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Effect of churning temperature on water content, rheology, microstructure and stability of butter during four weeks of storage

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ABSTRACT

The effect of churning temperature (10 °C vs. 22 °C) is evaluated with respect to water content, rheology, microstructure and stability of butter produced using the batch churning method with a temperature ramp of 4 °C/min. Using pulsed-nuclear magnetic resonance, an increase in relative solid fat content from 44% to 49.5% was observed when decreasing the churning temperature. Due to lower solid fat content formed upon churning at high temperatures, average water droplet size significantly increased from 5.5 μm to 18.5 μm and less water could be incorporated into the butter during mixing. Using differential scanning calorimetry, it was observed that water addition as well as churning at low temperatures induced a transition toward more stable crystal structures, as the melting point in the high melting fraction was slightly lower for butter churned at high temperature. This did, however, not reflect in any changes in terms of crystal polymorphism, and all butters contained primarily β′-crystals with traces of α- and β-crystals. Despite the observed changes, small deformation rheology revealed no difference as a function of churning temperature or water content. During isothermal storage at 5 °C, the solid fat content increased in all butters, but only butter churned at 10 °C showed an increase in hardness during storage. However, no difference in rheological behavior was observed among the butters. Thus it can be concluded that low temperature allows more water to be incorporated in the system without inducing any changes in rheological behavior or crystal polymorphism after four weeks of storage.

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Abbreviations: TH, butter churned at high temperature (22 °C); TL, butter churned at low temperature (10 °C); THW, butter churned at high temperature with water added (22 °C); TLW, butter churned at low temperature with water added; FITC, fluorescein-5-isothiocyanate; D307, 1,10-dioctadecyl-3,3,30,30-tetramethyl-indodicarbocyanine perchlorate.

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1. Introduction

Lately, the consumers demand and willingness to pay for milk fat based products with improved nutritional values has increased (Saulais & Ruffieux 2012). However, the key to develop such novel products is to fully understand the structure and physical properties of the fat. This is essential, since it largely determines the material and rheological behavior of the products, including spreadability, hardness and mouthfeel together with product shelf life and flavors perception (Marangoni et al., 2012; Narine & Marangoni, 1999).

In a series of papers, we have studied the effect of the different production steps on the microstructure and rheological properties of butter, when using the traditional batch churning, also referred to as the Fritz method (Frede & Buchheim, 1994). Moreover, the different production steps have been reviewed (Rønholt, Mortensen, & Knudsen, 2013a) and the rheological methods used to characterize the material properties of milk fat-based products evaluated (Rønholt, Mortensen, & Knudsen, 2013b). First step in the production process is pasteurization of the cream. Second, the cream is cooled to the churning temperature and eventually matured. By controlling the cooling rate, the rheological behavior of the butter can be changed, as a fast cooling rate results in a harder butter compared to butter produced using a slow cooling rate (Wiking, De Graef, Rasmussen, & Dewettinck, 2009). However, after 4 weeks of storage at 5 °C this effect is diminished (Rønholt, Kirkensgaard, Mortensen, & Knudsen, 2014a). Next step after cooling is churning, where the cream, an oil-in-water emulsion, is subjected to phase inversion. During churning, air bubbles surrounded by a protein film are formed. As a continuous shear is applied during churning, the milk fat globules will collapse allowing the liquid fat to surround the air bubbles. At a given point, the air bubbles will start to coalesce and larger aggregates of fat, also referred to as butter grains, are formed surrounded by buttermilk (King, 1953). The churning temperature is typically in the range of 10–15 °C. It has been discussed, that churning temperatures above 13 °C results in a relatively high amount of liquid fat, thus limiting the possibility of incorporating high amounts of water into the butter matrix (Nielsen, 1971). Additionally, more fat is lost in the buttermilk at high churning temperatures. The high temperature induces a partly melting of the fat, thus increasing the percentage of liquid fat. In liquid state, the fat is not incorporated in the butter grains and is then excreted in the buttermilk (Samuelsson, 1937). Churning at temperatures below 5 °C will on the other hand result in a solid fat content (SFC) so high, that only a small amount of liquid fat can be squeezed out of the milk fat globules during churning, resulting in long churning times and formation of very small butter grains (Samuelsson, 1937). While the cooling rate does not seem to influence the ability to incorporate water into the butter matrix (Rønholt, Kirkensgaard, Mortensen, et al., 2014), King (1953) states, that the small and hard butter grains formed upon churning at low temperatures have a large surface area hence much space for water entrapment in the crevices. On the other hand, such a rigid structure likely contains water channels facilitating drainage of the butter (King, 1953). After churning, the excess buttermilk is removed by draining, and thus water, oils or other components can be mixed with the butter matrix (Frede & Buchheim, 1994).

Since the studies by Samuelsson (1937) and Nielsen (1971), more advanced characterization techniques has been developed. Therefore, the aim of this study was to investigate to which extend a churning temperature of 10 °C and 22 °C and a water content ranging from 22% to 28% (weight/weight) affect the microstructure, rheology and stability of butter produced in a laboratory scale setup. The butter was stored for 4 weeks of at refrigerator condition (5 °C) and characterized multiple days during storage. Using light scattering, the cream was analyzed in terms of zeta potential and milk fat globule size before and after thermal treatment, and the fat content in the excess buttermilk was monitored using infrared analysis, adding valuable knowledge to previous stated hypotheses. Small deformation rheology was used to quantify the effect of churning temperature and water content on the fat crystal network. The experimental setup was based on a recent methodical evaluation for rheological properties of fat based products (Rønholt et al., 2013b), allowing a more detailed rheological profile compared to the work by Samuelsson (1937) and Nielsen (1971), while maintaining a constant temperature of 5 °C compared to room temperature in previous studies. By using fluorescent dyes, confocal laser scanning microscopy can now be used to visualize the fat crystal network and differential scanning calorimetry to evaluate the thermal behavior of the butter. Pulsed-nuclear magnetic resonance (p-NMR) was applied to quantify solid fat content and average water droplet size in the butter, whereas small- and wide-angle X-ray scattering (SAXS and WAXS) was used to study crystal polymorphism. Additionally for the SAXS and WAXS setup, a temperature stage allows time resolved studies at 5 °C.

Butter contains by definition maximum 16% water (Codex Alimentarius, 2011). However, to ease reading, samples produced in the present work will be referred to as butter despite a water content ranging from 22% to 28%.

2. Materials and methods

Due to logistic reasons, all measurements could not be conducted on the same days. An overview of the exact measurement days for each technique is given in Fig. 1 and is stated in each figure and table respectively. As the storage time for the purchased butter is unknown, it is listed separately in each figure.

