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Crystallization Behavior of Anhydrous Milk Fat−Sunflower Oil Wax Blends

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ABSTRACT: This research evaluates the effect of sunflower oil wax (SFOw) addition on the crystallization behavior and functional properties of anhydrous milk fat (AMF). Induction times of nucleation, melting behavior, microstructure of crystals, and hardness were evaluated for samples of pure AMF and AMF with 0.1 and 0.25% SFOw. Results from this research show that the addition of waxes induced the onset of crystallization of AMF by inducing its nucleation, as evidenced by decreased induction times of nucleation and the formation of smaller crystals. Crystal growth after tempering was also promoted by waxes, and significantly harder lipid networks were obtained. Results presented in this paper suggest that SFOw can be used as an additive to alter the physiochemical properties of low *trans*-fatty acid lipids.

KEYWORDS: Sunflower oil waxes, crystallization, induction times, nucleation, differential scanning calorimetry, polarized light microscopy, low field time domain nuclear magnetic resonance (TD-NMR)

INTRODUCTION

The quality and functional properties of lipid-based products strongly depend upon their crystallization behavior. Since the elimination of *trans*-fatty acids from food formulations, this area of research has become increasingly important. Researchers are exploring new technologies and processing conditions that, when used in low *trans*-fats, can generate optimal functional properties. Some of the strategies that are being explored are the structuring of vegetable oils using additives.[1−5] In general, the most efficient agents used to structure vegetable oils are small molecules, such as mono- and diglycerides, fatty acids, fatty alcohols, waxes, wax esters, and sorbitan esters, among others. Dassanayake et al. evaluated the physical properties of rice bran wax and their capability to form organogels.[6] These authors reported that, indeed, rice bran waxes show good organogel-forming qualities and attribute this behavior to the specific crystallization behavior of the waxes in the form of needle-like crystals in the liquid oil phase. Dr. Toro-Vazquez's group has extensively studied the formation of organogels through the addition of candelilla wax [7] and (*R*)-12-hydroxystearic acid.[8] They optimized the ratio of candelilla wax and high oleic safflower oil that will result in a transparent and stable organogel. These authors also evaluated the effect of tripalmitin co-crystallization on the stability of the gels. Results from their research show that the co-crystallization of tripalmitin and candelilla wax can be used to tailor the physicochemical properties of lipids. They also state that these systems (wax-based organogels) can be used to develop *trans*-free vegetable oil spreads. Other researchers have used monoglycerides in gel phases not only to structure a vegetable oil but also to control the release of lipids ingested by humans.[9]

Most of the existing research on structuring lipids has been performed in vegetable oils. Very little research analyzing the effect of structuring molecules, such as waxes, has been performed in semi-solid fats.[10] Anhydrous milk fat (AMF) is a semi-solid fat with ideal flavor and mouthfeel. The functional properties of AMF can be broadened by optimizing the processing conditions. A myriad of research has been performed on the effect of processing conditions on the crystallization behavior of milk fat. Some of the processing conditions explored were crystallization temperature, cooling rate, and addition of emulsifiers and waxes.[10−18]

Waxes are byproducts from the refining of some vegetable oils, such as sunflower and rice. The crystallization behavior of these molecules has been extensively studied to improve the separation efficiency during the refining process.[19−23] Sunflower oil wax (SFOw) contains a mixture of long-chain fatty acid ($C_{14}-C_{30}$) molecules esterified to long-chain alcohols ($C_{18}-C_{34}$), giving it some lipophilic characteristics. This characteristic suggests that SFOw might be a good molecule to alter the crystallization behavior of lipids.

The objective of this study was to evaluate the effect of SFOw on the crystallization behavior and functional properties of AMF. The crystallization behavior of the lipid systems were measured using induction times of nucleation, while the functional properties of the lipid network formed were quantified with the melting behavior, hardness, and microstructure of the lipid system.

