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2 **Effect of Waxes Addition on the Crystallization Behavior of Anhydrous Milk**

3 **Fat**

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22 Running title: Crystallization of AMF-wax blends

Abstract

Physicochemical characteristics of lipid-based foods depend, among other factors, on the microstructure and the characteristics of the lipid network formed during crystallization. The objective of this study was to evaluate the effect of sunflower-oil waxes' addition on the crystallization and melting behavior of anhydrous milk fat (AMF), a lipid with low content of palmitic and *trans*-fatty acids. The crystallization and melting behavior of AMF alone and with the addition of 0.25 and 0.5% of waxes was studied using a differential scanning calorimeter. The morphology of the crystallized samples was evaluated with a polarized light microscope. The addition of waxes induced and promoted the crystallization of AMF at high temperatures (> 25 °C) as evidenced by lower induction times of crystallization and higher crystallization and melting enthalpies. In addition, smaller crystals and different morphologies were obtained when AMF was crystallized with the addition of waxes. These results suggest that waxes could be used as an additive to modify lipid networks and their physicochemical characteristics, such as texture, smoothness and mouthfeel.

Key words: sunflower-oil waxes, crystallization, induction times, differential scanning calorimetry, polarized light microscopy, microstructure.

Introduction

Consumers' demand for *trans*-fat free products has increased over the years. Since January 2006, the United States requires *trans*-fat information to be included in nutrition labels. This requirement was a consequence of the association between *trans*-fatty acids, coronary heart disease (CHD), and the increase of undesirable LDL values in blood [1-3]. Due to the harmful effects of *trans*-fatty acids, healthy lipid alternatives are being sought.

From the functionality perspective, *trans*-fats are ideal lipids to be used in foods since they impart good physicochemical characteristics such as texture and hardness. Some highly saturated fats, such as palm oil (with more than 40% of palmitic fatty acids), meet similar physicochemical specifications as *trans*-fats. Although less severe, fats with high percentage of palmitic fatty acids have similar effect over CHD as *trans*-fats [4]. When using healthier fat alternatives, that is, lipids low in palmitic and *trans*-fatty acids, the desired physicochemical characteristics are hard to achieve. Some of the physicochemical characteristics expected in a lipid-based food product are texture, mouthfeel and hardness. These attributes depend, among other factors, on the microstructure and crystallization behavior of the lipid network. For example, small crystals are expected in margarine and butter to achieve a smooth texture. The desired functionality in a healthy lipid can be attained by using different processing conditions and/or additives that can modify the crystallization behavior of the lipid. The addition of lipophilic molecules to vegetable oils has gained popularity due to their ability to form gels when incorporated in an oil at low concentrations. Dr. Toro-Vazquez' group was able to induce the gelation of safflower oil by adding Candelilla waxes in concentrations above 1% [5]. These results were in the same line

as the ones obtained by Dr. Marangoni's group who studied the formation of organogels with ricinelaidic acid [6] and 12-hydroxysteric acids in vegetable oils [7, 8].

Anhydrous milk fat (AMF) has the potential to be used as *trans*-fatty acids replacement due to its low content in palmitic and *trans*-fatty acids. AMF is known to be high in stearic acid, which has been shown to have a neutral effect on CHD [2, 3]. Besides fat composition, AMF has good sensory attributes such as flavor and mouthfeel [9]. To explore the different functional properties of AMF and its possible use in different food products such as spreads, dressings and shortenings, several authors studied the effect of processing conditions and emulsifiers' addition on the crystallization behavior of AMF and AMF fractions [10-18].

Waxes are lipids consisting of esters of long chain fatty acids and long chain alcohols. Waxes can be found in nature in fruits and seeds. They are also a significant by-product during the refining process of several vegetable oils such as sunflower and rice oil [19-21]. In sunflower oil refining, waxes have low commercial value and are used for animal feeding. Finding new applications for waxes obtained from sunflower oil refining might increase their market value. Due to their lipophilic characteristics, waxes have the potential to alter the crystallization behavior of lipids, in this case, AMF.

The objective of this work is to evaluate the effect of sunflower waxes on the crystallization behavior of AMF. The effect of sunflower wax addition on the induction time of crystallization, the crystallization and melting profile, and the microstructure of AMF was investigated. Changes in the crystallization behavior of AMF due to the addition of waxes is the first step that needs to be investigated to evaluate the possibility of modifying the functional characteristics of AMF with the ultimate goal of using it as a shortening or in foods such as spreads and dressings.

Experimental Procedures

Starting Materials

AMF was a donation from KRAFT. Sunflower oil waxes (SFOw) were isolated and purified from tank settlings donated by ADM. For the isolation, approximately 60 g of the tank settlings were mixed with hexane. The mixture was heated to approximately 50 °C for 30 min. After this time, the solution was filtered while maintaining the system hot to eliminate insoluble particles. The hexane in the filtered solution was then evaporated using a Rotavapor system (Büchi 001). Waxes and oil were obtained after this process. This solution was kept at 4 °C for at least 48 h to allow waxes' precipitation and then centrifuged at 3,000g for 1 h at 4 °C. Waxes were obtained in the pellet. Waxes were purified by several washes with cold hexane. Purified waxes were mixed with AMF to obtain a final concentration of 0.25 and 0.5% (w/w) of waxes in AMF.

