

Formation Mechanisms and Edible Applications of Molecular Compound (MC) Forming Mixture Systems of Saturated-Unsaturated Mixed-Acid Triacylglycerols

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27 equivalent, Cocoa butter substitute

28

29 **Abstract**

30 The triacylglycerols (TAGs) containing saturated (S_{at}) -unsaturated (U) fatty acid moieties (Sat-U
31 mixed acid TAGs) are widely present in most natural fats and employed in many industrial applications.
32 The mixing behavior of different Sat-U mixed acid TAGs acts important roles in the physicochemical
33 properties TAG-based materials. Among the three main mixing states of miscible, eutectic and
34 molecular compound (MC) forming mixtures, fundamental research has been conducted on the MC
35 crystals formed by different Sat-U mixed acid TAGs to understand the structures, phase behavior and
36 crystallization properties. This article reviews recent studies on the complex thermodynamic, kinetic
37 and structural factors that affect the formation of MC crystals in binary and ternary mixtures of Sat-U
38 mixed acid TAGs ($S_{at}U_{Sat}$, $S_{at}S_{at}U$, $U_{Sat}U$ and UU_{Sat}) through specific molecular interactions among
39 the component TAGs. Furthermore, the application of the MC-forming mixtures containing cacao
40 butter to new types of cocoa butter alternative is reviewed.

INTRODUCTION

The physicochemical properties (e.g., texture, rheology, melting behavior, spreadability, appearance, *etc.*) of lipid-based products in the food, pharmaceutical and cosmetic industries are largely determined by the polymorphic and mixing behavior of the constituent triacylglycerols (TAGs) (Larsson et al., 2006, Floeter et al., 2018).

The polymorphic crystallization of TAGs, often based on complex simultaneous processes, and the phase transformation phenomena are determined by many different factors, such as the chemical nature of the fatty acid moieties in the TAG structures, and the crystallization conditions applied, which can be tailored in many different ways by the application of external factors (Bayés-García et al., 2015a), such as the use of dynamic thermal treatments (Bayés-García et al., 2013; Bayés-García et al., 2016; Bayés-García et al., 2018), sonication (Martini, 2013; Chen et al., 2013; Ye et al., 2014; Lee et al., 2015), shear (Sonwai et al., 2006; Mazzanti et al., 2011; Acevedo et al., 2012; Acevedo et al., 2014), emulsification (Povey et al., 2014; Douaire et al., 2014), additives (Smith et al., 2011; Yoshikawa et al., 2014; Bayés-García et al., 2022)). An in-depth understanding of the polymorphism and mixing behavior of TAG components in binary, ternary and even more complex systems is then required for the optimal control and design of industrial lipid crystallization procedures.

The mixing behavior of TAGs has been comprehensively reviewed by several authors (Floeter et al., 2018; Zhang et al., 2018; Macridachis et al., 2020). Among all TAG species, those with saturated-unsaturated mixed-acid compositions are widely present in most natural fats and used in industrial applications. Therefore, extensive research has been carried out in their binary (Koyano et al., 1992; Minato et al., 1997a; Minato et al., 1997b; Rousset et al., 1998; Takeuchi et al., 2002; Zhang et al., 2007; Zhang et al., 2009; Ikeda et al., 2010; Mizobe et al., 2013; Ikeda et al., 2014; Bayés-García et al., 2015b; Nakanishi et al., 2018; Cholakova et al., 2023; Alishevich et al., 2023; Wijarnprecha et al., 2023), ternary (Koyano et al., 1993; Sasaki et al., 2012; Watanabe et al., 2018; Ghazani et al., 2019a; Macridachis et al., 2021; Macridachis et al., 2022; Yoshikawa et al., 2022) and multicomponent

66 mixture systems (Bayés-García et al., 2017; Watanabe et al., 2021a). In parallel with the experimental
67 studies on the TAG mixtures, theoretical studies have been carried out to model and predict the mixing
68 behavior of different TAGs (Wesdorp et al., 2013; Seilert et al., 2021).

69 Although the number of molecules involved in complex end products is often on the order of
70 several hundred (Myher et al., 1988; Gresti et al., 1993), the study on the solid-state miscibility on a
71 smaller scale with a reduced number of TAG components, usually through binary or ternary mixtures,
72 has been shown to provide highly valuable information that can be extrapolated to real fats, such as
73 extra virgin olive oil (Bayés-García et al., 2017), cocoa butter (Sasaki et al., 2012; Ghazani et al.,
74 2019a; Ghazani et al., 2019b), cocoa butter and coconut oil (Joshi et al., 2020), palm oil (Gibon et al.,
75 1996; Minato et al., 1996; Minato et al., 1997a; Lu et al., 2019; Gibon & Danthine, 2020), milk fat/palm
76 oil/palm stearin mixtures (Mao et al., 2023) .

77 There are three main mixed states resulting from molecular interactions in binary systems of
78 TAGs. In a miscible mixture (Figure 1a), a solid solution is formed at all the mixing ratios between
79 the component TAGs with similar thermal stability and a high degree of isopolymorphism, as the
80 integration of one crystal phase into the other does not cause any significant disturbance in crystal
81 packing (Lusi, 2018). By contrast, dissimilar TAG components lead to eutectic behavior as shown in
82 Figure 1b, where the eutectic composition and the degree of partial solid miscibility can be determined
83 by, among other things, chain-length structures and melting behavior (Timms, 1984; Minato et al.,
84 1996; Floeter et al., 2018; Cholakova, 2023).

85 Of particular interest is the third mixed state of the molecular compound (MC) formation (Figure
86 1c). This stoichiometric compound is formed only at well-defined compositions by specific molecular
87 interactions between individual TAGs, resulting in unique structural and thermodynamic properties
88 (see below). The wide range of possibilities given by chemical nature and processing conditions of the
89 component TAGs may be further enhanced by the use of MC products due to their applicability in
90 edible fat structuring. For example, the potential of MC crystals as partial replacers for *trans* or

saturated fats has already been demonstrated by increasing melting temperature, hardness and solid fat content (Mykhaylyk et al., 2007).

In this review, a special emphasis has been given to the crucial role and potential applicability of MC-forming mixture systems to modify of the physicochemical properties of lipid structures. It should be noted that complex thermodynamic, kinetic and structural factors largely affect the formation of MC in saturated-unsaturated-saturated ($S_{at}U S_{at}$)/ $U S_{at}U$ and $S_{at}U S_{at}/S_{at}S_{at}U$ systems through specific molecular interactions among the component TAGs.

As a fundamental study to elucidate the formation mechanisms of MC, precise observation was performed on the crystallization behavior of palmitic-oleic-based MC of 1,3-dipalmitoyl-2-oleoyl-glycerol (POP)/1,3-dioleoyl-2-palmitoyl glycerol (OPO), POP/1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (*rac*-PPO) and POP/1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol (*sn*-PPO) when subjected to varied cooling rates. By comparing the crystallization behavior of POP/*sn*-PPO and POP/*rac*-PPO systems, the effects of glycerol structures and optical isomerization on MC crystal formation were also briefly described (Bayés-García et al., 2023). The analysis of the influence of the *cis-trans* isomerization on MC formation was performed by replacing *cis*-oleoyl to *trans*-elaidoyl (E) chains in the binary system of 1,3-dipalmitoyl-2-elaidoyl glycerol (PEP)/1,3-dielaidoyl-2-palmitoyl glycerol (EPE) (Zhang et al., 2020).

The effects of MCs as polymorphic stabilizers of a third TAG component were discovered by Yoshikawa et al., who observed the crystallization kinetics in ternary mixtures of 1,3-distearoyl-2-oleoyl glycerol (SOS)/1,3-dioleoyl-2-stearoyl glycerol (OSO)/1,2,3-trilauroyl glycerol (LLL) (Yoshikawa et al., 2022). It was found that the most stable β -form of LLL was activated by the presence of β -crystals of $MC_{SOS/OSO}$. It was indicated that this work has a possibility to develop a new type of cocoa butter substitute (CBS).