MATERIALS AND METHODS

Starting Materials. AMF was donated by Kraft (Glenview, IL). SFOw was isolated and purified from tank settlings donated by Archer Daniels Midland (Decatur, IL). The isolation and purification methods and the chemical composition of the waxes were reported elsewhere.[10]

Melting Point. The melting point of AMF, SFOw, and their blends was measured using differential scanning calorimetry (DSC, TA2910, TA Instruments). Between 4 and 15 mg of sample was hermetically

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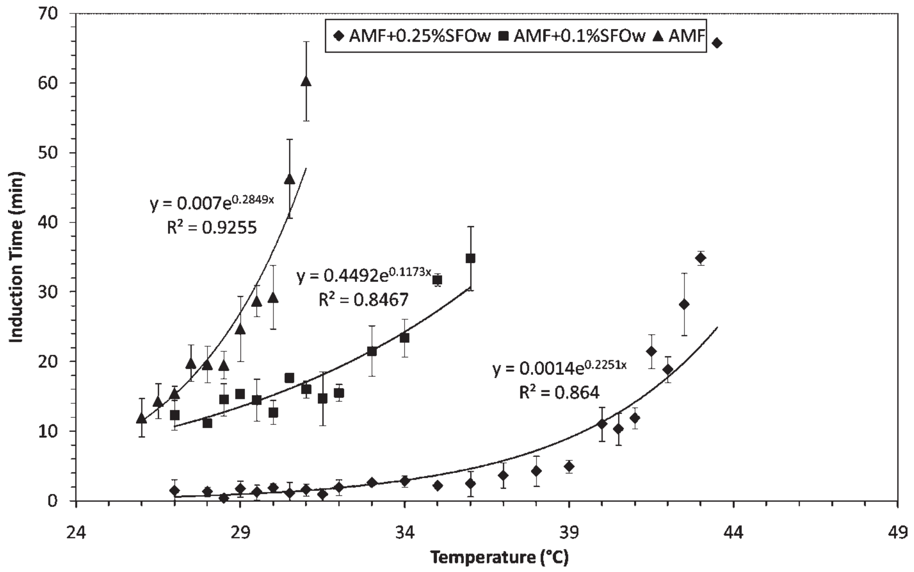

Figure 1. Induction time in minutes as a function of the crystallization temperature for AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw.

sealed in aluminum pans and placed in the DSC pans. Samples were held at 25 °C for 1 min, cooled at a rate of 5 °C/min to −20 °C, and held there for 1 h to allow for complete crystallization. Samples were then heated at 5 °C/min to 80 °C and held there for 1 min. The peak temperature obtained from the melting curve was used as the melting point.

Sample Preparation. The control sample was AMF, and the experimental samples were AMF with additions of 0.1 and 0.25% SFO wax by weight (AMF + 0.1% SFOw and AMF + 0.25% SFOw, respectively). The experimental samples were made by measuring 0.5 and 0.625 g of SFO wax into plastic trays. Waxes were transferred to a beaker, and the AMF was added to achieve 500 and 250 g of total weight. Samples were stirred and then stored in an oven at 65−75 °C from 30 min to 1 h until the waxes had completely dissolved. When samples were not in use, they were stored at 4 °C until the experiments were finalized.

Laser-Polarized Light Turbidimetry. A laser-polarized turbidimeter with a helium−neon laser as a light source was used to follow the occurrence of optically anisotropic fat crystals. Approximately 100 g of sample was completely melted at 90 °C, stored at 65−75 °C for 30 min to allow for temperature equilibration, and then placed in a water-jacketed glass cell, which was placed between the polarized laser and an analyzer. The absence of crystals at the beginning of the experiment was corroborated by laser turbidimetry and polarized light microscopy (PLM). The temperature of the cell (T_c) was controlled by water that was circulated through a water bath. Crystallization temperatures were set between 26 and 44 °C in 0.5 °C intervals. A magnetic stirring rod provided agitation at a rate of 100 rpm. A thermocouple was placed inside the sample between the path of the laser and the inside wall of the cell. The photosensor output and the cell temperature were recorded using LabView Software, version 8 (National Instruments Corp., Austin, TX).

From the temperature and photosensor graphs, the times when the sample reached crystallization temperature and started to crystallize were obtained and the induction time (τ) of nucleation was calculated as the time lapse between the moment the fat or fat/wax blend reaches the crystallization temperature and when crystallization begins. Repetitions were performed in triplicate, and induction times reported are the average value of those independent runs.