Chromatographic Standards

The following wax standards of almost 99% purity (Sigma Chemical Co., St. Louis, MO) were used for wax chromatographic analysis: C32 = lauric acid arachidyl ester ($C_{32}H_{64}O_2$), C36 = stearic acid stearyl ester ($C_{36}H_{72}O_2$), C38 = arachidic acid oleoyl ester ($C_{38}H_{74}O_2$), C40 = arachidic acid arachidyl ester ($C_{40}H_{80}O_2$), C42 = arachidic acid behenyl ester ($C_{42}H_{84}O_2$) and C44 = behenic acid behenyl ester ($C_{44}H_{88}O_2$). Standards of fatty-acid methyl esters and fatty alcohols with a purity of almost 99% over a wide range of carbon atom number (C14-C30), were acquired from Sigma (Sigma Chemical Co., St. Louis, MO).

Wax Chemical Characterization

The chemical composition of the waxes and of their constituents (fatty acids and alcohols) was determined using GC [22, 23]. Briefly, the experimental procedures were:.

GC of waxes: 2 mg of the material was directly dissolved in chromatographic grade n-heptane (3 mL), filtered through an organic-solvent 0.5- μ m filter and analyzed by capillary GLC [23]. A gas chromatograph Varian 3700 equipped with a FID and a temperature programmable on-column injector (Varian Associates Inc., Palo Alto, CA) was used for the final analysis. The capillary column was a HP5 (5% diphenyl and 95% dimethyl-polysiloxane), fused silica 11 m length x 0.32 mm i.d., 0.52 μ m film thickness (Hewlett-Packard, Palo Alto, CA). The operating conditions were: hydrogen at 3 mL/min and a pressure 8 psig as carrier gas; oven temperature programming: 80 °C-30 °C/min-200 °C (1 min) -3 °C/min-340 °C (20 min); on-column injector programmed from 80 °C to 320 °C at 40 °C/min and injection volume of 2 μ L; FID at 350 °C and attenuation 2×10^{-12} . A recorder-integrator Empower Pro (Waters, Milford, MA) was used for quantification. The analysis was performed by triplicate.

Fatty acids and fatty alcohols separation: The determination of the constituents of sunflower oil waxes required their saponification, extraction of both saponifiable and unsaponifiable matter, and separation of alcohols in the unsaponifiable matter by TLC , followed by the analysis of the fatty acids and separated alcohols by GC [22]. The separation technique was based in IOOC analytical method [24] with some modifications to secure a complete saponification and a good recovery of the analytes. Briefly, waxes were saponified with 4 mL of 2 N-KOH for 6 hs, followed by three washings with 4 mL of ethyl ether, and a subsequent washing of these joint

fractions with three-5mL portions of ethyl alcohol (50%). The hydro-alcoholic fraction contains the fatty acids while the ethyl fraction contains the fatty alcohols. Fatty acids were converted to methyl esters by acid-catalyzed esterification according to IUPAC standard method 2.301 [25]. Fatty alcohols were separated from other unsaponifiable matter by TLC as described in IOOC method [24].

GC of fatty acids: Fatty-acid composition was determined by gas chromatographic analysis according to IUPAC standard method 2.302 [25]. The fatty-acid methyl esters were separated on a SP-2380 (stabilized poly [90% biscyanopropyl / 10% cyanopropylphenylsiloxane]) fused silica capillary column, 30 m length x 0.25 mm i.d., 0.25 μ m film thickness (Supelco, Inc., Bellefonte, PA) maintained at a temperature of 170 °C for 15 min and then increased at 4 °C/min to 240 °C (held for 10 min), using hydrogen as the carrier gas.

GC of fatty alcohols: Standard solutions of alcohols and alcohol samples were turned into trimethylsilyl ethers and analyzed according to IOOC method [24]. Fatty-alcohols analysis was carried out on a SE-54 fused-silica capillary column (30 m x 0.25mm i.d.) of film thickness 0.25 μ m (Supelco, Inc., Bellefonte, PA), increasing the temperature at 7 °C/min from 170 °C to 300 °C (held for 15 min), using hydrogen as gas carrier.

Crystallization Assays

AMF, AMF with the addition of 0.25% (AMF + SFOw0.25) and 0.5% (AMF + SFOw0.5) of sunflower oil waxes were crystallized in a DSC pan and in a microscope slide.