Until recently, the application of MC formation to the end food products has been rather limited (Sibbald et al., 2016). However, systematic work was carried out to apply the MC-forming mixtures

to the development of cocoa butter equivalent (CBE) and cocoa butter substitute (CBS) formulations by forming the MC crystals in the ternary mixtures of cocoa butter, OSO, SSO and LLL fats (Watanabe et al., 2021; Watanabe et al., in submission). These studies may have indicated the high potential of the MC-forming TAG mixtures to produce the edible fats with novel functionality.

Molecular-level understanding of the formation mechanisms of MC crystals

Here briefly summarized are the main results of previous studies on the formation processes, structural properties and phase behavior of MC crystals.

It has been found that the following TAGs can form the MC crystals in their binary mixtures, in which the ratio of the component TAGs is always 50:50.

$S_{at}U_{sat}$: Symmetric saturated and unsaturated TAGs such as POP, SOS, PEP

$S_{at}S_{at}U$: asymmetric saturated and unsaturated TAGs such as PPO (or OPP), SSO

$U_{sat}U$: symmetric unsaturated and saturated TAGs such as OPO, OSO, EPE

$U_{sat}U_{sat}$: asymmetric unsaturated and saturated TAGs such as OOS (or SOO) and OOP (or POO)

Figure 2 illustrates the combination of the TAGs, noting the MC-forming and eutectic mixtures. In the binary mixtures of $S_{at}U_{sat}/S_{at}S_{at}U$ and $S_{at}U_{sat}/U_{sat}U$, the MC crystals are formed in the metastable α , β' and β polymorphs, as confirmed in the mixtures of SOS/OSO, SOS/SSO, POP/PPO, POP/PPO, PEP/EPE (see Introduction and section 3). However, the MC crystals are not formed and fully eutectic mixtures are formed in the combinations of $S_{at}U_{sat}/U_{sat}U_{sat}$ and $S_{at}S_{at}U/U_{sat}U$. The former result was confirmed in the mixtures of POP/OOP (Zhang et al., 2007) and SOS/OOS (Zhang et al., 2009), and the latter result was confirmed in the mixture of PPO/OPO (Bayés-García et al., 2015b).

Interestingly, the MC crystals were formed in the mixtures of $U_{sat}U/U_{sat}U_{sat}$ and $S_{at}S_{at}U/U_{sat}U_{sat}$ in their metastable states. For example, the MC crystals in the OPO/OOP and PPO/OOP mixtures occurred

only under metastable conditions and tended to separate into component TAGs to form eutectic mixture systems after 17 months of storage (Bayés-García et al., 2015b). These results were in contrast to those of previous studies on $S_{at}U_{sat}/S_{at}S_{at}U$ and $S_{at}U_{sat}/U_{sat}U$ in which the MC crystals are thermodynamically stable.

The molecular understanding of the ability and inability to form the MC crystals is still open to question. The main key factors may be the stabilization of chain packing between unsaturated and saturated chains, π - π interactions between the double bonds in the unsaturated chains and stabilization of glycerol conformations.

Figure 3 illustrates the glycerol conformations and structural models of the MC crystals by taking palmitic and oleic chains as S_{at} and U chains, respectively. The glycerol conformations are tuning fork in POP and OPO, and chair in PPO and OOP, and all the TAGs form triple chain length in their stable polymorphic forms of β -3 in POP and OPO, and β' -3 in PPO and OOP (Bayés-García et al., 2015b, Floeter et al., 2018). Three problems may arise in the formation of the MC crystals of the double chain-length structure: the chain packing between palmitic and oleic chains, stacking of glycerol groups between the tuning fork and chair conformations, and the π - π interactions between the unsaturated chains.

In the case of $MC_{POP/OPO}$, the stacking of the glycerol groups may be easily accomplished, since both component TAGs form the tuning fork conformation. The chain-chain packing including the π - π interactions may be easiest in $MC_{POP/OPO}$, and thus the formation of $MC_{POP/OPO}$ can be easily done.

However, the stacking of the glycerol groups in $MC_{POP/PPO}$ may be difficult since POP and PPO exhibit the tuning fork and chair conformations, respectively. In addition, steric hindrance between the oleic and palmitic chains may be a disturbing factor in the formation of $MC_{POP/PPO}$. Nevertheless, the metastable and stable polymorphs are formed in $MC_{POP/PPO}$. This suggests that the strong attractive interactions between palmitic chains may play dominant roles during the formation processes of $MC_{POP/PPO}$.

In the case of $MC_{OPO/OOP}$, the stacking of the tuning fork (OPO) and chair (OOP) glycerol conformations may not be easy, and the chain packing between the palmitic and oleic chains is rather unstable. Therefore, $MC_{OPO/OOP}$ is not thermodynamically stable. In the case of PPO/OOP, it seems that both the chain packing and

the stacking of the chair glycerol conformation of PPO and OOP may enable the formation of MC crystals, but MC_{PPO/OOP} is not thermodynamically stable. Finally, the structures of MC_{POP/OOP} and MC_{PPO/OPO} are unstable because the stacking of the glycerol groups and the chain packing may not stabilize the MC crystals. To conclude, the explanation of the formation of MC crystals at the molecular level seems to be incomplete except for MC_{POP/OPO} and MC_{POP/PPO}, and further research is needed.

The crystallization kinetics of MC depends on the TAG molecular structures as well as the rate of cooling. For example, quite rapid cooling (> 40 °C/min) of the binary mixture of POP/OPO resulted in separate crystallization of POP and OPO in the first, while MC_{POP/OPO} crystals started to form in addition to the POP and OPO crystals with decreasing cooling rates (<30 °C/min). The extent of the MC_{POP/OPO} crystals increased with decreasing cooling and heating rates after crystallization (Nakanishi et al., 2018). This result indicates that the formation of MC is a kinetic process involving competitive crystallization and structural rearrangement of the component TAGs.

Another interesting issue is the effect of racemization and optical isomerization of asymmetric S_{at}S_{at}U and UUS_{at} on the formation of MC when mixed with other TAGs. For example, the phase behavior of POP/*rac*-PPO and OPO/*sn*-PPO is somewhat different in terms of the melting points of MC_{POP/*rac*-PPO} and MC_{OPO/*sn*-PPO}, although both mixtures form stable polymorph of the double chain length structure (β-2). This may be related to the different crystallization kinetics of MC_{POP/*rac*-PPO} and MC_{POP/*sn*-PPO} (see below).

Formation of MC in binary mixtures of PEP/EPE

Replacing the *cis* oleoyl chain in a TAG with a *trans* isomeric elaidoyl chain drastically changes its physical and even nutritional properties. However, very little is known about the actual crystal structures and phase behavior of elaidoyl-containing TAGs. To clarify the effect of *cis-trans* isomerization on the crystallization behavior of TAGs, the phase behavior of binary mixtures of 1,3-

191 dipalmitoyl-2-elaidoyl-glycerol (PEP) and 1,3-dielaidoyl-2-palmitoyl-glycerol (EPE) was investigated
192 (Zhang et al., 2020). The binary mixtures of PEP and EPE were prepared at 10% intervals and
193 characterized by DSC, conventional powder X-ray diffraction, and synchrotron radiation X-ray
194 diffraction (SR-XRD). The effects of *cis-trans* isomerization on the crystallization behavior of
195 TAGs were examined by comparison with related *cis* and fully saturated counterparts.

196 The most stable polymorphic forms are β' for PEP, but β for EPE (Zhang et al., 2020). The
197 formation of MC crystals was first observed in the PEP/EPE mixture at a 50:50 ratio, as shown in the
198 phase diagram (Figure 4). The PEP-rich region exhibited a monotectic phase for β -form MC and
199 β' -form PEP, whereas the EPE-rich region exhibited a monotectic phase for β -form MC and β -
200 form EPE, which is quite similar to that observed for the POP/OPO (Minato et al. 1997).

