Rheological properties and microstructure of Cheddar cheese made with different fat contents

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ABSTRACT

Reduced- and low-fat cheeses are desired based on composition but often fall short on overall quality. One of the major problems with fat reduction in cheese is the development of a firm texture that does not break down during mastication, unlike that observed in full-fat cheeses. The objective of this investigation was to determine how the amount of fat affects the structure of Cheddar cheese from initial formation (2 wk) through 24 wk of aging. Cheeses were made with target fat contents of 3 to 33% (wt/wt) and moisture to protein ratios of 1.5:1. This allowed for comparisons based on relative amounts of fat and protein gel phases. Cheese microstructure was determined by confocal scanning laser microscopy combined with quantitative image analysis. Rheological analysis was used to determine changes in mechanical properties. Increasing fat content caused an increase in size of fat globules and a higher percentage of nonspherical globules. However, no changes in fat globules were observed with aging. Cheese rigidity (storage modulus) increased with fat content at 10°C, but differences attributable to fat were not apparent at 25°C. This was attributable to the storage modulus of fat approaching that of the protein gel; therefore, the amount of fat or gel phase did not have an effect on the cheese storage modulus. The rigidity of cheese decreased with storage and, because changes in the fat phase were not detected, it appeared to be attributable to changes in the gel network. It appeared that the diminished textural quality in low-fat Cheddar cheese is attributed to changes in the breakdown pattern during chewing, as altered by fat disrupting the cheese network.

Key words: cheese, low fat, microstructure, rheology

INTRODUCTION

The goal for an overall caloric reduction in the diet, sometimes specifically targeting calories from fat, has encouraged the development of low-fat alternatives to traditional products. Studies have shown that American consumers have a preference for lower fat dairy products, including cheese (Sandrou and Arvanitoyannis, 2000; Childs and Drake, 2009). Cheese is perceived as being high in fat and this discourages some consumers from including cheese in their diet, even though it is also a good source of dietary calcium (Johnson et al., 2009). However, because fat is a major contributor to flavor and textural quality of food products, reducing fat while maintaining sensory quality presents several challenges. Cheddar cheese contains about 32% (wt/wt) fat, and there exists a desire to produce a low-fat version with $\leq 6\%$ fat that maintains sensory quality so as to allow its continued use in school lunch programs (Johnson et al., 2009).

Many reports have shown that as the fat content of cheese is progressively reduced, the cheese develops an undesirable firm, rubbery texture (Bryant et al., 1995; Guinee et al., 2000; Mistry, 2001; Gwartney et al., 2002; Rogers et al., 2009). Likewise, numerous strategies have been applied to improve the texture of low-fat cheese and these have been reviewed (Drake and Swanson, 1995; Mistry, 2001; Banks, 2004, 2007; Johnson et al., 2009). However, it still remains a challenge to reduce fat and maintain the texture of a comparable full-fat cheese.

Sensory textural properties of cheese are the result of the temporal pattern of structural breakdown and mixing with saliva during oral processing (Hutthlings and Lillford, 1988). Therefore, when fat is removed, the low-fat structure needs to be altered such that it has a breakdown pattern and saliva interactions similar to that of full-fat cheese. Sensory texture terms can be separated into “first bite” and “chewdown” terms (Foegeding and Drake, 2007). First bite terms represent the initial deformation and fracture (e.g., firmness or hardness) of the cheese, and chewdown terms (e.g., degree of breakdown, cohesiveness, adhesiveness, and smoothness

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of mass) are determined after chewing and mixing with saliva. Reduced- and low-fat cheeses have increased first bite terms and decreased chewdown terms (Gwartney et al., 2002). Aging has a more dramatic effect on chewdown terms of Cheddar cheese, with sensory creaminess increasing from approximately 0.5 to 7 over 64 wk of aging and firmness changing only from 3.8 to 2.4 over the same period (relative values base on a 9-cm line scale; Hort and Le Grys, 2001). Cheddar cheeses containing 5 or 16% (wt/wt) fat are firmer and lower in chewdown terms than those containing 32% (wt/wt) fat at 2 wk after manufacture (Rogers et al., 2009). Upon aging, the largest changes in sensory texture are seen within the first 3 mo, with increases in the chewdown terms strongly differentiating cheeses based on fat level (Rogers et al., 2009). Therefore, understanding initial structural differences and how structural elements change over time is needed to determine how low-fat cheese texture can be brought closer to that of full-fat cheese.

