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ORIGINAL PAPER

The Effect of Phospholipids on Butter Physical and Sensory Properties

Yuliya Fedotova · Robert W. Lencki

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Abstract The addition of phospholipids (PL), either in the form of the milk fat globule membrane (MFGM) or soy lecithin, had a significant influence on butterfat crystal morphology. At low concentrations, PL addition increased spherulite size, but as PL levels reached 2 wt%, spherulite formation was inhibited and the microstructure consisted of well dispersed individual crystals. This change in crystal structure made the butter harder, less grainy at low temperatures, and less prone to oiling-off above room temperature. PL addition was also shown to affect the relative concentrations of the various species created during crystallization. Of the two 2L β' species that form at crystallization onset, the presence of PL shifted the balance towards the first species at the expense of the second. PL also facilitated the polymorphic transition of the 3L α species to a 2L β' structure. It is unclear, however, whether the inhibition of spherulite formation was due directly to the inhibition of secondary nucleation by PL at the crystal surface, or indirectly by the reduction of supercooling resulting from a more rapid polymorphic transition. Evidently, PL concentration must be properly controlled during manufacturing in order to optimize butter functional properties.

Keywords Anhydrous milk fat · Butter · Phospholipids · Functional properties

Introduction

Over the years, several approaches have been used for continuous butter production [12]. The 'New Way' or 'Alfa-Laval' processes as well as technology developed in Russia [13] involved concentrating cream to 80% fat via centrifugation followed by phase inversion. With others such as the 'Cherry-Burrell' or 'Creamery Package' methods, the cream was first partially oiled-off before being centrifuged to remove the desired amount of buttermilk. With the former processes, almost all of the phospholipids present in the cream are retained in the butter, whereas with the latter two, most of the phospholipids enter into the buttermilk phase. None of the above-mentioned processes achieved widespread commercial success, with large-scale production dominated by the continuous churning process. This method is an adaptation of the old batch method; the cream is first intensely beaten to create butter grains and then the buttermilk is expelled by slow churning followed by working with an Archimedean screw. With this method, known as the Fritz process, significant amounts of phospholipid (PL) are found in both the butter and buttermilk streams [19].

It is well known that the physical properties of butter are highly dependent on production method. For example, butter produced from phase inversion of highly concentrated cream is excessively hard with poor water dispersion [13]. On the other hand, removing buttermilk via centrifugation creates a butter that is grainy and has an oily appearance [13]. The success of the Fritz process can mainly be attributed to the superior appearance, functionality and sensory properties of the resulting butter.

Since the butter produced in each of these processes has different PL levels, we hypothesized that the butter quality differences observed between the various production methods was related to some extent to PL concentration. In a previous publication, we showed that PL concentration has a significant effect on butterfat crystal

Y. Fedotova · R. W. Lencki (✉)
Department of Food Science, University of Guelph,
Guelph, ON N1G 2W1, Canada
e-mail: rlencki@uoguelph.ca

structure [6]. At low PL concentrations, fat crystals formed large spherulitic structures, whereas at concentrations above approximately 1.5% PL, a more random network of evenly dispersed individual crystals was observed. The purpose of this work is to examine how the previously observed PL-induced changes in butter microstructure specifically affect the physical and sensory properties of butter.

Materials and Methods

Materials

Clarified butter (anhydrous milk fat) was purchased from Gay Lea Foods, Guelph, ON. Commercially made skim milk (0.1% milk fat) and buttermilk (1% milk fat) were purchased from a local grocery store. Raw milk was obtained from the University of Guelph Ponsonby Dairy Farm, Elora, ON. Laboratory grade soy lecithin containing 25% PC, 15% PI, 20% PE and 10% PA was purchased from Fisher Scientific (Nepean, ON, Canada).

Regular 35% cream was prepared by centrifuging raw milk at 1,500×g in a model IEC HN-SII centrifuge (Thermo Scientific, Waltham, MA, USA). The resulting cream was further concentrated at 40,000×g in an Optima LE-8K ultracentrifuge (Beckman Coulter, Fullerton, CA, USA) to produce a high fat content plastic cream containing approximately 80% milk fat (MF).

