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The microstructure and textural properties of Australian cream cheese with differing composition

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ABSTRACT

Confocal laser scanning microscopy was used to compare the microstructure of six Australian commercial cream cheese products. The optimal conditions for cryo scanning electron microscopy (cryo SEM) analysis of cream cheese microstructure were also examined. These complementary techniques revealed a typical cream cheese microstructure of homogenised fat globules embedded in a non-continuous protein network. The association between fat and protein within the microstructure was influenced by product composition (fat:protein ratio, moisture content) and ingredients. The addition of emulsifier led to a softer product with distinct microstructure. Cryo SEM also revealed a "honeycomb"-like structure, which was interpreted as a eutectic artefact formed by the addition of gum(s). Product hardness and gel strength generally correlated with high fat, low moisture content and a compact microstructure. Overall, this study shows how product composition affects the microstructure, texture and rheological properties of cream cheese.

1. Introduction

After many years of development, high resolution microscopy is now well established as a diagnostic tool in the development and quality control of many types of low-moisture foods and food ingredients (Morris & Groves, 2013). As these technologies continue to advance, their application is being extended into a wider range of foods, including more highly hydrated food matrices, such as cheese and cheese curd, where the use of cryo scanning electron microscopy (cryo SEM) and confocal laser scanning microscopy (CLSM) has been particularly successful (Everett & Auty, 2008; Oiseth & McKinnon, 2006). The parallel use of these methods can provide a wide range of information on the microstructure of cheese and cheese curd samples, distinguishing between different varieties, assisting with image interpretation and providing greater confidence in the identification of artefacts that occur due to sample preparation or imaging conditions (Ong, Dagastine, Kentish, & Gras, 2011). This type of microstructural analysis is now being applied to help optimise cheese production with respect to variables such as cheese milk composition and ripening time, by providing a better understanding of curd formation and physical characteristics (Nguyen, Ong, Kentish, & Gras, 2015; Ong, Soodam, Kentish, Powell, & Gras, 2015; Soodam, Ong, Powell, Kentish, & Gras, 2014; Yang, Watkinson, Gillies, & James, 2016). The objective of the present study was to apply this approach to cream cheese and to explore possible relationships between microstructural analysis, chemical composition and physical properties of six cream cheese product varieties.

In Australia, cream cheese production has increased by ~10% since 2009/2010. In 2014/2015, production of cream cheese in Australia accounted for 25% (approximately 85,000 tonnes) of total cheese manufactured and greatly exceeded the production of cottage, fetta, Neufchatel, ricotta and other fresh varieties (Dairy Australia Limited, 2015). A wide range of cream cheese and related products are commercially available and the reader is referred to the comprehensive review by Guinee and Hickey (2009) for further details regarding typical composition, product characteristics, manufacturing processes and product standards. It is well recognised that the moisture content, fat content and fat quality are key factors in determining the physical properties (notably hardness and spreadability) of cream cheese and related products (Brighenti, Govindasamy-Lucey, Lim, Nelson, & Lucey, 2008; Muir, Williams, Tamime, & Shenana, 1997; Roundy & Price, 1941; Wendin, Langton, Caous, & Hall, 2000).

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Recent studies have shown that, in common with many other dairy products, the interaction of protein with fat and moisture in the cream cheese product is also a critical determinant of product quality and shelf-life and that the participation of protein in the overall structure can be influenced by temperature, homogenisation conditions and type of hydrocolloid ingredients used (Brighenti et al., 2008; Guinee & Hickey, 2009). This more sophisticated view of the formation and stability of cream cheese structure is of particular importance for low-fat varieties, which are of increasing popularity in the market. Indeed, with most "light" cream cheese types, where the fat content has been reduced below 25%, the inclusion of additional ingredients such as hydrocolloids, is essential to maintain an acceptable structure, due to an increased water content (Guinee & Hickey, 2009).

Despite numerous studies investigating the microstructure of cream cheese using SEM, transmission electron microscopy (TEM) and CLSM techniques (Fenoul, Le Denmat, Hamdi, Cuvelier, & Michon, 2008; Kalab, 1985; Kalab & Modler, 1985; Kalab, Sargant, & Froelich, 1981; Monteiro, Tavares, Kindstedt, & Gigante, 2009; Ohashi et al., 1983; Sainani, Vyas, & Tong, 2004; Wendin et al., 2000), there appears to be limited representation of a typical cream cheese structure. It is anticipated that by utilising both CLSM and cryo SEM methodology (Ong et al., 2011), a robust and contemporary visualisation of the microstructure of cream cheese may be reported, including how the fat, moisture and protein components interact structurally and the role that hydrocolloids and other ingredients play in this system. Such information on the variety of structures formed would be useful to manufacturers and when combined with functionality testing can assist our understanding of the link between formulation, structure and physical functionality.

The commercial products selected for this study were all semi-soft, fresh acid curd cheeses, ranging in composition from 4.7 to 34.2 g 100 g^{-1} fat and from 56.1 to 75.4 g 100 g^{-1} moisture (Table 1). The range also includes samples with high protein content (e.g., 9.3 g 100 g^{-1} protein or 11.1 g 100 g^{-1} protein), as occurs within the Australian market. Each commercial product contained at least one type of hydrocolloid; an ingredient added to bind water, increase product stability and increase the viscosity of the aqueous phase, enhancing texture to achieve the desired viscoelastic and rheological properties and/or to replace fat in low fat varieties (Golding & Matia-Merino, 2013; Guinee & Hickey, 2009). In this study, cream cheese was also prepared at a laboratory scale, with and without the addition of hydrocolloid to further understand the distribution of hydrocolloid within the microstructure of the cream cheese product and to assist in the interpretation of the structure of hydrocolloids within these products.

2. Material and methods

2.1. Laboratory cream cheese production

The laboratory cream cheese was made according to the standard experimental procedure reported in a previous study (Ong, Kentish, & Gras, 2018). The milk was standardised to a concentration of 11.5 ± 0.9 g 100 g^{-1} fat and 3.3 ± 0.1 g 100 g^{-1} protein, homogenised at 12–14 MPa 55 °C and pasteurised at 72 °C for 15 s. The milk preparation was performed in a commercial plant (Victoria, Australia) and used in the laboratory within 1 week after collection. Milk (2 kg) was fermented with 0.3% mesophilic lactic acid starter culture (Chr Hansen, Victoria, Australia) at 31 °C until a pH of 4.5 was obtained. Whey separation using a centrifugation method reported by Ong et al. (2018) followed. The curd was then heated to 80 °C in a 2 L kettle and mixed at 500 rpm. Once a temperature of 80 °C was reached, a mixture of locust bean gum and guar gum (both from Sigma Aldrich, St. Louis, MO, USA) (1:1; 0.5 g 100 g^{-1}) and sodium chloride (1.0 g 100 g^{-1}) was added to the curd. The mixture was then further cooked for 25 min at 80 °C and hot filled into 250 mL square sample containers and stored at 4 °C. Cream cheese was also prepared without the addition of gum for comparison.

2.2. Commercial cream cheese samples

Six different commercial cream cheese products were purchased from various Australian retailers. A broad range of products was chosen, representing the diversity of cream cheese products on the Australian market. Compositional information was obtained from ingredient labelling on the packaging of each product, including fat, protein and carbohydrate, given as g 100 g^{-1}. Moisture was calculated by subtracting the combined mass of fat, protein and carbohydrate from 100 g. It should be noted that this value is an approximation and does not take into consideration the mass of sodium chloride (≥1%), gums (~0.2–0.5%) (Guinee & Hickey, 2009) and other ingredients used, the sum of which may equate to approximately 2 g 100 g^{-1}. A summary of the stated chemical composition of each commercial product is given in Table 1 (note that this is only an indication of composition, as seasonal and batch to batch variation are not typically considered in product declarations). All products were stored at 4 °C. All products were opened and examined within one week of purchase and before the expiry date.

