

1 Mixing phase behavior of tripalmitin and oleic-rich
2 molecular compound-forming triacylglycerols

3
4

5 Jorge Macridachis*, Laura Bayés-García and Teresa Calvet

6
7

8 *Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra,*
9 *Universitat de Barcelona, Martí i Franquès s/n, E-08028 Barcelona, Spain.*

10 * Correspondence: jmacridachis@ub.edu; Tel.: +34-934021350.

11
12
13
14

15 Keywords: polymorphism; crystallization; triacylglycerol, molecular compound; mixing
16 behavior

17 **ABSTRACT**

18

19 The binary phase behavior of triacylglycerol (TAG) mixtures constituted by PPP (tripalmitin)
20 and either POP (1,3-dipalmitoyl-2-oleoyl-glycerol), OPO (1,3-dioleoyl-2-palmitoyl-glycerol),
21 or MC_{POP/OPO} (molecular compound formed by POP/OPO at a 1:1 concentration ratio) was
22 determined by differential scanning calorimetry (DSC), synchrotron (SR-) and laboratory-scale
23 X-ray diffraction (XRD). Steric hindrance due to saturated-unsaturated acyl chain interactions
24 and differences in the thermal stability of TAGs seemed to dictate the independent polymorphic
25 crystallization and transformation observed under kinetic conditions, as well as the very
26 asymmetric eutectic-type phase diagrams obtained from the study of thermodynamically
27 stabilized PPP/POP, PPP/OPO, and PPP/MC_{POP/OPO} mixtures. Moreover, the solid integration
28 determined for MC_{POP/OPO} in the β_{PPP} crystalline phase (20-25%), halfway between that of OPO
29 (<5%) and POP (30-35%), showed the ability of molecular compounds to tune the
30 thermophysical properties of fat systems. This highlights the impact that molecular compound-
31 forming TAGs may have on the efficient industrial separation of lipids, and suggest their
32 potential applicability in the development of edible fat blends with tailored functionalities.

33 1. INTRODUCTION

34

35 The quality of several end-products delivered by the pharmaceutical, cosmetic, and food
36 industries is largely determined by lipid crystallization processes, which are performed during
37 their manufacture¹. More concretely, macroscopic properties such as melting behavior and
38 rheology are highly dependent on the constituent edible fats and oils through the polymorphism
39 (typically α , β' and β forms) and mixing phase behavior of their major TAGs.^{2,3} Therefore,
40 studies on the crystallization and polymorphic behavior of TAGs and their mixtures, with
41 particular emphasis on the molecular interactions (formation of eutectic, miscible or molecular
42 compound phases) that take place, are essential to gain a deeper understanding of the physical
43 properties of complex lipid systems.^{4,5}

44 In the food industry, unveiling the solid-state miscibility properties of TAGs becomes essential
45 for applications such as the fractionation of edible fats and oils or the improvement of the
46 mechanical and sensory properties of food products through fat structuring.⁶ Extensive research
47 carried out at a molecular level has made possible to relate the complex fractionation of palm oil
48 to the wide sort of mixing interactions that take place between its component TAGs.⁷⁻¹⁰ In this
49 connection, the prevailing eutectic behavior between palm oil major TAGs has been thoroughly
50 examined by differential scanning calorimetry (DSC) and thermodynamic modeling in a recent
51 report on the kinetic phase behavior of PPP/POP, PPP/OOP, and POP/OOP mixtures (with P
52 and O being palmitic and oleic fatty acids, respectively).¹¹ As to fat structuring applications, the
53 stable β' phases formed by eutectic PSS/PSP mixtures (with S being stearic acid) at all
54 concentration ratios, as well as by PPP and PSP or PSS at delimited composition ranges, have
55 been suggested as suitable hardeners for specific food applications.^{12,13}

56 The formation of either miscible or eutectic phases between TAGs strongly depends on their
57 degree of similarity regarding structural and thermal properties. Thus, complete solid-solution
58 phases have been reported for SOS/POS and SOS/SLiS systems (with SLiS being linoleic
59 acid),^{14,15} whereas mixtures of tri-saturated and saturated-unsaturated mixed-acid TAGs
60 typically exhibit immiscible behavior, with a eutectic composition which greatly depends on

61 their degree of molecular similarity. This was revealed in the stable phase diagrams of
62 SSS/OOO and PPP/OOO mixtures, for which a highly asymmetric eutectic composition and a
63 very low solubility of OOO in the tri-saturated TAGs below 10% could be estimated.^{16,17} The
64 miscibility in the high-melting TAG showed to be higher in PPP/OOP and PPP/POP eutectic
65 mixtures, indicating the key role of molecular resemblance on TAGs compatibility.^{7,18,19}
66 The influence of TAGs molecular mismatch on the symmetry of the eutectic composition was
67 further clarified by thermodynamically stabilized binary mixtures constituted by POP, PPO,
68 OPO, and OOP. The stable diagram of OPO/OOP showed to be eutectic at the 50/50
69 composition, whereas the higher difference in melting temperature between TAGs due to an
70 additional palmitic acid moiety caused the shifting of the eutectic point to a composition at 70-
71 80% OOP in POP/OOP mixtures, and above 90% OPO in the PPO/OPO system.¹⁹⁻²¹
72 Interestingly, a simple change in a palmitic acid *sn*- position in POP/OPO mixtures resulted in
73 the formation of a molecular compound at a 1:1 concentration ratio with its own unique
74 structural and thermodynamic properties.²² Equivalent mixing behavior was observed in
75 POP/PPO mixtures, as well as in their stearic acid-based counterparts, as a result of the specific
76 stabilizing interactions occurring between mixed-acid TAGs with a particular molecular
77 symmetry.^{9,23,24} Moreover, the ability of molecular compound-forming binary systems to form
78 stable β -2L (MC) ternary solid solutions has been recently reported for SOS/SSO/OSO
79 mixtures.²⁵
80 There is a growing interest to unveil the polymorphic crystallization and transformation
81 properties of molecular compound-forming TAGs due to their applicability in edible fats
82 structuring.^{26,27} The development of functional lipid blends through TAG-based agents results
83 advantageous given the wide range of possibilities in terms of physical properties that confer
84 TAGs chemical nature and processing conditions.²⁸ However, the desirable thermal, structural,
85 and functional properties provided by saturated TAGs difficult the development of suitable
86 healthier alternatives. In line with this, the increase in melting point, solid fat content, and
87 hardness displayed by blends of POP- and OPO-rich fat fractions have shown the potential of
88 molecular compound crystals as partial replacers of *trans* and saturated fats in lipid systems.²⁹

89 Therefore, with a view on the development of healthier oleic-rich TAG structuring agents, it
90 becomes relevant to gain a deeper molecular insight into the mixing interactions between fully
91 saturated high-melting TAGs and saturated-unsaturated TAGs, both in the pure form and
92 forming molecular compounds.