Preparation of Batch Churned Butter

A laboratory-scale churn with an approximate capacity of 1.0 L was used for conventional batch butter making. Sweet cream was first tempered at 8 °C for 1 day before churning. The tempered cream was churned until phase inversion occurred, which usually took less than a minute. The butter was then separated from the buttermilk and the granules were worked again with a spatula for an additional 1 min to release excess moisture and press the granules into a continuous mass.

Preparation of Concentrated Cream/AMF/Skim Milk Mixtures

AMF was first heated to 80 °C to destroy any previous crystal history, and was subsequently cooled to 23 °C. Ultracentrifuged cream and skim milk (both at 23 °C) were then combined with the AMF in various combinations in order to alter the globular-to-free milkfat ratio while maintaining the total lipid concentration at 80 wt%. The mixtures were then blended with a spatula until a homogeneous product was obtained.

Preparation of Lecithin/AMF/Skim Milk Mixtures

AMF was first heated to 80 °C to destroy any previous crystal history. After cooling to 60 °C, lecithin was added and the solution was stirred until the granules had completely dissolved. Mixtures were then cooled to 23 °C and 20 wt% of either 23 °C skim milk or buttermilk was added by continuous mixing with a spatula until a homogeneous product was obtained.

Fat Mixture Tempering

Butter mixtures were first placed in 2-in. diameter aluminum dishes, and then put inside a MIR-153 programmable incubator (Sanyo, Tokyo, JP) where they were exposed to various time–temperature treatments.

Polarized Light Microscopy (PLM) Analysis

An Olympus BX 60F5 (Olympus Optical, Tokyo, Japan) light microscope fitted with a Sensys HRD 060-NIK 0.60x camera (Photometrics, Tucson, AZ, USA) was employed. Image-Pro® Plus, version 4.5.1.29 (Media Cybernetics, Bethesda, MD, USA) was used for PLM image analysis.

Solid Fat Content (SFC) Determination

A Minispec mq20 pulse NMR analyzer equipped with Biospin software (Bruker Optics, The Woodlands, TX, USA) was used for the determination of SFC. Each data point represents the average and standard error of measurements obtained on three sample replicates.

Phospholipid Analysis

The phosphorous content of AMF was determined using a spectrophotometric measurement that involves the formation of a blue phosphomolybdic acid complex as outlined in AOCS Official Method Ca 12-55 [2]. The resulting phosphorus concentration was then multiplied by 30 to obtain the equivalent wt% phosphatide. Phosphorous analysis was performed on both the liquid and solid fractions of crystallized butterfat after the two phases were separated via vacuum filtration using Whatman No. 1 paper filter (Whatman, Florham Park, NJ, USA). Each analysis was performed in triplicate.

Differential Scanning Calorimetry (DSC)

Endothermic and exothermic heat flows were measured at a scan rate of 5 °C min^{-1} with a DuPont 2910 differential scanning calorimeter equipped with TA Universal Analysis software (TA Instruments, New Castle, DE, USA). All

crystallization and melting curves represent the average of three replicates.

Cone Penetrometry

The hardness of various butter samples was ascertained according to the AOCS Official Method Cc16-60 1989a using a Precision Penetrometer (Precision Scientific Co., Chicago, IL, USA) with an aluminum cone (#10, 25°) [1]. Each data point corresponds to the average and standard error of at least three replicates.

Bench-Top Tasting of Butter Samples

Samples were evaluated under normal daylight conditions in our laboratory at the University of Guelph. A group of untrained panelists consisting of four people were asked to descriptively evaluate the samples. Particular attributes such as oiliness, smoothness and graininess were taken into account during the taste tests. Regular butter, churned from 40% MF cream, was used as a reference and was stored for 3 h under the same temperature conditions as the other corresponding samples before each test. Samples stored at 4 °C and 23 °C were taken out 1–2 min before the evaluation, whereas those stored at 30 °C or 32 °C were tested right inside the incubator.

