Effect of cream aging temperature and agitation on butter properties

Jiwon Lee and Silvana Martini¹

Department of Nutrition, Dietetics, and Food Sciences, Utah State University, Logan 83422

ABSTRACT

Aging of cream is an important process to manage production time and to produce butter with consistent quality. The objective of this study was to evaluate the combined effect of temperature (5, 10, and 15°C) and agitation rate (0, 40, and 240 rpm) during aging of cream on the physical properties of cream and butter in a model system. Cream's solid fat content (SFC), melting behavior, and droplet size distribution were measured during and after 90 min of aging. Butter physical properties such as melting behavior, water content, and hardness were measured. The effects of agitation on SFC and droplet size are dependent on aging and churning temperature. Solid fat content increased faster at 5°C, and the maximum SFC was the highest at this temperature. An effect of agitation on SFC was observed only when cream was aged at 15°C. Agitating cream at 40 rpm increased the droplet size regardless of aging temperature. Two melting peaks, medium melting fraction (MMF) and high melting fraction (HMF), were found in cream samples aged at 5 and 10°C, but only a HMF melting peak was seen in the cream aged at 15°C. The enthalpy of MMF in the cream aged at 10°C with 40 rpm and without agitation was significantly lower than that in samples aged at 5°C regardless of agitation rate. Butter can be formed only from cream aged under certain conditions during 14.5 min of churning, which are 5°C with high agitation and 10°C regardless of agitation level. Butter produced with cream aged at 5°C with high agitation showed significantly higher MMF and total enthalpy values. However, no significant difference in enthalpy values was observed among the butter samples made from the cream aged at 10°C. Further crystallization of MMF occurred in the butter produced with cream aged at 10°C during 24 h of storage at 5°C, whereas no further crystallization occurred in the butter made with the cream aged at 5°C with high agitation. The hardest butter was obtained when cream was aged at 5°C with

240 rpm and at 10°C with 40 rpm. Softer butter was obtained when cream aged at 10°C with 240 rpm was used. This butter also had the highest water content. This study shows that butter hardness can be tailored by changing the aging conditions of the cream. Cream can be aged at higher temperature with low agitation without altering the hardness of butter. These results will help dairy producers to optimize butter making processes to obtain desired properties in the final product.

Key words: crystallization, aging, agitation, cream, butter

INTRODUCTION

Butter is a water-in-oil emulsion that contains at least 80% fat and no more than 16% water. Butter is made from cream, which is an oil-in-water emulsion, and it is obtained through a phase inversion from oil-in-water to water-in-oil emulsion that occurs during churning. Prior to phase inversion, pasteurized cream is aged to induce crystal formation. Crystallization of milk fat in cream is essential for butter making to form a continuous fat network during churning. Controlling crystallization of milk fat in cream is very important for the final quality of butter because crystal number and size, crystal size distribution, solid fat content (SFC), and polymorphic form affect the microstructure of butter (Rønholt et al., 2013). In bulk fats, crystallization can be controlled by processing conditions such as crystallization temperature, cooling rate, and agitation rate (Tang and Marangoni, 2007). High shear rates and low temperatures promote nucleation (Herrera and Hartel, 2000a; Pérez-Martínez et al., 2012). In addition, better heat transfer generated by high agitation rates may promote co-crystallization of triacylglycerols (TAG; Breitschuh and Windhab, 1996). Slow cooling rates can yield large and dense crystals, whereas high cooling rates generate narrow crystal size distributions (Herrera et al., 1999; Herrera and Hartel, 2000a; Wiking et al., 2009). High cooling rates can also result in high SFC of anhydrous milk fat (Herrera and Hartel, 2000b; Campos et al., 2002). Small size of crystals associated with high cooling rate will ultimately lead to harder materials (Kaufmann et al., 2012a,b).

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¹Corresponding author: silvana.martini@usu.edu

Crystallization in oil-in-water emulsions such as cream is more complicated than in bulk fat because crystallization occurs within the oil droplets. Crystallization in oil-in-water emulsions is often thought to be driven by primary nucleation because oil droplets are too small to contain impurities. Coupland (2002) pointed out that crystallization behavior of emulsions is affected by the TAG composition of the fat phase, the compatibility of TAG, and the type of emulsifier. In addition, emulsion droplet size can affect the crystallization behavior in oil-in-water emulsions. Walstra et al. (2005) mentioned that partial coalescence rate during churning increases with increasing milk fat droplet size in cream because the chance of colliding globules increases with increase of globule size. Tippetts and Martini (2009) and McClements et al. (1993) observed that smaller droplets hindered and inhibited crystallization in oil-in-water emulsions. More uniform small droplets can lead to homogeneous crystallization because nucleation is more likely to occur from the fat and not from impurities present in the emulsion (Rousseau, 2000; Coupland, 2002).