93 In connection with the above, the present work describes the phase behavior of binary and
94 ternary mixtures including PPP, POP, and OPO subjected to a long period of thermal
95 stabilization. The melting behavior of the mixtures was examined by DSC, whereas the
96 polymorphic identification could be attained by laboratory-scale X-ray diffraction (XRD). Phase
97 diagrams of PPP/OPO, PPP/POP, and PPP/POP/OPO, with POP and OPO at a 1:1 ratio to allow
98 complete $MC_{POP/OPO}$ formation, were compared on the basis of molecular-structure derived
99 properties. Additional experiments on selected mixtures, by using synchrotron radiation X-ray
100 diffraction (SR-XRD), were carried out in order to assess the polymorphic behavior in the
101 metastable state. The results here reported may be of practical interest in the engineering of
102 novel functional lipid mixtures in which molecular compound-forming TAGs may naturally
103 occur as a result of the combination of diverse vegetable oils, such as palm oil fractionation
104 products.

105

106 **2. MATERIALS AND METHODS**

107

108 **2.1. Sample preparation**

109

110 PPP (purity >99%), POP (99%), and OPO (99%) were purchased from Larodan AB (Solna,
111 Sweeden) and used without further purification.

112 Binary mixtures of PPP/POP and PPP/OPO at different molar mass intervals were prepared by
113 mixing both TAGs at 85 °C (temperature far above the melting point of the high melting TAG)
114 and stirring using a vortex mixer. To ensure the homogeneity of the samples, the heating-
115 stirring step was performed for several minutes, followed by a rapid cooling process of the
116 mixtures.

117 As for the ternary system, mixtures of PPP, POP, and OPO were prepared by keeping a constant
118 1:1 molar ratio of POP and OPO to allow the complete crystallization of both TAGs in the form
119 of a molecular compound. For this, an equimolecular stock mixture of POP and OPO was
120 prepared in the first place by mixing both components at 50 °C (for simplification, this mixture
121 will be termed as MC_{POP/OPO} in this work). Then, different molar mass fractions of the former
122 mixture were blended with PPP by following the same procedure as in binary systems.
123 To investigate the phase behavior of the PPP/OPO, PPP/POP, and PPP/MC_{POP/OPO} systems in
124 most stable polymorphs, melted mixtures were first held one week at an incubation temperature
125 of 40 °C to facilitate the crystallization of the most stable form of PPP.⁷ Then, the temperature
126 was gradually decreased to 12 °C for the PPP/OPO system, and to 27 °C for the PPP/POP and
127 PPP/MC_{POP/OPO} systems, at which mixtures were kept for several months (four in the case of
128 PPP/OPO, and six in PPP/POP and PPP/MC_{POP/OPO}) until polymorphic stability could be
129 experimentally confirmed.

130

131 **2.2. Differential Scanning Calorimetry (DSC)**

132

133 Crystallization and polymorphic transformations of the samples were determined at atmospheric
134 pressure using a PerkinElmer DSC-Diamond under a nitrogen flow of 20 cm³·min⁻¹. Melting
135 temperature and enthalpy of indium and n-decane standards were used for the calibration of the
136 equipment. An empty pan was used as a reference.

137 TAG samples were weighed (4.0-4.4 mg) into 50 µL aluminum pans and hermetically sealed.

138 For each system, after obtaining the thermodynamically most stable polymorphs, the melting
139 behavior of at least three independent samples (n=3) of the incubated mixtures was measured by
140 heating the samples from 0 to 80 °C at 2 °C·min⁻¹.

141 To evaluate the miscibility properties of PPP/OPO and PPP/MC_{POP/OPO} systems in the metastable
142 state, mixtures at a 1:1 ratio were subjected to different thermal treatments based on the
143 application of rapid (15 °C·min⁻¹) and intermediate (2 °C·min⁻¹) rates of cooling and heating.

144 Samples (n=3) were first held at 80 °C for several minutes to ensure its complete melting, then

145 cooled to a temperature far below the end of crystallization (-80 and -30 °C for PPP/OPO and
146 PPP/MC_{POP/OPO} mixtures, respectively) and reheated to 80 °C. DSC curves were processed by
147 Pyris software to obtain the onset (T_{onset}), peak top (T_{top}) and end (T_{end}) temperatures (°C), and
148 enthalpy (ΔH , J·g⁻¹) of the main phenomena observed. As the calibration of the equipment was
149 performed at 2 °C·min⁻¹, a correction described elsewhere³⁰ was applied to the samples
150 subjected to other rates. To attain a 95% confident interval, the standard error was estimated
151 using the Student's method approximation. In the present work, thermal events detected by DSC
152 will be defined by their characteristic T_{top} .

153

154 **2.3. X-ray diffraction experiments**

155

156 The same thermal processing implemented in DSC was applied to X-ray diffraction experiments
157 with both laboratory-scale (XRD) and synchrotron radiation (SR-XRD) sources in order to
158 attain polymorphic identification.

159 Laboratory-scale XRD measurements were carried out by using a PANalytical X'Pert Pro MPD
160 powder diffractometer equipped with a hybrid monochromator and a PIXcel detector. The
161 equipment operated with Debye-Scherrer transmission and temperature control was achieved
162 through an Oxford Cryostream Plus 220V (temperature 80-500K). The samples were introduced
163 in a 1 mm diameter Lindemann glass capillary, which was rotated about its axis in the course of
164 the experiment to minimize preferential crystalline orientation. The step size was 0.013° from 1°
165 to 28° 2 θ , and the measuring time 150 s per step. Diffraction data were analyzed with X'Pert
166 Highscore V2.2e software.

167 For kinetic experiments involving high cooling and/or heating rates (15 °C·min⁻¹), SR-XRD
168 measurements were carried out at beamline BL11-NCD-SWEET at the ALBA synchrotron
169 (Cerdanyola del Vallès, Barcelona, Spain) at 12.4 keV. The distance between the sample and the
170 detector was 2.2 m. X-ray scattering data were collected on a Pilatus 1M detector with a pixel
171 size of 172 X 172 μm^2 for the small-angle X-ray diffraction data (SR-SAXD) and on a LX255-
172 HS Rayonix detector with a pixel size of 44 X 44 μm^2 for the wide-angle X-ray diffraction data

173 (SR-WAXD). An aluminum cell with Kapton film windows was used to hold 2mm-thick
174 sample during the measurements, whose temperature was controlled by a Linkam stage. The q -
175 axis calibration was obtained by measuring silver behenate for SR-SAXD and Cr_2O_3 for SR-
176 WAXD. SR-XRD spectra were acquired at 20 s or 30 s intervals, depending on the cooling and
177 heating rates applied. The program pyFAI was used to integrate the 2D SR-WAXD into the 1D
178 data; the SR-SAXD data were processed with in-house software. Data analysis was performed
179 using Igor Pro V6.3.7.2 software.

180

181 **3. RESULTS**

182

183 **3.1. Phase behavior of PPP/OPO mixtures**

184

185 **3.1.1. Phase behavior of incubated PPP/OPO binary mixtures**

186

187 The mixing phase behavior of PPP/OPO mixtures was examined after a period of
188 thermodynamic stabilization consisting of a week at 40 °C, followed by four months at 12 °C.
189 The laboratory-scale XRD patterns obtained at 0 °C for all the compositions confirmed that
190 thermodynamic stability was achieved (Figure 1A). The small-angle reflection at 4.1 nm was
191 ascribed to β -2L (PPP) through the corresponding wide-angle peaks at 0.46, 0.45, 0.40, 0.38,
192 0.37, and 0.36 nm (denoted by ■), whereas the triple chain-length structure peak at 3.2 nm was
193 assigned to the stable β_1 -3L of OPO (wide-angle peaks at 0.46, 0.45, 0.39, 0.38, and 0.37 nm,
194 denoted by ▼ in pure OPO sample).