Results

Figure 1a shows a PLM image of butter (80% non-aqueous phase) crystallized at 18 °C with AMF as the sole source of fat. As previously observed by ourselves [6] and others [5], 1959), large spherulitic crystals were evident. However, as observed in our previous publication [6], when the non-aqueous phase was a combination of 60% globular fat (GF) from high-fat cream and 40% AMF, a much finer and more evenly distributed crystal structure was observed (Fig. 1b). Similar changes in crystal structure were obtained by simply adding 1.5% soy lecithin to the AMF (Fig. 1c); thus, it would appear that it is the phospholipids present in the high-fat cream that causes this change in fat morphology. Previous results have also shown that, as the percentage of GF in the non-aqueous phase of butter increases above 80%, the aqueous phase is no longer finely distributed, but begins to separate into micron-sized droplets [6].

At room temperature, the macroscopic appearance of the AMF butter and the 60% GF/40% AMF butter blend were also distinctly different (Fig. 2). Whereas the AMF butter looked very oily, the addition of GF produced a spread with better oil holding capacity and a less shiny appearance. Differences in appearance were less evident at fridge temperature, but qualitative examination indicated that the

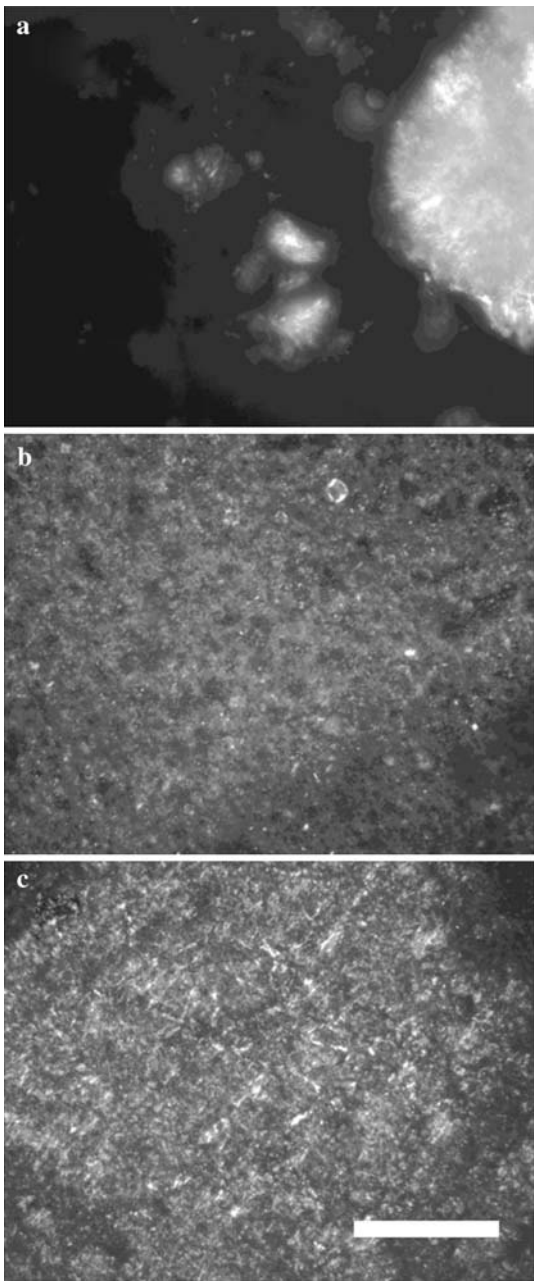

Fig. 1 Polarized light micrographs of butter with the non-aqueous phase consisting of : (**a**) 100% AMF; (**b**) 40% AMF + 60% GF; (**c**) 100% AMF + 2.0% soy lecithin

two butters had different consistencies: the 60% GF butter was more brittle and tended to fracture whereas the AMF butter was slightly more spreadable. Butters made with >80% GF had a creamy appearance and were slightly softer than the 60% GF butter.

