ABSTRACT

PATTON, JAMES QUINTON. Development and Application of Whey Protein Particles for the Improvement of Low Fat Cheddar Cheese Texture. (Under the Direction of E. Allen Foegeding.)

Increasing concern over the deleterious health effects associated with saturated fatty acids has precipitated an increased demand for low fat products. However, the reduction of fat in Cheddar cheese results in a concentration of the continuous casein phase, leading to several textural defects including increased firmness, rubberiness, and the breakdown process during chewing as compared to the full fat counterpart. Thus, the purpose of this research was to develop a whey protein based particle capable of functioning as a mimetic to provide a full fat like texture in Cheddar cheese.

Our method for particle production incorporated a 'top down' approach in which heat-set whey protein gels were first made with desired rheological and sensory attributes before particulation using a mechanical shearing device. This process allowed for the design of particles with a wide range of applications and functionality. At present, the primary mechanisms responsible for the breakdown pathway of Cheddar cheese are not well understood. Thus, our strategy was to first implement gel types with a diverse set of rheological and sensory attributes, and after evaluating their efficacy, further refine particle types for a more product specific design. Particles with stranded, particulate, or composite protein network structures were selected for incorporation into cheeses, with particle presence confirmed using confocal laser scanning microscopy.

Particles were incorporated into cheese in three separate experiments. The first was designed to validate our methodological approach. After 34 weeks ageing it was found that a

protein particle with embedded fat globules (composite-1) decreased fracture stress values of the cheese, but had little impact on sensory texture. Notably, we were unable to definitively quantify the volume fraction of particles retained in any of the three experiments, and thus it is plausible that the high degree of variation between replications of the same treatment is related to inconsistent particle delivery. Drawing from these findings, cheeses in experiment 2 were made with, among others, a modified composite type particle that provided a means to estimate the volume of particles incorporated. The native fat composite treatment was moderately effective in reducing fracture stress and improving sensory texture. The homogenized gel treatment in replication 1 and particulate gel treatment in replication 2 were both found to be highly efficacious in improving cheese texture, but these results were not consistent across replications. However, the amount of particles incorporated varied between replications. Experiment 3 tested the effects different filler volume fractions and suggested that a critical level may be necessary to emulate the breakdown pattern of fat in Cheddar cheese. While these results are not conclusive, our data suggests a strong potential for this methodology and the use of whey protein particles as a fat mimetic.

Development and Application of Whey Protein Particles for the Improvement of Low Fat Cheddar Cheese Texture

by
James Quinton Patton

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Food Science

Raleigh, North Carolina

2013

APPROVED BY:

_____	_____
Christopher Daubert, Ph.D.	MarryAnne Drake, Ph.D.

_____
E. Allen Foegeding, Ph.D.
Chair of Advisory Committee

DEDICATION

To my grandmother, Katharine "Kitty" Jenkins Sutherland, whose loving influence instilled me with a lifelong passion for learning.

BIOGRAPHY

Raised by two adept and caring parents, Quin Patton enjoyed much of his early youth venturing through the woods or spending time at his grandfather's farm in Alabama. In many ways a respect for the veracity of nature has never left him, and nurtured by his involvement with scouting, this has burgeoned into the hobby/wanderlust of high altitude climbing. At some point in his early adolescence it was observed that Quin had a penchant for running (rather) fast, and thus he found himself spending many of his cherished high school and college days running in circles. At age 18 Quin was sent out from home to continue his education in Davidson, NC. It was here that he pursued the tired and noble studies of ancient Greek and English literature. However, moved by the admonition of Garrison Keillor (*A Prairie Home Companion)* and the stark reality of fulfilling his English major destiny as a poetically inspired Starbucks barista, Quin decided to pursue other opportunities within the food industry. Upon meeting Dr. Allen Foegeding and his lab, Quin immediately became enamored with food science, and apart from the occasional mawkish adjective in research abstracts, has never looked back. In graduate school Quin, amongst many other things, gained a scientific perspective and mode of thinking, spent a summer as an intern at Frito-Lay, and challenged many tenured professors to a game of squash. He also became well acquainted with the researcher's conundrum: every answer asks (at minimum) two new questions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. REVIEW OF LITERATURE.

Department of Food, Bioprocessing, and Nutritional Sciences

North Carolina State University

1. INTRODUCTION

Cheese is one of the oldest known methods of food preservation, and though it is unclear who first made the discovery, records of consumption date back as early as 6000 BC (Fox, 1987). Popular lore attributes the 'invention' to a wondering sheppard, who, when attempting to store milk in a pouch made of lamb's stomach, found a rich curdled substance. This process was later refined and progressed along with human civilization both for its simplicity and substantive nutritional qualities. As an understanding of the chemical and biological mechanisms grew, so too did production and regional varietals (DRI, 2006). Cheese spread through the Roman Empire and into Medieval Europe; it visited the new world on the *May Flower* and has been produced industrially in the United States beginning in 1861 (Fox, 1987). Culturally entwined from Chaucer to Auden, cheese comprises a unique and protein rich piece of our human history.

2. MODERN CHEESE PRODUCTION

It is no surprise that cheese is still a major player in the modern food market. From its humble beginnings as a sheppard's snack, it has expanded to become a truly global product with over 500 varieties available worldwide (Fox, 1987). The United States, where more than 1/3 of all milk produced is used for cheese (DRI, 2006), is currently the world's largest manufacturer with 916 million pounds of cheese produced in May of 2012 alone (USDA, 2012). Advances in milking technology have bolstered this effort, and cheese milk can now be produced year round (USDEC, 2004). The U.S. market for cheese increased 6.1% in 2011 totaling $18.9 billion in sales (Mintel, 2012). Of that, Wisconsin produced 24% of all US

cheese in 2011 (Geisler, 2012). Due to the vast market for cheese worldwide, the U.S. is also a net exporter with a 29% increase from 2010 (USDA, 2012a). While many specialty cheese operations (defined as producing less than 40 million lbs a year) are growing, the majority of cheese production comes from highly mechanized industrial processes (PRWEB 2012). In just 450 U.S. plants, 30% of the world's cheese is produced (2004 USDEC). Mozzarella is the most popular varietal at 301 million lbs, closely followed by Cheddar at 278 million lbs (USDA, 2012).

2.1 Cheddar Cheese Process

The standard production method of Cheddar cheese has been well studied and is historically derived from the need to reduce the growth of gas-producing coliforms (Fox, 1987). Many strategies were directed at this problem, and often resulted in the development of new cheese varietals. This is evident in Gouda-type cheeses in which warm water is added to the curds to increase the rate of acid production and inhibit bacterial proliferation. Thus, Cheddaring is thought to have been developed near the middle of the 19th century in the small eponymous village in Somerset, England, and consists of successive steps of packing, turning, and piling the curd (Lawrence et al. 2004). This two hour process allows time for continued acid development dropping the pH to around 5.4, as well as inducing mechanical flow which squeezes out air pockets. However, while it was once believed this step was essential for the resultant texture of a Cheddar type cheese, it is now known that the texture develops due to acid development and the loss of calcium and phosphate from the protein matrix (Lawrence et al. 2004). As the piling step can be foregone given that sufficient time is still allowed for acid production, most modern mechanized cheese making systems use a

stirred curd method to provide a more continuous production process. Make procedure may vary by manufacturer, but the critical steps in a traditional Cheddar production schematic are included in Figure (1-1).

