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1 **Impact of triacylglycerol composition on shear-induced textural** 2 **changes in highly saturated fats**

3 Running title: Impact of TAG-composition on shear-induced textural changes in fats

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8 **Abstract**

9 This study demonstrates a strong interaction between triacylglycerol (TAG) composition and effects of shear
10 rate on the microstructure and texture of fats. Cocoa butter alternatives with similar content of saturated fat,
11 but different major TAGs (PPO-, PSO-, SSO-, POP- and SOS- rich blends) were evaluated. Results show how
12 shear can assist a harder texture in fat blends based on symmetric monounsaturated TAGs (up to ~200%),
13 primarily due to reduction in crystal size, whereas shear has little effect on the hardness of asymmetric
14 monounsaturated TAGs. Those differences could not be ascribed to differences in the degree of supercooling,
15 but was found to be a consequence of differences in the crystallisation behaviour of different TAGs. The fractal
16 dimension was evaluated by dimensional detrended fluctuation analysis and Fourier transformation of
17 microscopy images. However, the concept of fractal patterns was found to be insufficient to describe
18 microstructural changes of fat blends with high solid fat content.

19

1 Introduction

It is generally accepted that the textural properties of fat is determined by the amount of crystallised fat present and microstructural factors such as crystal morphology, crystal size and the spatial organisation of crystals. Vreeker et al. (1992) proposed that the fat crystal network has a fractal dimension. Based on the concept of fractability, Narine and Marangoni (1999) developed a model relating rheological properties of fats with the fractal dimension by adopting a model for colloid systems developed by Shih et al. (1990). The fractal dimension of the fat crystal network can be evaluated based on microscopy images by the use of box-counting or particle counting methods (Awad & Marangoni, 2005). However, those methods are not applicable at a high solid fat content. Instead, the fractal dimension may be determined by the use of Fourier transformation (Tang & Marangoni, 2006) or detrended fluctuation analysis (Franke et al., 2015)

Control of dynamic process factors can be a potential tool for improving the texture of fats by introducing changes in the microstructural characteristics. For instance, as a mean for replacing trans-fatty acid or decreasing the content of saturated fatty acid in fat-rich food products and still retain the original texture or even improving it. Already in 1962, Feuge et al. (1962) described how crystallisation and polymorphic transformation in cocoa butter and cocoa butter-like fats were influenced by mechanical processes. Since then, effects of shear rate on the crystallisation behaviour, microstructure and texture of fats have been widely studied, and the main findings are summarised in a recent review by Patel et al. (2015). Contradictory effects of shear rate on the texture of fats are reported in the literature. Some studies show a softening effect (Acevedo et al., 2012; Herrera & Hartel, 2000b; Narine & Humphrey 2004), whereas others show a hardening effect (Campos & Marangoni, 2014; Maleky & Marangoni, 2011; Reyes-Hernández et al., 2014). Furthermore, Kaufmann et al. (2012) show a higher complex modulus of milkfat blends for intermediate shear rates than for both static crystallisation and high shear rates.

Increased shear rate provides increased heat transfer rate, alignment of TAG molecules, enhanced collision frequency and promotion of secondary nucleation by breakage of existing crystals. Furthermore, shear force may favour a higher degree of mixed crystals (Campos & Marangoni, 2014) and result in an accelerated polymorphic transformation (Sato et al., 2013). In light of the multiple effects of shear rates on fat crystallisation, we hypothesise that a strong interaction exists between triacylglycerol (TAG) composition and the effect of shear rate on the microstructure and texture of fats.

The polymorphism and phase behaviour of pure TAG system has been widely described in the literature (Himawan et al., 2006; Sato 2001). However, mixtures of pure TAGs are usually too far from industrial food applications. On the other hand, most studies on mixtures of natural fats and fat fractions rarely focus on the TAG composition due to its complexity. Attempts have been made to model the solid–liquid equilibrium of more complex TAG systems (Hjorth et al., 2015a, 2015b; Rousset et al., 1998). A few recent, more empirically based studies have also been carried out to elucidate the link between TAG composition and the crystallisation behaviour, microstructure and texture of fats (De Graef et al., 2012; Vereecken, De Graef, et al., 2010; Vereecken et al., 2009; Vereecken, Foubert, et al., 2010). Those studies focus on effects of trisaturated TAGs, chain length (palmitic acid versus stearic acid) and the symmetry of TAGs (asymmetric versus symmetric monounsaturated TAGs), and a strong interaction between those structural factors are found. For instance, trisaturated TAGs provide a faster crystallisation, but this effect is more pronounced for palmitic acid-rich blends compared with stearic acid-rich blends (Vereecken et al., 2009). Vereecken et al. (2010) show very different crystallisation mechanisms for a SOS-rich blend and a SSO-rich blend, but similar crystallisation mechanisms for a POP-rich blend and a PPO-rich blend. Furthermore, the effect of TAGs symmetry on the microstructure is found to be greater for stearic acid based blends than for palmitic acid based blends (De Graef et al., 2012).