2.3. Microscopy

Cream cheese products were analysed using an inverted confocal laser scanning microscope (Leica TCS SP2; Leica Microsystems, Heidelberg, Germany) and a Quanta field emission gun scanning electron microscope (FEI, Hillsboro, OR, USA). The sample preparation methods for CLSM and cryo SEM were adapted from those developed for cheese curd, as described in detail by Ong et al. (2011). For CLSM, the lipid-specific stain Nile Red, prepared from stock solution (1 mg mL^{-1} in dimethyl sulphoxide) was dissolved in purified water prior to staining to a final concentration of 0.1 mg mL^{-1}. The protein-specific stain Fast Green FCF, prepared from a stock solution (1 mg mL^{-1} in dimethyl sulphoxide) was dissolved in purified water to a final concentration of 0.1 mg mL^{-1}. Samples (~5 mm × 2 mm × 2 mm) were stained on the slide to prevent sample damage. Samples were stained separately with Nile Red and Fast Green FCF for 10 min at 4 °C. Slides were kept on ice prior to observation.

Unless otherwise stated, cryo SEM samples (~1 mm × 2 mm × 2 mm) were rapidly immersed into freshly-prepared liquid nitrogen slush (−210 °C) for 5–10 s to ensure a cooling rate of >~10^5 °C s^{-1} (Severs, Terence, & Shotton, 1995). Samples were then fractured using a chilled surgical blade in a vacuum chamber kept at −140 °C, etched at −95 °C for 20 min and coated using a sputtered gold/palladium alloy for 120 s to a thickness of ~6 nm. Samples were observed at 10–12.5 kV using a secondary Everhart-Thornley detector.

Additional cryo SEM samples were prepared to assess the freezing, etching and sputter coating processes including: (i) the effect of direct immersion of sample to liquid nitrogen (approximately −196 °C), (ii) the effect of prolong etching at −95 °C for 60 min and (iii) the effect of coating of samples for 60 s to a thickness of ~3 nm.

2.4. Rheometry

The rheological properties of cream cheese were determined by small amplitude oscillatory rheology using a controlled-stress rheometer (AR-G2, TA instruments Ltd., New Castle, DE, USA). Strain sweeps and frequency sweeps were performed to determine the viscoelastic properties of the products including the elastic

properties or storage modulus (G′) and the viscous properties or loss modulus (G″) at 10 °C, which is understood to be a common temperature for the consumption of refrigerated cream cheese (Engelen et al., 2003). Cream cheese samples for amplitude strain sweeps were loaded into a 20 mL disposable plastic syringe, with the needle attachment removed. The product was allowed to equilibrate at 4 °C for ~24 h prior to the experiment. Approximately 3 mL of sample was extruded from the syringe and placed on the bottom plate. The parallel plate (40 mm in diameter) was then lowered to a 2 mm gap from the bottom plate and samples were conditioned at 10 °C for 10 min on the rheometer. Strain sweeps from 0.001 to 100% strain were performed at 10 °C with angular frequency set at 6.283 rad s^{-1}. Results presented are the average of two sweeps at 10 °C. G′ and G″ have been plotted against % strain for comparative purposes. Critical stress values (G′ = G″) for products 1–5 were also determined.

Amplitude frequency sweeps were also carried out at 10 °C to determine how products were affected by time during constant shearing. A strain setting of 0.01% was chosen for frequency sweeps, as strain sweep analysis had shown that this was within the linear viscoelastic region for all products tested (Fig. 8a). Samples were conditioned at 10 °C for 10 min and then 1–100 Hz frequency sweeps were performed at 10 °C. Results are presented as the average of two sweeps at 10 °C. G was plotted against frequency (rad s^{-1}) for comparative purposes.

2.5. Texture profile analysis

Texture profile (hardness) analysis of cream cheese products was performed using a TA.XT plus texture analyser (Stable Micro Systems, Godalming, UK). Before analysis, products were equilibrated at 10 °C for ~24 h. A 5 mm diameter stainless steel probe (P/5) was fitted to the texture analyser, which was calibrated using a 5 kg load cell. The sample was presented as a whole block (~11 cm × 6.5 cm) or tub (~9 cm in diameter) for analysis using the following settings: pre-test speed, 1 mm s^{-1}; test speed, 5 mm s^{-1}; post-test speed, 5 mm s^{-1}; distance, 10 mm; time, 5 s; automatic trigger force, 3 g. The results presented are the average of five measurements at 10 °C with standard deviations.

3. Results and discussion

3.1. Product composition

Retail cream cheese products are commonly sold in either block form, packaged in foil and cardboard (products 1 and 4; Table 1) or in plastic tubs (products 2, 3, 5 and 6). While full fat and light varieties are available in both types of packaging, “spreadable” varieties require more rigid and easily resealable packaging and are usually only available in tubs. The composition of each of the six commercial samples selected is shown in Table 1. Products were labelled on the packaging as either “full fat”, “light” or “extra light”, corresponding to their fat content. Four of the products were also labelled “spreadable”.

The declared fat content of these products varied widely, with full fat products (1 and 2) containing ~33 g 100 g^{-1} fat, spreadable and light varieties (products 3–5) containing between 13.7 and 25.7 g 100 g^{-1} fat and the “extra light” spreadable product (6) containing as little as 4.7 g 100 g^{-1} fat. There was less variation in the declared protein and carbohydrate content between products 1–5, with average values of 7.3 g 100 $g^{-1}$and 2.9 g 100 g^{-1}, respectively. Product 6, by contrast, had a significantly higher protein and carbohydrate content, with values of 11.1 g 100 $g^{-1}$and 8.8 g 100 g^{-1}, respectively. It is likely this increase in protein and carbohydrate content allowed the overall dry matter to be maintained in this product.

The moisture content also varied widely between all products and was negatively correlated with the fat content; that is, as the fat content is reduced the moisture content is increased (Fig. 1). This relationship was also noted by Liu, Xu, and Guo (2008) and Mistry (2001) in their studies of the formulation challenges with low fat cheeses in general, which commonly have higher moisture contents than their full fat counterparts. When investigating the influence of fat in cream cheese, Roundy and Price (1941) found that fat and moisture tended to impart similar characteristics on the physical properties of cream cheese and that moisture could

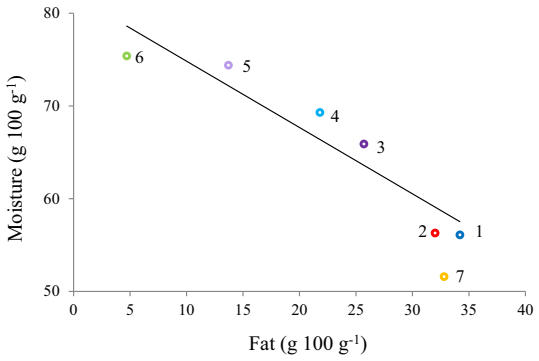

Fig. 1. The relationship between fat and moisture (g 100 g^{-1}) for the panel of cream cheese products 1–6. y = −0.8029x + 83.0561. R^2 = 0.8637. The open orange circle labelled 7 represents the composition of the laboratory produced product. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Labelled composition (as declared on the packaging) of six Australian commercial cream cheese products and comparison with a model cream cheese produced at laboratory scale.[a]