Differential Scanning Calorimetry (DSC): Five to fifteen milligrams of sample was placed in an aluminum DSC pan and hermetically sealed. Samples were placed in the DSC chamber and heated to 80 °C and kept at this temperature for 30 min to allow the complete melting of AMF and waxes. After this step, samples were cooled at 3 °C/min to crystallization temperature (T_c = 23, 24, 25, 26, 27, 28 °C), kept at T_c for 90 min to allow isothermal crystallization of the lipid system and heated at 5 °C/min to 80 °C to evaluate the melting behavior. The cooling rate of 3 °C/min was chosen to mimic the maximum cooling rate achievable on the microscope stage (see below for details). Crystallization temperatures were chosen to include a broad range of temperatures at which AMF and AMF with SFOW will crystallize isothermally. That is, at temperatures below 23 °C, the samples crystallized before reaching crystallization temperature (non-isothermal crystallization). At temperatures above 28 °C, AMF samples did not crystallize over the 90 min that they were held at T_c due to the low supercooling. The onset of crystallization was calculated as the time elapsed between the moment the sample reached T_c and the moment when crystallization occurred. This onset was expressed in minutes and was referred as induction times of crystallization. The melting behavior of the samples was evaluated by the onset temperature (T_{on}), peak temperature (T_p) and enthalpy (ΔH).

Isolated and purified sunflower waxes were placed in a DSC pan as described for the AMF samples and heated from room temperature to 100 °C to evaluate their melting profile. Onset and peak temperatures, and the change in enthalpy associated with the melting of the waxes were recorded.

Polarized light microscopy (PLM): In addition to the DSC crystallization experiments, samples were crystallized in a slide to evaluate the crystals' morphology. Samples were heated to 80 °C to allow complete melting of the lipids. A drop of the melted sample was placed in a

thermostated slide and cover slide and placed in the temperature-controlled stage adapted to the PLM. Once the sample was placed in the cooling stage (set at 60 °C), it was cooled to T_c (24, 26 and 28 °C) and the crystallization of the sample was followed using the PLM. The cooling rate achieved under these conditions was 3 °C/min. PLM images were taken at different times. The average crystal size and area fraction covered by the crystals were determined using Image J software (1.38x, NIH, USA <http://rsb.info.nih.gov/ij/Java> 1.5.0_07).

Statistical analysis: Values reported for the DSC and PLM measurements are mean values and standard deviations of duplicate experiments. Significant differences were analyzed using a two-way ANOVA and a Bonferroni post-test ($\alpha = 0.05$). Statistical analysis was performed using Graph Pad software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results and Discussion

Table 1 shows the chemical composition of the sunflower oil waxes in terms of their carbon number, fatty acid and alcohol content. The wax material consisted of C40-C56 waxes with higher percentages of C46, C48, C44 followed by C50 and C52. Bäumler et al. [23] found a similar wax profile analyzing the waxes present in the hull of sunflower seed that were obtained by sunflower-seed hexane washing. This is in accordance with the fact that the hull contribution to the wax content in sunflower oil reaches around 80% when only the crystallized fraction is considered [22]. Fatty acids were in the range of 14-30 carbon atoms with C18:1 (34.6%), C20:0 (11.64%) and C16:0 (9.39%) being most prevalent. Fatty alcohol distribution was in the range of 18-34 carbon atoms. The main alcohols found in the waxes were C24 (27.6%), C26 (26.9%) and

C28 (14.4%). Small quantities of odd-carbon compounds can explain the presence of odd-carbon waxes. These findings are in accordance to previous results from other authors [26-28].

In addition, the purified waxes were characterized by DSC giving the following melting profile:

T_{on} : 73.4 ± 2.3 °C, T_p : 76.6 ± 1.0 °C and ΔH : 160.1 ± 21.5 J/g.

Figure 1 shows the mean values and standard deviation for the induction times of crystallization for AMF, AMF + SFOw0.25 and AMF + SFOw0.5 when crystallized in the DSC pan at $T_c = 23, 24, 25, 26, 26.5, 27, 27.5$, and 28 °C. As expected, an exponential relationship between the induction time of crystallization and the crystallization temperature (T_c) was observed for all samples. This means that as the T_c increased the induction time of crystallization increased, and more time was needed for the sample to crystallize due to the lower supercooling. For a constant T_c , the induction time of crystallization decreased as the amount of added wax increased. That is, the addition of waxes induced the crystallization of AMF. This difference was more evident at higher T_c ($T_c \geq 27$ °C) than at lower T_c ($T_c < 27$ °C). For example, at 27.5 and 28.0 °C neither AMF nor AMF + SFOw0.25 crystallized during the 90 min at T_c . However, AMF + SFOw0.5 did crystallize at 27.5 and 28 °C with induction times of approximately 53 and 66 min, respectively. This indicates that the addition of 0.5% of SFOw induced the crystallization of AMF by at least 40 min.