These qualitative observations were confirmed using cone penetrometry (Fig. 3). When various butter blends were crystallized at 18 °C for 1 day, a large softness variation was observed. The 100% AMF butter was very soft, but the addition of 30% GF significantly increased

Fig. 2 Photographs of butter with the non-aqueous phase consisting of: (**a**) 100% AMF; (**b**) 40% AMF + 60% GF

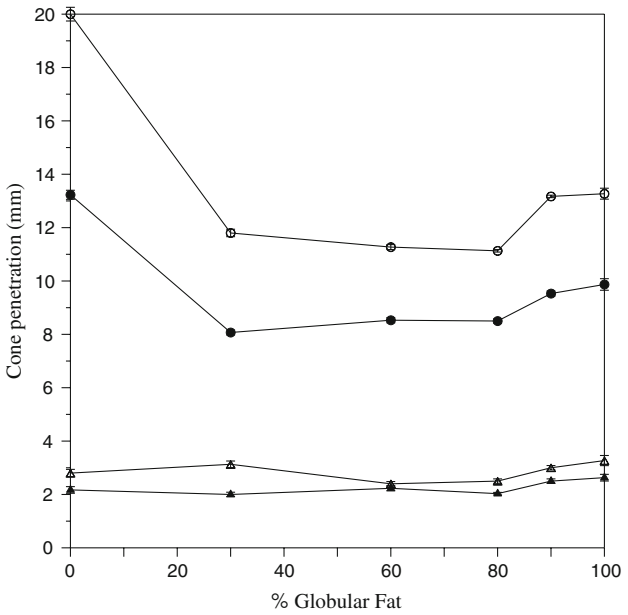

Fig. 3 Cone penetrometry measurements of butter with the non-aqueous phase consisting of blends of GF and AMF: *open circle*—crystallized at 18 °C for 1 day; *filled circle*—crystallized at 18 °C for 7 day; *open triangle* crystallized at 4 °C for 1 day; *filled triangle*—crystallized at 18 °C for 7 day

Table 1 Cone penetrometry results for AMF butter with and without the addition of lecithin

Name of the sample	Cone penetration (mm)	
	at 23 °C	at 4 °C
AMF	>37[a]	1.37 ± 0.13[b]
AMF + 2.0% soy lecithin	20.8 ± 0.12[c]	0.39 ± 0.08[d]

Means with the same letters are not significantly different ($p \leq 0.05$)

hardness. However, as the GF was further increased above 80%, the butter slightly softened, which roughly corresponds to the point where water coalescence began to occur. Evidently, phase inversion does not have as strong an effect on hardness as crystal morphology. Tempering the butter blends at 18 °C for 7 days hardened all samples but did not significantly alter their relative hardness. In contrast, the % GF had much less of an effect when the samples were crystallized at 4 °C (Fig. 3) and once again, tempering had little effect on relative hardness.

A similar hardening effect was also observed when soy lecithin was added to AMF butter (Table 1). At 23 °C, the cone penetrated right to the bottom of the 100% AMF butter sample. However, the butter was much harder after the addition of 2% lecithin. But once again, the relative hardness of the AMF versus AMF + lecithin butters was similar when the crystallization was performed at refrigeration temperature.

In our qualitative tests (Table 2), the amount of GF also appeared to have an effect on the taste and texture of the butter blends (although more rigorous sensory testing with a trained panel would be required to confirm these results). Compared to butter produced in the laboratory by batch churning and stored at 4 °C before sampling, the 100% GF butter seemed to have a wetter, cream cheese-like texture. This was likely due to the presence of a continuous water phase in the 100% GF butter. However, when tested at 23 or 30 °C, the 100% GF butter appeared to taste less oily than the churned butter control. Decreasing the GF fraction to 80% produced a butter that, at 4 °C, was similar in taste and texture to that of the churned control. This blend appeared slightly less oily when sampled at 23 or 30 °C. When the GF fraction was decreased to 60%, all panelists could detect graininess with a paraffin aftertaste at 4 °C. However, graininess was less evident as the sampling temperature increased. With only 30% GF, a pronounced grainy, waxy mouth-feel was evident. Finally, the 100% AMF butter had a very unpleasant sandy mouth-feel at

Table 2 Sensory evaluation of butter samples made from GF and AMF mixtures stored at 4° or 18 °C for 10 days