Product number	Product description	Fat	Protein	Carbohydrate	Moisture	Sodium	Other ingredients (starter culture and enzymes excluded)
1	Full fat (block)	34.2	7.4	2.3	56.1	358	Locust bean gum
2	Full fat/spreadable (tub)	32.0	8.8	2.9	56.3	320	Locust bean gum; Guar gum; Vegetable emulsifier (471)
3	Spreadable (tub)	25.7	5.7	2.7	65.9	349	Locust bean gum
4	Light (block)	21.8	6.1	2.8	69.3	376	Locust bean gum
5	Light/spreadable (tub)	13.7	8.0	3.9	74.4	264	Locust bean gum; Guar gum
6	Extra light/spreadable (tub)	4.7	11.1	8.8	75.4	281	Locust bean gum; Carrageenan; Thickener (1442); Preservative (200); Citric acid (330)
7	Model cream cheese produced at laboratory scale	32.8	9.3	ND	51.6	397	Locust bean gum

[a] Values are in g 100 g^{-1}; moisture was calculated by subtracting the combined mass of fat, protein and carbohydrate from 100 g; this value is an approximation and does not take into consideration the mass of sodium, gum and other ingredients used, the sum of which may equate to ~2 g 100 g^{-1}. ND, carbohydrate was not determined; a variant of this cheese was also produced without gum.

replace fat, to a certain extent, without changing these properties. It is therefore not surprising to see water used as a fat replacement in both light and spreadable cream cheese varieties. Water also contributes to the spreadable nature of a spreadable cream cheese product. This reduction in fat content and its effect on spreadability has been reported in previous studies (Brighenti et al., 2008; Glibowski, Zarzycki, & Krzepkowska, 2008; Liu et al., 2008; Muir et al., 1997; Wendin et al., 2000). Although there is no specific Australian compositional standard for cream cheese, it is evident that, with the exception of product 6, all of these products comply with the international Codex Standard for Cream Cheese, which requires minimum values of 25 g 100 g^{-1} milk fat in dry matter, 67 g 100 g^{-1} moisture on fat-free basis and 22 g 100 g^{-1} dry matter (FAO/WHO, 2018).

Ingredients in cream cheese other than milk, cream, salt, starter culture and enzymes are also important in influencing product functionality and stability. Hydrocolloids are paramount in commercial cream cheese production and are used for stabilisation, texture enhancement and fat/protein replacement (Golding & Matia-Merino, 2013). Due to their powerful ability to bind water, hydrocolloids can improve product texture by providing firmness, improving mouthfeel, enhancing spreadability, reducing syneresis and lengthening shelf-life (Guinee & Hickey, 2009; Wielinga, 2009a). Galactomannans, which are non-ionic, branched polysaccharides, such as locust bean gum (LBG) and guar gum, are used extensively in cream cheese production to increase the viscosity of the continuous phase and prevent syneresis (Goldstein, Alter, & Seaman, 2012; Guinee & Hickey, 2009; Rol, 2012). These gums also have the advantage of having low sensitivity to both salt and pH concentration (Wielinga, 2009a), allowing a wide range of formulations and process conditions. All the products tested in this study contained LBG, with products 2 and 5 also containing guar gum (Table 1).

Carrageenan, also a polysaccharide, is used less often in cream cheese and in this study was found only in the “extra light” product 6. Unlike the galactomannans, carrageenan is anionic and interacts with the positive charges on the surface of the casein micelles (Tasneem, Siddique, Ahmad, & Farooq, 2014). In cream cheese, it is generally used together with LBG, where it acts as a stabiliser, preventing aggregation and moisture separation. It also confers a smooth and creamy mouthfeel to the product, whilst also contributing to shape retention (Towle, 2012; Wielinga, 2009b). It is interesting to note that product 2 also contains vegetable emulsifier 471 (a mixture of glyceryl monostearate and glyceryl distearate), presumably as a means of improving spreadability, whilst maintaining a high fat content (Euston, 2008). Product 6 has the most complex formulation and appears to employ a combination of strategies to overcome the functionality challenges of a very low fat, high moisture product. These include the use of carrageenan, as well as LBG, thickener/stabiliser (hydroxypropyl distarch phosphate), preservative (sorbic acid) and citric acid, which probably acts as both an acidity regulator and emulsifier. Together, this panel of products provide a wide range of compositions resulting from different formulation strategies that are expected to differ in both their structural and physical properties.

Table 1 also lists the composition of a seventh product produced at laboratory scale for comparison with the range of commercial products (1–6). This cream cheese has a high fat content, similar to that of the commercial full fat products, as well as a high protein concentration and lower moisture content, placing the product in a similar position in Fig. 1 to products 1 and 2.

3.2. Microstructure

3.2.1. CLSM and cryo SEM of a model cream cheese

The microstructure of a cream cheese sample produced at laboratory scale and visualised by CLSM can be observed in Fig. 2. This image illustrates the characteristic corpuscular structure of cream cheese, with the discontinuous protein network and fat stained in green and red, respectively. This method allows for both simultaneous (Fig. 2a,d) and separate (Fig. 2b,c) visualisation of the protein and fat components. Three common microstructural features of this type of cream cheese can be seen particularly well at higher magnification (Fig. 2d).

Firstly, the corpuscular structures (indicated by the white circle) contain aggregates of protein and fat resulting from homogenisation during processing and comprise the majority of the microstructure. They range in size from ~2 to 10 μm in diameter. This cluster size is likely dependent upon the shearing and cooking of the curd after gum addition or the homogenisation conditions applied to the curd prior to packaging (Fenoul et al., 2008; Kalab et al., 1981). In agreement with the findings of Kalab et al. (1981), the fat globules appear to be more centrally located within these corpuscular clusters, while the protein aggregates are present towards the surface, forming an interface between the aqueous and lipid phases of the cream cheese.

The second characteristic feature is a relatively high number of large, “free-fat” globules dispersed between the corpuscular aggregates, generally ranging between 2 and 10 μm in diameter. These fat globules likely arise from the de-emulsification of fat that occurs due to extended shear in the final stages of processing at 80 °C (Lenze, Wolfschoon-Pombo, Schrader, & Kulozik, 2019), which may be followed by coalescence (Kalab et al., 1981). While processing conditions appear to be the major contributing factor influencing the relative size and amount of “free-fat”, product composition may also play a role.

Finally, the third characteristic feature is the unstained black areas (indicated by the white arrows) that contain the serum phase, made of water and various solutes such as salt, carbohydrates, gums and stabilisers. This feature depends on the moisture content of the product and appears less prominent in high-fat products with a low water content and more prominent in low-fat products with a high water content.

It is evident that while CLSM can provide a detailed insight into the interaction between protein and fat in cream cheese, potentially providing some valuable guidance for optimisation of the formulation and processing of the product, it provides little information about components within the serum phase, such as carbohydrates and solutes. Visualisation of gums, for example, would be particularly interesting, given their common use in cream cheese formulation and marked effects on product structure and rheology (Golding & Matia-Merino, 2013). The use of CLSM for this purpose presents serious technical challenges, however, as post-production staining of gums potentially requires covalent bonding to the fluorescent probe of interest (Goldstein et al., 2012; Laneuville & Turgeon, 2014; Monteiro, Rebelo, da Cruz e Silva, & Lopes-da-Silva, 2013; Tromp, van de Velde, van Riel, & Paques, 2001; van de Velde, Weinbreck, Edelman, van der Linden, & Tromp, 2003), unlike the simple staining mechanism of both Nile Red and Fast Green FCF, which rely on diffusion into fat and protein rich areas, respectively (Auty, Twomey, Guinee, & Mulvihill, 2001; Greenspan, Mayer, & Fowler, 1985). Furthermore, although highly effective in modulating cream cheese structure, gums are included at very low concentrations compared with fat, protein and carbohydrates and may be difficult to visualise microscopically against a dominant background of milk components.

