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Relationship between Crystallization Behavior, Microstructure, and Macroscopic Properties in trans-Containing and trans-Free Filling Fats and Fillings

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The objective of this study is to investigate the architecture to feature physical functionality of filling fats. This means an investigation of the different structure levels (crystallization, microstructure, macrostructure, etc.) that lead to good technological functionality. The isothermal crystallization behavior of two filling fats (one trans-containing and one trans-free) was examined by differential scanning calorimetry and microscopy. Furthermore, the hardness of the samples was examined after cooling in a water bath at two different temperatures and at three different storage times. The trans-containing filling fat crystallized faster and in smaller crystals as compared to the trans-free filling fat. The crystallization behavior of the trans-free filling fat was more complex, with the formation of different polymorphic forms. The hardness of the fillings was not only governed by the amount of solid fat present in the network but also by the structure of this network. The filling matrix components seem to have a pronounced influence on the microstructure and thus on the macroscopic properties.

KEYWORDS: Filling; crystallization; microstructure; hardness; fat

INTRODUCTION

Fillings are generally used in bakery applications (e.g., cream fillings) and confectionery (e.g., truffles). They are surrounded by a coating, a product that has already been studied by the same experimental setup as in this study (*1*). The general function of the filling fat is the same as for the coating fat: It provides the continuous matrix that holds the other ingredients and contributes to flavor, aroma, and color. The difference with the coating fats is that they need not be dry to touch at ambient temperatures. However, too low a solid fat content (SFC) can enhance fat migration with detrimental effects on the surrounding coating (*2*). Upon consumption, the fat matrix should melt away quickly and completely at or near mouth temperature. Failure of the filling to melt rapidly will result in poor flavor release and, probably, a waxy aftertaste (*3*). Another requirement of the filling is the absence of sandiness, which mainly occurs when fat crystallizes in the wrong polymorphic form or when too large fat crystals are formed (*4*).

Partial hydrogenation has been used as a technique to give fats the desired functionality. The problem with this process is the formation of trans fatty acids, which have received considerable attention in recent years, both in the scientific literature and in the popular press. Reports in the scientific literature indicate that high levels of trans fatty acids in the diet, as compared to high levels of cis fatty acids, result in unfavorable effects on both low-density lipoprotein and high-density lipoprotein cholesterol. In response to these reports, many organizations of health professionals have recommended reduced consumption of foods containing trans fatty acids (*5*). In the absence of partially hydrogenated oils—the major source of trans fatty acids—the manufacturers have to fall back to fats based on palm oil and fats based on lauric fats.

As already mentioned in the previous study on coating fats (*1*), few studies have involved all of the structure levels that lead to good technological functionality, a factor that is determined by the macroscopic properties of the fats. The amount of solids, the polymorphism of the solid state, and the microstructure of the network of crystalline particles all play a role in the development of the macroscopic properties, and all of these factors are influenced by processing conditions (*6*). Brunello et al. (*7*) and Campos et al. (*8*) examined the relationship between the different structure levels in the pure fats, cocoa butter, and milk fat and lard, respectively. Humphrey and Narine (*9*), Narine and Humphrey (*10*), and deMan et al. (*11*) on the other hand have studied the relationships in fat blends involved in the preparation of industrial shortenings and in commercial shortenings. Braipson-Danthine and Deroanne (*12*)

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Table 1. Composition of White Filling

ingredient	weight percentage (%)
filling fat	40
skimmed milk powder	15
sugar	45
lecithin	0.4

investigated the relationship between SFC and hardness and found a linear log–log relationship.

The objective of this study is to continue the work of Foubert et al. (*1*) by an investigation of the relationship between crystallization behavior, microstructure, and macroscopic properties in fat blends used for fillings, as these have not before been studied. To understand the difference between trans-containing and trans-free fats, a trans-containing filling fat (TC) (lauric-based) and a trans-free filling fat (TF) (palm-based) are the subjects of this research. Also, the macroscopic properties of the fillings are investigated to explore the effect of the fat phase and nonfat filling matrix components on the macroscopic properties of the fillings.

MATERIALS AND METHODS

Samples. The TC based on partially hydrogenated palm kernel oil as well as the trans-free alternative (TF) based on fractionated palm oil were supplied by Loders Croklaan (Wormerveer, Netherlands). With these filling fats, a white filling [trans-containing filling (TCF) and trans-free filling (TFF)] was produced by Loders Croklaan. The composition of the white filling is given in **Table 1**.

Fatty Acid Composition. Fatty acid methyl esters (FAME) were produced by dissolving 1 drop (10–20 mg) of sample in 2 mL of iso-octane and reacting it with 50 μL of 2 N KOH/methanol reagent. The mixture was shaken for 2 min at room temperature and then allowed to settle. The iso-octane layer was carefully removed and diluted two times. High-resolution FAME gas chromatography (GC) was carried out on a Thermo Finningan Trace GC fitted with a CP Select (CPSil88 bonded) CB 50 m $\times$ 0.25 mm i.d., 0.25 μm film. The helium carrier gas had an inlet pressure of 125 kPa. Two microliters of sample solution was injected via a PTV split/splitless injector (right inlet, 125 kPa; mode, CT split; and split ratio, 20:1). The oven temperature was programmed from 160 °C for 1 min, 160–200 °C at 1 °C/min, and 200–230 °C at 2 °C/min. Detection was via flame ionization detection (FID) set to 260 °C. The results were based on single measurements.