The lengthy ripening time required for Cheddar cheese potentiates several flavor and texture variations among batches, and thus much attention has been directed towards the development of commercially consistent cheese milk. An ideal composition is selected for low amounts of antibiotics, somatic cell content, and microbial pathogens. Many natural variations in stage of lactation, breed, health, season, and genetics can affect the quality of milk resulting in a standardizing step to increase processing and yield constancies (Frakye, 2004). For the production of Cheddar, a casein to fat ratio of 0.7 and total protein content of 3.5% - 4% in milk is most desirable (Lawrence et al. 2004). Additionally, pasteurization has a notable effect on indigenous microflora and enzymes and will result in a milder flavor and longer ripening time than Cheddars made from raw milk **(**McSweeney and Fox, 1993). Treatments to better control the microbial content of the milk include applications of H_2O_2, bactofugation (the removal of microbes via centrifuge), nitrate and lysozyme addition, as well as microfiltration (Frakye, 2004).

Once the milk has been sterilized and added to the vats, it is then inoculated with starter culture. In Cheddar cheese, the primary function of the starter bacteria is the acidification of milk, and thus the selection of cultures is highly predicated upon its rate of acid production. Other important considerations include the bacteria's phage resistance and contribution to flavor development (Lawrence et al. 2004). The most common starter strain used is *Lactococcus lactis* ssp. *cremoris* but *Lactococcus lactis* ssp. *lactis* is also used with a

high prevalence (Lane and Fox, 1996). After inoculating the milk, the starter culture can proliferate to concentrations as high as 10^9 cfu/g but are reduced to only 1% of their maximum within a month (McSweeney et al. 1994). The death of these bacteria then play an important role in proteolysis. As the cells lyse they release proteolytic enzymes which contribute to the subsequent flavor and texture of the cheese.

After the milk is inoculated with the starter, a coagulant is added to destabilize the casein micelles (Johnson and Law, 1999). These micelles are composed of phosphoproteins α_{s1}-, α_{s1}-, β-, and κ-casein, and associations among proteins are regulated by electrostatic and hydrophobic interactions (Lucey et al. 2003). The κ-casein is predominately present on the exterior of the micelle and functions to sterically stabilize the structure. Thus, the coagulant acts by hydrolyzing the glycomacropeptide of the κ-casein, effectively cleaving the hydrophilic coating of the micelle. The remaining para-casein is left with a highly hydrophobic surface and begins associating into a gel network (Srinivasan et al., 2008). This resulting coagulum entraps both water and fat and is a large contributor to the resulting texture of the cheese (Lawrence et al. 2004). While a host of different coagulants would be suitable for casein destabilization, Cheddar cheese has traditionally been made using calves rennet which is high in chymosin content. Recombinant and microbialy produced rennets are also used. The amount of rennet applied and the type affect the degree of proteolysis in the cheese, and so the concentration is generally targeted to the lowest possible volume to achieve a firm coagulum in the roughly 30 minutes spanning the time from set to cut (Lawrence et al. 2004). Once the coagulum has formed and has reached a sufficient strength as judged by the cheese maker, it is cut to promote syneresis. The size of the cut particles and

the subsequent stirring speed impact the eventual fat and protein content of the cheese. Thus, larger particles result in more moisture retained and a greater yield in the cheese (Whitehead and Harkness, 1954). However, curd sizing involves a delicate balance of considerations. If cut too large the curd will have a greater propensity to shatter and will ultimately result in many smaller particles and a significantly decreased yield for the cheese (Johnson et al. 1991).

After cutting, the curd is heated to approximately 38°C to induce further syneresis and control the rate of acid production (Lawrence et al. 2004). During this stage the curd becomes firm, dropping from approximately 87% to less than 39% percent moisture. Acid production in the vat is generally monitored as it is an important determinant of final pH and the rate of whey expulsion (Lawrence and Guiles, 1982). The whey is then drained and the curd is Cheddared while sufficient time elapses in order to induce the necessary acid development. Once a desired pH is reached, the curd is milled to provide greater surface area for additional whey drainage and to create a more uniform structure for salting.

The dry salting process likely originated from early attempts to preserve the curd and store it for a longer amount of time. Salt in moisture controls rate of bacterial growth and enzymatic activity and so a range between 4.5% and 5.5% is generally desired. Due to variations of moisture content and acidity, salt intake may vary, and thus titratable acidity at salting is assessed to ensure a uniform effect (Lawrence et al. 2004). After the salting, the curd is gently agitated for at least 15 minutes to maximize salt absorption (Breene e*t al.* 1965). The curds are then pressed together into a set geometry and stored while the cheese ripens.

2.2 Low Fat Cheddar

Americans eat an average of 33 pounds of cheese a year (Moss, 2010), 10 pounds of which are Cheddar (Geisler, 2012). This is also the number 2 source of calcium for Americans (Fulgoni, 2010). However, concern is growing over the impact that high amounts of saturated fat could have on obesity and public health. The American Heart Association recommends limiting saturated fats to just 7% of daily caloric intake (AHA, 2012), and over 70% percent of consumers report that they wish to reduce the amount of fat they ingest (Childs and Drake, 2009). It therefore follows that a low fat cheese product would be a welcomed addition to help lower fat intake. And this is not a new idea. Research into low fat cheese has been a timely endeavor, with over 50 years of research (Lawrenceet al. 1987). However, a negative consumer perception of low fat cheese persists. Over 44% of consumers describe low fat cheese as bland and less than 20% thought reduced-fat, low-fat, or fat free cheeses tasted as good as the full fat version (Mintel, 2011).

The removal of fat radically changes the structure of cheese as well as the development of a Cheddar flavor (Banks et al. 1989; Delahunty et al. 1996). At low fat concentrations, fat globule clumping and coalescence decrease, there is an increased volume fraction of the casein matrix, and the mean melt time significantly increases (Guinee et al. 2000). The dilution of salt in moisture phase plays large role in sensory changes (Mistry, 2001). With a lower fat content, there is also a lower yield from the milk (Banks et al., 1989). The changes is protein, fat, and moisture ratios in low fat cheese generally lead to lower flavor intensity, as well as bitter, astringent, and unclean flavors (Lee at al. 1992). With the reduction of fat, cheeses exhibit increased measures of harness and springiness, and

decreased values in sensory parameters such as adhesiveness and cohesiveness (Bryant et al. 1995).