Non-aqueous phase composition	Storage conditions/Sensory description		
	at 4 °C	at 18 °C then at 23 °C	at 18 °C then at 30 °C
Laboratory batch-churned butter control	Typical yellow color; firm texture; smooth mouth-feel	Darker yellow; smooth texture; slightly oily taste	Very soft with yellow oil phase separation; very oily taste
100% GF	White appearance; wet, cream cheese-like texture	Whiter, creamier and less oily than control	Soft but less oily than control; no oil phase separation
80% GF + 20% AMF	Slightly lighter but taste and texture similar to control	Color and texture similar to control but slightly less oily	Soft with some oil phase separation but less than control
60% GF + 40% AMF	Appearance similar to control; significant graininess; waxy aftertaste	Similar color but slightly oiler than control; significant graininess	Similar color but slightly oiler than control; slight graininess detectable
30% GF + 70% AMF	Yellower than control; very grainy with waxy aftertaste	Yellower and oilier than control; very grainy mouth-feel	Yellower and much oilier than control; significant graininess
100% AMF	Much yellower than control; grainy mouth-feel	Yellower and oilier than control; grainy mouth-feel	Yellower and oilier than control; significant graininess

Some samples were also tempered for 3 h at 23 °C or 30 °C immediately before evaluation

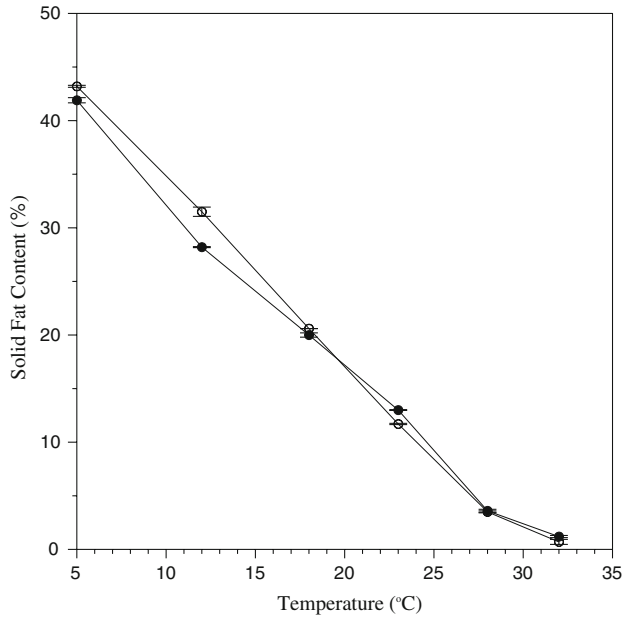

Fig. 4 Solid fat content versus temperature curves for: *open circles* pure AMF; *filled circles* AMF + 2 wt% soy lecithin

4 °C. This sandiness did decrease at 23 and 30 °C, but the samples then became even oilier than the churned butter control.

In order to determine if hardness changes were simply due to differences in total solids concentration, the SFC of AMF with and without 2% soy lecithin was determined. As illustrated in Fig. 4, lecithin addition had very little effect on the SFC versus temperature curves. After filter separation, phosphorous analysis was conducted on both the liquid phase and fat crystals formed at 23 °C. This analysis showed that less than 5% of the total added lecithin was found in the fat crystals, with the vast majority remaining in the liquid phase.

DSC scans were also performed on AMF with and without 2% lecithin (Fig. 5a, b). Lecithin addition did not appear to significantly affect the crystallization onset temperature when the melt was cooled (Fig. 5a). However, instead of a moderate-sized exothermic peak at 14 °C, a smaller peak at 12 °C, followed by a very large peak at 6 °C, which was observed with pure AMF, the first two exothermic peaks combined into one larger peak when 2% lecithin was added. When the crystallized AMF was reheated, five distinct endothermic events could be discerned, but with the 2% lecithin sample, only three endothermic peaks were observed (Fig. 5b).

Discussion

It is well known that crude fats crystallize quite differently from their final refined products. For example, refining of coconut TAG greatly reduces the rate of fat crystallization [8], and this change in kinetics has been attributed to the removal of minor components such as DAG, MAG, FA and PL during degumming, bleaching and deodorizing. These researchers also found that 1,2 DAG tend to have more of a retarding effect on crystallization kinetics than 1,3 DAG, so it would appear that minor component spatial structure also influences how it affects crystallization kinetics [8].