Triglyceride Composition. High-resolution separation for triglycerides was achieved using an Agilent 6890+ GC system fitted with an automated on-column injection onto a Quadrex 15 m $\times$ 0.25 mm, 0.1 μm film, 65% phenyl-methyl silicone GC column. The samples were dissolved in iso-octane at approximately 0.3 mg/mL. The injection volume was 0.1 μL. The helium carrier gas was set at constant flow of 1 mL/min. The oven program with the injector in the oven track mode was 80 °C for 0.5 min, ramping up to 330 °C at 50 °C/min, with triglyceride separation being achieved from 330 to 350 °C ramping at 1 °C/min. Detection was via FID. The results were based on single measurements.

SFC. SFC was measured by pulsed NMR with a Bruker Minispec pc 20 (Bruker, Karlsruhe, Germany). Melted fat was placed in NMR tubes (three replicates) and submitted to the tempering treatments of the IUPAC 2.150 serial-tempered method. The SFC was determined in the range of 0–45 °C at 5 °C intervals following 60 min incubations at each temperature.

Isothermal Crystallization Curves Via Stop-and-Return Technique [Differential Scanning Calorimetry (DSC)]. The isothermal crystallization curves were obtained with a TA Q1000 DSC (TA Instruments, New Castle, DE) with a Refrigerated Cooling System. The DSC was calibrated with indium (TA Instruments), azobenzene (Sigma-Aldrich, Bornem, Belgium), and undecane (Acros Organics, Geel, Belgium) before analyses. Nitrogen was used to purge the system. Fat (5–15 mg) was hermetically sealed in aluminum pans using sample preparation procedure B as described by Foubert et al. (*13*), and an empty pan was used as a reference. The time–temperature program applied was as follows: holding at 70 °C for 10 min to ensure a completely liquid state, cooling at 5 °C/min to the isothermal crystallization temperature (±0.05 °C), holding for the required crystallization time, and then heating at 20 °C/min to 70 °C (crystallization times were set at 1–60 min, depending on the stage of the crystallization). Thus, different amounts of crystallization were allowed to occur before remelting. The melting curves were integrated using a linear baseline with the end point determined by the calculation algorithm as described by Foubert et al. (*13*) and the starting point at the same *y*-value as the end point. Three repetitions were performed for each combination of temperature and time. The area of the melting peak thus increased with increasing time at the isothermal crystallization temperature as the degree of previous crystallization increased. Hence, the degree of crystallization as a function of time at the crystallization temperature could be determined, despite the fact that some crystallization might have occurred before the isothermal temperature was reached.

The heating rate was taken as 20 °C/min, because too low a heating rate may lead to polymorphic transitions during heating, making it impossible to describe the crystallization behavior. On the other hand, too high a heating rate will lead to a thermal lag of the melting peaks and a decrease in the resolution of the peaks.

Determination of Melting Curve after Penetration Test (DSC). These melting curves were obtained with a 2010 CE DSC (TA Instruments) with a refrigerated cooling system. Calibration was the same as for the TA Q1000 DSC described above. A small sample (5–15 mg) of the crystallized filling or fat was hermetically sealed in an aluminum pan and immediately placed in the DSC at the temperature of performance of the penetration test. The sample was then heated at a rate of 20 °C/min to 70 °C. The melting curves were integrated as described above. The melting curves were determined in triplicate

Microscopic Analyses. Microscopic analyses were conducted by the use of a Leitz Diaplan microscope (Leitz Diaplan, Leica, Germany) equipped with a Linkam PE 94 temperature control system (Linkam, Surrey, United Kingdom). The samples were melted, and one drop of the sample was put on a microscopic plate covered with a cover glass. Thereafter, the samples were placed on the temperature-controlled plate to investigate the microstructure directly after crystallization and after storage. Samples were imaged with a Nikon Coolpix 4500 (Nikon, Melville, NY).

Hardness (Penetration Test). Twenty milliliters of fat was placed in a plastic beaker and crystallized in a temperature-controlled water bath for 30 min at a specific temperature (5 or 10 °C) before storing at room temperature (19–21 °C). The hardness of the crystallized and stored samples was determined with a penetration test on a Texture Analyzer TA 500 (Lloyd Instruments, Hampshire, United Kingdom) with a cylindrical probe with a diameter of 4.51 mm (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 (±0.5 °C) (Lovibond, Dortmund, Germany). Hardness was defined as the maximum penetration force (*N*). The hardness experiments were performed in triplicate.

RESULTS AND DISCUSSION

Characterization of the Fat Samples. Table 2 shows the fatty acid composition of the two filling fats. The TC indeed contains around 10% elaidic acid (C18:1 trans), while the TF contains less than 1% of this trans fatty acid. It is also apparent that the TC contains a high amount of lauric and myristic acid, which can be explained by the fact that the fat blend is based on palm kernel oil. **Table 3** shows the triglyceride compositions of the two filling fats. The weight percentages of the 10 most abundant triglycerides are each time represented. The triglyceride composition shows that TC contains a lot of short chain trisaturated triglycerides, which is typically for lauric fats. The

Table 2. Fatty Acid Composition of Filling Fats[a]

	composition (wt %)	
fatty acid	TC	TF
8:0	4.9	
10:0	3.6	
12:0	40.6	0.2
14:0	11.1	1.0
16:0	9.8	50.3
18:0	12.4	5.0
18:1 cis	7.3	30.2
18:1 trans	9.7	0.2
18:2	0.2	7.4
others	0.5	0.8

[a] Note that single measurements were made on each sample.