195 Diffraction data agreed with the thermal behavior displayed by PPP/OPO mixtures during
196 heating from 0 to 80 °C at a rate of 2 °C·min⁻¹ (Figure 1B). Pure PPP and OPO showed single
197 well-defined endothermic signals at 67.8 °C and 22.4 °C, respectively, whereas the thermal
198 profile of the mixtures evidenced the successive melting of the stable eutectic phases formed by
199 both TAGs.

200 As illustrated in the PPP/OPO binary phase diagram constructed from the T_{top} of the
201 endothermic events observed by DSC (Figure 2), the melting temperature of β_1 -3L (OPO)

202 remained practically constant at around 22-23 °C for the full set of samples (see Table S1 in
203 supporting information). By contrast, melting peaks of PPP showed lower T_{top} and more
204 asymmetric shape towards the pure OPO sample due to the solvent-like behavior of the already
205 molten mixed-acid TAG. The great asymmetry of the eutectic point became clear through the
206 heating thermogram of the 10PPP/90OPO composition, in which the weak melting peak of β -2L
207 (PPP) ($\sim 16 \text{ J}\cdot\text{g}^{-1}$) was still identified at T_{top} of 54.2 °C, far above that of β_1 -3L (OPO) melting
208 (see the enlarged image in Figure 1B).

209 In PPP-rich mixtures, the presence of β_1 -3L (OPO) could not be confirmed by laboratory-scale
210 XRD in compositions at PPP content above 85%. Nevertheless, the endothermic phenomenon at
211 T_{top} of 22.9 °C detected by DSC in the 90PPP/10OPO composition, and the barely noticed one
212 at a close temperature in the 95PPP/5OPO mixture, suggested that the solubility limit of OPO in
213 PPP lied below 5%.

214

215 **3.1.2. Polymorphic behavior of PPP/OPO mixtures under kinetic conditions**

216

217 The study of TAG mixtures under the influence of dynamic temperature variations becomes
218 essential to gain a clear picture of their complex polymorphic behavior. Moreover, solid-state
219 miscibility is often enhanced in TAG systems when metastable forms are involved. To evaluate
220 the mixing behavior of PPP and OPO in the metastable state, additional DSC and X-ray
221 diffraction experiments were carried out on the 50PPP/50OPO mixture during two different
222 thermal treatments: 1) cooling and heating at $15 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$, and 2) cooling and heating at
223 $2 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$. The overall polymorphic behavior is depicted in Figure 3A.

224 The first DSC exothermic event displayed by the mixture when cooled at $15 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ (peak
225 with a shoulder at T_{top} of 32 °C in Figure 3B) corresponded to the crystallization of α -2L (PPP)
226 (small- and wide-angle peaks at 4.5 and 0.41 nm in SR-XRD patterns of Figure 3C) and β' -2L
227 (PPP) (peaks at 4.2, 0.45, 0.42, and 0.38 nm). Then, the following occurrence of β' -2L (OPO)
228 crystals (DSC peak at -8 °C and new SR-XRD reflections at 4.3 and 0.39 nm) confirmed the
229 immiscible behavior of these TAGs in the orthorhombic subcell packing. The polymorphic

230 behavior shown by OPO in combination with PPP contrasts with the α crystallization at T_{top}
231 below $-20\text{ }^{\circ}\text{C}$ reported for the pure mixed-acid TAG when a similar thermal protocol was
232 applied.³¹
233 During the subsequent heating at the same rate, the first melt-mediated process observed by
234 DSC at around $-2\text{ }^{\circ}\text{C}$ was due to the β' -2L (OPO) \rightarrow β_1 -3L (OPO) transformation (new SR-XRD
235 reflections at 6.3 and 0.46 nm clearly seen at $7\text{ }^{\circ}\text{C}$). Soon after, the most stable form of OPO
236 melted (endothermic peak at $23\text{ }^{\circ}\text{C}$), and metastable forms of PPP (α and β') transformed into
237 the β -2L polymorph (strong β -characteristic wide-angle peaks and new small-angle reflection at
238 4.0 nm), which finally melted at $64\text{ }^{\circ}\text{C}$.
239 As could be expected, the decrease in the cooling rate to $2\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ resulted in higher
240 crystallization temperatures and the occurrence of more stable polymorphic forms. The first
241 occurrence of β' (PPP) and β (PPP) was followed by the concurrent crystallization of β_1 -3L, β_2
242 (with undefined reported chain-length structure), and β' -2L forms of OPO (see corresponding
243 thermal and X-ray diffraction data in Figure S1 of supporting information), which during
244 heating did not exhibit substantial changes as compared to the mixture cooled at $15\text{ }^{\circ}\text{C}$. The
245 initial β' -2L (OPO) \rightarrow β_1 -3L (OPO) transition and the subsequent consecutive melting of β_2 and
246 β_1 forms of OPO was finally followed by the stabilization of PPP in β -2L crystals before the
247 whole sample became liquid.

248

249

250 **3.2. Phase behavior of incubated PPP/POP mixtures**

251

252 The work carried out by *Minato et al.*⁷ on PPP/POP mixtures, which were subjected to a two-
253 week thermodynamic stabilization process, confirmed the eutectic nature of the system and the
254 limited solubility of POP in PPP at around 40-50%. In the present study, the mixing behavior of
255 PPP/POP mixtures at specific concentration intervals was re-examined after six months of
256 thermal incubation in order to more precisely analyze the solubility limits of the binary system.

257 Diffraction patterns of thermodynamically stabilized PPP/POP mixtures obtained at 10 °C
258 confirmed the presence of β -2L (PPP) at all compositions (■ in Figure 4A). As to the pure POP
259 sample, the small-angle peak at 3.0 nm and wide-angle peaks at 0.46, 0.45, 0.40, 0.38, 0.37, and
260 0.36 nm (denoted by ♦) indicated the presence of the stable β ₁-3L (POP) form. However, the
261 emerging peak at 0.39 nm in the 5PPP/95POP composition (pointed by an arrow) suggested the
262 presence of β ₂-3L (POP),^{32,33} and evidenced that, even after 6 months, not all POP in the
263 mixtures was in the most stable polymorph. This seems to be due to the complicated
264 stabilization shown by symmetric saturated-*cis*-unsaturated components in binary mixtures of
265 TAGs.^{9,22–24} For simplification, POP β forms are referred to as β -3L (POP).
266 The distinct polymorphism and melting behavior displayed by the components of the mixtures
267 during their heating treatment (clarified in Figure 4B) were in agreement with published data, as
268 shown by the phase diagram depicted in Figure 5. In our work, however, the 60PPP/40POP
269 composition showed a clear DSC endothermic event associated with POP melting at around
270 38 °C (see detailed melting data in Table S2). Furthermore, a weak signal consisting of a double
271 endothermic peak (T_{top} of 31 and 36 °C) was still detected in the 65PPP/35POP mixture. Despite
272 this, no evident changes were observed in β -3L (POP) diffraction peaks during the thermal
273 processing before its complete melting (data not shown). Probably, some metastable POP was
274 still present at such a high concentration level of PPP, resulting in the first DSC endothermic
275 peak before the melting of β -3L (POP) crystals. Consequently, the amount of POP that showed
276 to be able to integrate into the crystalline phase of PPP was reduced to 30-35% in the present
277 study, which could be explained in part by the longer time allowed for the mixtures to stabilize.

