

## Research Article

# Effect of TAG composition on the solid fat content profile, microstructure, and hardness of model fat blends with identical saturated fatty acid content

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TAGs play an important role in determining the functional properties of fat-based food products such as margarines, chocolate, and spreads. Nowadays, special attention is given to the role of the TAG structure and how it affects functional properties such as mouth feel, texture, and plasticity. Key to this research is the need to develop more healthy fats with a reduced level of trans and saturated fatty acids (SFAs), while maintaining the desired properties. In this study, fat blends with identical levels of SFA (50%) but differing in the ratio asymmetric/symmetric blends were evaluated by pulsed NMR and texturometry as a function of storage time and storage temperature. A higher trisaturated TAG content gave rise to a higher solid fat content (SFC) at higher temperature and a lower SFC at lower temperature for both palmitic and stearic based blends. On the other hand, the effect of symmetry on the SFC-profile of the blends was only clear for the stearic based blends. At lower temperatures, the SFC of symmetric TAG based blend (blend SM) was markedly lower than that of asymmetric TAG based blend (blend iS). However, from 30°C onwards, the SFC of blend SM was clearly higher than that of blend iS. The microscopic analyses revealed a denser crystal network for a higher degree of trisaturated TAG and for symmetric stearic based blends. Moreover, some blends showed a clear evolution of the microstructure during storage with smaller crystals transforming into larger ones. Finally, texture analyses demonstrated the importance of the crystallization and storage temperature on the hardness of the blends.

**Keywords:** Hardness / Microstructure / SFC / Symmetry / Trisaturated

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## 1 Introduction

TAGs are the main constituents of fat-containing food products like butter, margarine, chocolate, ice cream, and spreads. The physical characteristics of the fat crystal network created by TAG determine the functional properties of these products, such as their texture, plasticity, and the mouth feel. Moreover, they are of paramount importance for the processing of the products, their application possibilities and consumers'

perception. Many sensory attributes, such as the spreadability of margarines and snap of chocolate, are dependent on the mechanical strength of the underlying fat crystal network [1, 2]. Small changes in the TAG composition can have a big impact on the crystallization and the further structure development of the fats, as described in the important reviews of Timms [3], Sato [4], and Himawan et al. [5]. Over recent years, fat modification is frequently proposed as a means to develop more healthy fat products and this has an important impact on the crystallization and subsequent structure development of fat-containing food products.

The most widespread modification processes applied today in the edible oil industry are hydrogenation, interesterification, and fractionation. The main purpose of these processes is to change the physico-chemical properties of the oil or fat either by reducing the degree of unsaturation of the acyl

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groups (hydrogenation), by redistributing the fatty acid chains (interesterification), or by a physical separation of the component TAG through selective crystallization and filtration to yield higher-, lower-, or sharper-melting fats (fractionation). Blending is perhaps the simplest fat modification process, in which different fats and oils are combined to obtain a blend with new physical properties. Kellens [6] has published a good review on fat modification processes.

Fat modification has an important impact on the solid fat content (SFC) profile of fat systems, which may be seen as a guideline in judging whether a certain oil, fat, or blend is suitable for a particular application [7]. For chocolate, the SFC is responsible for the hardness, the fast melting, and flavor release in the mouth and the possible waxy mouth feel at higher temperatures [8]. The SFC also has important implications for the overall melting characteristics of margarines and spreads at various temperatures. At low temperatures, i.e., 4–10°C, the SFC value gives an indication of the spreadability when the product is removed straight from the fridge. Higher temperatures—around a RT of 20–22°C—determine the product stability and resistance to oil exudation at table-top conditions, and the SFC should be at least 10% [9]. The SFC parameter is frequently used to select the ideal fat composition obtained following fat modification.

Different studies focus on the important link between SFC and fat structure parameters. Awad et al. [10] found a log–log relationship between the storage modulus  $G'$  and the solid volume fraction ( $\Phi = \text{SFC}/100$ ), which is related to structural characteristics of the network like the fractal dimension. Liang et al. [11] described correlations between compression modulus, SFC, and microstructure for different ratios of palm stearin (PO) and sunflower oil. Braipson-Danthine and Deroanne [12] also found a log–log relationship between hardness and SFC for blends of different vegetable oils.

It is not only the SFC that determines the mechanical properties (e.g., hardness and elastic modulus) of a fat crystal network, but the microstructure is also of utmost importance. Narine and Marangoni [13] used the fractal dimension to quantify the microstructure. For example, they found that systems with a lower fractal dimension had a stronger fat crystal network. In practice, this means that fat crystals with a smaller size generally lead to fat systems with a higher hardness, and also that the network between crystals is important.

When looking at the functionality and applications of fat-containing food products, the fat structure development directly after production should be investigated, and also the different crystallization related processes that proceed during storage in products like margarine, butter, and chocolate. These postcrystallization processes will influence the quality of the products. They include nucleation of new crystals and crystal growth, Ostwald Ripening (dissolution of small crystals and growth of big ones), polymorphic transformation, migration of oil, and migration of small crystals. In certain cases, postcrystallization is desired in order to produce

a harder consistency, for example, of low-fat products. In most other cases, postcrystallization processes are undesirable. For example, Ostwald Ripening may cause a loss in consistency because larger crystals give looser networks [14]. During postcrystallization, solid bridges can be formed as a consequence of simultaneous crystallization and aggregation [15]. Sintering between crystals will occur if some TAG molecules are incorporated in the lattices of both crystals. This is more likely to occur at crystal surfaces that have defects due to lattice mismatches. Therefore, it is more likely that sintering occurs in fats that contain a broad TAG composition (e.g., milk fat, palm oil, mixtures with lauric oils, etc.) [15].

