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Effect of cream cooling rate and water content on butter microstructure during four weeks of storage

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ABSTRACT

Crystallization, rheological properties and microstructure of butter with varying water content and subjected to different cooling rate were studied during four weeks of storage at 5 °C. Using small and large deformation rheology, the elastic modulus (G′) and Hencky strain at fracture was followed. When comparing samples with an equal water content, samples produced from fast cooled cream (7.5 °C/min) have a higher G′ compared to butter produced from slow cooled cream (0.4 °C/min), at day 1–7. However, no difference in G′ is observed as a function of time, even though the solid fat content increases. Increasing the water content from 20% to 32% decreases G′ at day 1–14, yet X-ray scattering and differential scanning calorimetry shows no difference in crystal polymorphism or crystallinity. After 21 days of storage, no difference in G′ is observed as a function of cream cooling rate or water content. For all samples, small angle X-ray scattering shows formation of 2L (41 Å) and 3L (57 Å) lamellar organization, while the wide angle spectra shows mainly β′-crystals (4.2 Å & 3.81 Å) together with traces of β (4.6 Å).

1. Introduction

In recent years one of the major challenges in food research has been to develop and create products covering the essential nutritional needs, being low fat based while maintaining an appealing texture and taste (Wassell, Bonwick, Smith, Almiron-Roig, & Young, 2010). In commercial butter-like products and spreads, vegetable oils, such as canola oil, have been added in order to obtain a soft and spreadable product (Kaufmann, Andersen, & Wiking, 2012). One way to lower the amount of fat per serving is to increase the water content in the products. The water percentage is closely related to the quality of the final product. Therefore, it has been discussed that presence of water might influence crystallization behavior, which in turn could influence the texture of the product (Vanhoutte, Dewettinck, Foubert, & Huyghebaert, 2002; Vereecken, Foubert, Meeussen, Lesaffer, & Dewettinck, 2009).

Butter, spreads and margarines are all multiphase water-in-oil emulsions, consisting of fat globules, crystalline fat and water droplets dispersed within a continuous oil phase (Juriaanse & Heertje, 1988). The fat crystal network strongly contributes to the product stability by physical stabilization of the water droplets dispersed within the fat phase, hence preventing microstructural changes (Rousseau, Zilnik, Khan, & Hodge, 2003). The organization of the fat crystals is in lamellar planes near the water and oil interfaces, almost parallel to the interface plane (Wassel et al., 2012). Both the properties of the fat crystals and the size of water droplets are crucial for the strength of the fat crystal network (Juriaanse & Heertje, 1988; Rousseau, Gosh, & Park, 2009). When the water content is increased, more interactions between the water droplets can occur and they might become deformed with a bimodal size distribution (van Dalen, 2002). Still, more knowledge is needed on how water impacts milk fat crystallization together with the colloidal stability of butter, butter-like products and spreads. Other factors, such as thermal history and storage conditions, also affect the properties of a fat crystal network (Kellens, Meeussen, & Reynaers, 1992; Rousseau, Marangoni, & Jeffreys, 1998) together with microstructure of the fat crystals (Litwinenko, Singh, & Marangoni, 2004; Narine & Humphrey, 2004).

In milk fat, the crystals primarily form three different polymorphs: α, β′ and β (Fig. 1) (Lopez, Bourgaux, Lesieur, & Ollivon, 2002). The triacylglycerol chains pack hexagonally in the α-crystals, orthorhombic in the β′-crystals and triclinic in the β-form (Chapman, 1962), in increasing order of stability. The crystalline structure formed in concentrated cream (40% fat) and anhydrous milk fat quenched from 60 °C to 4 °C has been studied during 6 days storage at 4 °C (Lopez et al., 2002). After 15 min of storage, α (4.14 Å & 4.17 Å) and β′-form (3.84, 4.26 & 4.28 Å) are formed in cream and anhydrous milk fat. In addition, traces of β-crystals (4.65 Å) were observed in the anhydrous milk fat. For long spacings, Lopez et al.

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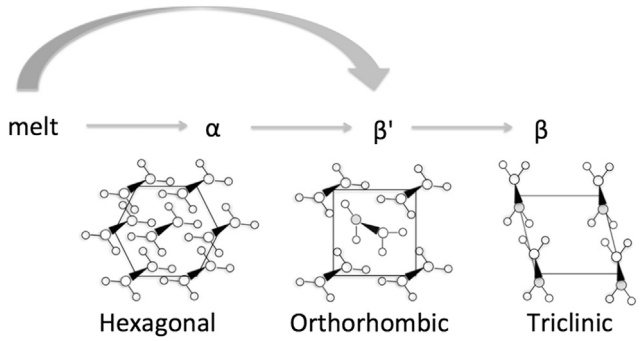

Fig. 1. Illustration of fat crystallization in milk fat. α-crystals (hexagonal subcell structure), forms directly from the melt, while β′-crystals (orthorhombic subcell) forms either via recrystallization of α to β′ or directly from the melt. β-crystals (triclinic subcell) are formed via recrystallization from β′.