201 The temperature dependence of the SR-XRD patterns and the DSC thermogram of PEP/EPE
202 at a 50:50 ratio taken during cooling and heating at a rate of 2 °C/min is shown in Figure 5. The
203 simultaneous crystallization of the β' form of MC_{PEP/EPE} along with the α form was confirmed in
204 the WAXS spectra by the appearance of β' peaks of 0.44 nm and 0.39 nm and an α peak of 0.42 nm,
205 as indicated by the arrows. With increasing temperature, the α form transformed into the β' form
206 and then further transformed to the thermodynamically stable β form via solid-state
207 transformation. Then, the β form of MC melted at approximately 48 °C. In the SAXS spectra, the
208 solid-state transition of $\alpha \rightarrow \beta' \rightarrow \beta$ was confirmed by the shifting of the peak from 4.62 nm to 4.32
209 nm and then to 4.25 nm.

210 Figure 6 shows the structural model of MC_{PEP/EPE} using the tuning fork conformation of
211 glycerol group, which allows the void (see arrow) at the methyl end of PEP in β' form due to the
212 chain length mismatch between neighboring P and E chains to be filled by the acyl chain of EPE, as

indicated by the dotted line. It is worth noting that when the “O” in certain binary mixtures of palmitic-oleic diacid TAGs such as POP and POO was replaced with “S”, or even “E”, the phase behavior remained the same. For example, a eutectic phase was found in both the POP/*rac*-POO (Zhang et al., 2007) and PSP/*rac*-PSS mixtures (Bhaggan et al., 2018), and the MC-forming mixture was formed in the POP/*rac*-PPO (Minato et al. 1997) and PSP/*rac*-PPS mixtures (Boodhoo et al., 2009). The MC-forming mixture was also found in the POP/OPO (Minato et al., 1997) and PEP/EPE mixtures (Zhang et al., 2020), and thus it is speculated that MC is formed in the PSP/SPS mixture.

Crystallization kinetics of MC crystals of POP/OPO and POP/PPO

To elucidate the effects of glycerol structures on the kinetic properties of MC-forming mixture phases, thermal analysis, X-ray diffraction and optical microscopy techniques were applied to the binary mixtures of POP/OPO, POP/*rac*-PPO and POP/1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol (*sn*-PPO) (Bayés-García et al., 2023). All the three mixtures exhibited the MC-forming mixture at a 1:1 ratio.

The mixture samples were subjected to dynamic conditions of cooling at low and intermediate rates of 0.1, 0.5 and 2 °C/min to complete crystallization and reheating at a constant rate of 2 °C/min, while monitoring complex crystallization and polymorphic transition phenomena, as summarized in Figure 7. The POP/OPO mixture formed only MC_{POP/OPO} crystals with double chain-length structure in its most stable β form when cooled under all the conditions analyzed, and they simply melted when heated. The occurrence of MC_{POP/OPO} β form was reported in both pure liquid (Minato et al., 1997b) and *n*-dodecane solution systems (Ikeda et al., 2010).

Similarly, the most stable β form of MC_{POP/*rac*-PPO} crystallized when cooled at 0.1 °C/min, although a metastable β' form was detected at 0.5 and 2 °C/min, which transformed to β when heated.

236 By contrast, the POP/*sn*-PPO mixture showed significantly complex polymorphic events in all
237 the experimental conditions studied, showing the coexistence of MC_{POP/*sn*-PPO} with polymorphs of pure
238 POP and *sn*-PPO component TAGs, even at the lowest cooling rate applied. In more detail, the 1:1
239 TAGs mixture crystallized into β' forms of *sn*-PPO (triple chain length), MC_{POP/*sn*-PPO} (double chain
240 length) and POP (double chain length) at 0.1 and 0.5 °C/min. When heated, the metastable β' of
241 MC_{POP/*sn*-PPO} transformed into its most stable β form before melting. At a higher cooling rate of
242 2 °C/min, the least stable polymorphs predominated, showing complicated simultaneous
243 crystallization processes of *sn*-PPO β' form (triple chain length) and α forms (double chain length) of
244 MC_{POP/*sn*-PPO}, POP and *sn*-PPO. Subsequent heating caused polymorphic transformations of $\alpha \rightarrow \beta' \rightarrow$
245 β for MC_{POP/*sn*-PPO}, and $\alpha \rightarrow L \rightarrow \beta'$ for POP (see Figure 7).

246 Regarding the effects of optical isomerization in mixed systems of TAGs, extensive work had
247 previously been carried out by Craven and Lenki (Craven et al., 2013). Additionally, Mizobe et al.
248 analyzed the polymorphic structures of R-PPO, S-OPP and their mixtures, and concluded that the two
249 optical isomers had identical structural properties, whereas the polymorphic characteristics of R-PPO
250 and *rac*-PPO became different, with the latter corresponding to the 1:1 mixture of R-PPO/S-OPP
251 (Mizobe et al., 2013).

252 To understand the differences in the crystallization behavior of MC_{POP/OPO} and MC_{POP/*sn*-OPO}, one
253 can pay attention to their structural models, which are mainly based on the glycerol structures of POP,
254 OPO and *sn*-PPO, as depicted in Figure 8. It can be assumed that the tuning fork glycerol conformation
255 is the most stable one for both POP and OPO β forms, since the palmitic and oleic acid chains are
256 located on different leaflets, favoring the packing of TAG molecules in triple chain-length structures
257 that avoid the steric hindrance between straight palmitic and bent oleic acid chains. By contrast, the
258 *sn*-PPO β' form may exhibit a chair-type glycerol conformation, which is also packed in a triple chain-
259 length structure for the same reasons explained above.

260 During the formation process of the MC crystals, the chain-length structures were changed from
261 triple to double, and close packing of glycerol groups and palmitic and oleic acid chains of neighboring
262 TAG molecules occurred. A higher ability of MC_{POP/OPO} to form and stabilize in β form compared to
263 other MCs was observed, since the tuning fork-type glycerol conformation of the two TAG
264 components POP and OPO can be maintained in MC_{POP/OPO}, resulting in a β -2 structure with palmitic
265 and oleic acid chains packed in separate leaflets. This ability was not observed in the POP/*sn*-PPO
266 mixture, as it did not form MC_{POP/*sn*-PPO} alone, but it coexisted with single POP and *sn*-PPO TAG
267 components.

268 Polarized light microscopy data confirmed a higher crystallization rate for *sn*-PPO crystals
269 compared to other single TAGs or MCs, which may explain the separate crystallization when the
270 POP/*sn*-PPO mixture was cooled, and this may also be explained from a structural point of view.
271 According to our model (Figure 8), an extra molecular rearrangement may occur during the MC<sub>POP/*sn*-
272 PPO</sub> formation, based on the change of the chair-type glycerol conformation of *sn*-PPO to the tuning
273 fork-type, leading to an MC with coexisting oleic and palmitic acid chains in the same leaflet.

274 Regarding the different crystallization behavior of MC_{POP/*sn*-PPO} and MC_{POP/*rac*-PPO}, one may
275 consider the ability of R-PPO and S-OPP to form a stable molecular packing (or racemic compound),
276 which may interact with POP molecules to form a stable MC structure. However, further work may be
277 required to understand the mechanisms involved.

278

279 **Interactive polymorphic crystallization of MC_{SOS/OSO} and LLL**

280

281 The effect of MC formation on the polymorphic crystallization of the other coexisting TAG was
282 examined using ternary TAG mixtures of LLL/SOS/OSO with a 1:1 weight ratio of SOS and OSO
283 (SOS/OSO = 1/1) (Yoshikawa et al., 2022).

284 Under a series of thermal conditions of cooling from the melt, isothermal holding, and heating,
285 pure LLL without containing SOS and OSO formed β' form crystals, which transformed to β form
286 during the heating process after the crystallization. In 1992, it was found that the binary TAG mixture
287 of SOS/OSO = 1/1 formed mainly β -form MC crystals of SOS and OSO ($MC_{SOS/OSO}$) during the
288 cooling process (Koyano et al., 1992). Based on these results, the ternary mixtures of LLL/SOS/OSO/
289 at various mixing ratios of LLL and SOS/OSO=1/1 were investigated and the immiscible eutectic
290 behavior was observed with retarded crystallization and lowered melting points of the formed crystals
291 (Figure 9). Quite interesting results were found in the crystallization kinetics of two β forms of
292 $MC_{SOS/OSO}$ and LLL; the β crystallization of $MC_{SOS/OSO}$ coincided with the β' to β transformation or
293 direct β melt-crystallization of LLL during the isothermal-holding process, as evident from the
294 synchrotron radiation X-ray diffraction (SR-XRD) data shown in Figure 10. This coincidence strongly
295 suggests the possibility that β crystallization of $MC_{SOS/OSO}$ triggered the β crystallization of LLL. As a
296 result, the spherulitic crystals of LLL deformed and changed their size distribution with increasing
297 concentration of SOS/OSO=1/1. These peculiar phenomena of the interrelationships between the
298 crystallization of β forms of $MC_{SOS/OSO}$ and LLL have been defined as “interactive polymorphic
299 crystallization” (Yoshikawa et al., 2022).