3. METHODS FOR ASSESSING CHEESE TEXTURE

As mentioned above, reduced texture quality is one of the hedonic limitations found in low fat Cheddar cheese. Cheese contains an inherently complex structure, and thus a wide variety of tests have been developed to different aspects of its texture. The most basic category of these include 'on site' methods such as probing the curd in vat to determine if sufficient set time has elapsed. However, while these tests provide valuable information to the cheese maker, they are largely intended for quality evaluations of the final product and are less comprehensive in scope. Instrumental and sensory tests have therefore been developed for a more targeted understanding of product structure. Instrumental tests can be either fundamental or empirical. Empirical tests are typically simple to conduct and are well suited to a manufacturing environment where speed is a premium (Mounsey and O'Riordan, 1999). However, they are also limited in that they do not provide information on material or rheological properties of the system. A prevalent example is the texture profile analysis (Bourne, 1968), which in specific instances has been found to correlate well to sensory properties (Breene, 1975). In comparison, fundamental tests are used to characterize specific material properties and composition.

3.1 Small Strain Tests

Small strain tests are generally designed to be fundamental in nature and utilize a sinusoidal pattern of oscillations to induce a stress or strain within the linear viscoelastic

region (LVR) at a known magnitude. Stress and strain are linearly proportional within the LVR, and so forces induced in this region do not permanently alter structure and are independent of rate (Steffe, 1996). The point at which this relationship deviates is that of critical stress or strain (depending on the force applied) and denotes the transition into the non-linear region (Subramanian and Gunasekaran, 1997). As cheese is a viscoelasitic material, frequency sweeps are often used to probe structural characteristics of the sample. The storage modulus (G') describes the in-phase component relating to the elastic elements in the sample, while the loss modulus (G") describe the out of phase components and relates to viscous elements in. Other parameters such as the phase angle (δ), loss modulus divided by storage modulus, and complex modulus (G*), a ratio of stress to strain amplitude, are used to provide additional information used to 'fingerprint' the specific structure of the material (Steffe, 1996).

3.2 Large Strain and Fracture Tests

Large strain and fracture pertain to tests assessing properties outside the LVR. These tests give less information on the structural consistency of the material, but can generally relate better to sensory characteristics as they characterize permanent deformations experienced through several concurrent forces (Luyten et al. 1992). Compressive tests such as the uniaxial compression are used to measure fracture stress and strain (Wium and Qvist, 1997). These properties can also be evaluated using a torsion method (Diehl et al., 1979) which does not induce volume changes in the sample shape, and thus induces pure strain without need of consideration for frictional forces, as is a concern in compression (Hamann 1983).

3.3 Sensory Tests

While mechanical methods of evaluation provide valuable information on the structure of food systems and their fundamental material properties, these still do not allow for a comprehensive analysis of the food. In order to best understand the way in which the human mouth perceives texture, a methodological approach which more closely matches this mode of analysis is often used. One of the earliest of these methods includes the dairy grading system. However, these types of tests remain largely traditional, and are best applied as a measure of quality control (Delahunty and Drake, 2004). Analytical tests, such as descriptive analysis, are therefore used to measure quantitative values. This method uses a panel of judges (often equated as a sensor) which are extensively trained on specific sensory attributes to evaluate the food system (Lawless and Heymann, 1999). Another type of test, the affective sensory test, uses untrained consumers to evaluate the product for acceptability. However, as noted by Foegeding and Drake (2007), this requires many more participants (upwards of 60 vs 6-12 for the descriptive analysis) and must also incorporate the consideration that consumers are unable to uncouple flavor and texture evaluations. Differences in one of these parameters are therefore likely to affect the other, and thus this test is less useful for determining specific attributes. When used for their appropriate function, sensory tests are an important and invaluable tool to accurately describe the resultant textures of a specific food product.

In order to gain a more comprehensive understanding of food structure, several studies have assessed the relationship between instrumental and sensory texture. Hort and Grys (1999) noted that compressive tests were well suited to model sensory terms of firmness

and hardness, as these are evaluated using similar compressive forces, but did not predict creaminess. Rheological values relating to sensory firmness have been found to possess the strongest relationships in Cheddar cheese. Gwartney et al. (2002) found correlations between fracture stress and sensory firmness and springiness . A relation of G' to firmness and cohesiveness has also been observed, but TPA measures were found to be better predictors of sensory texture overall (Drake et al. 1999). This was also noted by Wium and Qvist (1998) who cited that fracture stress had the greatest correlation to sensory firmness. Hort et al. (1996) also found significant correlations between fracture stress and strain and firmness but very little correlation between chemical (composition) and sensory values. Cheese is a viscoelstic material, and thus the authors suggested there may exist an optimum rate for relating instrumental properties to sensory results. Xiong et al. (2003) found deformation percentage and rate affected correlations between sensory and instrumental hardness. In another study, Green et al. (1981) found an increase in sensory and instrumental firmness with greater milk protein concentration factor, as well as a loss of sensory adhesiveness. The fracture modulus has been found to correlate well to sensory properties (Brown et al. 2003; Rogers et al. 2009). Cohesiveness and smoothness of mass, are two of the more difficult sensory properties to relate to texture as these properties involve interactions with saliva during oral processing. Interestingly, these terms largely account for differentiation among cheeses (Brown et al. 2003).

3.4 Confocal Laser Scanning Microscopy

Elucidating the arrangements of molecules at nano and micro scales is another way to view food structure and gain insight into texture mechanisms. Various methods have been

used to image the microstructure of Cheddar cheese. Confocal scanning laser microscopy (CSLM) is unique, and relatively novel (c.a. 1980), in that it allows for visualization of samples with a thickness greater than that of the focal plane. Out of focus light is blocked using a pinhole-sized aperture, and thus a clear resolution can be obtained at various focal positions within the sample (Durrenberger et al. 2001). An additional advantage of this method is that the sectional images can then be overlaid to provide a three dimensional rendering of the sample microstructure. Florescent tags are used to selectively dye compositional components. This approach is therefore well suited for the visualization of cheese as both the protein and fat phases can be targeted (Auty et al. 2001). A potential limitation of this technique is that resolution is limited by diffraction to a minimum of 0.2 μm. This limit may be lowered in some instances, but not without substantially lowering the signal to noise ratio (Auty et al. 1999). While this approach is not desirable for resolving individual proteins, it can provide valuable information on fat globule shape and structure (Everett and Olson, 2003).