2.1. *Materials*

Cream (38% fat), whole milk (3.5% fat) and skimmed milk (0.1% fat) from ARLA Foods, Slagelse Dairy, Denmark, was used for butter production. Sodium azide from Sigma Aldrich, St. Louis, USA, was added as preservative to the cream, using a concentration of 0.2 g/L. Commercial butter from ARLA Foods, Slagelse Dairy, Denmark, (Lurpak containing 81.5% fat, 16% water and 2.5% other components) was used as reference. Fluorescein-5-isothiocyanate (FITC) from Merck, Damstadt, Germany, Nile red and 1,10-dioctadecyl-3,3,30,30-tetramethyl-indodicarbocyanine perchlorate (D307) from Molecular Probes, Taastrup, Denmark were used as fluorescent dyes for the

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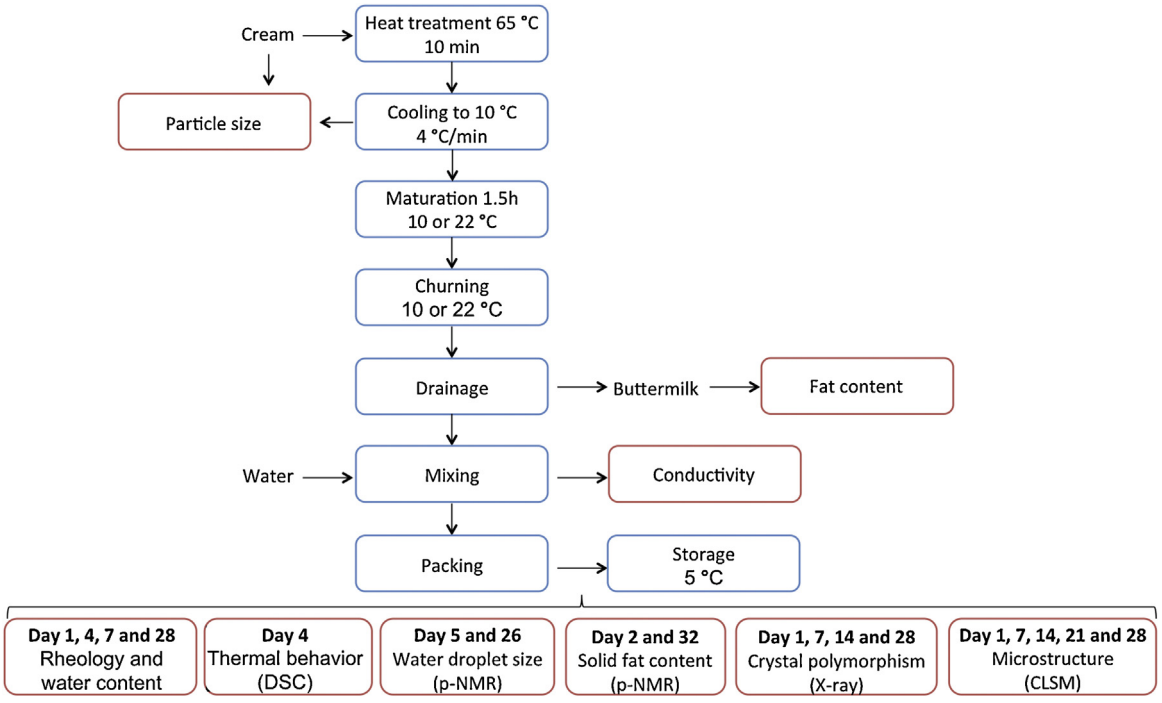

Fig. 1 – Working flow for butter production (blue boxes) and (red boxes). Addition of water during mixing was only applied for the samples with additional water (THW and TLW). DSC: differential scanning calorimetry, CLSM: confocal laser scanning microscopy, p-NMR: pulsed-field nuclear magnetic resonance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

confocal laser scanning microscopy images. Nile red and FITC were dissolved in acetone to a concentration of 0.01% (volume/volume) and D307 in ethanol, also to a concentration of 0.01%.

2.2. *Butter production*

Butter samples were produced in a modified version of a previously described small-scale laboratory setup (Rønholt, Kirkensgaard, Pedersen, Mortensen, & Knudsen, 2012), as schematically shown in Fig. 1. Two parameters were varied, the churning temperature (10 and 22 °C) and water content (22% and 26% or 28%). The butter with a water content of app. 22% will be referred to as TH (temperature high) for samples churned at 22 °C and TL (temperature low) for samples churned at 10 °C. Samples with high water content (26% and 28%) will be referred to as THW (temperature high water) and TLW (temperature low water).

The cream was incubated at 65 °C for 10 min, using a water circulation bath. This was done to erase crystal memory. To ensure the same cooling rate for all samples, the cream was subsequently transferred to a water bath tempered at 10 °C, while agitated with a magnet stirrer (100 rpm). The time from the beginning of the cooling to the cream reached a temperature of 10 °C was recorded and denoted as the cooling time. After reaching 10 °C, the cream was either kept in the water bath at 10 °C or incubated at 22 °C, both for 1.5 h. Next, the cream was churned using a kitchen machine equipped with a plastic bowl (2 L capacity) and universal blade running at maximum power (Braun Combimax 600, Kronberg, Germany). Churning time was defined as the time from onset to the point where a clear separation of the butter grains and buttermilk occurred. The cream temperature after churning was noted. To drain off the buttermilk, the obtained butter grains were gently squeezed in a mesh and water was added to the high water content samples, reaching final water content of 26–28% (based on the preliminary test). To avoid temperature fluctuations upon water addition, the temperature of the added water was adjusted to 10 °C and 22 °C respectively. The water addition was done by first adding half of the water, followed by mixing at low speed in the kitchen machine for 2 min, while adding the remaining water. The butter churned at high and low temperature (TH and TL) were also mixed for 2 min in the kitchen machine to ensure the same mechanical treatment. To minimize temperature fluctuations upon processing, churning, drainage and working were performed in a temperature controlled room at 5 °C for the samples churned at 10 °C and with a room temperature of 18 °C for the samples churned at 22 °C. Finally, to simulate the industrial butter production, working was performed in a food grinder (Beem Gigant ES 10/12, Rosbach, Germany) before transferring the butter to plastic containers. The containers were stored isothermally at 5 °C during four weeks. In order to obtain the same crystallization behavior, the butter was packed in the same amounts in each container. Each batch was prepared in triplicate ($N = 3$).

2.3. *Light scattering*

The milk fat globule size was measured before and after heating using light scattering (Malvern Mastersizer, Malvern

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Instrument Ltd., Malvern, UK). Moreover, the zeta potential of the cream was measured by a Malvern Zetasizer (Malvern Instrument Ltd., Malvern, UK), while the procedure and equipment was as described by Rønholt et al. (2012). All measurements were performed on the day of production.

2.4. Composition of buttermilk

The fat composition of the buttermilk was examined using a Milkoscan instrument from Foss, Hillerød, Denmark. Buttermilk samples were collected in duplicate subsequently to churning at both high and low temperatures. All measurements were run in triplicate. Skimmed and whole milk was used for calibration.

2.5. Conductivity

To ensure phase separation had occurred during churning, the conductivity was measured with a hand-held conductivity meter (Cond. 330i/SET, WTW Wissenschaftlich, Wilheim, Germany) on the freshly produced butter (Rønholt et al., 2012).

2.6. Water content

Water content was determined according to IDF 26A (IDF, 1993). Briefly, the water content was determined for all butter samples by placing 5 g butter on pumice stones in a porcelain crucible. The samples were incubated at 100 °C for 2 h and left to cool for 30 min at room temperature in an exicator. The water content was calculated as the percentage difference (weight/weight) before and after water evaporation. The measurements were run in triplicate.

2.7. Confocal laser scanning microscopy

Confocal laser scanning microscopy was conducted using an inverted Leica SP5 microscope (Leica Microsystems, Wetzlar, GmbH, Germany) equipped with krypton/argon and helium/neon lasers. A water immersion objective was used for 63× magnification. The dyes were added on a pre-cooled object glass one at a time when the former dye solvent had evaporated. Next, a chunk of the butter was placed on the objective glass and left to equilibrate for 30 min at 5 °C prior to analysis (Rønholt et al., 2013c). Sequential scanning was used for all samples.