300 As a possible mechanism underlying the interactive polymorphic crystallization, epitaxial
301 effects through triclinic-parallel ($T_{//}$) subcell matching between β crystals of LLL and $MC_{SOS/OSO}$ may
302 occur in the same manner that β' form seed crystals of tripalmitin or tristearin effectively accelerated
303 β' crystallization of coconut oil through orthorhombic-perpendicular (O_{\perp}) subcell matching
304 (Mahisanunt et al., 2020). Another possible mechanism is martensitic transformation via cooperative
305 displacement of atoms without diffusion in the crystal lattices, which can be initiated by the occurrence
306 of local stress through mechanical or thermal stimulation, as indicated for petroselinic acid (Kaneko
307 et al., 1997).

308 Elucidating the mechanism of interactive polymorphic crystallization requires further research,
309 such as SR-XRD experiments substituting the component TAGs (LLL, SOS, and OSO) with the other
310 corresponding TAGs (e.g., SSS, SSO, and SOO, respectively) and SR-XRD experiments using a
311 microbeam technique to analyze the local orientation of TAG molecules near the crystal-crystal
312 interfaces. For example, the SR-XRD data shown in Figure 11 indicate that the effect of facilitating β
313 crystallization of LLL in LLL/SOS/OSO was weakened by replacing OSO with rac-SOO, probably
314 because SOS and SOO crystallized separately in the less stable forms of SOS 5L (2L + 3L), in which
315 SOS formed in randomly packed double and triple chain-length structures (Mykhaylyk et al., 2007),
316 and SOO β' .

317 It may be worth noting that the study of LLL/SOS/OSO mixtures can be applied to solve the
318 problem of fat blooming in CBS-based compound coatings, which is partly caused by the polymorphic
319 transformation of lauric acid-based TAG crystals in CBS from $\beta'-2$ to $\beta-2$ (see below).

320

321 **Application of MC crystals to new types of cocoa butter equivalent (CBE) and cocoa butter** 322 **substitute (CBS)**

323

324 Cocoa butter (CB) is indispensable ingredient in chocolate, as it is responsible for the physical
325 properties such as hardness, texture, and melting behavior of chocolate products (Talbot, 2017).
326 However, CB is one of the most expensive ingredients of chocolate and its price is gradually
327 increasing due to several global issues such as low productivity of cacao due to climate change and
328 increasing demand for cacao products (Afoakwa, 2016). Therefore, the confectionery industry has
329 developed CB alternative fats (CBAs) with various functionalities to replace or blended with CB in
330 chocolate production.

331 CBAs can be classified into three groups based on their compositions: cocoa butter equivalent
332 (CBE), cocoa butter replacer (CBR) and cocoa butter substitute (CBS) (Timms, 2003). Recently, two
333 experimental studies on the physical properties and fat bloom stability of chocolate made with
334 S_{at}S_{at}U and U_{S_{at}}U type fats forming MC crystals with CB revealed that these fats can be used as new
335 types of CBA fats (Watanabe et al., 2021., Watanabe et al., in submission).

336

337 **CBE**

338 Systematic studies showed that the ternary mixtures of SOS, *rac*-SSO and OSO form MC
339 crystals of stable β -2 form at a concentration of SOS of 50% with different concentrations of SSO
340 and OSO (Watanabe et al., 2018). Figure 12 shows that the MC crystals with the stable β -2
341 polymorph are formed in a series of the ternary mixtures of SOS/SSO/OSO, in which the ratio of
342 SOS/(SSO+OSO) was set to 50/50 and the ratio of SSO/OSO was varied. Based on this study, it was
343 expected that the ternary mixtures can produce a new type of CBE with the stable polymorphic
344 structure when SOS, SSO and OSO are replaced with CB, SSO-fat and OSO-fat. The SSO-fat and
345 OSO-fat were prepared by full-hydrogenation, interesterification, and fractionation of canola oil high
346 oleic sunflower oil (Watanabe et al., 2021). The physical properties and fat bloom stability of
347 chocolates containing SSO-fat and OSO-fats, which were solidified by simple cooling without
348 tempering process, were then evaluated (Watanabe et al., 2021).

349 It should be noted that in the chocolate fat phases, the final ratio of the three types of TAG,
350 S_{at}U_{S_{at}}/S_{at}S_{at}U/U_{S_{at}}U, were 50/50/0–50/0/50. Figure 13 shows the temperature change in solid fat
351 content (SFC) values of six fat blends and CB, where the concentrations (%) of S_{at}U_{S_{at}}/S_{at}S_{at}U/U_{S_{at}}U
352 in the fat blends are 50/46/4 (sample A), 50/40/10 (B), 50/30/20 (C), 50/20/30 (D), 50/10/40 (E), and
353 50/3/47 (F). The SFC measurement of ternary fat blends of CB, SSO-fat and OSO-fat showed sharp

354 melting profiles around body temperature. Similarly, in the hardness measurement chocolate samples
355 prepared with fat blends of $S_{at}U S_{at}/S_{at}S_{at}U/U S_{at}U=50/30/20-50/20/30$ showed almost the same
356 hardness as that of pure chocolate. In addition, no fat bloom formation was observed in dark chocolate
357 with the fat blends of $S_{at}U S_{at}/S_{at}S_{at}U/U S_{at}U$ of $50/20/30-50/0/50$ during the one-year storage under
358 isothermal condition at 25 °C and thermal cycling condition between 20 and 30 °C. Overall, the CB,
359 SSO-fat, and OSO-fat blends can be used as a cocoa butter equivalent (CBE) without tempering
360 procedures.

361

362 CBS

363 The MC crystals made of CB and OSO-fat ($MC_{CB/OSO}$) were applied to compound chocolate
364 formulations by blending with lauric cocoa butter substitutes (CBS) (Watanabe et al., in submission).
365 Conventional CBSs have been produced by fractionation, hydrogenation and a combination of these
366 processes of lauric fats derived from palm kernel oil and other natural fats (Rossell, 1985). These fats
367 are composed of TAGs with lauric (L) and myristic (M) acid moieties including LLL, LLM, LMM,
368 and MMM which are crystallized into $\beta'-2$ form by simple cooling without tempering (Smith, 2012).
369 However, the CBS-based chocolate can potentially convert from the $\beta'-2$ to $\beta-2$ form, which leads to
370 fat bloom formation during long-term storage, because mono-acid TAGs such as LLL and MMM
371 have the potential to transform from $\beta'-2$ to the more stable $\beta-2$ form (β -tending; Koizumi et al.,
372 2022). In addition, cacao solids such as cacao powder and cacao liquor are often blended to improve
373 the flavour and taste of compound chocolate, resulting in more serious fat bloom due to the phase
374 separation of CBS and CB followed by polymorphic transformation of CB TAGs. This eutectic
375 effect makes it difficult to blend more than 5% cocoa butter in the compound chocolates (Laustsen,
376 1991).

As described in the previous section, studies on the polymorphic crystallization behavior of the ternary mixture system of LLL/SOS/OSO with the SOS/OSO ratio of 1/1 have revealed that the crystallization of β -2 form in MC_{SOS/OSO} promoted the crystallization and polymorphic transformation of β' -2 to β -2 in LLL (Yoshikawa et al., 2022). Based on these results, the physical properties and fat bloom stability of lauric-based compound chocolate with MC_{CB/OSO} were evaluated.