Siew and Ng [15] studied the effect of DAG at concentrations up to 10 wt% on the crystallization behavior of palm oleins with different degrees of unsaturation. In contrast to the results of Gordon and Rahman [8] with coconut, these researchers found that the presence of DAG

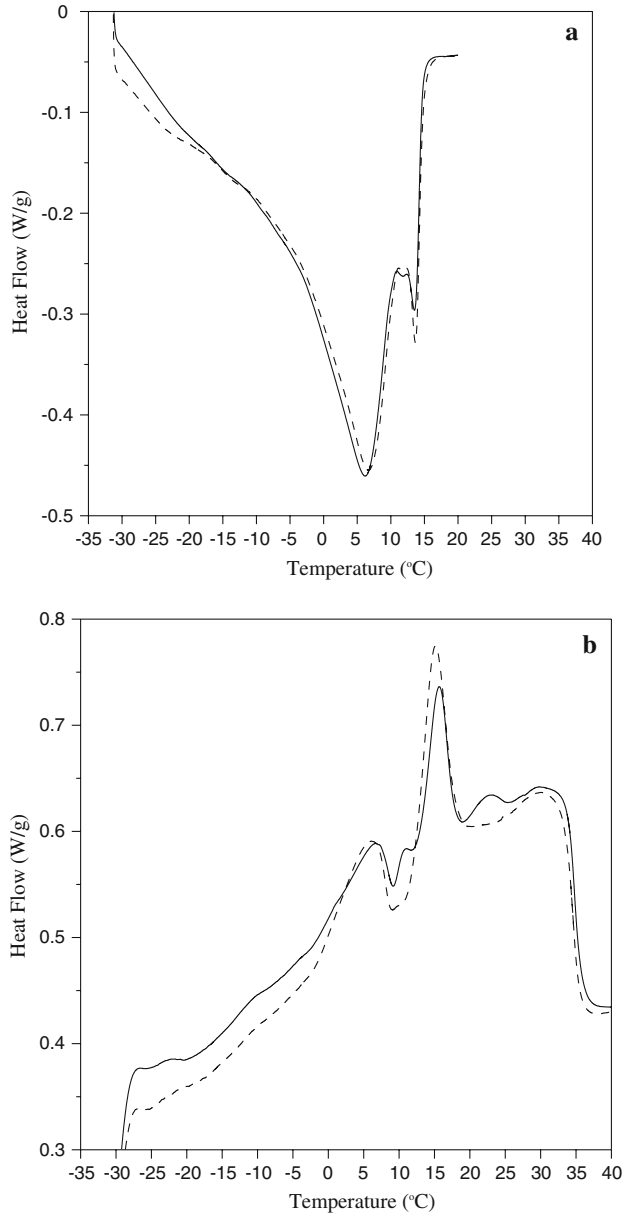

Fig. 5 Differential scanning calorimetry of AMF (*line*) and AMF + 2 wt% soy lecithin (*dashes*): (**a**) crystallization curves; (**b**) melting curves

accelerated palm crystallization kinetics, and 1,3 DAG appeared to be more effective than 1,2 DAG. Even though the authors of this work had no explanation for this apparent contradiction, one can speculate why such a different result was obtained. In coconut, minor component concentrations are typically in the ppm range, whereas DAG concentrations in palm oleins can attain levels of up to 10 wt%. At such high concentrations, DAG is no longer a minor component that cocrystallizes with TAG, but can form separate crystals. Since the melting points of these compounds would be in the order 1,3 DAG > 1,2 DAG > TAG, elevated concentrations of 1,3 DAG would accelerate crystallization simply because of a higher degree of supersaturation. Evidently, minor component concentrations must be maintained at appropriate levels in order to avoid such artifacts.

In a similar study, Smith [16] examined the effect of purified PL compounds on palm oil TAG crystallization. It was observed that the effect of PL on TAG crystal growth was very dependent on PL structure, with the resulting spherulite density and degree of branching strongly dependent on the PL subclass used. High hydrophilic–lipophilic balance (HLB) PL such as LPE and PI tended to increase spherulite density whereas low HLB PL like PC had little effect. Thus, PL with larger polar head groups have more of an inhibitory effect on secondary nucleation. Unfortunately, the maximum PL concentration used in this study was relatively low (<1 wt%), so complete inhibition of spherulite formation was not observed. In our previous work [6], we demonstrated that low PL concentrations can in fact increase spherulite size and density, but at elevated concentrations, spherulite formation is inhibited a minimum of 2.0 wt% soy lecithin was needed to completely break up AMF spherulites into individual crystals.

