1 Influence of monoglycerides, polyglycerol esters,
2 lecithin and their mixtures on the onset of non-
3 isothermal fat crystallization is concentration
4 dependent

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12 Influence of emulsifiers on fat crystallization

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21 agents, mixture design

22 **Abbreviations**

23	DSC	differential scanning calorimetry
24	ELSD	evaporative light scattering detector
25	GC-FID	gas chromatography-flame ionization detector
26	L	commercially available sunflower lecithin used in this study provided by
27		<i>Ingrizo N.V.</i> (De Pinte, Belgium)
28	M	commercially available monoacylglycerol used in this study provided by <i>Oleon</i>
29		<i>N.V.</i> (Oelegem, Belgium)
30	MAG	monoacylglycerol
31	P	commercially available polyglycerol esters used in this study provided by
32		<i>Ingrizo N.V.</i> (De Pinte, Belgium)
33	PGE	polyglycerol esters
34	PO	palm oil
35	RBD	refined, bleached, deodorized
36	SFC	solid fat content
37	TAG	triacylglycerol
38	T _{onset}	onset temperature of the high-melting fraction during crystallization

39 **1. Abstract**

40 The objective of this study was to investigate the influence of commercially available
41 monoacylglycerol (M), polyglycerol esters (P) and sunflower lecithin (L) on the onset of
42 crystallization of bulk fat as a function of the added concentration. Not only a single type of
43 emulsifier, but also binary and ternary mixtures of M, P and L were added to the two different
44 ‘host’ fats using a statistically sound mixture design. The obtained mixtures were analyzed for
45 their non-isothermal crystallization behavior by differential scanning calorimetry. If a single
46 type of emulsifier was added, the nucleation was accelerated when adding M or P, while L
47 addition had no significant effect. Furthermore it was shown that the minimum concentration
48 necessary to provoke a nucleation accelerating effect is lower for P than for M. Antagonistic
49 effects between M and L (and not between P and L) were suggested by the coefficient
50 estimates of the special cubic model.

Practical applications: Palm oil (PO) is one of the most consumed vegetable oils in the world because of its unique physical properties. It imparts a very high stability to the β' crystal form, yielding relatively small crystals which give a smooth texture to e.g. margarines and shortenings. Because PO is semi-solid at room temperature, it forms an ideal hard stock to be used in trans free food products. However PO has a slow crystallization rate and an unusually long α lifetime. These disadvantages can be overcome by the addition of an emulsifier acting as crystallization modifier (e.g. monoacylglycerols and diacylglycerols) [1]. Furthermore, from a nutritional point of view, the saturated fatty acids present in PO [2], are among the factors contributing to cardio-vascular diseases [3]. When using more unsaturated fats as hard stock, the use of additives in order to avoid compromising the functionality of the food product should be further investigated.

2. Introduction

Food emulsifiers are amphiphilic molecules, which means they contain both hydrophilic and hydrophobic groups [4]. Numerous manufactured food products contain emulsifiers which are added to improve the physicochemical characteristics: e.g. as foam stabilizer in whipping cream [5, 6], viscosity reducer in chocolate products [6, 7, 8, 9], dough strengthener in bread [6, 10] and aerator in cake [6, 11]. In these types of applications the emulsifiers act as surface-active compounds because they take position at the interface of multiphase systems where they lower the surface tension [4, 12, 13]. Additionally, industry's interest in the use of emulsifiers as minor components in the production of edible fat products to give them desirable melting and crystallization properties is continuously increasing [14]. In this study, the term emulsifier is used to describe amphiphilic molecules which influence the melting and crystallization properties of a fat rather than forming an emulsion of two or more substances.

Previous research has demonstrated that the addition of emulsifiers can modify (promote or inhibit) the nucleation, crystal growth, polymorphic transitions and melting profile of emulsified and bulk fat [14]. In this way, both the kinetics and thermodynamics of fat crystallization can be manipulated by emulsifier addition. The kinetics, usually described by the crystallization rate, are important for controlling the rate of operations in the food industry. The thermodynamics however, strongly influence the mechanical behavior of fats, including hardness and spreadability [15] and are mostly referred to by the equilibrium solid fat content (SFC), although this is not the only relevant determining characteristic (e.g. also the number and size of the crystals matter) [16].