Figure 2 shows the crystallization and melting enthalpies together with the melting onset (T_{on}) and melting peak temperature (T_p) for all the samples crystallized at different T_c . The crystallization enthalpy decreases as a function of T_c (Figure 2a). The higher the T_c (lower supercooling), the lower the crystallization enthalpy; indicating that fewer lipids are being crystallized. For T_c below 27 °C no significant differences ($p < 0.05$) were found between the crystallization enthalpies of the different samples. For $T_c = 27$ °C a significant difference ($p <$

0.05) was observed between crystallization enthalpies with a marked increase in the enthalpy value in samples with higher wax concentration. No crystallization was observed for AMF and AMF + SFOw0.25% at 27.5 and 28 °C. The lack of differences at low temperatures might be due to the high supercooling experienced by the sample. Such high supercoolings result in a fast crystallization of AMF even if no waxes are added. At high temperatures, that is lower supercooling, the crystallization of AMF is delayed but the presence of high melting point molecules in the waxes (Table 1) induces the crystallization in AMF + SFOw0.25% and AMF + SFOw0.5% samples.

After 90 min at T_c , melting enthalpies were significantly different between samples (Figure 2b). Two different behaviors were observed. When samples were crystallized at temperatures below 25 °C, the melting enthalpy of AMF + SFOw0.25 was significantly lower than the one observed for AMF and AMF + SFOw0.5 ($p < 0.05$) suggesting that when 0.25% of waxes were added to AMF, even though the crystallization was induced (Figure 1), a delay in the crystal growth was observed. This delay was probably due to the co-crystallization or adsorption of wax crystals on the surface of AMF. This crystallization mechanism was described by Garti [29] to explain the effect of emulsifiers on the crystallization behavior of fats. No significant differences were found at T_c between 25 and 26.5 °C among the melting enthalpies of the samples, while at temperatures above 26.5 °C enthalpies values of AMF + SFOw (0.25 and 0.5%) were significantly different from the ones obtained for AMF alone. The more waxes added to AMF, the higher the melting enthalpy. This suggests that for higher crystallization temperatures (lower supercoolings) the addition of sunflower waxes to AMF not only induced the crystallization (Figure 1) but also promoted the crystal growth (higher enthalpy values).

247 The melting T_{on} increased as a function of T_c (Figure 2c): the higher the T_c , the higher the T_{on} . In
248 general, no significant differences were found between the samples' T_{on} at a constant T_c , with the
249 exception of samples crystallized at $T_c \geq 27.5$ °C where a significant increase in the T_{on} value
250 was observed for AMF crystallized without the addition of waxes. For samples crystallized with
251 the addition of waxes, the same crystallization behavior observed at lower temperatures is
252 extended to temperatures above 27.5 °C. These results suggest that while only high melting point
253 triacylglycerides crystallize at high temperatures in AMF samples, the addition of waxes induces
254 the crystallization of lower melting point triacylglycerides resulting in lower T_{on} values.
255 Figure 2d shows a continuous increase in the melting T_p as a function of T_c for AMF crystallized
256 without the addition of waxes. T_p values for AMF crystallized with the addition of waxes
257 resulted in significantly lower values in samples crystallized at T_c above 23 °C ($p < 0.05$). At this
258 temperature, the lowest T_p was observed for AMF + SFOw0.25. At $T_c = 25$ °C the addition of
259 0.25 and 0.5% of waxes to AMF resulted in significantly lower T_p when compared to AMF alone
260 ($p < 0.001$). The lowest melting T_p was observed for AMF + SFOw0.25, while AMF + SFOw0.5
261 resulted in an intermediate melting T_p value. At $T_c > 25$ °C the addition of waxes resulted in
262 significantly lower T_p values ($p < 0.001$) but these values were not significantly different
263 between them. This behavior will be explained with Figure 3 description. It is interesting to note
264 here that even though no crystallization was detected by the DSC at 27.5 and 28 °C for AMF and
265 AMF + SFOw0.25% during 90 min at T_c (Figure 2a), some crystals were detected during melting
266 for AMF crystallized at 27.5 °C but not at 28 °C; while for AMF + SFOw0.25% crystals were
267 detected during melting when crystallized at both 27.5 °C and 28 °C (Figures 2 b-d).
268 As an example of the melting behavior of the samples used in this study, Figure 3 shows the
269 DSC melting profiles of samples crystallized at 23, 25 and 27 °C. This Figure explains the results

discussed in Figure 2d. It can be seen that for $T_c = 23\text{ }^{\circ}\text{C}$ the melting profiles of the three samples (AMF without and with the addition of sunflower oil waxes) are not significantly different ($p < 0.05$) (Figure 3a). A pronounced peak is observed at around $33\text{ }^{\circ}\text{C}$ with a small shoulder (see arrow in Figure 3) at lower temperatures ($\sim 27\text{ }^{\circ}\text{C}$). When samples were crystallized at $25\text{ }^{\circ}\text{C}$ (Figure 3b) this shoulder moves to higher temperatures and becomes more important especially in AMF samples crystallized with the addition of waxes. In addition, the use of 0.25% of waxes seems to have a greater effect on the development of this shoulder at $25\text{ }^{\circ}\text{C}$. This behavior results in the significant decrease in the T_p described in Figure 2d since in these samples, the shoulder becomes the predominant peak. When samples were crystallized at $T_c > 25\text{ }^{\circ}\text{C}$ (Figure 3c) the low temperature shoulder becomes even more important with no significant differences between the amounts of waxes added. This singular melting behavior as a function of T_c might be due to different polymorphic forms or molecular re-organizations that could be originated in AMF as a consequence of wax addition.

