1	A small scale laboratory process for cream cheese production and characterisation		
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15 Abstract

16 Cream cheese production is well established at large scale but an effective small scale laboratory 17 process could facilitate higher throughput and lower the cost of experimental studies. Whey was 18 separated using centrifugation or the cloth bag method and the effect of heating prior to separation 19 examined. Heat treatment and centrifugation resulted in cream cheese with a microstructure, 20 composition and rheological properties comparable to a commercial scale cream cheese. Heating was 21 necessary to achieve effective separation, the descried product microstructure and an adequate firmness 22 and viscosity, with the heat induced denaturation of some whey proteins contributing to these properties. 23 Whilst both whey separation methods resulted in a similar microstructure, centrifugation led to less fat 24 loss and an optimal product. These data provide new insights into the development of cream cheese 25 microstructure and provide a route to further understand and optimise this process.

26 Keywords: cream cheese, centrifugation, confocal, scanning electron microscope, microstructure

- 27 1. Introduction
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Cream cheese production has increased steadily in the last few decades, with more than 4.4×10^5 29 tonnes of cheese produced in the United States in 2015/16 (USDA, 2017) and 8.8 x 10⁴ tonnes produced 30 31 in Australia in 2015/16 (Dairy-Australia, 2017). Cream cheese is defined by the Food and Agriculture 32 Organization (FAO) as a soft, spreadable, unripened and rindless product (FAO, 2016). An increase in 33 the popularity of this cheese can be attributed to use in the food service sector, including in dips and 34 spreads and as an ingredient in the baking industry, where the product structure contributes to functional 35 properties including the rheological and textural properties. Whilst there are some existing studies on 36 cream cheese at pilot and commercial scale in the literature (Brighenti, 2009; Sanchez, Beauregard, 37 Chassagne, Bimbenet, & Hardy, 1994, 1996; Wendin, Langton, Caous, & Hall, 2000), there is no 38 documented process that can model commercial processes at a laboratory scale. Such an advance would 39 enable a detailed characterisation of the stages involved in cream cheese production and allow higher 40 throughput studies to test the role of ingredients and process parameters on product properties.

41 In general, cream cheese typically belongs to two product categories defined by their fat content: 42 double fat cream cheese made with milk standardized to at least 9-11% (w/w) fat and single fat cream 43 cheese made with milk standardized to 4.5-5% (w/w) fat (Guinee, Pudja, & Farkye, 1993). 44 Compositional requirements differ between countries and the food and drug administration in the US 45 regulates that cream cheese should have a minimum milk fat content of 33% (w/w), with a moisture content no greater than 55% (w/w) (FDA, 2016). The FAO Codex standard also specifies a minimum 46 47 dry matter of 22% (w/w) and minimum fat content of 5.5% (w/w) (FAO, 2016). In Australia, production 48 is not regulated in this way but the most common product is similar to a US double fat cream cheese 49 product.

The production of double fat cream cheese starts with the homogenization and pasteurization of standardized milk, followed by acidification with lactic acid bacteria (Phadungath, 2005). Fermentation leads to coagulation, where the milk protein aggregates to form a continuous protein network, commonly referred as an acidified milk gel. This gel is then subjected to heat treatment, which reduces the activity of the starter culture (Phadungath, 2005). The mesophillic starter cultures employed are typically inactivated by heating above 60°C. Inactivation occurs after ~2 min for *Lactococcus lactis*subsp. *lactis* (Kang, Jeon, Shin, Kwon, & So, 2015). *Lactococcus lactis* subsp. cremoris is similarly
expected to be inactivated at this temperature (Kim, Ren, & Dunn, 1999), although susceptibility may
vary between strains.

Heating is typically achieved at commercial scale by pumping the gel to a tubular or a plate heat exchanger prior to feeding the curd into a centrifugal separator. The shear involved during this transfer depends on many factors including: the type of pump, pipe length and pipe diameter, temperature of the feed, separator feed flow rate, type and size of separator and factory layout (Mezger, 2014; Schmitt, Sturm, Grupa, & Hensel, 2016; Steffe & Daubert, 2006). Despite these differences, the net effect of this unit operation is an increase in the temperature of the curd and breakdown of the curd into smaller sized particles.

66 The heat treatment applied to inactivate the starter culture also affects whey separation during 67 centrifugation, which can be assumed to follow Stokes' law (Heymann, 2011; McCarthy, 2011) :

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$$\upsilon = \frac{d^2(\rho_s - \rho_f) \left(\frac{\pi\omega}{30}\right)^2 r}{18\mu}$$

where v is the radial separating velocity (m s⁻¹), d is the diameter (m), ρ_s and ρ_f are the density of 69 70 particle and fluid respectively (kg m⁻³), r is the radius of rotation (m), μ is the viscosity of the continuous 71 phase (Pa s) and ω is the speed of rotation. Noting, however, that the particles will be in close proximity 72 so will be affected by hindered settling. Nevertheless, the simplified Stokes equation describes how an 73 increase in temperature decreases the viscosity and fluid density of the sample (Bakshi & Smith, 1984), 74 increasing the rate of the separation. Heat treatment can also denature whey proteins, altering 75 interactions with casein proteins (Nguyen & Anema, 2017) but the extent of whey protein denaturation 76 as a function of heat treatment in cream cheese production is unclear.

The continuous centrifugal separation employed at industrial scale ensures sufficient whey is removed to achieve a standard cream cheese moisture content of ~50-54% w/w (Brighenti, 2009; Phadungath, 2005). Such centrifugal separation allows for a faster continuous process, compared to traditional batch processing using cloth bags, allowing the hot sample to be pumped to a hopper where gums and salt are added, followed by shearing in a kettle and hot filling into packaging (Phadungath, 2005). Industrial separators have a throughput of 1500 L h⁻¹ to 13500 L h⁻¹ (GEA, 2016), making these
unsuitable for laboratory investigation. In this study, batch separation using a laboratory scale centrifuge
was investigated as a model of small scale separation. Comparisons were also made to a process where
whey was separated using the traditional cloth bag method and the properties of the resultant cream
cheese compared.

87 Rheological and textural assessment are typically used to measure the properties of cream cheese (Brighenti, Govindasamy-Lucey, Nelson, & Lucey, 2008; Brighenti, 2009; Sanchez et al., 1994, 1996). 88 89 While these tools have been coupled with structural analysis at the micron level to assess the effects of 90 processing in other dairy products (Auty, Twomey, Guinee, & Mulvihill, 2001; Everett, Ding, Olson, 91 & Sundaram, 1995; Nguyen, Ong, Kentish, & Gras, 2015; Ong, Dagastine, Kentish, & Gras, 2012), this 92 approach has not previously been adopted for cream cheese. A few studies have examined the final 93 cream cheese product using confocal laser scanning microscopy (CLSM), scanning electron microscopy 94 (SEM) or transmission electron microscopy (TEM) (Kalab & Modler, 1985; Monteiro, Tavares, 95 Kindstedt, & Gigante, 2009; Wendin et al., 2000) but the fat, which is a major component in cream 96 cheese is not typically retained within the microstructure when assessed using conventional SEM and 97 TEM. The current study employs complementary CLSM and cryo SEM techniques to preserve the fat, 98 protein and other components to investigate how the microstructure develops from milk to a continuous 99 gel structure, a concentrated curd and the final cream cheese product after cooking, stirring and gum 100 addition.