Table 1 shows six chocolate samples with the different relative concentrations (%) of CBS, OSO-fat and CB investigated. The SFC studies showed that the fat blends of CBS and MC_{CB/OSO} exhibited immiscible eutectic behavior, as did the fat blends of CBS and CB. The results of SFC value, crystallization rates and hardness of compound chocolate with MC_{CB/OSO} suggested that the compound chocolate is suitable for the chocolate production up to about 20% of MC_{CB/OSO} in the fat phases.

Table 1. Relative concentrations (%) of CBS, CB and OSO-fat in total chocolate fat in eight chocolate samples

Sample	CBS	OSO-fat	CB
A1	90.0	0.0	10.0
A2	85.0	0.0	15.0
A3	80.0	0.0	20.0
A4	75.0	0.0	25.0
B1	80.0	10.0	10.0
B2	70.0	15.0	15.0
B3	60.0	20.0	20.0
B4	50.0	25.0	25.0

391 In the fat bloom evaluation studies, while CBS/CB compound chocolate exhibited severe fat
 392 bloom within a few weeks, the CBS/CB/OSO-fat compound chocolate showed no fat bloom under any
 393 storage condition for 6 months (Table 2). In addition, the XRD studies revealed that no polymorphic
 394 transformation occurred for the CBS/CB/OSO-fat blends during the 6 months storage, while the
 395 polymorphic transformation from β' -2 to β -2 was observed for the fat blends of CBS/CB. The
 396 polymorphic transformations of CB TAGs and β -tending TAG in CBS cause fat blooming in CBS-
 397 based compound chocolate. Therefore, the mechanisms of fat blooming inhibition in compound
 398 chocolate made of CBS/CB/OSO-fat summarized in Table 1 can be assumed that β -tending TAG
 399 fractions in the CBS, such as LLL and MMM, may crystallize into β -form during the cooling process
 400 via interactive polymorphic crystallization with $MC_{CB/OSO}$. However, further studies are required to
 401 elucidate these mechanisms.

402

403 Table 2. Evaluation of fat bloom formation of eight dark chocolate samples after storage for 3 weeks
 404 (3W) and 15 weeks (15W) under different thermal conditions, -; no fat bloom, +; slightly bloomed,
 405 ++; fairly bloomed, +++: seriously bloomed

Chocolate	15°C		20°C		25°C		15-25°C	
sample	3W	15W	3W	15W	3W	15W	3W	15W
A1	+	+++	+	++	-	++	-	+
A2	+	+++	+	++	-	++	-	+
A3	+	+++	+	++	+	++	-	+
A4	+	+++	+	++	+	++	-	+
B1	-	-	-	-	-	-	-	-
B2	-	-	-	-	-	-	-	-
B3	-	-	-	-	-	-	-	-
B4	-	-	-	-	-	-	-	-

406

407 **CONCLUSION**

408

409 The study of the mixing behavior of principal TAGs and various natural and industrialized fats
410 is of great importance, as it is one of the most informative and applicable studies to improve the
411 physicochemical properties of lipid-based products using natural and technologically-produced lipid
412 materials. Until recently, the phases of MC-forming mixture have been studied from a fundamental
413 point of view using pure samples of saturated-unsaturated mixed-acid TAGs. However, the fat
414 materials produced by full-hydrogenation, interesterification and fractionation of vegetable fats and
415 oils have been used for the application of the MC crystals to end products such as fat spreads and
416 confectionery fats. The physical properties of the MC crystals are different from those of the
417 component TAGs and can improve the end products when applied; for example, the formation of MC
418 crystals containing the mixed acid TAGs with oleic acid moiety can reduce the use of saturated fats
419 (Sibbald et al., 2016), and the application of the MC crystals containing CB, OSO-fat, and SSO-fat to
420 CBE and CBS improved the crystallization kinetics and fat bloom stability. It is expected to explore
421 the molecular mechanisms of the ability and inability of MC formation, and the application of the MC
422 crystals to other end products such as emulsified and aerated systems.

423

424 **AUTHOR CONTRIBUTIONS**

425

426 LB, KT, LZ, SY, FK, YY and SW performed the experiments and published their original articles,
427 which are reviewed in this article. KS initiated and organized this work.

428

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430

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439 **Figure caption**

440

441 **Figure 1** Typical three binary mixing systems of triacylglycerols (TAGs). A and B: component
442 TAGs, L; liquid phase, S_A , S_B and S_{MC} ; solid phases of A, B and molecular compound (MC).

443

444 **Figure 2** Combination of saturated (S_{at})-unsaturated (U) mixed-acid TAGs exhibiting MC-
445 forming and eutectic binary mixtures.

446

447 **Figure 3** Key factors affecting the formation of MC crystals in binary mixtures of TAGs
448 containing palmitic (P) and oleic (O) acid moiety.

449

450 **Figure 4** Mixing phase behavior of PEP and EPE.

451

452 **Figure 5** DSC thermogram and SR-XRD patterns of PEP/EPE at a 50:50 ratio.

453

454 **Figure 6** Possible mechanism of $MC_{PEP/EPE}$ formation.

455

456 **Figure 7** Summary of temperature-dependent polymorphic crystallization in the binary mixtures
457 of POP/OPO, POP/*rac*-PPO and POP/*sn*-PPO.

458

459 **Figure 8** Effects of chain packing and glycerol conformation on the formation of MC crystals.

460

461 **Figure 9** Phase behavior of LLL/SOS/OSO mixtures with different weight fractions of
462 SOS/OSO = 1/1 ($W_{\text{SOS/OSO}}$): melting of $\text{MC}_{\text{SOS/OSO}} \beta\text{-2}$ (\square), transformation $\beta\text{-2} \rightarrow \beta\text{-2}$ of LLL (\blacktriangle),
463 and melting of LLL $\beta\text{-2}$ (\bullet).

464

465 **Figure 10** 3D surface plots and overhead projections of SR-XRD data on LLL/SOS/OSO =
466 2/1/1 (a) and 1/2/2 (b), taken during cooling at a rate of 2 °C/min, holding at 15 °C for 30 min, and
467 heating at a rate of 5 °C/min. Numbers hyphenated to Greek letters for crystal polymorphs represent
468 the chain-length structure. Unit: nm.

469

470 **Figure 11** 3D surface plots and overhead projections of SR-XRD data on LLL/SOS/*rac*-SOO =
471 2/1/1, taken during cooling at a rate of 2 °C/min, holding at 15 °C for 30 min, and heating at a rate of
472 5 °C/min. Numbers hyphenated to Greek letters for crystal polymorphs represent the chain-length
473 structure. Unit: nm.

474

475 **Figure 12** Phase diagram of ternary mixture systems of SOS/SSO/OSO. White symbols
476 represent MC crystals of $\beta\text{-2}$, black symbols represent eutectic mixtures of $\beta\text{-2}$ of MC crystals and
477 component TAG.

478

479 **Figure 13** SFC profiles of CB and CB/SSO-fat/OSO-fat blends. Blending ratio of A–F are as
480 follows. CB/SSO-fat/OSO-fat = (A) 50/50/0, (B) 50/40/10, (C) 50/30/20, (D) 50/20/30, (E) 50/10/40
481 and (F) 50/0/50.