The aim of this study was to elucidate the interaction between TAG-composition and shear rate on the microstructure and texture of fats. Five fat blends with similar level of saturated fats (50% w/w), but with either a high content of palmitic acid or stearic acid was evaluated. In addition, the fat blends were based on either symmetric (a POP-rich blend and a SOS-rich blend) or asymmetric monounsaturated TAGs (a PPO-rich blend, an SSO-rich blend and a PSO-rich blend).

69

2 Materials and Methods

2.1 Fat blends

Five different fats typically used in the manufacturing of cocoa butter alternatives were used for preparation of blends with similar levels of saturated fatty acids (50% w/w). In order to reach a level of 50% saturated fatty acids, the fats were diluted with high oleic sunflower oil (HOSFO) followed by mixing (250 rpm) for at least one

75 hour using a magnetic stirrer (~60°C). Table 1 gives the relative TAG composition of fats before dilution with
76 high oleic sunflower oil. All fats were kindly provided by AarhusKarlshamn Denmark A/S (Aarhus, Denmark).

77 **Table 1**

78

79 *2.2 Crystallisation procedure*

80 Before crystallisation, fat blends were heated to 60°C and kept at this temperature for at least one hour to
81 destroy all crystal memory. A control cooling from 60°C to a predefined crystallisation temperature (1°C/min)
82 was performed using a programmable water bath (Proline RP-845, Lauda, Germany). A crystallisation
83 temperature of 20°C was used for all fat blends and further to evaluate effects of the degree of supercooling,
84 the PPO-rich blend and the POP-rich blend were also crystallised at 15°C and the SOS-rich blend was
85 crystallised at 25°C. The choice of crystallisation temperatures was based on the difference in melting point
86 between stearic acid and palmitic acid. Based on the melting points of the most stable polymorphic forms of
87 the pure TAGs this provided an estimated supercooling of approximate ~20°C or ~15°C for the different fat
88 blends; ~20°C of supercooling for S-rich blends crystallisation at 20°C and for P-rich blends crystallised at
89 15°C and ~15°C of supercooling for P-rich blends crystallised at 20°C and the SOS-rich blend crystallised at
90 25°C.

91 The cooling period was followed by an isothermal crystallisation for 5 min. providing a slurry of crystallised and
92 liquid fat, which was transferred to appropriate storage containers. Crystallisation was conducted with different
93 agitation rates (0, 30, 40, 60, 80, 100, 120, 140 or 160 rpm) using a centrifugal stirrer (IKA Centrifugal stirrer,
94 Ø 60 mm, KA-Werke GmBh & Co, Staufen, Germany) coupled with a digital stirring unit (IKA Eurostar 20
95 digital, IKA-Werke GmBh & Co, Staufen, Germany).

96 *2.3 Solid fat content by pulsed NMR*

97 Solid fat content was analysed by pulsed NMR (pNMR). Crystallisation was performed as described in section
98 2.2 and the fat slurry was transferred to NMR tubes (Ø = 10mm). SFC was determined after 1 day and 14 days
99 of storage (20°C) by pNMR (Minispec mq20, Bruker, Germany) using the direct method (AOCS(Cd.16b-93)).
100 Three SFC determinations were conducted for each treatment (samples from the same batch).

101

102 *2.4 Differential scanning calorimetry*

103 The crystallisation behaviour of fat blends was evaluated by differential scanning calorimetry (DSC). Melted
104 fat of 10-14 mg was loaded into aluminium pans and analysed using a differential scanning calorimeter (Q1000
105 DSC, TA Instruments, New Castle, DE, USA) by heating at 60°C for 10 min followed by a controlled cooling at
106 1°C/min from 60°C to 20°C and a subsequent isothermal crystallisation for 5 min. An empty aluminium pan
107 was used as reference. DSC analyses were conducted in triplicates.

108 *2.5 Confocal scanning laser microscopy (CLSM)*

109 CLSM was used to study the microstructure of the crystallised fat. Fat blends with 0.0001 wt% Nile red were
110 prepared by dissolved the dye in liquid fat overnight at 60°C. Crystallisation was performed as described in
111 section 2.2 for agitation rates where the most pronounced effect on texture was observed for each fat. After
112 crystallisation, one droplet of the crystallised slurry was placed on an objective slide with a spherical depression
113 and a cover slide was placed on top of the sample. Three samples were prepared for each treatment (samples
114 from the same batch). After storage for 1 day at 20°C, samples were examined by CLSM (Nikon C2, Nikon
115 Instrument Inc, Tokyo, Japan) using a 60x objective lens. The argon laser beam was set at 568 nm excitation.
116 This method provided visualisation of the liquid fat phase, whereas the crystallised fat appeared dark. For each
117 sample, 5-7 images were obtained and subsequent evaluated. Different quantitative parameters were
118 determined based on CLSM images. The fractal dimension was calculated based on Fourier transformation
119 using the *MATLAB* routine *FDSURFFT*. This routine is based on the algorithm proposed by Russ (1995) and
120 has previously been tested by Mancardi et al. (2008) for quantification of the vascular architecture. The fractal
121 nature was also evaluated by determination of the scaling exponent from Two Dimensional Detrended
122 Fluctuation Analysis (2-D DFA) by implementation of the algorithm proposed by Yeh (2012) in *MATLAB*
123 (*MATLAB* Version R2014b, The Mathworks Inc., Natick, U.S.). Furthermore, the normalised grayscale variance
124 was evaluated as described in a previous study (Gregersen et al., 2015b).