Emulsifiers can promote or inhibit nucleation by respectively providing or shielding heterogeneous nucleation sites. The second stage of crystallization, crystal growth, may also be either promoted by emulsifiers forming additional growth sites or inhibited when the emulsifiers block crystal growth sites. Nucleation and crystal growth are two distinctly separated processes in terms of emulsifiers as one emulsifier may affect both processes in the same or in an opposite way or may affect only one process. A more complex aspect of fat crystallization is the phenomenon of polymorphism. Certain emulsifiers may be applied to delay undesired polymorphic transformations by increasing the energy barrier for transformation or by blocking specific faces at crystal growth sites. They may on the other hand also be used to stabilize an unstable polymorph or destabilize a stable polymorph. The effects of emulsifiers on crystal nucleation and growth and polymorphic transition are strongly dependent on the type of emulsifier, the degree of supercooling, the cooling rate, the concentration of the emulsifier in the bulk fat and the similarity in molecular structure, especially the fatty acid moiety, between the emulsifier and the fat. The strength of the effect increases with an increasing similarity [14, 17].

As this study focusses on the onset of crystallization, only the effects of the emulsifiers under study on the crystallization rate will now be discussed in more detail.

As monoacylglycerols (MAG) are considered to be one of the most important groups of emulsifiers, a lot of research has been published on their influence on the physical properties of fat systems. The main result commonly observed in these studies is that MAG can accelerate fat crystallization by inducing heterogeneous nucleation [1, 5, 18, 19, 20, 21, 22]. Some studies reported that the effect of MAG addition depends on the degree of supercooling [18], the concentration [18] and the type of MAG [5, 18, 19, 20]. For example the study of Fredrick et al. (2008) [19] showed that addition of saturated MAG (from palm oil itself) accelerated crystallization by promoting nucleation while unsaturated MAG (from sunflower oil) did not. Verstringe et al. (2014b) [23] were the first to unquestionably demonstrate the template effect of the MAG in PO crystallization through synchrotron radiation microbeam X-ray diffraction. They showed that the PO crystals were orientated by the previously crystallized MAG due to interactions through the palmitic acid moiety in PO and MAG. In comparison with MAG, polyglycerol esters (PGE) have much less frequently been investigated for their influence on fat crystallization. There are some reports examining the effects of PGE on the physical properties of fats in bulk phase [24, 21, 25] and emulsion systems [26, 27]. Analogous to MAG, PGE can accelerate fat crystallization by promoting

nucleation [24, 25, 26, 27]. Each study also noted that the strength of the effect on the crystallization rate increases with an increasing similarity between the ‘host’ fat and the emulsifier, especially where this similarity is in terms of the acyl chain characteristics, such as length and number of double bonds [24, 25, 26, 27]. For example Shimamura et al. (2013) [25] showed that PGE containing a palmitic acid moiety has a stronger effect on the crystallization rate of palm stearin than PGE with increasing concentrations of oleic acid moiety.

The influence of lecithin on the rate of fat crystallization has been studied several times, but with apparently contradicting results. The crystallization rate was observed to either increase [21, 28, 29], remain unaffected [15, 30] or decrease [31, 32, 33] due to lecithin addition. Svanberg et al. (2011) [29] and Dhonsi et al. (2006) [33] suggested that lecithin interferes with the nucleation process while Vanhoutte et al. (2002a) [31] showed that lecithin can also influence crystal growth. Only one study investigated the influence of type of lecithin on fat crystallization, but no significant effects could be observed [15].

Although mixtures of emulsifiers are often applied in industrial practice, fundamental research on this topic is very much lacking. The influence of the addition of emulsifier mixtures on fat crystallization in emulsions [34, 35] and in bulk phase [36, 37] has only a few times been the subject of investigation. A first study [34] showed that the addition of a combination of highly hydrophobic and highly hydrophilic sucrose oligoesters delays the crystallization of a palm-based fat more than the addition of only highly hydrophobic sucrose oligoesters. Another research [36] investigated which combination of different types of saturated and unsaturated MAG could be added to a fat system to increase its SFC to the desired value. Other studies are less relevant as their focus was more on the way of mixing the emulsifiers [37] and on the structural transformations of the emulsion [35].

The objective of this study was to investigate the influence of commercially available MAG, PGE and lecithin on the crystallization behavior of bulk fat as a function of the added concentration. Not only a single type of emulsifier, but also binary and ternary mixtures of MAG, PGE and lecithin were added to the two different ‘host’ fats using a statistically sound mixture design to obtain as much information as possible. The obtained mixtures were analyzed by differential scanning calorimetry (DSC) for their non-isothermal crystallization behavior.