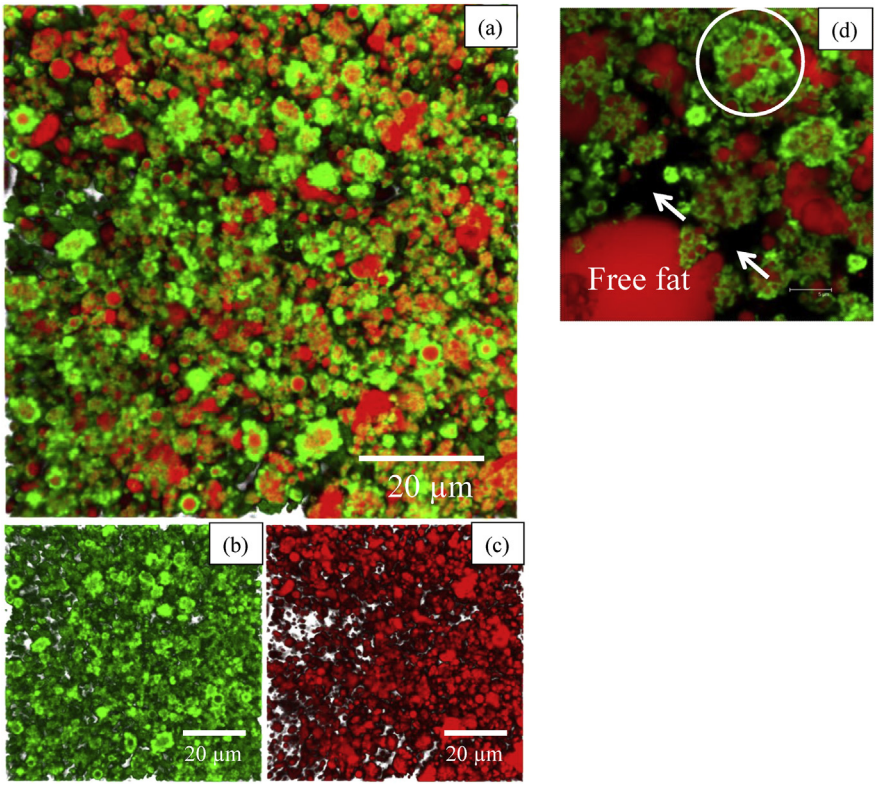

Fig. 2. Microstructure of cream cheese determined by CLSM imaging; fat, protein and serum phases appear red, green and black, respectively. Panel (a) illustrates the corpuscular structure of cream cheese, while panels (b) and (c) show the protein and fat phases of cream cheese, respectively; scale bars are 20 µm. Panel (d) presents cream cheese at a higher magnification and reveals (i) the corpuscular structure (white circle), (ii) free fat or fat not coated in protein and (iii) unstained black areas (white arrows) representative of the serum phase; the scale bar is 5 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Unlike CLSM, however, cryo SEM does allow observation of the spaces occupied by the serum phase after the aqueous phase is sublimed. This method therefore provides information on the distribution of water and solutes throughout the sample and its interaction with structural components, such as protein and fat. Care needs to be taken with the application of cryo SEM to high-moisture substrates, however, as the technique can generate experimental artefacts and provide misleading information on microstructural details (Guardeno et al., 2010; Llorca et al., 2005; Ong et al., 2011).

In developing the cryo SEM method for this study, the technique was first used to compare carefully controlled "model cream cheese" products made in the laboratory, both with LBG (Fig. 3a, b at low and high magnification, respectively) and without the inclusion of LBG (Fig. 3c, d at low and high magnification, respectively). Fig. 3 also illustrates a cryo SEM image for a pure solution of hydrated LBG gum (Fig. 3e). The honeycomb-like structures evident in both the hydrated gum sample (Fig. 3e) and the gum-containing cream cheese (Fig. 3a,b) are consistent with other published cryo SEM images of gels containing mixtures of LBG and κ-carrageenan (Dunstan et al., 2001).

Whilst the observation of such structures is a good indication of the presence of LBG or other gums in the serum phase (as these features are absent in Fig. 3c,d), the apparent honeycomb-like microstructure is likely to be an unavoidable eutectic artefact generated during the sample-etching step of the cryo SEM method and does not necessarily represent the intrinsic microstructure of this non-ionic gum network in the fresh sample (Dunstan et al., 2001; Guardeno et al., 2010; Llorca et al., 2005; Ong et al., 2011). In agreement with images observed using CLSM, the amount of honeycomb-like structure present provides information as to the moisture content of the cream cheese sample; this feature being less prominent in high-fat products with a low water content and more prominent in low-fat products with a high water content.

Like the CLSM images, the cryo SEM images in Fig. 3 illustrate the corpuscular structure of protein and fat aggregates (indicated by the white circles) inherent to cream cheese and the "free-fat" present due to processing conditions. The size of both features is comparable with that observed using CLSM. Despite the monochrome nature of the images and the inability to use differential staining to provide contrast, there are some advantages to using cryo SEM rather than CLSM. Cryo SEM allows for images to be taken with a higher resolution than CLSM, yielding images with greater clarity and with no requirement for fluorescent staining. It is evident from Figs. 2 and 3 that together, CLSM and cryo SEM can be powerful, complementary techniques for the microstructural analysis of high moisture foods.

3.2.2. *The potential artefacts in cryo SEM imaging of cream cheese*

In developing the cryo SEM method for the analysis of cream cheese, we also sought to investigate some of the variables used for sample preparation. These included (i) the freezing method, (ii) the length of etching/sublimation time and (iii) the amount of sputter coating used so that optimum conditions could be obtained for future reference and additional artefacts avoided (Fig. 4). The "light" cream cheese variety of product 4 was chosen for investigation, as it contains a high moisture content and therefore provides adequate visualisation of the honeycomb-like structure (i.e., eutectic artefact). Fig. 4 illustrates the sample preparation process

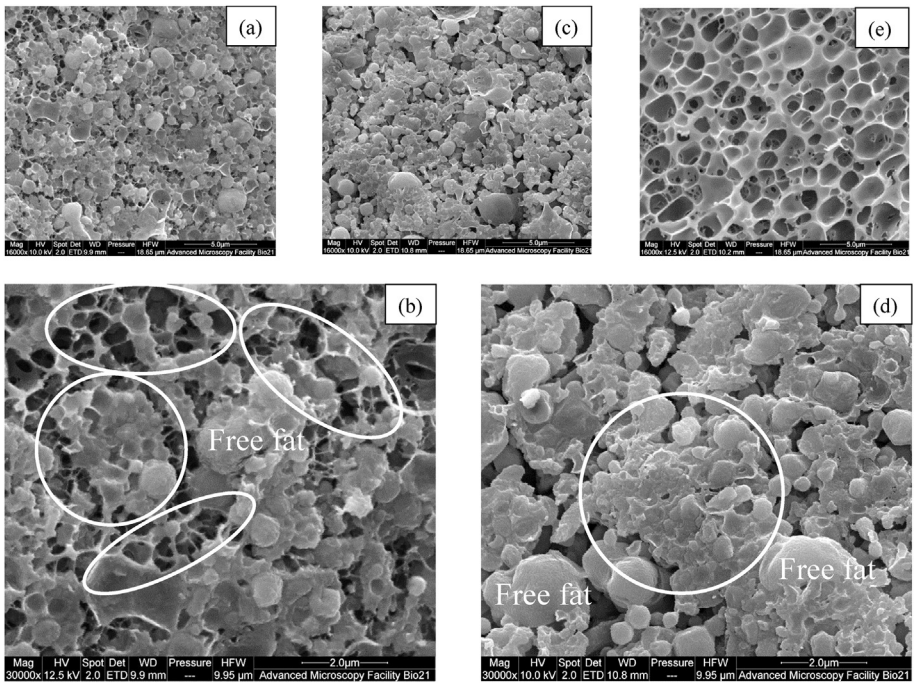

Fig. 3. Cryo SEM microstructure of cream cheese with gum (a,b), cream cheese without gum (c,d) and hydrated gum (e). Three characteristic features of cream cheese can be observed: (i) the corpuscular structure (white circles) representative of fat and protein aggregates, (ii) the eutectic artefact (white ovals) representative of the serum phase and (iii) free fat or fat that is not coated in protein. Images are 16,000× (a,c,e; scale bar 5 μm) and 30,000× (b,d; scale bar 2 μm).

and reveals four different representations of product 4 derived from the four different sample preparation pathways.

