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Polymorphism, microstructure and rheology of butter. Effects of cream heat treatment

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ABSTRACT

The effect of cream heat treatment prior to butter manufacturing, fluctuating temperatures during storage and presence of fat globules vs. no fat globules was examined in laboratory scale produced butter. X-ray diffraction and differential scanning calorimetry was used to study crystallization behaviour and nuclear magnetic resonance to measure solid fat content and water droplet size distribution. Furthermore, the crystal structure was linked to the rheological properties and microstructure of the butter using confocal laser scanning microscopy. Butter produced from non-matured cream mainly formed α- and β′-crystals with minor traces of β-crystals. Maturing of the cream caused a transition from α- to β′- and β-form. The rheological behaviour of slow cooled butter deviated from the matured ones by having a lower elastic modulus, caused by a weaker crystal network. Presence of fat globules did not affect the rheological properties significantly.

1. Introduction

Butter is produced by a mechanical phase inversion of cream, an oil-in-water emulsion, to reach a water-in-oil emulsion. More precisely, butter consists of a continuous fat phase in which water droplets; fat globules and a network of fat crystals are dispersed. The fat crystal network is essential, since it determines the spreadability, appearance and mouthfeel of the butter and is strongly related to the butter composition and overall structure. The ratio between the solid and liquid fat is of outmost importance for the rheological properties of butter and spreads: without solid fat, butter is fully liquid. Without liquid fat, the butter would appear hard and brittle (Narine & Marangoni, 1999). Even though the solid fat content is the same, fat can have very different physical characteristics (Haighton, 1965; Shama & Sherman, 1970). Since a greater part of the solid fat is inside the fat globules, not all fat crystals are able to form a network outside the globule. Due to the large volume fraction of fat globules in butter, their presence is thus believed to influence the firmness of the product although results are not conclusive as to what extent (Fedotova & Lencki, 2010; Mulder & Walstra, 1974).

Recently, there has been an increasing awareness on the nutritional aspects of milk fat. However, before changing fat content (and potentially the microstructure) of the butter, it is essential to gain more information on how the individual parameters, such as presence of milk fat globules, cream heat treatment and fluctuating temperature during storage, all contribute to the textural properties of butter. Therefore, we aim to simulate the industrially applied continuous butter making process (Fritz-method) to gain knowledge of the structural and rheological properties arising from such conditions. In the Fritz-method the cream is separated into buttergrains and buttermilk in a churning cylinder, followed by processing of the buttergrains and finally evacuation (Frede & Buchheim, 1994). Our aim was to investigate the effect of cream heat treatment on the final butter texture. Hence, we study how the thermal history of the cream affects rheological properties, solid fat content, microstructure and the crystallization characteristics of the butter, i.e. the structural organization of the solid fat. Further, we compare samples with and without fat globules.

The exact crystallization characteristics of the fat is influenced by many factors such as the way in which the sample is cooled from the bulk fat (Herrera & Hartel, 2000a, 2000b, 2000c; ten Grotenhuis, van Aken, van Malassen, & Schenk, 1999) and the mechanical treatment (Heertje, 1993). Also, the broad range of triacylglycerols found in milk fat results in different polymorphic forms as a result of varying chain length and degree of saturation of fatty acids. The polymorphism of the various constituents can be identified by X-ray diffraction and has been the topic of numerous studies (Fredrick et al., 2011; Lopez, Lavigne, Lesieur, Keller, & Ollivon, 2001; Lopez, Lesieur, Bourgaux, & Ollivon, 2005; Lopez et al., 2002; ten Grotenhuis et al., 1999; Wiking, De Graef,

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Rasmussen, & Dewettnick, 2009). The main structural characteristic of the molecular arrangements of triacylglycerols is that they pack into lamellar structures in the longitudinal direction; most often in stacks either two or three fatty acids long (termed 2L or 3L respectively). The chains lie perpendicular to the lamellae planes (possibly tilted) and the in-plane packing of the chains gives rise to various polymorphic forms. The three most important of these found in fats are denoted α, β′ and β in order of increasing stability (Mazzanti, Marangoni, & Idziak, 2009). The different polymorphic forms are characterized by the short and long *d*-spacings of their crystal lattice which constitutes an identifying structural fingerprint (Larsson, 1966; ten Grotenhuis et al., 1999; Vaeck, 1960). To determine this fingerprint and describe the crystal structure one needs knowledge of both the lateral packing and the longitudinal stacking.

In previous studies, the polymorphic characteristics of milk fat are typically studied in model systems with focus on the effect of temperature treatment and processing conditions on the crystallization kinetics. In the present study, we study polymorphism, microstructure and rheology of butter. Moreover, we study the crystal polymorphism in cream, subjected to different temperature treatments prior to butter making. We quantify the properties of the butter using a variety of characterization tools. Small and Wide Angle X-ray Scattering (SAXS and WAXS) is to describe the crystallization state before (i.e. of the cream) and after butter manufacturing. Further, we combine the SAXS and WAXS with Differential Scanning Calorimetry (DSC) to follow the thermal evolution of the crystallinity on subsequent heating. Light scattering is used to study milk fat globule size and zeta potential. Rheological measurements are used to quantify the mechanical properties of the fat crystal network and confocal laser scanning microscopy to visualize the microstructure of the butter. Finally, using Low Resolution Nuclear Magnetic Resonance (LR-NMR) we measure the water droplet size distribution and solid fat content of the butter.

2. Materials and methods

2.1. Materials

Cream (38% fat) and skimmed milk (0.1% fat) were collected from the local supermarket. They were all from ARLA Foods Dairy in Slagelse, Denmark. Sodium azide from Sigma Aldrich, St. Louis, USA was added to avoid microbial growth (0.2 g/L cream). Anhydrous milk fat from ARLA Foods, Götene, Sweden was used for the reference samples. Fluorescein-5-isothiocyanat (FITC) (Merck, Damstadt, Germany), Nile red and 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indodicarbocyanine perchlorate (D307) (Molecular Probes, Taastrup, Denmark) was used as fluorescent dyes for the confocal laser scanning microscopy images.

2.2. Sample preparation

Five butter samples with differing cream heat treatment were prepared in laboratory scale in triplicate. Furthermore, three reference samples of anhydrous milk fat and skimmed milk were prepared, also in triplicate, and subjected to different heat treatments according to the butter samples. The various treatments were: slow cooling (butter and reference), fast cooling (butter and reference), slow cooling and maturing (butter only), fast cooling and maturing (butter only) and finally fast cooling and storage at fluctuating temperatures (butter and reference). In order to control the heat treatment of the cream, a laboratory-scale butter making method was developed and systematically applied. To erase all crystal memory the cream was heated to 65 °C for 10 min followed by either fast (7.5 °C/min) or slow cooling (0.4 °C/min) to churning temperature (10 °C). For the matured samples, the cream was stored at 5 °C for 48 h. Non-matured samples were prepared from the cream immediately after reaching 10 °C. The samples stored at fluctuating were produced from fast cooled cream, and after manufacturing stored at 5 °C for 3 h, 20 °C for 3 h, followed by nine cycles of 1 h at 5 °C and 1 h at 20 °C. The cream was subjected to phase inversion in a kitchen machine and worked in a food grinder (Beem, Gigant ES-10/12) followed by vacuum treatment to remove air. The reference samples were prepared from anhydrous milk fat melted at 65 °C for 15 min and skimmed milk according to the water content in the butter samples. It should be noted, that butter by definition contains maximum 16% of water. In this work the water content varies from 24.6% to 27.3% (w/w). Even though our samples do not meet the formal requirements we will still refer to the samples as butter.