101 This study aimed to develop a laboratory scale production process that can facilitate higher 102 throughput and lower cost experimental studies. Four types of cream cheese were made using different 103 whey separation processes: a batch centrifugation process and cloth bag method with the addition or 104 absence of a heat treatment step prior to whey separation. These experiments sought to better understand 105 whey separation and the role of temperature in this unit operation. The ingredients and the processing 106 parameters used were selected to resemble commercial production, potentially allowing manufacturers 107 to evaluate the effect of different ingredients and processing parameters that have a critical impact on 108 the final product properties of cream cheese. A second aim of the study was to characterize the changes 109 that occur to the microstructure during the production of cream cheese.

111 **2.** Materials and Methods

Preliminary experiments including microstructure analysis, rheology and an evaluation of centrifugation (Section 2.1 – 2.4) were performed to obtain an indication of the optimum temperature and centrifugation speed for a laboratory scale separation of whey for batch cream cheese production. These parameters were then used for the cheese making trials described in Section 2.5.

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117 **2.1 Preparation of acid gel**

Milk was collected from a commercial plant (Victoria, Australia). The milk was standardized, homogenized and pasteurized in the factory. Fat was standardized by blending raw milk with cream to obtain a final concentration of 12.3% w/w fat and 3.4% w/w of protein. The composition of the milk was measured using a Milko Scan FT120 (FOSS, NSW, Australia). The milk was then homogenized at 14 MPa 55°C and pasteurized at 72°C for 15 s. The processed milk was stored at 4°C and used within 1 week.

The acid gel was prepared by fermentation with 0.3% w/w frozen mesophillic starter culture (Chr. Hansen, Victoria, Australia). Prior to the addition of the culture, the milk was heated to 31°C in a water bath. The cultured milk was then added to 250 mL plastic containers for the analysis of microstructure by CLSM and rheological assessment (Sections 2.2 and 2.3) or added to 1.8 mL centrifuge tubes for centrifugation tests (Section 2.4); all samples were incubated at 31°C in an incubator to assist fermentation (Thermoline, NSW, Australia). The fermentation was stopped when the pH of the sample reached pH 4.5 by cooling the sample in the fridge to 4°C.

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132 2.2 Characterization of milk and acid gel using CLSM

The microstructure of the standardized milk and acid gel was analysed using a Leica SP8 CLSM (Leica Microsystems, Heidelberg, Germany) following a published method used for milk and rennet gels (Ong, Dagastine, Kentish, & Gras, 2010). Six milk and acid gel images were collected at different magnifications for each sample and a representative image is presented.

138 **2.3 Rheological properties of acid gel**

139 Dynamic shear rheological analysis was performed for the acid gel using a rheometer (AR-G2, TA 140 Instrument, New Castle, USA). The strain limit was first determined in a strain sweep experiment to 141 obtain the linear viscoelastic region (LVR). A low strain of 0.1% was found to be within the LVR and was used for the temperature sweep experiment, where a sweep from 20°C to 90°C was performed 142 using a flat parallel plate 40 mm in diameter. The temperature increment was set at 5°C for 90 s at a 143 constant strain of 0.1% and angular frequency of 5 rad s^{-1} . Approximately 3 grams of gel sample was 144 used and the gap was set to 2 mm. Five acid gel samples were each analysed using the temperature 145 146 sweep experiment (n = 5).

The flow properties of the gel were assessed at 20°C and 70°C using a cone plate with a diameter of 40 mm and angle of 4°. The flow curve was obtained by increasing the shear rate from 0.1 s^{-1} to 100 s⁻¹ over 300 s followed by a holding time of 5 seconds. The shear rate was then decreased from 100 s⁻¹ to 0.1 s^{-1} . Three acid gel samples were analysed at each temperature (n = 3).

The broad shear rate selected here $(0.1 \text{ s}^{-1} \text{ to } 100 \text{ s}^{-1})$ covers the range that the sample is expected to experience as it passes through a pipe in the production plant, which was estimated to be 4 s^{-1} to 75 s⁻¹, calculated using an equation for non-Newtonian shear (shear rate = 2 u/d), where the mean velocity (u) of the sample was doubled and divided by the diameter (d) of the pipe (Steffe & Daubert, 2006). The pipe dimensions were assumed to range from 5 cm to 6.5 cm and the flow rate from 1350 – 13500 L h⁻¹.

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158 **2.4 Whey separation using centrifugation**

Preliminary centrifugation experiments were performed using a fixed angle Eppendorf 5424 centrifuge (Eppendorf, NSW, Australia). The gel samples were initially vortexed at 500 revolutions per minute (rpm) for 10 minutes and centrifuged at 3000 g, 5000 g, 10000 g, 12000 g or 15000 g for 10 min at 20°C. In a parallel experiment, gel samples were vortexed at 500 rpm, incubated at 70°C for 10 minutes follow by centrifugation at 3000 g, 5000 g, 10000 g, 12000 g or 15000 g for 10 min at 20°C. After centrifugation, the whey was carefully decanted and mass measured on a micro-balance (Mettler Toledo, Victoria, Australia). The results are presented as the mean together with the standard deviation of the mean (n = 4).

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168 **2.5 Cream cheese processing**

169 Four batches of cream cheese were made in one cheese making trial. Two independent trials were 170 conducted producing a total of eight batches of cream cheese samples. In addition, full fat commercial cream cheese samples were analysed for comparison. The milk composition, pre-treatment (i.e. 171 172 standardization, homogenization and pasteurization) and the fermentation process are described in 173 section 2.1, except 2.2 kg of milk was used for each batch. The composition of the milk for the cheese making trial was $10.6\% \pm 0.07$ fat and $3.4\% \pm 0.04$ protein. Whey separation was carried out by 174 centrifugation in a Sorvall RC6 Plus centrifuge (Thermo Scientific, Victoria, Australia) or using a cloth 175 176 bag. Samples were either heat treated or left at room temperature prior to whey separation.

The sample without heat treatment was mixed in the kettle at 500 rpm for 10 min prior to the whey separation using a batch centrifugation process (12000 g for 10 min) or using a cloth bag at room temperature overnight (16 hours). Where required, the gel was heated to 70°C with mixing at 500 rpm prior to whey separation using a centrifugation process (5000 g for 10 min) or using a cloth bag. The time required for the sample to reach 70°C was approximately 7-8 minutes but the total heat treatment time was standardized to 10 min.

After whey separation, the curds were heated to 80°C with mixing at 500 rpm prior to the addition of sodium chloride 1.0 % w/w and a mixture of locust bean gum and guar gum (1:1, 0.5% w/w). The mixing process continued for a further 25 minutes at 80°C. The total heat treatment and shearing of the curd was 30 min. The cream cheese samples were then hot filled into sample containers 120 mL in size before storage at 4°C.