One of the key aspects which determine the SFC and microstructural development of fat systems is the interaction between different TAG and their corresponding phase behavior. Indeed, each TAG has its own polymorphism and melting behavior. However, due to specific interactions, the mixture of TAG may behave in a totally different way, e.g., formation of molecular compounds with a different polymorphism [3]. Since natural fats consist of a broad range of TAG, all having their own specific properties and interactions between each other, it is quite difficult to understand their phase behavior. Therefore, most studies describe the phase behavior between pure TAG. For example, Minato et al. [16] mentioned the formation of a molecular compound for a 1:1 mixture of POP and PPO. An extensive compilation of phase diagrams of binary TAG mixtures mentioned in literature has been made by Wesdorp [17].

The studies which describe the phase behavior between pure TAG give valuable information, but have the disadvantage that they are too far removed from industrial applications. On the other hand, applied studies are available which focus on the physical behavior of natural oils and fats and their application in industry. They rarely focus on TAG composition and molecular interactions and they have not been designed specifically to study blends with the same level of saturated fat and a specific TAG composition. Therefore, the authors' group embarked on a study situated between fundamental and applied research, by investigating blends of natural fats and oils from a TAG point of view. The investigated blends all had a saturated fatty acid (SFA) content of 50%, which is similar to fats used in confectionery products (e.g., coatings) [18]. The fat blends were constructed in such a way that three different effects of TAG composition could be evaluated: the effect of trisaturated TAG, compared to disaturated monounsaturated TAG, by varying the amount of PPP and SSS, the effect of symmetry by varying the ratio of symmetric and asymmetric disaturated monounsaturated TAG and the effect of chain length by using palmitic based and stearic based fats. An earlier publication [19] discussed the primary crystallization behavior of such blends investigated by DSC and XRD, but did not look to the later structure development. Therefore, the objective of the present study was to investigate the fat structure development

of such blends. The SFC-profiles of the blends were recorded, while the fat structure development during crystallization and storage was followed by polarized light microscopy, pulsed NMR (pNMR), and texturometry.

## 2 Materials and methods

### 2.1 Samples

Palm-mid fraction (PMF, a POP source), interesterified palm-mid fraction (iPMF, a PPO/POP source), shea stearin (SHs, an SOS source), interesterified shea stearin (iSHs an SSO/SOS source), POs (a PPP-source), fully hydrogenated soybean oil (FHSBO, an SSS-source), and high-oleic sunflower oil (HOSF) were used as starting oils for the blends and were provided by Lodders Croklaan (Wormerveer, The Netherlands). The fatty acid and TAG composition of these starting oils are presented in Table 1. The methods for fatty acid and TAG analysis are described in a previous publication of the author's group [19].

### 2.2 Blend preparation

The blends were made based on the fatty acid and TAG composition of the starting oils [19]. Each starting oil was placed in an oven at 90°C till completely liquid. Then the appropriate masses for the blends were weighed into a glass beaker and the oils were mixed with a magnetic stirrer equipped with a heating block to ensure that no fat crystallized during the

**Table 1.** Fatty acid and TAG composition (%) of the initial oil with the amount of saturated fatty acids (SFA) in each oil (%)

Compound	POs	PMF	iPMF	FHSBO	SHs	iSHs	HOSF
<b>Fatty acid</b>							
C 16:0	80.59	57.75	57.65	9.82	4.49	3.35	3.82
C 18:0	5.48	6.09	5.86	86.54	58.08	58.99	3.00
C 18:1c	9.86	31.45	30.91	0.37	32.31	31.95	81.85
C 18:2n-6	2.03	2.95	2.69	0.00	3.17	2.66	8.92
SFA	87.89	65.36	64.92	99.60	64.34	63.77	8.03
<b>TAG</b>							
SatSatSat	77.2	4.2	28.3	99.9	1.2	27.7	0.0
SatOSat	12.4	77.6	13.4	0.1	83.7	13.5	0.4
OSatSat	2.1	6.9	26.9	0.0	1.0	27.0	0.0
SatLSat	1.6	6.2	1.3	0.0	7.6	1.2	0.1
SatSatL	0.2	0.5	2.7	0.0	0.0	2.5	0.0
SatOO	4	2.9	12.7	0.0	4.8	13.2	15.9
OSatO	0.1	0.1	6.4	0.0	0.0	6.6	0.0
SatOL/SatLO	1.1	0.6	3.8	0.0	0.4	3.6	1.3
OSatL	0.1	0.1	0.0	0.0	0.2	0.0	1.0
OOO	0.4	0.2	3.0	0.0	0.5	3.2	69.6
>3 d.b.	0.9	0.7	1.2	0.0	0.6	1.2	11.8

SFA, saturated fatty acids; Sat, saturated; O, oleic acid; L, linoleic acid.

mixing process. When the oils were completely mixed, the blend was poured into a plastic cup for further analysis.

### 2.3 Chemical composition of the blends

The compositions of the blends were reported in the previous publication of the authors' group [19].

Only the TAG composition will be referred to in the present work. A detailed description of the method used for the determination of the TAG composition is given by Vereecken *et al.* [19].

The TAG profile of the eight blends investigated is given in Table 2. As intended, the level of SatSatSat decreases from blend PH, through blend PM to blend PL, and from blend SH, through blend SM to blend SL. This makes it possible to evaluate the effect of trisaturated TAG. Furthermore, blends PM and iP contain almost the same level of trisaturated and disaturated mono-unsaturated TAG, enabling the assessment of the effect of symmetry by comparing these two blends. The same is valid for blends SM and iS. Finally, the effect of chain length can be investigated by comparing the palmitic based blends (PH, PM, PL, and iP) with their stearic based alternative (SH, SM, SL, and iS).

### 2.4 Solid fat content

Solid fat content (SFC) was measured by pNMR with a Bruker Minispec pc 20 (Bruker, Karlsruhe, Germany). Melted fat was placed in NMR tubes and submitted to the tempering treatments of the IUPAC 2.150 serial tempered method. The SFC was determined in the range of 5–70°C at 5°C intervals following 60-min incubations at each temperature. Three repetitions were performed for each sample.