Figure 4 shows the morphology of crystals when AMF was crystallized at $26\text{ }^{\circ}\text{C}$ without and with the addition of sunflower waxes. An increase in the number and size of AMF crystals as a function of time can be observed. The area fraction covered by the crystals increased from 0.6 to 12.6% for AMF crystallized at $26\text{ }^{\circ}\text{C}$ for 35 and 55 min, respectively. AMF crystallized forming spherulites with needle-like crystals organized radially outwards from the center. When AMF was crystallized with the addition of 0.25% of waxes, the crystals obtained were smaller; however the morphology was still the same. In addition, as discussed previously (Figure 1) an induction of crystallization was observed. The size and shape of the crystals did not change as a function of crystallization time, however a slight increase in the crystals' size can be observed when comparing the micrographs at 35 and 55 min showing an area fraction of 13.2 and 15.3%,

respectively. When AMF was crystallized with the addition of 0.5% of waxes, the crystals were smaller than the ones observed for AMF + SFOw0.25 (10.5 vs. 18.2 μm , respectively) due to the presence of more nucleation sites as a consequence of waxes' addition.

Figure 5 shows the morphology of crystals obtained when AMF was crystallized for 60 min without and with the addition of waxes at different crystallization temperatures ($T_c = 24, 26$ and $28\text{ }^\circ\text{C}$). As described before, when AMF was crystallized without the addition of waxes, needle-like crystals were obtained which were arranged in a spherulites-like manner. For lower T_c ($24\text{ }^\circ\text{C}$) both small and large spherulites were observed. For AMF crystallized with the addition of 0.25% of waxes, significantly smaller crystals are obtained. In addition, when this sample was crystallized at $24\text{ }^\circ\text{C}$ two evident crystal populations were observed representing different spherulites sizes. At intermediate ($26\text{ }^\circ\text{C}$) and high temperatures ($28\text{ }^\circ\text{C}$) the crystal distribution was more uniform and crystals were smaller. When AMF was crystallized with the addition of 0.5% of waxes, the crystals formed were even smaller, especially at higher temperatures (26 and $28\text{ }^\circ\text{C}$). In addition, at $28\text{ }^\circ\text{C}$ two different crystal populations were again observed with prevalence of smaller crystals. The presence of two different populations of crystals might be due to the differences in chemical composition between the SFOw and the AMF. The small crystals might be formed predominantly by wax molecules (high molecular weight molecules with high melting points), while the big spherulites could be constituted by triacylglycerols present in the AMF which at high temperatures crystallize very slowly forming big agglomerates.

In summary, sunflower oil waxes can be used to modify the crystallization behavior of AMF. This research showed that the addition of sunflower oil waxes can decrease the induction time of crystallization, promoting crystallization, especially at high crystallization temperatures.

Moreover, waxes' addition significantly affected the microstructure of the AMF. The higher the amount of waxes added, the smaller the crystals formed. Due to the effect of waxes on AMF crystallization behavior and microstructure, these findings show that the addition of waxes to AMF might have significant implications on the texture, polymorphism and mouthfeel of the lipid system.

The induction of crystallization provoked by sunflower waxes suggests that these molecules could be used to structure lipids and modify their functional properties for food applications. An induction in the crystallization usually results in harder materials with higher solid fat contents. In addition, microstructural differences must be taken into account when evaluating the functional properties of lipids. The possibility of different polymorphic forms must not be neglected either. More research is necessary to evaluate the effect of sunflower waxes on the functional properties of AMF such as solid fat content, texture and sensory profile.

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413 **Figures Legends**

414 **Figure 1:** Induction times of crystallization for AMF (■), AMF + SFOW0.25 (○) and AMF +
415 SFOW0.5 (▽) when crystallized in the DSC.

416 **Figure 2:** Crystallization (a) and melting enthalpy (b); melting onset (T_{on}) and melting peak (T_p)
417 temperature (c and d, respectively) for samples crystallized at different T_c . AMF (■), AMF +
418 SFOW0.25 (○) and AMF + SFOW0.5 (▽)

419 **Figure 3:** DSC melting profiles of AMF crystallized without and with the addition of waxes. a)
420 $T_c = 23\text{ }^{\circ}\text{C}$, b) $T_c = 25\text{ }^{\circ}\text{C}$, c) $T_c = 27\text{ }^{\circ}\text{C}$

421 **Figure 4:** Morphology of crystals obtained when AMF is crystallized at $26\text{ }^{\circ}\text{C}$ without and with
422 the addition of waxes: Variation as a function of time

423 **Figure 5:** Morphology of crystals obtained after crystallizing AMF without and with the addition
424 of sunflower waxes for 60 min at 24, 26 and $28\text{ }^{\circ}\text{C}$.