2.3. Light scattering

Initially, the milk fat globule size and zeta potential of the cream was measured using light scattering (Malvern Mastersizer connected to a 50 ml stirring unit and Malvern Zetasizer, Malvern Instruments Ltd., Malvern, UK). For determination of zeta potential the refractive index (RI) of the cream must be known. For all samples it was assumed that the RI was 1.39 (Calhoun, Maeta, Roy, Bali, & Bali, 2010). The zeta potential generated from the samples is a combination of the signal from the fat globules as serum phase in which they are dispersed. Consequently, the signal obtained from the serum phase must be subtracted to get the zeta potential of the fat globules. A serum phase was therefore prepared by centrifugation at 16,100 rpm for 1 h at 4 °C (SL16R centrifuge from Holm&Halby, Brøndby, Denmark). The serum phase was removed with a syringe. Any proteins remaining in the bottom of the centrifuge tube were removed and redispersed in the plasma phase during ultrasonication for 30 min followed by centrifugation at 3400 rpm for 0.5 h, as described by Wade and Beattie (1997). All measurements were done in triplicate on samples diluted 1:10 with water. The distributions of fat globule sizes were derived from measurements on cream diluted in deionised water. The volume-surface mean diameter ($d_{3,2}$) was calculated using the Malvern Mastersizer software. The measurements of zeta potential and globule sizes were conducted the day the samples were prepared.

2.4. Dry matter

Water content (dry matter) was measured in duplicate on all samples. The samples were placed in an oven at 100 °C for 2 h followed by 30 min in an exicator at room temperature. The water content was calculated as the % w/w difference before and after heating.

2.5. Conductivity

Conductivity was measured with a hand-held conduct meter in the final butter sample (Cond.330i/SET, WTW Wissenschaftlich-Technische Werstätten GmbH, Weilheim, Germany). The results are the average of three measurements.

2.6. Low resolution nuclear magnetic resonance

The solid fat content and water droplet size distribution were determined in all butter and reference samples using a Bruker wide line LR-NMR system (Bruker Minispec mq 20, Bruker Optik GmbH, Ettlingen, Germany) equipped with a pulsed gradient field unit, operated at 5 °C. The samples were obtained by pressing the NMR tubes (0.8 cm in diameter for water droplet size distribution

and 0.5 cm in diameter for solid fat content) to a height of 2 cm into the samples at random locations by hand, as described by Rousseau, Gosh, and Park (2009). The samples were loaded carefully to avoid any air space. The shown results are the average of three runs. The size distribution is given by the volume-weighted geometric mean diameter ($d_{3,3}$), as defined by Alderliesten (1990).

2.7. Rheology

An AR G2 Rheometer (TA Instrument, West Sussex, England) equipped with a temperature controlled fluted cup (radius 30 mm) and vane (radius 28 mm, height 42 mm) geometry was used in all measurements. Time sweeps were conducted for 21 h at 5 °C (frequency 1 rad/s, strain of 0.1%) and 3 h at 5 °C, 3 h at 20 °C followed by nine cycles of 1 h at 20 °C and 1 h at 5 °C for the sample stored at fluctuating temperatures. The frequency sweeps were performed in an interval of 500–0.05 rad/s divided into 21 steps. The critical strain was constant at 0.1%. The amplitude sweeps were performed in an interval of 0.001–10% strain divided into 21 steps. All measurements were performed within the linear viscoelastic region (tested, data not shown). Strain at fracture was defined as the strain value where a 10% and 50% decrease of the elastic modulus relative to the plateau in the linear viscoelastic region was seen. Results are the average of three runs.

2.8. Confocal laser scanning microscopy

The Leica SP5 (Leica Microsystems, Wetzlar GmbH, Wetzlar, Germany) confocal laser scanning microscope with krypton/argon and helium/neon laser was used to capture images. A water immersion objective was used for a 63 × magnification. FITC, Nile red and D307 were used as fluorescent dyes in a 0.01% (v/v) solution. The dyes were immersed on a cooled object glass until the solvent evaporated; the sample was placed and equilibrated at 5 °C for 30 min.

2.9. X-ray scattering

X-ray scattering was performed at the SAXSLab instrument (JJ-Xray, Denmark) at the University of Copenhagen, and equipped with a 100XL + micro-focus sealed X-ray tube from Rigaku and a 2D 300 K 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} with the magnitude of the scattering vector 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 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 $d = 2\pi/q^*$, where q^* is the Bragg peak position. 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. A temperature ramp of 2 °C/min was used with simultaneous 60 s long scattering measurements following the DSC temperature scans.

2.10. Differential scanning calorimetry

Isothermal information of the crystallization kinetics was obtained by measuring with DSC using a Mettler Toledo DSC (Mettler Toledo, Greifensee, Switzerland). DSC measurements were performed in the 5–60 °C temperature range during heating with a scan rate of 2 °C/min. Prior to heating, the samples were held for 10 min at 5 °C to ensure a sample temperature of 5 °C. Sample masses were 20–30 mg. An empty sealed pan was used as a reference.

2.11. Statistical analysis

Statistical analysis was carried out using GraphPad Prism (Version 5.02, GraphPad Software, Inc., La Jolla, CA, USA). A one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test was used in the data analysis. ANOVA was applied on the repeated measurements (at least triplicate) of all eight samples using average diameter of both average size of milk fat globules in cream and water droplets in butter, zeta potential, conductivity, solid fat content, water content, strain at 10% and 50% decrease in G', respectively and finally G' as variables. In addition, the data structure was analysed by a Principal Components Analysis (PCA) on the mean responses of average water droplet size, water content, solid fat content, strain at 10% fracture and G' at 5 rad/s). Unscrambler (Version 9.2, CAMO A/S, Trondheim, Norway) was used for the PCA analysis, where all variables were standardised (1/SD). A full cross validation was used as model validation criterion.

3. Results and discussion

It has been found that mechanical treatment of milk can disrupt the fat globules leading to changes of the fat globule membrane (Morin, Jiménez-Flores, & Pouliot, 2007). Since the surface of the fat globules can act as a catalytic impurity, changes in the membrane can lead to differences in nucleation properties and thereby affect the fat crystal network. Also, such changes would enable the milk proteins to adsorb on the damaged spot. This will change the surface composition and hereby affect the zeta potential (Wade & Beattie, 1997). To ensure that the fat globules remained intact after heat treatment, zeta potential and fat globule size distributions were measured (Table 1). No significant changes were observed in zeta potential or size distribution (Fig. 1) after heating or maturing of the cream. The average fat globule size in commercial cream was found to 2.48 μm corresponding to the sizes found in our test cream. As a result, we conclude that the heat treatment of the cream did not cause any changes to the fat globules. Previously, Wade and Beattie (1997) measured the zeta potential to −22 mV in commercial cream, which agree with our findings.