482

483 REFERENCES

484

485 Acevedo NC, Block JM, Marangoni AG. Unsaturated emulsifier mediated modification of the
486 mechanical strength and oil binding capacity of a model edible fat crystallized under shear.
487 *Langmuir*. 2012; 28:16207–16217. <https://doi.org/10.1021/la303365d>

488

489 Acevedo NC, Marangoni AG. Functionalization of noninteresterified mixtures of fully hydrogenated
490 fats using shear processing. *Food Bioprocess Technology*. 2014;7:575–587.
491 <https://doi.org/10.1007/s11947-013-1110-z>

492

493 Afoakwa EO. World cocoa production, processing and chocolate consumption pattern. In: Afoakwa
494 EO. editor. *Chocolate Science and Technology*. West Sussex, UK: Wiley & Blackwell; 2016. p.
495 17–48

496

497 Alishevich K, Berčíková M, Kyselka J, Sasínová K, Honzíková T, Šimicová P, Šmidrkal J, Rohlíček
498 J, Filip V. Binary Phase Behavior of 2-oleoyl-1-palmitoyl-3-stearoyl-rac-glycerol (POS) and
499 2-linoleoyl-1-palmitoyl-3-stearoyl-rac-glycerol (PLS). *Food Biophysics*. 2023;18:161–173.
500 <https://doi-org.sire.ub.edu/10.1007/s11483-022-09761-8>

501

502 Bhaggan K, Smith KW, Blecker C, Danthine S. Polymorphism and Kinetic Behavior of Binary
 503 Mixtures of Trisaturated Triacylglycerols Containing Palmitic and Stearic Acid Under Non-
 504 Isothermal Conditions. *European Journal of Lipid Science and Technology*. 2018;120:1800072.
 505 <https://doi.org/10.1002/ejlt.201800072>
 506
 507 Bayés-García L, Calvet T, Cuevas-Diarte MA, Ueno S, Sato K. Crystallization and Transformation of
 508 Polymorphic Forms of Trioleoyl Glycerol and 1,2-Dioleoyl-3-rac-linoleoyl Glycerol. *Journal of*
 509 *Physical Chemistry B*. 2013;117:9170–9181. <https://doi.org/10.1021/jp403872a>
 510
 511 Bayés-García L, Patel AR, Dewettinck K, Rousseau D, Sato K, Ueno S. Lipid crystallization kinetics
 512 - roles of external factors influencing functionality of end products. *Current Opinion in Food*
 513 *Science*. 2015a;4: 32–38. <https://doi.org/10.1016/j.cofs.2015.04.005>
 514
 515 Bayés-García L, Calvet T, Cuevas-Diarte MA, Ueno S, Sato K. Phase Behavior of Binary Mixture
 516 Systems of Saturated-Unsaturated Mixed-Acid Triacylglycerols: Effects of Glycerol Structures
 517 and Chain-Chain Interactions. *Journal of Physical Chemistry B*. 2015b;119:4417–4427.
 518 <https://doi.org/10.1021/acs.jpcb.5b00673>
 519
 520 Bayés-García L, Calvet T, Cuevas-Diarte MA, Ueno S. In situ crystallization and transformation
 521 kinetics of polymorphic forms of saturated-unsaturated-unsaturated triacylglycerols: 1-palmitoyl-
 522 2,3-dioleoyl glycerol, 1-stearoyl-2,3-dioleoyl glycerol, and 1-palmitoyl-2-oleoyl-3-linoleoyl
 523 glycerol. *Food Research International*. 2016;85:244–258.
 524 <https://doi.org/10.1016/j.foodres.2016.05.011>
 525

526 Bayés-García L, Calvet T, Cuevas-Diarte MA, Ueno S. From Trioleoyl glycerol to extra virgin olive
 527 oil through multicomponent triacylglycerol mixtures: Crystallization and polymorphic
 528 transformation examined with differential scanning calorimetry and X-ray diffraction techniques.
 529 Food Research International. 2017;99: 476–484. <https://doi.org/10.1016/j.foodres.2017.06.015>
 530

531 Bayés-García L, Calvet T, & Cuevas-Diarte MA. Effects of dynamic temperature variations on
 532 microstructure and polymorphic behavior of lipid systems. In Sato K editor. Crystallization of
 533 lipids. Fundamentals and applications in food, cosmetics and pharmaceuticals. Hoboken, NJ,
 534 USA: Wiley-Blackwell; 2018. p. 183–210. <https://doi.org/10.1002/9781118593882.ch6>
 535

536 Bayés-García L, Yoshikawa S, Aguilar-Jiménez M, Ishibashi C, Ueno S, Calvet T. Heterogeneous
 537 Nucleation Effects of Talc Particles on Polymorphic Crystallization of Cocoa Butter. Crystal
 538 Growth & Design. 2022;22:213–227. <https://doi.org/10.1021/acs.cgd.1c00859>
 539

540 Bayés-García L, Fukao K, Konishi T, Sato K, & Taguchi K. Crystallization and Transformation
 541 Behavior of Triacylglycerol Binary Mixtures Forming Molecular Compounds of POP/OPO,
 542 POP/rac-PPO, and POP/sn-PPO. Crystal Growth & Design. 2023;23:2870–2881.
 543 <https://doi.org/10.1021/acs.cgd.3c00038>
 544

545 Boodhoo MV, Bouzidi L, Narine SS. The binary phase behavior of 1, 3-dipalmitoyl-2-stearoyl-sn-
 546 glycerol and 1, 2-dipalmitoyl-3-stearoyl-sn-glycerol. Chemistry and Physics of Lipids.
 547 2009;160:11–32. <https://doi.org/10.1016/j.chemphyslip>
 548

549 Chen F, Zhang H, Sun X, Wang X, & Xu X. Effects of ultrasonic parameters on the crystallization
550 behavior of palm oil. *Journal of the American Oil Chemists' Society*. 2013;90:941–949.
551 <https://doi.org/10.1007/s11746-013-2243-y>
552

553 Cholakova, D, Tcholakova, S, & Denkov, N. Polymorphic Phase Transitions in Bulk Triglyceride
554 Mixture. *Crystal Growth & Design*. 2023;23:2075–2091. <https://doi.org/10.1021/acs.cgd.2c01021>.
555

556 Craven RJ, Lencki RW. Polymorphism of Acylglycerols: A Stereochemical Perspective. *Chemical*
557 *Review*. 2013;113:7402–7420. <https://doi.org/10.1021/cr400212r>
558

559 Douaire M, di Bari V, Norton JE, Sullo A, Lillford P, Norton IT. Fat crystallisation at oil-water
560 interfaces. *Advances in Colloid and Interface Science*. 2014;203:1–10.
561 <https://doi.org/10.1021/acs.cgd.2c01021>
562

563 Floeter E, Haeupler M, Sato K. Molecular interactions and mixing phase behavior of lipid crystals. In
564 Sato K editor. *Crystallization of lipids. Fundamentals and applications in food, cosmetics and*
565 *pharmaceuticals*; Hoboken, NJ, USA: Wiley-Blackwell; 2018. p. 61–104.
566 <https://doi.org/10.1002/9781118593882.ch3>
567

568 Gibon V, Durant F, Deroanne C. Polymorphism and intersolubility of some palmitic, stearic and oleic
569 triglycerides: PPP, PSP and POP. *Journal of the American Oil Chemists' Society*. 1986;63:1047–
570 1055. <https://doi.org/10.1007/BF02673796>
571

572 Gibon V, Danthine S. Systematic Investigation of Co-Crystallization Properties in Binary and Ternary
 573 Mixtures of Triacylglycerols Containing Palmitic and Oleic Acids in Relation with Palm Oil Dry
 574 Fractionation. *Foods*. 2020;9:1891. <https://doi.org/10.3390/foods9121891>
 575

576 Ghazani SM, Marangoni AG. The ternary solid state phase behavior of triclinic POP, POS and SOS
 577 and its relationship to CB and CBE properties. *Crystal Growth & Design*. 2019a;19:704–713.
 578 <https://doi.org/10.1021/acs.cgd.8b01273>
 579

580 Ghazani, S.M., & Marangoni, A. G. The triclinic polymorphism of cocoa butter is dictated by its major
 581 molecular species, 1-palmitoyl, 2-oleoyl, 3-stearoyl glycerol (POS). *Crystal Growth & Design*.
 582 2019b;19: 90–97. <https://doi.org/10.1021/acs.cgd.8b00973>
 583

584 Gresti J, Bugaut M, Maniongui C, Bezard, J. Composition of molecular species of triacylglycerols in
 585 bovine milk fat. *Journal of Dairy Science*. 1993;76:1850–1869. [https://doi.org/10.3168/jds.S0022-](https://doi.org/10.3168/jds.S0022-0302(93)77518-9)
 586 0302(93)77518-9
 587

588 Ikeda E, Ueno S, Miyamoto R, Sato K. Phase Behavior of a Binary Mixture of 1,3-dipalmitoyl-2-
 589 oleoyl-sn-glycerol and 1,3-dioleoyl-2-palmitoyl-sn-glycerol in n-dodecane Solution. *Journal of*
 590 *Physical Chemistry B*. 2010;114:10961–10969. <https://doi.org/10.1021/jp101821c>
 591