### 2.5 Storage experiments

To evaluate the structure development during storage, samples were fully melted and subsequently crystallized at

**Table 2.** TAG profile (%) of the eight blends investigated

TAG	PH	PM	PL	iP	SH	SM	SL	iS
SatSatSat	40.6	23.9	3.1	20.9	40.5	23.0	0.9	20.9
SatOSat	6.7	29.1	56.9	10.0	7.7	31.8	62.5	10.3
OSatSat	1.1	2.9	5.1	19.8	0.1	0.4	0.7	20.3
SatLSat	0.9	2.5	4.6	1.0	0.7	2.9	5.7	0.9
SatSatL	0.1	0.2	0.4	2.0	0.0	0.0	0.0	1.9
SatOO	9.6	8.2	6.4	13.6	8.5	8.1	7.6	13.9
OSatO	0.1	0.1	0.1	4.7	0.0	0.0	0.0	5.0
SatOL/SatLO	1.2	1.0	0.8	3.1	0.7	0.7	0.6	3.0
OSatL	0.5	0.4	0.3	0.3	0.5	0.5	0.4	0.2
OOO	33.2	26.8	18.8	20.5	35.3	27.8	18.1	19.6
>3 d.b.	6.1	5.0	3.7	4.0	6.0	4.9	3.5	3.8

Sat, saturated; O, oleic acid; L, linoleic acid; d.b., double bonds.

15 and 20°C using a thermostatic cabinet (for the microscopic analyses) or a water bath (for the SFC and hardness measurements), after which they were stored at 20 and 25°C. The microstructure, SFC, and hardness were then monitored during storage.

### 2.5.1 Microscopic analyses

Microscopic analyses were conducted using a Leitz Diaplan microscope (Leitz Diaplan, Leica, Germany) equipped with a Linkam PE 94 temperature control system (Linkam, Surrey, UK). The samples were melted, and one drop of the sample was put on a microscope slide. In order to permit three-dimensional crystallization to take place, two coverslips were placed on the slide and the fat drop deposited between them and then covered with a third coverslip. Thereafter, the samples were placed on a temperature-controlled metal plate (constructed in-house) to investigate the microstructure directly after crystallization (after 2 h of crystallization at 15 and 20°C in a thermostatic cabinet) and after storage at 20 and 25°C (using a thermostatic cabinet). The samples were stored together and, consequently, experienced the same temperature fluctuations. Samples were imaged with an Olympus Color View camera (Olympus, Aartstelaar, Belgium) equipped with CellD software (Olympus). The pictures were taken at a magnification of 100×.

### 2.5.2 Solid fat content

The SFC after 2 h crystallization in a water bath at 15 and 20°C and after 1 month of storage in a thermostatic cabinet at 20 and 25°C was measured using the same equipment as for the determination of the SFC-profile (see Section 2.4). The experiments were performed in triplicate.

### 2.5.3 Hardness measurement (penetration test)

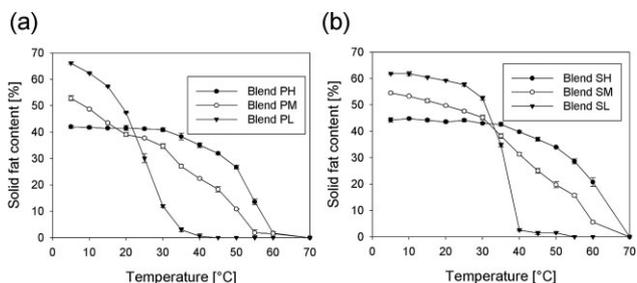
Fat (20 mL) was placed in a plastic beaker and crystallized in a temperature-controlled water bath for 2 h at the specified temperatures (15 and 20°C) before storing for 1 month at 20 and 25°C (in a thermostatic cabinet). The hardness of the crystallized and stored samples was determined with a penetration test using a Texture Analyzer TA500 (Lloyd Instruments, Hampshire, United Kingdom) with a cylindrical probe of 8.5 mm diameter (CNS Farnell, Hertfordshire, United Kingdom). The probe penetrated the product at a constant, optimized speed of 10 mm/min to a distance of 10 mm. To ensure measurement of the hardness at the specified temperature, the texture analyzer was placed in a temperature-controlled cabinet ( $\pm 0.5^\circ\text{C}$ ) (Lovibond, Dortmund, Germany). The hardness was defined as the maximum penetration force (N). Five repetitions were performed for each sample.

## 3 Results and discussion

### 3.1 Effect of trisaturated TAG, and of acyl chain length

Figure 1 demonstrates the effect of trisaturated TAG on the SFC-profile of the investigated blends by comparing blend PH with blend PM and blend PL (Fig. 1a) and by comparing blend SH with blend SM and blend SL (Fig. 1b). Not surprisingly, it demonstrates that a higher amount of SatSatSat (see also Table 1) gives rise to a higher SFC at higher temperatures ( $T > 20\text{--}25^\circ\text{C}$  in Fig. 1a and  $T > 30\text{--}35^\circ\text{C}$  in Fig. 1b). This is because the SatSatSat provides the solid fat at the higher temperatures (where most of the other TAG are liquid). Since it has a finite solubility in the liquid phase, the amount of solid fat increases as the concentration of SatSatSat rises. It is well known that fats with a higher melting point have a lower solubility in low-melting fat, which is governed by the Hildebrand Equation [20, 21]. Similarly, the NMR melting point (the temperature where the SFC becomes 0%) is clearly higher for blends with a higher content of SatSatSat. This can be explained by the lower solubility of SatSatSat in the liquid phase and its higher melting point compared to disaturated TAG [22].