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189 **2.6 Protein profiles of whey from different whey separation techniques**

The protein within whey was analysed using Sodium Dodecyl Sulphate–Polyacrylamide Gel
Electrophoresis (SDS-PAGE). Whey samples 8 μL in volume were mixed with 10 μL of 4x lithium
dodecyl sulfate (LDS) sample buffer solution and 4 μL of Bolt reducing agent (both from Life

193 Technologies, Victoria, Australia), together with 18 µL of milli-Q water before boiling at 100°C for 3 194 min. The sample was then loaded onto a 4-12% tris glycine gel (Life Technologies) in duplicate, where 195 each well contained $\sim 20 \,\mu\text{L}$ of the sample solution. A molecular weight standard 7 μL (Precision plus, 196 Bio-Rad, Vic, Australia) was also loaded into the gel. The electrophoresis running conditions and the 197 staining and de-staining followed the method reported in a previous study (Ong et al., 2010). The gel was then scanned using a Fuji Film Dark Box II and Fuji Film LAS3000 V2.2 software (Brookvale, 198 199 Australia) and proteins identified based on molecular weight standards.

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2.7 Microstructure of curd obtained after whey separation and cream cheese

202 The curd and cheese samples for CLSM were prepared in a cold room at 4°C where samples were 203 cut with a surgical blade to approximately 2 mm x 2 mm x 2 mm in size and stained with Nile red and 204 Fast Green FCF as described in a previously published method (Ong et al., 2010). Two samples were 205 prepared for each treatment in each trial. Two sets of three dimensional (3D) images were collected 206 from each sample giving a total of four sets of 3D data for each of the four processes.

207 The 3D image reconstruction was performed using Imaris image analysis software (Bitplane, USA). 208 Each 3D image was processed to obtain the total volume of the fat and the protein, as described in a 209 previous method (Ong, Dagastine, Kentish, & Gras, 2011a). The unstained section was calculated from 210 the total sample volume minus the total volume of fat and protein, as shown in Supplementary Figure 211 1.

212 The cream cheese samples were also analysed using cryo SEM (Quanta, Fei Company, Oregon, 213 USA) following an established method (Ong et al., 2011a). Two samples were prepared for each of the 214 four processes. SEM images were collected from different magnifications (2000 - 16000x) and one 215 representative image is presented.

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217 2.8 Composition of whey and cream cheese

218 The fat content of the whey and cream cheese was analysed using the Babcock method (AOAC, 2002) and the protein content using the Kjeldahl method (IDF, 1993). The moisture content was 219 220 analyzed by oven drying (IDF, 1982).

222

2.9 Rheological properties of cream cheese

Cream cheese samples were added to a 20 mL disposable plastic syringe where the end of the syringe was removed. This formed samples in a cylindrical shape (20 mm in diameter and 10 mm in height) that could be pushed from the cut end and dispensed onto the rheometer. The sample was placed on the temperature controlled base plate set at 10°C. The gap was adjusted by lowering the 40 mm stainless steel parallel plate to 2 mm followed by a conditioning step at 10°C for 10 minutes. A strain sweep was then performed (0.001% - 100%) at 10°C at a constant angular frequency of 6.283 rad s⁻¹.

A frequency sweep was also performed for the fresh samples at 10°C from 1 Hz -100 Hz at a constant strain of 0.01%. The gap used was also 2 mm. A total of eight batches of laboratory cream cheese were made, two for each process. At least two measurements were performed for each batch and the data averaged. The data is presented as the mean \pm the standard deviation of the two average values for each process (n = 2).

234 2.10 Firmness and spreadability of cream cheese

The firmness of cream cheese at $10^{\circ}C \pm 1^{\circ}C$ was determined using a compression test on a TA-XT Plus texture analyser (Stable Micro Systems, Surrey, UK). Samples stored in the fridge were placed in a 10°C incubator for 1 hour prior to analysis. A 5 kg load cell and a 5 mm diameter flat cylindrical probe were used for compression with the trigger force set to 3 g and a test speed of 5 mm s⁻¹. The firmness of the cheese was defined as the maximum force required by the probe to compress the cream cheese by 10 mm.

A spreadability test was also performed on the cream cheese samples at 10° C following a previous method with some modification (Brighenti et al., 2008). A 40° Perspex cone was used to penetrate the cream cheese samples by 12 mm at a test speed of 2 mm s⁻¹.

A total of eight batches of laboratory cream cheese were made, two for each treatment. At least three measurements were performed for each batch and the data averaged. The data is presented as the mean \pm standard deviation of the two average values for each process (n = 2).

248 2.11 Data analysis

The results were analysed using a statistical package from Minitab (Minitab Inc, PA, USA). Analysis of Variance (ANOVA) and Tukey pairwise comparisons were performed with a significance level of P=0.05. Linear regression analysis was used to determine a relationship between hardness and moisture or fat content.

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254 **3. Results and Discussion**

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256 **3.1 Microstructure of the milk and acid gel**

257 The microstructure of the milk standardised prior to cream cheese manufacture was found to contain 258 'fat clusters' or 'homogenization clusters' when assessed by CLSM (Figure 1a1). This clustering was 259 expected, as there is insufficient milk protein to cover the surface of the newly formed homogenized fat globules (Walstra, Geurts, Noomen, Jellema, & Boekel, 1999) in this high fat milk stream. The 260 261 appearance of homogenization clusters resembles that previously reported for high fat products, such 262 as cream, which contained 42% w/w fat and 2.1% w/w protein (Chandrapala et al. 2016). The size of 263 the particles in the standardized and homogenized milk measured by image analysis (D[3,0]) ranged 264 from $0.88 - 0.92 \mu m$, similar to the D[4,3] of milk particles measured by light scattering after homogenisation at 10 MPa at 65°C (Brighenti, 2009). 265

A three dimensional network formed after 5 hours of acidification when the pH of the milk coagulum reached 4.5 (**Figure 1a2**). Both the fat globules and protein appear to contribute to this network structure; this arrangement is quite distinct from the gel prepared for Cheddar cheese making, where the unhomogenized fat globules act as inert fillers (Ong et al., 2011a).

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3.2 Heat treatment and shearing; the effect on gel properties and the whey separation

The acid milk gel is typically heated in cream cheese production to aid whey separation, so the effect of heat treatment on gel properties was assessed using a small amplitude temperature sweep from 20° C to 90°C. The gel was more elastic than viscous for the range examined, as the storage modulus (G') was greater than the loss modulus (G'') (**Figure 1b**). The G', G'' and viscosity (μ) decreased as the temperature increased from 20°C to 60°C (Figure 1b and 1c), indicating a weakening of the gel structure, which is likely due to the liquefaction of fat as the temperature increases. Coalescence of fat at higher temperatures (Lefevere, Dewettinck, & Huyghebaert, 2000; Ong, Dagastine, Kentish, & Gras, 2011b), could also reduce structural support. The lower modulus and lower viscosity of high fat products at higher temperatures is known to increase sample flow and increase the efficiency of separation during centrifugation (Hoffmann, 2011).

A temperature of 70°C was selected for further study, as there was no further significant reduction in G', G" or μ between 60-80°C. The tan delta (δ), which is calculated from the ratio of G"/G', did decrease above 60°C, however, indicating an increase in elasticity as the gel is further heated (**Figure 1b**). The variability in G' and μ data increased above 80°C, potentially due to protein aggregation, such aggregation could affect separation and the final product properties providing a further reason to separate the whey at a lower temperature of 70°C.