Savage and Dimick [14] attempted to explain hardness variations of cocoa obtained from various countries on differences in their natural PL content. Hard butters tended to crystallize more rapidly whereas soft butters were observed to have much longer induction times. Interestingly, soft butters contained higher concentrations of high HLB PL such as PI and PG, whereas low HLB PL such as PE and PC where more prevalent in hard butters. Thus, at the relatively low concentrations naturally found in cocoa butter, polar PL will increase spherulite size since larger crystals will typically produce a softer fat matrix. This finding is consistent with what Smith [16] observed with palm, and with our previous results with AMF at low concentrations of globular fat [6].

Toro-Vazquez et al. [18] investigated the crystallization behavior of TAG in unrefined cocoa butter and in cocoa butter without polar lipids. These researchers also found that the presence of low concentrations of PL increased the size of crystal spherulites. Furthermore, they observed that PL slowed the α-crystal growth rate and delayed the α to β' polymorphic transition [18]. Both Savage and Dimick [14] and Toro-Vazquez et al. [18] observed that PL (and PC and PE in particular) are present in high concentrations in seed nuclei; thus, it is likely that these minor components also influence nucleation behavior.

The role of minor components on crystallization of cocoa butter/milk fat blends was examined by Tietz and Hartel [17]. Surprisingly, they observed that the removal as well as the doubling of AMF minor components both increased nucleation onset times and had a significant effect on spherulite structure. Wright and Marangoni [20]

also found that the effect of minor component removal on AMF crystallization changes quite significantly depending on what time of the year the AMF was purchased. Perhaps this is not surprising because AMF has a very wide range of TAG, the structures of which are seasonally dependent; as a result, the DAG present would also be diverse, and as previously noted, how DAG influences crystallization is very dependent on spatial structure. The work of Foubert et al. [7] further supports this conjecture. They observed that distearin slows the growth of AMF, whereas monostearin or diolein both accelerate nucleation. Cerdeira et al. [4] also observed that surfactants with similar HLB but with different spatial structures can have widely different effects on crystallization behavior.

Ishii [9] examined the effect of PL structure on oil–water emulsion stability. In general, low HLB surfactants (<9) form water-in-oil emulsions whereas surfactants with an HLB > 9 form oil-in-water emulsions. Thus, lyophosphatidyl choline (LPC) (HLB = 12.9) and SM (9.2) tend to form micelles whereas PC (7.4), PE (7.4) and PA (6.5) form reverse micelles. Surfactant shape is also important—LPC is an inverted cone, SM and PC are cylinders, and PE and PA are cones. Therefore, LPC and PC are more stable in convex surfaces but PE and PA prefer a concave configuration. One can hypothesize that PL with shapes similar to those of TAG (which tend to be lamellar) would tend to affect crystallization differently than those with very different spatial configurations.

The principle PL components in MFGM are: PC, 32 wt%: PE, 31 wt%; sphingomyelin (SM), 20 wt%; PI, 7 wt%; PS, 5 wt%; and lactosyl-cerebroside (LS), 5 wt% [19]. By comparison, soy lecithin is a mixture of approximately 28 wt% PC, 30 wt% PI, 26 wt% PE and 16 wt% LPC [3]. It is interesting that, even though one mixture is derived from an animal source while the other is from a plant, in both, the ratio of low to high HLB PL is approximately 4:1. The low HLB PL would concentrate in the oil phase, and would more likely have a stronger influence on fat crystallization, whereas the high HLB PL would have more of an effect on the stability of the butter water-in-oil emulsion. Thus, crystallization was mostly affected at low to moderate total PL concentrations, while water phase coalescence was observed at higher concentrations of MFGM PL.