125 *2.6 Hardness measurements*

126 Needle penetration test was used to evaluate the hardness. Fat slurry was transferred to plastic cups ($\varnothing=3$
127 cm, sample height ~3 cm) after crystallisation as described in Section 2.2. Hardness was determined after 1
128 day and 14 days of storage (20°C) using a Texture Analyser (FTC TMS-Touch, Food Technology Corporation,
129 USA) in compression mode. Penetration was performed to a depth of 7 mm with a constant speed of 0.5 mm/s
130 using a needle probe (Stable Micro Systems: P/2N, $\varnothing$ 2mm). The force versus distance was recorded and the

131 hardness was defined as the maximum penetration force. Five determinations on each sample were carried
132 out and three samples were evaluated for each treatment (samples from the same batch). Measurements were
133 performed at room temperature.

134 *2.7 Brittleness analysis*

135 Cone penetration test was used to characterise brittleness of the fat as described in a previous study
136 (Gregersen et al., 2015b). Samples were prepared as described for hardness measurements (Section 2.6).
137 Analysis was performed on a Texture Analyser (FTC TMS-Touch, Food Technology Corporation, USA) using
138 a cone probe (Stable Micro Systems: P/30C, 30° conical probe). Each sample was penetrated to a depth of 8
139 mm with a constant speed of 0.2 mm/s. The force versus distance was recorded. A second order polynomial
140 curve was fitted to the obtained data. The area difference between the fitted polynomial curve and the actual
141 penetration curve was used as a measure for the brittleness, i.e. a large difference between fitted and recorded
142 curves indicates a more brittle sample. Three samples were analysed for each treatment (samples from the
143 same batch) and measurements were performed at room temperature.

144 **3 Results and discussion**

145 *3.1 Crystallisation behaviour and solid fat content*

146 Fat blends with the same level of saturated fatty acid but different levels of palmitic acid and steric acid, and
147 either based on symmetric or asymmetric monounsaturated TAGs were evaluated; a POP-rich blend, a SOS-
148 rich blend, a PPO-rich blend, a PSO-rich blend and a SSO-rich blend. Thus, representing the main TAGs of
149 vegetable oils used in the chocolate and confectionery industry. SOS and POP are the main constituents of
150 cocoa butter equivalents, whereas SSO, PSO and PPO are important in low-*trans* cocoa butter replacers
151 (Talbot, 2009). Like all fats from natural sources, the present fat blends have a relative broad TAG composition
152 (Table 1).

153 DSC analysis showed large differences in the crystallisation behaviour of the present fat blends (Figure 1).
154 The PPO-rich fat crystallised at a high temperature (shorter time prior to crystallisation onset) compared with
155 the other fat blends, most likely as a consequence of the minor component, PPP, acting as a seeding agent.
156 A very fast initial crystallisation was observed for the PSO-rich fat, but at a higher degree of supercooling than
157 the PPO-rich blend (longer time prior to crystallisation onset). Compound crystal formation might explain this

behaviour as compound crystals are known to crystallise faster than the pure components of the same polymorph (Himawan et al., 2006). The PSO-rich blend has a complex composition with a relative high amount of PPO (10.2%), POP (4.3%), SOS (5.5%) and SSO (14.5%), which all have been shown to form compound crystals (Minato et al., 1997; Takeuchi et al., 2002). Furthermore, PSO and POS (22.3% and 9.3% of the PSO-rich blend, respectively) may also form compound crystals. The SSO-rich blend also showed a very fast initial crystallisation, as observed by a steep crystallisation curve, which again indicates compound crystal formation. The SSO-rich blend has a high content of SSO (49.1%) and SOS (21.9%). DSC thermograms indicate a slightly slower initial crystallisation for the SOS-rich blend and the POP-rich blend compared with fat blends based on asymmetric monounsaturated TAGs as observed by less steep crystallisation curves (Figure 1). This was followed by a relative fast crystallisation of a small fraction for both the SOS-rich fat and the POP-rich fat. The crystallisation behaviour of the individual fat blends was very similar to what we have shown previously for the different fats without dilution with HOSFO (Gregersen et al., 2015a; Gregersen et al., 2015b).

