolate [4]. 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 transitions, crystal structure models are essential. In a previous paper we presented a crystal structure model of the β_2-polymorph of SOS and the (similar) β-V polymorph of Ivory Coast cocoa butter [5]. More recently, we also solved crystal structures of the β_1-polymorph of several monounsaturated TAGs and the (similar) β-VI polymorph of cocoa butter [1]. The experience gained with the structure determination and refinement of the β_1-polymorphs has incited us to re-examine the initial β_2 structure determination. This has led to new insight in the β_2 conformation and a novel β_2 structure model has been obtained for symmetric mono unsaturated TAGs [2].

Experimental

Pure TAG samples of 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,3-distearoyl-2-oleoylglycerol (SOS), 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS) and 1-stearoyl-2-oleoyl-3-arachidoylglycerol (SOA) were obtained in the β_1 form from Unilever Research Laboratorium (Vlaardingen, The Netherlands) and Unilever R&D Colworth (Sharnbrook, UK). The samples were loaded in borosilicate glass capillaries for data collection. The Ivory Coast cocoa butter sample, obtained from ADM (Koog a/d Zaan, The Netherlands), was crystallized in the β_1 form directly in a capillary. All β_2 samples have been crystallized from the smelt in a capillary. Most of the samples have been measured at the high resolution powder diffraction beam lines at ESRF (BM01b, ID31) and the X'pert Pro α_1 diffractometer provided by PANalytical was also employed for additional measurements (β_2-SOS, β_2-SOA, β-VI-ivory coast cocoa butter). Further details about the sample preparation and data collection are reported elsewhere [1, 2].

Indexing, structure solution and refinement

With the standard autoindexing programs such as Treor, Dicvol, ITO and McMaille no satisfactory solution could be found due to dominant zones and peak overlap problems. The β_1 polymorphs have been indexed with the program LSQDETC [6] by a systematic search for unit cells in a limited solution space. The obtained solutions were verified with program Chekcell [7]. The monoclinic unit cell has Z=4 which limits the most probable space groups to those with a general position with a multiplicity of 4. The acentric groups from this selection could be excluded by systematic extinctions. From the remaining centric space groups the correct space group was selected by a trial and error procedure. The β_1 polymorph was solved in $P2_1/n$ with the program FOX [8]. The degrees of freedom in the structure solution process correspond to the dihedral angles around the central glycerol moiety and around the double bond in the oleic chain as well as the rotation and translation of the whole molecule.
Indexing of the β_2 polymorph samples was done with the program Chekcell. Starting from the cell of the β_1 polymorph and by connecting reflection markers to the appropriate calculated reflections the correct solution was found. By changing the space group of the β_1 polymorph in FOX from $P2_1/n$ to Cc a calculated diffraction trace was generated that turned out

to be similar to the β_2 trace. The β_2 polymorph has been solved in this space group using program FOX with the same degrees of freedom considered for the β_1 polymorph.

The GSAS software [9] was used for structure refinements. H-atoms were involved in Rietveld analysis. Application of planar, distance and angle restraints was necessary for reliable refinements. Tables 1 and 2 summarize the structural results of the β_1 and β_2 polymorphs respectively. As an example for both of the sample series the diffraction patterns of β_1-SOA and β_2-SOA are shown in figure 1.

Table 1. Summary of β_1 refinement results.

	β_1-POP	β_1-SOS	β_1-POS	β_1-SOA	β-VI-Ivory
station	BM01B	BM01B	BM01B	BM01B	X'PertPro
λ [Å]	0.79889	0.75003	0.75003	0.85019	1.54059
a [Å]	5.450(1)	5.442(1)	5.445(1)	5.439(1)	5.448(1)
b [Å]	121.32(1)	129.90(1)	125.98(2)	134.65(2)	126.71(1)
c [Å]	8.209(1)	8.184(2)	8.195(1)	8.199(1)	8.201(2)
β[°]	88.85(1)	88.71(1)	88.79(1)	88.73(1)	88.61(1)
V [Å^3]	5426.6(5)	5784.1(5)	5620.3(3)	6003.7(4)	5648.5(1)
Rp	6.37	5.52	6.16	6.97	4.3
Rwp	8.61	6.84	7.06	8.79	5.91
GOF	6.02	3.66	2.64	3.45	5.09
ρ (g/cm^3)	1.02	1.021	1.018	1.015	1.00
Form.	$C_{53}H_{100}O_6$	$C_{57}H_{108}O_6$	$C_{55}H_{104}O_6$	$C_{59}H_{112}O_6$	$C_{55}H_{108}O_6$

Table 2. Summary of β_2 refinement results.