Cheese structure can be viewed as a continuous protein gel network disrupted with interdispersed fat globules. From a materials science perspective, this is a 2-phase material comprising a continuous protein gel phase (accounting for protein, water, and dissolved solids) with an interdispersed fat phase. This so-called "filled gel" model has been used to describe the role of fat in hard and semi-hard cheeses (Visser, 1991). The storage modulus (G') of a filled gel network is determined by gel network elasticity and phase volume (ϕ_{gel}), filler particle elasticity and phase volume (ϕ_{filler}), and interactions (or lack of) between the filler particle and the gel network (van Vliet, 1988; Dickinson and Chen, 1999; Sala et al., 2009). Stiffer filler particles produce a firmer material as a result of a reinforcing effect provided by the filler (Brownsey et al., 1987). This stiffening effect is seen in cheese and rennet casein gels where the firmness of the fat filler particles is affected by temperature (Visser, 1991; Zhou and Mulvaney, 1998).

Removal of fat is associated with a lower fat to protein ratio, resulting in a more dense protein structure, thus making the cheese firmer (Johnson and Chen, 1995). Density of cheese structure can be viewed from 2 perspectives. First, viewing overall cheese structure, a decrease in ϕ_{filler} will increase ϕ_{gel} when fat is reduced (note that $\phi_{\text{filler}} + \phi_{\text{gel}} = 1$). Alternatively, or coinciding, a reduction in fat without a proportionate increase in water also will increase the concentration of protein in the gel phase. For most protein gels, an increase in protein concentration coincides with a more rigid (increased gel network elasticity) and stronger network because of a greater density of proteins in the network. This relationship was the case in the investigations of

Bryant et al. (1995) and Guinee et al. (2000), where a decrease in fat content coincided with a decrease in the moisture to protein ratio and an increase in cheese hardness. Likewise, Ustunol et al. (1995) showed that decreasing fat content (34 to 13% wt/wt) with a coinciding decrease in moisture to protein ratio caused an increase in the G' of a filled gel network. Therefore, the combination of a decreased percentage of fat phase and increased protein concentration in the gel phase is expected to result in a firmer cheese based on filled gel considerations. However, this assumes that the only differences are in the amount of protein gel and fat phases and concentration of protein in the gel phase, without taking into account other factors determining gel network formation. Factors during processing, such as pH, will alter cheese texture and this reflects changes in the protein gel network (Johnson and Chen, 1995; Mistry, 2001). Besides composition and pH, aging also plays a major role in cheese structure and texture. Cheese aging results from several processes, including protein hydrolysis and reequilibration of ions (Lawrence et al., 1987; Altemueller and Rosenberg, 1996; Fenelon et al., 2000; Guinee et al., 2000; Lucey et al., 2003; Banks, 2004, 2007). Reequilibration of ions and hydrolysis would affect all levels of protein structure (molecular mass to quaternary structures) and thereby alter the array of molecules available to form the gel network and their interactions.

The general approaches that have been used to improve low-fat cheese texture involve decreasing protein concentration (i.e., increasing moisture to protein ratio of the gel phase), causing greater hydrolysis of the proteins, altering protein-protein interactions, or creating a bigger filler phase (Drake and Swanson, 1995; Mistry, 2001; Banks, 2004, 2007; Johnson et al., 2009). Comparing among investigations, it is difficult to sort out the individual effects of pH, proteolysis, and protein concentration because each tends to be convoluted when altering the fat content of cheese. This problem was noted by Zhou and Mulvaney (1998), who took the approach of making a model gel using rennet casein and unsalted butter to look at the effects of casein to water ratio and milk fat.

In a previous investigation, Cheddar cheeses were prepared with similar moisture to protein ratios (~ 1.5) and varying amounts of fat (5 to 32% wt/wt) to examine the effect of a constant gel phase composition (Rogers et al., 2009). Although that investigation established changes in sensory texture and rheological properties, the limited range of fat content (5, 16, and 32% wt/wt), broad sampling times (0.5, 3, 6, and 9 mo), and lack of microstructure determination did not allow for establishing changes in cheese structure during the initial phase of aging. The objective of this investigation

was to determine structural changes among Cheddar cheeses with a range of fat contents over 24 wk of aging. Cheddar cheeses were made with target values of 3, 8, 13, 18, 23, 28, and 33% (wt/wt) fat. Changes in rheological and microstructural properties were measured at 2, 4, 8, 12, and 24 wk. A subsequent report will discuss large deformation, fracture, and sensory properties of the cheeses.