Figure 1

Effects of agitation rate on the melting behaviour of fat blends after storage for 14 days were also evaluated by DSC. However, only small differences were observed in the melting behaviour of the fat blends for different agitation rates (Supplementary material). Thus, the final polymorphic form of the present fat blends is most likely unaffected by agitation rate. However, a shift to a higher melting temperature for increased agitation rate was observed for the SOS-rich blend after 1 day of storage, indicating a faster polymorphic transformation (Supplementary material). This was not observed for any of the other fat blends.

SFC was analysed after 1 day and 14 days of storage (20°C) for different agitation rate. No significant effect of agitation rate on the SFC was observed for any of the fat blends (data not shown), which is in agreement with previous findings (Herrera & Hartel, 2000b; Kaufmann et al., 2012; Maleky et al., 2011). The final SFC after 14 days of storage was different for the fat blends, despite similar levels of saturated fat; ~57% for the PPO-rich blend, ~55% for the PSO-rich blend, ~63% for the SSO-rich, ~68% for the POP-rich fat and ~72% for the SOS-rich fat ($T_c = 20^\circ\text{C}$). This could only partly be explained by the higher melting point of stearic acid than of palmitic acid, and differences in the level of minor TAGs as well as the fractional behaviour or polymorphism might be of importance.

3.2 Microstructure of the crystallised fats

186 The microstructure was evaluated by CLSM after crystallisation with different agitation levels. A strong
187 interaction between agitation rate and TAG composition was found, but no general effect of agitation rate on
188 crystal morphology, crystal/crystal cluster size or the spatial organisation of crystals/crystal clusters was
189 observed (Figure 2).

190 For fat blends based on symmetric monounsaturated TAGs (POP-rich and SOS-rich blends), increased
191 agitation provided smaller crystal clusters. This is in agreement with most previous findings in the literature
192 (Campos & Marangoni, 2014; Herrera & Hartel, 2000a; Kaufmann et al., 2012; Martini et al., 2002). The
193 decrease in crystal cluster size was more pronounced for the SOS-rich blend than the POP-rich blend. For the
194 SOS-rich blend, longer needle-shaped boundaries and a higher degree of aggregation between crystals was
195 also observed at higher agitation rate. The POP-rich blend formed irregular crystal clusters with a less dense
196 core and needle shaped boundaries independently of agitation rate. The effect of agitation rate on the crystal
197 cluster size was slightly more pronounced when lowering the crystallisation temperature for both the POP-rich
198 fat and the SOS-rich fat, i.e. 15°C compared with 20°C for the POP-rich blend, and 20°C compared with 25°C
199 for the SOS-rich blend. The PPO-rich fat formed a homogenous network of crystal clusters composed of
200 filament shaped crystals at static crystallisation, but upon increased agitation larger crystal clusters with a
201 dense core was observed. This effect was more pronounced at a crystallisation temperature of 20°C than at
202 15°C. The crystal cluster size was unaffected by agitation rate for the SSO-rich blend and the PSO-rich blend.
203 However, agitation resulted in a less homogenous network structure for the PSO-rich fat i.e. formation of larger
204 disorder aggregates of filament shaped crystals. The SSO-rich fat was observed to form an inhomogeneous
205 network structure of large dense crystal clusters connected by smaller and less dense crystals independently
206 of agitation rate.

207 **Figure 2**

208 The contradictory effects of agitation on the microstructure of fat blends could not be ascribed to differences
209 in the degree of supercooling. All blends was crystallised at an estimated supercooling of approximate ~20°C;
210 S-rich blends crystallisation at 20°C and P-rich blends crystallised at 15°C. Furthermore, an estimated
211 supercooling of ~15°C was evaluated for P-rich blends crystallised at 20°C and the SOS-rich blend crystallised
212 at 25°C.

213 Shear introduced by agitation may influence all stages of the crystallisation process. For instance, increased
214 shear can induce the primary nucleation due to an increase in heat and mass transfer rate, alignment of TAG

215 molecules or better distribution of impurities. Furthermore, shear forces may break existing crystal, thereby
216 promoting secondary nucleation. Increased shear also provides an increased collision frequency, which can
217 enhance crystal growth and aggregation of crystals. Thus, the effect of shear rate on nucleation, crystal growth
218 and aggregation is most likely a balance between the different mechanisms, which most likely is susceptible
219 to the crystallisation conditions, the magnitude of the shear rate and the nature of the system, explaining the
220 contradictory effect on the microstructure observed in this study, as well as in the literature.

221 The pronounced effect of agitation on the crystal size of the symmetric monounsaturated fat blends might be
222 ascribed to a relative slow nucleation, as observed by less steep crystallisation curves (Figure 1), but a narrow
223 crystallisation time, which without agitation results in large crystals. However, these are easier to break up by
224 agitation forces. Small crystal fragments from the breakings will induce more secondary nucleation, thus,
225 promoting smaller crystals/crystal clusters. The asymmetric TAG blends have a more steep crystallisation
226 curve, but longer crystallisation times, providing rather small crystals under static conditions, which apparently
227 are more difficult to break up, but seem to cluster a bit more upon agitation, presumably due to higher collision
228 frequency.