Table 3. Triglyceride Compositions of Filling Fat TC and Filling Fat TF[a]

triglyceride	composition (wt %)
filling fat TC	
CCLa	7.6
CLaLa	9.2
LaLaLa	17.3
LaLaM	9.9
LaMM	5.4
LaOLa / LaELa	4.5
LaOM / LaEM	4.3
MMM	4.5
MOM / MEM	3.9
MOP / MEP	3.5
filling fat TF	
MOP	1.8
PPP	2.4
POP	47.3
PLP	9.3
POS	9.2
PLS	1.9
PLO	4.9
POO	12.7
OOO	2.1
SOO + SLS	1.6

[a] Note that positional isomers have not been separated and that single measurements were made on each sample.

triglyceride composition of TF corresponds to a palm fraction, which is often used as a cocoa butter equivalent (*14*). **Figure 1** shows the SFC curves of both filling fats. The difference between the two fats is especially apparent between 20 and 25 °C. Despite the difference in chemical composition, both fats possess the same melting point based on SFC (temperature where the SFC becomes zero).

Isothermal Crystallization Behavior of Filling Fats. Using DSC, the crystallization in a cooling tunnel was simulated. The fats were completely melted and then cooled to a certain temperature, where the samples were kept isothermally for a certain period of time. In some experiments, the fat began to crystallize during cooling to the isothermal temperature (which also happens in reality in a cooling tunnel), making it impossible to integrate the isothermal crystallization curves. This was solved by heating the fat after certain isothermal periods and integrating the melting curve to obtain the melting enthalpy, which is related to the amount of fat that had crystallized at the moment that the heating was started. This way of obtaining crystallization curves is called the "stop-and-return" technique (as described above).

Figure 2 shows the melting enthalpy (as a measure of the crystallinity) as a function of the isothermal time for TC and TF. In contrast to coatings (*1*), fillings need not be dry as they leave the cooling tunnel (after 10–30 min of crystallization), but too low an amount of crystallized filling fat can lead to postcrystallization, leading to a release of crystallization heat. This can also occur when the fat recrystallizes from an unstable to a more stable polymorph. Such release of crystallization heat can have a detrimental effect on the surrounding coating, for example, blooming of the coating. Therefore, the temperature range studied for each fat was around the temperature at which the fat was almost completely crystallized within a period between 30 and 45 min.

Figure 2a shows that TC had a high crystallization rate, which is typical for lauric fats (*15*). At 19 °C, the fat was completely crystallized after 30 min. From this temperature onward, no significant differences were found between the melting enthalpy at 30 min and the melting enthalpy at longer isothermal times. To get more insight in the crystallization mechanism, the peak maxima of the melting profiles were investigated as a function of time for each of the temperatures. For TC, only one peak could be observed for the different crystallization temperatures and isothermal times, as presented in **Figure 3**. The values of the peak maxima probably (no X-ray diffraction was performed) correspond to a β'-polymorph (*16*), as palm kernel oil (on which TC is based) is very stable in the β'-polymorph and a β-polymorph only occurs after months of storage (*17*).

Figure 2b shows that the crystallization rate of TF was much lower in comparison with TC, which can be explained by the higher amount of cis unsaturated fatty acids (see **Table 2**). To have a complete crystallization after 30 min, the TF needed to be crystallized at a crystallization temperature of 8 °C or lower. There was a big difference in crystallization rate between a crystallization temperature of 17.5 and 20 °C and between a temperature of 16 and 17.5 °C. To try to explain this, the crystallization behavior was again investigated by studying the peak maxima of the melting profiles as a function of time for each of the temperatures. The crystallization behavior of TF was much more complex than the crystallization behavior of TC. For a crystallization temperature of 5 °C, two peaks were visible at an isothermal time of 0 min. At longer isothermal times, only one peak could be observed, which coincides with the original second peak. This is illustrated in **Figure 4a**. For a crystallization temperature of 8 °C, only one peak could be observed that coincides with the peak at longer isothermal times for a crystallization temperature of 5 °C (see **Figure 4b**). Between a crystallization temperature of 12 and 17.5 °C, two peaks could be observed for short isothermal times (represented by a crystallization temperature of 16 °C in **Figure 4c**). The low-melting peak disappeared at longer isothermal times. The low-melting peak at short isothermal times coincides with the peak for crystallization at 8 °C. For a crystallization temperature of 20 °C or higher, only one peak could be observed (see **Figure 4d**), which coincided with the peak for longer isothermal times at a crystallization temperature between 12 and 17.5 °C. The difference between 17.5 and 20 °C could also be observed in **Figure 2b** by the clear difference in crystallization rate between these two temperatures. The difference between the crystallization rate at 16 and at 17.5 °C could not be explained by the peak maxima. A possible explanation is the higher amount of α-crystals present at short isothermal times for crystallization at 16 °C.

On the basis of these results, the following crystallization mechanism can be hypothesized (no X-ray diffraction performed). At a crystallization temperature of 5 °C, a sub-α-mediated α-crystallization was supposed. The sub-α- and the α-polymorph are indeed observed in palm oil (*18*), and the temperatures for the peak maxima are too low for a β'- or a β-polymorph (*16*). On the basis of the hypotheses for crystal-

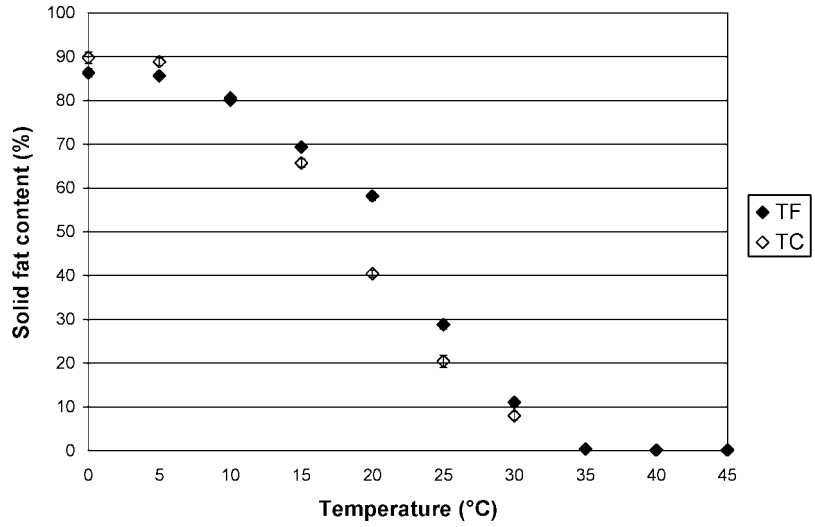

Figure 1. SFC as a function of temperature for both filling fats.