3. Materials and methods

3.1. Starting materials

Two different vegetable fats were obtained from *Wouters N.V.* (Lokeren, Belgium) and *Fuji Oil Europe* (Gent, Belgium). The former was a commercial palm based margarine fat (fat A), while the latter was a non-hydrogenated fat not based on palm or palm kernel oil which is used in fat rich products with a low amount of saturated FA (fat B). Three commercially available emulsifiers were selected for this study: MAG and PGE obtained from *Oleon N.V.* (Oelegem, Belgium) (M, P) and sunflower lecithin provided by *Ingrizo N.V.* (De Pinte, Belgium) (L).

3.2. Determination of FA composition

The FA composition of fat A, fat B, M, P and L was determined by methylating the fat to form fatty acid methyl esters (FAME) which were subsequently separated by gas chromatography-flame ionization detector (GC-FID). The methylation was performed by heating a mixture of 5 mg lipid sample, 1 ml toluene and 2 mL 1% sulfuric acid overnight at 50 °C. Aqueous sodium chloride solution (5 mL, 5%) was then added and the FAME were extracted with 3 mL hexane. Separation and quantification of the FAME was obtained by GC-FID (Trace GC Ultra, Thermo Scientific, *Interscience*, Louvain-la-Neuve, Belgium) with cold on-column injection. The FAME were separated on an EC Wax column (length: 30m, ID 0.32mm, film: 0.25µm) (*Grace*, Lokeren, Belgium) by using a specific time-temperature program (70 °C – 180 °C (5 °C/min), 180 °C – 235 °C (2 °C/min), 235 °C (9.5 min)). Peak identification was achieved using a standard series of 35 different FAME (*Nu-check*, Elysian, USA). Quantification of the peak areas was performed with Chromcard for Windows software (*Interscience*, Louvain-la-Neuve, Belgium). Determinations were carried out in triplicate.

3.3. Determination of triglyceride composition

The analysis of the triglyceride (TAG) composition of fat A and fat B was performed with a HPLC system (*Thermo Electron Corporation*, Brussels, Belgium) which was coupled to an evaporative laser light scattering detector (ELSD) (*Grace*, Lokeren, Belgium) according to the method of Rombaut et al. (2009) [38]. Due to a non-linear response of the detector, co-elution of some TAG and the presence of non-identifiable TAG, detailed numerical values of the TAG species in fat A and fat B are not reported and only a more qualitative composition description is given.

3.4. Mixture design

A statistically sound simplex lattice mixture design was used to study the influence of varying concentrations of M, P and L on fat crystallization. The emulsifier composition of the thirteen combinations obtained from the software (Statistica 12, *StatSoft Inc.*, Tulsa, USA) are shown in Table 1. The total amount of emulsifiers in each blend was set to 1.5%. The special cubic model was fit to the mixture data:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3,$$

where Y is the dependent variable, β_1 , β_2 , etc., are the coefficients for each term of the model and X_1 , X_2 and X_3 are the concentrations of M, P and L respectively. For each parameter the predicted equation was obtained with Statistica 12.

3.5. Preparation of the mixtures

In order to prepare the thirteen combinations obtained in the mixture design, solid emulsifiers were added to the melted ‘host’ fat and subsequently heated to 85 °C. When the mixture was fully melted, it was kept at 85 °C for 30 min and regularly stirred to ensure complete homogeneity.

3.6. Differential scanning calorimetry

A TA differential scanning calorimeter Q2000 (*TA Instruments*, Brussels, Belgium) equipped with an autosampler was used to analyze the non-isothermal crystallization behavior of fat A and fat B, with and without emulsifiers added. About 5-15 mg of melted fat was sealed into aluminum pans (*TA Instruments*, Brussels, Belgium). The following time-temperature program was used: equilibration at 80 °C during 15 min to ensure complete melting and to erase the crystal memory, followed by cooling at 10 °C/min to -60 °C to obtain the crystallization profile. The DSC profiles were analyzed with the Universal analysis software version 4.5A (*TA Instruments*, New Castle, USA). Onset temperatures of crystallization of the high-melting fraction (T_{onset}) were determined by the intersection of the baseline of the crystallization curve with the absolute highest tangent of the crystallization peak at the high temperature side. Measurements were carried out in quadruplicate.

3.7. Statistical analysis

To determine if T_{onset} changes significantly by addition of emulsifiers (apart from the mixture design), the DSC data were statistically analyzed by one-way ANOVA with SigmaPlot. Furthermore Tukey tests were applied to determine significant differences between the mean values of the different mixtures, at a level of $\alpha=0.05$.