(2002) observed peaks corresponding to 2L (39 Å) and 3L (70.5 Å) lamellar packing in cream. In the milk fat, a transition occurs from 3L (70.5 Å) → 2L (39 Å) and 3L (66 Å). After four days of storage, both α, β′ and β crystals are present in cream and anhydrous milk fat. In the long spacings, a coexistence of peaks corresponding to 2L (40.5 Å) and 3L (54.2 Å) is observed, demonstrating presence of a fast and slow transition in milk fat during storage at 4 °C, as more stable crystals are formed during storage.

In the present work, butter with different water content was prepared from fast and slow cooled cream. The objective of the study was to understand how water content and cream cooling rate affects the butter microstructure during storage. Therefore, butter was produced with a water content of 20%, 26% and 32% respectively. The butter was stored at 5 °C and characterized at day 1, 4, 7, 14, 21 and 28 after production. Low resolution nuclear magnetic resonance (LR-NMR) was used to study water droplet stability and solid fat content of the butter. Rheological characterization was used to quantify the effect of water content on the fat crystal network and confocal laser scanning microscopy to visualize the microstructure of the samples. Finally, the crystal polymorphism and stability was followed by small and wide angle X-ray scattering (SAXS and WAXS) and thermograms of the samples were obtained using differential scanning calorimetry.

By definition, butter contains maximum 16% of water (Codex Alimentarius, 2011). In the present study, the water content of the samples varies from 20 to 32%. However, to ease the reading all samples produced in this study will be referred to as butter.

2. Materials and methods

2.1. Materials

Pasteurized cream (38% fat) from ARLA Foods (Denmark) was used to butter manufacturing. To prevent microbial growth, 0.2 g/L sodium azide from Sigma Aldrich (St Louis, USA) was added. For the confocal laser scanning microscopy fluorescein-5-isothiocyanat (FITC) (Merck, Damstadt, Germany), Nile red and 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indodicarbocyanine perchlorate (D307) (Molecular Probes, Paisley, UK) were used as fluorescent dyes.

2.2. Sample preparation

The butter samples were prepared in laboratory scale, all in triplicate. Two parameters were varied: the water content (20%, 26% and 32%) and the cream cooling rate (fast cooling at 7.5 °C/min and slow cooling at 0.4 °C/min) resulting in six different samples. A laboratory-scale butter making method developed in a previous study was systematically applied (Rønholt, Kirkensgaard, Pedersen, Mortensen, & Knudsen, 2012). First, the cream was kept at 65 °C for 10 min to erase all crystal memory. Afterward, the cream was cooled either slow or fast to a churning temperature of 10 °C. Finally, the cream was subjected to phase inversion at room temperature in a kitchen machine (CombiMax 600, Braun, Kronberg, Germany), equipped with a 2.0 L work bowl and a universal chopping blade. Churning time was defined as the time from the start of churning to phase inversion. The churning was considered complete, when butter grains appeared together with a liquid phase, the buttermilk. Water was then squeezed out of the butter to reach the desired water content. Finally, the butter was packed in plastic containers intended for butter, to avoid moisture loss during storage and placed at 5 °C in a refrigerator.

2.3. Water content

For each measurement, 5 g sample was spread on small pumice stones, placed in a porcelain crucible, in order to enhance water evaporation. The samples were placed in an oven at 100 °C for 2 h followed by 30 min in an exicator at room temperature. The procedure was continued until a stable weight was achieved e.g. all water was evaporated. Water content was obtained as the % difference in weight/weight before and after evaporation of the water. Water content (dry matter) was measured in duplicate on day 1, 4, 7, 14, 21 and 28 after production.

2.4. Low resolution nuclear magnetic resonance

Solid fat content and water droplet size distribution were determined during 28 days of storage at 5 °C. The measurements were conducted using a Bruker wide line low resolution nuclear magnetic resonance system (LR-NMR) (Bruker Minispec mq 20, Bruker Optik GmbH, Ettlingen, Germany) equipped with a pulsed gradient field unit, operated at 5 °C. The samples were prepared by punching a cylindrical glass (0.8 cm in diameter) into the sample at random locations, as described by Rousseau et al. (2009). Then, the samples were placed in an NMR tube. At least three replicates were prepared for each sample. The size is given by the volume-weighted geometric mean diameter ($d_{3,3}$), as defined by Alderliesten (1990). The determination of solid fat content by LR-NMR is possible since the transverse magnetization of solid fat decays faster than oil, resulting in faster spin–spin relaxation time for solid fat compared to oil (Balinov, Mariette, & Söderman, 2004). Even though the relaxation of water is faster than for oil, it is not possible to distinguish between their contributions to the signal (Balinov et al., 2004). Thus, the measured liquid content is the sum of water and oil. However, as the percentage water content is known for all samples in the present work, the reported solid fat content is corrected for the amount of water in each sample. In this way, the solid fat content represents the amount of solid fat relative to liquid fat.