In contrast, Fig. 1a and b demonstrates that, at lower temperatures ( $T < 20\text{--}25^\circ\text{C}$  in Fig. 1a and  $T < 30\text{--}35^\circ\text{C}$  in Fig. 1b), the SFC is lower for blends with a higher content of SatSatSat. In this temperature range, both the disaturated mono-unsaturated and monosaturated di-unsaturated TAG provide solid fat as well as the trisaturated TAG. However, the SFA content of the disaturated mono-unsaturated and monosaturated di-unsaturated TAG is lower than that of the trisaturated TAG (see also Table 1) so that more of these TAG can be present (compared to the trisaturated TAG) for the same SFA content, thus contributing more to the solid phase. Indeed, for the same SFA content, 1% SatSatSat can be replaced with 1.5% SatOSat. Blends with a low amount of trisaturated TAG (and a high amount of monosaturated and disaturated TAG) will thus have a higher SFC at lower temperatures. It is clear that the SFC curves of the stearic



**Figure 1.** Effect of trisaturated TAG on the SFC-profile of the investigated blends: (a) palmitic based blends and (b) stearic based blends.

**Table 3.** Melting points of the various polymorphs of the palmitic and stearic based TAG (adapted from Hagemann [22])

TAG	$\alpha$ (°C)	$\beta'$ (°C)	B (°C)
PPP	46–44.7	53.57–56.6	66–66.4
SSS	55–54.7	61.64–63.2	73–73.5
POP	8.8–21.5	20.8–37	35–38.3
SOS	22.3–29.5	29–41.5	41.8–43
PPO	18.1–18.5	29.8–34	34.5–34.8
SSO	26.7–30.6	30.3–43.5	38.5–42.5

based blends are higher for all temperatures than the SFC curves of the palmitic based blends. Also, the NMR melting point (the temperature where the SFC becomes 0%) is clearly higher for the stearic based blends compared to the palmitic based blends. This is due to the higher melting point of stearic based TAG compared to palmitic based TAG (Table 3), leading to a lower solubility in the liquid phase and thus a higher SFC value [21, 22].

During the further discussion of the results, the SFC-values at 15, 20, and 25°C are especially important, since these temperatures correspond to the crystallization and storage temperatures used. Therefore, Table 4 summarizes the SFC-values at these temperatures. For each temperature, significant ( $p < 0.05$ ) differences between the blends are indicated (a, b, c, etc.). Blends with the same letter are not significantly ( $p < 0.05$ ) different at a specific temperature. For each temperature, a significant difference in SFC was found between the blends, except for blends PH and iP at 15°C.

While the previously reported work [23] described the crystallization characteristics of these fat blends, the current paper records the changes occurring in the fats during storage. As noted earlier, these include Ostwald Ripening,

**Table 4.** SFC (%) of the investigated blends at 15, 20, and 25°C, based on their SFC-profile given in Fig. 1

Blend	Temperature (°C)		
	15	20	25
PH	41.50 ± 0.26 <sup>a</sup>	41.53 ± 0.95 <sup>a</sup>	41.27 ± 0.42 <sup>a</sup>
PM	43.50 ± 0.36 <sup>b</sup>	39.07 ± 0.76 <sup>b</sup>	37.73 ± 0.32 <sup>b</sup>
PL	57.40 ± 0.26 <sup>c</sup>	47.50 ± 0.26 <sup>c</sup>	30.07 ± 1.67 <sup>c</sup>
iP	41.87 ± 0.23 <sup>a</sup>	35.90 ± 0.66 <sup>d</sup>	33.57 ± 0.06 <sup>d</sup>
SH	44.23 ± 0.51 <sup>b</sup>	43.60 ± 0.36 <sup>c</sup>	44.17 ± 0.50 <sup>c</sup>
SM	51.57 ± 0.67 <sup>d</sup>	49.73 ± 0.23 <sup>f</sup>	47.63 ± 0.40 <sup>f</sup>
SL	60.50 ± 0.20 <sup>c</sup>	59.30 ± 0.20 <sup>g</sup>	57.73 ± 0.64 <sup>g</sup>
iS	59.40 ± 0.66 <sup>f</sup>	57.00 ± 0.30 <sup>h</sup>	53.40 ± 0.26 <sup>h</sup>

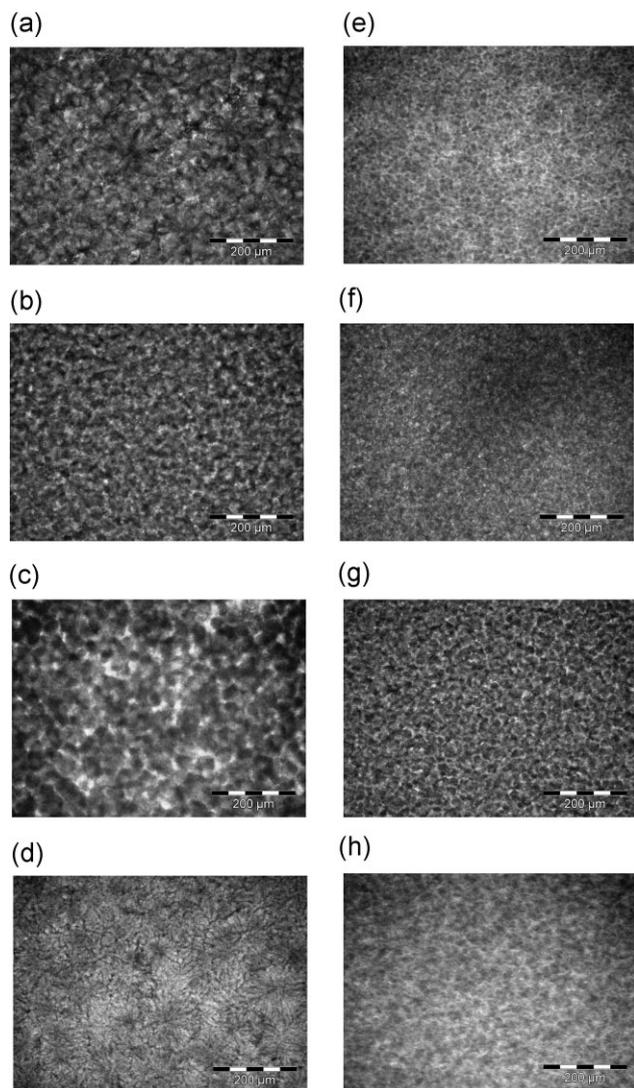
For each temperature significant ( $p < 0.05$ ) differences between the blends are indicated (a, b, c, etc.). Blends with the same letter are not significantly ( $p < 0.05$ ) different at a specific temperature.

polymorphic transformation, and crystal sintering (network formation). There is no reason why these processes may not occur simultaneously, and each will have an impact on the structure development in the fat. In order to explore the changes taking place during storage, SFC, hardness measurement, and microscopy were used to investigate changes following crystallization at 15 and 20°C and subsequent storage at 20 and 25°C.