2.8. Differential scanning calorimetry

The thermal behavior of the butter was examined after 4 days of storage at 5 °C using a differential scanning calorimeter from Mettler Toledo, Greifense, Switzerland. For sampling, 20–30 mg butter was placed in pre-cooled aluminum pans, while an empty, hermetically sealed pan was used as reference. The pans were inserted at 5 °C, and held at this temperature for 5 min followed by a temperature ramp step of 2 °C/min to a temperature of 60 °C.

2.9. Pulsed-nuclear magnetic resonance

Pulsed-nuclear magnetic resonance (p-NMR) was used to determine both average water droplet size and solid fat content (SFC) for all butter samples during storage. The water droplet size measurements were run on a Bruker Minispec mq20 from Bruker Optik GmbH, Germany. The samples were prepared by punching a glass tube with a height of 4 cm and an inner diameter of 0.8 mm into random locations in the butter. The tube was then placed in an NMR tube and held at 5 °C until analysis. The measurements were run in quadruplicate. The average water droplet size is given as the volume-weighted mean diameter ($d_{3,3}$).

Solid fat content, SFC, was measured by the direct method using a p-NMR from Maran Ultra, UK-based. Here, the samples were prepared by punching a pre-cooled cylindrical metal tube into the samples at random locations, and then transfer the samples to NMR glass tubes to a sample height of 4–5 cm. The samples were allowed to equilibrate for at least 30 min prior to analysis. SFC is represented as the ratio of solid fat relative to liquid fat, and corrected for the water content using the equation

$$\mathrm{SFC} = \frac{\mathrm{SFC}_{\mathrm{measured}}}{1 - \mathrm{SFC}_{\mathrm{water}}} \quad (1)$$

as described by Rønholt, Kirkensgaard, Mortensen, et al. (2014).

2.10. Small deformation rheology

Small deformation rheology was performed on an AR G2 Rheometer from TA instruments, West Sussex, England. The rheometer was equipped with serrated parallel plates, both temperature controlled, with a diameter of 25 mm. This setup is shown to be the most reliable configuration for rheological characterization of fat-based samples (Rønholt et al., 2013b). The sampling method was done as earlier described (Rønholt et al., 2013b). Briefly, cylindrical discs with a height of 4 mm and diameter of 25 mm was prepared and loaded in the rheometer. The upper plate was manually lowered until visual contact between the sample and the plate was established and then automatically lowered to achieve a normal force of 10 N (Awad, Rogers, & Marangoni, 2004). The rheological properties of the butter were evaluated with a frequency sweep in the interval 500–0.05 rad/s followed by a stress sweep with an angular frequency of 5 rad/s. All the measurements were performed in the linear viscoelastic region (tested, data not shown). The shown results are the average of at least six independent measurements.

2.11. X-ray scattering

X-ray scattering was run at the SAXSLab instrument (JJXray, Denmark) installed at the University of Copenhagen, using the experimental setup as described in Rønholt et al. (2012).

2.12. Statistical analysis

Statistical analyses were performed using GraphPad Prism (Version5.02, GraphPad Software, Inc., La Jolla, USA). One-way analysis of variance (ANOVA) was applied on the replicates, followed by Tukey's test. Water content, water droplet size, SFC, the elastic modulus (G'), storage modulus (G''), strain at 10% and 50% decrease G' and time were used as variables.

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Table 1 – Physical parameters characterizing the cream before and after churning, the buttermilk after churning and butter after phase inversion. TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. ΔT is the product temperature difference before and after churning of the cream. The error bar denotes the standard deviation.

	Cream					Butter milk	Butter
	Average diameter of fat globules [μm]	Zeta potential [mV]	Cooling rate [°C/min]	Churning time [s]	ΔT [°C]	Fat content, buttermilk [%]	Conductivity [μS/cm]
Commercial cream	1.0 ± 0.1	1.7 ± 0.8	–	–	–	–	–
TH	1.25 ± 0.0	1.1 ± 3.0	4.27 ± 0.20	59 ± 10	0.3 ± 0.3	6.1 ± 0.4	0.01 ± 0.00
TL	1.10 ± 0.1	3.6 ± 2.3	4.04 ± 0.09	85 ± 7	1.3 ± 0.3	2.0 ± 0.4	0.02 ± 0.01
THW	–	–	3.90 ± 0.24	54 ± 16	0.1 ± 0.1	–	0.02 ± 0.01
TLW	–	–	3.95 ± 0.08	92 ± 16	1.0 ± 0.1	–	0.01 ± 0.00

3. Results and discussion

3.1. *Butter production*

Previous work has shown, that mechanical treatment of cream is likely to cause disruption of the milk fat globule membrane (Morin, Jiménez-Flores, & Pouliot, 2007), thus potentially affecting nucleation and crystallization behavior. Such changes enables protein absorption to the membrane surface and are likely to alter both membrane composition and zeta potential (Wade, & Beattie, 1997). Therefore, the zeta potential was monitored in addition to the average diameter of the fat globules, to ensure the integrity of the milk fat globules after thermal treatment (Table 1). According to Table 1, no changes were observed in zeta potential or size distribution (Table 1) as a function of thermal treatment. It is therefore suggested, that the milk fat globules remained intact. The zeta potential listed in Table 1 is, however, higher than the −22 mV previously reported for commercial cream (Wade, & Beattie, 1997). The discrepancy is likely due to a difference in dissolution media (water in previous studies and milk serum in the present work), as the zeta potential is measured by the electrophoretic mobility of the particles, hence significantly depends on dissolution media and dilution factor.

Both the temperature of the cream during cooling and churning, and the churning time was monitored during butter preparation (Table 1). The data shows, that a reproducible cooling rate of 4 °C/min was obtained. Moreover, the churning time is shown to depend on churning temperature, as a churning temperature at 10 °C results in a churning time of 89 s and a temperature increase during churning of 1.2 °C, whereas churning temperature at 22 °C gives a churning time of 57 s and a temperature increase of 0.2 °C (Table 1). This confirms the finding by Samuelsson (1937), stating that a low churning temperature decreases SFC to such an extent, that only a small fraction of liquid fat can escape the milk fat globules resulting in a long churning time.

Previously, it has been shown that upon churning at 10 °C, when using the same equipment as in the present work, fast cooled cream (7.5 °C/min) has a churning time of 220 s, whereas the churning time of slow cooled cream (0.4 °C/min) is 120 s (Rønholt, Kirkensgaard, Mortensen, et al., 2014). The slow cooling process facilitating the presence of large

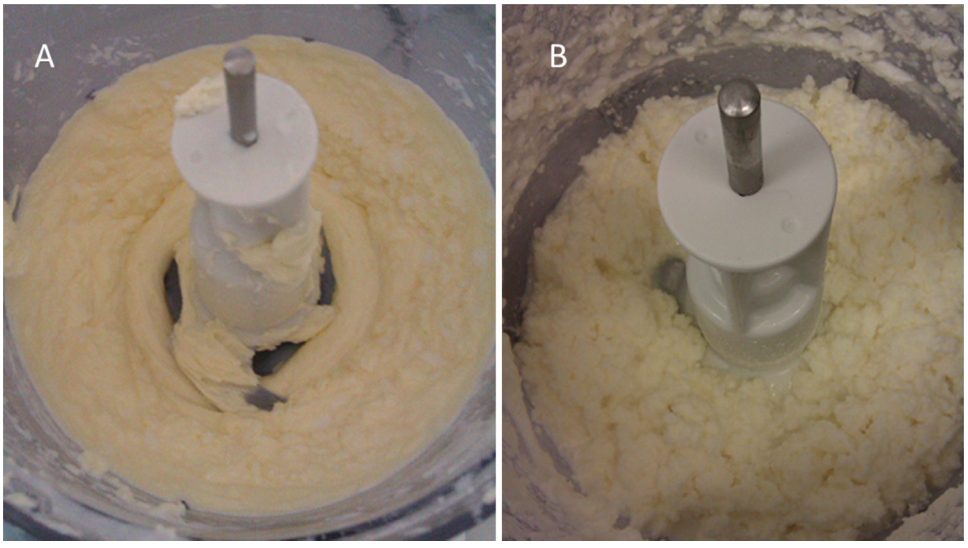

Fig. 2 – The appearance of butter grains churned at 22 °C (A) and 10 °C (B) respectively.