4. Results and discussion

4.1. Determination of FA and TAG composition

Table 2 shows the FA composition of fat A, fat B, M, P and L. As fat A is a palm based margarine fat, its FA composition is very similar to that of refined, bleached, deodorized (RBD) PO reported in literature [2]. The primary FA of fat A were palmitic, oleic and linoleic acid, in amounts of $41.3 \pm 0.3\%$; $39.6 \pm 0.2\%$ and $11.2 \pm 0.3\%$. The main triglycerides in fat A were POO and POP, together accounting for 50% of the TAG, followed by PPP + SOO (10%). The TAG composition of fat A largely corresponds to that of PO, although the PPP concentration in fat A seems to be somewhat higher than in PO [2, 13]. Fat B had more unsaturated FA than fat A with $60.1 \pm 0.3\%$ oleic acid. It also contained a substantial amount of stearic acid ($20.23 \pm 0.12\%$). The main triglycerides in fat B were OOO (alone accounting for 50% of the TAG), SOO + PPP (about 15%) and POO + SLO (about 10%). In general fat B had less than 5% trisaturated TAG. M and P both had palmitic and stearic acid as their main FA, but in an opposite proportion. The palmitic and stearic acid concentration in M was $59.8 \pm 0.2\%$ and $36.5 \pm 0.8\%$ respectively, while the quantities of these FA in P were $31.64 \pm 0.07\%$ and $66.93 \pm 0.10\%$ respectively. As L is derived from sunflower oil, it has the same primary FA: oleic ($20.80 \pm 0.04\%$) and linoleic acid ($62.07 \pm 0.03\%$) [39].

4.2. Crystallization profile

DSC analysis was used to investigate the non-isothermal crystallization behavior of fat A and fat B, without and with emulsifiers added. The DSC crystallization profiles of pure fat A and pure fat B are shown in Figure 1. The crystallization profiles for both fats with emulsifiers added were similar but not exactly the same as those without emulsifiers added (DSC profiles not shown). Fat A and fat B showed considerably different crystallization profiles. In accordance with the literature on RBD PO, the crystallization profile of fat A contained two distinct peaks: one located in the high temperature region, representing the transition of the fraction rich in di- and trisaturated TAG (stearin fraction) and a second in the lower temperature region corresponding to the transition of the phase rich in di- and triunsaturated TAG (olein fraction) [40, 41, 42].

On the other hand, the crystallization profiles of fat B displayed three distinct peaks: two small peaks in the high and medium temperature region and one large peak in the low temperature region. The peak in the high temperature region can probably be attributed to the transition of trisaturated TAG and SOS, while the peak in the medium temperature region may correspond to the crystallization of SOO. The large peak in the high temperature region

is attributable to the high percentage of OOO [43]. As in this study, only the influence of emulsifier addition on the onset of crystallization is investigated, the focus is on the peak in the high-melting region. Both for fat A and fat B, this peak is caused by the crystallization of di- and trisaturated TAG, which are mostly palmitic based in fat A, while in fat B they are mainly stearic based.

As a high cooling rate is applied in this study, it can be expected that almost all crystallization of fat A and fat B will take place in the α form [1, 23, 44].

4.3. Onset temperature of crystallization of the high-melting fraction (T_{onset})

4.3.1. Combinations mixture design

Table 1 shows T_{onset} of the 13 combinations of the mixture design and of the ‘host’ fat for fat A and fat B. T_{onset} of fat A is $23.0 \pm 0.2^{\circ}\text{C}$, which is slightly higher than the onset temperature of PO ($18.4 \pm 0.2^{\circ}\text{C}$) observed by Verstringe et al. (2013) [22]. This could be due to the higher concentration of the high-melting TAG PPP. T_{onset} of fat B is $25.3 \pm 0.3^{\circ}\text{C}$ which is higher than that of fat A which could be expected due to the fact that the di- and trisaturated TAG are mostly palmitic based in fat A, while in fat B they are mainly stearic based.

When adding emulsifiers, the observed trends were similar for both ‘host’ fats. If a single type of emulsifier was added, it was observed that T_{onset} significantly increased to a similar extent by adding 1.5% M or 1.5% P (samples 1 and 2), which corresponds to literature [5, 18, 19, 20, 21, 24, 26]. No significant effect could be determined when adding 1.5% L (sample 3). This coincides with some literature on lecithin addition [15], but disagrees with some of the other apparently conflicting results [21, 28, 31, 32]. When adding emulsifier mixtures, T_{onset} increased the most in case of a high concentration (1%) of M and P, but it was never higher than when 1.5% pure M or P were added.