229 *3.3 Textural properties of the crystallised fats*

230 The effect of agitation rate on the texture of fat blends was evaluated by needle penetration (hardness) and
231 cone penetration (brittleness). Increased agitation influenced both hardness and brittleness differently for the
232 different fat blends (Figure 3 and Figure 4).

233 The effect of agitation rate on hardness was more pronounced for fat blends based on symmetric
234 monounsaturated TAGs than for fat blends based on asymmetric monounsaturated TAGs. The hardness
235 increased ~200% upon high agitation rates compared with static crystallisation for the SOS-rich fat (Figure
236 3E), and approximately 60% for the POP-rich blend, but here intermediate agitation was enough to obtain the
237 effect (Figure 3D). The effect of agitation rate on the hardness of the SOS-rich blend and the POP-rich blend
238 was, however, strongly depended on crystallisation temperature and storage time (Figure 3D and 3E).
239 Furthermore, higher agitation rates provided an increase in the brittleness of the SOS-rich blend (Figure 4E),
240 whereas the POP-rich blend showed a low degree of brittleness independently of agitation rate (Figure 4D).
241 The increase in hardness of the POP-rich blend and the SOS-rich blend with increased agitation rate is
242 correlated with the reduction in crystal size revealed by the microscopy analyses (Figure 2). A reduction in
243 crystal size results in an increase in the number of interaction sites between microstructural elements, thus,

correspond with a harder texture. Increased aggregation and more needle-shaped boundaries may also contribute to the large increase in hardness observed for the SOS-rich blend. This may also partly explain the increase in brittleness of the SOS-rich blend at high agitation rates. A more rigid structure due to strong interparticle links is expected to provide a more brittle texture (Gregersen et al., 2015b).

The hardness of fat blends based on asymmetric monounsaturated TAGs was nearly unaffected by agitation rate (Figure 3A, 3B and 3C), despite the pronounced effect of agitation on the microstructure of the PPO-rich fat and the PSO-rich fat (Figure 2). This may be due to contradictory effects on the strength of the interaction within the fat crystal network i.e. larger but denser crystals for the PPO-rich fat and a less homogenous network structure but with a higher degree of aggregation for the PSO-rich fat. The latter may also contribute to an increase in brittleness of the PSO-rich fat at high agitation rates (Figure 4B). Brittleness of the PPO-rich blend and the SSO-rich blend was unaffected by agitation rate (Figure 4A and 4C).

Independently of the symmetry of the major TAGs, the texture of S-rich blends was harder than the P-rich blends. For instance, the SSO-rich blend was >60% harder than the PPO-rich blend, and for an intermediate agitation rate (100 rpm) the SOS-rich blend was approximate 30% harder than the POP-rich blend. This is in agreement with previous findings by De Graef et al. (2012) and may be due to the similarity in chain length between stearic acid and oleic acid allowing a denser packing of the monounsaturated TAGs. S-rich blends (PSO-rich blend, SSO-blend and SOS-rich blend) also had a more brittle texture compared with P-rich blends (PPO-rich blend and POP-rich blend), which supports the findings in our previous study (Gregersen et al., 2015b). However, the brittleness of the PSO-rich blend and the SOS-rich blend was depended strongly on the agitation rate and crystallisation temperature. Furthermore, brittleness increased during storage for the PSO-rich blend, the SSO-rich blend and the SOS-rich blend, most likely because of increased formation of solid bridges.

For the symmetric TAG blends, a more pronounced effect of agitation rate on the textural characteristic was observed at a lower crystallisation temperature than at a higher crystallisation temperature (15°C compared with 20°C for the POP-rich blend and 20°C compared with 25°C for the SOS-rich blend). In contrast, for the PPO-rich blend a more pronounced effect of agitation was observed at a higher crystallisation temperature.

Figure 3

Figure 4

272 3.4 Image analysis of microstructure in relation to texture

273 The quantitative relation between microstructure and texture was examined by three different types of image
274 analysis; fractal dimension by Fourier transformation, scaling exponent from 2-D DFA and the greyscale
275 normalized variance (Figure 5).