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crystals within the milk fat globules explains this behavior. Large crystals are likely to work as eroding agents, piercing the milk fat globule membrane. Contrary, fast cooling would result in formation of many, but smaller crystals and consequently a longer churning time (Boode, Walstra, & de Groot-Mostert, 1993). In the present work, the cream churned at 10 °C is subjected to a larger degree of super cooling, facilitating formation of small, heterogeneous crystals (Heertje, van Bendenburg, Cornelissen, & Juriaanse, 1988; Rønholt, Kirkensgaard, Mortensen, et al., 2014) compared to the expected larger crystals formed upon maturing and churning at 22 °C. Thus, the churning time following a given thermal treatment of the cream can be listed as: cooling rate at 4 °C/min, maturing 1.5 h (22 °C) and churning at 22 °C < cooling rate at 4 °C/min, maturing 1.5 h (10 °C) and churning at 10 °C < cooling rate at 0.4 °C/min and a churning temperature of 10 °C < cooling rate at 7.5 °C/min and a churning temperature of 10 °C.

The macroscopic appearance of the butter grains was different as a result of the two churning temperatures applied (Fig. 2). Whereas churning at 22 °C resulted in a coherent, smooth and glossy surface (Fig. 2A), churning at 10 °C gave a lumpy structure of butter grains (Fig. 2B). This difference in appearance is in line with earlier studies, where the smooth and glossy surface is seen as a result of the high percentage of liquid fat not incorporated in the butter grains (Samuelsson, 1937; Nielsen, 1971), whereas the lumpy structure are expectedly formed by the very small butter grains (Samuelsson, 1937). This hypothesis is now confirmed by the fat content in the buttermilk (Table 1), showing that a higher amount of fat is excreted from the butter grains upon churning at 22 °C compared to 10 °C. Thus it can be confirmed, that the high SFC in the butter churned at 10 °C results in a harder structure of the milk fat globules, as more fat is crystallized. Due to the hard crystal shell of the milk fat globules, more energy must be applied to the crystals (i.e. shear) during churning before phase inversion occurs, explaining the long churning time (Table 1).

In the produced butter, conductivity was measured to ensure complete phase inversion from an oil-in-water to a water-in-oil emulsion. In all butters produced, the conductivity appeared close to zero (Table 1), confirming that water-in-oil emulsions were formed (Bordi et al., 1996).

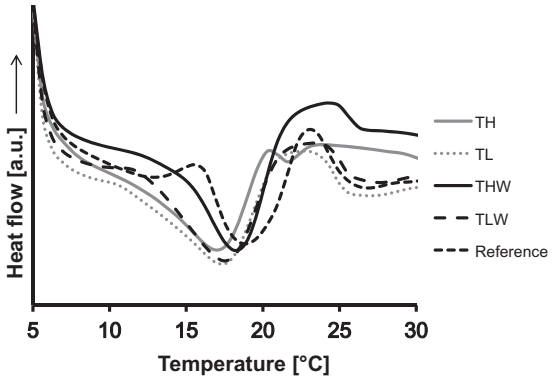

Fig. 3 – Representative thermogram of butter churned at 22 °C (TH), 10 °C (TL), 22 °C with water added (THW) and 10 °C with water added (TLW). A commercial available butter was used as reference. The measurement is performed after 4 days of storage at 5 °C.

3.2. *Effect of churning temperature and water content*

3.2.1. *Crystallization*

In the present study, cream was matured for 1.5 h at either 10 °C or 22 °C and subsequently churned at the maturing temperature. Such increase in churning temperature led to a decreased churning time and consequently a lower degree of shear during churning compared to a churning temperature of 10 °C (Table 1), as further discussed in Section 3.1. Thus, the

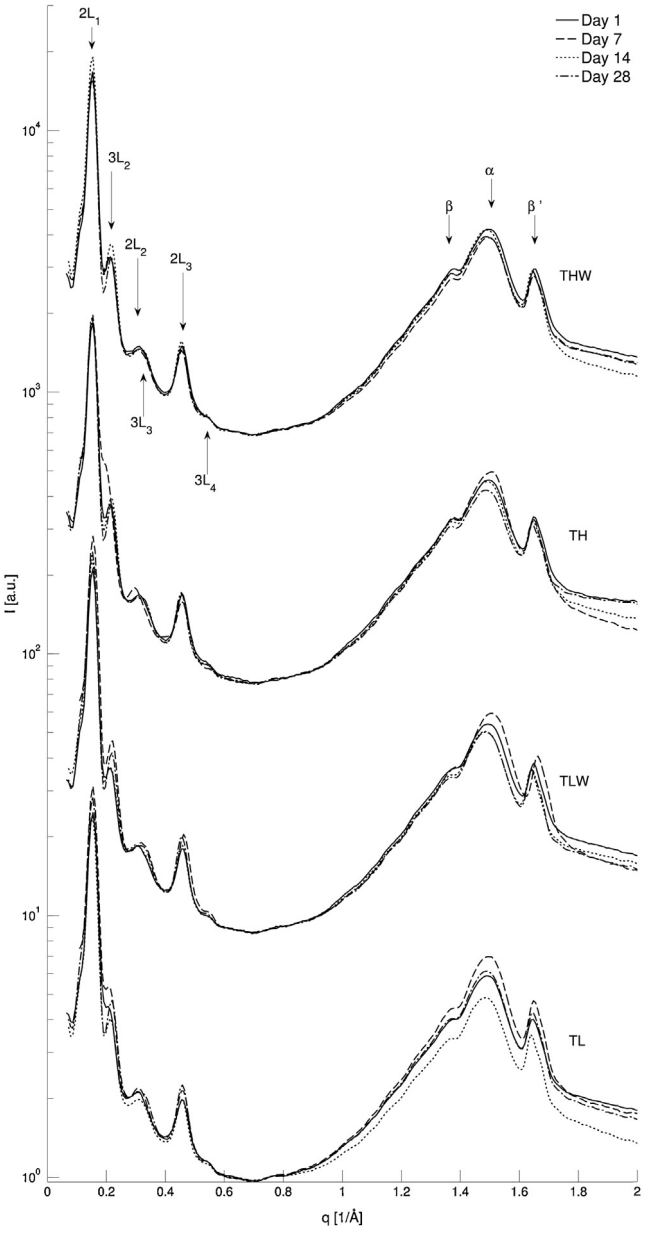

Fig. 4 – Spectra obtained form small and wide angle X-ray scattering (SAXS and WAXS). TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. The day refers to days of storage at 5 °C. See text for further details.