In order to begin understanding the structural components responsible for imparting a characteristic full fat cheese texture, it is important to model the system so that all major compositional elements are considered. As such, cheese is best understood as a composite or "filled gel" with a continuous protein network, and discontinuous filler phase. The storage modulus of the system is therefore dependent upon the phase volume and respective G' of each phase (van Vilet, 1988). An additional consideration however is the role of interactions between the two phases. Incorporating a proteinaceous filler particle, as is the case in our study, into a casein matrix invites potential interactions differing from those intrinsically

imparted by milk fat. An 'active' particle will exhibit interactions between the filler and continuous phases, while an 'inactive' particle will not. This condition can affect the storage modulus of the cheese as a reflection of its total volume fraction. Theoretically, an inactive filler will decrease the storage modulus of the system, as it serves to disrupt the continuous nature of the network. The effects of an active filler depend on its storage modulus relative to the network. If the G' prime of the active filler is greater than, equal to, or less than the continuous phase it will serve to increase, not effect, or reduce the G' of the gel network (Dickinson and Chen, 1999). Work conducted on the effects of lipid structure, i.e. native vs. homogenized globules in composite gels observed that a whey protein isolate stabilized emulsion functions as an active filler. The fat globules become emulsified with whey protein adhering to their surface and interact with the casein network to actively increase the storage modulus of the system (McClements et al. 1993; Chen and Dickinson, 1998). Yang et al. (2011) evaluated a number of filled-gel models to predict G' based on fat content at specific temperatures. While notably they did not account for globule size and shape, many were able to adequately predict G' based on the volume of gel and fat phases and their rheological properties.

4. STRATEGIES FOR THE PRODUCTION OF LOW FAT CHEESE

To meet the inherent challenges of creating a low fat product, cheese producers have employed several strategies which generally involve one of three methodological approaches. These include the introduction of adjunct cultures, the inclusion of fat mimetics, and modifications to the cheese make procedure (Drake et al. 1995).

4.1 Adjunct Cultures

Cheese flavor and proteolytic activity are largely resultant from intercellular enzymes introduced via lactic acid bacteria (Soda et al., 2000). The proliferation of starter culture and the subsequent development of nonstarter lactic acid bacteria (nsLAB) is therefore an important consideration in the production of Cheddar cheese. Introduction of nsLAB may originate from the cheese milk itself or from ambient bacteria present in the manufacturing facility. The nsLAB start at relatively low concentrations of 10^2 - 10^3 cfu/g in cheese but can proliferate to as high as 10^8 cfu/g after 12 weeks ageing (Fox et al., 1998). However, due to the compositional differences in a low fat cheese, the development of nsLAB is often greatly reduced (Haque et al., 1997). Adjuncts of select bacteria strains are therefore incorporated into the milk to improve the development of cheese sensory texture and flavor (Soda, 2000). There are now several commercially available cultures selected for improvement of low fat cheese. These can be generally characterized as contributing low acid production, sustained proliferation, and low proteolytic activity (Banks, 2004).

Much research has been conducted on the use of adjunct cultures and enzymes, and it has been amply demonstrated that non-starter lactobacilli have the potential to increase flavor intensity in Cheddar cheese (Puchades et al. 1989). Using elevated salt levels and a direct to vat adjunct culture in conjunction, Banks et al. (1993) produced a reduced fat Cheddar (c.a. 16% fat) with similar flavor attributes as a commercial full fat control. However, they noted the treatment was unable to improve upon textural defects inherent to low fat cheese. Several others have found flavor and ripening improvements from this strategy. Fenelon et al. (2002) assessed Cheddar cheese (c.a. 18% fat) made with adjunct cultures of *Lactobacillus*

helviticus and found an increase in free amino acid concentration and an improvement in low fat cheddar flavor at both 90 and 180 days. Drake et al. (1997) also conducted a study on adjunct strains of *Lactobacillus helviticus* in 33% reduced fat Cheddar, and found the treatments had lower bitterness scores and significantly higher rates of proteolysis through 6 months ageing. In this study the low fat cheese with adjunct culture was rated similarly in flavor to the full fat control by trained dairy judges and received a higher consumer acceptance score than the full fat control. A study by Weimer et al. (1997) found flavor improvements in 60% reduced fat Cheddar from the use of adjunct cultures of *Brevibacterium linens* and identified methanethiol producing capacity, as opposed to aminopeptidase, as the primary driver for increasing consumer preference scores. Viable adjuncts have potential for flavor improvement in low fat Cheddar, but are limited in that the cells must first die off before their enzymatic potential is realized.

Many studies have therefore assessed attenuated and highly autolytic cultures which can increase enzymatic activity and provide an increased rate of flavor development and proteolysis without affecting acid production (El Soda, 2000). Cultures are generally attenuated using physical methods such as freeze drying, freeze shocking, or spray drying (Fox et al., 1996). However, these methods often enact differing levels of enzymatic viability and release. El Soda et al. (1999) reviewed various methods and found freeze shock resulted in the highest levels of autolysis. Using a strand of highly autolytic *Lactobacillus helviticus* as both a starter and adjunct they produced a strong Cheddar flavor and demonstrated a potential for this treatment as a ripening accelerator. Johnson et al. (1995) also found increased flavor intensity with attenuated cultures of *Lactobacillus helviticus*.

Guinee et al. (1991) observed increased rates of proteolysis using elevated ripening temperatures and enzymes, but detected an increase in soapy flavor and an overly soft, brittle, and adhesive texture for full fat cheese. Increased proteolytic rates were also observed by Hannon et al. (2003). Work by Benech et al. (2003) found that incorporation of a nisin producing culture enhanced proteolysis but did not affect cheese fracturability or cohesiveness. It has been noted that ripening accelerators can alter cheese texture by lowering hardness and increasing fracturability (Sallami et al., 2004). Fenelon et al. (1999) found that an elevated ripening temperature (12°C) in conjunction with a Lacticin producing starter increased proteolysis but contributed no effects to flavor or texture. The use of mesophilic *Lactobacillus* adjuncts induced negligible proteolytic changes but did alter aroma and flavor from the control (Lynch et al. 1999). Encapsulated enzymes have been used to effectively increase proteolysis and accelerate cheese ripening, but cheeses using this treatment received lower overall sensory texture scores (Kailasapthy and Lam, 2005). Kilcawley et al. (2012) assessed three commercially available enzyme preparations and found that the treatments increased the rate of flavor development. They also observed significantly lower fracture stress values for treatment cheeses, as well as reductions in hand springiness, hand firmness, and first bite firmness. The authors reported these changes as defects which rendered the cheese well below the acceptable range for a full fat cheddar and attributed these effects to higher moisture, greater primary proteolysis, and a lower pH. However, while perhaps not desirable in a full fat cheese, the change in texture does illuminate a potential application in low fat Cheddar where decreased fracture stress and firmness can be desirable.