278

279 **3.3. Phase behavior of PPP and POP/OPO molecular compound system**

280

281 **3.3.1. Phase behavior of incubated PPP/MC_{POP/OPO} mixtures**

282

283 Figure 6A shows laboratory-scale X-ray diffraction patterns of incubated PPP/MC_{POP/OPO}
284 mixtures obtained at 10 °C. Structural similarities between stable β -2L forms of PPP and

285 $MC_{POP/OPO}$ caused significant difficulties in the differentiation of both polymorphs by only
286 considering wide-angle X-ray diffraction data, especially in mixtures in which one of the
287 components was at a very low concentration. In the same manner, the similar long spacing
288 values determined (4.2 and 4.1 nm) resulted in the overlapping of diffraction peaks, preventing
289 the accurate detection of low-intensity peaks. Then, in order to deliver a more precise data
290 analysis, 003 reflections of the small-angle region were also used to support our observations.
291 For pure $MC_{POP/OPO}$ sample, small-angle peaks at 4.2 nm and its 003 reflection at 1.4 nm were
292 associated with the most stable β -2L ($MC_{POP/OPO}$) form together with its strong characteristic
293 wide-angle peaks at 0.46, 0.44, 0.41, 0.40, 0.38, 0.37 and 0.36 nm, \blacklozenge), which is in agreement
294 with previous work.^{22,26} DSC melting data obtained during the heating treatment of $MC_{POP/OPO}$ at
295 $2\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ also confirmed the only presence of this form through a single endothermic event at
296 around $33\text{ }^{\circ}\text{C}$ (DSC heating thermograms and peak top melting temperatures for all the mixtures
297 are gathered in Figure 6B and Table S3, respectively).

298 At the low addition of 5% PPP, the melting peak of β -2L ($MC_{POP/OPO}$) was followed by a second
299 endothermic event at around $50\text{ }^{\circ}\text{C}$ (see the enlarged image in Figure 6B). No discernible
300 changes were observed in the wide-angle XRD pattern at $10\text{ }^{\circ}\text{C}$. However, a low-intensity
301 shoulder appeared on the small-angle peak at 4.2 nm (see the enlarged image in Figure 6A).
302 Furthermore, a new low-intense one showed up at 1.3 nm, very close to the β -2L ($MC_{POP/OPO}$)
303 003 reflection at 1.4 nm. In the pure PPP XRD pattern, this reflection was associated with β -2L
304 (PPP), together with its characteristic β wide-angle peaks (\blacksquare) and the single 001 reflection at
305 4.1 nm. Except for the pure $MC_{POP/OPO}$ sample, the former peaks were detected in XRD patterns
306 at all the mixtures, evidencing the immiscible nature of PPP and $MC_{POP/OPO}$.

307 At the 15PPP/85 $MC_{POP/OPO}$ composition, the presence of PPP could also be confirmed by the
308 occurrence of a new peak near that of $MC_{POP/OPO}$ at 0.38 nm (arrow in the corresponding wide-
309 angle pattern). Simultaneously, the weak shoulder detected at low concentrations of PPP
310 increased its intensity, exhibiting a clear d -spacing of 4.1 nm at 20% PPP. A further increase in
311 the PPP content caused a broadening in diffraction peaks due to the overlapping of the
312 reflections of both polymorphic forms in the diffraction patterns, more noticeable in those at

313 higher angles. Furthermore, close peaks at d -spacing around 0.38 nm became a wider single
314 one, making both polymorphs indistinguishable in mixtures at 40% PPP and above. From this
315 mixture on, again the concurrent presence of both polymorphic forms could only be confirmed
316 by small-angle data.

317 Regarding the thermal behavior of β -2L ($MC_{POP/OPO}$), the mixtures exhibited melting
318 endotherms at temperatures slightly lower than the single peak observed in the pure $MC_{POP/OPO}$
319 sample, as shown by the corresponding DSC curves. In mixtures up to 50% PPP, this
320 temperature remained above 30 °C. However, in the PPP-rich region, the peak top temperature
321 dropped to 29 °C at the 65PPP/35 $MC_{POP/OPO}$ composition. At this composition, a weak peak at
322 1.4 nm, associated with β -2L ($MC_{POP/OPO}$), was still detected in the small-angle XRD patterns,
323 although the signal seemed to be lost in mixtures of 70%-75% PPP. Despite this, we concluded
324 that some non-solubilized $MC_{POP/OPO}$ was still present at the 75PPP/25 $MC_{POP/OPO}$ composition,
325 according to the subtle endothermic peak still noticeable in the corresponding DSC thermogram
326 in a similar temperature range to that of other samples (see amplification in Figure 6B). This
327 highlights the importance of the combination of DSC and X-ray diffraction techniques for the
328 greater accuracy of studies involving TAGs crystallization and phase behavior.

329 As for PPP, a substantial increase in the melting temperature of β -2L (PPP) from the mixture at
330 5% PPP towards the equimolecular composition was clearly noticed (difference of ~15 °C).
331 Although not so markedly due to the decrease in the amount of liquid media surrounding β -2L
332 (PPP) crystals, this tendency continued until reaching the pure PPP sample. As a result, the
333 corresponding phase diagram (Figure 7) evidenced a similar behavior of the liquidus line
334 compared to that observed in the PPP/OPO and PPP/POP systems.

335 To sum up, mixtures of PPP with POP and OPO at a concentration ratio allowing the formation
336 of a stable molecular compound showed eutectic phase behavior after six months of thermal
337 incubation. Moreover, according to DSC and X-ray diffraction data, PPP exhibited no solubility
338 in $MC_{POP/OPO}$, whereas the latter was able to integrate into the crystalline phase of PPP up to 20-
339 25%, a value that lies between that reported in the present work for pure OPO and POP in PPP.

340

341 **3.3.2. Polymorphic behavior of 50PPP/50MC_{POP/OPO} mixtures under kinetic conditions**

342

343 To observe the polymorphic occurrence and miscibility properties of the PPP/MC_{POP/OPO} system
344 under metastable conditions, DSC and SR-XRD experiments were conducted on mixtures at
345 equimolecular composition during a thermal process consisting of cooling at different rates (15
346 and 2 °C·min⁻¹) and subsequently heating at a constant rate of 2 °C·min⁻¹. The sequence of
347 polymorphic events detected under these conditions is clarified in Figure 8A.