MATERIALS AND METHODS

Cheddar Cheese Production

Materials. A starter culture consisting of frozen pellets of a blend of *Lactococcus lactis* ssp. *lactis/cremoris* (DVS850) was obtained from Chr. Hansen Inc. (Milwaukee, WI). Double strength (~650 international milk clotting units/mL) chymosin rennet (Maxiren) and single strength annatto color were obtained from DSM Food Specialties USA Inc. (Eagleview, PA). Fresh cow's milk was obtained from Utah State University's Caine Dairy Research and Teaching Center (Wellsville, UT). Plastic hoop liners and bags (V7-400) were obtained from Vilutis and Co. Inc. (Frankfort, IL).

Cheese Manufacture. Cold milk was transported to the Gary Haight Richardson Dairy Products Laboratory at Utah State University (Logan) and 700-kg batches were standardized to protein to fat ratios described in Table 1 so as to obtain nominal fat levels of 3, 8, 13, 18, 23, 28, and 33% (wt/wt) in the cheese. The milk was pasteurized (73°C for 15 s) and pumped into a Tetra Scherping horizontal cheese vat (Tetra Pak Cheese & Powder Systems Inc., Winsted, MN) and heated to 31°C. Starter culture was added at 20 g/100 kg of milk and annatto was added at 9 mL/100 kg of milk, and the milk was allowed to ripen for 40 min. The milk was set by chymosin using 8 mL/100 kg of milk, stirred for 2 min, and then allowed to coagulate without stirring until a firm set was reached (30 min).

Cheesemaking parameters were then varied based upon the fat level in the milk with the aim of increasing the moisture content as fat content decreased so as to maintain a constant moisture to protein ratio as well as a constant salt to moisture ratio in the final cheese. The curd was cut at 14 rpm for up to 2 min as shown in Table 1, then gently agitated starting at 2, 3, 4, 5, and 6 rpm for 1 min each to allow curd healing, and then stirring was increased to 14 rpm over a 12-min period. Fifty minutes after renneting, the curd and whey was heated at rates and temperatures shown in Table 1. After stirring for 35 min more, the curd and whey were pumped to a drain table (Kusel Equipment Co., Watertown, WI) and stirring continued until the curd pH reached 6.3, and whey was drained. The curd was washed for 10 min with sufficient water (60 to 100 kg) to adjust the curd temperature as shown in Table 1 and to remove a portion of lactose from the curd to prevent overacidification. After draining the water and whey, the curd was stirred until pH 5.90 was reached, and the curd was weighed and salted at rates shown in Table 1 using 3 applications spaced 5 min apart. Twelve kilograms of salt curd was placed in plastic cheesecloth-lined stainless steel hoops and pressed at 80 kPa overnight (~18 h) at room temperature (~20°C) into nominal 10-kg blocks. The cheeses were vacuumed packaged and then stored at 6°C for aging. Cheese was manufactured on individual days and 2 replications of each cheese were produced. The cheeses were kept at Utah State University for aging at 8°C for 6 mo. Sample blocks were shipped to North Carolina State University at 2, 4, 8, 12, and 24 wk of age for testing. Two complete replications of cheeses were made.

Proximate Analysis

Moisture content was determined in triplicate by weight loss using a microwave oven (CEM Corp., Indian Trail, NC) at 70% power with an endpoint set-

Table 1. Manufacturing parameters modified to obtain similar moisture-to-protein and salt-to-moisture ratios in Cheddar cheese targeted at containing 3, 8, 13, 18, 23, 28, and 33% (wt/wt) fat