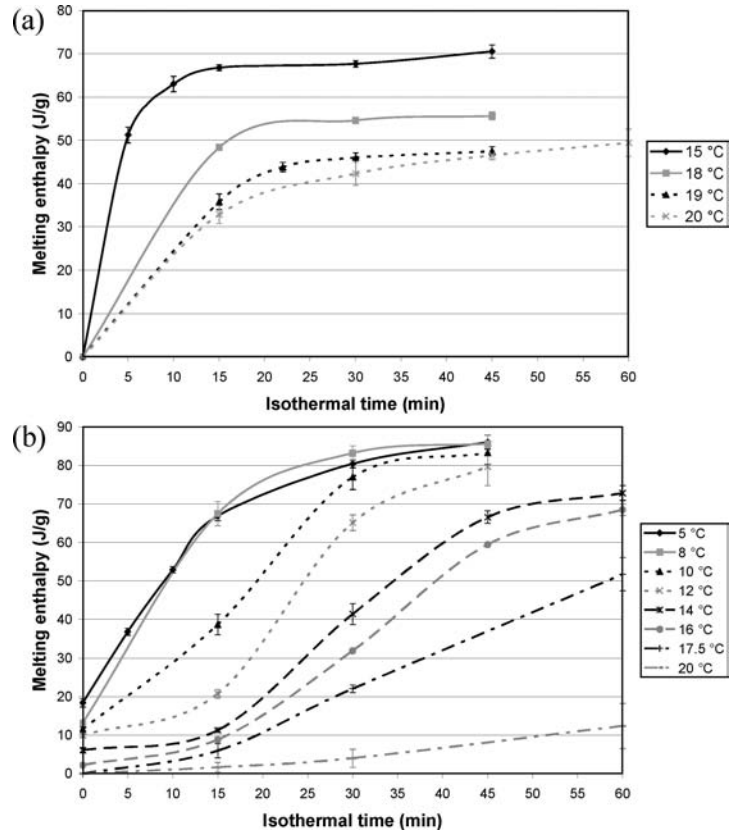

Figure 2. Melting enthalpy of TC (**a**) and TF (**b**) as a function of isothermal time at different crystallization temperatures.

lization at 5 °C, the following forms were probably occurring when crystallizing at other temperatures: an α-crystallization at 8 °C, an α-mediated β'-crystallization between 12 and 17.5 °C, and a direct crystallization in a β'-polymorph for crystallization at 20 °C and higher. Some authors have indeed proven that palm midfraction is stable in a β'-polymorph, due to the high amounts of POP, POO, and PPP (*2*).

The above-mentioned results of the DSC analysis can be used to select temperatures to be used in a cooling tunnel. The most important thing is to crystallize the correct polymorphic form, which is the β'-polymorph. A β-polymorph is unwanted, because it can give sandiness in the filling. The sub-α- and α-polymorph are unstable forms, which can recrystallize in a more stable polymorphic form with a release of unwanted recrystallization heat. For TC, the selection of the correct cooling tunnel temperature is easy, because it crystallizes directly in the β'-polymorph for all of the crystallization temperatures. The selection will be more difficult for TF because it crystallizes in different polymorphic forms as a function of the crystallization temperature. Crystallization at higher temperatures will give crystallization in the wanted β'-polymorphic form but will also decrease the crystallization rate, so a longer time in the cooling tunnel is required to inhibit postcrystallization. Too high a temperature will lead to formation of a β-polymorph.

Microscopic Analysis of Filling Fats. The microstructure of the filling fats was investigated after 30 min of crystallization at 5 °C (**Figure 5**). There was a clear difference between both fats directly after the crystallization. TC forms smaller crystals than