PLM. PLM was used to follow the morphology of the fat crystals or crystal clusters during the crystallization process. When the first crystals were observed with the naked eye in the crystallization cell, a drop of sample was placed between a slide and cover slide and placed under a Leitz microscope model Ortholux II (Ernest Leitz Co., Wetzlas, Germany). Photographs were taken under polarized light with a Leitz-Vario-Orthomat camera. Optimas 6.1 software was used to collect the images. Aliquots were taken from the crystallization cell as a function of time for 90 min with a 20× objective.

Texture Profile Analysis (TPA). The texture of the crystallized samples was measured using a double compression test with a TA-XT plus texture analyzer (Texture Technologies, Scarsdale, NY). Samples were crystallized at T_c (27, 29, and 30 °C), kept at T_c for 90 min, then placed in 1 cm diameter cylindrical tubes, and stored at 5 °C for 48 h. For measurement, the solid fat was cut into 1 cm length cylindrical samples. A 5 cm diameter cylindrical probe compresses the sample at a constant speed of 5 mm/s. Hardness was defined as the maximum peak force obtained during the first compression. Samples were penetrated to 25% strain.

DSC. The melting behavior of the samples used for TPA analysis was evaluated using DSC (TA2910, TA Instruments). That is, the melting behavior of the crystal network formed after 90 min in the crystallization cell and after storage for 48 h at 5 °C was measured using DSC. Between 4 and 15 mg of each sample was hermetically sealed in aluminum pans and placed in DSC. DSC pans were precooled at 5 °C by placing the pans in the fridge. Samples were placed in the DSC pans and immediately placed in DSC already set at 5 °C. Samples were held in DSC for 1 min at 5 °C and then heated at a rate of 5 °C/min to 80 °C. The melting profile was analyzed in terms of the peak temperature and melting enthalpy.

Solid Fat Content (SFC). The SFC was evaluated using a pulsed ^{1}H time-domain nuclear magnetic resonance spectrometer (TD-NMR, the minispec, Bruker Corp., Billerica, MA), operating at a resonance frequency of 20 MHz with a 10 mm probe diameter. During TD-NMR experiments, all hydrogen nuclei in fats/oil blends are excited by radio frequency pulses in resonance with the Larmor frequency of hydrogen. These nuclei return to their original state once the pulse is stopped, emitting a decaying NMR signal. The initial amplitude of the signal is proportional to the total number of hydrogen nuclei in the sample. The nuclei in solid phases decay much faster than those in liquid phases, and this difference is used to non-invasively determine the percent SFC. The minispec is calibrated for this application using three certified SFC standards of plastic in oil with known solid content: 0, 31.5, and 72.6% solids (supplied by Bruker).

Samples of AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw were measured for SFC at 27, 29, and 30 °C. To perform the SFC measurements, samples were melted at 90 °C using a microwave, placed in NMR tubes, and left at 60 °C for 30 min. Melted samples were transferred to a dry bath that had been set at T_c (27, 29, and 30 °C). SFC measurements were collected every 1 min for the first 10 min, every 5 min up to 30 min, and every 15 min until a total of 90 min.