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cream churned at 22 °C is subjected to less shear than cream churned at 10 °C. It is well documented, that both thermal treatment (Wiking et al., 2009) and shear affects the crystallization behavior of milk fat-based products (Mazzanti, Gutherie, Sirota, Marangoni, & Idziak, 2003; Kaufmann, de Graef, Dewettinck, & Wiking, 2012; Kaufmann, Kirkensgaard, Andersen, & Wiking, 2013). Therefore, differential scanning calorimetry, small and wide angle X-ray scattering (SAXS and WAXS) were applied together with pulsed nuclear magnetic resonance (p-NMR) to evaluate the crystallization behavior (Figs. 3–5).

Milk fat is typically described by dividing the fatty acids present into the low (LMF), middle (MMF) and high (HMF) melting fraction with a melting point below 10 °C, within the range from 10 °C to 19 °C and above 20 °C respectively (Deffense, 1993). The main components of LMF being butyric acid (4.3%), hexanoic acid (2.5%) and linoleic acid (2.28%), for MMF being caprylic acid (1.5%) and oleic acid (22.59%) and finally for HMF being capric acid (3.3%), lauric acid (3.7%), mysteric acid (11.8%), pentadecanoic acid (1.48%), palmitic acid (31.3%), palmitoleic acid (1.92%), heptadecanoic acid (1.25%) and stearic acid (10.3%) (Couvreur, Hurtaud, Lopez, Delaby, & Peyraud, 2006). The values listed are given as fatty acid g/100 g of total fatty acids, and are reported for cows fed with corn silage replacements (Couvreur et al., 2006). In the present work, the thermograms of the butter including a reference show two melting fractions (Fig. 3). For the butters with no water added (TH and TL) and the butter churned at 10 °C with water added (TLW), an endothermic peak in the range of MMF is observed. The peak is characterized by an average melting point of 17 °C and appears relatively sharp, in accordance with previous thermograms recorded on milk-fat based systems (ten Grotenhuis, van Aken, van Malssen, & Schenk, 1999). Butter churned at 22 °C with water added (THW) and the reference, a commercial butter, are also characterized by an endothermic peak in the range of MMF, having an average melting point of 18 °C and 19 °C respectively. Again, the peaks appear sharp and well defined. Additionally for all butters tested, an endothermic peak appears in the range of HMF. For TH, the melting point is found at 21.5 °C, whereas the remaining butters all have a pronounced shoulder before a more broad endothermic peak, with an average melting point at 26.5 °C. For milk fat-based systems, peak broadening is seen as a result of a variety of transition-temperatures originating from the more than 200 identified even numbered triglycerides found in milk fat, as the multiple thermal events occurring leads to partial overlapping peaks (Gresti, Bugaut, Maniongui, & Bezard, 1993; Lopez, Bourgaux, Lesieur, & Ollivon, 2007; ten Grotenhuis et al., 1999). A corresponding, additional peak is seen for the reference in the MMF, having a melting point of 13 °C. In the range of the HMF, the lower melting point of TH compared to the other butters might be explained by the higher churning temperature inducing formation of less stable crystals with a lower melting point compared with the more stable ones (Vithanage, Grimson, & Smith, 2009). When comparing the peak values in the MMF, no systematic variation is observed.

In milk fat, three primary crystal polymorphs are present namely the α-, β′- and β-crystals with increasing order of stability (Larsson, 1966). What differentiates the crystals is their subcellular structure, as the α-crystals have a hexagonal structure, the β′-crystals an orthorhombic structure and the β-crystals a triclinic structure. Moreover, the crystalline chain structure is arranged in either double chain length (2L) or triple chain length (3L) structures (Lopez, Bourgaux, Lesieur, & Ollivon, 2002; Mazzanti, Gutherie, Sirota, Marangoni, & Idziak, 2004). For more details about parameters affecting crystal polymorphism and the link between crystal polymorphism and structural behavior of butter and other milk fat-based systems, the reader is referred to a recent review (Rønholt et al., 2013a).

For the present work, the SAXS and WAXS spectra are shown in Fig. 4. For TL, a 2L structure with a repeat distance of 41.2 Å and a 3L structure at 61 Å are observed at day 1. In the WAXS spectra, primarily β′-crystals are present together with traces of β-crystals and minor α contribution. Similarly for TH, THW and TLW, the SAXS peaks reveal a 2L structure at 41.2 Å complementary to a 3L structure at 58 Å. In the WAXS spectra

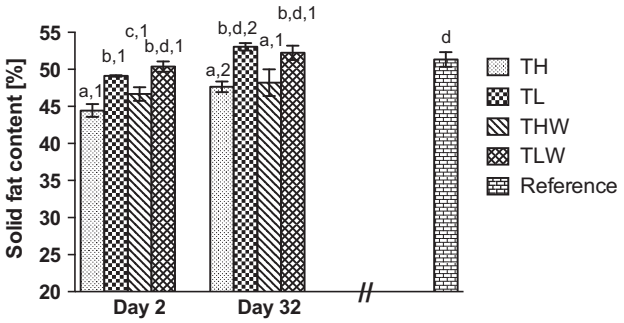

Fig. 5 – Percentage Solid fat content, SFC, relative to the total amount of fat in the system. TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. The day refers to days of storage at 5 °C. The error bar denotes the standard deviation, letters difference between treatments within the same day of measurement and letters difference within the same treatment as a function of time.

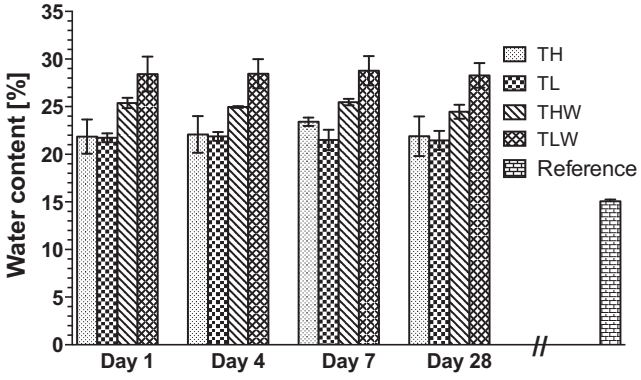

Fig. 6 – Average water content in the butter. TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. The day refers to days of storage at 5 °C. The error bar denotes the standard deviation. At all days of measurement, the water content of TLW was higher than of TH and TL.

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presence of primarily β'-crystals with minor traces of both α- and β-crystals are observed for TH, THW and TLW. From the thermograms (Fig. 3), it was concluded that TH are characterized with the least stable, mixed crystals among the butters studied. The SAXS and WAXS spectra suggest, that this behavior is not directly related to the crystal polymorphic state (Fig. 4), as α-crystals are present in all samples.