Table 3 shows the coefficient estimates of the special cubic model that was fitted for T_{onset} of fat A and B. For both fats the same trends were visible. In agreement with previous direct observation of the data, these results indicated that M and P influenced T_{onset} to a similar extent as their coefficients had a comparable value. The interactions between M and P and P and L were not significant ($\alpha=0.05$), while M and L worked antagonistically, which can be deduced from the negative value of the coefficient estimate. This difference in interaction between M and L on the one hand and P and L on the other hand can also be seen when looking at the results in Table 1. When adding 1% M or P together with 0.5% L (sample 8 and 9) instead of 1.5% M or P (sample 1 and 2), T_{onset} decreased but this effect was much more pronounced in the combination of M and L compared to the combination of P and L. When

adding 0.5% M or P together with 1% L (samples 5 and 6), this effect was even more clear: the combination of M and L did not lead to a significant increase of T_{onset} compared to the ‘host’ fat while the combination of P and L did. When mixing the three emulsifiers a synergistic effect was observed, although it was not significant. T_{onset} was however not higher than when pure M or P were added.

4.3.2. Analyses of extra samples

In order to further investigate the difference in interaction between M and L and P and L, eight extra samples were prepared by adding 0.5% M, 1% M, 0.5% P or 1% P to both fat A and fat B and analyzed by DSC (in duplicate) for their T_{onset} . In Table 4 the results of T_{onset} of the extra mixtures with 0.5% M and 1% M added to both ‘host’ fats are compared with certain strategically selected combinations of the mixture design. In Table 5 the same is shown for the extra mixtures with 0.5% P and 1% P added. Several trends valid for both fat A and fat B could be deduced from Table 4. First, it was observed that addition of 0.5% M had no significant effect on T_{onset} of the ‘host’ fat, while adding 1% M led to a significant increase of T_{onset} and adding 1.5% M led to an even higher increase. This observation can be linked to the results of Foubert et al. (2004) [18], who reported that the induction time of milk fat crystallization reduced as a function of the added concentration of monostearin. Secondly, when also considering the emulsifier combinations (combinations 5 and 8 of the mixture design), the results indicate that addition of 1% M could also significantly increase T_{onset} of the ‘host’ fat in the presence of 0.5% L, although to a significantly lesser extent than when only 1% M was added. As could be expected from the results of the pure emulsifier additions, addition of 0.5% M in the presence of 1% L did not lead to a significant increase of T_{onset} .

When subsequently focusing on P addition to fat A and fat B (Table 5), a very striking difference was observed compared to the results for M addition. Unlike addition of 0.5% M, adding 0.5% P already led to a significant increase in T_{onset} of the ‘host’ fat. This indicates that the minimum concentration necessary to provoke a crystallization accelerating effect due to heterogeneous nucleation is lower for P than for M. In other words, crystals of P act more effective as catalytic impurities for heterogeneous nucleation than crystals of M. So not only the quantity of catalytic impurities seems to be relevant for the nucleation accelerating effect, but also the type of impurity. Fig. 2 illustrates this trend. Furthermore it can also be deduced from Table 5 that addition of L to the 0.5% P as well as to the 1% P mixture led to a significant decrease of the T_{onset} (like in the case of the 1% M addition). On the other hand, both addition of 0.5% P in the presence of 1% L and addition of 1% P in the presence of 0.5%

L still resulted in a significant increase in T_{onset} compared to the pure ‘host’ fat (unlike in the case of the 0.5 and 1% M addition). Therefore it can be hypothesized that the antagonistic effect between M and L (in comparison to the not significant interaction between P and L), suggested earlier by the results in Table 3, is also related to a difference in concentration effect between M and P.