Similarly, crystallization of milk fat globules in cream is influenced by many factors such as cooling rate, aging time and temperature, churning temperature, and phase inversion that occurs during churning (Walstra et al., 2005; Rønholt et al., 2012, 2014a,b; Buldo et al., 2013). Few studies have evaluated the effect of various processing conditions in cream to change butter quality. For example, to understand how processing of cream influences butter quality, some studies have studied the effect of aging time and temperature, cooling rate, and churning temperature on the physical properties of cream and butter. Bylund (2003) and Walstra et al. (2005) stated that the heat treatment history before churning including aging time and temperature, and temperature fluctuations affect the rate of partial coalescence and crystallization behavior in the globules. Walstra et al. (2005) stated that solid fat is required for partial coalescence, but too much solid fat in globules delays partial coalescence due to the lack of liquid oil phase to develop a bridge between droplets. Rønholt et al. (2012) reported that cream temperature during aging can influence the crystallization behavior. These authors reported that more stable crystals could be formed when cream is aged at lower temperature due to a higher degree of shear by a longer churning time. Rønholt et al. (2014b) also examined the effect of churning temperature (22.8 and 10.8°C) on water droplet size and water content and on rheological properties of the butter. When cream was aged and churned at 22.8°C, the butter had bigger water droplets and lower SFC compared with the cream aged and churned at 10.8°C; however, churning temperature did not affect

the water content of butter. In addition, cooling rate of the cream before churning can affect the water content and the rheological properties of butter. Rønholt et al. (2014) reported that a harder butter was produced with a fast cooling rate (7.5°C/min) compared with butter produced with a slow cooling rate (0.4°C/min) because of a shorter churning time of slowly cooled cream. A shorter churning time can be expected in slow-cooled cream because larger crystals formed at a slow cooling rate are more prone to damage the milk fat globule membrane than smaller crystals for partial coalescence, which is required for phase conversion (Boode and Walstra, 1993; Rønholt et al., 2014a). No difference was found in water content between the butters that were made with slow- and fast-cooled cream (Rønholt et al., 2014a). Even though these studies provide valuable information on how processing conditions of cream affect the physical properties of butter, no research has assessed the combined effect of agitation rate and aging temperature on the quality of butter.

Thus, the objective of this study was to evaluate the combined effect of agitation rate and aging temperature before churning on the physical properties of butter such as hardness, thermal behavior, and water content in a model system. Agitation and temperature are 2 important processing parameters that affect fat crystallization; therefore, it was our hypothesis that the combination of these 2 parameters will affect churning time and therefore physical properties of butter.

MATERIALS AND METHODS

Butter Making

Pasteurized heavy whipping cream (40% fat; Organic Valley, La Farge, WI) without stabilizer was purchased at a local grocery store. Cream (100 g) was heated to 55°C for 30 min in an oven to erase crystal memory and then transferred into a jar in a water bath that was set up to 1 of 3 aging temperatures (5, 10, and 15°C). These 3 temperatures were within normal aging temperatures (Chandan et al., 2015). The cream was aged for 90 min while agitated with an overhead stirrer (IKA RW basic 16, IKA, Wilmington, NC) at 0 rpm (no agitation; **NA**), 40 rpm (low agitation; **LA**), or 240 rpm (high agitation; **HA**). The slowest agitation level chosen in our study was the minimum speed that was allowed by the stirrer, and the highest agitation level chosen was the maximum speed allowed without splashing cream. These agitation speeds are within the speeds used in the dairy industry, where cream is commonly agitated at speeds that provide efficient heat transfer but avoid splashing and churning of the cream. Aging time of 90 min was chosen to allow for complete

crystallization of the fat in the cream. The aging process was repeated 3 times to collect a total of 300 g of each sample for churning. Each batch was transferred to a beaker and stored at aging temperature until collecting 300 g. After collecting 300 g of cream, the cream was transferred to a KitchenAid (KICA0WH; Benton Harbor, MI) 2-quart ice cream maker stand mixer attachment. The surface of the ice cream making bowl was punctured at 2 different spots to remove the chemical that was inside the bowl and to connect to a water bath for temperature control. The cream was churned using a KitchenAid stand mixer (model K5-A, Troy, OH; speed setting 9) at its aging temperature for 14.5 min maximum. This maximum churning time was used because it was the time required to produce butter at 10°C but was not extensively long. After churning, buttermilk was drained from butter grains and weighed. Butter grains were collected in cheesecloth and were gently squeezed using a butter press (The Cheesemaker, Maple & Cherry Butter Press, Mequon, WI) to remove buttermilk. Fresh butter was weighed and tested for melting behavior and water content. The remaining butter was stored at 5°C for 24 h and then tested for melting behavior and texture. Butter was prepared in duplicate.

According to the international standard (Codex Standard 279–1971; CAC, 2010), butter must contain no more than 16% moisture. The water content of the samples formulated in this study was higher than 16%, and therefore the samples cannot be referred to as butter. However, for simplification we still use the term “butter” even though the samples are truly butter-like spreads.

Fat Droplet Size Distribution

Droplet size and distribution were quantified for all the cream samples using Beckman Coulter particle characterization equipment (LS230 version 3.19; Beckman Coulter Inc., Indianapolis, IN). After 90 min of aging, a cream sample (15 mL) was collected and used for measurement. Droplet size was expressed as $D_{3,2}$ values (surface weighted), also called Sauter mean diameter, calculated as $D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$, where d_i is the

diameter of the particles in each size class and n_i is the number of particles in each size per class unit volume of emulsion (McClements, 2004). Each sample was measured in triplicate.

Melting Behavior

A differential scanning calorimeter (Q20, TA Instruments, Castle, DE) was used to evaluate the melting

behavior of cream aged for 90 min, fresh butter, and butter stored for 24 h at 5°C. Between 7 and 11 mg of sample was placed into aluminum hermetic pans and sealed. An empty cell was used as a reference. Cream was heated from the aging temperature to 60°C at a ramp rate of 5°C/min. Melting behavior was quantified with the onset melting temperature (T_{on} ; °C), peak melting temperature (°C), and change in enthalpy (J/g) associated with the melting process. These parameters were measured using TA universal analysis software (TA Instruments).