The microstructure of the gel samples at the end of the temperature sweep is shown in **Figure 1a3**, to provide an indication of the structure of the heated sample prior to whey separation. The gel appears in dense clumps, possibly due to the contraction of the gel network upon heating. Some fat globules have coalesced and others appear on the surface of the gel particles, where they may be lost to the whey during centrifugal separation.

The acid gel was found to be irreversibly altered when subject to shear $(0.1 \text{ s}^{-1} - 100 \text{ s}^{-1})$, a range that includes the shear likely experienced within factory pipes (4 s⁻¹ to 75 s⁻¹, see section 2.3), at the temperature of interest for separation of 70°C, (**Figure 2a**). These results indicate that once the acid gel structure is altered, i.e. during shearing in a pump or within narrow pipes, the structure may not recover to its original state. Similar deformation was also observed for cooler samples sheared at 20°C, although the viscosity was higher across the shear range (**Figure 2b**).

The ~10-fold low viscosity induced by heat treatment at 70°C compared to 20°C (**Figure 2b**) allowed for effective separation of the whey (65% (w/w)) at lower centrifugation speeds (e.g. 3000 g to 15000 g, **Figure 3**), while a speed of at least 12000 g to 15000 g was required to induce separation at room temperature. Notably, a higher variation in the efficiency of whey separation was also observed at a lower temperature with lower speeds of separation.

305 **3.3 Whey separation and microstructure of the curd**

Four processes were next examined at 2.2 kg scale to determine the best method for small scale cream cheese production. The effect of heat treatment was examined together with two methods of whey separation: centrifugation or cloth bag separation. These experiments employed the conditions described above where the curd was heated to 70°C. Separation of ~65% (w/w) whey was targeted by centrifugation at 5000 g for heated samples; a higher force of 12000 g was required for unheated sample to achieve a similar level of whey separation (**Figure 3**).

Heating improved separation and whey separation was most effective and closest to the 65% (w/w) target when heat was applied with either the centrifugation or cloth bag method (**Figure 4a**). In contrast, whey separation was least effective in the absence of heat using the cloth bag method. A further disadvantage of the traditional cloth bag process, is that the volume of whey separated cannot easily be adjusted, whereas the centrifugation process allows greater flexibility.

317 Heating appeared to qualitatively increase the density of the curd (Figure 4b), consistent with the 318 higher volume of whey separation (Figure 4a). Samples that were centrifuged and heated had a reduced 319 unstained volume within CLSM images (Figure 4c), which can be attributed to the greater expulsion 320 of whey in these samples. The protein network and fat also occupied a greater fraction of the sample 321 volume examined by CLSM in samples that were centrifuged and heat treated compared to unheated, 322 cloth bag separated curd samples (Figure 4c). A further change was the greater coalescence of fat globules induced by heat and this was most notable for curd separated by both centrifugation and the 323 324 cloth bag method (Figure 4b).

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326 **3.4 Composition and protein profiles of whey**

Batch centrifugation of heated samples was found to provide the most effective model for current manufacturing processes, resulting in smaller fat losses to the whey $(0.2 \pm 0.0 \% \text{ w/w})$ than the cloth bag method with heat treatment $(1.3 \pm 0.3 \% \text{ w/w})$. This lower fat loss was also reflected in the composition of the whey for these treatments (**Figure 5a**). This difference in fat loss was possibly due to the higher extent of fat coalescence within (+) cloth samples (**Figure 4b**), although some coalescence was also observed for the centrifugation process with heat ((+) Centrifugation) that did not lead to such losses, possibly as centrifugation is better at capturing this fat within the curd. The total fat loss in samples without heat treatment was also similar to the preferred method of centrifugation with heating ($0.4 \pm 0.2 \%$ w/w (-) centrifuge and $0.49 \pm 0.2 \%$ w/w (-) cloth), although the composition of the whey varied (**Figure 5a**).

Heat treatment denatured some whey proteins including lactoferrin, serum albumin and α lactalbumin (la), as the concentration of these proteins was reduced in the whey of heat treated samples analysed by SDS-PAGE (**Figure 5**, indicated by the dotted boxes). Lactoferrin and serum albumin could not be detected by staining after heat treatment and the intensity of the band for α -la reduced by 53 ± 6% of the intensity observed without heat treatment (averaged across the two processes, i.e. centrifugation and cloth bag methods). β -Lactoglobulin (lg) was less affected by heat treatment and the band intensity reduced by 12 ± 16% of the intensity observed without heat treatment.

344 The differences in denaturation observed here are expected, as the temperature applied here is less 345 than the denaturation temperature of β -lg (73°C) but above the temperature reported for other whey proteins, including α -lactalbumin (65°C), as assessed by differential scanning calorimetry in model 346 347 simulated milk ultrafiltrate (SMUF) at pH 6.7 (Ruegg & Moor, 1977). Partial denaturation of β -lg and 348 α -la is known to occur in milk at pH 6.7 for temperatures similar to those applied here (74°C for 30 349 min), with full denaturation only occurring at higher temperatures of 90°C (Larson & Rolleri, 1955), 350 although protein denaturation varies depending on the pH, calcium, mineral and protein concentration, 351 often in a synergistic manner (Corredig & Dalgleish, 1999; Dissanayake, Ramchandran, Donkor, & 352 Vasiljevic, 2013; Ju, Hettiarachchy, & Kilara, 1999; Ruegg & Moor, 1977), making direct comparisons 353 to this study difficult. Such denatured proteins are known to associate with casein micelles (Corredig 354 & Dalgleish, 1999; Oldfield, Singh, Taylor, & Pearce, 2000), with greater association occurring when 355 elevated temperatures are applied together with a decrease in pH (association occurs within 3-4 min at 356 90°C at pH 6.2) (Corredig & Dalgleish, 1999). Here, the pH of 4.5 is considerably lower than in milk and the pH and temperature applied decrease native structure and promote association with the protein 357 358 network formed by casein proteins.

359 The interaction between heat denatured whey proteins and the casein network is thought to be 360 mediated by intermolecular disulphide bonding, non-covalent hydrophobic interactions and physical 361 entrapment (Dissanayake et al., 2013; Hollar & Parris, 1995). Specifically, β -lg interacts with κ - and α_{s2} - case through disulphide bonds but some fraction also forms pure whey protein aggregates 362 363 (Vasbinder & De Kruif, 2003). Heat also induces complexes between α -la complexes with β -lg (Corredig & Dalgleish, 1999). Whilst these heat induced differences were observed by SDS-PAGE, 364 365 however, they represent only a small fraction of the total protein present in the curd and did not impact significantly on the total protein concentration in the whey (P > 0.05) (Figure 5a). Nevertheless, 366 367 complexes between the whey protein and casein network within the curd could affect the functional 368 properties of the cheese, as increased interactions between casein and whey proteins are known to lead 369 to a higher storage modules in acid skim milk gels (Lucey, Munro, & Singh, 1998, 1999).