348 At a cooling rate of 15 °C·min⁻¹, two main strong exothermic signals at T_{top} of 33 and 10 °C
349 were observed by DSC (Figure 8B). According to SR-XRD data (Figure 8C), the first α -2L
350 (PPP) crystallization (small- and wide-angle peaks at 4.5 and 0.41 nm) was then followed by the
351 occurrence of β -2L (MC_{POP/OPO}) forms (small-angle peak at 4.2 nm and wide-angle peaks at 0.46
352 and 0.38 nm). This molecular arrangement of MC_{POP/OPO} in the stable β -2L form seemed to be
353 favored by the presence of the tri-saturated TAG since α -2L (MC_{POP/OPO}) forms would be
354 expected to crystallize under the fast cooling conditions applied.²²

355 During the subsequent heating at 2 °C·min⁻¹, no clear polymorphic events could be discerned by
356 DSC before the endothermic peak detected at around 30 °C. However, SR-XRD patterns
357 between 20 and 25 °C showed an increase in the intensity of small- and wide-angle signals at
358 4.1 and 0.38 nm, respectively, at the expense of those associated with α -2L (PPP). Thus, it
359 became clear that the α -2L (PPP) \rightarrow β' -2L (PPP) transformation was the first one to occur. The
360 endothermic event was then ascribed to the subsequent melting of β (MC_{POP/OPO}) crystals. Since
361 the characteristic β peak at 0.46 nm did not completely disappear in diffraction patterns until the
362 end of the heating treatment, we concluded that the further β' -2L (PPP) \rightarrow β -2L (PPP)
363 transformation (better defined β -like wide-angle peaks before final melting) initiated at a close
364 temperature, likely promoted by the presence of molten mixed-acid TAGs in the mixture.

365 As expected given the rapid stabilization in β -2L (MC_{POP/OPO}) shown by the mixed-acid
366 components upon fast cooling, except for the favored occurrence of β' -2L (PPP) crystals, the
367 polymorphic and mixing behavior displayed by the 50PPP/50MC_{POP/OPO} mixture when cooled

368 at 2 °C·min⁻¹ resulted practically similar (the corresponding DSC and X-ray diffraction can be
369 found in Figure S2).

370

371 **4. DISCUSSION**

372

373 Miscibility properties of binary mixtures of TAGs strongly depend on their polymorphism,
374 chain-length structure, and melting behavior. Furthermore, the saturated-unsaturated fatty acids
375 proportion and position in the glycerol backbone may promote or hinder specific interactions
376 between TAGs.² Thus, the phase behavior displayed by PPP/OPO, PPP/POP, and
377 PPP/MC_{POP/OPO} mixtures may be explained on this basis.

378 In agreement with previous reports on mixtures of tri-saturated and saturated-unsaturated
379 mixed-acid TAGs,^{7,16,17} stabilized PPP/OPO, PPP/POP, and PPP/MC_{POP/OPO} mixtures displayed
380 eutectic phase behavior with limited solubility of oleic-rich TAGs and MC_{POP/OPO} in the
381 crystalline phase of PPP. Furthermore, the higher melting temperature of β -2L (PPP) (T_{top} of ~
382 68 °C) as compared to β ₁-3L (OPO), β ₁-3L (POP), and β -2L (MC_{POP/OPO}) (22, 38, and 33 °C,
383 respectively) even in the compositions at the lowest PPP concentration (up to 10 °C higher),
384 pointed the eutectic equilibrium of the three mixture systems in a concentration range very near
385 to 0% PPP. Previous research on binary mixtures of TAGs with different configurations (tri-
386 saturated/tri-saturated, tri-saturated/saturated-unsaturated, and saturated-unsaturated/saturated-
387 unsaturated)^{7,21,34} evidenced the shifting of eutectic points towards pure lower-melting
388 components at larger differences in thermal stability between TAGs.

389 The ability of chemically different TAGs to exchange crystallographic positions at all
390 concentration ratios to form binary solid solutions is favored by a close melting point and a high
391 degree of isomorphism of the components¹⁷, as reported for SOS/POS and SOS/SLiS binary
392 systems.^{14,15} This kind of mixing behavior is prevented in PPP/OPO, PPP/POP, and
393 PPP/MC_{POP/OPO} mixtures due to the great steric hindrance between the palmitoyl chains of PPP
394 and the oleoyl chains of the mixed-acid TAGs (see molecular models³⁵ in Figure 9), which
395 ultimately limits the formation of mixed-crystals to PPP-rich mixtures. Likewise, the formation

396 of molecular compounds in these systems appears highly unlikely, since strong affinitive
 397 aliphatic interactions (unsaturated-unsaturated, saturated-saturated) between components seem
 398 to be a requisite for the occurrence of molecular compounds including saturated-unsaturated
 399 mixed-acid TAGs.^{9,22–25,36}

400 Concerning the miscibility areas delimited in the PPP-rich side of the phase diagrams
 401 (summarized in Table 1), melting temperatures of polymorphic forms involved in PPP/OPO,
 402 PPP/POP, and PPP/MC_{POP/OPO} systems seemed to have a major role in their extent. However,
 403 the contribution of molecular geometry should not be disregarded. DSC data on PPP/OPO
 404 mixtures confirmed a very low solubility of OPO in PPP below 5%, whereas up to 10-20% OOP
 405 was able to integrate into the β crystalline phase of the tri-saturated TAG.^{8,11} In this connection,
 406 binary mixtures of tri-saturated TAGs also showed a positive relationship between the
 407 asymmetric *sn*- distribution of fatty acids in one mixed-acid component and increased solid-
 408 state miscibility.³⁷ As for POP, the solubility limit in PPP lied at about 35%, a value slightly
 409 lower than that reported for PPP/POP mixtures after two weeks of thermodynamic
 410 stabilization.⁷ Both OPO and POP stabilized in a β -3L form, but the exchange of oleic acid by
 411 palmitic acid in POP increased thermal stability of the TAG molecule and reduced the number
 412 of bent sites due to unsaturation, which may facilitate its insertion in the crystal lattice of PPP
 413 through a reduction of steric hindrance.

414

415 **Table 1.** Solubility of saturated-unsaturated mixed-acid TAGs in β -2L (PPP) crystals.

416

TAG	Solubility in PPP	Reference
OOP	10-20%	[8, 11*]
OPO	<5%	Present work
POP	40-50%	[7]
	30-35%	Present work
MC _{POP/OPO}	20-25%	Present work

417

* Miscibility observed without a preceding stabilization process.

418

419 In PPP/MC_{POP/OPO} mixtures, up to 20-25% of the mixed-acid components were solubilized in
 420 solid PPP. By considering the low miscibility of OPO, which led to DSC thermal signals even at
 421 the 95PPP/5OPO composition, these results suggest that neither POP nor OPO was

422 preferentially adsorbed in PPP/MC_{POP/OPO} mixed crystals. Therefore, it is assumed that, after
423 stabilization, the saturated-unsaturated mixed-acid TAGs were probably embedded closely
424 packed in the crystal lattice of PPP. This would be facilitated by the strong specific interactions
425 occurring between POP and OPO molecules, which also showed to be unaffected by solute-
426 solvent interactions in the presence of up to 98% *n*-dodecane.³⁸

427 The integration of POP and OPO in the form of MC_{POP/OPO} could explain in part the different
428 solubility of OPO and MC_{POP/OPO} in PPP. Along with the increased melting point of MC_{POP/OPO},
429 the high degree of isopolymorphism of MC_{POP/OPO} and PPP (both with stable β -2L forms) might
430 favor miscibility through a reduction of crystal lattice distortion. Still, the presence of two oleic
431 fatty acid moieties in OPO is expected to cause a greater increase in the free energy of mixed
432 crystals than the single one of POP, which could explain the lower integration of MC_{POP/OPO} in
433 the β crystalline phase of PPP compared to that of POP (Figure 10). Nevertheless, further
434 precise analysis on TAGs lateral packing, giving a special emphasis on the glycerol
435 configuration, chain-chain interactions, and methyl-end stacking is needed for better
436 clarification of the structural factors involved in the extent of miscibility gaps in
437 saturated/saturated-unsaturated TAG systems.