4.2 Fat Mimetics

Another prevalent strategy for the production of low fat cheese is the incorporation of filler particles, conventionally categorized into fat substitutes and fat mimetics. The former are generally non-polar compounds possessing the same physical properties as fat with a reduced caloric potential. The latter, fat mimetics, are polar, water soluble compounds intended to replicate the sensory and functional characteristics of fat (Jones, 1995). While several commercial versions of both these filler particle types are currently available, this review will address those mimetics of both protein and polysaccharide component structures. Many of the mimetics particles are manufactured through a variant of the process outlined by Singer and Dunn (1990) which uses a homogenizing pasteurization treatment to form microparticulated proteins. The particles can then be spray dried for extended storage and rehydrated for incorporation into a food system. Commercial mimetics are generally designed to be used in a wide variety of dairy products including ice-cream, sour cream, and yoghurt (Yazici and Akgun, 2004), but while cheese is an apparent potential application, only a handful of studies have been conducted to evaluate the efficacy of mimetics in Cheddar.

Due to their hydrophilic structure, mimetics can deftly bind water, increasing both moisture retention and yield in a product. This is considered an important mechanism for the purpose of improving the texture of a low fat cheese (Drake and Swanson, 1995). Of the studies conducted on Cheddar, most have found microstructural effects from mimetics. Aryana and Haque (2001) attempted to differentiate commercial fat mimetics based on their microstructural effects on low fat cheese. Using transition election microscopy and scanning electron microscopy, they assessed protein based Diary-Lo and Simplesse, as well as

polysaccharide based structures Novagel and Stellar. Their findings reflect that ripples in the delipidated indentions (artifacts from microscopy) differed between mimetic, with Dairy-Lo and Stellar exhibiting significantly fewer ripples than the low fat control. Relating these structures to cheese texture, the researchers suggested the protein layers (ripples) may provide a mechanism to resist crushing during compression (Aryana and Haque, 2001). A study by Konuklar et al. (2004a) used scanning electron microscopy to examine cheeses containing Nutrim. They noted that treatment cheeses had an altered microstructure with more uniform droplet voids than the low fat control. These differences were also observed by Drake et al. (1996), as cheeses containing mimetics exhibited a smoother protein phase and more finely dispersed fat network than the control.

A few studies have also examined the mechanical and sensory effects of mimetics on cheese texture (Table 1-2). Fenelon and Guinee (1997) found that reduced fat Cheddar cheeses containing Dairy-Lo exhibited higher moisture retention which resulted in a softer mechanical structure than the reduced fat control through the first 4 months of ageing, but found no sensory improvement as determined by a trained grading panel. Kucukoner et al. (1998) also found that Dairy-Lo, along with several other fat mimetics including Novagel and Simplesse, increased moisture retention and decreased fracture stress values in the cheese. Among the mimetics examined in their study, Lipro was the most efficacious. It exhibited fracture stress values very similar to the full fat control as well as an altered microstructure as characterized by scanning electron microscopy. The study concluded that protein based fat replacers exhibited moderate efficacy in improving rheological properties (i.e. reducing fracture stress) from the low fat control. Rudan et al. (1998) found that Salatrim

exhibited little effects save whitening in low fat mozzarella. In order to incorporate Salatrim into the cheese, the milk had to first be homogenized. The researchers therefore speculate the slightly higher yields found in cheese treated with Salatrim were likely the result of the homogenization treatment. McMahon et al. (1996) also found little efficacy, save moisture retention, using both protein and carbohydrate based fat replacers in mozzarella cheese. Konuklar et al. (2004b) found Nutritrim had lower mechanical texture values of hardness and fracturability than the low fat control but also that the cheese exhibited a bitter taste and a starchy, less creamy mouth feel. Drake et al., (1996) investigated the effect on various fat mimetics on 60% reduced fat Cheddar. The study found Novagel, Dairy-Lo, and ALACO PALS all increased moisture retention in the cheese. An attribute specific sensory panel found all three mimetics had significantly greater firmness scores than the low fat control but also exhibited reduced values of rubberiness. A consumer acceptance panel rated cheeses containing Novagel and ALACOPALS as significantly improved from the low fat (but not equal to the full fat) control. Ma et al. (1997) investigated the rheology of Cheddars (60% reduced fat) containing Dairy-Lo, Novagel, and ALACOPALS. As evaluated by small strain rheology, they found Novagel to be the most improved treatment in mimicking the full fat control, although the researchers note that none of the mimetics were similar to the full fat control.

In summary, mimetics have been demonstrated to affect cheese microstructure, yet few studies have found significant mechanical textural improvements using fat replacers in Cheddar cheese and even fewer have confirmed these finding with consumer sensory acceptance. The literature on this subject is far from exhaustive but suggests only moderate

efficacy from the commercial mimetics assessed. Clearly more work is needed both in characterizing the effects of these mimetics when applied to cheese and in understanding their effects on cheese microstructure, mechanical values, and texture.

4.3 Make Procedure Modifications

It is well known that producing a Cheddar cheese with a reduced fat content alters both its texture and flavor, and thus several adjustments in make procedure have been assessed in an attempt to alleviate these effects.

4.3.1 Acid and Moisture Regulation

While there exist several conceivable options for procedural modifications in Cheddar cheese manufacture, Drake and Swanson (1995) report that the majority of these adjustments affect the two critical factors of acid production and moisture content. Control of acid production can help mitigate development of bitter flavors and increase moisture retention with a higher pH at mill. Acid levels at drain are an important regulator for the percentage of chymosin, plasmin, and Ca^{2+} retained in the curd, and a high pH at this point can lead to lowered plasmin activity (Lawrence et al. 1984). It is generally held that a greater fat content in the curd makes it more difficult to remove moisture through syneresis (Lawrence and Giles, 1987). Increasing moisture content, generally accomplished by lowered scald and stir times (Banks et al. 1989; Mistry et al. 1998), is used to increase yield. However, a significant drawback of increased moisture is a shorter shelf life and lower keeping quality.

Regulation of lactose, which is the substrate for the production of lactic acid, concentration can help to adjust the pH of the cheese. Lactose typically comprises 4.65% (w/w) of milk, 2% of which is trapped in the cheese and fermented into acid (Rehman et al.

2004). Curd washing has been a frequently enlisted method to remove excess lactose (Drake and Swanson, 1995) but is of questionable efficacy as it has been suggested that the curd skin at this stage of production has sufficiently hardened to disallow significant permeation. Washing may also reduce the rate of syneresis by removing residual calcium. This would provide for greater hydration of the caseins and a softer resultant curd texture (Banks, 2004). Rehman et al. (2004) found that adjusting the amount of lactose in the milk affected finished cheese texture and flavor. High lactose milk resulted in a cheese with lower flavor scores, while the low lactose treatment exhibited a softer, more crumbly texture with mild flavor. A study by Guinee et al. (2007) found that a higher pH at milling increased moisture retention and lowered rheological firmness in cheese, though these changes were undistinguished by a grading panel.