	β_2-POP	β_2-SOS	β_2-POS	β_2-SOA	β- V-Ivory
Station	ID31	X'pert	BM01B	X'pert	ID31
λ [Å]	1.249926	1.54059	0.79948	1.54059	1.24993
a [Å]	5.447(1)	5.440(1)	5.424(1)	5.438(1)	5.442(1)
b [Å]	121.62(2)	130.30(1)	126.53(2)	135.29(1)	127.64(1)
c [Å]	8.220(1)	8.221(1)	8.121(2)	8.213(2)	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(4)	6040.1(1)	5704.0(4)
Rp	6.65	3.82	7.9	6.31	5.41
Rwp	9.85	5.84	8.57	8.45	6.52
GOF	4.37	3.86	5.07	2.7	3.14
ρ (g/cm^3)	1.017	1.014	1.027	1.015	1.008
Form.	$C_{53}H_{100}O_6$	$C_{57}H_{108}O_6$	$C_{55}H_{104}O_6$	$C_{59}H_{112}O_6$	$C_{55}H_{108}O_6$

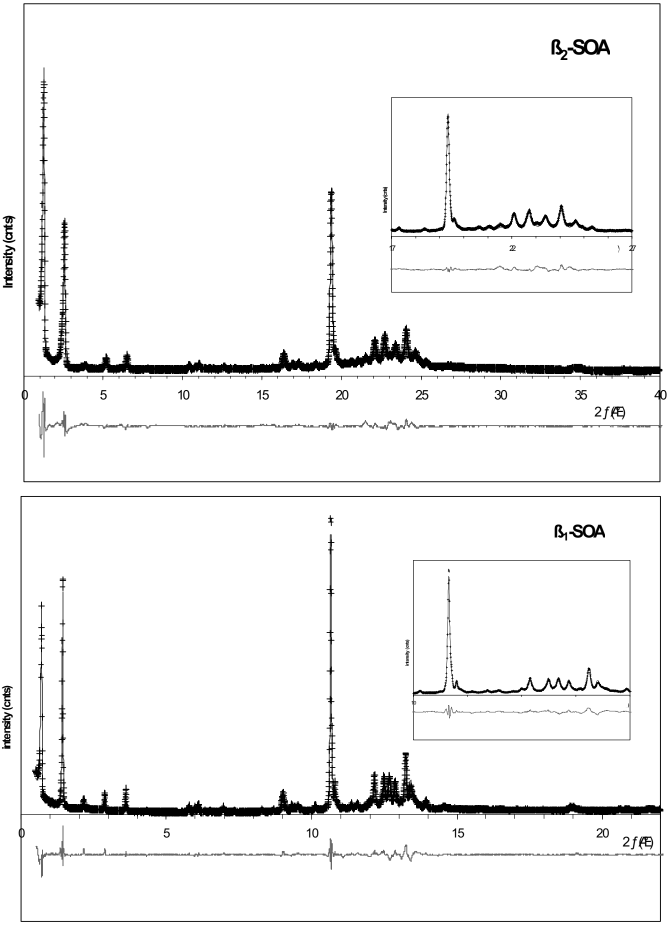

Figure 1. Final Rietveld refinements of β_2-SOA (top) and β_1-SOA(bottom).

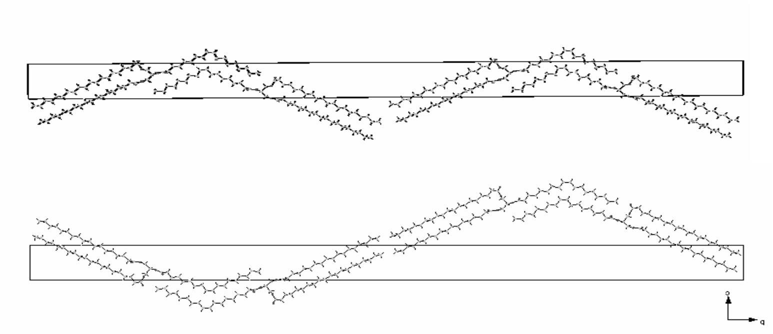

Figure 2. Comparison of the molecule packing in β_2-SOA (top) and β_1-SOA(bottom).

Discussion

The molecules in both polymorphs have the expected conformation [3,10,11] with the two saturated chains parallel and the mono unsaturated oleic chain pointing in the opposite direction. When viewed along the short axis (figure 2), the packing of the molecules consists of a sequence of six acyl-chain layers or, more precisely, of two sets of tri-acyl chain layers, from now on referred to as three-packs, which are symmetry related. Each three-pack consists of an unsaturated zone, in which the oleic chains are packed parallel and side-by-side, that is sandwiched by two saturated zones. Interestingly, Filer et al. [12] had proposed the existence of a six-acyl chain packing with saturated and unsaturated chain zones as early as 1946. The three-packs are identical in both β_1 and β_2 polymorphs. The difference between the two polymorphs is in the symmetry relation between the three-packs. In β_1 the three-packs are related by an inversion centre whereas in β_2 they are related by a translation (½,½,0). This relation indicates a simple explanation of the mechanism responsible for the formation of fat bloom on chocolate. The underlying phase transition from β_2 (=β-V) to β_1 (=β-VI) is just a rotation of 180° of one three-pack relative to the other one along the longest axis of the unit cell as can easily be seen in figure 2.

Conclusions

More than 60 years after discussion in the literature started about how the β structure(s) of monounsaturated triacylglycerols could look like [12] we have been able to solve the structures of the β_1 and β_2 polymorphs from high resolution powder diffraction data. Our structural results for the two polymorphs suggest a simple mechanism responsible for the fat bloom formation in chocolate.

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