Statistical Analysis. Average and standard deviation values obtained from triplicate experiments are reported in the paper. Statistical analysis was performed with GraphPad Prism 4.03 (La Jolla, CA) using two-way analysis of variation (ANOVA) tests using a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the induction times of nucleation for each type of sample as a function of the crystallization temperature. As expected, all samples showed an exponential relationship between the induction time of nucleation and T_c. As T_c increased, the supercooling in the sample decreased, and therefore, more time was needed to induce nucleation (longer induction times). These differences were more pronounced at higher T_c, where fewer crystals were formed and crystal growth was favored. Similar relationships between induction times of nucleation and crystallization as a function of the crystallization temperature for lipid systems were described by other authors.[11,12] The addition of SFOw resulted in a significant reduction in the induction time of nucleation for all of the T_c assayed and for a constant supercooling. For example, at 30 °C, average induction times were 46.3, 12.7, and 1.8 min for AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw, respectively. The melting point (MP) of the AMF used in this study is 35.1 ± 1.2 °C (MP_{AMF}). No significant difference ($p > 0.05$) in the melting point of samples with and without waxes was observed, and therefore, the reported value is an average of the melting points obtained for all of the samples. This significant decrease in the induction time of nucleation for samples with added waxes suggests that these lipophilic molecules are capable of inducing the crystallization of AMF. The high melting point of waxes (78.5 ± 1.1 °C) is probably responsible for this behavior. The crystallization temperatures used in this study generate a very high supercooling for the waxes to crystallize (supercoolings in the order of 45 °C). These high supercoolings are responsible for the fast crystallization of waxes, which, in turn, act as nuclei for AMF crystallization. Wax solubility in AMF might also be responsible for the crystallization behavior of the system. As the temperature decreases, the solubility of SFOw in AMF decreases, and therefore, the crystallization kinetics is faster. The kinetics of crystallization of waxes in sunflower has been previously studied by Martini and Añón,[22] who reported activation free energies of nucleation, microstructure, and melting behavior of these compounds. These authors reported that the crystallization of waxes is a function of the crystallization temperature and, therefore, the supercooling, but it also depends upon the concentration of the waxes or supersaturation. Therefore, the reduction in the induction times of nucleation observed in this paper is a consequence of the supercooling in the sample and also the supersaturation, resulting from the wax concentration in the system. That is, for a constant T_c, the supercooling is constant, and the higher the wax concentration added to AMF, the shorter the induction time for nucleation (higher supersaturation in the system). On the other hand, for a constant wax concentration, a higher temperature will not only result in a lower supercooling but will also be translated into a lower supersaturation (because the concentration of waxes in equilibrium increases as a function of the temperature), all of which contribute to a longer induction time of nucleation.

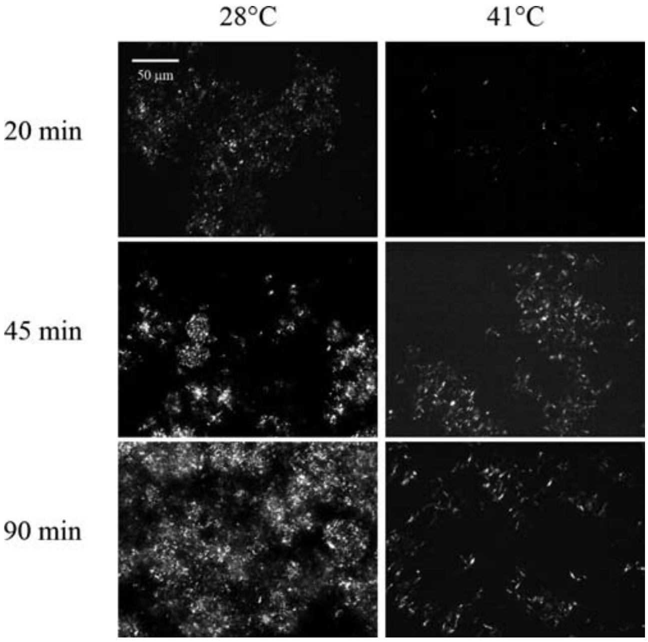

Figure 2. Microstructure of AMF + 0.25% SFOw at temperatures below (28 °C) and above (41 °C) the melting point of AMF (35.1 °C) at 20, 45, and 90 min. White bar = 50 μm.

It is interesting to note from Figure 1 that, in AMF + 0.1% SFOw and AMF + 0.25% SFOw samples, crystallization occurred even at temperatures where AMF alone did not crystallize within time constraints ($T_c > MP_{AMF}$). At these temperatures, only wax crystallization is observed after 90 min of crystallization with minimal or no AMF crystallization observed (Figure 2). Figure 2 shows the microstructure of crystals obtained at two specific temperatures (28 and 41 °C; below and above MP_{AMF}) for AMF + 0.25% SFOw. Similar results were obtained for the AMF + 0.1% SFOw samples. The presence of characteristic wax crystals at low T_c (28 °C) and short crystallization times (20 min) and the crystallization of AMF on top of wax crystals at longer crystallization periods (after 45 and 90 min) is evident in Figure 2. On the other hand, when samples were crystallized at higher temperatures ($T_c > MP_{AMF}$), only wax crystals are observed, with no AMF crystals formed even after 90 min into the crystallization process. This is an expected result because AMF is not expected to crystallize at $T_c > MP_{AMF}$. These observations suggest that the induction in the crystallization of AMF samples observed at temperatures below MP_{AMF} is driven by the presence of wax crystals that act as nuclei for the AMF to crystallize.