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371 **3.5 Microstructure and composition of cream cheese**

372 Two of the four processes examined produced cream cheese with a microstructure comparable to a 373 commercial cream cheese; these were batch centrifugation or the cloth bag method with the addition of 374 heat (Figure 6a, b vs e). The cooking and shearing process creates a typical corpuscular structure 375 characterized by clusters of small fat globules coated extensively with protein aggregates. In contrast, 376 cream cheese produced by either the centrifugation of cloth bag method in the absence of heat resulted 377 in a qualitatively less dense network structure, with greater non-stained regions corresponding to cheese serum and possibly polysaccharides, such as gums or stabilizers. This observation is consistent with the 378 379 higher moisture in these samples (Table 1), due to less effective whey separation.

The structure observed here is similar to previous microscopy analysis of cream cheese using TEM (Kalab, 1985) and CLSM (Wendin et al., 2000), which have also revealed a structure of protein-coated fat globules within cream cheese. Previous studies have not directly characterized the polysaccharide components present in cream cheese (Wendin et al., 2000), possibly due to the difficulty in obtaining fluorescence stains specific for polysaccharides or the limited availability of SEM instruments with cryo preservation attachments. Conventional SEM requires significant dehydration of samples prior to 386 observation, which often leads to the loss of the hydrated serum and the structure associated with 387 pockets of polysaccharide present within the cream cheese.

Inspection of the samples by cryo-SEM reveals the presences of flat sheet-like structures that likely arise from the presence of pockets of polysaccharides within the serum or protein network of the cheese that form structure during the sublimation process of cryo-SEM preparation (**Figure 6f-j**, indicated by arrows). Similar structures formed by methylcellulose in wheat batter are reported to be eutectic artefacts that form due to the accumulation of soluble solutes during sample etching (Llorca et al., 2005), where a larger cell structure indicates less interaction between components. A similar flat sheet structure has also been observed in yoghurt made with gelatin (Fiszman et al., 1999).

The greatest area of flat sheet structures was observed in samples that were not heat treated prior to whey separation ((-) Cloth and (-) Centrifuge, **Figure 6h** and **i**). Whilst these samples had a similar concentration of mixed locust bean gum and guar gum (1:1), they had a higher moisture content (**Table 1**), which could increase gum hydration, decrease interactions gums and other components and altering the distribution of gum. The distribution and structure of gums play an important role in cream cheese, as this component can prevent syneresis throughout an extended shelf life, making these observations important for the current study.

402 Image analysis confirmed visual observations that a high volume fraction of unstained serum was 403 present in unheated samples produced by the cloth bag drainage method (Figure 6k), although unheated 404 and centrifuged samples were not found to be quantitatively different to samples produced with heating. 405 The microstructure of this later sample ((-) Centrifuge) did not appear qualitatively as dense as the 406 cheeses with heat treatment prior to whey separation and these samples could possibly be improved by 407 applying a higher centrifugation speed to increase the whey separation. The volume of fat in both 408 unheated samples (centrifuged and cloth bag) was also less than in heated samples, potentially reflecting 409 the density of the network, rather than fat loss.

The composition of cream cheese produced with heat treatment prior to whey separation (**Table 1**) by either the batch centrifugation or the cloth bag process was within the range reported for commercial cream cheese (Phadungath, 2005). The lower amount of whey separated in the sample without heat treatment resulted in a cream cheese with very high moisture content (**Figure 6**). The fat and protein 414 content in the samples produced by the cloth bag method without heat were also significantly reduced,

415 consistent with the altered microstructure in these cheeses. Samples produced by centrifugation without

416 heat were also similarly compromised, with reduced protein content.

417

418 **3.6 The texture and rheological properties of cream cheese**

419 The cream cheese produced by batch centrifugation and heat treatment ((+) Centrifuge) displayed a hardness comparable to a commercial sample (Figure 7a). The cream cheeses produced using batch 420 421 centrifugation or the cloth bag process with the addition of heat ((+) Centrifuge or (+) Cloth) were 422 firmer than the cheeses without heat treatment prior, possibly due to the different moisture contents of 423 these cheeses (Table 1). The hardness of the cheese increased as the moisture content decreased (r=-424 0.77, P=0.023) or as the fat content increased (r = 0.74, P=0.035). These results are consistent with 425 previous reports of commercial samples, where firmness increased with fat (Brighenti et al., 2008). 426 The spreadability, defined as the ease at which a product can be spread, was assessed using the adhesive 427 force of a 40° Perspex cone during compression. A higher negative area in this test indicates a product 428 that is stickier and more difficult to spread (Figure 7b). Both the (+) Centrifuge and commercial sample 429 were sticker, consistent with previous reports that cream cheese that is hard or has a low water content 430 is more difficult to spread (Brighenti et al., 2008).

The textural properties of the cream cheese produced using batch centrifugation or the cloth bag process with heat ((+) Centrifuge or (+) Cloth) differed despite a similar moisture content (**Table 1**). This difference likely arise from the different processing times; the curd obtained after centrifugation was processed immediately to obtain a final cream cheese product whereas the process of whey separation takes 18 hours by the cloth bag method, which may contribute to the differences observed in texture.

All of the cream cheese samples produced had a similar linear viscoelastic region (LVR, 0.001 –
0.1% strain) when subject to a strain sweep (Figure 8a). Within this region, the modulus of the cheese
(G' or G'') was constant during shearing. The magnitude of the modulus, however, differed between
treatments and followed a similar trend to the hardness of the cheese samples (Figure 7a), as expected.

The rheological properties of the (+) Centrifuge cream cheese sample most closely resembled the commercial sample when a frequency sweep was performed at a constant 0.01% strain (**Figure 8b**). All samples displayed a positive slope, which is expected for a viscoelastic solid. The magnitude of the slope varied between samples and the sample produced with heat and centrifugation displayed the highest storage modulus, indicating stronger bonds with the network of these cheese samples.

The difference in moisture content between cheeses is thought to be a major contributor to 446 447 differences in structure, texture and rheology for the four cheese samples. Heat treatment, which 448 affected the interactions between denatured whey proteins and casein proteins also contributed to the 449 increased firmness of the cheese (i.e. increased hardness during the compression test and higher storage 450 modulus during the small amplitude rheological test). Cream cheese firmness can similarly be 451 improved by homogenization of the curd during the final stages of manufacture (Sanchez et al., 1994). 452 A homogenization pressure of 15-20 MPa was reported by Sanchez and colleagues to increase the 453 storage modulus and the shear stress of cream cheese measured during extrusion. This past study did 454 not show any visual changes in microstructure but the increase in shear stress and elasticity measured 455 indicates suggested stronger interactions between curd particles after homogenisation was applied; 456 thought to be due to a reduction of particle size, a change in the re-emulsification of the fat and a 457 structural reorganization of cream cheese components. Future experiments could seek to examine 458 cream cheese with a standardized moisture content but varied heat treatment to give further insights 459 into the role of whey proteins and casein networks in determining the final texture of cream cheese.

460 4. Conclusion

A laboratory scale process was developed using heat treatment and centrifugation that results in a cream cheese product with a microstructure, composition and rheological properties comparable to cream cheese produced at a commercial scale.