All blends were crystallized quite rapidly. Polarized light microscopy showed a contrast between the crystal structure development between crystallization at 15 and 20°C. At the lower temperature, in particular, where crystal growth was very rapid, a dense structure of very small crystals was formed. This factor meant that details of the structure and crystal network were unclear (Fig. 2). During storage, little change in microstructure was visible. At the higher temperature, the crystallization rate was slower and greater differences were visible in the microstructure directly after crystallization. Blends with the highest levels of SatSatSat (i.e., blends PH and SH) formed the smallest crystals initially (Fig. 3). This is likely to be due to the SatSatSat TAG, which crystallize rapidly at higher temperatures than the other TAG, providing many seeds on which subsequent crystallization can occur [24].

Concerning changes taking place during storage, blend SL (low SSS, high SOS) showed clear differences (Fig. 4). Immediately following crystallization, a mass of small crystals was observed. However, after just 1 day storage at 20°C, very large spherulitic crystals had appeared amongst the small crystals. These grew so that after 1 month only large spherulites were present. This is likely to be due to initial crystallization of an unstable polymorph, followed by the nucleation of a more stable polymorph [25]. The fact that the palmitic analog (blend PL, Fig. 4) did not perform in a similar manner may be due to a number of reasons. Firstly, POP has a lower melting point than SOS so that the undercooling at a given crystallization temperature is lower, meaning that more stable polymorphs might be nucleated. Secondly, although an unstable polymorph might form, it might be meta-stable under the storage conditions, leading to a decreased rate of polymorphic transition. Thirdly, the undercooling of the stable polymorph at the storage temperatures will be less for POP than SOS, with an associated lower rate of nucleation amongst the unstable crystals.

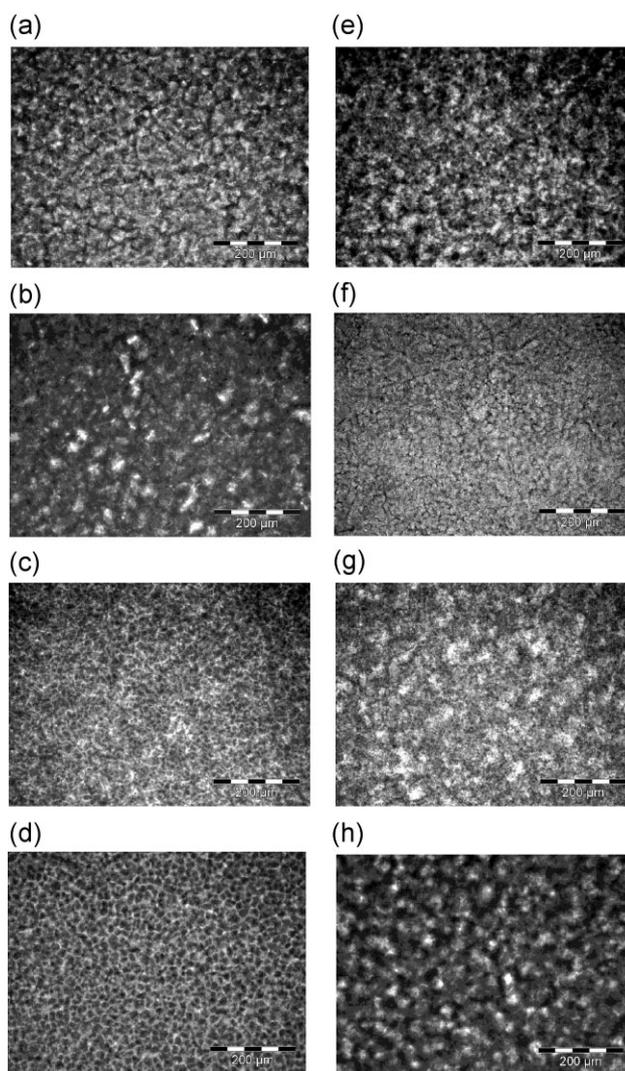
Hardness of the fat is not dependent solely on the SFC, as is evident from Fig. 5. The SFC measured 2 h after crystallization showed an increasing tendency for lower levels of SatSatSat at 15°C with PH < PM < PL = SH < SM < SL ( $p < 0.05$ ). However, at 20°C, the opposite trend could be observed (PL < PH < PM < SH < SM < SL with  $p = 0.05$ ), with the blend with the lowest SatSatSat (blend PL) having half the SFC of the other blends (Fig. 4a). After storage at 20°C for 1 month, SFC values were similar to those after 2 h (Fig. 4b), except blend PL. Blend PL, which had the lowest SFC after crystallization at 20°C, now



**Figure 2.** Effect of TAG composition on the microstructure of the investigated blends after 2 h crystallization at 15°C: (a) blend PH, (b) blend PM, (c) blend PL, (d) blend SH, (e) blend SM, (f) blend SL, (g) blend iP, and (h) blend iS (100× magnification).

showed the significantly ( $p < 0.05$ ) highest SFC for storage after crystallization at both 15 and 20°C. This is despite the fact that microscopy showed little visible difference.