The first pathway shown on the left gives rise to Fig. 4a and represents product 4 prepared using steps 1–5. As observed for previous cryo SEM images, this set of variables provides a clear image highlighting the dense network of protein and fat aggregates (indicated by the white circle), the honeycomb-like serum phase (indicated by the white oval) and "free-fat" (indicated by arrows). The micrograph is clear, evenly charged and does not appear to display any artefacts other than the appearance of the gum.

The second pathway (steps 1–4, 5*) differs from the first pathway in that the sputter coating time is halved to 60 s to give a thickness of only ~3 nm, as shown in Fig. 4b. An artefact due to insufficient coating, such as charging due to static charge accumulation is evident and appears as a bright white region on this image (white oval) (Kalab, 1984; Ong et al., 2011). This artefact, however, does not appear to influence the typical features of the product, as both protein and fat aggregates and the honeycomb-like serum phase are apparent. Therefore, while sputter coating for 60 s is not recommended due to charging artefacts and potential damage to the SEM, typical cream cheese features can still be observed.

The third pathway (steps 1–3, 4*, 5) highlights the effect that an increase in etching time (60 min versus 20 min) can have on the cream cheese product (Fig. 4c). An increase in regions of the honeycomb-like structure is immediately apparent and supports similar findings in milk gels subjected to increased etching times (Ong et al., 2011). Larger pore sizes could also be observed and may be the result of larger ice crystals, resulting from the more lengthy etching process. Ice recrystallisation and the formation of cubic ice have been reported during etching (Severs et al., 1995). Water molecules that sublimed from the surface could possibly recondense back on the sample with prolonged sublimation. This artefact could lead to misinterpretation of the sample, by suggesting a product of high water content due to the increased honeycomb-like structures and therefore, shorter etching times of 20 min are recommended as this limits damage to the cream cheese structure. At 20 min etching time, the microstructure obtained by cryo SEM is also comparable with the microstructure obtained by CLSM (Fig. 5), providing consistency between the two methods.

Finally, the fourth pathway (steps 1, 2*, 3–5) illustrates the effect that slow freezing at −196 °C can have on the cream cheese product (Fig. 4d). The slow freezing rate (~10^3 °Cs^{-1}) is the result of the direct immersion of sample to liquid nitrogen at its boiling point of −196 °C, in which the heat transfer is limited by an insulating layer of the evaporated nitrogen gas, a phenomenon known as the Leidenfrost effect (Severs et al., 1995). A significant reduction of the honeycomb-like structure is apparent and appears to be replaced by large open areas (indicated by the white oval). Interestingly, while this eutectic artefact has been affected, possibly by the growth of large ice crystals during the slower freezing process, the protein and fat aggregates appear undamaged (white circle) and capable of withstanding this freezing process. This procedure is not ideal, as this artefact could again lead to the misinterpretation of the sample by suggesting a less dense product with a significantly reduced gum network or lack of gum. This simple study highlights the many options available when preparing samples for cryo SEM and the importance of choosing the correct conditions to avoid artefacts and obtain reliable images characteristic of the product. The use of complementary microscopy techniques is also recommended.

3.2.3. CLSM and cryo SEM of cream cheese products 1–6

The CLSM and cryo SEM images of products 1–6 are shown in Figs. 5 and 6 at low and high magnification, respectively. CLSM images of products 1 (Figs. 5a and 6a) and 3–6 (Figs. 5c–f and 6c–f) display a typical microstructure for cream cheese, with fat globule clusters (red) surrounded by a non-continuous protein (green) network, interspersed with "free fat" and the serum phase (Kalab et al., 1981; Monteiro, Tawares, Kindstedt, & Gigante, 2009; Ohashi et al., 1983). The characteristic corpuscular-like structures (white

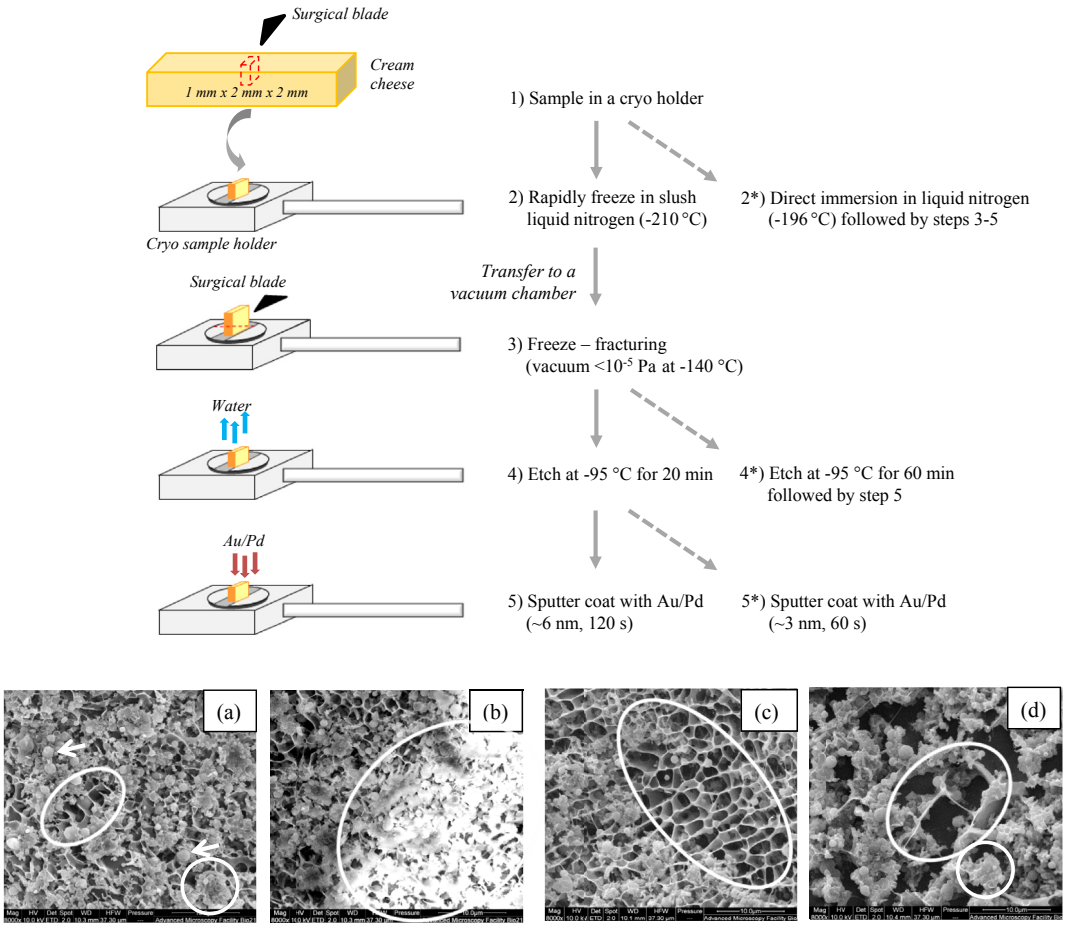

Fig. 4. Schematic showing four different pathways in the sample preparation of cream cheese samples for cryo SEM and the respective images (a–d; 8000×; scale bars 10 μm; white ovals indicate artefacts and arrows indicate "free fat"). The first pathway (1-2-3-4-5; panel a) shows the optimum method, the second pathway (1-2-3-4-5*; panel b) shows the method with reduced sputter coating time, the third pathway (1-2-3-4*-5; panel c) shows the method with an increased etching time and the fourth pathway (1-2*-3-4-5; panel d) shows the method with a slower rate of freezing.

circles) can be more easily observed at higher magnification (Fig. 6) and appear to range in shape and size depending on the product.