An optimal temperature range for whey separation of 60-80°C was identified from rheological analysis. Heat treatment was necessary to achieve effective whey separation, leading to an acceptable moisture content and resulting in a denser curd microstructure compared to unheated samples, where the unstained volume in the cheese was higher when quantified by CLSM image analysis and the gum 468 or stabilisers were visible, as flat sheet-like structures by cryo-SEM, potentially leading to changes in 469 shelf-stability. Heating denatured some whey proteins, altering interactions between these proteins and 470 the casein network and contributing to a firmer more viscous product, although it also induced some 471 coalescence of fat.

472 Batch centrifugation was superior to the cloth bag method, when applied with heating. Whilst both 473 processes gave a similar microstructure, centrifugation resulted in reduced fat loss to the whey and 474 produced a firmer, more viscous product that more closely resembled cheese from a commercial 475 process.

These observations provide new insights into the factors affecting cream cheese properties. The development of a characteristic cream cheese microstructure was documented at key stages of the production process and this benchmarked process can potentially be applied to understand and optimise the effect of various process parameters on the microstructure and properties of cream cheese.

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490 **References**

491 AOAC. (2002). Association of Official Analytical Chemists: Official Methods of Analysis 989.04-

492 2000 (18th ed.). Gaithersburg, MD.: AOAC International.

Auty, M. A. E., Twomey, M., Guinee, T. P., & Mulvihill, D. M. (2001). Development and application
of confocal scanning laser microscopy methods for studying the distribution of fat and protein
in selected dairy products. *Journal of Dairy Research*, 68, 417-427.

496	Bakshi, A. S., & Smith, D. E. (1984). Effect of fat content and temperature on viscosity in relation to			
497	pumping requirements of fluid milk products. Journal of Dairy Science, 67, 1157-1160.			
498	Brighenti, M., Govindasamy-Lucey, S., Nelson, K., & Lucey, J. A. (2008). Characterization of the			
499	rheological, textural and sensory properties of samples of commercial US cream cheese with			
500	different fat contents. Journal of Dairy Science, 91, 4501-4517.			
501	Brighenti, M. (2009). Texture and functionality of cream cheese. University of Wisconsin-Madison			
502	PhD Thesis.			
503	Corredig, M., & Dalgleish, D. G. (1999). The mechanisms of the heat-induced interaction of whey			
504	proteins with casein micelles in milk. International Dairy Journal, 9, 233-236.			
505	Dairy-Australia. (2017). Australian Dairy Industry in Focus. Dairy Australia Annual Bulletin.			
506	Dissanayake, M., Ramchandran, L., Donkor, O. N., & Vasiljevic, T. (2013). Denaturation of whey			
507	proteins as a function of heat, pH and protein concentration. International Dairy Journal, 31,			
508	93-99.			
509	Everett, D. W., Ding, K., Olson, N. F., & Sundaram, G. (1995). Applications of confocal microscopy			
510	to fat globule structure in cheese. Advances in Experimental Medicine and Biology, 367, 321-			
511	330.			
512	FAO. (2016). Standard for cream cheese. Codex Alimentarius International Food Standards, CODEX			
513	STAN 275-1973, Amendment 2016.			
514	FDA. (2016). Title 21: Food and drugs. Part 133: cheese and related cheese products. Subpart B:			
515	requirements for sepcific standardized cheese and related products: cream cheese. In: Code of			
516	Federal Regulations.			
517	GEA. (2016). Process lines from GEA Westfalia separator for the production of soft cheese. GEA			
518	Westfalia, accessed from <u>www.gea.com</u> (1/07/2016).			
519	Guinee, T. P., Pudja, P. D., & Farkye, N. Y. (1993). Fresh acid-curd cheese varieties. In P. F. Fox			
520	(Ed.), Cheese: Chemistry, Physics and Microbiology (pp. 363-419). London: Chapman &			
521	Hall.			

- Heymann, B. (2011). Plant and equipment | Centrifuges and separators: types and design. In J. W.
 Fuquay (Ed.), *Encyclopedia of Dairy Sciences (Second Edition)* (pp. 166-174). San Diego:
 Academic Press.
- Hoffmann, W. (2011). Cream | Manufacture. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences*(Second Edition) (pp. 912-919). San Diego: Academic Press.
- Hollar, C. M., & Parris, N. (1995). Factors affecting the denaturation and aggregation of whey
 proteins in heated whey protein concentrate mixtures. *Journal of Dairy Science*, 78, 260-267.
- 529 IDF. (1982). *Determination of the total solids content (cheese and processed cheese)*. Brussels:
- 530 International Dairy Federation

- IDF. (1993). *Milk: determination of the nitrogen content (Kjeldahl method) and calculation of crude protein content*. Brussels: International Dairy Federation.
- Ju, Z. Y., Hettiarachchy, N., & Kilara, A. (1999). Thermal properties of whey protein aggregates.
 Jornal of Dairy Science, 82, 1882-1889.
- Kalab, M. (1985). Microstructure of dairy foods. 2. Milk products based on fat. *Journal of Dairy Science*, 68, 3234-3248.
- Kalab, M., & Modler, H. W. (1985). Development of microstructure in cream cheese based on Queso
 Blanco cheese. *Food Microstructure*, *4*, 89-98.
- Kang, C.-H., Jeon, H., Shin, Y., Kwon, Y.-J., & So, J.-S. (2015). Heat adaptation improves viability
 of Lactococcus lactis subsp. lactis HE-1 after heat stress. *Food Science and Biotechnology*,
 24, 1823-1827.
- Kim, W. S., Ren, J., & Dunn, N. W. (1999). Differentiation of Lactococcus lactis subspecies lactis
 and subspecies cremoris strains by their adaptive response to stresses. *FEMS Microbiology Letter, 171*, 57-65.
- Larson, B. L., & Rolleri, G. D. (1955). Heat denaturation of the specific serum proteins in milk.

Journal of Dairy Science, 38, 351-360.

547 Lefevere, I., Dewettinck, K., & Huyghebaert, A. (2000). Cheese fat as driving force in cheese flow
548 upon melting. *Milchwissenschaft*, 55, 563-565.