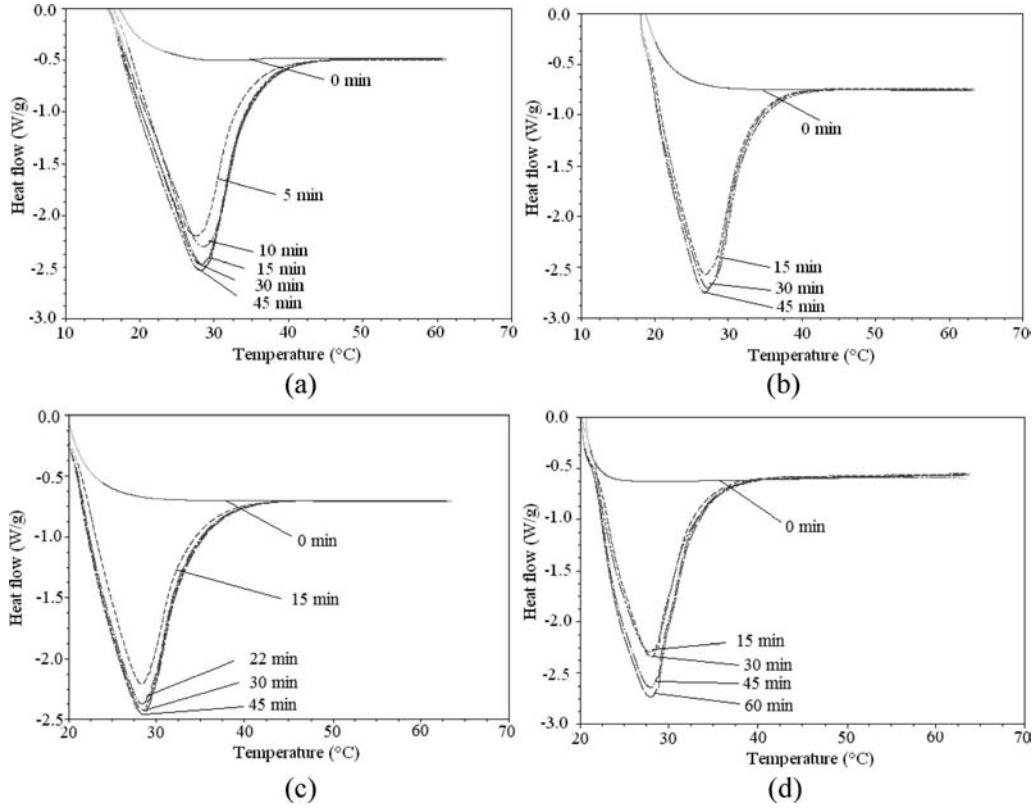

Figure 3. Melting curves of TC as a function of isothermal time at a crystallization temperature of 15 (**a**), 18 (**b**), 19 (**c**), and 20 °C (**d**).

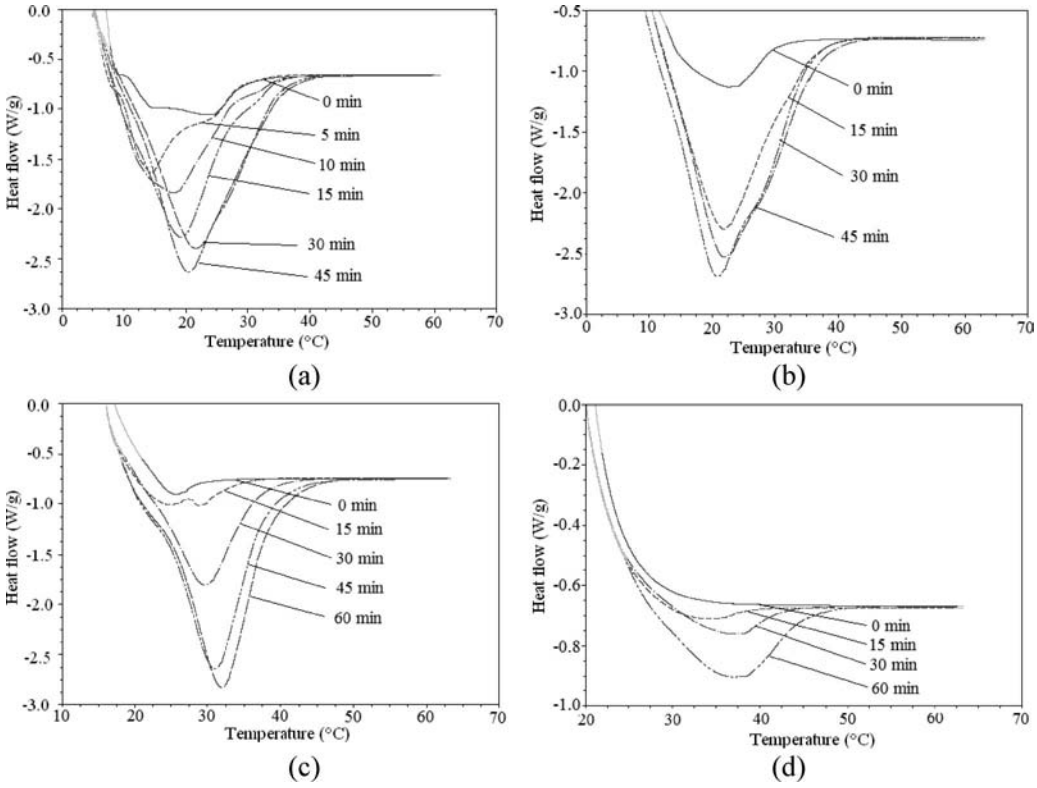

Figure 4. Melting curves of TF as a function of isothermal time at a crystallization temperature of 5 (**a**), 8 (**b**), 16 (**c**), and 20 °C (**d**).

TF, which could be explained by the faster crystallization of TC (see above) with instantaneous formation of a large number of nuclei. Fast crystallization is accompanied by a rapid increase in viscosity, thus limiting molecular diffusion and crystal growth (*19*).

The difference in microstructure became smaller when both samples were stored at room temperature (19–21 °C) after their crystallization at 5 °C for 30 min (**Figure 5**). The microstructure of TC did not change after storage, which can be explained by the occurrence of one polymorpic form (β') for different temperatures. Polymorphism is one of the important factors that