Products 1, 3 and 4 (Fig. 6a,c,d, respectively) share protein and fat clusters of a similar size (~2–10 μm in diameter). The size, number and distribution of "free fat" (~2–10 μm) is also similar. These structural similarities occur despite product 4 having less fat than products 1 and 3 and are likely due to a combination of factors, including processing conditions and gum type (all contain LBG). In contrast, products 5 and 6 appear to share "free fat" of a reduced size, with a diameter of ~3 μm (Fig. 6e,f). While product 6 shows a cluster size similar to products 1, 3 and 4, the cluster size for product 5 appears to have increased to a diameter of ~2–15 μm, again likely due to differences in processing conditions and ingredients.

The CLSM images clearly depict the serum phase (black) for products 1 and 3–6 (Figs. 5 and 6). A limited serum phase can be observed for product 1, which is a densely packed high fat product (Figs. 5a and 6a). Products 3 (Figs. 5c and 6c) and 4 (Figs. 5d and 6d) by contrast, display a higher amount of serum interspersed between the protein and fat clusters, as they share a higher water content, which contributes to their spreadable and reduced fat properties, respectively. Finally, an even greater amount of serum can be observed for product 5 (Figs. 5e and 6e) and 6 (Figs. 5f and 6f), surrounding a loosely packed protein and fat network. This increase in moisture again contributes to the functionality of the product and allows for both a reduction in the fat content and increase in spreadability.

In contrast to the typical cream cheese microstructure observed for products 1 and 3–6, product 2 appears quite different in structure (Figs. 5b and 6b). Protein and fat aggregates appear more closely packed and are smaller, with a diameter of ~1–6 μm. While the size of the "free fat" is comparable with that observed for products 5 and 6 (~3 μm in diameter), there is a significant increase in "free fat" compared with all other products examined. The fat globules seem more evenly dispersed throughout the clusters and a significant reduction in the serum phase is also evident. Products 1 and 2 have similar protein and fat content (Table 1) so could be expected to share a similar microstructure, however, this is not the case. Although processing effects, such as differences in homogenisation pressures, cannot be discounted, it is suggested that the apparently atypical microstructure of product 2 is most likely due to the effects of an added vegetable emulsifier, that acts to disperse the fat more homogeneously through the product. Such emulsifiers are absent from all other products.

The cryo SEM images complement the CLSM images and similarly reveal the characteristic microstructure of cream cheese for products 1 and 3–6. The high fat product 1 displays densely packed protein and fat aggregates, interspersed with "free fat" and a

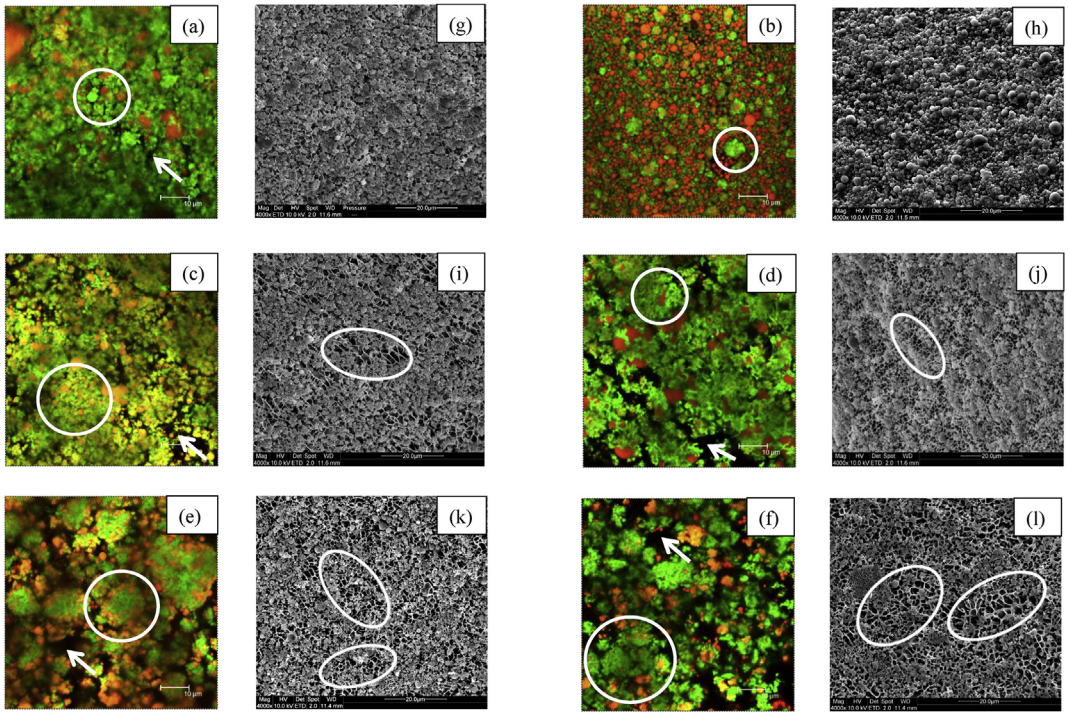

Fig. 5. The microstructure of cream cheese products 1–6 using CLSM (a–f) and cryo SEM (g–l). In the CLSM images the fat, protein and serum phases appear red, green and black, respectively; the scale bars are 10 μm in length, white circles indicate the characteristic corpuscular structure (aggregates of protein and fat) of cream cheese and arrows indicate the serum phase. Cryo SEM images are 4000× and the scale bars are 20 μm in length; white ovals indicate parts of the serum phase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

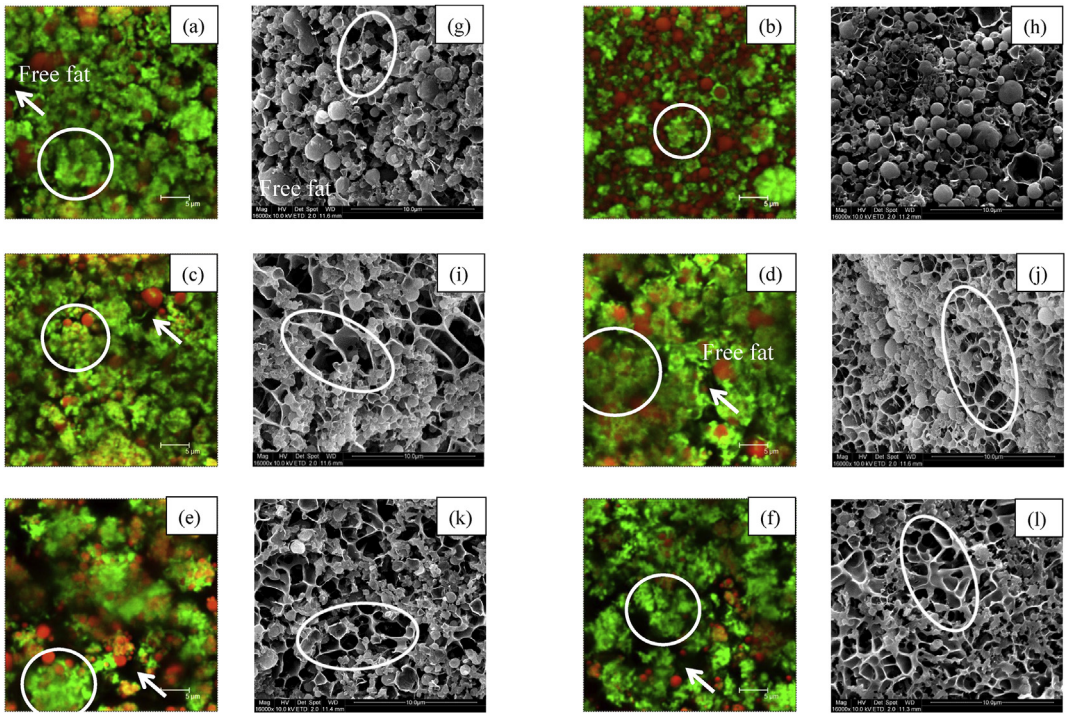

Fig. 6. The microstructure of cream cheese products 1–6 using CLSM (a–f) and cryo SEM (g–l) at higher magnification. In the CLSM images the fat, protein and serum phases appear red, green and black, respectively; the scale bars are 5 μm in length, white circles indicate the characteristic corpuscular structure (aggregates of protein and fat) of cream cheese and arrows indicate the serum phase. Cryo SEM images are 16,000× and the scale bars are either 5 or 10 μm in length; white ovals indicate parts of the serum phase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