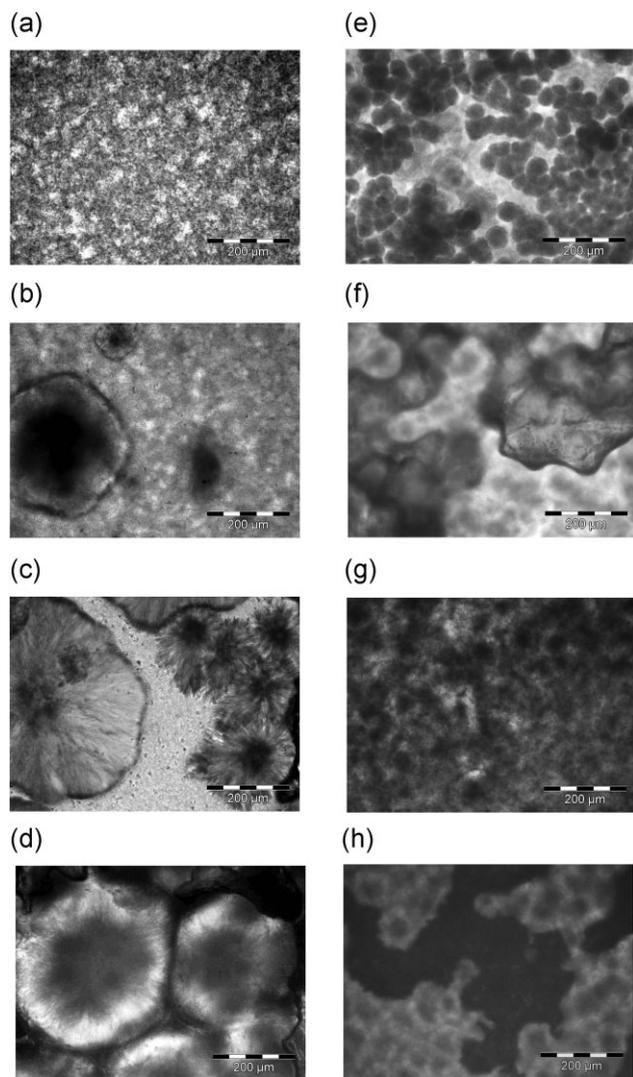
Although the SFC values were similar for palmitic blends, hardness values were quite different. Hardness of samples crystallized at 15°C increased significantly ( $p < 0.05$ ) as the SatSatSat content decreased; the hardest fat was the blend with the least PPP (Fig. 5c). However, those crystallized at 20°C were much softer, and hardness decreased significantly ( $p < 0.05$ ) in line with the SatSatSat content (PH < PM < PL). Interestingly, although the SFC remained similar during storage (or even increased for blend PL), the hardness



**Figure 3.** Effect of TAG composition on the microstructure of the investigated blends after 2 h crystallization at 20°C: (a) blend PH, (b) blend PM, (c) blend PL, (d) blend SH, (e) blend SM, (f) blend SL, (g) blend iP, and (h) blend iS (100× magnification).

decreased (Fig. 5d). Thus, although there was no visible change in microstructure of blend PL, the SFC increased (after crystallization at 20°C) but the hardness decreased. Since the SFC was the same, or greater, the decrease in hardness must be due to reduced sintering or interaction between the individual crystals. Indeed, Schaink and van Malssen [26] proved that only a small amount of fat (possibly not noticeable by SFC) is necessary to bridge between crystals and to form a continuous fat crystal network.

Solid fat content of the stearic based blends were similar, with a slight but significant ( $p < 0.05$ ) increase as SatSatSat content decreased (SFC SH < SM < SL), and remained more or less the same during storage (Fig. 5a and b). However, the



**Figure 4.** Evolution of the microstructure of blend SL (left column) and PL (right column) during storage at 20°C: (a,e) directly after crystallization at 20°C, (b,f) after 1 day of storage, (c,g) after 1 wk of storage, and (d,h) after 1 month of storage.

hardness after 2 h was greatest for the blend with the highest SatSatSat content (SFC  $SL < SM < SH$  with  $p < 0.05$ ). Hence, in this series, there was an inverse relationship between the SFC and the hardness. This may be related to the crystal size, with the highest SatSatSat blend having many small crystals (Fig. 3). Interestingly, during storage, the hardness of the blends became more similar, with that of the highest SSS (blend SH) decreasing and that of the lowest SSS (blend SL) increasing.

Storage temperature (20°C vs. 25°C) had a marked effect on the SFC of the palmitic blends, but not so much for the stearic blends. This is likely to be due to the higher melting point of SOS compared to POP. Despite the similarity in SFC between stearic based blends stored at 20 or 25°C, the

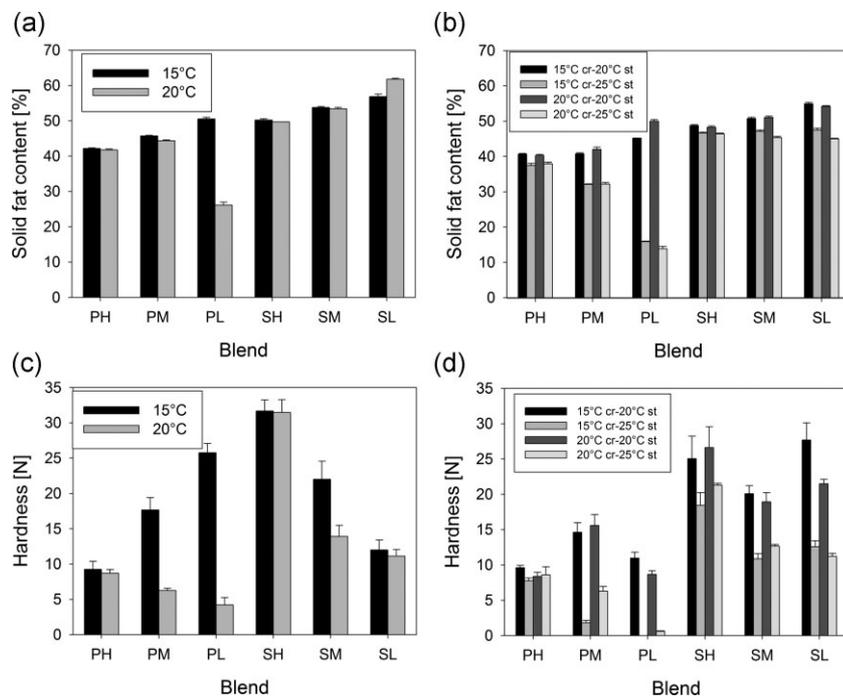
hardness was quite different, particularly for the blend lowest in SSS (blend SL) (Fig. 5b and d), with  $SM < SL < SH$  ( $p < 0.05$ ) when stored at 20°C and  $SL < SM < SH$  ( $p < 0.05$ ) when stored at 25°C. Like the effect on SFC, storage temperature had a great influence on the hardness of palmitic blends, with decreasing hardness for lower levels of PPP ( $PL < PM < PH$  with  $p < 0.05$ ) after storage at 25°C. This suggests a marked melting of the crystals, particularly of the bridges between them. This was less noticeable for the stearic based blends, probably due to the higher melting point of SOS compared to POP (Table 3).