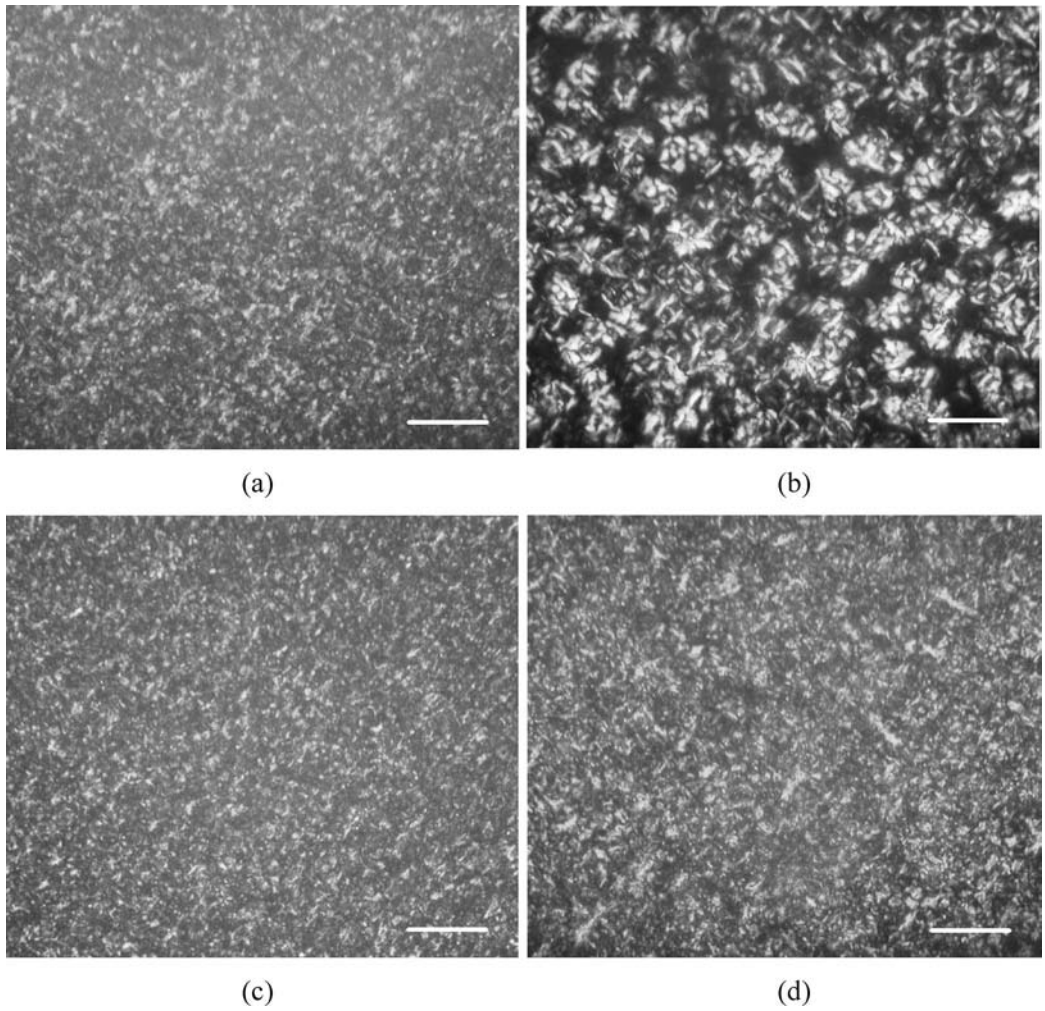

Figure 5. Microscopic images (500× magnification) of TC crystallized for 30 min at 5 °C (**a**), TF crystallized for 30 min at 5 °C (**b**), TC crystallized for 30 min at 5 °C and after 1 week of storage at room temperature (**c**), and TF crystallized for 30 min at 5 °C and after 1 week of storage at room temperature (**d**). The white bars represent 500 μm.

influence the microstructure of fats (*20*). Another possible explanation is that TC already formed a very dense network after crystallization at 5 °C, which did not give space for changes in the microstructure.

The microstructure of TF changed a lot after storage during 1 week at room temperature (**Figure 5**). This can be explained by the different crystallization mechanism of TF as compared with TC (see above). The crystallization rate of TF was very low, which meant that the fat would crystallize further during storage at room temperature leading to a denser crystal network. Also, the polymorphism could change during storage. TF showed an evolution in polymorphic forms when the crystallization temperature was changed (see above). At a crystallization temperature of 5 °C, sub-α-mediated α-crystallization was hypothesized, and at a crystallization temperature of 20 °C, direct crystallization in a β'-polymorph was supposed (as already explained in the paragraph about the isothermal crystallization behavior). This can lead to different polymorphic forms directly after crystallization at 5 °C and after storage at 20 °C. Different polymorphic forms often show different microstructures (*10*), but some authors also mention that one polymorphic form can lead to the formation of different microstructures depending on the processing conditions.

Hardness of Filling Fats as a Function of Crystallization Temperature and Storage Combined with Melting Profile/Microscopy. The hardness of the filling fats was examined after cooling in a thermostated water bath at 5 and 10 °C for 30 min. Thus, the exact crystallization temperatures cannot be compared with the isothermal crystallization behavior. However, trends as a function of temperature were assumed to remain valid for the different crystallization volumes. The hardness was examined immediately after the cooling process and also after 1 day and 1 week of storage at room temperature (19–21 °C). To be able to explain better certain phenomena, the hardness tests were coupled with measurements of the melting profile and with microscopic analyses under the same conditions.

Figure 6 shows the hardness as well as the melting enthalpy of both filling fats as a function of storage time after crystallization at 5 and 10 °C. After crystallization at 5 °C, the hardness of TC decreased significantly during storage, which was also the case for the melting enthalpy. The decrease in melting enthalpy can be explained by the melting of the sample during storage at room temperature, which can also be observed in the big reduction in solid fat (**Figure 1**) between 5 (the crystallization temperature) and 20 °C (the storage temperature).