Table 1: Chemical composition of sunflower oil waxes in terms of their carbon number, fatty acid and alcohol content

Wax ^a	(wt %)	Fatty Acid ^b	(wt %)	Fatty Alcohol ^b	(wt %)
C40	Tr.	C14:0	1.19	C18	1.79
C41	Tr.	C16:0	9.39	C20	1.38
C42	1.92	C16:1	0.82	C21	0.22
C43	0.45	C17:0	1.10	C22	8.99
C44	17.89	C18:0	6.65	C23	0.87
C45	1.80	C18:1	34.63	C24	27.60
C46	25.89	C18:2	4.51	C25	2.32
C47	1.61	C20:0	11.64	C26	26.90
C48	18.81	C20:1	0.53	C27	0.88
C49	1.16	C20:2	6.39	C28	14.45
C50	12.69	C22:0	6.19	C29	0.77
C51	1.86	C22:1	1.21	C30	7.67
C52	8.35	C23:0	1.03	C32	5.40
C53	0.68	C24:0	1.68	C34	0.76
C54	4.26	C24:1	0.39		
C55	0.33	C25:0	0.81		
C56	2.30	C26:0	0.74		
		C27:0	1.07		
		C28:0	2.64		
		C29:0	3.84		
		C30:0	3.45		

^aAverage values of three determinations (n=3), ^bAverage values of two determinations (n=2),

tr = traces (< 0.1%)

Figure 1: Induction times of crystallization for AMF (■), AMF + SFOw0.25 (○) and AMF + SFOw0.5 (▽) when crystallized in the DSC

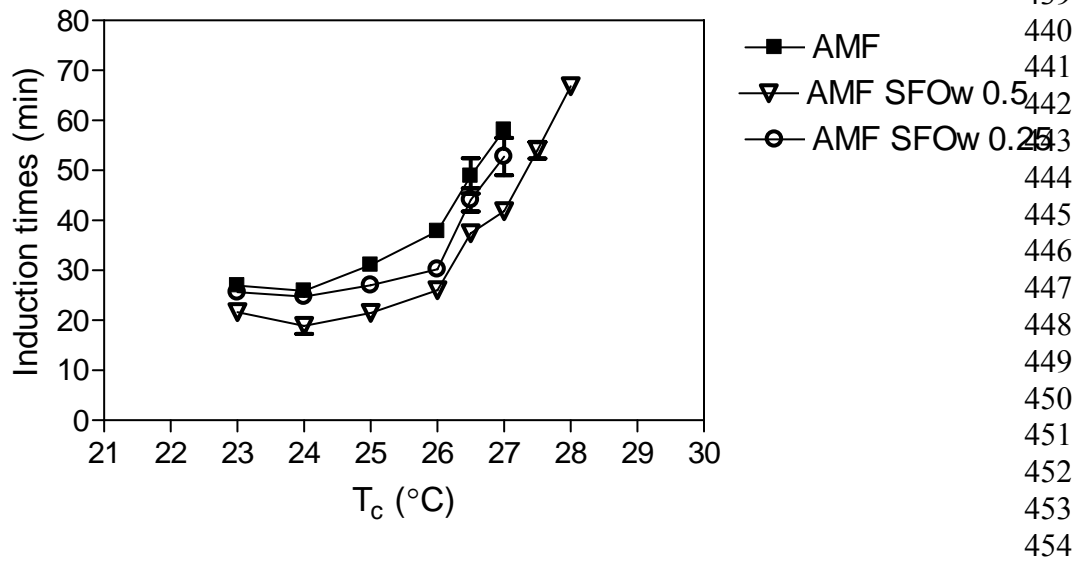


Figure 2: Crystallization and melting enthalpy; melting onset (T_{on}) and melting peak (T_p) temperature for samples crystallized at different T_c . AMF (■), AMF + SFOw0.25 (○) and AMF + SFOw0.5 (▽)