Parameter	Target cheese fat content, %						
	3	8	13	18	23	28	33
Milk fat level, g/100 g	0.4	0.9	1.4	2.0	2.3	3.1	3.7
Milk protein to fat ratio	6.6	3.5	2.3	3.3	1.4	1.0	0.83
Cutting revolutions ¹	14	18	21	21	21	21	42
Heating rate, °C/min	0.26	0.30	0.30	0.33	0.28	0.27	0.22
Cooking temperature, °C	35	36	36	36	37	38	39
Set-to-drain time, min	107	107	125	125	130	135	145
Wash water temperature, °C	15	17	16	17	18	20	21
Curd wash temperature, °C	25	26	27	28	28	30	31
Set-to-salt time, min	180	180	180	180	180	185	195
Salt, g/kg of curd	29	27	27	26	25	24	22

¹Number of vat agitator revolutions at 14 rpm during curd cutting mode.

ting of <0.4 mg of weight change over 2 s. Fat content was determined in duplicate using a modified Babcock method (Richardson, 1985). Salt was measured by homogenizing grated cheese with distilled water for 4 min at 260 rpm in a Stomacher 400 (Seward, Worthing, UK). The slurry was filtered through a Whatman #1 filter paper, and the filtrate was analyzed for sodium chloride using a chloride analyzer (model 926, Corning, Medfield, MA). The pH was measured using a glass electrode after stomaching 20 g of grated cheese with 10 g of distilled water for 1 min at 260 rpm. Protein was calculated from N measured by combustion (triplicate samples) and multiplied by 6.38.

Rheological Analysis

Determination of Linear Viscoelastic Region.

Stress sweeps of cheeses were done at 25°C using a Stress Tech controlled stress rheometer (ATS Rheosystems, Bordentown, NJ) fitted with a 20-mm smooth parallel plate geometry, according to the method of Rogers et al. (2009). The cheese samples were sliced 4 mm thick, trimmed to the size of the plate, and glued to both plates to prevent slip using cyanoacrylate glue (Loctite 401, Loctite Corp., Rocky Hill, CT). A thin layer of synthetic lubricant (SuperLube, Synco Chemical, Bohemia, NY) was applied to exposed edges of the sample to minimize sample dehydration during testing. Tests were conducted at 10 Hz with a stress range from 1 to 1,000 Pa on each cheese for each replication. The limit of the linear viscoelastic region was defined as the point where consecutive measurements (taken every 26.3 s) showed decreasing complex modulus (G^*) in sequential measurements.

Controlled Temperature Frequency Sweeps.

Frequency sweeps for all cheeses were conducted within the linear viscoelastic region using the basic rheometer conditions and sample preparation described above. Sweeps were conducted from 0.1 to 10 Hz at 150 Pa. All cheeses were tested at 4 controlled temperatures (10, 15, 20, and 25 ± 0.1°C). Only 2 decades of frequency were used because the testing time required for 3 or more decades was not possible within the timeframe to analyze all treatments at one sampling time.

Microstructural Analysis

Microscopy. Cheese microstructure was imaged using confocal scanning laser microscopy (CSLM). The method for imaging cheese samples with CSLM was based on that of Auty et al. (2001). Cheese samples were kept at 10°C until sliced into sections approximately 5 mm × 5 mm × 1 mm thick using a razor blade. Fluorescent dyes Nile Red and Rhodamine B

Figure 1. Mechanical spectra for cheeses containing 3.0% (a) and 33.0% fat (b). Properties at 10°C: ● = storage modulus (G'), ○ = loss modulus (G''), and ■ = phase angle. Properties at 25°C: ▼ = G' , △ = G'' , and □ = phase angle.

(Invitrogen Molecular Probes, Eugene, OR) were used to image the fat and protein phases of the cheese, respectively. The dyes were pipetted on the cut surface of the cheese: 15 µL of Nile Red solution (2% solids) and 15 µL of Rhodamine B solution (0.2% wt/vol in deionized water). Samples stood for at least 10 min to absorb the dyes and return to room temperature. Cheese samples were then inverted onto a single-welled slide with a #1.5 coverslip attached to the bottom with silicone grease. Samples were imaged using an inverted Leica TCS SP1 CSLM using a PL Fluotar 40.0× 1.0 oil UV objective (Leica Microsystems GmbH, Wetzlar, Germany). A zoom of 2.5× was used, resulting in a field size of 100 µm × 100 µm. Excitation of the sample was performed using a 488-nm laser (fat phase) and

a 561-nm laser (protein phase) sequentially. For each cheese treatment, 2 samples were prepared and 5 images were taken per sample, resulting in 10 images per treatment.