2.5. Small deformation rheology

An AR G2 Rheometer (TA Instrument, West Sussex, England) equipped with a plate–plate geometry was used for all measurements, as described by Rønholt et al. (2012). Both upper and lower plate are temperature controlled and with serrated surfaces (25 mm in diameter). The frequency sweeps were performed in an interval of 500–0.05 rad/s divided into 21 steps. The oscillation stress was held constant at 500 Pa. The stress sweeps were performed in an interval of 1–800 Pa divided into 21 steps. Angular frequency was constant at 1.0 rad/s. All measurements were performed within the linear viscoelastic region (data not shown) at 10 °C. Cylindrical samples (25 mm in diameter) were punched out

from all samples and cut to three discs, 4 mm in height, using a wire cutter. Results are the average of three runs.

2.6. Large deformation rheology

Stress and Hencky strain at fracture was measured by uniaxial compression using a texture analyser (Instron 6654, Massachusetts, USA) equipped with a plate–plate geometry (70 mm in diameter). 9 cylindrical samples with a radius of 15 mm were punched out from each batch, and cut to a height of 15 mm using a wire cutter. All samples were stored at 5 °C until tested. Stress and Hencky strain were calculated from the compression curves.

2.7. Confocal laser scanning microscopy

A confocal laser scanning microscope (SP5) from Leica Microsystems, Wetzlar, Germany, was used to study the microstructure of the samples. The krypton/argon and helium/neon laser was used together with a 63× magnification water immersion objective. The dyes, FITC, Nile red and D307 (0.01% (volume/volume) solution), were immersed on a cooled object glass until the solvent evaporated. Thereafter, the samples were placed on the glass slides and equilibrated at 5 °C for 30 min, as described by Buldo and Wiking (2012).

2.8. X-ray diffraction

X-ray scattering was performed at the SAXSLAB instrument (JJ-Xray, Denmark) installed at the University of Copenhagen. The instrument is equipped with a 100XL + micro-focus sealed X-ray tube from Rigaku and a 2D 300K Pilatus detector from Dectris. Measurements were performed with a pin-hole collimated beam with the detector positioned asymmetrically to yield a single measurement q-range of 0.05–2.8 $Å^{-1}$. The magnitude of the scattering vector is defined by $q = 4\pi/\lambda \sin\theta$, where $\lambda = 1.54$ Å is the X-ray wavelength and θ is half of the scattering angle. In this setting small and wide angle X-ray scattering (SAXS and WAXS) are measured simultaneously so that all relevant peak information for both short and long spacings can be obtained in a single measurement. The d-spacings are calculated as $d = 2\pi n/q^*$, where q^* is the Bragg peak position and n the order of the Bragg reflection. The samples were loaded at 5 °C in cooled sample holders and sealed between 5 and 7 μm thick mica windows. The background scattering from the mica was subtracted from the sample spectra. The cooled sample holders were loaded onto a temperature controlled sample stage from Linkam.

2.9. Differential scanning calorimetry

Differential scanning calorimetry measurements were performed using a Mettler Toledo differential scanning calorimeter (Mettler Toledo, Greifensee, Switzerland) in order to study the crystallization kinetics. The samples were held at 5°C for 10 min, followed by heating to 65 °C using a scan rate of 2 °C/min. Sample masses were 20–30 mg. An empty sealed pan was used as a reference.

2.10. Statistical analysis

The experimental design was multifactorial, using a split-plot with three replicates. Water content and cream cooling rate make up the whole plot, while storage time is the subplot treatment. The data was analyzed accordingly: a one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison tests were carried out using GraphPad Prism (Version 5.02, GraphPad Software, Inc., La Jolla, CA, USA). ANOVA was applied on the repeated measurements (at least triplicate) of all samples using the following as variables: water droplet size, solid fat content, water content, Hencky strain at fracture and elastic modulus (G′) at 5 rad/s. Furthermore, the variation within each sample was analyzed using ANOVA. To improve clarity, only statistically significant results are reported.

3. Results

3.1. Sample preparation

Table 1 shows churning time for fast and slow cooled cream. Fast cooled cream have a longer churning time compared to slow cooled cream.

3.2. Water content and low resolution nuclear magnetic resonance

Fig. 2 shows a plot of evolution in solid fat content (A), water droplet size (B) and water content (C) during storage. Accordingly, the statistical analysis is listed in Table 2. For the solid fat content and water droplet size, no significant differences are observed between the samples. For solid fat content, an increase is observed in all samples as a function of time (Fig. 2A, Table 2), as is the case for water droplet size (Fig. 2B, Table 2).

No difference in water content was observed as a function of cooling rate. Furthermore, only samples containing 26% water have water loss during storage (Table 2 & Fig. 2C).

3.3. Small and large deformation rheology

Fig. 3 shows the development in Hencky strain at fracture during storage. No significant differences in Hencky strain at fracture are observed as a function of cream cooling rate or water content. During storage however, an increase is observed in Hencky strain at fracture from day 1–4. From day 7–28, however, there is a tendency toward a decrease in Hencky strain at fracture.