- 549 Llorca, E., Hernando, I., Perez-Díaz, I., Quiles, A., Larrea, V., Fiszman, S. M., & Lluch, M. A.
- 550 (2005). Microstructural study of frozen batter-coated squid rings prepared by an innovative
 551 process without a pre-fying step. *Food Hydrocolloids*, *19*, 297-302.
- Lucey, J. A., Munro, P. A., & Singh, H. (1998). Rheological properties and microstructure of acid
 milk gels as affected by fat content and heat treatment. *Journal of food science*, *63*, 660-664.
- 554 Lucey, J. A., Munro, P. A., & Singh, H. (1999). Effects of heat treatment and whey protein addition
- on the rheological properties and structure of acid skim milk gels. *International Dairy Journal*, 9, 275-279.
- McCarthy, O. J. (2011). Plant and Equipment | Centrifuges and separators: applications in the dairy
 industry. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences (Second Edition)* (pp. 175183). San Diego: Academic Press.
- 560 Mezger, T. G. (2014). *The rheology handbook*. Hanover, Germany: Vincentz.
- Monteiro, R. R., Tavares, D. Q., Kindstedt, P. S., & Gigante, M. L. (2009). Effect of pH on
 microstructure and characteristics of cream cheese. *Journal of Food Science*, 74, C112-C117.
- Nguyen, H. A., & Anema, S. G. (2017). Ultrasonication of reconstituted whole milk and its effect on
 acid gelation. *Food Chemistry*, 217, 593-601.
- 565 Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2015). Homogenisation improves the
- 566 microstructure, syneresis and rheological properties of buffalo yoghurt. *International Dairy*567 *Journal*, 46, 78-87.
- Oldfield, D. J., Singh, H., Taylor, M. W., & Pearce, K. N. (2000). Heat-induced interactions of βlactoglobulin and α-lactalbumin with the casein micelle in pH-adjusted skim milk. *International Dairy Journal, 10*, 509-518.
- Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2010). The effect of milk processing on the
 microstructure of the milk fat globule and rennet induced gel observed using confocal laser
 scanning microscopy. *Journal of food science*, *75*, E135-E145.
- Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2011a). Microstructure of gel and cheese curd
 observed using cryo scanning electron microscopy and confocal microscopy. *LWT Food Sci. Technol.*, 44, 1291-1302.

- Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2011b). Coagulation temperature affects the
 microstructure and composition of full fat Cheddar cheese. *Dairy Science and Technology*,
 91, 739-758.
- 580 Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2012). The effect of pH at renneting on the
- 581 microstructure, composition and texture of Cheddar cheese. *Food Research International*, 48,
 582 119-130.
- Phadungath, C. (2005). Cream cheese products: A review. Songklanakarin Journal of Science and
 Technology, 27, 191-199.
- Ruegg, M., & Moor, U. (1977). Calorimetric study of the thermal denaturation of whey proteins in
 simulated milk ulrafiltrate. *Journal of Dairy Research*, 44, 509-520.
- Sanchez, C., Beauregard, J. L., Chassagne, M. H., Bimbenet, J. J., & Hardy, J. (1994). Rheological
 and textural behaviour of double cream cheese. Part I: Effect of curd homogenization. *Journal of food engineering*, 23, 579-594.
- Sanchez, C., Beauregard, J. L., Chassagne, M. H., Bimbenet, J. J., & Hardy, J. (1996). Effects of
 processing on rheology and structure of double cream cheese. *Food Research International*,
 28, 547-552.
- Schmitt, C., Sturm, B., Grupa, U., & Hensel, O. (2016). Analysis of the influence of different factors
 on the quality of a curd-whey mixture during pumping. *LWT Food Science and Technology*,
 65, 88-97.
- Steffe, J. F., & Daubert, C. R. (2006). *Bioprocessing pipelines: rheology and analysis*. USA: Freeman
 Press.
- 598 USDA. (2017). Dairy products 2016 Summary. In: United States Department of Agriculture, National
 599 Agricultural Statistic Service.
- Vasbinder, A. J., & De Kruif, C. G. (2003). Casein-whey protein interactions in heated milk: the
 influence of pH. *International Dairy Journal*, *13*, 669-677.
- Walstra, P., Geurts, T. J., Noomen, A., Jellema, A., & Boekel, M. A. J. S. (1999). Dairy Technology:
- 603 *Principles of milk properties and processes.* New York: Marcel Dekker.

- 604 Wendin, K., Langton, M., Caous, L., & Hall, G. (2000). Dynamic analyses of sensory and
- 605 microstructural properties of cream cheese. *Food Chemistry*, 71, 363-378.

606 Figure Legend

607 Figure 1. CLSM images of key stages during cream cheese production: standardized milk prepared 608 for cream cheese making (a1), the acidified milk gel obtained after 5 hours of fermentation (a2) and 609 the cooked gel particles obtained after small amplitude rheological testing with a temperature sweep 610 from 20°C to 90°C (a2). The microscopic images are representative of at least 6 2D-images taken for 611 each sample. The modulus of the gel (b) including the storage modulus (Δ), the loss modulus (\blacksquare) and 612 the tan delta δ (x) are presented for the sample examined during the temperature sweep analysis. The 613 viscosity of this sample during the temperature sweep analysis is also presented (c). Data are the mean 614 \pm the standard deviation of the mean (n = 5). The milk for CLSM analysis was diluted 1:10 times in 615 SMUF before observation on a cavity slide. Nile red stained fat appears red and Fast Green FCF 616 stained protein appears green. The scale bars are 5 µm in length. 617

Figure 2. The viscosity of the gel as a function of increasing and decreasing shear $(0.1 - 100 \text{ s}^{-1})$ at a constant temperature of (a) 70°C or (b) 20°C. Data are the mean ± the standard deviation of the mean (n = 3).

621

Figure 3. The percentage of whey separated as a function of centrifugation speed for samples heated for 10 min at 70°C (\Box) or samples separated without heating at 20°C (\bullet). The data presented are the mean \pm the standard deviation of the mean (n = 4).

625

Figure 4. (a) The mass of whey expelled and (b) the microstructure of the curd samples produced 626 627 using the four processes examined by CLSM. Samples were produced using either batch centrifugation or the cloth bag process and received either heat treatment (+) or no heat treatment (-) 628 prior to whey separation. Nile red stained fat appears red. Fast Green FCF stained protein appears 629 630 green. The scale bars are 5 μ m in length. (c) The percentage of fat (\Box), protein (\blacksquare) or unstained volume (\blacksquare) obtained from image analysis of the CLSM images. Data are the mean \pm the standard 631 deviation of mean from at least 2 reconstructed z-stack images for each process. ^{abc} Data from 632 633 different processes with different superscripts are significantly different (P < 0.05).

634 **Figure 5.** (a) The concentration of fat (\Box) and protein (\blacksquare) in the whey obtained during cream cheese production (n = 2) using either the batch centrifugation or cloth bag process where samples received 635 heat treatment (+) or no heat treatment (-) prior to whey separation. ^{abc} The data between different 636 processes with different superscripts are significantly different (P < 0.05). (b) A representative SDS-637 638 PAGE profile of the protein found in the whey on separation. The molecular weight standard (Mw 639 std.) appears in the first lane. The whey proteins: serum albumin (66.4 kDa), lactoferrin (78.1 kDa), α - lactalbumin (14.2 kDa) and β - lactoglobulin (18.3 kDa) were identified based on their molecular 640 641 weight. The dotted lines highlight the lower intensity of the bands corresponding to serum albumin, 642 lactoferrin and α - lactalbumin in samples that received heat treatment prior to whey separation.

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644 Figure 6. The microstructure observed by CLSM (a-e) or cryo SEM (f-j) for the four processes (a-d 645 and f-i) or obtained from a commercial cream cheese process (e, j). The four processes involved either batch centrifugation or the cloth bag process where samples were heat treated (+) or unheated (-646) prior to whey separation. (k) The percentage of fat (\Box) , protein (\blacksquare) or unstained volume (\blacksquare) obtained 647 from image analysis of the CLSM images. Data are the mean \pm the standard deviation of the mean 648 from at least 2 reconstructed z-stack images for each process. ^{abc} The data for processes with different 649 superscripts are significantly different (P<0.05). Nile red stained fat appears red. Fast Green FCF 650 651 stained protein appears green. The scale bars are 5 μ m in length for the CLSM images (a-e) and 2-5 652 μm in length for cryo SEM images (f-j).