To ensure that a phase inversion from oil-in-water to water-in-oil had occurred, conductivity was measured in all butter samples. In all cases the conductivity appeared very low (see Table 2). This confirms formation of a non-conducting water-in-oil emulsion, with a continuous fat phase (Bordi et al., 1996).

Also the water content was measured in all samples (Table 2), and a variation less than 3% was observed. Even though the water content in the reference slow cooled and fluctuating samples was statistically different from the other samples (Table 2), this variation did not affect the rheological properties of the butter (data not shown).

Table 1
Average diameter ($d_{3,2}$) and zeta potentials (ζ) for fat globules from commercial cream and cream with a controlled temperature history.

Cream	ζ (mV)	$d_{3,2}$ (m)
Commercial cream	−27 ± 6	2.48 ± 0.1
Fast cooled cream	−28 ± 5	2.46 ± 0.02
Slow cooled cream	−28 ± 4	2.53 ± 0.03
Matured fast cooled cream	−30 ± 2	2.48 ± 0.05
Matured slow cooled cream	−27 ± 4	2.54 ± 0.1
Fluctuating temp. cream	−33 ± 4	2.39 ± 0.08

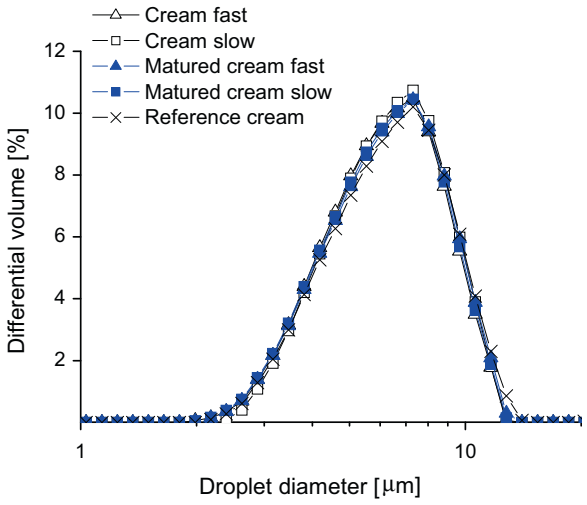

Fig. 1. Size distribution of milk fat globules obtained from light scattering measurements of cream. The cream samples were cooled from 65 to 10 °C either fast, 7.5 °C/min or slow 0.4 °C/min. Two samples were stored at 5 °C/min for 48 h after fast and slow cooling, respectively, while one was stored at fluctuating temperatures (3 h at 5 °C, 3 h at 20 °C, 8 cycles of 1 h at 20 °C and 1 h at 5 °C). Furthermore, a reference cream was analysed.

The water droplets are dispersed within the continuous fat phase of butter, and form a network together with the fat globules. So far, it is not fully established how the water content affects butter microstructure. It is likely that increasing the water content in the samples reduces the number of contact points between the fat crystals hence weakening the fat crystal network. Furthermore, since the viscosity of water is lower than that of the crystallized fat, one could speculate that increasing the water content might decrease the hardness of the butter, while a decreasing water droplet size would increase butter hardness. By changing the heat treatment of the cream, we can affect the fat crystals and thereby the network within the butter including the water droplet size. Also, the size of the water droplets in butter influences the microbial stability, as well as the sensorial properties of butter such as spreadability and mouthfeel (Van lent, Vanlerberghe, Van Oostveldt, Thas, & Van der Meeren, 2008). In this work LR-NMR was used to determine the water droplet size distribution in butter (Table 2). The measured $d_{3,3}$ was in the range from 8.9 to 14.40 μm in butter samples, and 28.65 to 37.00 μm in the reference samples. No statistically significant difference was observed between the varying heat treatments. We measured $d_{3,3}$ in commercial butter to 1.90 μm, which is in agreement with the literature, where $d_{3,3}$ is measured in commercial non-salted butter, with a fat content of 82%, to 2.3–6.4 μm (Van lent et al., 2008) and 2.6–10.6 μm in commercial spreads with 40–80% fat (van Dalen, 2002). The water droplet sizes in this work are slightly higher than the values reported in the literature, which is likely due to the smaller scale manufacturing compared to the industrial and also an increased water content compared to the samples measured in previous studies (Van lent et al., 2008 and van Dalen, 2002).

The solid fat content determination shows no significant changes as a result of the cream cooling rate (Table 2). However, with respect to the reference samples a significant difference was observed between the fast cooled sample and the sample stored at fluctuating temperatures. Furthermore, the solid fat content in commercial butter was significantly higher compared to our samples. Our samples were measured 24 h post manufacturing while the commercial butter was measured 3 weeks post manufacturing and consequently allowing more liquid fat to solidify. In our samples, the observed rheological differences can fully be explained by the different physical characteristics in the various samples, with an exception of the difference between the fast cooled reference sample and the reference sample stored at fluctuating temperatures. This confirms the findings of Wiking et al. (2009), where milk fat subjected to different cooling rates had a similar solid fat content, but differed in rheological properties and microstructure. In addition, variables such as size distributions of both water droplets and fat globules together with chemical composition of the samples are also expected to affect the rheological properties of the product (Afoakwa, Paterson, & Fowler, 2007).

3.1. Effect of cream cooling rate and maturing time

In the present study, the cream was cooled at either fast or slow rate prior to butter manufacturing. In addition, the effects of 48 h maturing of the cream at 5 °C were studied after fast and slow cooling, respectively. Small and large amplitude oscillatory shear rheology was applied to determine the elastic modulus of the butter (Fig. 2).

It has been shown, that the elastic modulus of a fat crystal network is directly related to the hardness index (as determined by cone penetrometry). This makes the elastic modulus a reliable indicator of the macroscopic consistency of butter (Narine & Marangoni, 1999). To evaluate the strain brittleness of the samples, the strain applied to obtain a 10% and 50% decrease in the elastic modulus relative to the elastic modulus in the linear viscoelastic region was recorded (Table 3).

In the non-matured butter samples, no significant differences were observed in elastic modulus (Table 4) or brittleness (Table 3). Interestingly, maturing of the slow cooled cream significantly increased the elastic modulus from 0.30 to 0.38 MPa compared to butter produced from non-matured cream. Maturing of the cream enhances crystal growth, as shown in Fig 3. Here, the microstructure 21 h after manufacturing is revealed by confocal laser scanning microscopy.

Table 2
Conductivity, solid fat content, average water droplet diameter ($d_{3,3}$) and water content in the butter (B) and reference samples (R).