SFC

The amount of crystalline fat present in the cream was measured using a pulsed nuclear magnetic resonance (Minispec mq-20, Bruker Inc., Billerica, MA) following the AOCS Cd 16b-93 method (AOCS, 2009). While cream was being aged, SFC was measured every 5 min for 60 min and then every 10 min until 90 min. The SFC was measured by transferring approximately 2 mL of cream to a nuclear magnetic resonance tube (10 mm in diameter and 180 mm in height).

Water Content

Water content was measured as described in Rønholt et al. (2012). Fresh butter (5 g) was placed in a porcelain cup and was stored in an oven at 100°C for 2 h. After that, the cup was placed in a desiccator at room temperature for 30 min. When the sample reached room temperature, the cup was weighed and water loss from the sample was calculated as a percentage of the initial weight. Water content was measured in triplicate.

Texture

The texture (hardness) of the samples was measured as described by Cisneros Estevez et al. (2013) with modification. A penetrometry test was performed on the butter that was stored at 5°C for 24 h using a texture profile analyzer (TA-XT Plus texture analyzer, Texture Technologies Corp., Hamilton, MA) with a spreadability rig TA-425 TTC (Texture Technologies Corp.). The rig comprised a matched cup and 90° cone probe. For height calibration, the empty cup was mounted on the bottom, and the probe was calibrated to be positioned at 25 mm (the starting position) from the cup, having a touch force of 10 g. For this hardness test, the probe was set up to travel 23 mm (leaving a 2-mm gap between the bottom of the cup and the cone probe) from the starting position (25 mm) at a rate of 3 mm/s to reach the maximum penetration depth and return to the starting position. After the calibration

and program setup, the butter sample was placed into 5 cups. Excessive butter on the cup was scraped for flattening the top surface. The cups were kept at room temperature (23°C) for 60 min before performing the test. The force (g) required for the probe to travel 23 mm as a function of time (s) was plotted. The peak force that was required to reach the maximum penetration depth was used for hardness of the butter sample, which was then converted to Newtons. Five measurements were performed for each sample.

Statistical Analysis

All data were analyzed using Prism 7.0 (GraphPad Software, San Diego, CA). Tukey's multiple comparison was used for 1-way ANOVA for $D_{3,2}$ values of fat droplet size of cream and hardness, melting behavior, water content, and SFC of butter. Two-way ANOVA was used to evaluate the effect of agitation level and aging temperature. Statistical differences were evaluated at $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Effect of Aging Temperature and Agitation Rate on the Physical Properties of Cream

SFC. Solid fat content increased as fat crystallized in the cream. Figure 1 shows SFC increase in cream aged at 5, 10, and 15°C with different agitation levels (NA, LA, and HA) during 90 min. Increase in SFC slowed down and reached a plateau under all the experimental conditions within 90 min. The highest SFC values were observed at 5°C followed by 10 and 15°C. The SFC values were 18.0, 18.6, and 19.2% at 5°C after 90 min of cream aging at 5°C for NA, LA, and HA, respectively, and these values were not significantly different from each other ($P > 0.05$). The SFC values of the cream samples after 90 min at 10°C were 12.5, 13.5, and 13.6% for LA, NA, and HA, respectively. Significantly lower SFC values were obtained with LA conditions compared with HA conditions ($P < 0.05$), but no difference was observed in SFC of samples aged with NA and LA or NA and HA. It is unclear why SFC values of LA creams were lower compared with NA and HA creams. Later it is described that LA samples had a slightly higher droplet size. It is likely that the heat transfer in a bigger droplet is slower and therefore the crystallization is slightly delayed. Even though a slight induction in crystallization was observed in the HA samples as evidenced by higher SFC values at initial times of the crystallization process (Figure 1; approximately 5 min), a significant difference in the final SFC was not observed between the HA and NA samples. This lack

of difference found in SFC for creams treated at 5°C or the presence of small differences in creams treated at 10°C is somehow an expected result because the high

Figure 1. Solid fat content (SFC) in cream aged at 5, 10, or 15°C with no agitation (NA, ○), low agitation (40 rpm; LA, ■), and high agitation (240 rpm; HA, ▲) for 90 min. The error bars represent the SD of the mean ($n = 2$).

supercooling in the sample is the main processing factor that drives crystallization.

No difference ($P > 0.05$) was observed in SFC of samples aged with NA and LA at 15°C, and a significantly higher SFC ($P < 0.0001$) was observed for the HA condition after 90 min of aging. The SFC values were 5.0, 5.0, and 6.4% at 15°C after 90 min for NA, LA, and HA, respectively. The SFC in cream samples aged with HA increased faster than in those aged with NA and LA at all 3 aging temperatures. Similar to the previous discussion, the supercooling at 15°C is lower, and therefore processing conditions such as agitation play an important role in the induction of crystallization. It is possible that secondary nucleation occurs within the oil droplet at this high temperature due to the breaking of existing crystals caused by HA.

The SFC values increased over time, showing a sigmoidal shape for cream crystallized at 5 and 10°C, whereas a nonsigmoidal increase in SFC was observed at 15°C (Figure 1). Nucleation occurs more potently at low crystallization temperature (high supercooling) than at high crystallization temperature. Thus, SFC increases faster at lower temperature with an increase in viscosity. When viscosity increases, molecules become less mobile, nucleation rate decreases, and SFC reaches a plateau. In the case of the cream aged at 15°C, the supercooling was too low and the increase in SFC occurred gradually and never reached a plateau. Induction time of crystallization is independent from agitation rate when aging temperature is low. Dhonsi and Stapley (2006) reported that shear decreased the induction time of cocoa butter crystallization at high temperature (20 and 23°C), but shear did not affect induction time at a lower temperature (13 and 17°C).