Image Analysis. Confocal scanning laser microscopy images of the fat phase of cheese samples were analyzed using Metamorph Imaging System software (Molecular Devices, Downingtown, PA). The area of each fat globule was measured and the shape factor was calculated, which was defined as follows: shape factor = $4\pi \text{ area/perimeter}^2$. A shape factor of 1 indicated a perfect sphere, whereas lower shape factors showed a deviation from roundness. Fat globules touching the edge of the frame were eliminated from analysis.

RESULTS AND DISCUSSION

Proximate Analysis

Actual fat levels were very close to target levels (Table 2). The most different were the 13% (15.5% actual) and 18% (20.3% actual) cheeses. Because the goal was to provide a range of fat levels rather than precise fat levels, these differences did not alter the investigation. All fat levels presented in figures are actual values. The moisture to protein ratio was very similar among cheeses, ranging from 1.41:1 to 1.69:1, with an average of 1.57:1 (Table 2). This coincides well with our previous investigation that ranged from 1.5:1 to 1.6:1 and an average of 1.53:1 (Rogers et al., 2009). This similarity allowed for application of the filled gel model where the differences in composition are primarily attributed to the amount of gel (water and protein) and fat phase. However, it should be noted that changes within the gel phase were not measured.

Rheological Properties

The mechanical spectra provides a rheological fingerprint of the cheeses by presenting elastic (G') and viscous (loss modulus; G'') elements as a function of time (frequency; Figure 1). The overall spectra for 3% (Figure 1a) and 33% (Figure 1b) fat were similar; show-

ing an increase in G' and G'' as frequency increased and maintaining the relative magnitude of $G' > G''$ (as seen in the relatively constant values for phase angle). This response is a characteristic pattern of a viscoelastic gel, where frequency-dependent moduli are observed, whereas the overall phase angle remains relatively unchanged. Because the major changes in G' between 10 and 25°C coincided with minimal change in phase angle, and phase angle remained $<20^\circ$, differences were attributed to the general stiffness of the network rather than a melting transition (i.e., no fluid to solid transition observed). Similar transitions were observed by Lucey et al. (2003). Therefore, it appears that the overall softening of cheese with increased temperature is attributed to a combined melting (at least to a degree) of the dispersed fat and weakening of the gel network but no overall melting at 10 to 25°C. This observation is consistent with temperature effects observed with a model casein gel made from rennet casein filled with milk fat from butter (Zhou and Mulvaney, 1998).

The mechanical spectra of cheeses varied with temperature, age, and fat content. At 10°C, the G' values were generally higher at 2 than 24 wk (Figure 2). By 24 wk, the G' values showed a general trend of increasing with fat content; however, some cheeses had different fat content but similar G' values (e.g., 8.5, 15.5, and 20.3% wt/wt fat cheeses; Figure 2b). If we assume that the rheological properties of milk fat are similar among treatments and would therefore scale by fat level, then the lack of a clear scaling of cheese G' values with fat content at 10°C likely reflects differences in the gel phase. However, when cheeses were measured at 25°C, a reasonable temperature for oral processing, the differences among fat levels were minimal. The cheeses showed minor differences at 2 wk (Figure 2c) and no major differences at 24 wk (Figure 2d). This behavior was explored further by looking at how G' changed with temperature at the 4 different aging times (Figure 3). A major decrease in G' occurred as temperature increased from 10 to 15°C, and all values among treatments started to converge at 20°C and were very similar at 25°C. This temperature behavior can be attributed

Table 2. Compositional analysis of cheese

Fat content		Moisture content (% wt/wt)	Moisture to protein ratio	Protein content (% wt/wt)	pH
Target (% wt/wt)	Actual (% wt/wt)				
3.0	3.0	53.0	1.65:1	32.2	5.4
8.0	8.5	49.6	1.41:1	35.2	5.3
13.0	15.5	46.8	1.58:1	29.6	5.6
18.0	20.3	45.4	1.69:1	26.9	5.5
23.0	23.0	43.4	1.56:1	27.8	5.4
28.0	28.8	40.0	1.52:1	26.3	5.5
33.0	33.0	37.5	1.56:1	24.1	5.5

Figure 2. Storage modulus (G') of Cheddar cheeses of various fat contents over a range of frequencies, showing lowest (10°C) and highest (25°C) temperature for both 2 wk (a, 10°C ; c, 25°C) and 24 wk (b, 10°C ; d, 25°C) of aging.