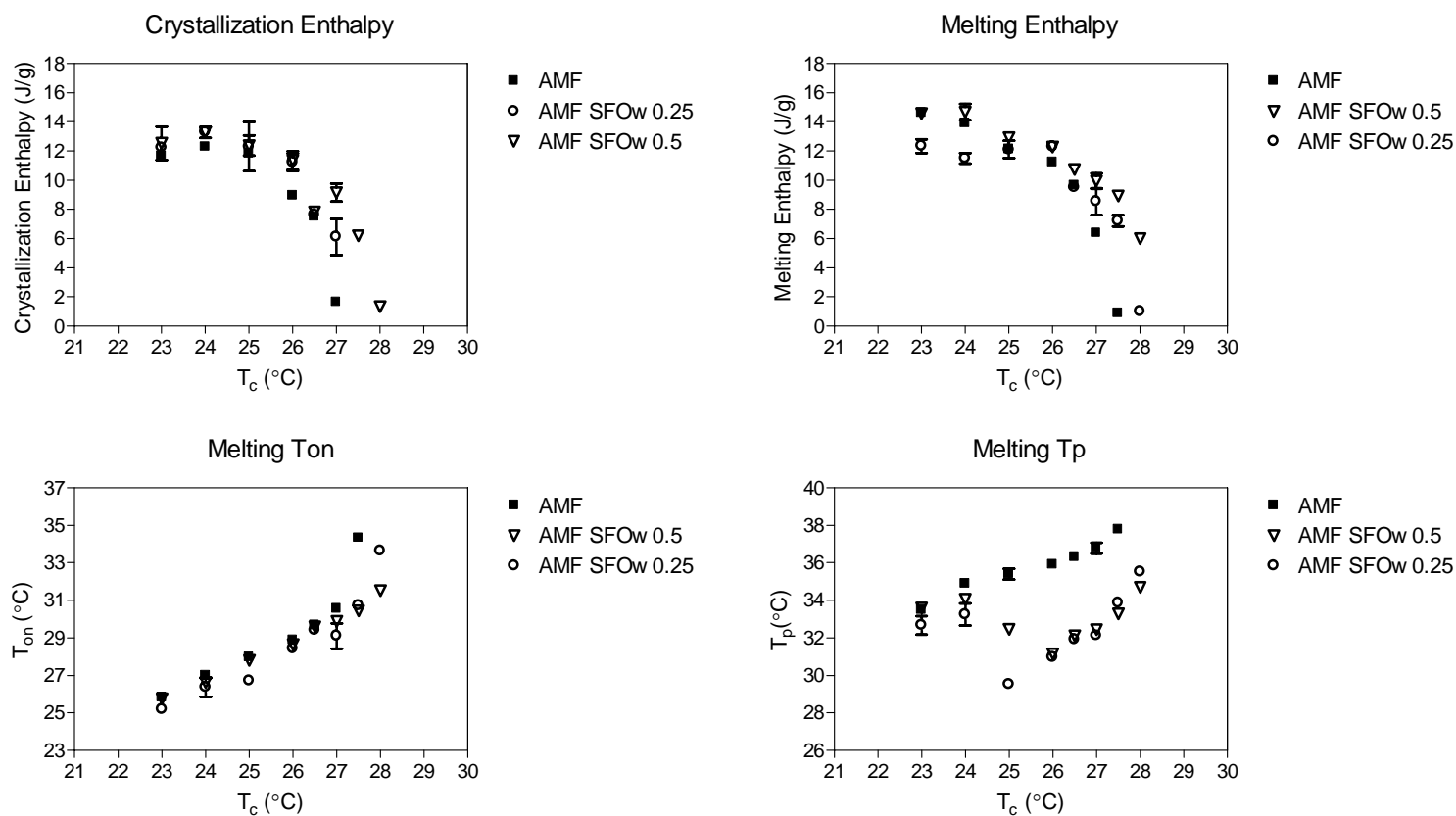
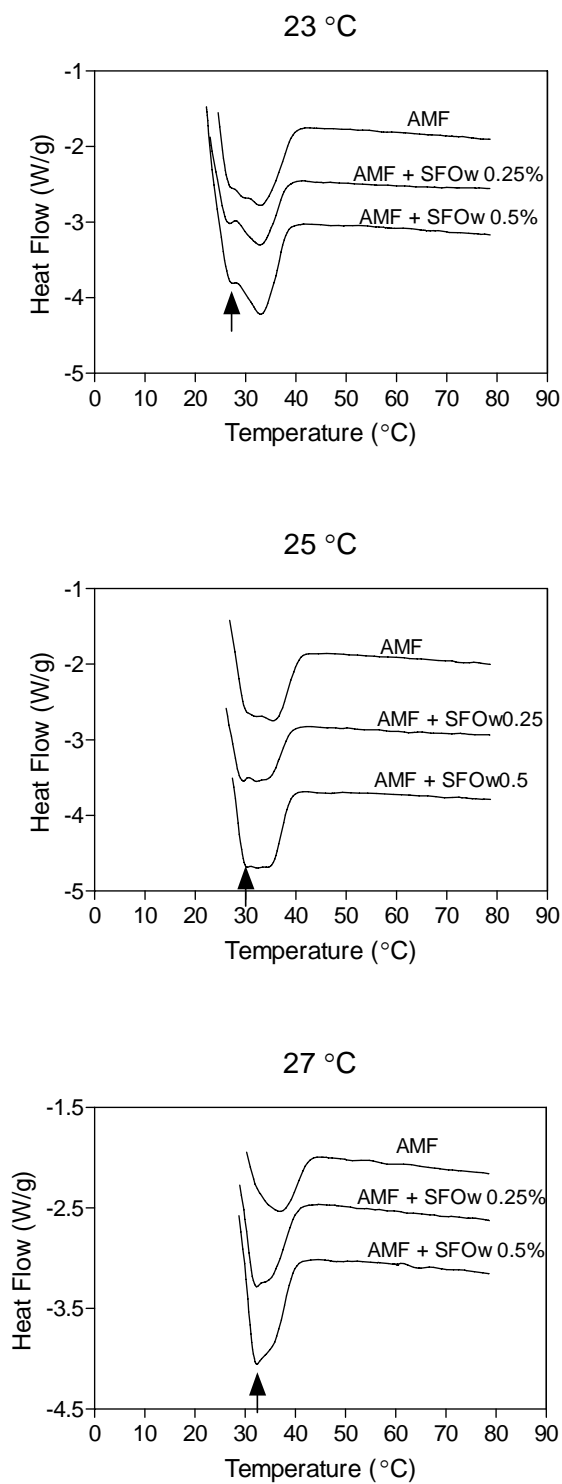