Droplet Size. Figure 2 shows the surface volume mean diameter, $D_{3,2}$, of 300 g of cream samples collected after aging at different temperatures with different agitation before churning. There was a tendency for LA to increase average droplet size in cream samples regardless of aging temperature. In particular, cream aged with LA at 5°C had a significantly higher $D_{3,2}$ value compared with that aged with HA. At 15°C, cream aged with LA had significantly higher $D_{3,2}$ than that aged without agitation (NA). Droplet size can be used to measure the degree of coalescence in an emulsion: the bigger the droplet size, the more coalescence in the emulsion. Coalescence occurs when 2 droplets aggregate, forming a big droplet, and it is a sign of cream instability. Shear or agitation can cause instability or stability of emulsion. Nandi et al. (2001) reported that coalescence decreased with increasing shear rate. However, our results show that larger $D_{3,2}$ values were obtained in LA samples compared with those agitated with HA at all the aging temperatures, indicating that

Figure 2. The $D_{3,2}$ values (Sauter mean diameter) of fat droplets in cream aged at 5, 10, or 15°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) for 90 min. The error bars represent the standard deviation of the mean ($n = 2$). Values sharing letters (a–d) are not significantly different ($P > 0.05$).

LA promoted coalescence, probably due to a higher degree of droplet collision. However, when agitation was not applied to cream, $D_{3,2}$ values were similar to those of the samples that was aged with HA (Figure 2). This is because coalescence occurs relatively slowly in cream without agitation. In addition, it is possible that even though droplet collision in HA cream was higher, the agitation was high enough to allow for droplets to “bounce back” after colliding, and therefore coalescence was not induced.

Droplet size distributions of creams measured after aging are shown in Figure 3A (NA), Figure 3B (LA), and Figure 3C (HA). No difference was observed between the cream samples that were agitated with NA and HA at 5, 10, and 15°C, but a slight difference was seen in the cream samples aged at different temperatures agitated with LA. A higher number of larger droplets is seen in the cream aged at 15°C with LA than in creams aged at 5 and 10°C with LA. This is because more coalescence is prone to occur at higher temperature. Hinrichs and Kessler (1997) found that fat globules are more sensitive to shear stress at higher temperature because at lower temperatures a higher SFC enhances the stability of fat globules.

In addition, it is important to consider that agitation can damage the milk fat globule membrane and cause coalescence. Depending on the crystallization temperature, damage of the milk fat globule membrane can cause partial coalescence or coalescence. Lack of SFC at a higher temperature causes coalescence rather than partial coalescence because there is not enough SFC to form partial coalescence. Both partial coalescence and coalescence will result in a higher number of bigger droplets in the droplet size distribution data. A higher level of agitation is more prone to damage the

Figure 3. Droplet size distribution of fat droplets in cream aged at 5, 10, or 15°C with (A) no agitation, (B) low agitation (40 rpm), and (C) high agitation (240 rpm) for 90 min.

milk fat globule membrane; thus, a higher number of large droplets is expected at all temperatures. However only LA at 15°C showed bigger droplets (Figure 3B), suggesting that the milk fat globule membrane is not being ruptured by agitation.

Melting Behavior. In general, 3 melting fractions can be obtained in anhydrous milk fat. These include low (<10°C), medium (10–19°C), and high (>20°C) melting fractions (Deffense, 1993; Bhaskar et al., 1998) comprising TAG that melt over a wide range of temperatures (low, medium, and high). In our study, 2 melting peaks were observed in the cream samples aged at 5 and 10°C (Table 1; Figure 4A and 4B). The first peak represents the melting of medium melting fraction (MMF), and the second peak shows the melting of the high melting fraction (HMF). A single melting peak (HMF) was observed in cream samples aged at 15°C (Figure 4C) because TAG present in the MMF do not crystallize at this high temperature. All cream samples crystallized at 5°C had similar melting behavior ($P > 0.05$) in terms of onset temperature and melting peak temperature regardless of agitation rates (Table 1). A similar trend was observed at 10°C with some exceptions. For example, the total enthalpy in creams aged at 10°C was affected by agitation with higher enthalpy values obtained at higher agitation rates. When comparing the effect of aging temperature on melting behavior, a significantly higher onset temperature ($P < 0.05$) was observed in the MMF of the cream aged at 10°C without agitation (NA) than in the cream aged at 5°C with HA. This indicates that a wider range of medium-melting TAG was present in the cream aged at 5°C with HA than at 10°C without agitation. Cream aged at 10°C without agitation also had a significantly lower melting peak ($P < 0.05$) in the MMF compared with the cream that was aged at 5°C without agitation. Cream samples aged at 10°C had lower MMF enthalpy values compared with those aged at 5°C. Significantly lower enthalpy values of MMF are observed in the cream aged at 10°C with NA ($P < 0.01$) and LA ($P < 0.05$) compared with the values in the cream aged at 5°C with HA.