Sample	Conductivity (μS/cm)	Solid fat content (%)	$d_{3,3}$ (μm)	Water content (% w/w)
Commercial butter	0.03 ± 0.00	60.09 ± 0.153^{a}	1.90 ± 0.00^{a}	16.00 ± 0.02^{a}
B Fast cooled	1.41 ± 0.23	51.74 ± 0.866^{b,c}	11.46 ± 1.33^{b,c}	27.15 ± 0.32^{b}
B Slow cooled	1.62 ± 0.27	50.19 ± 2.18^{b,c}	11.63 ± 1.56^{b,c}	27.33 ± 0.89^{b}
B Matured fast cooled	1.48 ± 0.17	49.81 ± 2.42^{b,c}	11.19 ± 3.47^{b,c}	27.15 ± 0.32^{b}
B Matured slow cooled	1.64 ± 0.21	47.75 ± 3.17^{b,c}	8.920 ± 2.04^{b,c}	26.39 ± 0.26^{b}
B Fluctuation	0.28 ± 0.18	47.47 ± 0.916^{b,c}	14.40 ± 1.47^{b,c}	25.99 ± 0.21^{b,c}
R Fast cooled	0.59 ± 0.10	46.04 ± 2.05^{b}	28.65 ± 12.88^{b,c,d}	26.10 ± 0.78^{b,c}
R Slow cooled	0.44 ± 0.14	48.20 ± 2.19^{b,c}	37.00 ± 3.91^{d}	26.87 ± 0.91^{b}
R Fluctuation	0.56 ± 0.20	53.92 ± 3.00^{c}	35.00 ± 1.38^{d}	24.64 ± 0.48^{c}

The superscript letters within each column indicates significant differences among the values ($P < 0.05$) according the one-way ANOVA analysis. Samples that are NOT significantly different from each other are denoted with the SAME superscript letters.

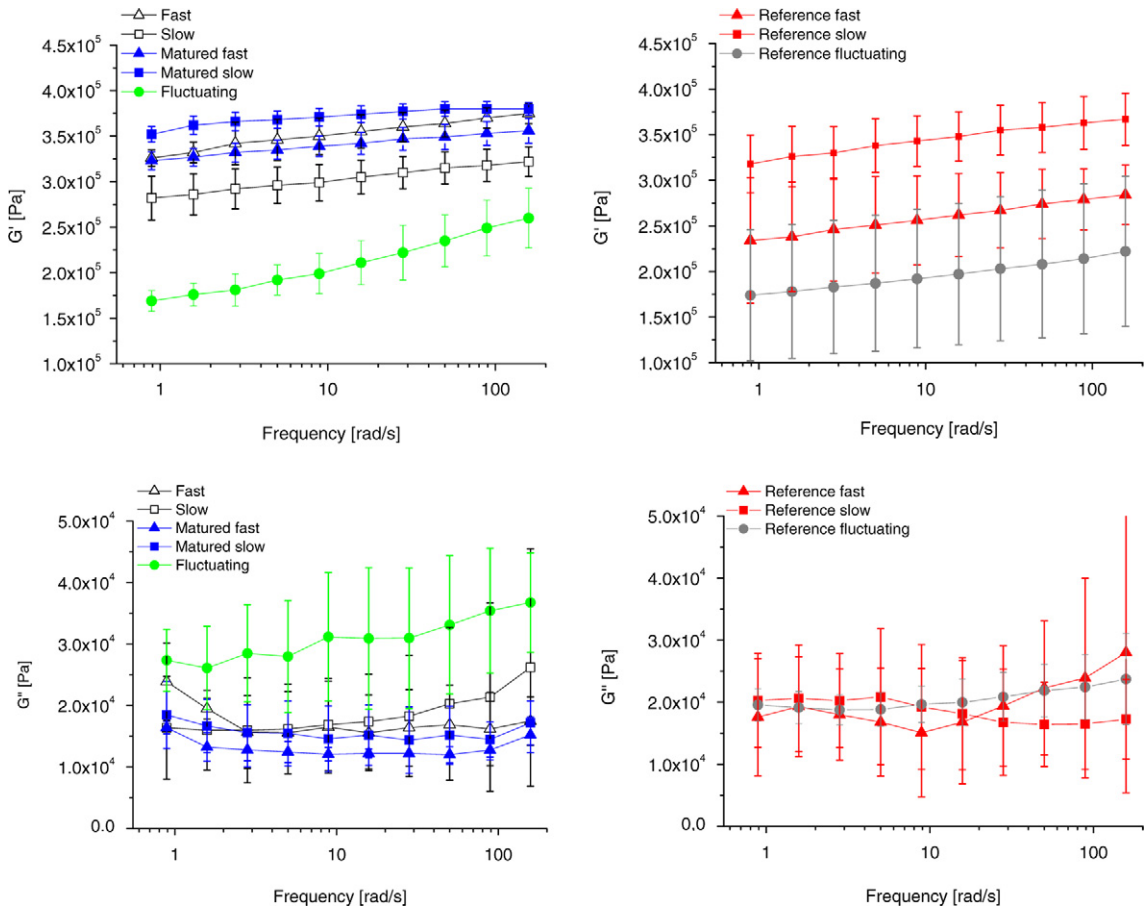

Fig. 2. Frequency sweep of butter produced from cream with different temperature history (left column). The elastic modulus (G') is shown in the top and the viscous modulus (G'') at the bottom. Two cooling rates are used: slow cooled (0.4 °C/min) and fast cooled (7.5 °C/min). The reference samples are prepared from anhydrous milk fat and water (right column). The butter and reference sample stored at fluctuating temperatures was prepared from fast cooled cream and after production subjected to 3 h at 5 °C followed by 3 h at 20 °C and nine cycles of 1 h at 5 °C and 1 h at 20 °C.

Table 3
Strain applied to obtain a 10% and 50% decrease in the elastic modulus relative to the elastic modulis in the linear viscoelastic regime. Both butter (B) and reference samples (R) were studied.

Sample	Strain at 10% decrease G' (%)	Strain at 50% decrease G' (%)
B Fast cooled	0.04[a]	1.0[a]
B Slow cooled	0.04[a]	1.0[a]
B Matured fast cooled	0.04[a]	1.3[b]
B Matured slow cooled	0.04[a]	1.0[a]
B Fluctuation	0.01[b]	0.19[c]
R Fast cooled	0.02[c]	0.19[c]
R Slow cooled	0.02[c]	0.13[d]
R Fluctuation	0.02[c]	0.18[e]

The superscript letters within each column indicates significant differences among the values ($P < 0.05$) according the one-way ANOVA analysis. Samples that are NOT significantly different from each other are denoted with the SAME superscript letters.