limited amount of serum, observed as honeycomb-like structures (indicated by the white ovals, Figs. 5g and 6g). As the fat content decreases and the moisture increases, the protein and fat aggregates become less densely packed and the honeycomb-like structures attributed to the presence of gums appear more prominent (products 3–6; Figs. 5i–l and 6i–l), where they play a particularly important role in microstructural reinforcement. This increasing openness of the cream cheese structure with decreasing total solids and increasing moisture content is complementary to the black serum observed in the CLSM images. The shape and size of the pores within these honeycomb-like structures appear to vary between products and is likely due to differences in both processing conditions and product composition.

Product 2 has an atypical microstructure for cream cheese when observed by cryo SEM (Figs. 5h and 6h), consistent with observations by CLSM (Figs. 5b and 6b). Protein and fat clusters are densely packed in this sample and appear smaller than all other products. "Free fat" is again significantly more prominent than observed in other products, leading to a very globular-like structure. A significant reduction in the serum phase is also observed. These cryo SEM images of product 2 (Figs. 5h and 6h) provide further insight into the unusual structure of this product, which apparently differs from the other products in containing a larger proportion of emulsified fat globules.

Differences in the protein structure between products 1–6 were less evident in the CSLM and cryo SEM images, compared with differences observed in fat and moisture (Figs. 5 and 6). While product 1 contains 7.4 g 100 g^{-1} protein, the protein in this sample appears similar to the protein in product 6, which contains a much larger protein content of 11.1 g 100 g^{-1} (Figs. 5f,l and 6f,l versus Figs. 5a,g and 6a,g). Nevertheless, some differences in protein distribution and association can be observed.

A simple schematic for the general microstructure of cream cheese is proposed, based on microstructural observations of fat and moisture made in this study (Fig. 7). A high fat, low moisture cream cheese product is illustrated in Fig. 7a, b corresponding to typical CLSM and cryo SEM images, respectively. The protein and fat clusters appear densely packed and there is "free fat" present. Serum (indicated in blue) is limited and is observed as a continuous phase surrounding the aggregates for CLSM images (Fig. 7a) or a honeycomb-like structure for cryo SEM images (Fig. 7b). In comparison, a low fat and high moisture cream cheese product is illustrated in Fig. 7c, d corresponding to CLSM and cryo SEM images, respectively. The protein and fat clusters are less densely packed in these figures and there is significant more surrounding serum. There appears to be no significant difference in the "free fat" observed between the samples.

3.3. Dynamic rheology of products 1–6

Amplitude strain sweeps of products 1–6 were performed to determine the linear viscoelastic region. Strain sweeps for all products revealed G > G, indicating an elastic and gel-like behaviour characteristic of low-viscosity gels (Fig. 8a). These observations are in agreement with previous rheological investigations and are considered to be typical for cream cheese products (Kealy, 2006; Sanchez, Beauregard, Chassagne, Duquenoy, & Hardy, 1994b; Sanchez, Beauregard, Chassagne, Bimbenet, & Hardy, 1994a, 1996).

With the exception of product 2, Fig. 8a also shows that the viscoelastic properties of these products correlated linearly with fat content in the linear viscoelastic region, that is as G′/G″ values decrease, the fat content also decreases. These results reflect the general trend for simply formulated dairy products, whereas the fat content is reduced the moisture content is increased, leading to a less structured and more spreadable product. Similar observations

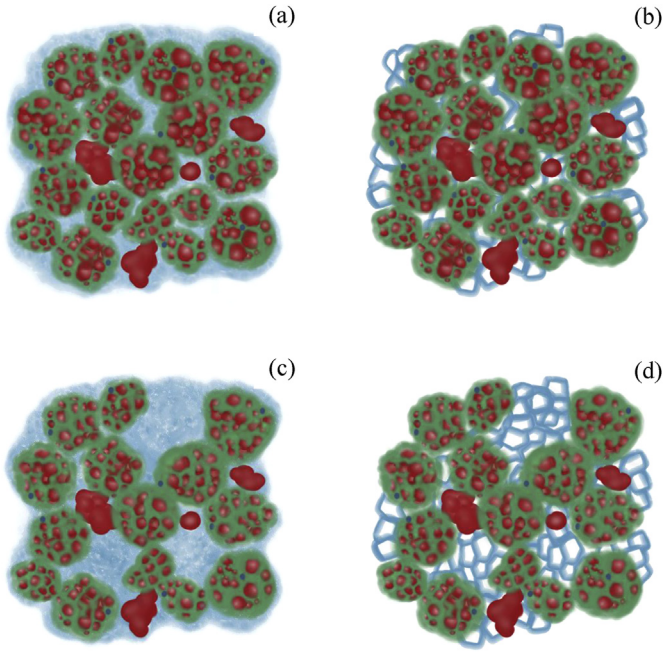

Fig. 7. Schematic for the typical microstructure of cream cheese as a function of fat and moisture content. A high fat, low moisture cream cheese product is illustrated in panels a and b, corresponding to the typical microstructures observed by CLSM and cryo SEM, respectively. A low fat and high moisture cream cheese product is illustrated in panels c and d, corresponding to the typical microstructure observed by CLSM and cryo SEM images, respectively. Protein is shown in green, fat is shown in red and the serum phase is shown either as solid blue for CLSM images or as a blue honeycomb-like structure for cryo SEM images, representing the eutectic freeze artefact observed with this type of imaging. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

have previously been reported for acid gels (Lucey, Munro, & Singh, 1988), cheese analogues (Liu et al., 2008), processed cheese spreads (Dunstan et al., 2001; Lee, Anema, & Klostermeyer, 2004; Pereira, Bennet, & Hemar, 2001) and cream cheese (Brighenti et al., 2008; Kealy, 2006). These rheological findings also support both CLSM and cryo SEM images obtained for products 1 and 3–6 and together provide insight into the functionality of the various cream cheese products. The high fat product (product 1) contains little free serum, has a low moisture content and a high G′ value. Reduced fat and/or spreadable products (products 3–5) appear less densely packed, have a higher moisture content with greater observable serum and a lower G′ value while the "extra light" product (product 6) is very loosely packed, has a very high moisture content and the greatest volume of observable serum and a very low G′ value.