An induction in lipid crystallization usually results in changes in the physicochemical properties and functional properties of the lipid network formed. Some of these properties include the microstructure, texture, and melting profile of the crystals. In particular, the microstructure is a very important property responsible for providing specific texture characteristics of lipid-based products. Smaller crystals lead to a smoother and harder product, and crystals uniform in size are desirable for a product with optimum texture and mouthfeel. Figure 3 shows the microstructure of AMF alone and with the addition of 0.1 and 0.25% SFOw after 90 min of crystallization at 27, 29, and 30 °C.

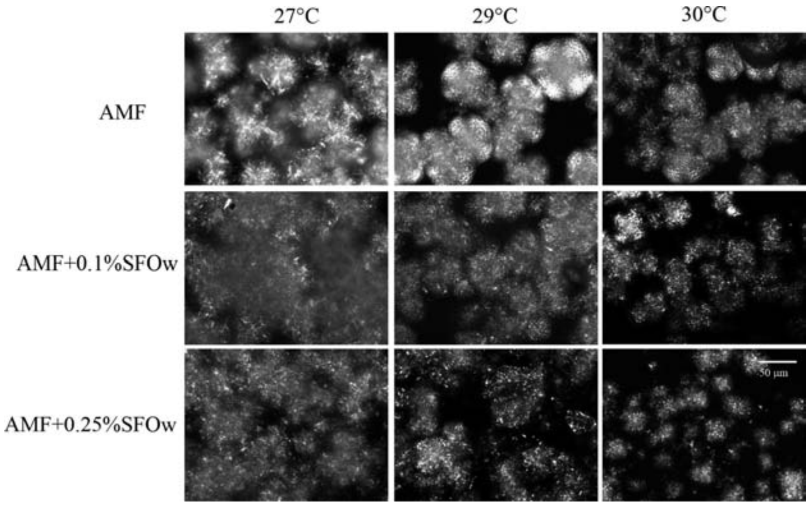

Figure 3. Microstructure of AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw after 90 min at 27, 29, and 30 °C. White bar = 50 μm.

As expected, fewer crystals were observed in each sample with increasing temperatures. Also, as the amount of SFOw increased, the size of crystals decreased. This effect is more evident for 30 °C. This shows a significant effect of wax addition on crystal microstructure. The smaller crystals obtained for a specific T_c as a consequence of wax addition supports the hypothesis that waxes act as heteronuclei during AMF crystallization and, therefore, accelerate the nucleation process, resulting in smaller and more crystals. The effect of processing conditions on the microstructure of lipid networks has been extensively studied. Research has shown that the cooling rate, agitation, and use of emulsifiers are some of the processing parameters that can be changed to tailor a specific microstructure in the lipid crystal network formed.[11,12,17,24,25] Novel technologies, such as high intensity ultrasound, have also been reported to change the microstructure of lipid networks.[26,27] Results reported in this paper provide evidence of an additional additive that can be used to modify the microstructure of lipid networks and, therefore, change their functional properties.