to the solid to fluid phase change of the fat in the cheese (Glignem et al., 2009; Vithanage et al., 2009) and alterations in the protein network. Visser (1991) also described this behavior, where differences in the range of 14 to 26°C were attributed to change in rigidity of the fat particles because of crystallization. However, it is seen that the lowest fat cheeses (3 to 15% wt/wt) have a fairly linear decrease in G' as temperature increases, suggesting a contribution mainly from the gel network. This coincides to G' transitions observed in comparing low- and full-fat cream cheese (Brighenti et al., 2008). Whereas there is variation with age, a break point between 20.3 and 23.0% (wt/wt) fat was observed regarding temperature-induced changes in the elasticity of the cheese network.

Changes in G' were grouped by fat and protein contents to determine which property best represented the

cheeses (Figure 4). At 10°C , a higher fat, lower protein content resulted in a higher storage modulus (Figure 4a, b), supporting the idea that the fat is dominating the rheological properties at this temperature. These differences were markedly reduced when the temperature was increased 25°C (Figure 4c, d). The network rigidity was highly dependent on temperature and shows the importance of matching temperatures when comparing mechanical and sensory evaluation of texture. Moreover, it shows that temperature and moisture to protein ratio, rather than protein content per se, determines the overall firmness of Cheddar cheese.

Microstructure

Cheeses from replications 1 and 2 exhibited very similar microstructures, so all images presented are

Figure 3. Storage modulus (G') of Cheddar cheeses of varying fat contents and temperatures. Values were determined at 1 Hz at 2 wk (a), 4 wk (b), 8 wk (c), 12 wk (d), and 24 wk (e) of aging.

Figure 4. Storage modulus (G') of Cheddar cheeses with respect to fat (a) and protein (b) contents at 10°C and fat (c) and protein (d) contents at 25°C and measured at 1 Hz.

from replication 1 and are representative of each treatment. Numerous features were observed in the Cheddar cheeses that correlate with previously reported cheese microstructure. Curd junctions could be visualized in some microstructural images (Figure 5). When the curd is cut, fat globules tend to separate from the surface of the curd during washing, leaving a protein-rich surface. Curds are subsequently pressed together and, when fused, form curd junctions with a high density of protein matrix and very little fat (Kalab et al., 1982). A micrograph of a curd junction is shown in row A of Figure 5, which is similar to curd junctions imaged in Emmental cheese using CSLM (Lopez et al., 2007). Previous studies have identified 4 forms of fat present in Emmental cheese: 1) intact milk fat globules; 2) aggregates or clusters of milk fat globules; 3) coalesced milk fat globules, resulting in a large globule, and 4)

nonglobular fat or free fat (Lopez et al., 2007). Similar forms of fat were seen in images of Cheddar cheese, which are identified in Figure 5. Intact milk fat globules are the small, spherical globules dispersed throughout cheeses, especially visible in the lower fat cheeses. Aggregates of milk fat globules appear as clumps of circular globules (row B of Figure 5), whereas coalesced globules are spherical but larger than typical milk fat globules (row C of Figure 5). Nonglobular fat appears in the highest fat content cheeses, identifiable as large, nonspherical fat areas in the cheese (row D of Figure 5).

The CSLM images of cheese microstructure showed increasing fat globule size with increasing cheese fat content (Figure 6) and also showed changes in the shape of fat globules. Low-fat cheeses (3 and 8% wt/wt fat) had more spherical fat globules dispersed through-

out the protein matrix; in contrast, fat globules in the highest fat cheeses (28 and 33% wt/wt fat) appear clumped and coalesced into nonspherical shapes. This observation is consistent with previous observations of Cheddar cheeses with varying fat levels (Guinee et al.,

Figure 5. Confocal scanning laser microscopy images for Cheddar cheese showing examples of cheese features. All images show the fat (left) and protein (right) phases as bright against a dark background. Curd junctions (row A), aggregated fat globules (row B), coalesced fat globules (row C), and nonglobular fat (row D) are identified with arrows. Field size is 100 $\mu\text{m} \times 100 \mu\text{m}$ for each image.