Overall, in terms of hardness as a function of SatSatSat content, palmitic blends exhibited the opposite trend to stearic blends. In both case, however, there was not a consistent trend with SatSatSat level. In the case of the palmitic blends, that with an intermediate PPP level had the greatest hardness within the series. In the case of the stearic blends, the blend with the intermediate level of SSS had the lowest hardness. Although not observed, this may be related to the influence of PPP on the crystal morphology and crystal interactions of POP compared to that of SSS on SOS.

### 3.2 Effect of symmetry, and of acyl chain length

Figure 6 demonstrates the effect of symmetry on the SFC-profile of the blends by comparing blends with a high amount of symmetric TAG (blend PM and SM) with blends with a high amount of asymmetric TAG (blend iP and iS). For the palmitic based blends, there was only a small difference in the SFC profile of the symmetric TAG based blend and that of the asymmetric TAG based blend. The melting point of the most stable polymorphic form of POP is around 36°C while the corresponding melting point of PPO is around 34.5°C (see also Table 3). The blends were tempered and, although the polymorphic form was not established to be the most stable [19], the small difference in melting point between the symmetric and asymmetric TAG appears to lead to a small difference in the SFC profile. As described by Minato *et al.* [16], POP and PPO form a molecular compound at a 1:1 concentration (melting point around 31.2°C), and this is likely to occur in blend iP, although having an excess of PPO. However, this was not apparent from the SFC results, which may be due to the rather coarse nature of the measurement, and the presence of other TAG.

On the other hand, a clear difference in SFC profile was seen for the stearic based blends (Fig. 6b). For temperature lower than 30°C, the SFC of the symmetric TAG based blend (blend SM) was markedly lower than the SFC of asymmetric TAG based blend (blend iS). However, from 30°C onwards, the SFC of blend SM was clearly higher than the SFC of blend iS. This may occur if the SFC tempering procedure is not sufficient to stabilize blend iS. Indeed, the SFC profile of blend iS is more typical of an unstable polymorphs, namely higher SFC values at low temperatures and a strong decrease, due to their lower melting points, at higher temperatures



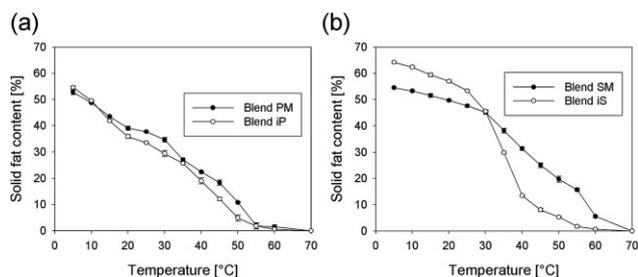
**Figure 5.** Effect of trisaturated TAG on the SFC and hardness of the investigated blends: (a) SFC after 2 h crystallization at 15 and 20°C, (b) SFC after 1 month of storage at 20 and 25°C (using 15 and 20°C as crystallization temperatures), (c) hardness after 2 h crystallization at 15 and 20°C, and (d) hardness after 1 month of storage at 20 and 25°C (15 and 20°C crystallization).

[18]. As shown in Table 2, the composition of blend iS is broader (due to the presence of both asymmetric and symmetric TAG) than the composition of blend SM, possibly making it more difficult to obtain a stable polymorph with the tempering procedure (40 h at 26°C, see Section 2.5.2). In the unstable polymorphic form, since molecules are less closely packed, it is possible that there is a greater intersolubility of the TAG [27, 28]. From the previous work with these blends [19], it was clear that blend iS had a very slow crystallization rate. This slower crystallization may be another explanation for the difference in SFC-profile between blend iS and SM.

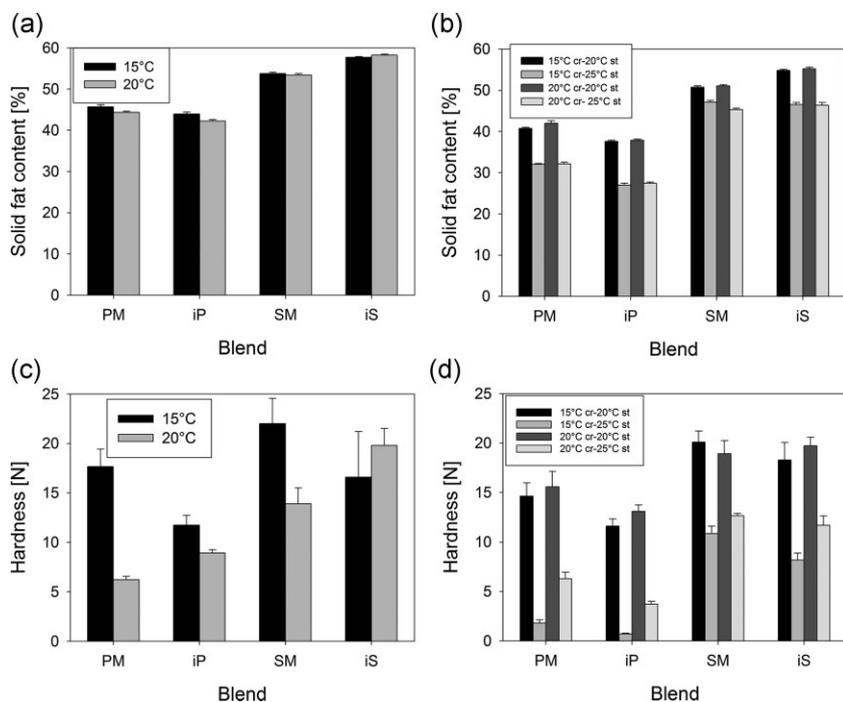
From Figs. 2 and 3 the effect of symmetry on the microstructure of fat blends after 2 h crystallization at 15 and 20°C, respectively, can be deduced by comparing symmetric TAG based blends (blend PM and SM) with asymmetric TAG