438 In agreement with the prevalent eutectic phases formed after thermodynamic stabilization, the
439 kinetic experiments carried out on equimolecular PPP/OPO and PPP/MC_{POP/OPO} mixtures
440 evidenced independent polymorphic crystallization and transformation of PPP and the mixed-
441 acid TAGs. In addition, cooling experiments at 15 °C·min⁻¹ showed that PPP promoted the
442 crystallization of OPO and MC_{POP/OPO} at a higher temperature (increase up to 10 °C) and in more
443 stable forms (β' before α in OPO, and β before α forms in MC_{POP/OPO}) compared to the pure
444 mixed-acid components under thermal processing.^{22,31} Accordingly, the mixing behavior in the
445 α polymorph could not be clarified for any of the mixtures at the conditions applied in the
446 present study. Understanding the miscibility of TAGs in metastable forms is of practical
447 industrial value since their occurrence governs subsequent stabilization processes or may be
448 desired for specific applications. Thus, the preliminary data obtained in the present work should
449 be expanded in future studies aimed to clarify the kinetic influence on miscibility properties.

450 Due to the great diversity of TAGs in terms of fatty acid composition and distribution, most of
451 the reported binary phase diagrams exhibit eutectic phases with partial miscibility in a delimited
452 compositional range. Therefore, a detailed study of these regions, together with the influence of
453 additional TAG components on their greater or lesser extent, is essential in crystal engineering
454 research addressed to the design of TAG-based crystalline materials for specific food
455 applications.

456 In the present work, PPP/MC_{POP/OPO} mixtures served as a model system to clarify the impact of a
457 third component on the physical behavior displayed by two-TAG component systems. The
458 increased molecular integration exhibited in the sequence OPO → MC_{POP/OPO} → POP in the
459 stable crystalline phase of PPP showed how the physical properties of lipid blends may vary due
460 to intermolecular interactions resulting from the presence of an additional TAG. From a more
461 applied perspective, this has direct implications for industrial oil fractionation processes, in
462 which the optimum separation of low- and high-melting components may be compromised by
463 the formation of molecular compound crystals. Moreover, the increased miscibility area from
464 the PPP/OPO to the PPP/MC_{POP/OPO} system suggests the incorporation of molecular compound
465 crystals in mixtures of fully saturated and saturated-unsaturated TAGs as a worthy to explore
466 strategy for the development of tunable oleic-rich TAG-based structuring agents. In order to
467 validate the former statement, further research on ternary mixtures of TAGs should analyze the
468 influence of chemically diverse molecular compound-forming systems, as well as the variability
469 in chain length of component saturated fatty acids, on the width of the compositional ranges
470 able to form solid solutions.

471

472 **5. CONCLUDING REMARKS**

473

474 DSC and X-ray diffraction techniques were used to evaluate the polymorphism and mixing
475 phase behavior of binary and ternary mixtures of PPP, POP, and OPO under kinetic conditions
476 and after several months of thermodynamic stabilization. Experiments on PPP/OPO and
477 PPP/MC_{POP/OPO} equimolecular mixtures under varying cooling and heating rates showed the

478 independent polymorphic crystallization and transformation behavior of the tri-saturated and the
479 saturated-unsaturated TAGs. As to thermodynamically stabilized PPP/OPO, PPP/POP, and
480 PPP/MC_{POP/OPO} mixtures, all three systems exhibited similar eutectic behavior with a very
481 asymmetric eutectic composition but differed in the extent of the partial miscibility area in the
482 PPP-rich region. In this connection, the increased ability to integrate into the PPP crystal lattice
483 observed in the sequence OPO → MC_{POP/OPO} → POP seemed to be influenced by the higher
484 melting point exhibited by the saturated-unsaturated components of the mixture at decreasing
485 content in oleic acid. Furthermore, the lower miscibility observed for mixtures including the
486 oleic-rich OPO also indicated that TAGs lateral packing was additionally hindered in these
487 mixtures, probably due to a higher degree of unsaturation and the subsequent enhanced
488 destabilizing effect of palmitoyl-oleoyl interactions. These results underline the importance of
489 studies on multi-component TAG mixtures to obtain valuable information for the optimal
490 handling of food industrial application processes, such as oil fractionation, in which the physical
491 properties of many raw materials and end products might be influenced by molecular
492 compound-forming TAGs. Moreover, considering the unique properties exhibited by molecular
493 compounds alone and in combination with a third component, additional studies at a molecular
494 level may clarify in greater detail the suitability of molecular compound crystals as a source of
495 oleic acid in tailored lipid blends intended to be used as TAG-based structuring agents.

496

497 **ACKNOWLEDGEMENTS**

498

499 The authors acknowledge the financial support of the Ministerio de Economía y Competitividad
500 through Project MAT2015-65756-R and the “Ayudas para contratos predoctorales para la
501 formación de doctores 2016” program (BES-2016-076612). Funding from the Alba synchrotron
502 facility for performing SR- XRD experiments is gratefully acknowledged. SR-XRD
503 experiments were conducted with the approval of the Alba Scientific Advisory Committee
504 (proposals 2017022003 and 2019023268). The authors thank Dr. Marc Malfois, responsible for
505 BL11-NCD-SWEET at Alba, for his help.

506 **SUPPORTING INFORMATION**

507

508 **Table S1.** DSC peak top melting temperatures ($^{\circ}\text{C}$) of PPP and OPO when stabilized PPP/OPO
509 mixtures were heated from 0 to 80 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

510 **Table S2.** DSC peak top melting temperatures ($^{\circ}\text{C}$) of PPP and POP when stabilized PPP/POP
511 mixtures were heated from 0 to 80 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

512 **Table S3.** DSC peak top melting temperatures ($^{\circ}\text{C}$) of PPP and $\text{MC}_{\text{POP/OPO}}$ when stabilized
513 PPP/ $\text{MC}_{\text{POP/OPO}}$ mixtures were heated from 0 to 80 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

514 **Figure S1.** DSC and laboratory-scale XRD patterns of the 50PPP/50OPO mixture obtained
515 during cooling and subsequent heating at 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

516 **Figure S2.** DSC and SR-XRD patterns of the 50PPP/50 $\text{MC}_{\text{POP/OPO}}$ mixture obtained during
517 cooling and subsequent heating at 2 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

518

519 **REFERENCES**

520

- 521 (1) Bayés-García, L.; Patel, A. R.; Dewettinck, K.; Rousseau, D.; Sato, K.; Ueno, S. Lipid
522 Crystallization Kinetics - Roles of External Factors Influencing Functionality of End
523 Products. *Curr. Opin. Food Sci.* **2015**, *4*, 32–38.
524 <https://doi.org/10.1016/j.cofs.2015.04.005>.
- 525 (2) Floeter, E.; Haeupler, M.; Sato, K. Molecular Interactions and Mixing Phase Behavior of
526 Lipid Crystals. In *Crystallization of lipids. Fundamentals and applications in food,*
527 *cosmetics and pharmaceuticals*; Sato, K., Ed.; Wiley-Blackwell: Hoboken, NJ, USA,
528 2018; pp 61–104.
- 529 (3) Larsson, K.; Quinn, P.; Sato, K.; Tiberg, F. *Lipids: Structure, Physical Properties and*
530 *Functionality*; The Oily Press: Bridgwater, UK, 2006.
- 531 (4) Zhang, L.; Ueno, S.; Sato, K. Binary Phase Behavior of Saturated-Unsaturated Mixed-
532 Acid Triacylglycerols—A Review. *J. Oleo Sci.* **2018**, *67* (6), 679–687.
533 <https://doi.org/10.5650/jos.ess17263>.