4.3.3. Pure emulsifiers

To further explain the behavior of the emulsifiers in the blends with the ‘host’ fats, it was decided to obtain insight into the crystallization behavior of the pure emulsifier substances. Therefore, nine extra samples consisting of only emulsifiers (pure and in binary blends) were analyzed by DSC (in duplicate) for their T_{onset} . The emulsifier composition and the T_{onset} of these extra samples are presented in Table 6. When focusing on the pure emulsifiers, T_{onset} of M and P was high in comparison to that of L and was also higher than the T_{onset} of pure fat A and pure fat B (reported in Table 1). The observed T_{onset} of M is similar to that of the commercial MAG blend studied by Verstringe et al. (2014a) [1] which also had monopalmitin and monostearin as main constituents. Data on the crystallization behavior of pure lecithin or PGE have not yet been published in literature. The relatively low T_{onset} of L may be explained by its origin, being sunflower oil, which itself crystallizes between -45 °C and -15 °C [39]. The difference in T_{onset} also coincides with the difference in FA profile as reported in Table 2: over 95% of the FA in L are unsaturated whereas both M and P consist of over 95% saturated FA. The observed T_{onset} of the pure emulsifiers probably explains the origin of the nucleation accelerating effect of M and P which was deduced earlier from the results in Table 1: M and P start to crystallize before the ‘host’ fat does and thus form heterogeneous nuclei that serve as ‘seeds’ or ‘templates’ for heterogeneous nucleation of the ‘host’ fat molecules and facilitate nucleation of the ‘host’ fat. This hypothesis about seeding properties of high-melting components is supported by the research of Verstringe et al. (2014b) [23], who demonstrated the template effect of the MAG in PO crystallization through synchrotron radiation microbeam X-ray diffraction. They showed that the PO crystals were orientated by the previously crystallized MAG due to interactions through the palmitic acid moiety in PO and MAG [23]. This mechanism also explains why addition of L had no effect on the nucleation rate of the ‘host’ fat. T_{onset} of L is indeed much lower than that of the ‘host’ fat.

When taking into account the binary mixtures of pure emulsifiers, it could be observed that when 67% M or P and 33% L were mixed, the T_{onset} was significantly lower than that of pure M and P respectively. However, when looking at the 67% L 33% P or M mixtures a clear

difference was observed: 67% L 33% M showed a remarkably lower T_{onset} than the 67% L 33% P mixture although in the 67% M or P 33% L mixtures this was reversed. These results show that not only a difference in concentration effect between M and P, but also different interactions between M and L and P and L are responsible for the observed antagonistic effects between M and L when compared to the non-significant interaction between P and L. Further research is needed to investigate the mechanism that is at the origin of the difference in interaction between M and L and P and L.

5. Conclusions

The addition of some emulsifiers could positively influence the crystallization properties of the two ‘host’ fats, depending on the quantity and the type of emulsifier. The high-melting emulsifiers monoacylglycerols (M) and polyglycerol esters (P) accelerated nucleation as a function of the added concentration. The minimum concentration necessary to provoke a nucleation accelerating effect due to heterogeneous nucleation was lower for P than for M. It was however not possible to obtain a stronger effect on the crystallization behavior by mixing different types of emulsifiers. In addition, antagonistic effects between M and L (and not between P and L) were suggested by the coefficient estimates of the special cubic model. This is probably caused by a combination of the difference in concentration effect between M and P on the one hand and a different interaction of M and P with L on the other hand. Furthermore no differences in effects between fat A and fat B could be observed. This can probably be explained by the similar TAG composition of their high-melting fraction.

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497 **8. Tables**

498 **Table 1.** Emulsifier composition of the 13 different combinations obtained in the mixture design and mean value $\pm$ standard deviation of onset
 499 temperature of crystallization of the high-melting fraction (T_{onset}) of fat A and fat B, with and without the 13 different emulsifier blends added.
 500 Results with the same uppercase character are not significantly different ($\alpha=0.05$). Abbreviations: *M* commercial mixture of MAG, *P* commercial
 501 mixture of PGE, *L* commercial mixture of sunflower lecithin.