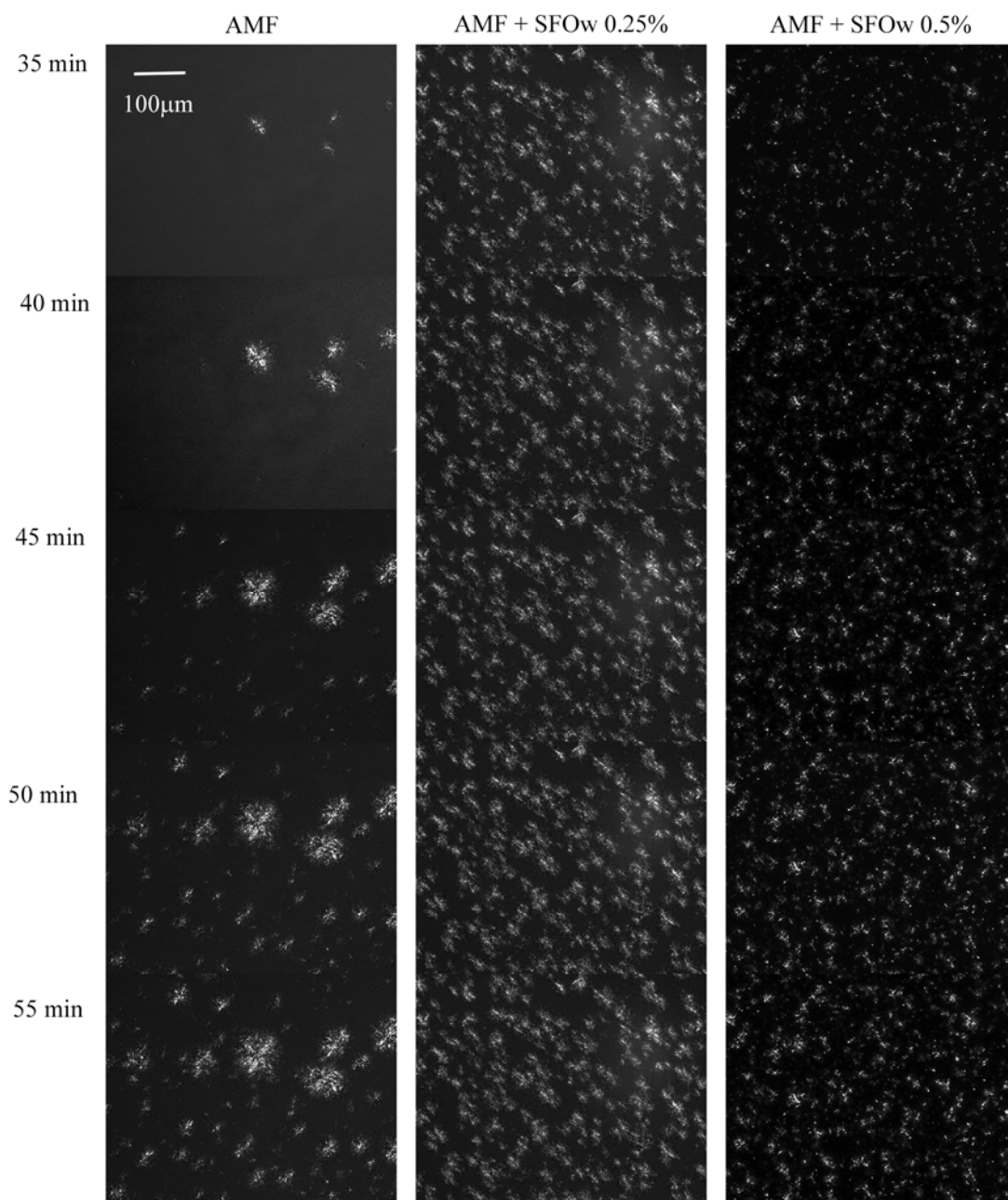
Figure 3: DSC melting profiles of AMF crystallized without and with the addition of waxes. A) $T_c = 23\text{ }^{\circ}\text{C}$, B) $T_c = 25\text{ }^{\circ}\text{C}$, C) $27.5\text{ }^{\circ}\text{C}$



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494 **Figure 4:** Morphology of crystals obtained when AMF is crystallized at 26 °C without and with
495 the addition of waxes: Variation as a function of time.

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518 **Figure 5:** Morphology of crystals obtained after crystallizing AMF without and with the addition
519 of sunflower waxes for 60min at 24, 26 and 28 °C.
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