592 Ikeda-Naito E, Hondoh H, Ueno S, Sato K. Mixing phase behavior of 1,3-dipalmitoyl-2-oleoyl-
 593 sn-glycerol (POP) and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol (PPO) in n-dodecane solution.
 594 *Journal of the American Oil Chemists' Society*. 2014;91:1837–1848.
 595 <https://doi.org/10.1007/s11746-014-2534-y>
 596

597 Joshi BL, Zielbauer BI, Vilgis TA. Comparative Study on Mixing Behavior of Binary Mixtures of
 598 Cocoa Butter/Tristearin (CB/TS) and Cocoa Butter/Coconut Oil (CB/CO). *Foods*. 2020;9: 327.
 599 <https://doi.org/10.3390/foods9030327>
 600
 601 Kaneko F, Kobayashi M, Sato K, Suzuki M. Martensitic phase transition of petroselinic acid:
 602 influence of polytypic structure. *Journal of Physical Chemistry B*. 1997;101:285–292.
 603 <https://doi.org/10.1021/jp9625927>
 604
 605 Koizumi H, Takagi M, Hondoh H, Michikawa S, Hirai Y, Ueno S. Control of Phase Separation for
 606 CBS-Based Compound Chocolates Focusing on Growth Kinetics. *Crystal Growth & Design*.
 607 2022;22:6879–6885. <https://doi.org/10.1021/acs.cgd.2c00317>
 608
 609 Koyano T, Hachiya I, Sato K. Phase Behavior of Mixed Systems of SOS and OSO. *Journal of Physical*
 610 *Chemistry*. 1992;96:10514–10520. <https://doi.org/10.1021/j100204a072>
 611
 612 Koyano T, Kato Y, Hachiya I, Unemura R. Crystallization behavior of ternary mixture of
 613 POP/POS/SOS. *Journal of the Japanese Oil Chemists' Society*. 1993;42:453–457.
 614 <https://doi.org/10.5650/jos1956.42.453>
 615
 616 Larsson K, Quinn P, Sato K, Tiberg F. *Lipids: Structure, Physical Properties and Functionality*. Oily
 617 Press: Bridgwater, U.K.; 2006
 618
 619 Laustsen K. The nature of fat bloom in molded compound coatings. *The Manufacturing*
 620 *Confectioner*. 1991;71,137–144

621

622 Lee J, Ye Y, Martini S. Physicochemical and oxidative changes in sonicated interesterified soybean
623 oil. *Journal of the American Oil Chemists' Society*. 2015;92:305–308.
624 <https://doi.org/10.1007/s11746-014-2585-0>

625

626 Lu C, Zhang B, Zhang H, Guo Y, Dang L, Liu Z, Shu Q, Wang Z. Solid–liquid phase equilibrium and
627 phase behaviors for binary mixtures composed of tripalmitoylglycerol (PPP), 1,3-dipalmitoyl-2-
628 oleoylglycerol (POP), and 1,2-dioleoyl-3-palmitoyl-glycerol (POO). *Industrial and Engineering*
629 *Chemical Research*. 2019;58:10044–10052. <https://doi.org/10.1021/acs.iecr.9b01947>

630

631 Lusi M. A rough guide to molecular solid solution: Design, synthesis and characterization of mixed
632 crystals. *Crystal Engineering Communication*. 2018;20:7042–7052.
633 <https://doi.org/10.1039/C8CE00691A>

634

635 Macridachis J, Bayés-García L, Calvet T. An Insight into the Solid-State Miscibility of Triacylglycerol
636 Crystals. *Molecules*, 2020;25:4562. <https://doi.org/10.3390/molecules25194562>

637

638 Macridachis J, Bayés-García L, Calvet T. Mixing Phase Behavior of Tripalmitin and Oleic-Rich
639 Molecular Compound-Forming Triacylglycerols. *Industrial and Engineering Chemical Research*.
640 2021;60:5374–5384. <https://doi.org/10.1021/acs.iecr.1c00324>

641

642 Macridachis J, Bayés-García L, Calvet T. Solid phase behavior of mixture systems based on
643 tripalmitoyl glycerol and monounsaturated triacylglycerols forming a molecular compound.
644 *Physical Chemistry and Chemical Physics*. 2022;24:3749–3760.
645 <https://doi.org/10.1039/D1CP05361B>

646

647 Mahisanunt B, Hondoh H, Ueno S. Coconut oil crystallization on tripalmitin and tristearin seed

648 crystals with different polymorphs. *Crystal Growth & Design*. 2021;20:4980–4990.

649 <https://doi.org/10.1021/acs.cgd.0c00064>

650

651 Mao J, Gao Y, Meng Z. Crystallization and phase behavior in mixture systems of anhydrous milk

652 fat, palm stearin, and palm oil: Formation of eutectic crystals. *Food Chemistry*. 2023;399:133877.

653 <https://doi.org/10.1016/j.foodchem.2022.133877>

654

655 Martini S. Sonocrystallization of fats. In: *Sonocrystallization of Fats*. SpringerBriefs in Food, Health,

656 and Nutrition. Springer, New York, NY; 2013. https://doi.org/10.1007/978-1-4614-7693-1_6

657

658 Mazzanti G, Li M, Marangoni AG, Idziak SHJ. Effects of shear rate variation on the nanostructure of

659 crystallizing triglycerides. *Crystal Growth & Design*. 2011;11:4544–4550.

660 <https://doi.org/10.1021/cg200786k>

661

662 Minato A, Ueno S, Yano J, Wang ZH, Seto H, Amemiya Y, Sato K. Synchrotron radiation X-ray

663 diffractions study on phase behavior of PPP-POP binary mixtures. *Journal of the American Oil*

664 *Chemists' Society*. 1996;73:1567–1572. <https://doi.org/10.1007/BF02523526>

665

666 Minato A, Ueno S, Smith K, Amemiya Y, Sato K. Thermodynamic and Kinetic Study on Phase

667 Behavior of Binary Mixtures of POP and PPO Forming Molecular Compound Systems. *Journal*

668 *of Physical Chemistry B*. 1997a;101: 3498–3505. <https://doi.org/10.1021/jp962956v>

669

670 Minato A, Ueno S, Yano J, Smith K, Seto H, Amemiya Y, Sato K. Thermal and Structural Properties
 671 of sn-1,3-dioleoyl-2-palmitoylglycerol Binary Mixtures Examined with Synchrotron Radiation X-
 672 Ray Diffraction. *Journal of the American Oil Chemists' Society*. 1997b;74:1213–1220.
 673 <https://doi.org/10.1007/s11746-997-0047-7>
 674

675 Minato A, Yano J, Ueno S, Smith K, Sato K. FT-IR study on microscopic structures and conformations
 676 of POP-PPO and POP-OPO molecular compounds. *Chemistry and Physics of Lipids*.
 677 1997c;88:63–71. [https://doi.org/10.1016/S0009-3084\(97\)00045-5](https://doi.org/10.1016/S0009-3084(97)00045-5)
 678

679 Mizobe H, Tanaka T, Hatakeyama N, Nagai T, Ichioka K, Hondoh H, Ueno S, Sato K. Structures and
 680 binary mixing characteristics of enantiomers of 1-oleoyl-2,3-dipalmitoyl-sn-glycerol (S-OPP) and
 681 1,2-dipalmitoyl-3-oleoyl-sn-glycerol (R-PPO). *Journal of the American Oil Chemists' Society*.
 682 2013;90:1809–1817. <https://doi.org/10.1007/s11746-013-2339-4>
 683

684 Myher JJ, Kuksis A, Marai L, Sandra P. Identification of the more complex triacylglycerols in bovine
 685 milk fat by gas chromatography-mass spectrometry using polar capillary columns. *Journal of*
 686 *Chromatography*. 1988;452: 93–118. [https://doi.org/10.1016/S0021-9673\(01\)81440-0](https://doi.org/10.1016/S0021-9673(01)81440-0)
 687

688 Mykhaylyk OO, Smith KW, Martin CM, Ryan AJ. Structural models of metastable phases occurring
 689 during the crystallization process of saturated/unsaturated triacylglycerols. *Journal of Applied*.
 690 *Crystallography*. 2007;40: 297–302. <https://doi.org/10.1107/S0021889806055191>
 691

692 Nakanishi K, Mikiya Y, Ishiguro T, Ueno S. Crystallization behavior of molecular compound in binary
 693 mixture system of 1,3-dioleoyl-2-palmitoyl-sn-glycerol and 1,3-dipalmitoyl-2-oleoyl-sn-glycerol.