Since the dyes are only soluble in liquid phases, the fat crystals appear as grey–black zones in the images. In all non-matured slow cooled samples, the crystals showed a wide crystal size distribution, while the fast cooled samples had a narrow distribution. The difference in the number of crystals and contact points between them is also noticeable. The fast cooled samples had a more dense crystal network with more contact points, compared to the slow cooled samples. Interestingly, no difference in microstructure was observed between the matured samples, which all revealed a dense crystalline network. This explains the observed rheological properties. Maturing of the slow cooled sample results in a more dense crystal network with an increased number of contact points giving significant increase in elastic modulus compared to non matured butter (Table 4). The observed structural differences in the non-matured samples are most likely a consequence of the changes in cooling rate. One could speculate that a faster cooling rate accelerate nucleation rate and thereby crystal growth. Consequently, the crystals will be smaller in the early stages and have a somewhat faster aggregation rate (Walstra, Kloek, & van Vliet, 2001). Smaller crystals give a firmer network of fat products, conversely larger crystals give a sandy mouthfeel (Mulder & Walstra, 1974). Aggregation of fat crystals occurs due to Van der Waals attraction as soon as they have obtained a given size. This explains why no difference is observed between the samples after 48 h of cream maturing; all crystals may have been reached the critical size for aggregation at this point.

With respect to brittleness, maturing of the fast cooled sample decreased the brittleness compared to the non-matured. In the reference samples, the fast cooled had a significantly lower elastic modulus compared to the slow cooled, according to the ANOVA. Previously, Herrera and Hartel studied the effect of fast (5.5 °C/min) and slow (0.2 °C/min) cooling on the rheological properties (2000c) and microstructure (2000a and 2000b) of fractionated milk fat during agitation. Slow cooling caused formation crystals with a wide size distribution and more contact points compared to fast cooling, with smaller crystals separated by a liquid phase. In agreement with our findings, slow cooling caused a higher modulus

Table 4
ANOVA conducted on the elastic modulus (G') of all samples. The samples are denoted RF: fast cooled reference, RS: slow cooled reference, BF: fast cooled butter, BS: slow cooled butter, BMF: fast cooled and matured butter, BMS: slow cooled and matured butter, RFL: Reference stored at fluctuating temperatures and BFL: fast cooled butter stored at fluctuating temperatures.

	ANOVA analysis of G'						
	BF	BS	BMF	BMS	BFL	RF	RS
BF							
BS	NS						
BMF	NS	*					
BMS	NS	***	NS				
BFL	***	***	***	***			
RF	***	**	***	***	***		
RS	***	NS	**	***	***	**	
RFL	***	***	***	***	NS	***	***

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

compared to fast cooled samples. Later, Wiking et al. (2009) studied the relation between crystallization mechanism and microstructure in milk fat. The milk fat was cooled at without agitation either 0.1 or 10 °C/min to 20 °C followed by isothermal crystallization. They found a firmer crystal network in the fast cooled sample resulting in a higher complex modulus compared to the slower cooled milk. Similar to our findings and those by Herrera and Hartel (2000c) and Wiking et al. (2009) are, that an increased number of contact points between the crystals increases the harness of the samples.

Besides the rheological profile, other factors contribute to the consistency and appearance of a fat crystal network. According to the literature, the elastic modulus is related to solid fat content via the fractal dimension of the fat crystal network (Narine & Marangoni, 1999). The fractal dimension describes how the mass and size of the crystal aggregates grows. Since the solid fat content did not change as a consequence of varying the thermal history of the samples, the observed changes in the elastic modulus must be largely related to changes in the fractal dimension. Therefore, we used SAXS and WAXS to study the crystal polymorphism.

Milk fat crystallizes in three polymorphic forms: α, β′ and β, where α is least stable and β most stable. We identified the polymorphism of the crystals using SAXS and WAXS. From the SAXS and WAXS spectra (Fig. 4), we can identify the polymorphic forms of the four cream precursor samples. The WAXS data show that the non-matured cream samples mainly form α and β′ with minor traces of β, as also found by Lopez et al. (2005). Maturing of the cream leads to a transition from α to β′ and β. The same conclusion can be drawn from the SAXS data where the non-matured samples show an α-related 3L structure around 67 Å combined with the typical β′ 2L lamellae around 41 Å (Lopez et al., 2005). Upon maturing, the 3L arrangement changes to a 57 Å stacking indicating crystal rearrangement from α to β′, which agree with our findings.

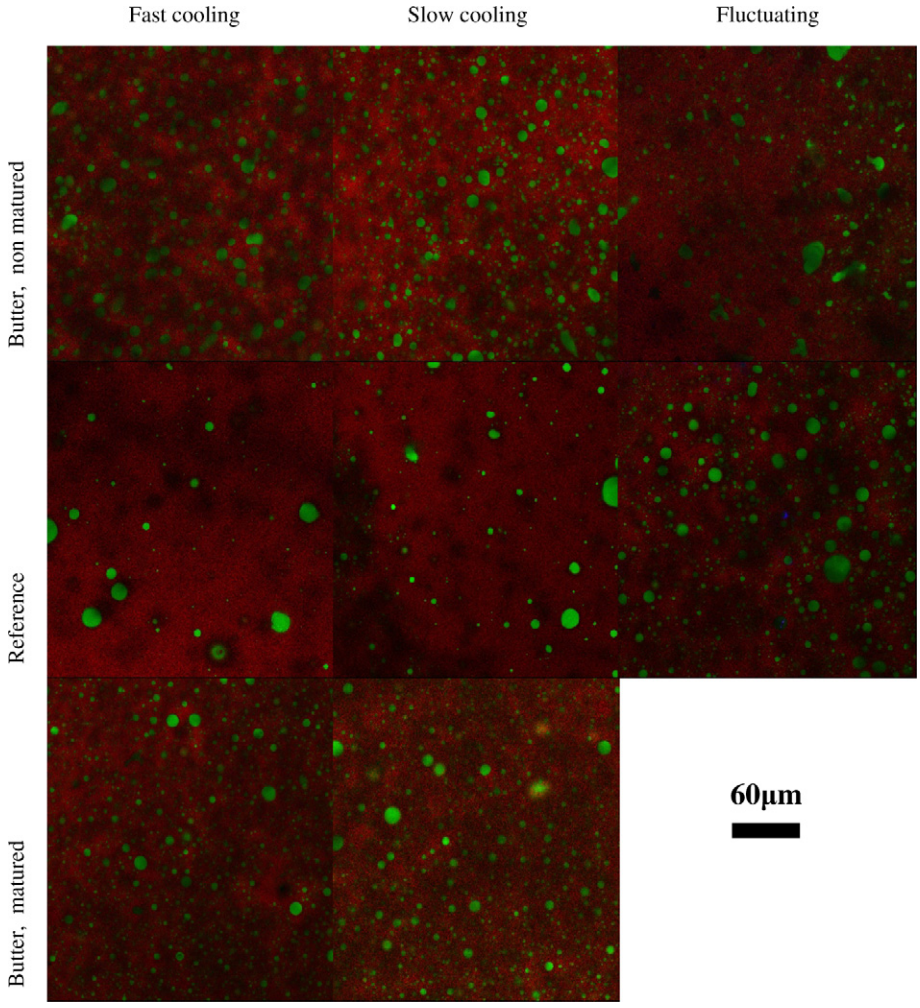

Fig. 3. Confocal images of butter samples. The green colour illustrates the water phase (FITC), the red phase is fat (Nile red) and blue the phospholipids (DiD oil). Fat crystals are colour negative (black shadows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