Fig. 4 shows the elastic modulus (G′) during storage, with the statistical analysis listed in Table 3. Comparing the different samples within one day, the same phenomenon is observed at all days of measurement: increasing water content decreases G′ (indicated with an arrow in Fig. 4). Furthermore, the samples produced from fast cooled cream (20% water) have a higher G′ compared to butter produced from slow cooled cream (20% water) at day 1–7, while the same is observed for the samples containing 32% water, but for day 21 and 28. During storage, no significant changes in G′ is observed.

3.4. Confocal laser scanning microscopy

Fig. 5 shows representative confocal laser scanning microscopy images of the butter microstructure. Since fat crystals are color negative, they appear as dark shadows in the images. Comparing images at day 1 with day 28, an increasing number of fat crystal clusters can be observed (more dark shadows, as indicated with an arrow).

Table 1
Churning time for fast and slow cooled cream.

Sample	Churning time (min:sec)
Fast cooled cream	3:40 ± 0:12
Slow cooled cream	2:00 ± 0:10

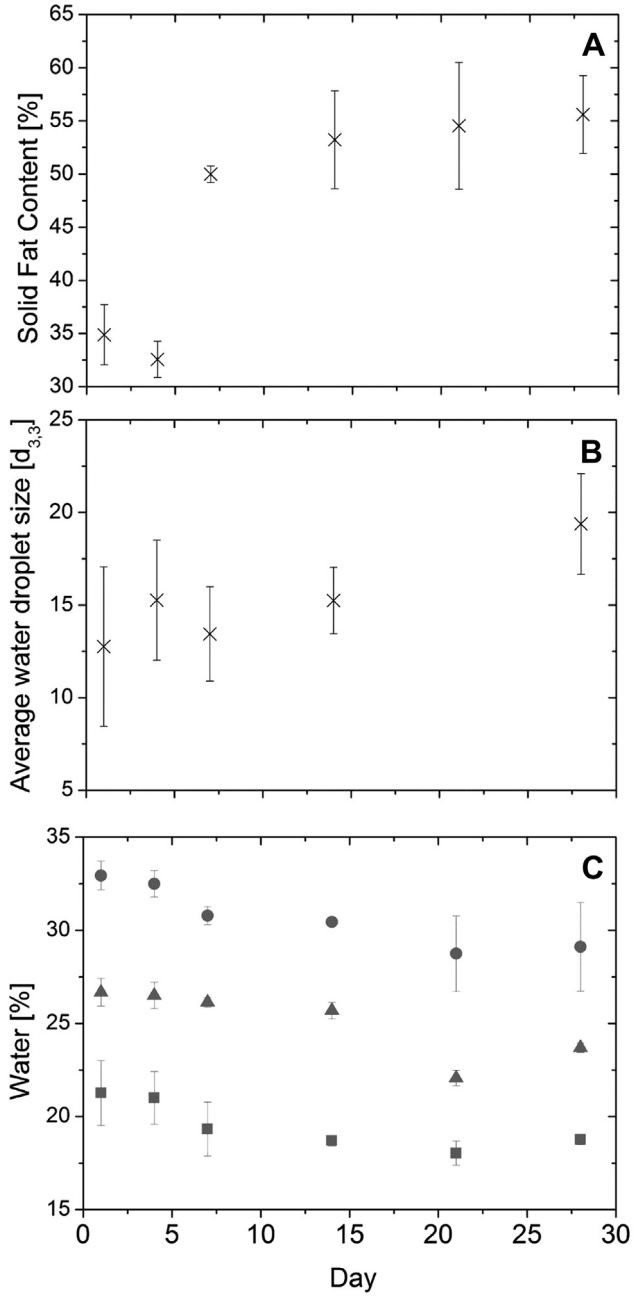

Fig. 2. Solid fat content (A), water droplet size (B) and water % (C) measured in the butter at day 1, 4, 7, 14, 21 and 28 after production. In graph A and B, no differences were observed between the samples. x represents the average of all six samples (fast and slow cooled cream with 20%, 26% or 32% water). The solid fat content (A) is corrected for water content, and reflects the solid fat vs. oil ratio. In graph C, no difference was observed between fast and slow cooled cream with respect to water content. ■ The symbols represent average of slow and fast cooled cream with ■ 20%, ▲ 26% and ● 32% water.

3.5. X-ray diffraction

Fig. 6 summarizes the time evolution of both small and wide angle X-ray scattering (SAXS and WAXS) as measured at day 1, 4, 7, 14, 21 and 28 post production. The SAXS and WAXS data reveals similar spectra for all samples (Fig. 6, bottom graph). At wide angles, two strong peaks are observed at 4.2 Å and 3.81 Å, which corresponds to β′-polymorph, as shown in Fig. 1 (Lopez, Lesieur, Bourgaux, Keller, & Ollivon, 2001). Furthermore, a small peak at 4.6 Å is observed, corresponding to traces of β (Lopez, Lesieur, et al., 2001). At small angles, a peak at 41 Å indicating double layer (2L) packing of the triacylglycerols is observed together with at peak at 57 Å, corresponding to a triple layer (3L) packing (Lopez, Lesieur, et al., 2001). From day 1–28, no changes are observed in the WAXS pattern (Fig. 6, right). In the SAXS spectra, an increase in the 3L peak (57 Å) is observed during storage (Fig. 6, left).