Remarkably higher enthalpy values were observed in the MMF of the cream aged at 5°C compared with at 10°C. Even though no statistical difference was observed in the onset temperatures and melting peaks of HMF, HA decreased the onset temperatures of cream samples at 5 and 10°C, suggesting that HA slightly induces the crystallization of TAG with lower melting points within the HMF. Agitation and aging temperature did not significantly influence the onset temperature and melting peak of HMF ($P > 0.05$). However, aging temperature had a significant effect on the enthalpy of the HMF ($P < 0.01$). The enthalpies of HMF decreased in the order

Table 1. Melting behavior of cream aged at 5, 10, or 15°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) for 90 min¹

Parameter	5°C			10°C			15°C		
	NA	LA	HA	NA	LA	HA	NA	LA	HA
T _{on-1} (°C)	12.6 ± 1.0 ^{ab}	12.3 ± 0.3 ^{ab}	12.2 ± 0.9 ^{bc}	14.4 ± 0.2 ^a	13.9 ± 0.1 ^{ab}	13.7 ± 0.2 ^{ab}	ND	ND	ND
T _{p-1} (°C)	16.3 ± 0.3 ^b	16.5 ± 0.1 ^{ab}	16.4 ± 0.3 ^{ab}	17.1 ± 0.1 ^a	16.8 ± 0.0 ^{ab}	16.4 ± 0.1 ^{ab}	ND	ND	ND
ΔH ₋₁ (J/g of fat)	6.0 ± 1.7 ^{ab}	7.5 ± 1.0 ^a	7.9 ± 0.6 ^a	2.0 ± 0.3 ^c	3.6 ± 0.4 ^{b,c}	4.5 ± 0.3 ^{abc}	ND	ND	ND
T _{on-2} (°C)	25.1 ± 1.2 ^a	25.1 ± 0.3 ^a	22.2 ± 0.1 ^a	25.6 ± 0.8 ^a	25.3 ± 1.1 ^a	23.8 ± 0.1 ^a	24.8 ± 0.3 ^a	24.7 ± 1.1 ^a	25.0 ± 2.1 ^a
T _{p-2} (°C)	30.6 ± 3.3 ^a	32.8 ± 0.0 ^a	32.9 ± 0.5 ^a	31.4 ± 2.2 ^a	31.1 ± 2.7 ^a	28.9 ± 0.1 ^a	30.0 ± 0.4 ^a	30.0 ± 0.8 ^a	30.7 ± 1.5 ^a
ΔH ₋₂ (J/g of fat)	15.5 ± 2.1 ^{ab}	15.8 ± 0.5 ^{ab}	15.1 ± 0.9 ^{ab}	15.0 ± 0.4 ^{ab}	17.4 ± 0.7 ^{ab}	21.3 ± 0.2 ^a	12.3 ± 0.6 ^b	13.2 ± 1.7 ^b	13.3 ± 4.2 ^b
ΔH _{total} (J/g of fat)	21.4 ± 3.8 ^{abc}	23.2 ± 0.5 ^{ab}	23.0 ± 1.5 ^{ab}	17.0 ± 0.1 ^{bcd}	21.1 ± 0.3 ^{abcd}	25.7 ± 0.5 ^a	12.3 ± 0.6 ^c	13.2 ± 1.7 ^{de}	13.3 ± 4.2 ^{de}

^{a-c}Values sharing letters within the row are not significantly different ($P > 0.05$).

¹Melting behavior was expressed as onset melting temperature (T_{on}) and peak melting temperature (T_p) and the change in enthalpy associated with the melting process (ΔH). Subscript 1 indicates parameters obtained from the first melting peak, and subscript 2 refers to parameters obtained from the second melting peak. ND indicates that data were not detected. Mean values and standard deviations are reported.

Figure 4. Thermal behavior of cream measured by differential scanning calorimetry. Cream was aged at (A) 5°C, (B) 10°C, or (C) 15°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) for 90 min. Endo = endothermic transition.

of aging temperatures 10, 5, and 15°C. Cream aged at 10°C with HA had the highest enthalpy of HMF among all the samples but was not statistically different from the values obtained at 5°C regardless of the agitation condition. However, these values were significantly higher than those obtained when the cream was aged at 15°C regardless of agitation level ($P < 0.05$). The enthalpy of the MMF at 10°C is lower than that observed at 5°C, whereas the enthalpy of HMF at 10°C is similar to or higher than that obtained at 5°C. This suggests that low temperatures induce the crystallization of low-melting TAG. However, the total enthalpy of the cream sample aged at 5°C (sum of the enthalpies of MMF and HMF) is similar to the one observed in cream aged

at 10°C without agitation. When a higher temperature such as 10°C and agitation are used, a lower amount of MMF and a greater amount of HMF are crystallized. It is very likely that this higher temperature does not allow for the MMF TAG to crystallize by themselves (not enough driving force), but they can co-crystallize with the HMF TAG. This is an interesting result that suggests that the amount of crystallized material obtained at 5°C can be matched and even increased by crystallizing the cream at a higher temperature (10°C) and by using agitation.

Aging temperature had a significant effect on the total enthalpy values ($P < 0.0001$). All the total enthalpy values at 15°C with HA, LA, and NA were significantly lower than those at 10°C with HA ($P < 0.01$). More obvious differences in the total enthalpy values were observed between the samples with different agitation levels at 10°C than at 5 and 15°C, and cream aged at 10°C with HA had significantly higher enthalpy values than that aged without agitation. These results suggest that the agitation applied to the cream aged at 15°C was not enough to induce the crystallization of fat and match the final enthalpy values obtained at 5 and 10°C. It is important to reiterate here that MMF TAG were not present in creams aged at 15°C. Further analysis of the data showed that SFC was highly correlated with the enthalpy values of MMF (Pearson $r = 0.9$; $P = 0.015$) and with total enthalpy values (Pearson $r = 0.856$; $P = 0.003$). Total enthalpy values were highly correlated with the enthalpy values of HMF (Pearson $r = 0.832$; $P = 0.005$).