2000). Increasing size of the fat globules was attributed to shearing effects during the milk handling and cheesemaking processes, causing a rupture in the milk fat globule membrane and coalescence (Guinee et al., 2000). Image analysis allowed for measuring the changes seen in milk fat globules. Changes in mean shape factor and fat globule area were consistent with fat globules getting larger and less spherical as fat content increased from 3.0 to 33.0% (wt/wt) fat (Figure 7). The shape factor distribution provides more information on fat globule morphology change. Spherical particles represented 42 to 47% of the particles in cheeses with 3 or 8.5% fat whereas they accounted for only 26 to 30% of the fat in cheeses at 23 to 33% fat. In contrast, the least spherical particles (shape factor <0.9) accounted for 34 to 47% in the lower fat cheeses (3 or 8.5% wt/wt fat) and 62 to 70% in higher fat cheeses (23 to 33% wt/wt fat). Because a spherical geometry has the lowest surface areas to volume, the transition to less spherical fat globules in higher fat cheeses adds to the increase in fat-protein interfacial area. However, total surface area at a fixed volume would increase as spherical diameter decreased, so it is difficult to say whether higher fat content has greater fat surface area.

Aging of the cheese did not result in major changes in the cheese microstructure; images were similar for all treatments across aging time (2 to 24 wk; Figure 8). Minimal changes in microstructure over time could be expected because the fat phase would be mainly solid and less prone to clumping or coalescence at the storage temperature of 8°C (Guinee et al., 2000). Therefore, the overall decrease in G' with aging is most likely attributable to changes in the protein network and not to changes in the dispersed fat phase.

Major Structural Changes Associated with Fat Reduction

Previous investigations (e.g., Bryant et al., 1995; Guinea et al., 2000) have reduced the fat content of Cheddar cheese and not held the moisture to protein ratio constant. This resulted in increased protein concentration in the gel phase as the amount of fat (filler phase) was decreased and an increase in G' (Ustunol et al., 1995). Therefore, cheese firmness was related to decreasing the amount of fat and increasing protein concentration in the gel phase. In our investigation, changes in the filler phase volume were apparent in the microstructure, along with rheological properties determined at 10 and 15°C. The combination of fat melting and the casein gel weakening made the rigidities of the cheeses roughly equivalent at 25°C. Our effects of temperature on G' of Cheddar cheese are very similar to those Zhou and Mulvaney (1998) observed

Figure 6. Confocal scanning laser microscopy images for all fat contents for Cheddar cheese at 12 wk of age. Column A represents the fat phase and column B represents the protein phase of the cheeses. Field size is $100\ \mu\text{m} \times 100\ \mu\text{m}$ for each image.

with casein gels. They showed a neutral fat effect at 20 to 25°C and a weakening at higher temperatures. Therefore, the contribution of fat to G' will progress from positive to neutral to negative as fat moves from the solid to the liquid state (Zhou and Mulvaney, 1998; Gliguem et al., 2009). However, maintaining moisture to protein ratio in the gel phase and decreasing fat content still produces cheese with undesirable textural properties (Rogers et al., 2009).

CONCLUSIONS

The basic model for sensory texture proposed by Huttchings and Lillford (1988) indicates that a starting “structure” is broken down during mastication and mixed with saliva for lubrication and bolus formation. These processes determine sensory texture. When the moisture to protein ratio was held constant, no differences were found in the structures of Cheddar cheeses containing 3 to 33% fat at 25°C, as indicated by similar rheological properties (magnitude of G' and frequency dependence). However, the magnitude of G' decreased over time, indicating a change in the fine structure of the protein network (i.e., changes at the nano scale). In contrast, clear differences were observed in microstructure at the micrometer level regarding fat, with differ-

ences in amount, size, and shape of fat globules but no age-associated changes. It would appear that the textural properties of Cheddar cheese depend on how the initial distribution of fat globules, and changes in

Figure 7. Image analysis results from confocal scanning laser microscopy images showing mean fat globule shape factor and area for cheeses aged 12 wk. Error bars represent 1 standard deviation above and below the mean.

Figure 8. Confocal scanning laser microscopy images for Cheddar cheeses at 3.0 and 33.0% fat content at 2, 4, 8, 12, 24 wk of aging. Column A represents the fat phase and column B represents the protein phase. Field size is 100 μm $\times$ 100 μm for each image.

the protein gel network over time, alter the breakdown pathway and particle lubrication during oral processing.

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