Table 2
ANOVA conducted on solid fat content (SFC) (corrected for water), water droplet size ($d_{3,3}$) and water content within all samples, by comparing day of storage. No differences is observed between the samples within the same day of measurement for SFC and $d_{3,3}$. With respect to water content, no differences is observed between fast and slow cooled samples when comparing samples with 20%, 26% and 32% water respectively.

Cooling rate	H_2O %	Var.	Day					
			1 vs 0.4	4 vs. 7	7 vs. 14	14 vs. 21	21 vs. 28	14 vs. 28
Fast	20	SFC	***	***	***	***	***	–
		$d_{3,3}$	*	ns	ns	–	–	ns
		water	ns	ns	ns	ns	ns	–
	26	SFC	***	***	***	***	***	–
		$d_{3,3}$	ns	ns	*	–	–	ns
		water	ns	ns	ns	**	ns	–
	32	SFC	ns	***	**	**	**	–
		$d_{3,3}$	***	***	***	–	–	ns
		water	ns	ns	ns	ns	ns	–
Slow	20	SFC	***	***	**	***	***	–
		$d_{3,3}$	*	ns	ns	–	–	*
		water	ns	ns	ns	ns	ns	–
	26	SFC	***	***	***	***	***	–
		$d_{3,3}$	ns	ns	ns	–	–	*
		water	ns	ns	ns	**	ns	–
	32	SFC	ns	***	ns	***	*	–
		$d_{3,3}$	ns	ns	ns	–	–	*
		water	ns	ns	ns	ns	ns	–

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = no significant difference.

3.6. Differential scanning calorimetry

Fig. 7 shows the heat flow during melting of butter. The shown samples contained 32% of water and were produced from fast cooled cream. The first endothermic event in the thermograms occurs around 17 °C, corresponding to melting of β′-crystals (Lopez,

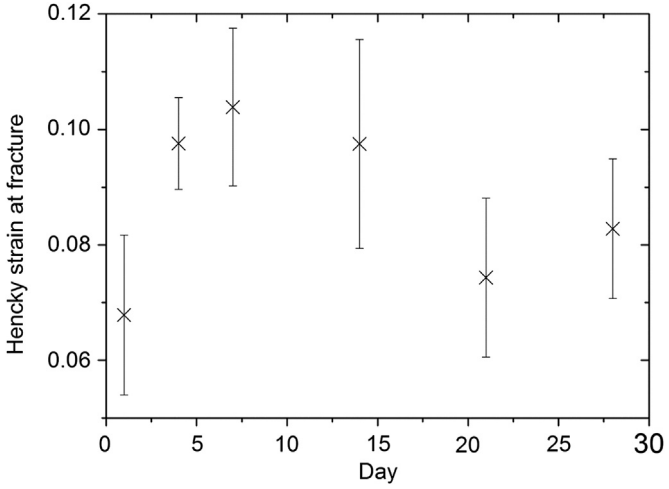

Fig. 3. Hencky strain at fracture measured in all samples during storage, using uniaxial compression test. During storage, an increase is observed from day 1–4. However, no differences are observed as a function of cream cooling rate or water content. x represents the average of all six samples (fast and slow cooled cream with 20%, 26% or 32% water).

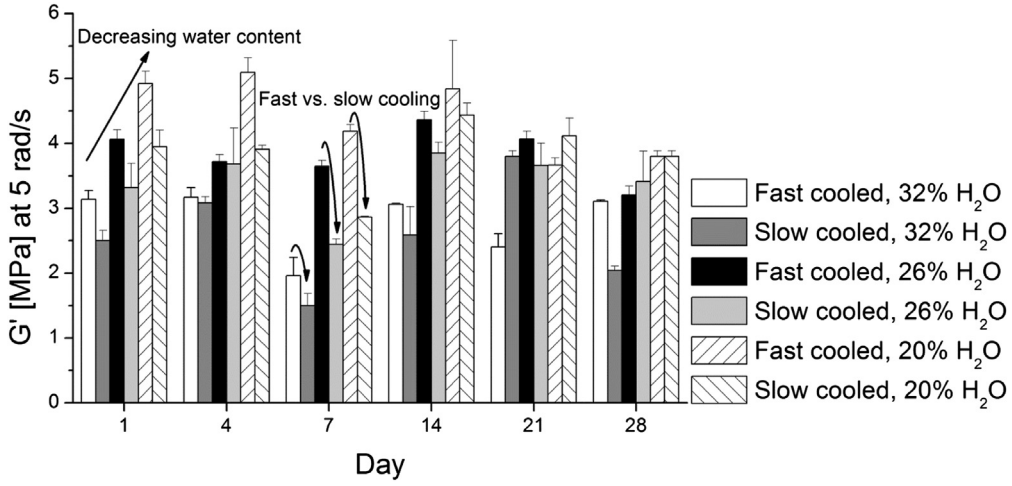

Fig. 4. Elastic modulus, G′, of the samples measured at 5 rad/s. The samples were produced from either slow cooled (0.4 °C/min) or fast cooled (7.5 °C/min) cream, with a final water percentage of either 20, 26 or 32. The samples are measured on day 1–28 after production.