276 The fat crystal network is suggested to have a fractal nature (Vreeker et al., 1992) and the fractal dimension
277 has been used to model the rheological properties of fats (Narine & Marangoni 1999). However, no
278 significantly correlation was found between the fractal dimension determined by Fourier transformation of
279 CLSM images and the hardness for any of the present fat blends ($p > 0.05$) (Figure 5A). The 2-D DFA has
280 more recently been introduced to determine the fractal dimension of images based on different algorithms
281 (Alvarez-Ramirez et al., 2006; Wang et al., 2015; Yeh et al., 2012), and Franke et al. (2015) reported a high
282 positive correlation between the scaling exponent from 2-D DFA and the firmness of fractions of palm oil, palm
283 kernel fat and milk fat. In agreement, results from this study indicate a positive relation between hardness and
284 the scaling exponent (Figure 5B), with exception of one outlier (SOS-rich fat with high hardness value >6 N).
285 This was, however, not significant ($p > 0.05$) and for the individual fat blends, a high correlation between the
286 scaling exponent and the hardness was only observed for the POP-rich blend (Pearson correlation coefficient
287 = 0.9). The high scaling exponents observed for the SSO-rich blend, the PPO-rich blend and the SOS-rich
288 blend indicate a lower degree of self-similarity and some samples may not even be considered as fractal. Thus,
289 the concept of fractal patterns is insufficient to describe microstructural changes for fat blends with high SFC.
290 We have in a previous study shown how the normalised grey scale variance of microscopy images of fats are
291 related to the size of the microstructural elements (Gregersen et al., 2015b). A relative high negative correlation
292 was observed between the hardness of the SOS-rich blend and the normalised grayscale variance (Figure 4C,
293 Pearson correlation = -0.71), in agreement with the observed decrease in crystal size for increased agitation.
294 However, results show a low correlation between the normalised grayscale variance and the hardness of the
295 POP-rich blend, although reduction in crystal cluster size most was found to be of importance for the effect of
296 agitation on the hardness of the POP-rich blend. The normalised grayscale variance of a greyscale image is
297 very sensitive to both resolution of the image and noise (de Bont et al., 2002). Thus, this type of evaluation
298 may not be sensitive enough to reflect the small differences in crystal size observed for the POP-rich blend.
299 The textural characteristic of the SSO-rich fat was unaffected by agitation rate and in agreement no effect on
300 the different microstructural parameters was observed. For the PPO-rich blend and PSO-rich blend the

301 determined microstructural parameters were strongly affected by agitation rate, but this was not reflected in
302 the textural characteristics, most likely as a consequence of contradictory effects on the strength of the fat
303 crystal network.

304 Nanocrystalline platelets is suggest to make up the mesoscale crystals observed on confocal laser scanning
305 microscopy images of fat (Acevedo & Marangoni 2014), and effects at the nanoscale level may also be of
306 importance for the contradictory effects of shear rate on asymmetric TAG blends and symmetric TAG blends.
307 Symmetric TAG systems may form larger nanocrystalline platelets compared with the asymmetric TAG blends,
308 which makes it easier to break op the resulting mesoscale crystals. However, evaluation of the fat crystal
309 network at the nanoscale is needed to verify such effects.

310 **Figure 5**

311 **4 Conclusion**

312 Results from this study demonstrate a strong interaction between TAG-composition and effects of shear rate
313 on the microstructure and texture of fats. Shear can assist a harder texture in fat blends based on symmetric
314 monounsaturated TAGs, whereas introduction of shear has little effect on the hardness of asymmetric
315 monounsaturated TAGs. Shear most likely enhances secondary nucleation for symmetric TAG blends; hence
316 providing smaller crystals. In contrast, shear may affect crystal growth and aggregation of the asymmetric TAG
317 blends to a higher extent then the initial nucleation, thereby, providing larger crystals/crystal clusters and a
318 less homogenous network. In general, the S-rich blends obtained a harder but more brittle texture than the P-
319 rich blends.

320 In conclusion, this study demonstrates that it is difficult to predict effects of shear rate on the functionality of
321 fats due to a strong interaction between TAG-composition and shear rate. Thus, improving the functionality of
322 fat by control of shear forces has to rely on experimental data for the fat and processing system of interest.