- 534 (5) Macridachis-González, J.; Bayés-García, L.; Calvet, T. An Insight into the Solid-State
535 Miscibility of Triacylglycerol Crystals. *Molecules* **2020**, *25* (19), 4562.
536 <https://doi.org/10.3390/molecules25194562>.
- 537 (6) Sato, K. *Crystallization of lipids. Fundamentals and applications in food, cosmetics and*
538 *pharmaceuticals*; Wiley-Blackwell: Hoboken, NJ, USA, 2018; pp 1–16.
- 539 (7) Minato, A.; Ueno, S.; Yano, J.; Wang, Z. H.; Seto, H.; Amemiya, Y.; Sato, K.
540 Synchrotron Radiation X-Ray Diffraction Study on Phase Behavior of PPP-POP Binary
541 Mixtures. *J. Am. Oil Chem. Soc.* **1996**, *73* (11), 1567–1572.
542 <https://doi.org/10.1007/BF02523526>.
- 543 (8) Gibon, V.; Danthine, S. Systematic Investigation of Co-Crystallization Properties in
544 Binary and Ternary Mixtures of Triacylglycerols Containing Palmitic and Oleic Acids in
545 Relation with Palm Oil Dry Fractionation. *Foods* **2020**, *9* (12), 1891.
546 <https://doi.org/10.3390/foods9121891>.
- 547 (9) Minato, A.; Ueno, S.; Smith, K.; Amemiya, Y.; Sato, K. Thermodynamic and Kinetic
548 Study on Phase Behavior of Binary Mixtures of POP and PPO Forming Molecular
549 Compound Systems. *J. Phys. Chem. B* **1997**, *101* (18), 3498–3505.
550 <https://doi.org/10.1021/jp962956v>.
- 551 (10) Ikeda-Naito, E.; Hondoh, H.; Ueno, S.; Sato, K. Mixing Phase Behavior of 1,3-
552 Dipalmitoyl-2-Oleoyl-sn-Glycerol (POP) and 1,2-Dipalmitoyl-3-Oleoyl-rac-Glycerol
553 (PPO) in n-Dodecane Solution. *J. Am. Oil Chem. Soc.* **2014**, *91* (11), 1837–1848.
554 <https://doi.org/10.1007/s11746-014-2534-y>.
- 555 (11) Lu, C.; Zhang, B.; Zhang, H.; Guo, Y.; Dang, L.; Liu, Z.; Shu, Q.; Wang, Z. Solid–
556 Liquid Phase Equilibrium and Phase Behaviors for Binary Mixtures Composed of
557 Tripalmitoylglycerol (PPP), 1,3-Dipalmitoyl-2-Oleoyl-Glycerol (POP), and 1,2-
558 Dioleoyl-3-Palmitoyl-Glycerol (POO). *Ind. Eng. Chem. Res.* **2019**, *58* (23), 10044–
559 10052. <https://doi.org/10.1021/acs.iecr.9b01947>.
- 560 (12) Bhaggan, K.; Smith, K. W.; Blecker, C.; Danthine, S. Binary Mixtures of
561 Tripalmitoylglycerol (PPP) and 1,3-Dipalmitoyl-2-Stearoyl-sn-Glycerol (PSP):

- 562 Polymorphism and Kinetic Phase Behavior. *Eur. J. Lipid Sci. Technol.* **2018**, *120* (3),
563 1700306. <https://doi.org/10.1002/ejlt.201700306>.
- 564 (13) Bhaggan, K.; Smith, K. W.; Blecker, C.; Danthine, S. Polymorphism and Kinetic
565 Behavior of Binary Mixtures of Trisaturated Triacylglycerols Containing Palmitic and
566 Stearic Acid under Non-Isothermal Conditions. *Eur. J. Lipid Sci. Technol.* **2018**, *120* (9),
567 1800072. <https://doi.org/10.1002/ejlt.201800072>.
- 568 (14) Rousset, P.; Rappaz, M.; Minner, E. Polymorphism and Solidification Kinetics of the
569 Binary System POS-SOS. *J. Am. Oil Chem. Soc.* **1998**, *75* (7), 857–864.
570 <https://doi.org/10.1007/s11746-998-0237-y>.
- 571 (15) Takeuchi, M.; Ueno, S.; Flöter, E.; Sato, K. Binary Phase Behavior of 1,3-Distearoyl-2-
572 Oleoyl-sn-Glycerol (SOS) and 1,3-Distearoyl-2-Linoleoyl-sn-Glycerol (SLS). *J. Am. Oil*
573 *Chem. Soc.* **2002**, *79* (7), 627–632. <https://doi.org/10.1007/s11746-002-0535-1>.
- 574 (16) Costa, M. C.; Boros, L. A. D.; Souza, J. A.; Rolemberg, M. P.; Krähenbühl, M. A.;
575 Meirelles, A. J. A. Solid-Liquid Equilibrium of Binary Mixtures Containing Fatty Acids
576 and Triacylglycerols. *J. Chem. Eng. Data* **2011**, *56* (8), 3277–3284.
577 <https://doi.org/10.1021/je200033b>.
- 578 (17) Wesdorp, L. H.; Van Meeteren, J. A.; de Jong, S.; van der Giessen, R.; Overbosch, P.;
579 Grootsholten, P. A. M.; Struik, M.; Royers, E.; Don, A.; de Loos, T.; Peters, C.;
580 Gandasmita, I. Liquid–Multiple Solid Phase Equilibria in Fats. Theory and
581 Experiments. In *Structure and properties of fat crystal networks*; Marangoni, A. G.,
582 Wesdorp, L. H., Eds.; CRC Press: Boca Raton, FL, USA, 2013; pp 241–418.
- 583 (18) Kerridge, K. Melting-Point Diagrams for Binary Triglyceride Systems. *J. Chem. Soc.*
584 **1952**, No. 0, 4577–4579. <https://doi.org/10.1039/JR9520004577>.
- 585 (19) Moran, D. P. J. Phase Behaviour of Some Palmito-Oleo Triglyceride Systems. *J. Appl.*
586 *Chem.* **1963**, *13* (2), 91–100. <https://doi.org/10.1002/jctb.5010130207>.
- 587 (20) Zhang, L.; Ueno, S.; Miura, S.; Sato, K. Binary Phase Behavior of 1,3-Dipalmitoyl-2-
588 Oleoyl-sn-Glycerol and 1,2-Dioleoyl-3-Palmitoyl-rac-Glycerol. *J. Am. Oil Chem. Soc.*
589 **2007**, *84* (3), 219–227. <https://doi.org/10.1007/s11746-006-1034-0>.