The melting behavior of the crystallized samples after 90 min into the crystallization process and after tempering at 5 °C for 48 h was measured using DSC, as described in the Materials and Methods. Figure 4 shows the melting profiles of AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw after 90 min at T_c (27, 29, and 30 °C) and after tempering for 48 h at 5 °C. This figure shows that, after holding the samples at T_c for 90 min (panels a, b, and c of Figure 4), a single melting peak is observed with peak temperatures of approximately 35.5 ± 1.6 °C. These melting profiles show that, at higher T_c, a slight fractionation in the crystal network is present, as evidenced by the small shoulder present at the left of the melting peak (see arrow), as the SFOw concentration increases. A similar behavior was reported by Martini et al.[10] As expected, after tempering the samples for 48 h at 5 °C (panels d, e, and f of Figure 4), two melting peaks are obtained. The first melting peak, with a peak temperature of approximately 16.2 ± 1.8 °C, is a consequence of secondary crystallization occurring during the tempering. The second melting peak, with a peak temperature of approximately 32.8 ± 2.5 °C, represents the melting behavior of crystals formed during 90 min at T_c and the crystal growth and/or recrystallization of the crystal network after tempering at 5 °C for 48 h. Similar to the discussion presented in panels a−c of Figure 4, melting profiles shown in panels d−f of Figure 4 suggest that the addition of waxes induce some fractionation after tempering, as evidenced by the presence of a small shoulder in the second melting peak. Considering that the second melting peak is a representation of the melting profile of the crystal network formed during crystallization at T_c and after tempering, the change in enthalpy (ΔH_m) obtained from this second melting peak is used to quantify the amount of crystalline material formed during the crystallization and during tempering (Figure 5). Figure 5a shows the change in enthalpy after 90 min at T_c, while Figure 5b shows the change in enthalpy after tempering the samples. As expected, a significant decrease ($p < 0.05$) in ΔH_m was observed after 90 min at T_c with increasing temperatures (Figure 5a). No significant differences were found between ΔH_m during the melting of the samples with and without SFOw addition. This indicates that, even though SFOw induced the crystallization of AMF, crystal growth was not promoted, as evidenced by the lack of change in ΔH_m. Interestingly, when the melting profile of the crystal networks was evaluated after tempering the samples at 5 °C for 48 h (Figure 5b), no significant differences ($p > 0.05$) were observed among ΔH_m from crystal networks obtained at different T_c. However, significant differences ($p < 0.05$) were observed among samples, with a significant increase in the enthalpy values with wax addition, especially at high temperatures and with the higher SFOw concentration (Figure 5b). Statistics shown in Figure 5b are to indicate significant differences among samples. These results suggest that the addition of waxes, especially at a concentration of 0.25%, not only induces the onset of crystallization, as evidenced by a shorter induction time (Figure 1), but can also promote the crystallization of AMF (crystal growth) during tempering, as demonstrated by a higher ΔH_m (Figure 5b), especially at higher crystallization temperatures (29 and 30 °C). It is also interesting to note that crystallization continues during the tempering period for higher temperatures (29 and 30 °C), as evidenced by the higher ΔH_m observed in Figure 5b. This was especially observed in the samples that had waxes added. This again suggests that waxes promote crystal growth in the long term (during tempering). Even though differences were found in the enthalpy values of the samples crystallized with and without the addition of the waxes, the SFC of the samples crystallized at 27, 29, and 30 °C was not significantly different (data not shown). This can be explained considering that the changes