Effect of Cream Aging Temperature and Agitation Rate on Churning Time and Physical Properties of Butter

Butter Making. Cream was churned for a maximum of 14.5 min, and butter was obtained only from the cream that was aged with NA, LA, and HA at 10°C and with HA at 5°C. Rønholt et al. (2014a) showed that slow cooling (0.4°C/min) resulted in bigger crystals in cream compared with a cream cooled under fast-cooling (7.5°C/min) conditions. Bigger crystals are more favored for partial coalescence, and less churning time is required for a cream that has been cooled under slow-cooling conditions. In our study, cooling rates were not remarkably different among cream samples that were agitated or not agitated. Cooling rates decreased as aging temperature increased. Cooling rates were 10.2, 10.7, and 11.0°C/min at 5°C; 10.5, 11.0, and 12.2°C/min at 10°C, and 9.5, 9.7, and 10.5°C/min at 15°C with NA, LA, and HA, respectively. The slowest cooling rate was observed at 15°C followed by 10 and 5°C. Despite the small differences in the cooling rates in our ex-

periments, these can be considered fast cooling rates compared with the ones used by Rønholt et al. (2014a). Rønholt et al. (2014b) also found that churning time was dependent on aging and churning temperature. These authors reported that cream aged for 90 min at 10°C and churned at 10°C had a longer churning time than cream aged for 90 min at 22°C and churned at 22°C (3.4 vs. 2.0 min). Low churning temperature can cause long churning time because of high SFC. When SFC is high, liquid oil is not available for formation of the butter grain. In the present study, aging at 5°C required a longer churning time than aging at 10°C to obtain buttermilk, and no butter was obtained after churning for 14.5 min for the NA and LA conditions. The time that was required to obtain butter grain for cream aged at 5°C with HA was approximately 14.5 min. Buttermilk leaked from butter grains at approximately 8.5 to 10 min (HA), 10.3 to 12.3 min (LA), and 11 to 14 min (NA) when cream samples were aged and churned at 10°C, whereas buttermilk leaked from butter grains at approximately 14.5 min (HA) and 15 to 15.5 min (LA and NA) at 5°C. Cream that was aged at 5°C with HA was turned into butter faster than that aged at 5°C with LA and NA. As previously mentioned, these results show that longer churning times are needed when cream is aged and churned at lower temperatures. This is probably due to the higher SFC generated at these low temperatures. However, when the cream is aged under agitation, churning time is significantly reduced. It is likely that an induction in crystallization of TAG with lower melting point (as evidenced by the slightly lower T_{on} in the HMF; Table 1) contributes to this shorter churning time for the cream crystallized at 5°C under HA conditions. These TAG with a lower melting point could stabilize the butter grains formed during churning. In addition, the smaller droplet size of creams aged at 5°C under HA might have played a role in inducing partial coalescence. Smaller droplets have higher surface area and therefore more points of contact to induce partial coalescence. This effect is more evident in creams aged and churned at 10°C, where higher agitation rates reduced churning times. Table 1 shows lower T_{on} for the MMF and the HMF and a higher enthalpy value in MMF as agitation increases, suggesting that more TAG with a low melting point are present in the cream.

Cream aged at 15°C did not turn into butter within 14.5 min of churning regardless of agitation or agitation rate because SFC was too low for partial coalescence to occur. Table 1 shows that these creams do not contain an MMF peak. Therefore, it is likely that MMF TAG must be present in the crystalline form to obtain butter. To form a continuous fat phase during butter making, partial crystallization within the milk

fat globule is essential because crystals in the milk fat globule can function as a penetrating agent that can pierce the membrane of other milk fat globules to form a continuous fat phase. Buldo et al. (2013) observed a significant reduction of the churning time (almost 7-min reduction) by increasing SFC from 15 to 23%. They thought that churning time was influenced by SFC that determined the rate of partial coalescence. Buldo et al. (2013) explained the importance of SFC level for partial coalescence, referencing the study of Boode et al. (1993). Partial coalescence can take place when SFC is not too high or too low (Boode et al., 1993; Walstra et al., 2005). Partial coalescence occurs when 2 partially crystallized milk fat globules are close and when crystals within a globule can protrude the second globule membrane and adsorb the layer of that globule. At the same time, liquid oil is necessary because the crystal penetrating the second globule is wetted by the oil that supports the bond between 2 droplets (Boode and Walstra, 1993). Thus, for partial coalescence to occur, both partial crystalline and liquid oil are required and a certain level of SFC is essential. Furthermore, the rate of partial coalescence increases with higher SFC, but SFC that is too high impedes partial coalescence due to the lack of liquid oil to form partial coalescence (Boode et al., 1993). Globule size and crystal size are the determining factors for the minimum SFC that is required for partial coalescence to occur (Boode et al., 1993). For example, partial coalescence would less likely occur when milk fat globules are too small because of a decreased chance to meet each other. Thus, when small globules are present in cream or other types of oil-in-water emulsions, high SFC and bigger crystals are necessary for partial coalescence (Boode et al., 1993; Fredrick et al., 2010). However, if small droplets are present and agitation is applied, these small droplets might encounter each other faster and their higher surface area might promote partial coalescence. Our study shows that in addition to considering SFC and droplet size, agitation and the type of TAG that form the solid material might play a role in butter formation.

The highest SFC in the cream aged at 15°C was 6.4% with HA after 90 min of aging. If the cream was churned longer than 14.5 min, a shear motion from churning promoted crystal formation and assisted aggregation of fat globules and formation of a 3-dimensional crystal network. In fact, butter could be formed after 15 to 15.5 min with the cream samples that were aged at 5°C with NA and LA, unlike the cream sample that was aged at 5°C with HA. However, it took longer to churn cream to make butter from cream samples that were aged at 15°C with any agitation.