Lavigne, Lesieur, Bourgaux, & Ollivon, 2001). Most likely, those endothermic peaks are the low- and middle-melting fraction of the milk fat (Lopez, Lavigne, et al., 2001; Lopez, Lesieur, Bourgaux, & Ollivon, 2005; Lopez, Lesieur, et al., 2001). The fraction of high melting triacylglycerols melt around 23 °C, corresponding to both β′- and β-crystals (Lopez, Lavigne, et al., 2001). No significant change occurs in thermograms as a function of either time or water content.

4. Discussion

4.1. Effect of cream cooling rate

As a result of the cream cooling rate, a remarkably large difference in churning time during sample preparation is observed between the fast and slow cooled cream (Table 1). Most likely, the shorter churning time can be explained by presence of larger crystals within oil droplets in slow cooled cream compared to the crystals in fast cooled cream. During churning, the crystals work as eroding agents penetrating the milk fat globule membrane. Larger crystals will facilitate more rupture of the milk fat globule membrane; hence in turn facilitate phase inversion from oil-in-water to a water-in-oil emulsion (Boode, Walstra, & de Groot-Mostert, 1993).

It is known, that fast cooling favors formation of small crystals, hence a firmer crystal network compared to slow cooling, where larger crystals with less contact points are formed, resulting in a soft network (Wiking, De Graef, Rasmussen, & Dewettinck, 2009). This confirms our finding, as samples produced from fast cooled cream had a higher elastic modulus (G′) compared to those produced from slow cooled cream, at day 1–7 (Fig. 4 (marked with an arrow) & Table 3).

Table 3
The elastic modulus, G′, at 5 rad/s were analyzed using ANOVA. As an example, the difference between samples is shown for day 7.

Day 7	H_2O %	Fast			Slow		
		20	26	32	20	26	32
Fast	20	–	–	–	–	–	–
	26	ns	–	–	–	–	–
	32	***	***	–	–	–	–
Slow	20	***	*	**	–	–	–
	26	***	***	ns	ns	–	–
	32	***	***	ns	***	**	–

*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = no significant difference.

4.2. Effect of water content

The strength of a fat crystal network depends crucially on the water droplet size and the amount of crystallized fat (Juriaanse & Heertje, 1988). To prevent destabilization of a fat network, such as butter, a minimum amount of 9% solid fat content is needed (Rousseau et al., 2003). Below this value, the water coalesces (Rousseau et al., 2003). Our results show an increase in solid fat content during storage. Between samples, no significant difference is observed in the ratio of solid fat relative to oil (Fig. 2A and Table 2). It is not solid fat content itself, but the ratio between solid and liquid (both water and liquid oil) that strongly affects the hardness and spreadability of a butter-like product (Pothiraj, Zuñiga, Simonin, Chevallier, & Le-Bail, 2012). As the water content is increased in the present study, the ratio of solid fat relative to the liquid phase (oil and water) shifts toward more liquid. Consequently, less fat contributes to the fat crystal network, hence directly affecting product firmness (Fig. 4) as well as indirectly affecting water droplet stability (Mulder & Walstra, 1974). The stability of the water droplets was measured using low resolution nuclear magnetic resonance (LR-NMR) (Fig. 2B). The results show no differences in water droplet size between samples (Table 2), despite an increase in water droplet size during storage for the samples containing 26% of water. It can therefore be concluded, that varying the water content from 20% to 32% does not alter the stability of the fat crystal networks ability to stabilize the water droplets.

Still, it is not fully understood to which extent presence of water influences crystallization behavior (Vanhoutte et al., 2002; Vereecken et al., 2009). The results reported in the present study demonstrates that increasing the water content in butter from 20% to 32% decreases the modulus and thus hardness of the product from day 1–14 (Fig. 4), without affecting crystal polymorphism (Fig. 6). In addition, the obtained thermograms are similar for all samples, at the same day of measurement (Fig. 7), suggesting that water does not affect the thermal behavior of the fat crystals.

4.3. Crystal polymorphism

Milk fat crystallizes in either α, β′ or β-form, in increasing order of stability (Chapman, 1962). In the present study, the X-ray data shows, in all samples, primarily formation of β′-crystals with traces of β (Fig. 6). This is in agreement with the literature, that reports presence of β′-crystals together with traces of β in anhydrous milk