4.3.2 Cheese Milk Modifications

Milk fat is highly complex in structure and consists of several hundred species of triacylglycerols, the concentration of which vary by season and animal species (Wright and Marangoni, 2006). Mistry et al. (1996) supplemented cheese milk with 5% filtered sweet buttermilk which contains a high milk fat globule membrane concentration. The researchers observed a slight decrease in hardness at 4 weeks but no differentiation from the reduced fat control at 24 weeks aging. A 5% addition of buttermilk was used because the authors found that higher concentrations resulted in unacceptably high moisture retention and poor curd fusion. Romeih et al. (2012) found that buttermilk powder reduced cheese mechanical hardness at 24 weeks and resulted in a smoother microstructure with small voids and a uniform fat globule distribution. In comparison the low fat control, produced with skim milk

powder, appeared coarse with irregular large voids. The authors recommend this modification for commercial production of low fat cheese, necessitating future work for a greater understanding of buttermilk powder's effect on sensory texture and consumer acceptance.

Another approach by Molina et al. (2000) employed a pressurized homogenization treatment (400 MPa for 15 min.) and found that it induced a faster coagulation time. This resulted in an increased moisture and protein retention as well as a decreased hardness in the finished cheese. The researchers also noted increased proteolytic activity, but ceded this effect may be attributed to higher levels of residual rennet. McGregor and White (1990) found that ultrafiltration (5x concentration of solids) resulted in higher moisture, salt, and total protein, as well as lower lactic acid, but did not significantly improve the texture or flavor of the finished cheese. Oommen et al., (2000) found cheese made with ultrafiltrated and homogenized milk in conjunction exhibited more desirable texture scores, as determined by an experienced dairy panel, than concentrated milk alone. In a comparison of microfiltration and ultrafiltration, Rodriguez et al. (1999) found ultrafiltration to result in greater yields, while mircrofiltration resulted in a firmer mechanical texture due to lower retention of whey.

Goudedranche et al. (2000) conducted preliminary work on the effect of fat particle size in dairy products. While their results were inconclusive, it suggested a smaller globule size may play a role in texture and mouth feel attributes. Michalski et al. (2004) further explored this area, and using a microfiltration process, separated native milk fat globules into small (3μm) and large (6 μm) distributions. For Emmental cheese they found that the smaller

globules increased moisture in the cheese and contributed to a more desirable melting texture, an effect thought to be the result of a thinner casein coating on the globules.

4.3.3 Homogenization of Cheese Milk/Cream

Alterations on milk fat globule size and structure have frequently been employed as strategies for texture improvement in low fat Cheddar cheese. Among the most well studied processes to elicit these effects is the homogenization of milk prior to renneting. Homogenization both reduces fat globule size (with a concomitant increase in surface area by a factor of 4-10x) (Nair *et al.* 2000) and disrupts the milk fat globule membrane, increasing membrane-casein interactions (Mistry, 2001). The former of these alterations increases fat retention in the renneted gel (Oommen, *et al.* 2000; Banks, 2004), and the later contributes to a more rigid curd structure. However, along with an increased moisture retention in cheese (Emmons *et al.* 1980), homogenization of milk can also induce an undesirable flavor in hard cheeses and contribute to processing defects such as curd matting and shattering (Peters, 1955; McGregor and White, 1990). For this reason, the homogenization of milk is not frequently employed in the commercial manufacture of hard cheeses (the exception being blue cheese in which homogenization is used for flavor development by lipid hydrolysis).

Yet, while the homogenization of milk has been found to offer little efficacy in the manufacture of low fat Cheddar cheese, Metzger and Mistry (1994) employed a strategy of homogenizing cream (later standardized with skim milk) which minimizes affected proteins and produces a cheese with few of the defects typically found when using homogenized milk. Additionally, they found that reduced fat Cheddar cheese made with homogenized cream exhibits improved texture (lower hardness and fracturability scores compared with reduced

fat non-homogenized control) as well as improved yields and increased moisture. However, these improvements were still unable to match the texture of the full fat control. In a similar study on reduced fat Mozzarella, Rudan *et al.* (1998) found homogenized cream treatments elicited minimal curd shattering and provided an increased moisture content and whiter color. Nair *et al.* (2000) also assessed homogenization of cream to improve body and mouth feel and noted that, as fat has a greater tendency to clump once the milk fat membrane is disrupted, there is an optimum fat content to homogenization pressure ratio for commercial applications of Cheddar cheese made using this strategy. The study also found homogenized cream did not increase rennet clotting time, as has been observed with homogenized milk, and suggested this difference emphasizes the lesser degree of κ-casein coating on the fat globule surface for homogenized cream. Metzer and Mistry (1995) examined the microstructure of reduced fat Cheddar cheeses using Scanning Electron Microscopy and confirmed a uniform distribution of smaller sized fat globules in cheeses made with homogenized cream. Additionally, they found this treatment decreased the levels of free oil in the cheese, suggesting improved emulsifying properties resulting in an increased yield.

A study exploring the effects of fat distribution found that inhomogenously distributing fat in emulsion filled gels improved sensory mouth feel attributes. The researchers constructed gels with various layers of high and low fat, and it was found that in layering, especially when high fat layers were on the outside, the perception of fat in the gel was greater than if the fat was homogeneously distributed (Mosca et al., 2012). Fenelon et al. (1999) assessed this effect in cheese and modeled a production strategy after the manufacture of cheeses, such as Colby-Jack, which use curd blending to achieve an aesthetic marbled

effect. In this study, curds of high and low fat cheese were blended to produce a reduced-fat Cheddar. Notably, the low fat curds contained the fat mimetic Dairy-Lo (at the manufacture's recommended level, 2% w/w). An additional treatment, which was shredded after milling, was found to significantly reduced cheese firmness in comparison to the low fat control. Both the blended and shredded-blended curd reduced yield stress. A trained grading panel however found no differences between treatments and the low fat control cheeses.

5. CONCLUSIONS

Clear differences exist between low and full fat texture, and the limited success gained from current methodological approaches illustrates the need for additional gains. One approach that has not been taken is to replace the phase volume of fat with a particle that has properties consistent with the function of fat in cheese texture. The objective of this research was to improve the texture of low fat Cheddar cheese using a whey protein based microgel particle. As a secondary objective we have also moved to garner an improved understanding of the product specific breakdown structure of Cheddar cheese. This could potentially allow for the design of microgels with the physical and rheological capacity to affect a desired functionality. Cheese is highly complex system and by demonstrating the potential application of our approach in Cheddar, additional parallels might be made to provide a greater understanding of breakdown pathways in viscoelastic foods.

6. REFERENCES

Aryana, K. J., & Haque, Z. U. (2001). Effect of commercial fat replacers on the microstructure of low-fat cheddar cheese. *International Journal of Food Science & Technology, 36*(2), 169-177.

Banks, J. M. (2004). The technology of low-fat cheese manufacture. *International Journal of Dairy Technology, 57*(4), 199-207.