In Rønholt et al. (2012), butter produced from matured (48 h) and non-matured fast (7.5 °C/min) and slow cooled cream (0.4 °C/min) was analyzed using X-ray diffraction. While the butter produced from non-matured cream contained α and β′-crystals with minor traces of β-crystals, butter produced from matured cream contained only β′ and β-crystals (Rønholt et al., 2012). Likewise for puff pastry butter, primarily β′-crystals are found with a population of α-crystals after 2 days of storage at 20 °C (Rønholt, Kirkensgaard, Høyer, Mortensen, & Knudsen, 2014b). According to Ostwald's rule of stages, the first crystals formed are of the least stable polymorph (i.e. α-crystals in milk fat) existing at the given crystallization temperature, in this case 22 °C and 10 °C respectively. This behavior is explained by an increasing nucleation and growth time as a function of increased stability of the crystal polymorph (ten Grotenhuis et al., 1999). Consequently, more stable crystals are formed upon maturing at 10 °C due to the long churning time and thus a higher degree of shear applied to the system, when compared to a maturing and churning time of 22 °C. Shear rates in the range from 0 to 2800 s^{-1}, when applied for 30 min to milk fat and milk fat triglycerides held at 50 °C, have been shown to accelerate the transition from α to β′-crystals, when measured after subsequently cooling at 3 °C/min and maintaining the shear, to a crystallization temperature of 17–17.5 °C (Mazzanti, Marangoni, & Idziak, 2009). This behavior is explained by the fact that the applied shear changes the already formed crystallites, thereby inducing more nucleation sites for β′-crystals. Based on the present work, we can via the thermograms add to the finding of Mazzanti et al. (2009), that not only shear applied at 50 °C but also an increased shear applied to milk fat at lower temperatures combined with a high water content seems to induce a transition toward more stable crystals and must thereby be taken into account when designing such new products.

This is a relevant and unexpected finding, since presence of β-crystals is attempted eliminated in milk fat-based products, and β-crystals tend to form large, platelet-like crystals with a grainy macroscopic structure, resulting in sandiness-like mouthfeel (Madsen, 1971; Sato, 1999). However, a low churning temperature allows more water to be incorporated into the system and a higher SFC, without affecting the rheological properties of the product when comparing to a high churning temperature. One could speculate, that an intermediate churning temperature could be of industrial interest, if β-crystals could be avoided and on the same time much water incorporated into the system.

The high water content in THW and TLW (Fig. 6), reveals no significant effect on the polymorphic behavior in the butter, thus confirming our previous findings, where butter with a water content from 20% to 32% all showed presence of mainly β′-crystals with traces of β (Rønholt, Kirkensgaard, Mortensen, et al., 2014). Similarly for the SAXS spectrum a 2L (41 Å) and 3L (57 Å) lamellar organization was observed (Rønholt, Kirkensgaard, Mortensen, et al., 2014), as is the case in the present work for all butters studied independently of water content.

3.2.2. *Solid fat content*

The solid fat content depicted in Fig. 5 is corrected for water content (Eq. (1)), and therefore reflects SFC as the percentage of the total fat (Rønholt, Kirkensgaard, Mortensen, et al., 2014). As expected for day 2, a churning temperature of 10 °C significantly increases the SFC when compared to a churning temperature of 22 °C (Fig. 5) due to the higher degree of supercooling for TL compared to TH during production. Moreover, at day 2, SFC for TLW is the same as for the reference, a commercial butter. The results show, that increasing the water content in the butter from 22% to 28% (weight/weight) (Fig. 6) does not affect the solid fat content after water correction. However, butter churned at 10 °C contains more solid fat facilitating incorporation of more water in the butter matrix, when compared to butter churned at 22 °C (Fig. 6). Based on this, we are now able to experimentally confirm previous speculations (King, 1953; Nielsen, 1971), stating that low churning temperatures impede a large surface area allowing entrapment of more water in the butter matrix (King, 1953). Yet, no tendencies of water loss is observed in the present work, thus rejecting the hypothesis saying that such rigid structure formed as a consequence of low churning temperatures on the contrary allows formation of water channels facilitating drainage of the butter (King, 1953). If comparing with the literature, the level of SFC found at day 2 is in agreement with previous studies of butter having a similar water content and storage profile (Rønholt et al., 2012).

3.2.3. *Water droplet size and microstructure*

According to previous studies, a solid fat content above 9% is needed for milk fat-based systems to prevent coalescence of

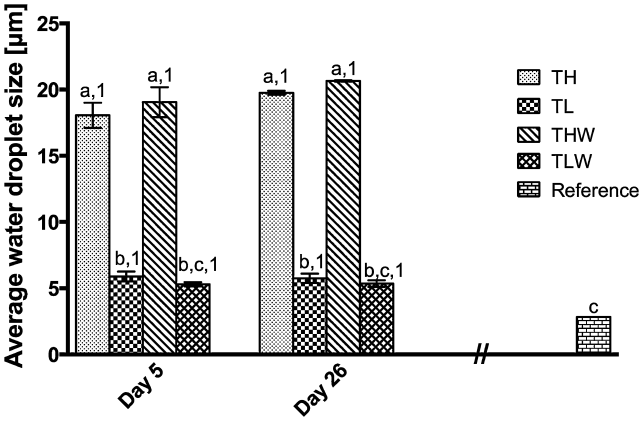

Fig. 7 – Average size of the water droplets dispersed within the butter. TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. The day refers to days of storage at 5 °C. The error bar denotes the standard deviation, letters difference between treatments within the same day of measurement and letters difference within the same treatment as a function of time.