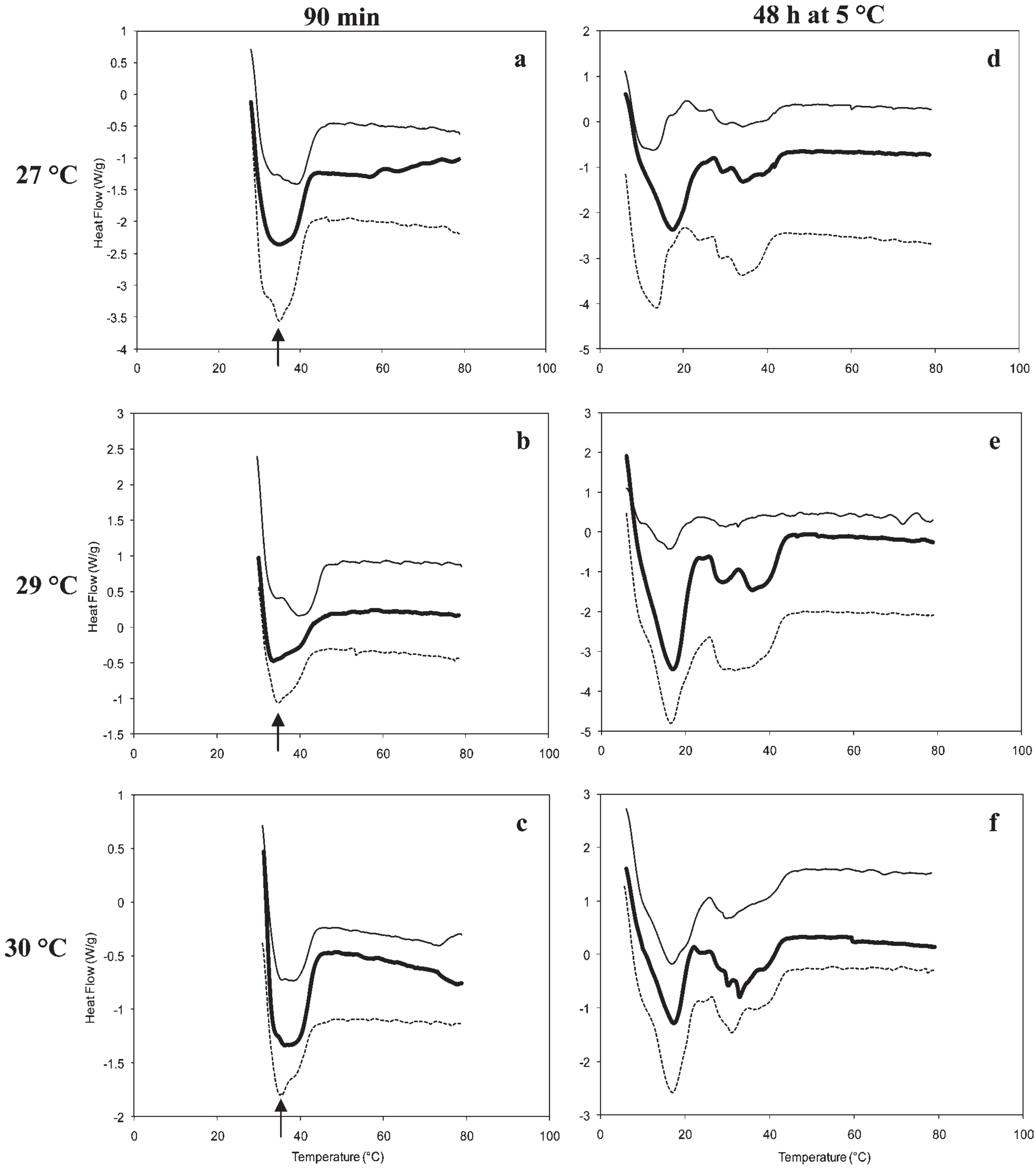

Figure 4. Melting profile of AMF (thin line), AMF + 0.1% SFOw (thick line), and AMF + 0.25% SFOw (dashed line) after crystallization for 90 min (a, b, and c) at T_c = 27, 29, and 30 °C, respectively, and after tempering for 48 h at 5 °C (d, e, and f).

observed in the enthalpy values does not significantly affect the proportion of total liquid and solid phases in the sample and are only evidenced after storing the samples at 5 °C for 48 h. It is important to note here that the differences observed in the ΔH_m as the differences reported in Figure 5 could also be due to the contribution of the enthalpy values of the waxes. Melting enthalpy values for pure waxes at the T_c used in this study are approximately 142.1 ± 12.0 J/g. Therefore, the contribution of the enthalpy of the waxes to the total enthalpy values in the AMF samples is in the order of 0.142 J/g of sample and 0.355 J/g of sample for AMF + 0.1% SFOw and AMF + 0.25% SFOw, respectively. These values are within the standard error of the enthalpy of the samples, and we therefore consider this effect insignificant.

Figure 6 shows the average hardness values for AMF and AMF with 0.1 and 0.25% wax for 27, 29, and 30 °C. Hardness values show a general decrease with increasing T_c for each type of sample (AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw); however, this decrease is not statistically significant. This is an expected result, because higher temperatures are associated with