Sample code	Emulsifier composition			Fat A	Fat B
	<i>M</i> (%)	<i>P</i> (%)	<i>L</i> (%)		
bulk fat	- ¹	-	-	23.0 ± 0.2^a	$25.3 \pm 0.3^{a,b,c}$
1	1.50	0.00	0.00	27.9 ± 0.1^b	$30.3 \pm 0.1^{d,e}$
2	0.00	1.50	0.00	27.9 ± 0.1^b	$30.4 \pm 0.1^{d,e}$
3	0.00	0.00	1.50	23.2 ± 0.0^a	$24.5 \pm 0.0^{a,b}$
4	0.50	1.00	0.00	28.0 ± 0.1^b	30.7 ± 0.0^e
5	0.50	0.00	1.00	22.9 ± 0.1^a	$24.5 \pm 0.1^{a,b}$
6	0.00	0.50	1.00	25.0 ± 0.4^c	$26.9 \pm 1.5^{c,f,g}$
7	1.00	0.50	0.00	27.9 ± 0.2^c	$30.3 \pm 0.6^{d,e}$
8	1.00	0.00	0.50	$25.0 \pm 0.1^{c,d}$	$26.4 \pm 0.2^{b,c,f}$
9	0.00	1.00	0.50	$25.7 \pm 0.1^{c,d,e}$	$27.8 \pm 0.1^{f,g,h}$
10	0.50	0.50	0.50	$26.2 \pm 0.2^{e,f}$	$27.9 \pm 0.1^{f,g,h}$
11	1.00	0.25	0.25	26.8 ± 0.5^f	$29.3 \pm 0.2^{d,e,f}$
12	0.25	1.00	0.25	$24.9 \pm 0.4^{d,e,f}$	$28.7 \pm 0.1^{g,h}$
13	0.25	0.25	1.00	23.2 ± 0.2^a	24.1 ± 0.1^a

¹Not relevant

503 **Table 2.** FA composition (%) of fat A, fat B, commercial mixture of MAG (M) commercial mixture of PGE (P) and commercial mixture of
504 sunflower lecithin (L)

Fatty acid ¹	Fat A	Fat B	M	P	L
C12:0	0.21 ± 0.04	6.85 ± 0.03	0.272 ± 0.007	0.028 ± 0.002	-
C14:0	0.846 ± 0.013	2.48 ± 0.04	1.172 ± 0.013	0.362 ± 0.006	0.0729 ± 0.0013
C15:0	0.038 ± 0.007	-	0.045 ± 0.008	0.0198 ± 0.0007	0.0297 ± 0.0013
C16:0	41.3 ± 0.3	4.942 ± 0.019	59.8 ± 0.2	31.64 ± 0.07	0.0297 ± 0.0013
C16:1	0.7 ± 0.5	0.048 ± 0.009	-	-	12.04 ± 0.04
C17:0	- ⁴	0.032 ± 0.005	0.104 ± 0.008	0.174 ± 0.015	0.082 ± 0.004
C18:0	4.1 ± 0.2	20.23 ± 0.12	36.5 ± 0.8	66.93 ± 0.10	3.700 ± 0.005
C18:1	39.6 ± 0.2	60.1 ± 0.3	1.7 ± 0.6	-	20.80 ± 0.04
C18:2	11.2 ± 0.3	4.65 ± 0.13	-	-	62.07 ± 0.03
C18:3	1.42 ± 0.04	0.060 ± 0.006	-	0.023 ± 0.002	0.115 ± 0.010
C20:0	0.27 ± 0.05	0.058 ± 0.006	0.256 ± 0.004	0.42 ± 0.02	0.112 ± 0.010
C20:1	0.26 ± 0.04	0.116 ± 0.006	0.245 ± 0.004	-	0.081 ± 0.012
C22:0	0.08 ± 0.03	0.42 ± 0.02	-	-	0.81 ± 0.03
C22:1	0.022 ± 0.005	-	-	-	-
SFA ²	46.8 ± 0.3	35.00 ± 0.13	98.2 ± 0.8	99.58 ± 0.13	4.84 ± 0.03
UFA ³	53.2 ± 0.6	65.0 ± 0.3	2.0 ± 0.6	0.023 ± 0.002	95.11 ± 0.07

505 ¹Values show mean ± standard deviation of three replicates

506 ²Saturated fatty acid

507 ³Unsaturated fatty acid

508 ⁴Not detected

509

Table 3. Estimated special cubic model coefficients $\pm$ standard deviation for the onset temperature of crystallization of the high-melting fraction (T_{onset}) of fat A and fat B, including the determination coefficient R^2 for the calculated models. Abbreviations: *M* commercial mixture of MAG, *P* commercial mixture of PGE, *L* commercial mixture of sunflower lecithin.