On the basis of having very similar fat and moisture contents (Table 1), it might be expected that products 1 and 2 would have similar rheological properties. Product 2 has a G′ value almost two orders of magnitude less that product 1, with, however, spreadability properties similar to those of low-fat products rather than product 1 (Fig. 8a). Analysis by CLSM and cryo SEM showed that these two products also differed markedly in microstructure, with product 2 having a more homogenous structure, with smaller and less aggregated fat and protein clusters, compared with product 1 (Figs. 5 and 6). Although the physical processing history of these products is not known, the inclusion of a vegetable emulsifier in product 2 (not present in product 1; see Table 1) suggests that the observed differences in microstructure and rheology may be explained by the greater degree of emulsification of fat and protein in product 2. As discussed above, emulsifiers are commonly used to improve the spreadability and stability of cream cheeses, processed

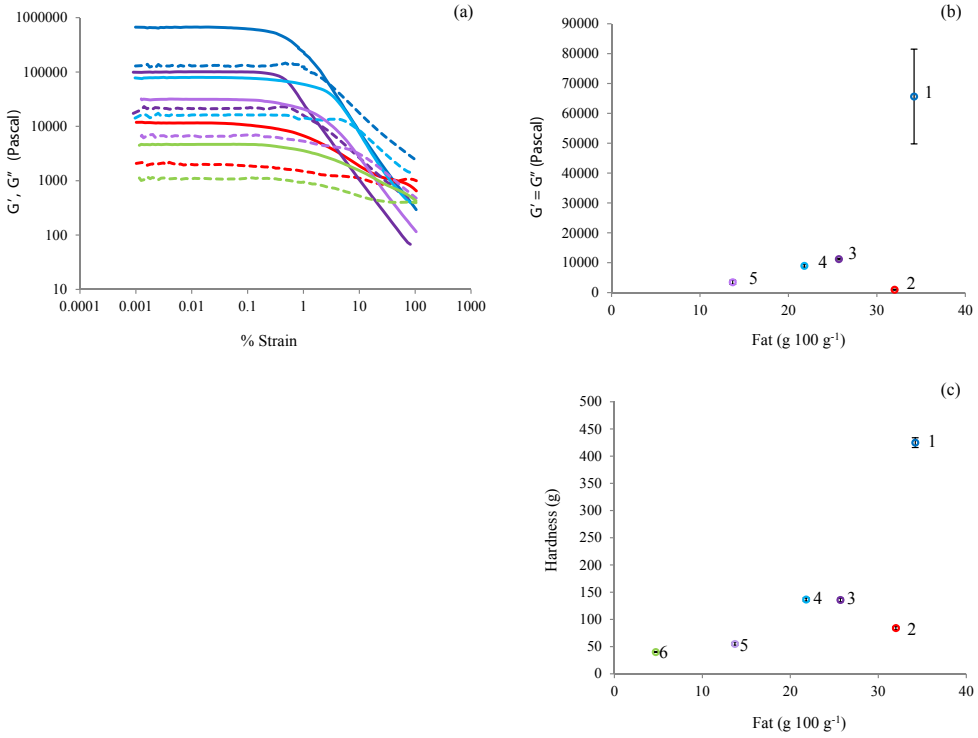

Fig. 8. Amplitude strain sweeps of cream cheese products 1–6 at 10 °C (a). Storage modulus, (G′) is shown as a solid line for products 1 (——), 2 (——), 3 (——), 4 (——), 5 (——) and 6 (——) and loss modulus (G″) is shown as a dashed line from products 1 (------), 2 (------), 3 (------), 4 (------), 5 (------) and 6 (------). Critical stress values (G′ = G″) were obtained for products 1–5 (a critical stress value could not be determined for product 6) and are expressed as a function of fat content (b); the error bars represent the standard deviation, where n = 2. Panel c gives a scatter plot representing the hardness (g) of cream cheese products 1–6 at 10 °C expressed as a function of fat content; the error bars represent the standard deviation, where n = 5 ($y = 8.4613x - 40.273$; $R^2 = 0.4455$).

cheeses and dairy products in general (Euston, 2008; Guinee & Hickey, 2009; Wendin et al., 2000).

Critical stress values or the crossover point of G′ and G″ were determined from strain sweeps for products 1–5, and shown as a function of fat content in Fig. 7b. Again, with the exception of product 2, these values appear to show a correlation with fat content, with critical stress values decreasing as the fat content is reduced. As products 3 and 4 have similar fat and moisture contents and use only LBG as an additional ingredient, it is not surprising that they have comparable critical stress values. The critical stress value for product 2 is extremely low and reflects a product of limited structure. The weakest structure, however, is observed for product 6, for which a critical stress value could not be obtained. The variability observed for the critical stress value of product 1 for this data set also reflects the higher modulus for this high fat sample.

A strain of 0.01% was selected for frequency sweeps, as strain sweeps showed that this was within the linear viscoelastic region for all products tested (Fig. 8a). Frequency sweeps showed that all cream cheese products exhibited viscoelastic properties representative of strong viscoelastic solids or weak gel systems (Kealy, 2006; Lucey et al., 1988). These data were consistent with the strain sweeps (Supplementary material Fig. S1).

3.4. Texture profile analysis of products 1–6

While sensory methods permit more complex attributes to be evaluated and are in many ways the ultimate test of consumer acceptance, these tests are also time-consuming and expensive. Routine commercial quality control of products usually relies on simpler but reproducible instrumental techniques, such as texture profile analysis. Texture analysers like the one used in this study are commonly used in cream cheese assessment, where hardness measurements can give a useful indication of spreadability as well as resistance to biting and chewing (Brighenti et al., 2008; Glibowski et al., 2008; Wendin et al., 2000).

The hardness of products 1–6, as determined by texture profile analysis, is shown in Fig. 8c. Presented as a function of fat content, these data appear very comparable with those from the rheological analysis, Fig. 8b. With the exception of the highly emulsified product 2, they show a clear positive correlation between product hardness and fat content, which is in general agreement with previous investigations (Brighenti et al., 2008; Kealy, 2006; Lee et al., 2004). Other studies of this product type have also shown a general correlation of hardness with total solids content, illustrating the important role of protein, protein-water interactions and fat content, in determining cheese product structure (Brighenti et al., 2008; Jack & Paterson, 1993; Lee et al., 2004; Pereira et al., 2001). Indeed, it is likely that the inclusion of a higher level of gum, carbohydrate and thickener in products 5 and 6 (Table 1) is required to compensate for a reduction in combined fat and protein content to a level that would be insufficient to maintain an acceptable structure.

These hardness data similarly support CLSM and cryo SEM images obtained for products 1 and 3–6 and provide further insight into the functionality of the various cream cheese products. The high fat, low moisture product (product 1) appears densely packed, has a high G′ value and is very hard. The reduced fat and/or spreadable products with a higher moisture content (products 3–5) appear less densely packed, have a lower G′ value and are considerably softer, while the “extra light” product with a very high moisture content (product 6) is very loosely packed, has a very low G′ value and is very soft.

4. Conclusion

This microstructural investigation of a model cream cheese and six Australian commercial products has highlighted the benefit of using both CLSM and cryo SEM techniques in parallel. Cryo SEM may be used to locate the gum in these products, using the eutectic artefacts that form in areas of high moisture associated with the presence of gum. Other avoidable artefacts arising from sample preparation are also investigated, as a guide for optimal conditions when examining cream cheese or similar dairy products by cryo SEM.

This study shows how microstructural analysis can provide valuable insights into the complex relationship between formulation, processing, structure and functionality of cream cheese, when employed in conjunction with physical testing and compositional analysis. Microstructure analysis can help to explain why two products with similar fat and water content (products 1 and 2) have very different physical properties, such as hardness. Product microstructure, together with fat and moisture content, also correlated well with rheological properties and hardness for the six commercial products examined, with dense high fat products being more viscous and harder, while lighter, less dense and more moist products were less viscous and very soft. Together, these microscopy methods can also potentially be used to help predict product functionality, which is difficult to achieve by compositional and physical analysis alone.

The methodology presented here may have broad potential in product development, helping to optimise cream cheese formulation, processing conditions, ingredient substitution and in the development of “clean label” products that rely less on gums and stabilisers. Although there may be some interest in the use of this approach to help “reverse engineer” a product, whose detailed manufacturing history is unknown, microstructural analysis is likely to be of greatest value when used in conjunction with a detailed knowledge of product formulation and processing history. Further systematic studies on model cream cheese systems would be useful in helping to establish these relationships.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2019.104548.

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