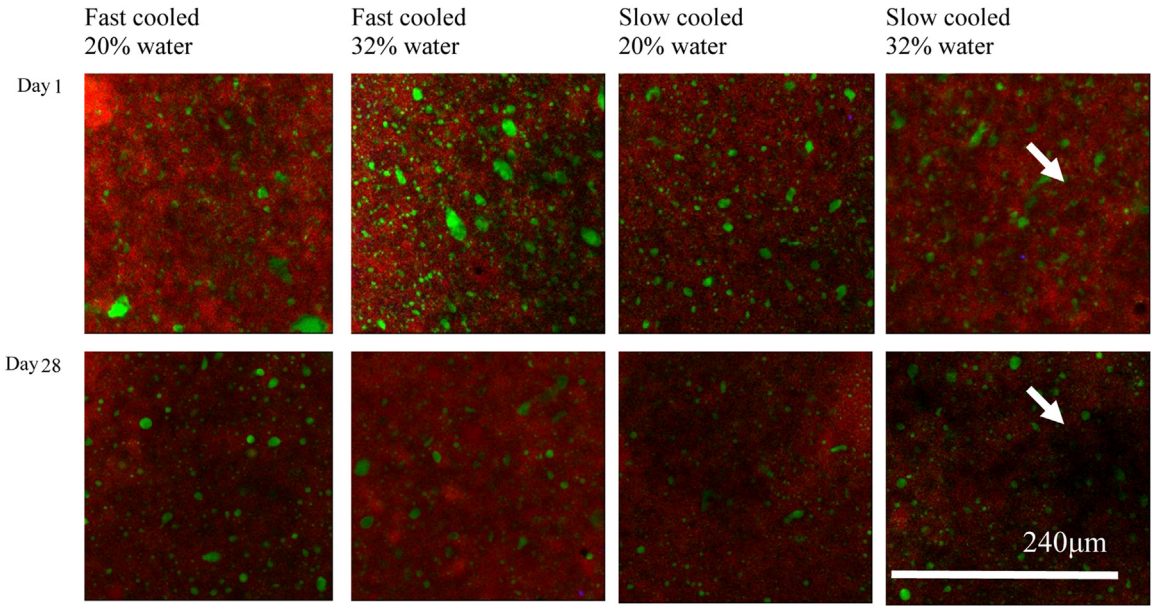

Fig. 5. Images of butter samples captured using confocal laser scanning microscopy. The vertical numbers are days after production. The water phase (green) is colored with FITC, the fat phase (red) is colored using Nile red and phospholipids (blue) with DiD oil. The fat crystals are color negative, and therefore appears as dark shadows (indicated with a white arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fat and in cream after four days of storage at 4 °C (Lopez et al., 2005). Lopez et al. (2005) observes a transition in milk fat during storage, as more stable crystal forms are formed. A recent study focused on the effect of cream heat treatment on polymorphism. In both fast and slow cooled cream, mainly β′-crystals are found with minor traces of α and β (Rønholt et al., 2012). In the present study, after only one day of storage at 5 °C, no α-crystals are present. This indicates that a transition from α → β′ occurs between the time where the cream reached 10 °C during preparation and until day 1 after production. Still, further studies are needed to identify exactly when the recrystallization occurs. During further storage, no polymorphic transitions occur (Fig. 6). As no α-crystals are present, no formation from α- to β′-form can occur. For the long spacings, an increase in 3L is observed during storage, indicating that the samples get slightly more ordered during storage.

4.4. Texture during storage

An increase in solid fat content is observed during the 28 days of storage. This corresponds to formation of a more dense crystal

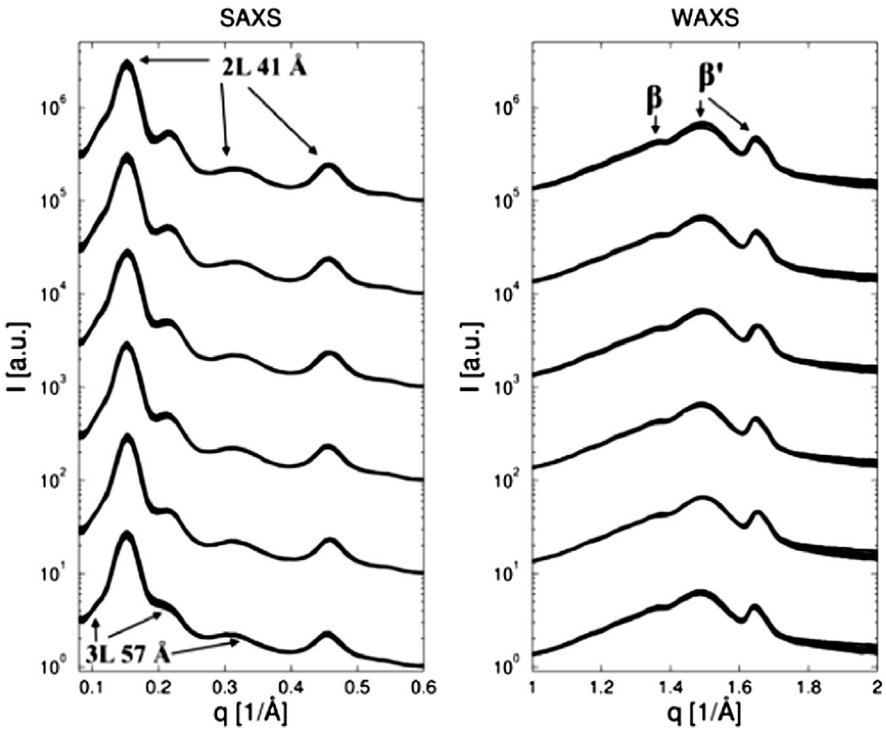

Fig. 6. SAXS (left) and WAXS (right) spectra from all samples from day 1 (bottom curve) to day 28 (top curve) after production. Long spacings (1st, 2nd and 3rd order peaks are indicated): 41 Å 2L packing and 57 Å 3L packing (1st, 2nd and 3rd order peaks are indicated). Short spacings: β-4.6 Å and β′ - 3.81 Å and 4.2 Å.