323

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References

- Acevedo, N. C., Block, J. M., & Marangoni, A. G. (2012). Critical laminar shear-temperature effects on the nano-and mesoscale structure of a model fat and its relationship to oil binding and rheological properties. *Faraday discussions*, 158, 171-194.
- Acevedo, N. C., & Marangoni, A. G. (2014). Nanostructured Fat Crystal Systems. *Annual review of food science and technology*, 6, 71-96.
- Alvarez-Ramirez, J., Rodriguez, E., Cervantes, I., & Carlos Echeverria, J. (2006). Scaling properties of image textures: A detrending fluctuation analysis approach. *Physica A: Statistical Mechanics and its Applications*, 361, 677-698.
- AOCS(Cd.16b-93). Solid fat content (SFC) by low-resolution nuclear magnetic resonance - the direct method. Official methods and Recommended Practices of the AOCS, 6th Ed. 2009. 2011-2012 Methods and Additions and Revisions.
- Awad, & Marangoni. (2005). Chapter 11, Comparison between image analyses methods for the determination of the fractal dimension of fat crystal networks. In A. G. Marangoni (Ed.), *Fat Crystal Networks* (pp. 381-386). New York: Marcel Decker.
- Campos, R., & Marangoni, A. G. (2014). Crystallization Dynamics of Shear Worked Cocoa Butter. *Crystal Growth & Design*, 14, 1199-1210.
- de Bont, P. W., van Kempen, G. M., & Vreeker, R. (2002). Phase separation in milk protein and amylopectin mixtures. *Food Hydrocolloids*, 16, 127-138.
- De Graef, V., Vereecken, J., Smith, K. W., Bhaggan, K., & Dewettinck, K. (2012). Effect of TAG composition on the solid fat content profile, microstructure, and hardness of model fat blends with identical saturated fatty acid content. *European Journal of Lipid Science and Technology*, 114, 592-601.
- Feuge, R. O., Landmann, W., Mitcham, D., & Lovegren, N. V. (1962). Tempering triglycerides by mechanical working. *Journal of the American Oil Chemists Society*, 39, 310-313.
- Franke, K., Bindrich, U., & Heinz, V. (2015). Fat crystal network structures have a strong influence on properties of fat-based barrier layers. *European Journal of Lipid Science and Technology*(117), 1792-1800.
- Gregersen, S. B., Miller, R. L., Andersen, M. D., Hammershøj, M., & Wiking, L. (2015a). Inhomogeneous consistency of crystallized fat. *European Journal of Lipid Science and Technology*, 117, 1782-1791.
- Gregersen, S. B., Miller, R. L., Hammershøj, M., Andersen, M. D., & Wiking, L. (2015b). Texture and microstructure of cocoa butter replacers: Influence of composition and cooling rate. *Food Structure*, 4, 2-15.
- Herrera, M., & Hartel, R. (2000a). Effect of processing conditions on physical properties of a milk fat model system: microstructure. *Journal of the American Oil Chemists' Society*, 77, 1197-1205.
- Herrera, M., & Hartel, R. (2000b). Effect of processing conditions on physical properties of a milk fat model system: Rheology. *Journal of the American Oil Chemists' Society*, 77, 1189-1196.
- Himawan, C., Starov, V. M., & Stapley, A. G. F. (2006). Thermodynamic and Kinetic Aspects of Fat Crystallization. *Advances in Colloid and Interface Science*, 122, 3-33.
- Hjorth, J. L., Miller, R. L., Woodley, J. M., & Kiil, S. (2015a). Thermodynamic Modeling of Multi-phase Solid-Liquid Equilibria in Industrial-Grade Oils and Fats. *Journal of the American Oil Chemists' Society*, 92, 17-28.
- Hjorth, J. L., Miller, R. L., Woodley, J. M., & Kiil, S. (2015b). Kinetic modeling of multi-component crystallization of industrial-grade oils and fats. *European Journal of Lipid Science and Technology*, 117, 1066-1078.
- Kaufmann, N., De Graef, V., Dewettinck, K., & Wiking, L. (2012). Shear-induced crystal structure formation in milk fat and blends with rapeseed oil. *Food Biophysics*, 7, 308-316.
- Maleky, F., & Marangoni, A. (2011). Thermal and mechanical properties of cocoa butter crystallized under an external laminar shear field. *Crystal Growth & Design*, 11, 2429-2437.
- Maleky, F., Smith, A. K., & Marangoni, A. (2011). Laminar shear effects on crystalline alignments and nanostructure of a triacylglycerol crystal network. *Crystal Growth & Design*, 11, 2335-2345.
- Mancardi, D., Varetto, G., Bucci, E., Maniero, F., & Guiot, C. (2008). Theoretical Biology and Medical Modelling. *Theoretical Biology and Medical Modelling*, 5, 12.
- Martini, S., Herrera, M., & Hartel, R. (2002). Effect of processing conditions on microstructure of milk fat fraction/sunflower oil blends. *Journal of the American Oil Chemists' Society*, 79, 1063-1068.
- Minato, A., Ueno, S., Smith, K., Amemiya, Y., & Sato, K. (1997). Thermodynamic and kinetic study on phase behavior of binary mixtures of POP and PPO forming molecular compound systems. *The Journal of Physical Chemistry B*, 101, 3498-3505.

384 Narine , S. S., & Humphrey , K. L. (2004). A comparison of lipid shortening functionality as a function of
385 molecular ensemble and shear: microstructure, polymorphism, solid fat content and texture. *Food*
386 *research international*, 37, 28-38.

387 Narine , S. S., & Marangoni , A. G. (1999). Fractal nature of fat crystal networks. *Physical Review E*, 59,
388 1908-1920.

389 Patel, A. R., & Dewettinck, K. (2015). Current update on the influence of minor lipid components, shear and
390 presence of interfaces on fat crystallization. *Current Opinion in Food Science*, 3, 65-70.

391 Reyes-Hernández, J., Pérez-Martínez, J. D., & Toro-Vazquez, J. F. (2014). Influence of Processing
392 Conditions on the Physicochemical Properties of Complex Fat Systems. *Journal of the American Oil*
393 *Chemists' Society*, 91, 1247-1259.