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Figure 7. The textural properties of the cream cheese from the four processes or for cream cheese from a commercial process including measurements of **(a)** hardness and **(b)** spreadability at 10°C. Samples were produced using either batch centrifugation or the cloth bag process where samples were heat treated (+) or unheated (-) prior to whey separation. A total of eight batches of cream cheese were made, i.e. two for each of the four processes. Data are the mean \pm the standard deviation of the mean for each process (n = 2). ^{abc} The data for the different processes with different superscripts are significantly different (*P*<0.05).

Figure 8. The rheological properties of cream cheese from the four processes $(\circ, \Box, \bullet, \bullet)$ or for cream cheese from a commercial process (x) measured at 10°C. (a) The storage modulus of the cream cheese during a strain sweep from 0.001 – 100% and (b) during a frequency sweep from 1 – 100 Hz at a constant 0.01% strain. Cream cheeses were produced using either batch centrifugation (\bullet , \circ) or the cloth bag process (\bullet , \Box) and heat treated (\Box , \circ) or unheated (\bullet , \bullet) prior to whey separation. A total of eight batches of laboratory cream cheese were made, i.e. two for each of the four processes. At least two measurements were performed for each batch and the data averaged. Data are the mean \pm the

standard deviation of the two average values for each process (n = 2).







Figure 2. The viscosity of the gel as a function of increasing and decreasing shear $(0.1 - 100 \text{ s}^{-1})$ at a constant temperature of **(a)** 70°C or **(b)** 20°C. Data are the mean ± the standard deviation of the mean (n = 3).



Figure 3. The percentage of whey separated as a function of centrifugation speed for samples heated for 10 min at 70°C
 (□) or samples separated without heating at 20°C (●). The data presented are the mean ± the standard deviation of the mean (n = 4).





Figure 4. (a) The mass of whey expelled and (b) the microstructure of the curd samples produced using the four processes examined by CLSM. Samples were produced using either batch centrifugation or the cloth bag process and received either heat treatment (+) or no heat treatment (-) prior to whey separation. Nile red stained fat appears red. Fast Green FCF stained protein appears green. The scale bars are 5 μ m in length. (c) The percentage of fat (\Box), protein (\blacksquare) or unstained volume (\blacksquare) obtained from image analysis of the CLSM images. Data are the mean \pm the standard deviation of mean from at least 2 reconstructed z-stack images for each process. ^{abc} Data from different processes with different superscripts are significantly different (*P*<0.05).



Figure 5. (a) The concentration of fat (\Box) and protein (**\blacksquare**) in the whey obtained during cream cheese production (n = 2) using either the batch centrifugation or cloth bag process where samples received heat treatment (+) or no heat treatment (-) prior to whey separation. ^{abc} The data between different processes with different superscripts are significantly different (*P*<0.05). (**b**) A representative SDS-PAGE profile of the protein found in the whey on separation. The molecular weight standard (Mw std.) appears in the first lane. The whey proteins: serum albumin (66.4 kDa), lactoferrin (78.1 kDa), α - lactalbumin (14.2 kDa) and β - lactoglobulin (18.3 kDa) were identified based on their molecular weight. The dotted lines highlight the lower intensity of the bands corresponding to serum albumin, lactoferrin and α - lactalbumin in samples that received heat treatment prior to whey separation.



Figure 6. The microstructure observed by CLSM (**a-e**) or cryo SEM (**f-j**) for the four processes (**a-d and f-i**) or obtained from a commercial cream cheese process (**e, j**). The four processes involved either batch centrifugation or the cloth bag process where samples were heat treated (+) or unheated (-) prior to whey separation. (**k**) The percentage of fat (\Box), protein (**a**) or unstained volume (**a**) obtained from image analysis of the CLSM images. Data are the mean ± the standard deviation of the mean from at least 2 reconstructed z-stack images for each process. ^{abc} The data for processes with different superscripts are significantly different (*P*<0.05). Nile red stained fat appears red. Fast Green FCF stained protein appears green. The scale bars are 5 µm in length for the CLSM images (**a-e**) and 2-5 µm in length for cryo SEM images (**f-j**).

Cheese samples	Moisture	Fat	Protein
	% (w/w)	% (w/w)	% (w/w)
(+) Centrifuge	$53.0\pm2.3^{\text{b}}$	$32.0\pm2.2^{\rm a}$	9.0 ± 0.7^{a}
(+) Cloth	54.8 ± 2.3^{b}	$31.0\pm2.5^{\rm a}$	8.7 ± 0.3^{ab}
(-) Centrifuge	60.0 ± 0.7^{ab}	$26.6\pm0.4^{\rm a}$	7.1 ± 0.0^{bc}
(-) Cloth	66.0 ± 3.6^{a}	$16.9\pm2.8^{\text{b}}$	6.1 ± 0.5^{c}
Commercial cream cheese#	53.2 ± 0.1^{b}	33.5 ^a	9.5ª

Table 1. Composition of cream cheese produced using the four processes*

* Two batches of cheese were prepared for each of the four processes. The results presented are the mean \pm the standard deviation of the mean (n=2).

^{abc} The data for processes with different superscripts are significantly different (*P*<0.05).

[#] The fat and protein composition of commercial cream cheese was obtained from the label on the packaging.



Figure 7. The textural properties of the cream cheese from the four processes or for cream cheese from a commercial process including measurements of (a) hardness and (b) spreadability at 10°C. Samples were produced using either batch centrifugation or the cloth bag process where samples were heat treated (+) or unheated (-) prior to whey separation. A total of eight batches of cream cheese were made, i.e. two for each of the four processes. Data are the mean \pm the standard deviation of the mean for each process (n = 2). ^{abc} The data for the different processes with different superscripts are significantly different (*P*<0.05).

(b)

(a)



Figure 8. The rheological properties of cream cheese from the four processes (\circ , \square , \bullet , \blacksquare) or for cream cheese from a commercial process (x) measured at 10°C. (a) The storage modulus of the cream cheese during a strain sweep from 0.001 – 100% and (b) during a frequency sweep from 1 – 100 Hz at a constant 0.01% strain. Cream cheeses were produced using either batch centrifugation (\bullet , \circ) or the cloth bag process (\blacksquare , \square) and heat treated (\square , \circ) or unheated (\blacksquare , \bullet) prior to whey separation. A total of eight batches of laboratory cream cheese were made, i.e. two for each of the four processes. At least two measurements were performed for each batch and the data averaged. Data are the mean ± the standard deviation of the two average values for each process (n = 2).



Supplementary Figure 1. Representative 3D CLSM image reconstructions of cheese produced by the cloth bag process without heat treatment (a1) and with heat treatment (b1). Each 3D image was processed to obtain the total volume of the fat and the total volume of the protein as shown in (a2) and (b2). The unstained volume was then calculated from the difference between the total sample volume and the sum of the total volume of fat and protein. Nile red stained fat appears red and fast green stained protein appears green. The scale bars are 10 μm in length.