Journal of the American Oil Chemists' Society. 2018;95:51–59.
<https://doi.org/10.1002/aocs.12005>

Povey MJW. Crystal nucleation in food colloids. *Food Hydrocolloids*. 2014;42:118–129.
<https://doi.org/10.1016/j.foodhyd.2014.01.016>

Rossell JB. Fractionation of lauric oils. *Journal of the American Oil Chemists' Society*. 1985;62:385–390. <https://doi.org/10.1007/BF02541409>

Rousset P, Rappaz M, Minner E. Polymorphism and solidification kinetics of the binary system POS/SOS. *Journal of the American Oil Chemists' Society*. 1998;75:857–864.
<https://doi.org/doi:10.1007/s11746-998-0237-y>

Sasaki M, Ueno S, Sato K. Polymorphism and mixing phase behavior of major triacylglycerols of cocoa butter. In: Garti N, Widlak NR, editors. *Cocoa butter and related compounds*. AOCS Press: Urbana, IL, USA; 2012. p. 151–172. <https://doi.org/10.1016/B978-0-9830791-2-5.50009-8>

Seilert J, Moorthy AS, Kearsley AJ, Floeter E. Revisiting a model to predict pure triglyceride properties: parameter optimization and performance. *Journal of the American Oil Chemists' Society*. 2021;98:837–850. <https://doi.org/10.1002/aocs.12515>

Sibbald AN, Carney JR, Marangoni AG. Enhanced Structuring of Fat with Reduced Saturates Using Mixed Molecular Compounds. *Journal of the American Oil Chemists' Society*. 2016;93:1441–1452. <https://doi.org/10.1007/s11746-015-2718-0>

718

719 Smith KW. Confectionery Fats. In: Garti N, Widlak NR, editors. Cocoa butter and related
720 compounds. AOCS Press: Urbana, IL, USA; 2012. p. 475–495. [https://doi.org/10.1016/B978-0-](https://doi.org/10.1016/B978-0-9830791-2-5.50022-0)
721 9830791-2-5.50022-0

722

723 Smith KW, Bhagga K, Talbot G, van Malssen K. Crystallization of lipids: influence of minor
724 components and additives. *Journal of the American Oil Chemists' Society*. 2011;88:1085–1101.
725 <https://doi.org/10.1007/s11746-011-1819-7>

726

727 Sonwai S, Mackley MR. The effect of shear on the crystallization of cocoa butter. *Journal of the*
728 *American Oil Chemists' Society*. 2006;83:583–596. <https://doi.org/10.1007/s11746-006-1243-6>

729

730 Takeuchi M, Ueno S, Sato K. Crystallization kinetics of polymorphic forms of a molecular compound
731 constructed by SOS (1,3-distearoyl-2-oleoyl-sn-glycerol) and SSO (1,2-distearoyl-3-oleoyl-rac-
732 glycerol). *Food Research International*. 2002;35:919–926. [https://doi.org/10.1016/S0963-](https://doi.org/10.1016/S0963-9969(02)00154-0)
733 9969(02)00154-0

734

735 Talbot G. Properties of cocoa butter and vegetable fats. In: Beckett ST, Fowler MS, Ziegler GR
736 editors. *Beckett's industrial chocolate manufacture and use*. 5th ed. Chichester: Wiley-
737 Blackwell; 2017. p. 153–184. <https://doi.org/10.1002/9781118923597.ch7>

738

739 Timms RE. Phase behaviour of fats and their mixtures. *Progress in Lipid Research*. 1984;23:1–38.
740 [https://doi.org/10.1016/0163-7827\(84\)90004-3](https://doi.org/10.1016/0163-7827(84)90004-3)

741

742 Timms RE. Confectionery fats handbook. Woodhead Publishing Limited; 2003

743

744 Watanabe S, Yoshikawa S, Arishima T, Sato K. Polymorphism and mixing phase behavior in ternary
 745 mixture systems of SOS-SSO-OSO: Formation of molecular compound crystals. Journal of the
 746 American Oil Chemists' Society. 2018;95: 447–460. <https://doi.org/10.1002/aocs.12054>

747

748 Watanabe S, Yoshikawa S, Sato K. Formation and properties of dark chocolate prepared using fat
 749 mixtures of cocoa butter and symmetric/asymmetric stearic-oleic mixed-acid triacylglycerols:
 750 Impact of molecular compound crystals, Food Chemistry. 2021;339:127808.
 751 <https://doi.org/10.1016/j.foodchem.2020.127808>

752

753 Wesdorp LH, van Meeteren J, de Jon S, Giessen R, Overbosch P, Grootscholten P, Liquid-multiple
 754 solid phase equilibria in fats: theory and experiments. In: Marangoni AG, Wesdorp LH, editors.
 755 Structure and properties of fat crystal networks. Volume 2. 2nd ed. Boca Raton: CRC Press; 2013.
 756 p. 241–418. <https://doi.org/10.1201/b12883>

757

758 Wijarnprecha K, West R, Rousseau D. Temperature-Dependent Mixing Behavior of Tristearin and
 759 1,2-Distearoyl-3-oleoyl-rac-glycerol (SSO). Crystal Growth & Design. 2023;23:4807–4814.
 760 <https://doi.org/10.1021/acs.cgd.2c01514>

761

762 Ye Y, Tan CY, Kim DA, Martini S. Application of high-intensity ultrasound to a zero-trans shortening
 763 during temperature cycling at different cooling rates. Journal of the American Oil Chemists'
 764 Society. 2014;91:1155–1169. <https://doi.org/10.1007/s11746-014-2458-6>

765

766 Yoshikawa S, Kida H, Sato K. Promotional effects of new types of additives on lipid crystallization.
 767 Journal of Oleo Science. 2014;63:333–345. <https://doi.org/10.5650/jos.ess13155>
 768

769 Yoshikawa S, Watanabe S, Yamamoto Y, Kaneko F, Sato K. Interactive Polymorphic Crystallization
 770 Behavior in Eutectic Triacylglycerol Mixtures Containing Molecular Compound Crystals. Crystal
 771 Growth & Design. 2022;22:1753–1763. <https://doi.org/10.1021/acs.cgd.1c01340>
 772

773 Zhang L, Ueno S, Miura S, Sato K. Binary Phase Behavior of 1,3-dipalmitoyl-2-oleoyl-sn-glycerol
 774 and 1,2-dioleoyl-3-palmitoyl-racglycerol. Journal of the American Oil Chemists' Society.
 775 2007;84:219–227. <https://doi.org/10.1007/s11746-006-1034-0>
 776

777 Zhang L, Ueno S, Sato K, Adlof RO, List GR. Thermal and structural properties of binary mixtures of
 778 1,3-distearoyl-2-oleoyl-glycerol (SOS) and 1,2-dioleoyl-3-stearoyl-sn-glycerol (*sn*-OOS). Journal
 779 of Thermal Analyses and Calorimetry. 2009;98:105–111. [https://doi.org/10.1007/s10973-009-](https://doi.org/10.1007/s10973-009-0451-3)
 780 0451-3
 781

782 Zhang L, Ueno S, Sato K. Binary phase behavior of saturated-unsaturated mixed-acid triacylglycerols
 783 - A review. Journal of Oleo Science. 2018;67:679–687. <https://doi.org/10.5650/jos.ess17263>
 784

785 Zhang L, Wei KJ, Chen JC, Xiong M, Li X, Hondoh H, Ueno S. Effect of Cis–Trans Isomerization on
 786 the Crystallization Behavior of Triacylglycerols. Crystal Growth & Design. 2020;20:1655–1664.
 787 <https://doi.org/10.1021/acs.cgd.9b01406>