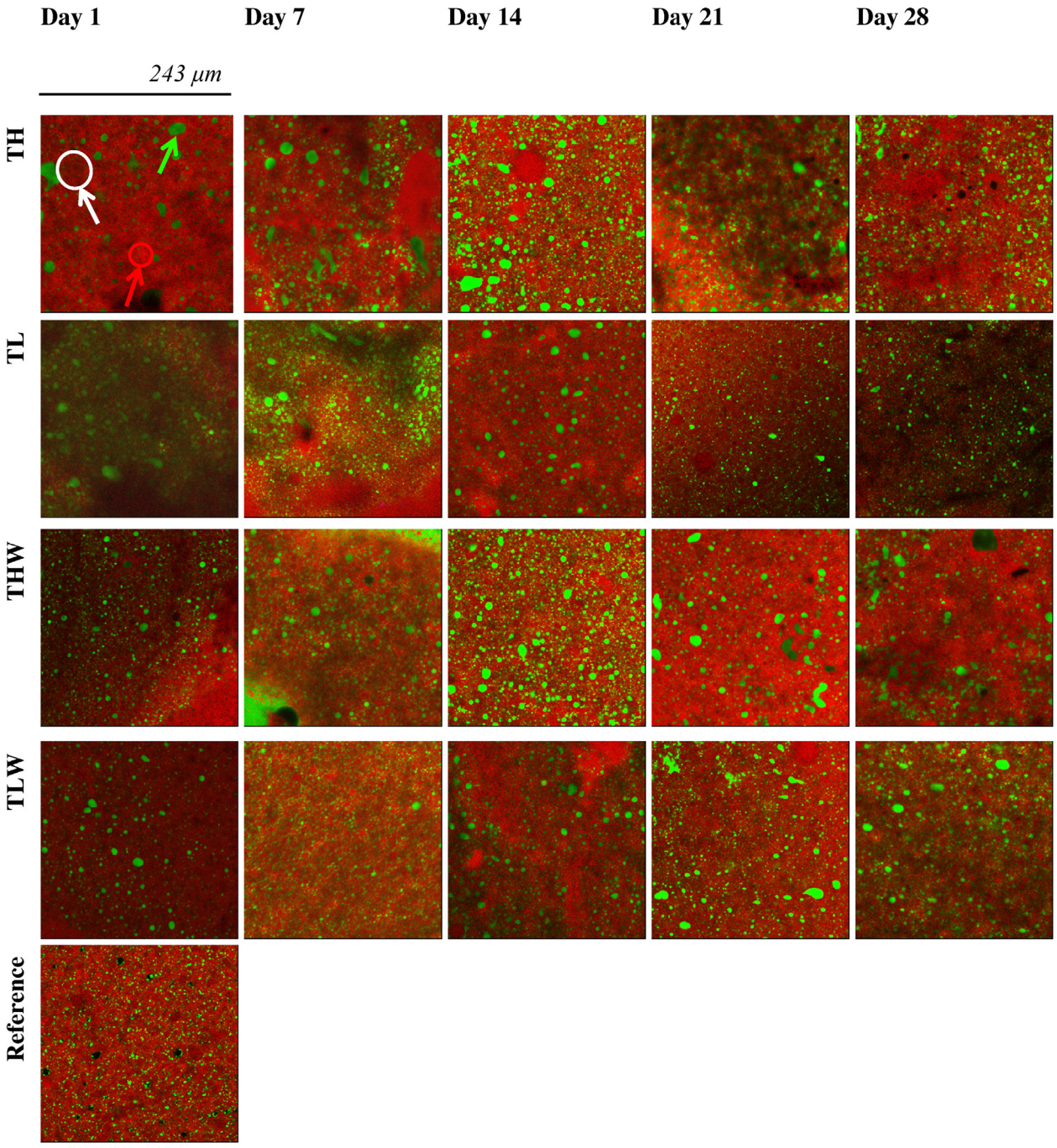

Fig. 8 – Microstructural images obtained using confocal laser scanning microscopy, CLSM, during storage. TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. The day refers to days of storage at 5 °C. The reference is a commercial butter studied after undefined storage time. The bright red spherical shaped areas represents the intact fat globules (red arrow), the red background the continuous fat phase, the black/gray shadows the fat crystals (white arrow), the blue/purple the phospholipids and the green color being the water droplets (green arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the water droplets (Rousseau, Zilnik, Khan, & Hodge, 2003). For the butters produced in the present study, SFC is well above 9% (Fig. 5). Churning at low temperature (TL and TLW) results in butter with an average water droplet size at day 5 of 5.5 μm, while commercial butter had an average diameter of 2.8 μm (Fig. 7). An increase in the churning temperature from 10 to 22 °C leads to a significant increase in water droplet size, with an average value of 18.5 μm, corresponding to a decreased SFC. Similar high values for average water droplet size are previously reported in butter produced at laboratory scale

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containing 20–32% water (Rønholt, Kirkensgaard, Mortensen, et al., 2014). Most likely, this is due to a less intense degree of mixing in a laboratory scale setup compared to industrial production. Such differences in water droplet size between butters produced at different temperatures are likely to affect both microbacterial growth and sensory evaluation of the butter (Frede & Buchheim, 1994; Delmarre & Batt, 1999). Those parameters will not be discussed further as they are not within the scope of the present work. No significant difference is, however, seen in the microstructure of the different butters (Fig. 8).

3.2.4. *Rheological behavior*

After nucleation and crystal formation, the fat crystals grow forming a 3D fat crystal network. The crystal–crystal interactions are typically characterized as either reversible (secondary) bonds occurring due to van der Waals interactions between the crystals or as the stronger irreversible (primary) bonds formed upon mechanical interlinkage of the crystals (van den Tempel, 1958; Haighton, 1965). The properties of the fat crystal network can be quantified using rheological analysis (Narine, & Marangoni, 1999). In the present work, we determined the strain applied to obtain a 10% and 50% decrease in the elastic modulus (G') (Fig. 9). This strain is related to brittleness of the butter (Rønholt et al., 2013b). Also, the storage modulus G' and the loss modulus (G'') were monitored during a frequency sweep (Fig. 10). Whereas G' is shown to be directly linked to product hardness for milk fat-based products (Narine, & Marangoni, 1999), the relationship between G' and G'' shows whether the sample behaves solid or liquid-like. For both strain at fracture, G' and G'' no difference was seen as a function of churning temperature or water content when compared to the reference, despite a higher SFC for butter churned at 10 °C (Figs. 5, 9 and 10). This finding again highlights, that SFC cannot be used as the single parameter to predict hardness of milk fat based systems, as hardness depends on the interplay between microstructure and SFC (Narine, & Humphrey, 2004; Rønholt et al., 2012, 2013c; Rønholt, Kirkensgaard, Mortensen, et al., 2014). All butters tested showed a frequency depending manner and with $G' > G''$, indicating a solid-like behavior (Fig. 9). Similar frequency dependent behavior has previously been reported for G' measured on butter (Rohm & Weidinger, 1993; Shukla & Rizvi, 1995). How working temperature affects crystallization and hardness of butter has previously been studied using the cone penetration method (Taylor, Dolby, & Russell, 1973). Taylor et al. (1973) found an increased temperature from 12.5 °C to 15 °C during working and temperature changes from 12.5 °C to 18 °C during production to induce crystallization. Contrary, a decrease in temperature from 12.5 °C to 7 °C (30 min) did not induce any changes in crystallization behavior compared to a stable temperature of 12.5 °C. The effect of water content on the rheological properties of butter has previously been studied, showing that an increase in water content from 20% to 32% (weight/weight) results in an decrease in G', whereas an increase in water content from 20% to 26% only decreases G' for butters produced from slow cooled cream (0.4 °C/min) when measured the day after production.

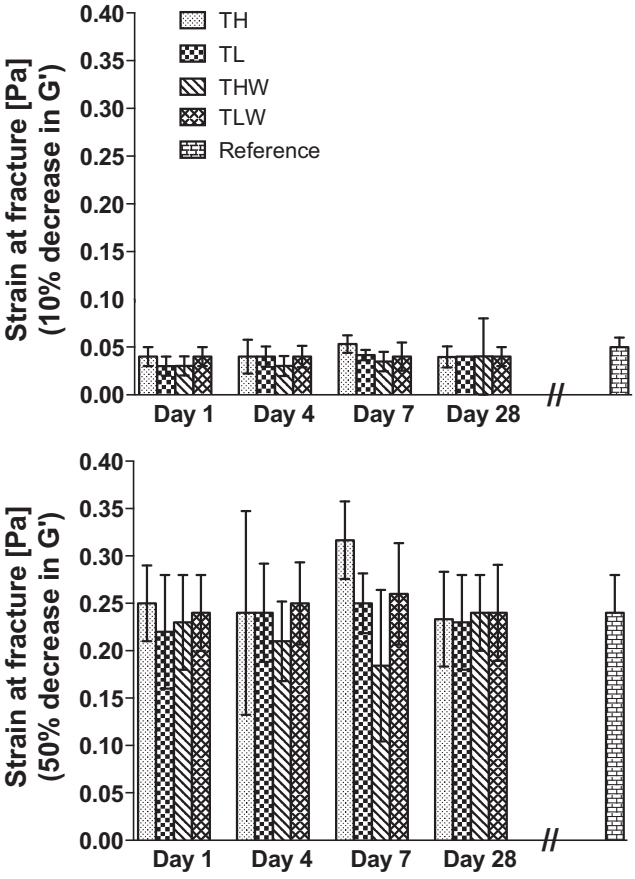

Fig. 9 – Strain applied to obtain a decrease of 10 and 50% respectively in the elastic modulus relative to the elastic modulus, G', in the linear region. TH: churning at 22 °C, TL: churning at 10 °C, THW: churning at 22 °C with water added TLW: churning at 10 °C with water added. The day refers to days of storage at 5 °C. At day 7, butter churned at 22 °C requires a higher strain compared to butter churned at 22 °C with water added before reaching a both 10% and 50% decrease in G'.