The decrease in the amount of solid fat can be an explanation for the decrease in hardness, as already demonstrated by different studies (*8, 12, 21, 22*). Polymorphism cannot be an explanation, as the peak maximum of the melting curve did not change during storage. The stabilization of the microstructure of TC was rather slow, which can be observed by the significant difference in hardness and melting

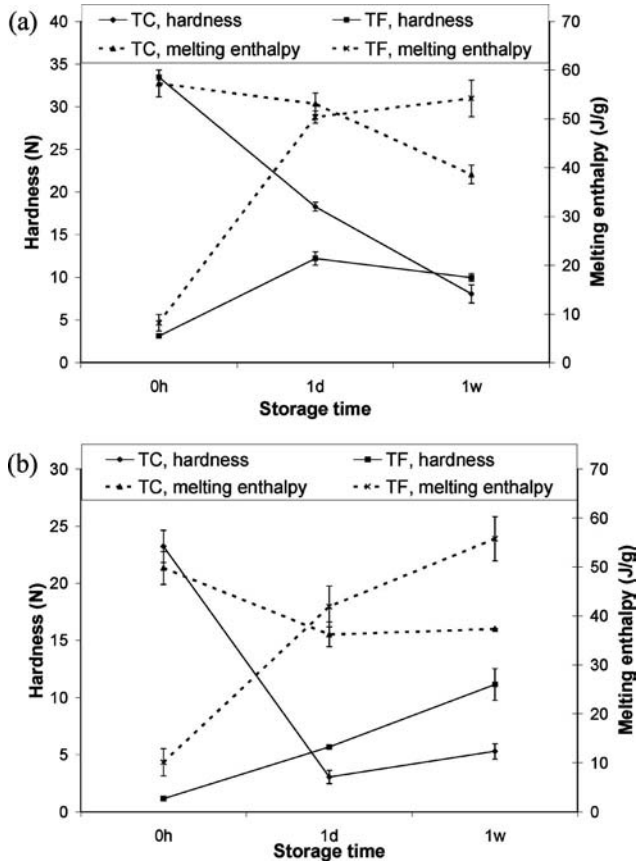

Figure 6. Hardness and melting enthalpy of the filling fats as a function of storage time after crystallization at 5 (**a**) and 10 °C (**b**).

enthalpy between 1 day and 1 week of storage. No clear differences between the different storage times could be detected in the microscopic analyses performed (**Figure 5**), but it should be stressed that the crystallization volume is very much different (1 drop vs 20 mL), as is the mode of crystallization (two-dimensional crystallization vs three-dimensional crystallization). When the crystallization temperature of TC was increased to 10 °C, the hardness and the melting enthalpy decreased significantly during the first day of storage. Also, the melting enthalpy decreased during the first day of storage, so the decrease in hardness again can be explained by a decrease in the amount of solid fat. In contrast, the hardness and melting enthalpy did not decrease between 1 day and 1 week of storage, meaning that the microstructure was stabilized more quickly than for a crystallization temperature of 5 °C. This can be explained by the lower amount of solid fat after crystallization at 10 °C, which can be seen by the lower value of the melting enthalpy directly after crystallization at 10 °C as compared with the melting enthalpy for 5 °C crystallization. Because of this lower amount of solid fat, less fat can melt down and the fat structure will be stabilized more quickly.

After crystallization at 5 °C, the hardness of TF increased significantly during the first day of storage, which is also the case for the melting enthalpy. Because of the rather slow crystallization of TF (see above), the crystallization was not complete after the cooling process, and thus, the sample crystallized further during storage, leading to a higher melting enthalpy and a higher hardness. Different studies (*8, 12, 21, 22*) have already demonstrated that higher amounts of crystallinity lead to harder networks. After 1 day of storage, the sample had reached equilibrium as there were no significant differences between 1 day and 1 week of storage, neither for the melting enthalpy nor for the hardness. The results of the microstructural investigation (see above) agreed with the results of the hardness measurements (see **Figure 5**). Narine and Marangoni (*20*) have indeed proven that the microstructure of fats has an enormous influence on the macroscopic properties of the fat network.

When the crystallization temperature was increased to 10 °C, the hardness and melting enthalpy continued to increase between 1 day and 1 week of storage. This can be explained by the lower amount of fat that was crystallized directly after the crystallization, because of the higher crystallization temperature. More fat needed to crystallize further and needed to be stabilized, leading to a longer equilibrium time.

The big difference in hardness between both filling fats directly after crystallization can be explained by the different nature of the fats. TC is a lauric fat with a faster crystallization rate (see above) as compared with TF. Another important characteristic of these lauric fats is their small melting range (*14*). TF is based on a palm fraction and showed a slower crystallization rate. Because of these reasons, TC will melt during storage at 20 °C, while TF will crystallize further during storage. This leads to a hardness of both filling fats that is not much different from each other after crystallization at 5 °C. This was also observed for the microstructure (see **Figure 5**). The direct crystallization of TF at 5 °C gave larger crystals, which are known to have weaker attractive forces (*8*) and thus a lower value for the hardness. After storage, the microstructure of both fats is the same, which explains the same value of the hardness of both samples.

Also, for a crystallization temperature of 10 °C, the difference in microstructure was very big directly after crystallization, because of the difference in crystallization rate. In contrast to a crystallization temperature of 5 °C, the hardness after 1 week of storage at room temperature was different for both filling fats. Presumably, the microstructure was also different between TC and TF after 1 week of storage. Unfortunately, these differences could not be observed by the microscopic analyses. It can be concluded that the crystallization temperature plays an important role in the development of the microstructure and the macroscopic properties of fat systems. A big difference in hardness between two samples directly after crystallization does not mean that the equilibrium hardness between two samples will differ a lot after a long storage time.