Factor	Coefficient estimates					
	Fat A			Fat B		
	T_{onset} ($^{\circ}\text{C}$)			T_{onset} ($^{\circ}\text{C}$)		
M	28.1	$\pm$ 0.3	*	30.4	$\pm$ 0.5	*
P	27.5	$\pm$ 0.3	*	30.2	$\pm$ 0.4	*
L	23.1	$\pm$ 0.3	*	24.4	$\pm$ 0.5	*
MP	0.4	$\pm$ 1.5		1	$\pm$ 2	
ML	-7.5	$\pm$ 1.4	*	-9	$\pm$ 2	*
PL	-0.4	$\pm$ 1.5		0	$\pm$ 2	
MPL	10	$\pm$ 9		4	$\pm$ 13	
R^2	0.935			0.9158		

*These coefficients are statistically significant ($\alpha=0.05$)

Table 4. Mean value $\pm$ standard deviation of onset temperature of crystallization of the high-melting fraction (T_{onset}) of fat A and fat B with different concentrations of a commercial mixture of MAG (M) added, compared with certain combinations of the mixture design. Results with the same uppercase character for the same crystallization parameter are not significantly different ($\alpha=0.05$). Abbreviations: L commercial mixture of sunflower lecithin.

Sample code		Fat A T_{onset} ($^{\circ}\text{C}$)	Fat B T_{onset} ($^{\circ}\text{C}$)
‘host’ fat		23.0 $\pm$ 0.2 ^a	25.3 $\pm$ 0.3 ^a
extra mixtures			
0.5% M		23.12 $\pm$ 0.04 ^a	24.62 $\pm$ 0.08 ^{a,b}
1% M		25.78 $\pm$ 0.02 ^b	27.4 $\pm$ 0.9 ^c
combinations mixture design			
1	1.5% M	27.90 $\pm$ 0.09 ^c	30.30 $\pm$ 0.07 ^d
5	0.5% M 1% L	22.86 $\pm$ 0.05 ^a	24.50 $\pm$ 0.12 ^b
8	1% M 0.5% L	25.05 $\pm$ 0.13 ^d	26.4 $\pm$ 0.2 ^e

Table 5. Mean value $\pm$ standard deviation of onset temperature of crystallization of the high-melting fraction during crystallization (T_{onset}) of fat A and fat B with different concentrations of a commercial mixture of PGE (P) added, compared with certain combinations of the mixture design. Results with the same uppercase character for the same crystallization parameter are not significantly different ($\alpha=0.05$). Abbreviations: *L* commercial mixture of sunflower lecithin.

Sample code		Fat A T_{onset} ($^{\circ}\text{C}$)	Fat B T_{onset} ($^{\circ}\text{C}$)
'host' fat		23.0 $\pm$ 0.2 ^a	25.3 $\pm$ 0.3 ^a
extra mixtures			
0.5% P		26.37 $\pm$ 0.09 ^b	29.31 $\pm$ 0.05 ^{b,c}
1% P		26.94 $\pm$ 0.03 ^b	30.14 $\pm$ 0.06 ^b
combinations mixture design			
2	1.5% P	27.86 $\pm$ 0.10 ^c	30.41 $\pm$ 0.07 ^b
6	0.5% P 1% L	25.0 $\pm$ 0.4 ^d	26.9 $\pm$ 1.5 ^d
9	1% P 0.5% L	25.67 $\pm$ 0.13 ^e	27.78 $\pm$ 0.11 ^c

Table 6. Emulsifier composition and mean value $\pm$ standard deviation of onset temperature of crystallization (T_{onset}) of the extra mixtures consisting of only emulsifiers (so without ‘host’ fat). Results with the same uppercase character are not significantly different ($\alpha=0.05$). Abbreviations: *M* commercial mixture of MAG, *P* commercial mixture of PGE, *L* commercial mixture of sunflower lecithin.

Sample code	Emulsifier composition			T_{onset} (°C)
	<i>M</i> (%)	<i>P</i> (%)	<i>L</i> (%)	
E1	100	0	0	65.3 $\pm$ 0.014 ^a
E2	0	100	0	54.3 $\pm$ 0.03 ^b
E3	0	0	100	18.0 $\pm$ 0.08 ^c
E4	67	33	0	56.5 $\pm$ 4.1 ^b
E5	67	0	33	58.7 $\pm$ 0.06 ^b
E6	33	67	0	56.1 $\pm$ 0.06 ^b
E7	0	67	33	49.1 $\pm$ 0.06
E8	0	33	67	50.0 $\pm$ 0.3 ^e
E9	33	0	67	39.9 $\pm$ 0.5 ^f

546 **9. Figures**

547

548 **Figure 1.** DSC-curves of 'host' fat A (full line) and 'host' fat B (dashed line).

549

Figure 2. Onset temperature of crystallization of the high-melting fraction (T_{onset}) of ‘host’ fat A as a function of the concentration of the added commercial mixture of MAG (M) (▲) or the added commercial mixture of PGE (P) (■).