Banks, J. M., Brechany, E. Y., & Christie, W. (1989). The production of low fat cheddar-type cheese. *International Journal of Dairy Technology, 42*(1), 6-9.

Banks, J. M., Hunter, E. A., & Muir, D. D. (1993). Sensory properties of low fat cheddar cheese:Effect of salt content and adjunct culture. *International Journal of Dairy Technology, 46*(4), 119-123.

Benech, R., Kheadr, E. E., Lacroix, C., & Fliss, I. (2003). Impact of nisin producing culture and liposome-encapsulated nisin on ripening of lactobacillus added-cheddar cheese. *Journal of Dairy Science, 86*(6), 1895-1909.

Bryant, A., Ustunol, Z., & Steffe, J. (1995). Texture of cheddar cheese as influenced by fat reduction. *Journal of Food Science, 60*(6), 1216-1219.

Childs, J. L., & Drake, M. (2009). Consumer Perception of Fat Reduction in Cheese. *Journal of Sensory Studies, 24*(6), 902-921

Delahunty, C. M., Piggott, J. R., Conner, J. M., & Paterson, A. (1996). Comparison of dynamic flavour release from hard cheeses and analysis of headspace volatiles from the mouth with flavour perception during consumption. *Journal of the Science of Food and Agriculture, 71*(3), 273-281.

Drake, M. A., Boylston, T. D., Spence, K. D., & Swanson, B. G. (1997). Improvement of sensory quality of reduced fat cheddar cheese by a lactobacillus adjunct. *Food Research International, 30*(1), 35-40.

Drake, M. A., Bolyston, T. D., & Swanson, B. G. (1996). Fat mimetics in low-fat cheddar cheese. *Journal of Food Science, 61*(6), 1267-1271.

Drake, M. A., & Swanson, B. G. (1995). Reduced- and low-fat cheese technology: A review. *Trends in Food Science & Technology, 6*(11), 366-369.

El Soda, M., Madkor, S. A., & Tong, P. S. (2000). Adjunct cultures: Recent developments and potential significance to the cheese industry. *Journal of Dairy Science, 83*(4), 609-619.

Fenelon, M. A., Beresford, T. P., & Guinee, T. P. (2002). Comparison of different bacterial culture systems for the production of reduced-fat cheddar cheese. *International Journal of Dairy Technology, 55*(4), 194-203.

Fenelon, M. A., Ryan, M. P., Rea, M. C., Guinee, T. P., Ross, R. P., Hill, C., et al. (1999). Elevated temperature ripening of reduced fat cheddar made with or without lacticin 3147-producing starter culture. *Journal of Dairy Science, 82*(1), 10-22.

Guinee, T. P., Fenelon, M. A., Mulholland, E. O., O'kennedy, B. T., O'brien, N., & Reville, W. J. (1998). The influence of milk pasteurization temperature and pH at curd milling on the composition, texture and maturation of reduced fat cheddar cheese. *International Journal of Dairy Technology,51*(1), 1-10.

Guinee, T. P., Auty, M. A. E., & Fenelon, M. A. (2000). The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of cheddar cheese. *International Dairy Journal, 10*(4), 277-288.

Hannon, J. A., Wilkinson, M. G., Delahunty, C. M., Wallace, J. M., Morrissey, P. A., & Beresford, T. P. (2003). Use of autolytic starter systems to accelerate the ripening of cheddar cheese. *International Dairy Journal, 13*(4), 313-323.

Johnson, J. A. C., Etzel, M. R., Chen, C. M., & Johnson, M. E. (1995). Accelerated ripening of reduced-fat cheddar cheese using four attenuated lactobacillus helveticus CNRZ-32 adjuncts. *Journal of Dairy Science, 78*(4), 769-776.

Kailasapathy, K., & Lam, S. H. (2005). Application of encapsulated enzymes to accelerate cheese ripening. *International Dairy Journal, 15*(6–9), 929-939.

Kilcawley, K. N., Nongonierma, A. B., Hannon, J. A., Doolan, I. A., & Wilkinson, M. G. (2012). Evaluation of commercial enzyme systems to accelerate cheddar cheese ripening. *International Dairy Journal, 26*(1), 50-57.

Konuklar, G., Inglett, G. E., Carriere, C. J., & Felker, F. C. (2004). Use of a β-glucan hydrocolloidal suspension in the manufacture of low-fat cheddar cheese: Manufacture, composition, yield and microstructure. *International Journal of Food Science & Technology, 39*(1), 109-119.

Konuklar, G., Inglett, G. E., Warner, K., & Carriere, C. J. (2004). Use of a β-glucan hydrocolloidal suspension in the manufacture of low-fat cheddar cheeses: Textural properties by instrumental methods and sensory panels. *Food Hydrocolloids, 18*(4), 535-545.

Lane, C. N., & Fox, P. F. (1996). Contribution of starter and adjunct lactobacilli to proteolysis in cheddar cheese during ripening. *International Dairy Journal, 6*(7), 715-728.

Lawrence, R. C., Gilles, J., Creamer, L. K., Crow, V. L., Heap, H. A., & Honoré, C. G. Cheddar cheese and related dry-salted cheese varieties. *Cheese: Chemistry, physics and microbiology* (pp. 71-102).

Lucey, J. A., Johnson, M. E., & Horne, D. S. (2003). Invited review: Perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science, 86*(9), 2725-2743.

Lucey, J. A., Johnson, M. E., & Horne, D. S. (2003). Invited review: Perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science, 86*(9), 2725-2743.

Ma, L., Drake, M. A., Barbosa-Canovas, G. V., & Swanson, B. G. (1997). Rheology of full-fat and low-fat cheddar cheeses as related to type of fat mimetic. *Journal of Food Science, 62*(4), 748-752.

McGregor, J. U., & White, C. H. (1990). Effect of enzyme treatment and ultrafiltration on the quality of lowfat cheddar cheese. *Journal of Dairy Science, 73*(3), 571-578.

McMahon, D. J., Alleyne, M. C., Fife, R. L., & Oberg, C. J. (1996). Use of fat replacers in low fat mozzarella cheese. *Journal of Dairy Science, 79*(11), 1911-1921.

McSweeney, P. L. H., Fox, P. F., Lucey, J. A., Jordan, K. N., & Cogan, T. M. (1993). Contribution of the indigenous microflora to the maturation of cheddar cheese. *International Dairy Journal, 3*(7), 613-634.

Metzger, L. E., & Mistry, V. V. (1994). A new approach using homogenization of cream in the manufacture of reduced fat cheddar cheese. 1. manufacture, composition, and yield. *Journal of Dairy Science, 77*(12), 3506-3515.

Metzger, L. E., & Mistry, V. V. (1995). A new approach using homogenization of cream in the manufacture of reduced fat cheddar cheese. 2. microstructure, fat globule distribution, and free oil. *Journal of Dairy Science, 78*(9), 1883-1895.