Melting Behavior. Butter melting behavior was measured by differential scanning calorimeter in fresh

butter and butter stored at 5°C for 24 h (Table 2; Figure 5A and 5B). As previously described for cream, 2 melting peaks were observed in both fresh and day-old butter samples that were made from creams aged at 5 and 10°C (Figure 5A and 5B). Most differences in melting behavior were observed in the first melting peak that corresponds to the melting of MMF TAG. Significantly lower onset temperature ($P < 0.05$) was observed in fresh butter when cream aged at 5°C with HA was used. This low T_{on} is a result of low aging and churning temperature, which promoted crystallization of lower melting TAG. Aging at 10°C significantly lowered the enthalpy of the first peak in fresh butter ($P < 0.05$), similar to the results found for the cream (Table 1). In addition, aging at 10°C with HA significantly increased the enthalpy of the first peak in fresh butter compared with aging at 10°C with NA ($P = 0.01$), indicating that HA induces crystallization of low-melting TAG. During butter storage at 5°C for 24 h, further crystallization occurred in butter samples churned and aged at 10°C as the first peak enthalpy values increased. However, butter made with cream aged at 5°C with HA does not show much increase in enthalpy for both melting peaks after storage, indicating that most crystallization of fat occurred when fresh butter was prepared. Moreover, significantly higher total enthalpy ($P < 0.05$) was observed in fresh butter when cream was aged at 5°C with HA.

The SFC of cream shows correlation with MMF enthalpy values of fresh butter (Pearson $r = 0.977$; $P = 0.023$). The MMF enthalpy values of fresh butter are also correlated with the total enthalpy values of fresh butter (Pearson $r = 0.997$; $P = 0.003$) and 1-d-old butter (Pearson $r = 0.981$; $P = 0.019$). The MMF enthalpy values of fresh butter are also correlated with MMF enthalpy values of cream (Pearson $r = 0.966$; $P = 0.034$) and 1-d-old butter (Pearson $r = 0.970$; $P = 0.030$). Total enthalpy values are correlated with the SFC of cream (Pearson $r = 0.988$, $P = 0.012$) and the total enthalpy values of 1-d-old butter (Pearson $r = 0.988$, $P = 0.012$). The total enthalpy values are also correlated with the MMF enthalpy values of cream (Pearson $r = 0.950$, $P = 0.050$).

Hardness. Hardness of butter was measured with spreadability measurement rigs on the produced butter stored at 5°C for 24 h and stabilized at room temperature (23°C) for 1 h (Figure 6). The least spreadable butter (harder butter) was obtained from the cream sample that was aged at 10°C with LA and at 5°C with HA (Figure 6). According to Herrera and Hartel (2000b), a high number of small crystals can produce a harder material. Agitation and aging or churning temperature are influential factors that control the number of crystals. Introducing high shear rate or agitation

Table 2. Melting behavior of fresh butter and day-old butter stored at 5°C¹

Butter sample	Parameter	5°C	10°C		
		HA	NA	LA	HA
Fresh butter	T _{on-1} (°C)	9.7 ± 0.4 ^b	14.0 ± 0.3 ^a	14.7 ± 0.2 ^a	13.8 ± 0.0 ^a
	T _{p-1} (°C)	17.0 ± 0.5 ^a	16.3 ± 0.8 ^a	17.7 ± 0.0 ^a	16.7 ± 0.0 ^a
	ΔH ₋₁ (J/g of fat)	22.0 ± 0.6 ^a	3.3 ± 0.5 ^c	4.5 ± 0.9 ^{bc}	7.1 ± 0.5 ^b
	T _{on-2} (°C)	24.8 ± 0.3 ^a	24.6 ± 0.4 ^a	25.5 ± 1.0 ^a	24.2 ± 0.1 ^a
	T _{p-2} (°C)	32.0 ± 1.0 ^a	33.2 ± 1.1 ^a	34.2 ± 0.6 ^a	34.3 ± 0.1 ^a
	ΔH ₋₂ (J/g of fat)	18.2 ± 0.9 ^a	19.5 ± 0.2 ^a	18.1 ± 3.0 ^a	19.0 ± 0.2 ^a
1-d-old butter	ΔH _{total} (J/g of fat)	40.0 ± 1.5 ^a	22.9 ± 0.3 ^b	22.4 ± 3.9 ^b	25.9 ± 0.7 ^b
	T _{on-1} (°C)	9.4 ± 0.6 ^b	12.2 ± 0.1 ^a	12.2 ± 0.4 ^a	10.9 ± 0.7 ^{ab}
	T _{p-1} (°C)	16.6 ± 0.2 ^a	16.6 ± 0.1 ^a	16.4 ± 0.0 ^a	16.8 ± 0.2 ^a
	ΔH ₋₁ (J/g of fat)	22.9 ± 1.2 ^a	12.3 ± 0.2 ^b	14.7 ± 0.8 ^b	16.8 ± 1.1 ^b
	T _{on-2} (°C)	24.0 ± 0.0 ^a	24.3 ± 0.2 ^a	25.1 ± 0.0 ^a	25.4 ± 1.0 ^a
	T _{p-2} (°C)	34.2 ± 0.3 ^a	33.8 ± 0.7 ^a	34.2 ± 0.3 ^a	34.5 ± 0.6 ^a
	ΔH ₋₂ (J/g of fat)	19.3 ± 0.1 ^a	20.3 ± 1.0 ^a	17.1 ± 1.2 ^a	16.3 ± 2.5 ^a
	ΔH _{total} (J/g of fat)	42.2 ± 1.1 ^a	32.6 ± 0.8 ^a	31.7 ± 2.0 ^a	33.0 ± 3.6 ^a