based blends (blends iP and iS). From this figure it is clear that there was a different effect of symmetry for the palmitic based compared to stearic based blends. Both palmitic based blends (see Fig. 3b and g) showed quite a fast crystallization, leading to a dense network of quite small crystals. Two hours after crystallization, there are clear differences between symmetric and asymmetric rich samples (compare Fig. 3b with g and Fig. 3e with h). Both palmitic blends crystallized rapidly [19], leading to a dense mass of small crystals. Those of blend iP (PPO rich) were a little larger than those of blend PM (POP rich) (compare Fig. 3b with e). It may be that the potential presence of the POP–PPO compound, which has a lower melting point than POP, experiences lower undercooling, lower nucleation rate, and hence fewer, larger crystals. A similar effect was noted for the stearic based blends. However, there was a more pronounced difference between blends rich in SOS or SSO (blends SM and iS); the latter blend showed much larger crystal clusters (compare Fig. 3e with h).

The difference in visible microstructure is reflected, to some degree, in the hardness results. Both palmitic and stearic asymmetric TAG rich blends show lower hardness after crystallization at 15°C compared to the symmetric TAG rich blends (Fig. 7) (iP<iS<PM<SM with  $p<0.05$ ). However, this is more marked for the palmitic blends than for the stearic blends, which indicates that it is not solely due to the crystal size. It is interesting to note that, although the SFC is almost the same for the palmitic blends (PM and iP) crystallized at both 15 and 20°C, the hardness is notably



**Figure 6.** Effect of TAG symmetry on the SFC-profile of the investigated blends: (a) palmitic based blends and (b) stearic based blends.



**Figure 7.** Effect of TAG symmetry on the SFC and hardness of the investigated blends: (a) SFC after 2 h crystallization at 15 and 20°C, (b) SFC after 1 month of storage at 20 and 25°C (using 15 and 20°C as crystallization temperatures), (c) hardness after 2 h crystallization at 15 and 20°C, and (d) hardness after 1 month of storage at 20 and 25°C (15 and 20°C crystallization).

different. The POP rich blend is harder than the PPO rich blend when crystallized at 15°C ( $iP < PM$  with  $p < 0.05$ ), but softer than it when crystallized at 20°C ( $PM < iP$  with  $p < 0.05$ ) (SFC all very similar). At the lower temperature, it appears that the larger crystal size of blend iP leads to lower hardness [2]. At the higher temperature, it may be that the sintering of the crystals becomes more important, and this may be aided by blend iP's broader TAG composition providing intermediate melting material for bridges [14]. Again, it is clear that SFC is not the only contributor to hardness.

After 1 month storage, the SFC of both symmetric and asymmetric blends remained about the same (Fig. 7b), although a little less for samples stored at 25°C (especially for palmitic based blends). Despite the significant differences in hardness between symmetric and asymmetric blends observed soon after crystallization, values were similar after 1 month at 20°C, although the hardness of the PPO rich blend was still significantly lower ( $p < 0.05$ ) than the POP rich blend. Palmitic blends both showed low hardness after storage at 25°C, reflecting their lower melting point compared to the stearic based blends ( $iP < PM < iS = SM$  for crystallization at 20°C and  $iP < PM < SM < iS$  for crystallization at 15°C with  $p < 0.05$ ).

## 4 Conclusions

In this study, the SFC-profile and the fat structure development of eight blends containing the same amount of SFA (50%), but having different TAG composition, were

investigated and compared by pNMR, microscopy, and texturometry. Three different effects were investigated, namely the effect of the amount of trisaturated TAG, the effect of symmetry, and the effect of chain length.

The determination of the SFC-profiles revealed totally different shapes for different levels of trisaturated TAG, although the level of SFA was the same. A high level of trisaturated TAG led to a flat SFC-profile while a lower level resulted in a steeper profile. As anticipated, the presence of trisaturated TAG led to smaller crystals when observing the microstructure of the blends. Furthermore, storage experiments revealed that the selected crystallization and storage temperature especially influenced the SFC and hardness of blends with a lower level of trisaturated TAG. In this way, the effect of trisaturated TAG depended on the crystallization and storage temperature selected.

The effect of symmetry was less clear from the different analyses. Almost no difference in the SFC-profile was observed between the symmetric TAG based and asymmetric TAG based blends, especially not for the palmitic based blends. In contrast, the microstructure analyses revealed a larger crystal size for the SSO based blend. The storage experiments revealed a somewhat higher hardness for the symmetric TAG based blends, although the differences were almost negligible. Finally, the effect of chain length was investigated by comparing palmitic based blends with stearic based blends, but this did not lead to surprising results. Stearic based blends were generally harder and had a higher SFC than their palmitic based alternatives.

The results demonstrate the importance of formulating the TAG composition of a fat in order to achieve the desired structure development, which might be important for certain applications (e.g., to obtain the desired plasticity of margarines or the desired snap of chocolate). Although the blends were all constructed with the same saturated fat level, they showed quite divergent results.

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