In the present work, it was possible to increase the water content to a larger extend (6%) for butter churned at 10 °C compared to 22 °C (4%) without affecting rheological properties and crystal polymorphism. The water droplets are, however, larger for butter churned at 22 °C compared to 10 °C, as less solid fat is present to prevent the water droplets from coalescence. This does, however, not affect the hardness of the butter. One might speculate, that if comparing butter containing less than 22% water, the interactions between the fat crystals are less hindered compared to butter containing more water. Differences in microstructure as a function of water content might change the rheological properties, in this case induced as a result of churning temperature.

3.3. *Effect of storage*

For TL, the SAXS data revealed that both the 2L structure observed in the SAXS with a repeat distance of 41.2 Å and the

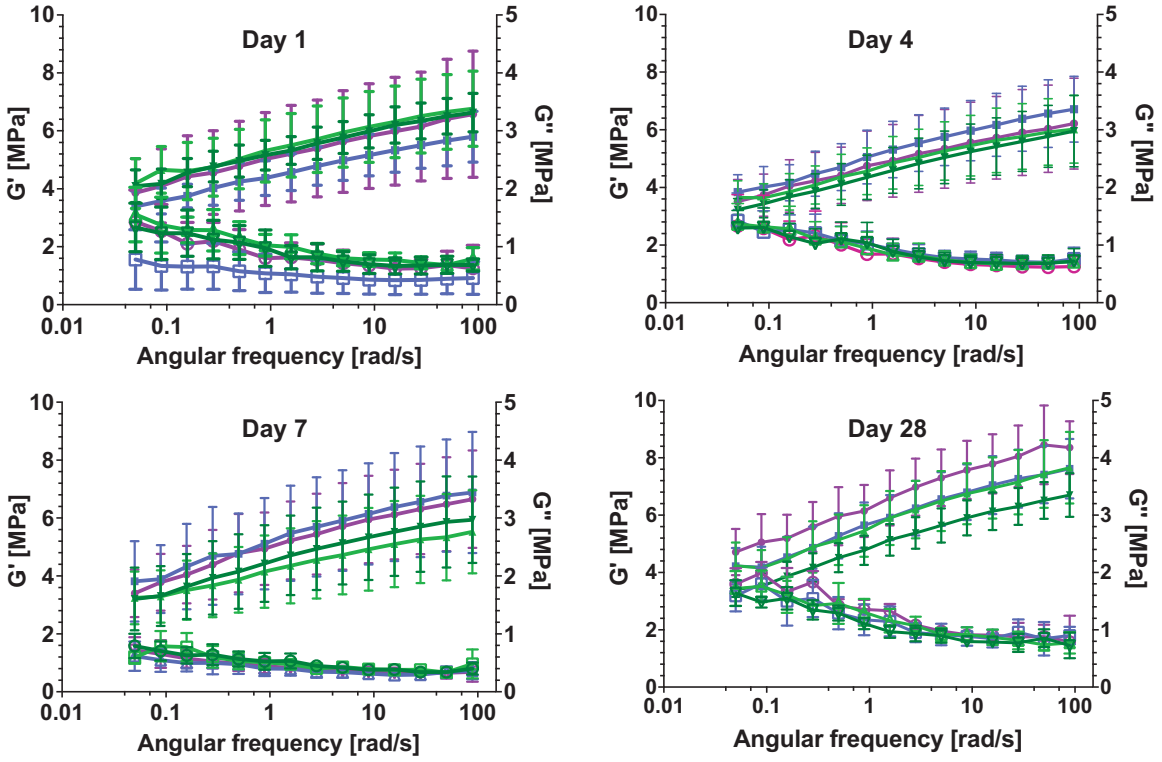

Fig. 10 – Frequency sweep of butter churned at 22 °C (○), 10 °C (□) and the butter with added water churned at 22 °C (△) and 10 °C (▽). The elastic modulus, G', is seen on the left axis and corresponding graphs are shown using closed symbols. The storage modulus, G″, is seen on the right y-axis and corresponding graphs are shown using open symbols. The day refers to days of storage at 5 °C. The error bar denotes the standard deviation. No changes in G′ or G″ at 5 rad/s are observed as a function cream treatment or water addition. However, G′ increases for the butter churned at 10 °C from day 1 to 28.

3L structure gradually changes from day 1 to day 14 from ca. 61 Å to 58 Å and then stays constant (Fig. 4). The WAXS peaks do not change significantly over the time course of the experiment, as primarily β′-crystals are observed with traces of β. At day 1 and 7, minor populations of α-crystals are observed. For TLW and TH, the only difference seen as a function of time is at day 7, where both the WAXS peaks and the 2L packing unexpectedly seems to be shifted a little toward a more tightly packed structure. This is, however, likely an artifact caused by a minor temperature drift during this measurement (Rønholt, Kirkensgaard, Høyer, et al., 2014). Both the SAXS and WAXS spectra are stable for THW throughout the storage period. SFC did, however, increase as a function of time, still the butter churned at low temperature having a higher SFC compared to butter churned at high temperature (Fig. 5). This increase in SFC as a function of time is also reflected in the microstructure of the butter (Fig. 8), where more black-gray shadows i.e. more solid fat occurs during storage. Although SFC increased from day 2 to 32, both strain at fracture, G′ and G″ remained stable. The only exception is TL, showing an increase in G′ from day 1 to 28. Neither the average water droplet size (Fig. 7) nor water content (Fig. 6) showed any difference as a function of time, indicating that no destabilization of the system occurred during the storage period. Consequently, our work shows that the churning temperature is not a crucial parameter to maintain stable structure during butter production. It should be mentioned, though, that increasing the temperature increases the fat content of the buttermilk, thus a lower yield is expected.

4. Conclusions

The butter produced at the two different churning temperatures, 10 °C and 22 °C, did not show significant difference in the water content (~22%), but more water could be incorporated upon mixing for butter churned at 10 °C. This allows us to confirm a previously posted hypothesis, stating that the high fat content remaining in the butter grains upon churning at 10 °C compared to 22 °C impedes formation of smaller and harder butter grains, thus allowing more water to be entrapped in the butter matrix. On the contrary, a high amount of fat is excreted from the butter grains upon churning at high temperature compared to low temperature, also confirming a previous hypothesis. Moreover, differences in crystal stability, solid fat content and water droplet size were established. No difference was observed in crystal polymorphism, as all butters contained primarily β′-crystals with traces of α- and β-crystals throughout the storage period, together with presence of 2L and 3L lamellar packing. Moreover, churning temperature of 22 °C induced a lower solid fat content and an increased average water droplet size

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compared to butter churned at 10 °C. Nonetheless, the changes in the water and solid fat content did not affect the hardness or brittleness of butter. During the storage at 5 °C for 28 days, all the produced butter had an increase in the solid fat content, while the average water droplet size was constant. Yet, neither the hardness nor the brittleness increases during the storage. These findings suggest that the churning temperature and the amount of water do not affect the hardness or the brittleness of butter. Conclusively, the churning temperature can be used as a tool to incorporate more water into butter-like systems, without affecting the rheological behavior and crystal polymorphism after four weeks of storage.

Industrial relevance

Recently, there has been an increased consumer interest for and focus on butter and butter like products with lower fat content and an improved nutritional profile. However, for the industry to develop such new and innovative products it is important to understand how the different production steps affect the structure and functionality of butter and butter like products. This paper addresses churning temperature of cream and how this production step affects structural and rheological properties of butter. The results add new knowledge of potential interest to the industry and especially for the product development phase. The results may form a basis for further shaping and design of production setup according to desired structure and functionality hence saving time and money.

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