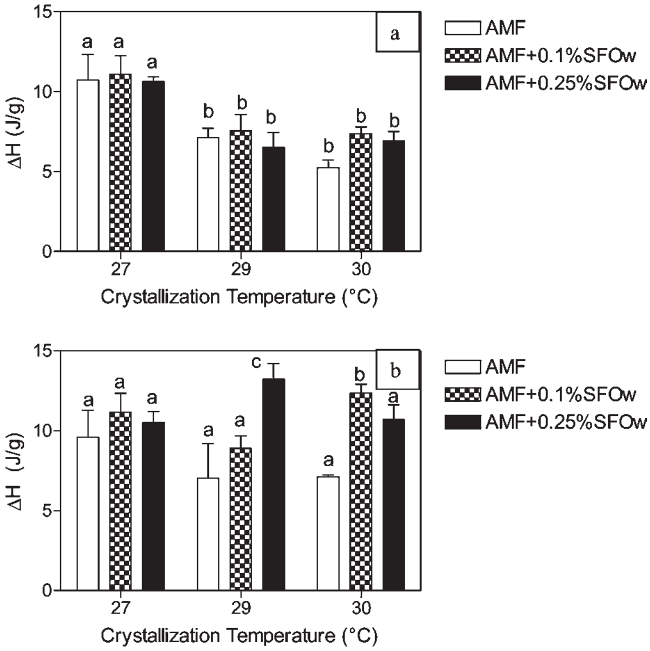

Figure 5. Average enthalpy values for AMF, AMF + 01% SFOw, and AMF + 0.25% SFOw: (a) melting enthalpy values after 90 min at T_c and (b) melting enthalpy values after tempering for 48 h at 5 °C. Error bars with the same letters in panel a are not significantly different ($p < 0.05$) among T_c. Error bars with the same letters in panel b are not significantly different ($p < 0.05$) among samples.

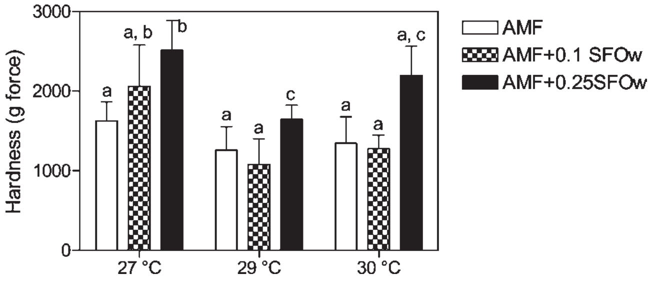

Figure 6. Average hardness values for AMF, AMF + 0.1% SFOw, and AMF + 0.25% SFOw at specific T_c. Error bars with the same letters are not significantly different ($p < 0.05$).

lower degrees of crystallization and, therefore, softer materials. Note that the range of temperatures tested (27−30 °C) is very small, and therefore, small differences in texture, as the ones reported in this research, are expected. It can be seen that the addition of SFOw significantly ($p < 0.05$) increased the hardness of the lipid crystal network at the different temperatures assayed, especially when 0.25% of waxes was used. The increase in hardness can be attributed to two different factors: (a) the smaller crystals generated by the presence of waxes (Figure 3) and (b) the induction on the crystal growth (more crystalline material generated) as a consequence of wax addition (Figure 5b). The effect of crystal size on the hardness of a lipid network was previously reported,[26,27] where harder materials were obtained as a consequence of the smaller crystals formed during ultrasound application. The effect of crystal size on the network structure can be explained considering that, at higher temperatures, bigger and fewer crystals are formed. Networks formed by bigger and fewer crystals have fewer intercluster bondings, which results in less structured networks and softer materials. Results reported in this paper show that, at higher crystallization temperatures (29 and 30 °C), both effects (crystal size and crystallized material) are responsible for the harder network formed, while at lower temperatures (27 °C), the crystal size will most likely be the only factor responsible for the changes in texture, because no changes were observed in the enthalpy values at these temperatures (Figure 5b).

In summary, this study demonstrates how the functional properties of a lipid system, such as AMF, can be influenced by processing conditions, such as crystallization temperature, and the use of different additives, such as SFOw. This study shows that the addition of waxes to AMF can modify the crystallization behavior of the system by inducing its nucleation, generating smaller crystals, and as a consequence, increasing the hardness of the crystal network formed. The degree of the changes observed is dependent upon the crystallization temperature used during processing and the amount of wax added. These results introduce an additional processing tool that can be used to structure semisolid fats and broaden their use as *trans*-fat replacements.

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