As has been observed previously by several researchers [20], the influence of PL on fat crystallization was more a kinetic than a thermodynamic phenomenon. As a result, PL addition had little effect on the final SFC concentration (Fig. 4). Furthermore, PL kinetic effects were minimal at elevated degrees of supercooling, when crystallization rates were very high (e.g., at 4 °C). How the addition of soy PL specifically affects AMF crystallization kinetics can be seen in the DSC experiments (Fig. 5a, b). Lopez et al. [11] identified four different crystallizing species during simultaneous DSC and x-ray diffraction (XRD) analysis of AMF. At crystallization onset, a moderate sized peak is followed by a much smaller one, with both species forming 2L β' crystals. Next, the largest crystallization peak is observed, which has a 3L α structure. Lopez et al. [11] also observed a very small peak at around 2 °C that corresponded to a third 2L β' crystal. The first two 2L β' as well as the 3L α peaks are evident in our DSC cooling curve (Fig. 5a), but it is hard to discern the low temperature 2L β' peak. Either our AMF did not have a significant amount of this species or the DSC system was not sensitive enough to distinguish it.

The addition of 2 wt% soy lecithin tended to increase the first 2L β' peak at the expense of the second and perhaps third crystallization peaks (Fig. 5a). The first 2L β' crystal has a smaller (4.15 nm) double-chain length as compared to the second 2L β' crystal (4.83 nm). It is possible that the presence of PL allows the high-melting TAG to be more accommodating, allowing more TAG species to enter into the tighter crystal packing.

Lecithin addition also had a significant effect on the AMF melting curve (Fig. 5b). At moderate scan rates like the one used in our study (5 °C min^{-1}), a slight endotherm followed by a large exotherm and large endotherm are typically observed with AMF at around 10 °C [10]. This complex group of peaks is believed to correspond to the melting of the 3L α species and its conversion into a 2L β' crystal [10]. The broad endotherm observed between 20 and 38 °C with AMF involves the melting of the two 2L β' species formed during cooling, as well as the third 2L β' crystals resulting from the previously described polymorphic transition. Adding 2 wt% lecithin appeared to significantly affect the polymorphic transition of the 3L α species. PL are known to facilitate polymorphic transitions [7]. Furthermore, the endothermic peak at 23 °C observed with AMF was missing in the AMF + lecithin sample. Perhaps this peak corresponds to the melting of the small second 2L β' species formed during cooling. As discussed above, the formation of this crystal species was inhibited in the presence of soy PL.

Dramatic changes in crystal morphology were also evident when 2 wt% lecithin was added to AMF (Fig. 1). The repression of spherulite formation could be the direct result of secondary nucleation inhibition by PL adsorbed onto fat crystals. On the other hand, the PL facilitated polymorphic transitions that were observed in the DSC scans could also reduce the degree of supercooling, thereby indirectly affecting secondary nucleation and spherulite formation.

The addition of PL to AMF, either in the form of fat globules or as soy lecithin, appears to have several effects on butter functionality: it increases hardness, particularly at

room temperature, and reduces the tendency to oil-off at elevated temperatures. Too little PL creates a very grainy butter whereas too much GF leads to the coalescence of the aqueous phase, and an undesirably wet mouth-feel. Optimal butter functionality can be achieved with blends containing 60–80% GF.

With this in mind, perhaps it is not surprising that some previously developed butter making technologies were not highly successful. Using 80% concentrated cream to produce butter has the advantage that the cream does not need to be tempered but produces a hard product with a 'wet' taste, likely due to elevated levels of high HLB PL like SM that induce coalescence of the aqueous phase. In contrast, oiling-off the cream and removing buttermilk via centrifugation would likely remove too much PL, resulting in large spherulite formation that gives an oily product with a grainy texture. Batch or continuous butter churning methods do appear to yield butter with the appropriate amount of PL, but these methods require that the cream is first tempered for an extended period of time. Nevertheless, even with the currently used continuous churning processes, PL concentration is not well controlled and is likely not always at the optimal level. One could speculate that the tempering stage could be bypassed while still maintaining optimal PL concentration by developing a hybrid process. Combining oiled-off AMF and high-fat cream at the appropriate ratio could be another method to effectively control PL concentration and optimize butter functional properties.

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