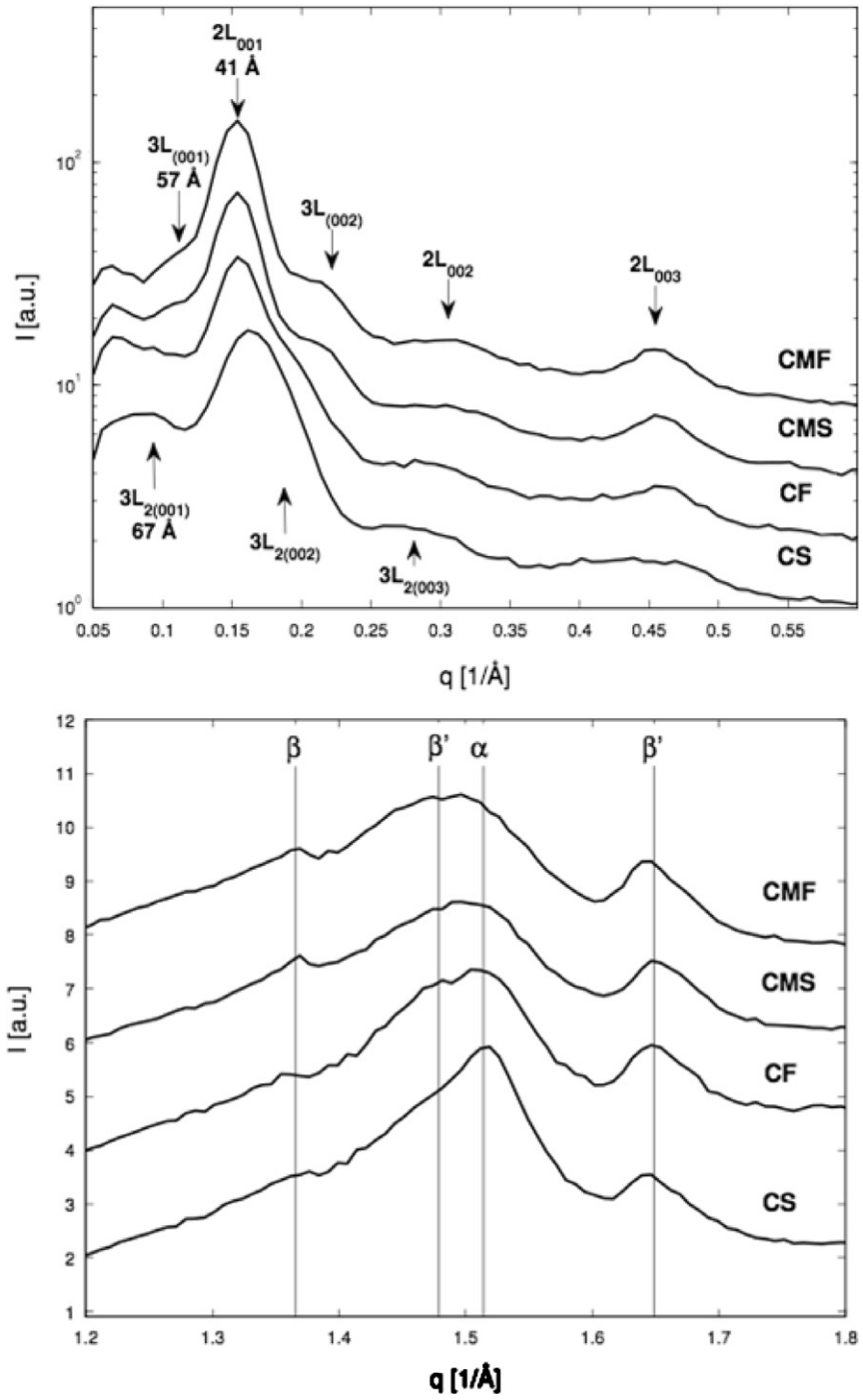

Fig. 4. SAXS (Top) and WAXS (Bottom) spectra from cream at 5 °C subject to different temperature treatments. CS: slow cooled cream, CF: fast cooled cream, CMS: slow cooled and matured cream and CMF: fast cooled and matured cream. Vertical lines indicate the positions of the characteristic peaks from the polymorphs forms: α: 4.15 Å, β': 3.81 Å, 4.2 Å, β: 4.6 Å (Lopez et al., 2005).

After sufficiently long crystallization time, milk fat tends to form β′-crystals and sometimes also β-crystals are reported (de-Man, 1961; Lopez et al., 2005; ten Grotenhuis et al., 1999), which confirms our findings in the matured cream. The butter samples were analysed with SAXS and WAXS 21 h after manufacturing (Fig. 5). Despite the fact, that one would expect the crystallization to evolve further after the cream is subjected to phase-inversion during butter manufacturing and subsequently stored at 5 °C for 21 h, the SAXS and WAXS patterns were identical in cream and butter subjected to the same thermal history. This is in accordance with Fredrick et al. (2011), who found that milk fat in bulk and cream had identical crystallization mechanisms when rapidly cooled. They observed first α-crystals immediately after fast cooling and secondly β′-crystals during further storage at 5 °C, or when slow cooled (de-Man, 1961; Fredrick et al., 2011). A possible explanation for this behaviour is that the nucleation of the fat crystals primarily occurs in the fat globules (Fredrick et al., 2011). During phase inversion, a larger fraction of the fat globules are ruptured hence full crystallization cannot be achieved. Those findings highlight the importance of the heat treatment of the cream prior to butter manufacturing.

In Fig. 6, the intensity of each diffraction peak (WAXS middle and SAXS bottom) is plotted as a function of temperature during heating together with DSC recording. The DSC recordings reveal the transition of α to β′-crystals. Comparing the cream heat treatments, the DSC measurements showed no changes in the melting of the α-crystals between butter produced from fast and matured fast cream, whereas the β′-crystals melted at higher temperatures for the matured samples. This indicates a higher stability of the α-crystals in the matured samples compared to non-matured, since more driving force is needed to transformation into β′-crystals (ten Grotenhuis et al., 1999).

Summing up, heat treatment of cream prior to butter manufacturing does not significantly affect the rheological properties of the butter or solid fat content. However, the microstructure is affected. Maturing of the slow cooled cream significantly increases the hardness of the butter whereas maturing of fast cooled cream significantly decreases the fragility of the butter.