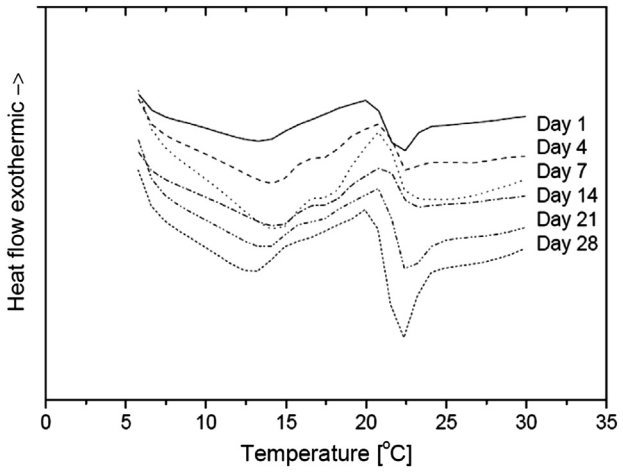

Fig. 7. DSC melting curve of butter produced from fast cooled cream, containing 32% of water. The sample was measured on day 1, 4, 7, 14, 21 and 28 after production. The temperature ramp was at 2 °C/min.

network upon 28 days of storage, compared to day 1, as observed using microscopy (Fig. 5). However, no increase in G′ is observed as a function of time, yet Hencky strain at fracture increases from day 1–4 (Figs. 3 and 4). Litwinenko et al. (2004) studied the effect of adding glycerol or Tween 60 to a mixture of triolein and fractionated milk fat. They show that even though the solid fat content differs between the samples when adding 3% glycerol/Tween 60, no difference in breaking force is observed. This behavior is explained by differences in crystal size and crystal-melt interfacial tension (Litwinenko et al., 2004). Accordingly, Narine and Humphrey (2004) demonstrates, that solid fat content is not necessarily predictive of final hardness of shortenings, neither is the microstructure.

The effect of storage time is discussed in a recent study, where the texture of spreads was monitored during 9 days of storage at 4 °C (Pothiraj et al., 2012). They observe a small increase in hardness only within the first two days of storage, thereafter the crystal particles are trapped in a rigid fat crystal network. In the present work, no difference is observed in neither G′ nor Hencky strain at fracture as a function of neither cream cooling rate nor water content after 21 days of storage (Figs. 3 and 4). In accordance, no further increase in solid fat content is observed (Fig. 2A) and the microstructure of the samples is similar (Fig. 5). After 21 days of storage, the crystal clusters are at their ideal size, hence the initial difference in size according to the cream cooling rate is eliminated. Likewise, the increase in G′ observed at day 1 when decreasing the water content is diminished after 21 days of storage. At this point the fat crystal network is fully developed (no increase in solid fat content or polymorphic changes), and the rheological behavior is no longer strongly affected by water content.

During storage, however, an increase in variation of G′ within samples is observed, as more outliers appear in the dataset. A similar increase in variation is observed by Pothiraj et al. (2012), during a 9 days aging study of spreads. Likely, this increased variation between samples can be explained by the tendency observed in Hencky strain at fracture data from day 14–28, indicating that the samples become more brittle after 14 days of storage (Fig. 3). From Fig. 2C, a water loss is identified during the four weeks of storage, and average water droplet size increases during storage (Fig. 2B). This decrease in moisture and water migration within the sample might explain why the butter gets more brittle after two weeks of storage. While the samples become more brittle, the risk of small cracks within the samples increases, hence a larger standard deviation in G′.

5. Conclusions

This study shows an increase in solid fat content during 28 days of storage, resulting in a denser fat crystal network, as observed in the confocal laser scanning microscopy images. Even though more solid fat is formed, the elastic modulus (G′) remains constant during storage. Contrary, Hencky strain at fracture increases from day 1–4, signifying a decrease in brittleness. Those findings show that solid fat content and microstructure is not necessarily predictive for the hardness and brittleness of the final product. Changing the water content in butter affects the rheological properties of the butter, as an increase in the water content decreases the modulus and thus hardness of butter within the first 14 days of storage. In addition, changing the cooling rate of the cream clearly affected the rheological properties of the butter, as butter produced from fast cooled cream had a higher G′ compared to butter produced from slow cooled cream, during the first 14 days of storage. However after 21 days of storage, G′ appears the same for all samples independently of cream cooling rate and water content. Also, the nanostructure seems unaffected when changing the water content from 20% to 32%; in all samples, mainly β′-crystals is observed together with traces of β. Furthermore, no differences were observed in polymorphism of the milk fat crystals as a result of cooling rate. Likewise, identical water content is observed in butter produced from fast and slow cooled cream respectively.

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