- 590 (21) Bayés-García, L.; Calvet, T.; Cuevas-Diarte, M. À.; Ueno, S.; Sato, K. Phase Behavior
591 of Binary Mixture Systems of Saturated-Unsaturated Mixed-Acid Triacylglycerols:
592 Effects of Glycerol Structures and Chain-Chain Interactions. *J. Phys. Chem. B* **2015**, *119*
593 (12), 4417–4427. <https://doi.org/10.1021/acs.jpcc.5b00673>.
- 594 (22) Minato, A.; Ueno, S.; Yano, J.; Smith, K.; Seto, H.; Amemiya, Y.; Sato, K. Thermal and
595 Structural Properties of sn-1,3-Dipalmitoyl-Oleoylglycerol and sn-1,3-Dioleoyl-2-
596 Palmitoylglycerol Binary Mixtures Examined with Synchrotron Radiation X-Ray
597 Diffraction. *J. Am. Oil Chem. Soc.* **1997**, *74* (10), 1213–1220.
598 <https://doi.org/10.1007/s11746-997-0047-7>.
- 599 (23) Koyano, T.; Hachiya, I.; Sato, K. Phase Behavior of Mixed Systems of SOS and OSO. *J.*
600 *Phys. Chem.* **1992**, *96* (25), 10514–10520. <https://doi.org/10.1021/j100204a072>.
- 601 (24) Takeuchi, M.; Ueno, S.; Sato, K. Crystallization Kinetics of Polymorphic Forms of a
602 Molecular Compound Constructed by SOS (1,3-Distearoyl-2-Oleoyl-sn-Glycerol) and
603 SSO (1,2-Distearoyl-3-Oleoyl-rac-Glycerol). *Food Res. Int.* **2002**, *35* (10), 919–926.
604 [https://doi.org/10.1016/S0963-9969\(02\)00154-0](https://doi.org/10.1016/S0963-9969(02)00154-0).
- 605 (25) Watanabe, S.; Yoshikawa, S.; Arishima, T.; Sato, K. Polymorphism and Mixing Phase
606 Behavior in Ternary Mixture Systems of SOS-SSO-OSO: Formation of Molecular
607 Compound Crystals. *J. Am. Oil Chem. Soc.* **2018**, *95* (4), 447–460.
608 <https://doi.org/10.1002/aocs.12054>.
- 609 (26) Nakanishi, K.; Mikiya, Y.; Ishiguro, T.; Ueno, S. Crystallization Behavior of Molecular
610 Compound in Binary Mixture System of 1,3-Dioleoyl-2-Palmitoyl-sn-Glycerol and 1,3-
611 Dipalmitoyl-2-Oleoyl-sn-Glycerol. *J. Am. Oil Chem. Soc.* **2018**, *95* (1), 51–59.
612 <https://doi.org/10.1002/aocs.12005>.
- 613 (27) Nakanishi, K.; Ueno, S. Mixing Ratio and Cooling Rate Dependence of Molecular
614 Compound Formation in OPO/POP Binary Mixture. *Molecules* **2020**, *25* (22), 5253.
615 <https://doi.org/10.3390/molecules25225253>.
- 616 (28) Rogers, M. A. Novel Structuring Strategies for Unsaturated Fats - Meeting the Zero-
617 Trans, Zero-Saturated Fat Challenge: A Review. *Food Res. Int.* **2009**, *42* (7), 747–753.

- 618 <https://doi.org/10.1016/j.foodres.2009.02.024>.
- 619 (29) Sibbald, A. N.; Carney, J. R.; Marangoni, A. G. Enhanced Structuring of Fat with
620 Reduced Saturates Using Mixed Molecular Compounds. *J. Am. Oil Chem. Soc.* **2016**, *93*
621 (11), 1441–1452. <https://doi.org/10.1007/s11746-015-2718-0>.
- 622 (30) PerkinElmer. *Instructions Model DSC-4*; Norwalk, Connecticut, USA, 1982.
- 623 (31) Bayés-García, L.; Calvet, T.; Cuevas-Diarte, M. À.; Ueno, S.; Sato, K. In Situ
624 Synchrotron Radiation X-Ray Diffraction Study of Crystallization Kinetics of
625 Polymorphs of 1,3-Dioleoyl-2-Palmitoyl Glycerol (OPO). *CrystEngComm* **2011**, *13*,
626 3592–3599. <https://doi.org/10.1039/c1ce05024a>.
- 627 (32) Sato, K.; Arishima, T.; Wang, Z. H.; Okima, K.; Sagi, N.; Mori, H. Polymorphism of
628 POP and SOS. I. Occurrence and Polymorphic Transformation. *J. Am. Oil Chem. Soc.*
629 **1989**, *66* (5), 664–674. <https://doi.org/10.1007/BF02662157>.
- 630 (33) Bayés-García, L.; Calvet, T.; Cuevas-Diarte, M. À.; Ueno, S.; Sato, K. In Situ
631 Observation of Transformation Pathways of Polymorphic Forms of 1,3-Dipalmitoyl-2-
632 Oleoyl Glycerol (POP) Examined with Synchrotron Radiation X-Ray Diffraction and
633 DSC. *CrystEngComm* **2013**, *15* (2), 302–314. <https://doi.org/10.1039/c2ce26522b>.
- 634 (34) Takeuchi, M.; Ueno, S.; Sato, K. Synchrotron Radiation SAXS/WAXS Study of
635 Polymorph-Dependent Phase Behavior of Binary Mixtures of Saturated Monoacid
636 Triacylglycerols. *Cryst. Growth Des.* **2003**, *3* (3), 369–374.
637 <https://doi.org/10.1021/cg025594r>.
- 638 (35) Minato, A.; Yano, J.; Ueno, S.; Smith, K.; Sato, K. FT-IR Study on Microscopic
639 Structures and Conformations of POP-PPO and POP-OPO Molecular Compounds.
640 *Chem. Phys. Lipids* **1997**, *88* (1), 63–71. [https://doi.org/10.1016/S0009-3084\(97\)00045-](https://doi.org/10.1016/S0009-3084(97)00045-5)
641 [5](https://doi.org/10.1016/S0009-3084(97)00045-5).
- 642 (36) Zhang, L.; Wei, K. J.; Chen, J. chun; Xiong, M.; Li, X.; Hondoh, H.; Ueno, S. Effect of
643 Cis–Trans Isomerization on the Crystallization Behavior of Triacylglycerols. *Cryst.*
644 *Growth Des.* **2020**, *20* (3), 1655–1664. <https://doi.org/10.1021/acs.cgd.9b01406>.
- 645 (37) Knoester, M.; De Bruijne, P.; van Den Tempel, M. The Solid-Liquid Equilibrium of

646 Binary Mixtures of Triglycerides with Palmitic and Stearic Chains. *Chem. Phys. Lipids*
647 **1972**, 9 (4), 309–319. [https://doi.org/10.1016/0009-3084\(72\)90017-5](https://doi.org/10.1016/0009-3084(72)90017-5).
648 (38) Ikeda, E.; Ueno, S.; Miyamoto, R.; Sato, K. Phase Behavior of a Binary Mixture of 1,3-
649 Dipalmitoyl-2-Oleoyl-sn-Glycerol and 1,3-Dioleoyl-2-Palmitoyl-sn-Glycerol in n-
650 Dodecane Solution. *J. Phys. Chem. B* **2010**, 114 (34), 10961–10969.
651 <https://doi.org/10.1021/jp101821c>.
652
653

654 **GRAPHIC ABSTRACT**

655