394 Rousset, P., Rappaz, M., & Minner, E. (1998). Polymorphism and solidification kinetics of the binary system
395 POS-SOS. *Journal of the American Oil Chemists' Society*, 75, 857-864.

396 Russ, J. C. (1995). *The image processing handbook* (2nd Ed). Boca Raton: CRC Press.

397 Sato , K. (2001). Crystallization behaviour of fats and lipids - a review. *Chemical Engineering Science*, 56,
398 2255-2265.

399 Sato, K., Bayés-García, L., Calvet, T., Cuevas-Diarte, M. À., & Ueno, S. (2013). External factors affecting
400 polymorphic crystallization of lipids. *European Journal of Lipid Science and Technology*, 115, 1224-
401 1238.

402 Shih, W.-H., Shih, W. Y., Kim, S.-I., Liu, J., & Aksay, I. A. (1990). Scaling behavior of the elastic properties of
403 colloidal gels. *Physical Review A*, 42, 4772.

404 Takeuchi, M., Ueno, S., & Sato, K. (2002). Crystallization kinetics of polymorphic forms of a molecular
405 compound constructed by SOS (1,3-distearoyl-2-oleoyl-sn-glycerol) and SSO (1,2-distearoyl-3-
406 oleoyl-rac-glycerol). *Food Research International*, 35, 919-926.

407 Talbot, G. (2009). Vegetable Fats. In S. T. Beckett (Ed.), *Industrial Chocolate Manufacture and Use* (4 ed.,
408 pp. 415-433). Oxford: Wiley-Blackwell.

409 Tang, D., & Marangoni, A. G. (2006). Computer simulation of fractal dimensions of fat crystal networks.
410 *Journal of the American Oil Chemists' Society*, 83, 309-314.

411 Vereecken, J., De Graef, V., Smith, K. W., Wouters, J., & Dewettinck, K. (2010). Effect of TAG composition
412 on the crystallization behaviour of model fat blends with the same saturated fat content. *Food*
413 *Research International*, 43, 2057-2067.

414 Vereecken, J., Foubert, I., Smith, K. W., & Dewettinck, K. (2009). Effect of SatSatSat and SatOSat on
415 crystallization of model fat blends. *European journal of lipid science and technology*, 111, 243-258.

416 Vereecken, J., Foubert, I., Smith, K. W., Sassano, G. J., & Dewettinck, K. (2010). Crystallization of model fat
417 blends containing symmetric and asymmetric monounsaturated triacylglycerols. *European Journal of*
418 *Lipid Science and Technology*, 112, 233-245.

419 Vreeker, R., Hoekstra, L., Den Boer, D., & Agterof, W. (1992). The fractal nature of fat crystal networks.
420 *Colloids and surfaces*, 65, 185-189.

421 Wang, F., Liao, D.-w., Li, J.-w., & Liao, G.-p. (2015). Two-dimensional multifractal detrended fluctuation
422 analysis for plant identification. *Plant methods*, 11, 12.

423 Yeh, R.-G., Lin, C.-W., Abbod, M. F., & Shieh, J.-S. (2012). Two-dimensional matrix algorithm using
424 detrended fluctuation analysis to distinguish burkitt and diffuse large b-cell lymphoma. *Computational*
425 *and mathematical methods in medicine*, 2012. doi: 10.1155/2012/947191

427 **Figure captions**

428

429 **Figure 1. Crystallisation curves.** DSC crystallization curves of fat blends shifted relative to each other.
430 Samples was heating at 60°C for 10 min followed by a controlled cooling at 1°C/min from 60°C to 20°C and a
431 subsequent isothermal crystallisation for 5 min. Curves are representative examples from 3 replicates. The
432 crystallization curves are shifted relative to each other.

433

434 **Figure 2. CLSM images.** CLSM images of PPO-rich blend, PSO-rich blend, POP-rich blend and SOS-rich
435 blend cooled from 60°C to a crystallisation temperature (15°C, 20°C and 25°C) static or dynamic (different
436 agitation rate) and analysed after 1 day of storage (20°C)

437 **Figure 3. Hardness.** Hardness of fats blends determined by needle penetration test after 1 day or 14 days
438 of storage (20°C) for different agitation rates and crystallisation temperatures (T_c). Error bars shows standard
439 variation between replicates from the same batch ($n = 3$).

440 **Figure 4. Brittleness.** Brittleness of fat blends determined by cone penetration test after 1 day or 14 days of
441 storage (20°C) for different agitation rates and crystallisation temperatures (T_c). Error bars shows standard
442 variation between replicates from the same batch ($n = 3$).

443 **Figure 5. Correlation of hardness and quantitative parameters from CLSM images.** Scatter plots of the
444 hardness determined by the needle penetration test and the fractal dimension determined by Fourier
445 transformation (A), the scaling exponent from 2-D DFA (B) and the normalised greyscale variance (C) of CLSM
446 images. Image parameters are average values from 4–5 images for each of two samples.

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