Hardness of Fillings as a Function of Crystallization Temperature and Storage Combined with Melting Profile. **Figure 7** shows the hardness as well as the melting enthalpy of both fillings as a function of storage time after crystallization at 5 °C and 10 °C.

After crystallization at 5 °C, the hardness and the melting enthalpy of TCF decreased during storage, which was also observed for the TC. The reason for the decrease in hardness of TCF is thus most probably a decrease in the amount of solid fat. Also, for crystallization at 10 °C, the hardness and melting enthalpy of TCF show the same evolution as the corresponding fat. During the first day of storage, the hardness decreased significantly, but between 1 day and 1 week of storage, the hardness was stabilized and even slightly increased. The melting enthalpy follows the same pattern. The agreement in evolution of the melting enthalpy and hardness between the TCF and the corresponding TC can be explained by the good fit of the short chain triglycerides or the smaller crystals of TC within the solid

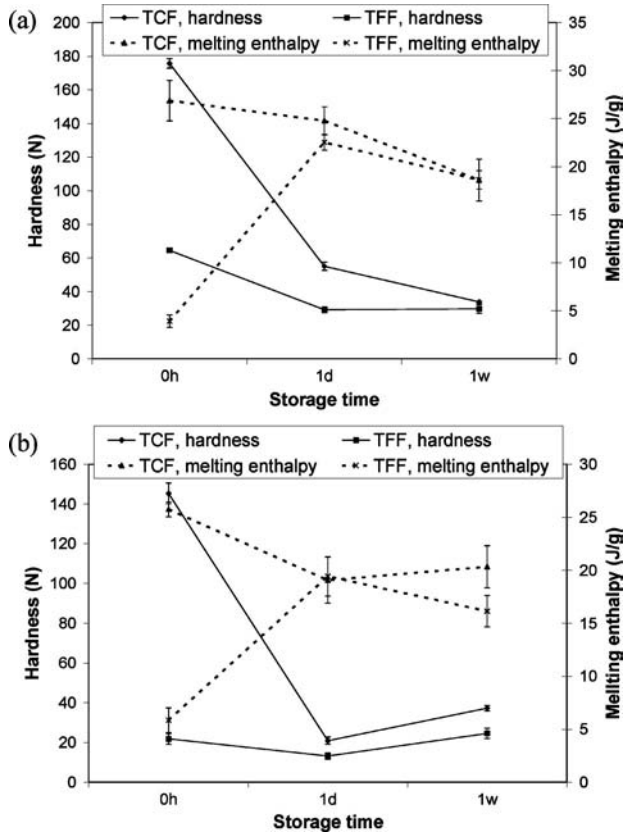

Figure 7. Hardness and melting enthalpy of the fillings as a function of storage time after crystallization at 5 (**a**) and 10 °C (**b**).

dispersed phase (sugar crystals and milk solids; see **Table 1**) of TCF, leading to the same behavior for the TCF as for the TC.

In contrast to TC, the hardness of TFF did not follow the same pattern as the corresponding fat (TF). After crystallization at 5 °C, the hardness of TFF decreased during the first day of storage, while the melting enthalpy increased. The decrease in hardness thus could not be explained by a change in the amount of solid fat, because then the melting enthalpy should decrease as a function of storage time. Probably, the microstructure and the interactions between the fat phase and the other ingredients in the filling will lead to the decrease in hardness. Liang and Hartel (*23*) have proven that different types of milk powders can influence chocolate properties, so it could be that milk powder also influences the filling properties of TCF. Important parameters of milk powder are the strength of the particles, their shape, and the amount of air included in void spaces (*23*). It could be that TF interacts in such a way with the solid dispersed phase (sugar and milk solids) that the hardness decreases during storage. Also, for a crystallization temperature of 10 °C, the hardness and the melting enthalpy did not correspond to each other. The hardness did not change a lot during storage (slight decrease during the first day of storage and slight increase between 1 day and 1 week of storage), while the melting enthalpy increased a lot during the first day of storage (further crystallization) and decreased slightly between 1 day and 1 week of storage. Polymorphism could not be an explanation for the change in hardness, because the peak maximum of the melting peak did not change during storage. Thus, a higher amount of solid fat (derived from the melting enthalpy) does not contribute to a higher value for the hardness of the filling. This is in contrast with the evolution of the hardness of the corresponding fat, where the hardness and the melting enthalpy increased simultaneously as a function of storage time. Probably, the microstructure changes a lot during storage, and also, the interactions with the solid dispersed phase vary, which are the main reasons for the changes in hardness and thus for the macroscopic properties of the filling. The same results were found for the analysis of coating fats and coatings (*1*). The triglycerides of TF have a longer chain length than the triglycerides of TC and give rise to larger crystals (see **Table 3**), and this could be the reason for the worse interaction with the solid dispersed phase and the reason for different evolution of the hardness during storage. The hardness of TFF remains lower than the hardness of TCF during storage, which does not correspond with the hardness of the filling fats (higher hardness for TF after storage). This means that for TC the interactions between the fat phase and the solid dispersed phase create a firmer network with a higher hardness than the interactions between the TF and the solid dispersed phase of the corresponding TFF. Alternatively, the crystals of TC are able to form a stronger network around the matrix than can the TF.

Thus, it can be concluded that the different filling matrix components can have a very pronounced influence on the microstructure and thus on the macroscopic properties such as the hardness. This conclusion was also demonstrated previously for coatings used in confectionery (*1*).

ABBREVIATIONS USED

TC, trans-containing filling fat; TF, trans-free filling fat; TCF, trans-containing filling; TFF, trans-free filling; FAME, fatty acid methyl ester; SFC, solid fat content; DSC, differential scanning calorimetry.

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