3.2. Effect of globular structure

Butter contains a smaller or larger fraction of milk fat globules, depending on the processing conditions. It is a widely accepted that increasing the number of fat globules in butter decreases the hardness of the product: when the fat globules are destroyed more crystalline interglobular phase is formed, hereby increasing the hardness of the butter (Juriaanse & Heertje, 1988; Mulder & Walstra, 1974). Nevertheless, Fedotova and Lencki (2010) found butter containing 60% globular fat to be more brittle and less spreadable compared to butter made from anhydrous milk fat, when tested with cone penetrometry. Accordingly, we compared butter produced from fast and slow cooled cream with reference samples of anhydrous milk fat subjected to the same heat treatment. Skimmed milk was added to the reference samples, to ensure equal water content in all samples. The images captured with confocal laser scanning microscopy (Fig. 3) clearly show a different microstructure in the reference vs. the butter samples. All butter samples contained similar amounts of fat globules, while none were observed in the reference samples. The mean diameter of fat globules was about 2–3 μm (see Table 1 (number weighted diameter) and Fig. 1 (volume weighted diameter)), which is on the order of the size found by Mulder and Walstra (1974). The average diameter of the fat globules observed by confocal laser scanning microscopy is well within this size range.

The frequency sweep (Fig. 2 and Table 4) shows no significant difference between the slow cooled butter and reference. However, the fast cooled reference has a significantly lower elastic modulus compared to the fast cooled butter. Furthermore, a significant difference is observed in brittleness (Tables 3 and 4), the reference samples fractures at a fivefold lower strain compared to the butter samples. With respect to microstructure, it is not possible to distinguish between the crystal size between the reference samples (Fig. 3), likewise the observed WAXS pattern were identical for not only the fast and slow cooled reference samples, but also similar to the fast and slow cooled butter.

In the microstructure, more contact points between the crystals are observed in the samples containing fat globules (Fig. 3). The fat globules take up some space in the microstructure, moving the fat crystals closer together facilitating crystal aggregation and thereby development of a strong, and less brittle crystal network. In addition, the composition of triacylglycerols is expected to be similar in the anhydrous milk fat and the cream used for butter manufacturing. Hence, the nucleation rate is likely the same in the reference and butter sample subjected to the same heat treatment. Since fat crystallization primarily occurs within the fat globules, rupture leads to imperfect crystallization resulting in a softer

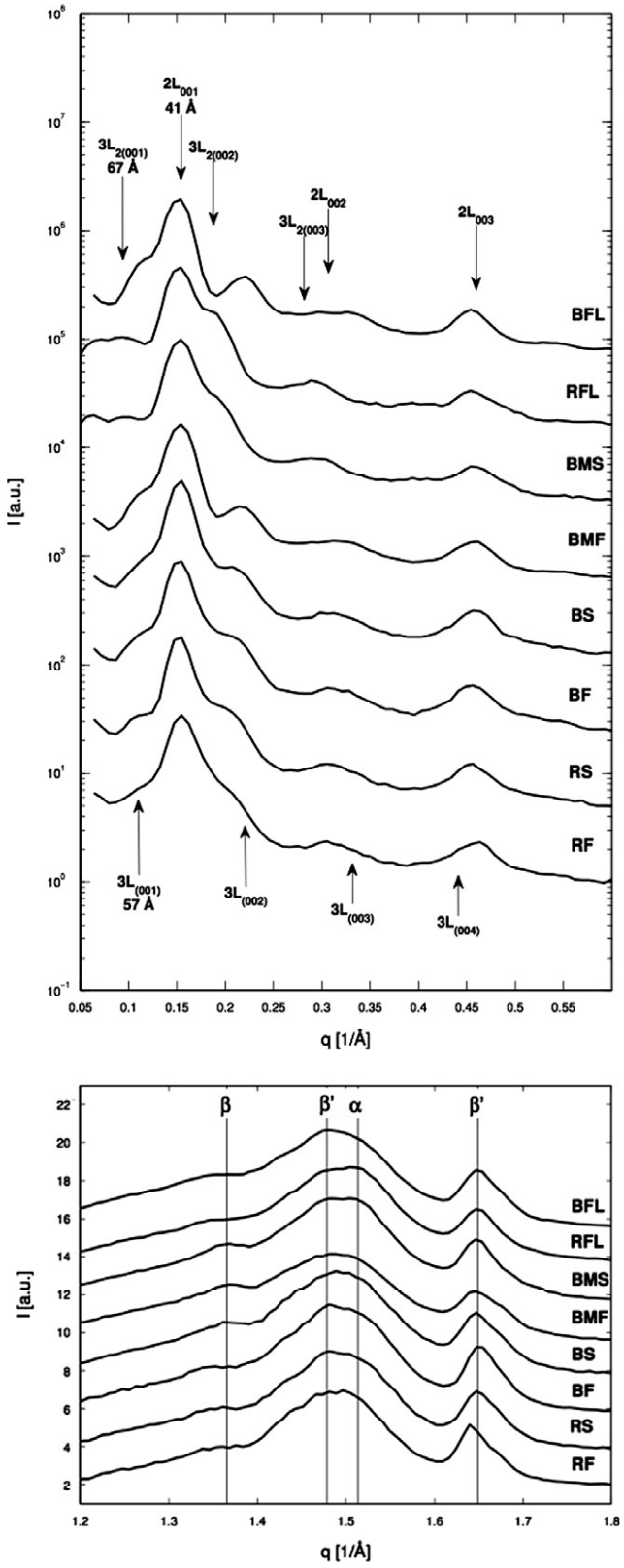

Fig. 5. SAXS (Top) and WAXS (Bottom) spectra from butter and reference samples at 5 °C subject to different temperature treatments. RF: fast cooled reference, RS: slow cooled reference, BF: fast cooled butter, BS: slow cooled butter, BMF: fast cooled and matured butter, BMS: slow cooled and matured butter, RFL: Reference stored at fluctuating temperatures and BFL: fast cooled butter stored at fluctuating temperatures. Vertical lines indicate the positions of the characteristic peaks from the polymorphs: α: 4.15 Å, β′: 3.81 Å, 4.2 Å, β: 4.6 Å (Lopez et al., 2005).