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Butter was made with cream aged at 5 or 10°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) for 90 min and stored at 5°C for 24 h. Melting behavior was expressed as onset melting temperature (T_{on}) and peak melting temperature (T_p) and the change in enthalpy (ΔH). Subscript 1 indicates parameters obtained from the first melting peak, and subscript 2 refers to parameters obtained from the second melting peak. Mean values and standard deviations are reported.

induces a high number of smaller crystals. In addition, low aging or churning temperature increases the churning time because of high supercooling (Rønholt et al., 2014a). A longer churning time promotes smaller crystal formation due to longer exposure to shear (Rønholt et al., 2014a). Smaller crystals can hold more water in the network. However, this tendency was not observed in our study or in the study of Rønholt et al. (2014b). Cream aged under HA at 5°C is expected to have a higher number of smaller crystals than other cream samples. Significantly higher enthalpy was detected in the butter that was made with the cream sample aged at 5°C with HA. However, the water content of this sample was not significantly higher than that of other samples aged at 10°C, which are churned for a shorter time (Table 3). This indicates that water content was not dependent on churning temperature. The low temperature and high amount of solids explain the harder consistency of butters made with cream aged and churned at 5°C under HA conditions.

Aging at 10°C with LA produced the firmest butter. A softer butter would have been expected when aging at this higher temperature because lower SFC and bigger crystals should have been formed under this condition. This is actually the case of butter obtained from cream aged at 10°C under NA conditions. The use of agitation (LA condition) resulted in a harder butter and butter with the lowest water content (Table 3). Fresh butters processed with cream aged and churned at 10°C under LA conditions have a lower melting enthalpy for the MMF compared with butters processed at the same temperature under NA and HA conditions (Table 2). However, after storage at 5°C, all butters

had similar melting enthalpies and the butter processed under the 10°C LA condition was the one that suffered the greatest secondary crystallization during storage. This secondary crystallization might have resulted in bigger crystals that retained less water, resulting in a harder butter.

In this experiment, cream was churned until buttermilk leaked out from butter grains completely. However, only cream samples that could form butter within 14.5 min were collected for further measurement. As previously mentioned, buttermilk leaked from butter grains completely (which is equal to the churning time) at approximately 8.5 to 10 min (HA), 10.3 to 12.3 min (LA), and 11 to 14 min (NA) when cream samples were aged and churned at 10°C. Shear force by churning can induce crystallization, but it can also break the existing crystal structure and network, weakening the crystal network. Cream that was aged with NA at 10°C was churned longer than other cream samples at 10°C, resulting in the least firm butter. In summary, softer butter was observed when either NA or HA conditions were used at 10°C. This could be explained by the higher amount of water in these samples, even though no statistical correlation ($P > 0.05$) was observed between water content and hardness. In addition, hardness of butter is not statistically correlated with MMF, HMF, or total enthalpy values of cream, fresh butter, and 1-d-old butter. Further studies about the microstructure under these conditions would help explain how crystal shape and size distribution affected the hardness of butter produced in this study.

Ratio of the Amount of Butter and Buttermilk. No difference was found in the ratio of the amount of

Table 3. Water content of fresh butter¹

Measurement	5°C		10°C	
	HA	NA	LA	HA
Water content	17.5 ± 0.8 ^{ab}	18.4 ± 1.6 ^a	16.2 ± 0.6 ^b	18.4 ± 1.0 ^a
Buttermilk:butter ratio	0.9 ± 0.1 ^a	0.9 ± 0.0 ^a	1.0 ± 0.03 ^a	0.9 ± 0.0 ^a

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).
¹Butter was made with cream aged at 5 or 10°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) for 90 min and churned for 14.5 min at its aging temperature. Mean values and standard deviations are reported.

butter and buttermilk produced after churning. Previous research has shown that high churning temperature resulted in more liquid oil in buttermilk because fat is partly melted by the high temperature (Rønholt et al., 2014b). Because more liquid oil will be present in

buttermilk, it is expected to produce more buttermilk and less butter. However, this was not observed in our study.

CONCLUSIONS

This study shows that aging and churning conditions of cream, such as agitation and temperature, affect butter formation. Previous studies have shown that aging of cream at low temperature delays butter formation due to a high level of crystallized fat that inhibits the leakage of liquid fat from the oil droplet. Our study shows that butter can be formed at low temperature when agitation is applied during aging. This induction in butter formation is probably due to the induction in the crystallization of TAG with lower melting points in the cream that allow for a better and more stable fat crystalline network. In addition, this study shows that the hardness of the butter can be controlled by the aging conditions of the cream. For a constant aging temperature (10°C), harder butters were obtained when the cream was aged under LA, which probably

Figure 5. Thermal behavior of fresh and 1-d-old butter measured by differential scanning calorimetry. Cream was aged at 5 or 10°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) for 90 min; (A) churned for 14.5 min at its aging temperature; and (B) stored for 24 h at 5°C. Endo = endothermic transition.

Figure 6. Hardness (N) of butter stored at 5°C for 24 h. Butter was made with cream aged at 5 or 10°C with no agitation (NA), low agitation (40 rpm; LA), and high agitation (240 rpm; HA) and churned for 14.5 min at its aging temperature. The error bars represent the standard deviation of the mean ($n = 2$). Values sharing letters (a–c) are not significantly different ($P > 0.05$).

promoted partial coalescence in the cream and had bigger fat crystals that would retain less water. The results provide valuable information regarding the effect of cream aging temperature and agitation on lipid crystallization and how these changes influence the melting behavior and hardness of butter. Thus, this study can help the dairy industry understand the relationship between aging conditions of cream and physical properties of butter. Further research is required to explore microstructure of butter for crystallization behavior under different aging conditions and to reproduce these results in butter production at the industrial scale.

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