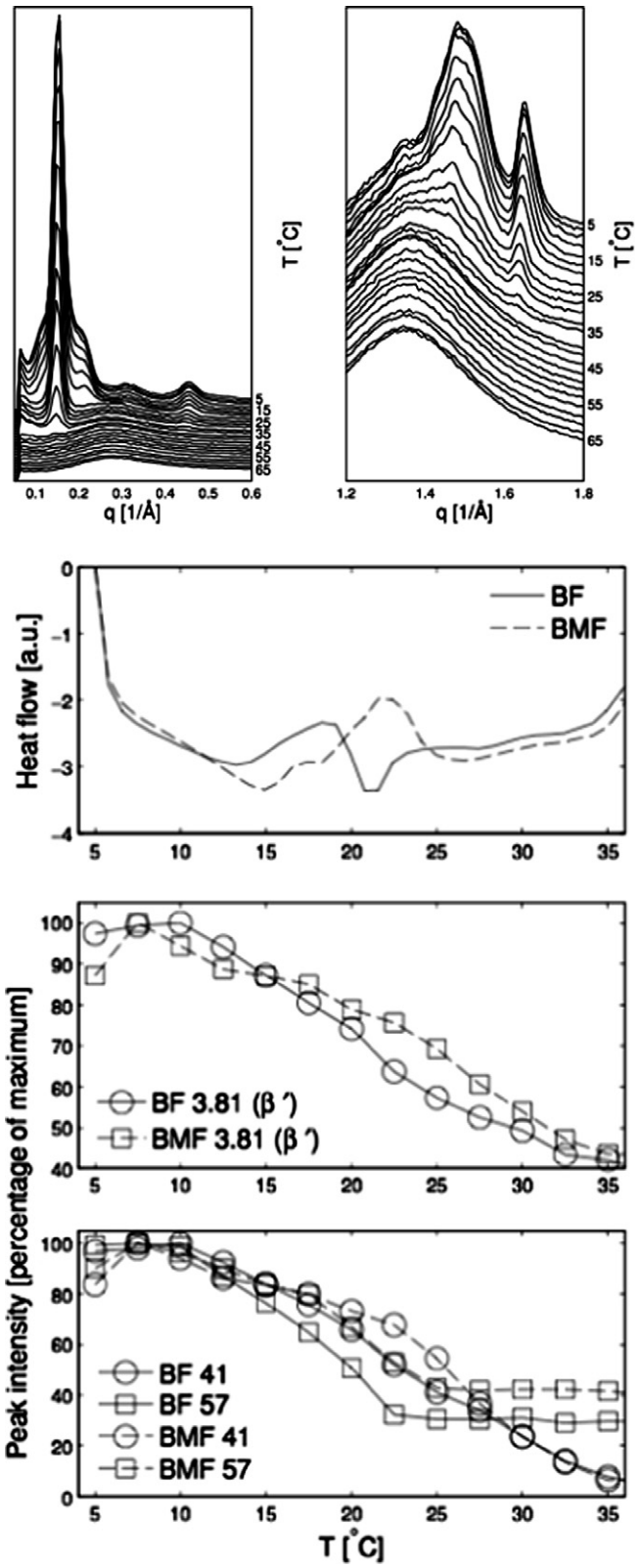

Fig. 6. Comparing peak evolution upon heating between fast cooled (BF) and matured (BMF) butter. (Top) Typical SAXS and WAXS peak progression curve upon heating from 5 to 65 °C. Data from BF. (Bottom) Combined plot of DSC and evolution of selected WAXS and SAXS peaks. The peak *d*-spacings are indicated in the legends in Å. The DSC spectra represent a typical evolution plot upon heating of milk fat.

product (Mulder & Walstra, 1974). This, however, is not reflected in the WAXS data, since no differences were recorded.

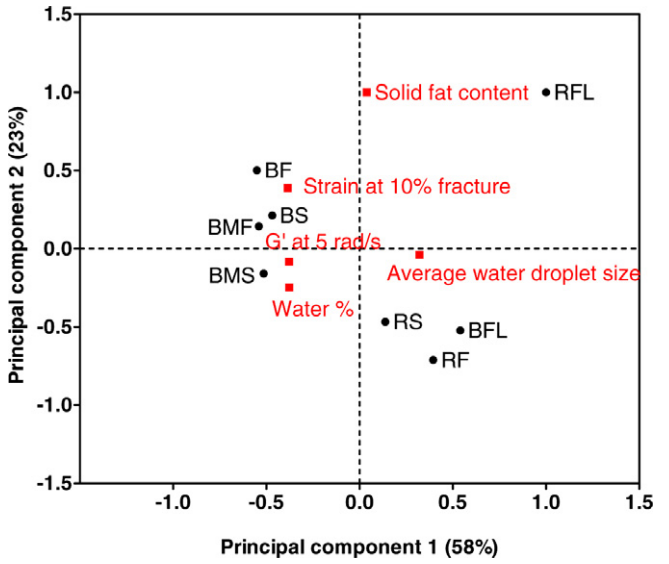

Fig. 7. Principal component analysis (bi-plot of scores and correlation loadings) of variables related to the rheological properties of the samples. Notice the difference in scale of principal component 1 and 2, since it leads to visually overemphasising the influence of principal component 2.

3.3. Effect of fluctuating temperature during storage

We aimed to test how taking the butter in and out of the refridgerator affects the rheology, micro- and nano-structure of the butter. Heating of the butter will affect and might even melt a smaller or larger fraction of the fat crystals. That implies disappearance of some fat globules and eventually coalescence of water droplets, hence changes in the microstructure that are not necessarily reversible upon cooling of the butter. To further investigate this, we tested the effect of fluctuating temperature during storage. Butter and reference produced from fast cooled cream were subjected to 3 h at 5 °C followed by nine cycles of 1 h at 20 °C and 1 h at 5 °C. Fig. 2 shows that the samples stored at fluctuating temperatures had a significantly decreased elastic modulus compared to all the other samples (Table 4).

From confocal laser scanning microscopy images it is difficult to quantify differences between the samples stored at fluctuating temperatures compared to the ones stored at 5 °C. Nevertheless, the rheological data indicates that the microstructure is possibly changed as a result of the fluctuating temperature. Previous studies have focused on the effect of cold/warm/cold treatment of cream prior to butter manufacturing (Frede & Buchheim, 1994; Ulberth, 1989). They concluded that temperature and length of the warm period significantly affects the spreadability of the butter, while increasing temperature and length of heating period decreases the hardness of the butter. In conclusion, fluctuating temperatures during both cream heat treatment as well as during storage seems to decrease the hardness of the butter.

The variables discussed above, can be structured in a principal component analysis (Fig. 7). The first principal component explained 58% of the variation in strain at fracture, G', water content and average water droplet size. A high strain at fracture, G' and water content is related to the butter samples while high average water droplet size is characteristic for the reference samples together with butter stored at fluctuating temperature. The second principal component provides information on variation in solid fat content, where the reference sample stored at fluctuating temperature is characterized by a higher number.

4. Conclusion

The heat treatment of cream prior to butter manufacturing largely determines the final textural characteristics, such as spreadability and mouthfeel, of the butter. Slow and fast cooling of the cream may result in similar rheological properties and polymorphic forms, α- and β′-crystals, but differ in microstructure. Butter produced from slow cooled cream had fewer crystals with a wider size distribution whereas the butter produced from fast cooled cream consisted of more uniform crystals. Maturing of the cream may lead to a transition from β′ to β. Maturing of the fast cooled cream does not cause any changes in the rheological profile nor microstructure. However, maturing of the slow cooled cream significantly increases the hardness of the produced butter, as a result of a more dense crystal network.

Fat globules seem to affect not only the rheological properties of butter, but also how butter behaves when subjected to fluctuating temperature. Absence of fat globules results in a significantly more brittle butter product compared to when fat globules are present. Further studies are needed to explore the impact of fat globules in a fat crystal network, which is the case in butter. Such studies could expand the previous knowledge obtained from simpler model systems with no fat globules present.

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