Mistry, V. V., Metzger, L. E., & Maubois, J. L. (1996). Use of ultrafiltered sweet buttermilk in the manufacture of reduced fat cheddar cheese. *Journal of Dairy Science, 79*(7), 1137-1145.

Mistry, V. V. (2001). Low fat cheese technology. *International Dairy Journal, 11*(4–7), 413-422.

Molina, E., Dolores Álvarez, M., Ramos, M., Agustín Olano, & López-Fandiño, R. (2000). Use of high-pressure-treated milk for the production of reduced-fat cheese. *International Dairy Journal, 10*(7), 467-475.

Mosca, A. C., Rocha, J. A., Sala, G., van de Velde, F., & Stieger, M. (2012). Inhomogeneous distribution of fat enhances the perception of fat-related sensory attributes in gelled foods. *Food Hydrocolloids, 27*(2), 448-455.

Nair, M. G., Mistry, V. V., & Oommen, B. S. (2000). Yield and functionality of cheddar cheese as influenced by homogenization of cream. *International Dairy Journal, 10*(9), 647-657.

Romeih, E. A., Moe, K. M., & Skeie, S. The influence of fat globule membrane material on the microstructure of low-fat cheddar cheese. *International Dairy Journal*.

Rudan, M. A., Barbano, D. M., & Kindstedt, P. S. (1998). Effect of fat replacer (salatrim®) on chemical composition, proteolysis, functionality, appearance, and yield of reduced fat mozzarella cheese. *Journal of Dairy Science, 81*(8), 2077-2088.

Sallami, L., Kheadr, E. E., Fliss, I., & Vuillemard, J. C. (2004). Impact of autolytic, proteolytic, and nisin-producing adjunct cultures on biochemical and textural properties of cheddar cheese. *Journal of Dairy Science, 87*(6), 1585-1594.

Shakeel-Ur-Rehman, Waldron, D., & Fox, P. F. (2004). Effect of modifying lactose concentration in cheese curd on proteolysis and in quality of cheddar cheese. *International Dairy Journal, 14*(7), 591-597.

Zamora, A., Ferragut, V., Guamis, B., & Trujillo, A. J. (2012). Changes in the surface protein of the fat globules during ultra-high pressure homogenization and conventional treatments of milk. *Food Hydrocolloids, 29*(1), 135-143.

Table 1-1. CHEDDAR CHEESE CATEGORY DEFINITIONS

Category	Definition
Fat-free	Less than 0.5 g per serving
Low fat	Maximum 3 g total fat per serving
Light	If less than 50% of the calories come from fat
Reduced fat	Minimum 25% reduction in total fat per reference amount
Full fat	45–60% fat on dry basis

Table 1-2. SUMMARY OF SOME COMMERCIALLY AVAILABLE FAT MIMETICS**.**

Commercial Name	Base Composition	Vendor
Dairy Lo	whey protein, lactose	Parmalat SpA. Collecchio, Italy
Simpless	milk, egg protein	CPKelco. Atlanta, GA
Novagel	Microcystaline cellulose, guar gum	FMC Corp. Philadelphia, PA, USA

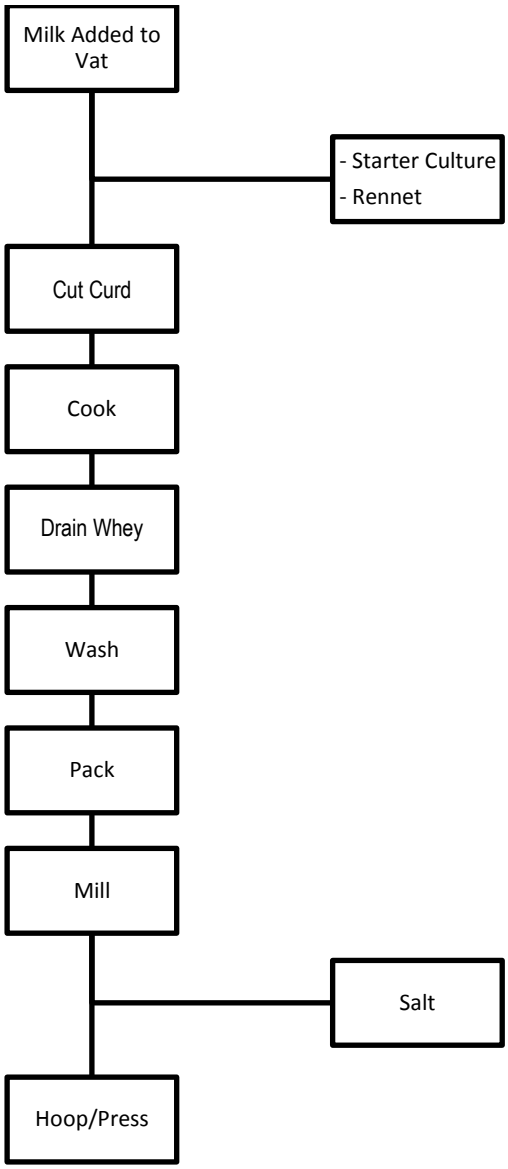


Figure 1-1. Production Schematic of Cheddar Cheese.

Figure adapted from (Banks et al. 2007)

CHAPTER 2. DEVELOPMENT AND MANUFACTURE OF WHEY PROTEIN PARTICLE PARTICLES.

Department of Food, Bioprocessing, and Nutritional Sciences

North Carolina State University

1. ABSTRACT

A series of whey protein gel based fat mimetics were developed for application into Cheddar cheese. It was hypothesized that whey protein gel particles could function as fat mimetics if they were both substituted at a phase volume equal to the fat removed and had similar interactions with the casein gel network. This study enlisted a novel approach for fat mimetic particle production which enables the design of a particle with specific rheological and textural properties. Protein, salt, and fat concentrations significantly affected the rheological and sensory texture characteristics of whey gels. Particle size and appearance, as determined using confocal laser scanning microscopy, were found to be dictated by gel composition. Fat globule condition (native vs. homogenized) had no effect on gel fracture rheology. This work illustrates the suitability of a function oriented approach for creating a Cheddar cheese specific fat mimetic.

2. INTRODUCTION

As reviewed in chapter 1, concern over fat-related health effects remains a prevalent dietary consideration for consumers. United States dietary guidelines for now over a decade have recommended a decrease in consumption of saturated fatty acids as a strategy in risk factor reduction for cardiovascular disease (Laatikainen *et al.* 2010). Cheese, which accounts for 16% of saturated fat in the US diet (DRI, 2006), is therefore pinnacled as a food in which a reduction in saturated fat would help to reduce consumers' saturated fat intake. However, cheese texture is governed by a series of highly complex and largely unknown mechanisms so that in the past 8,000 years it is estimated humans have been enjoying dairy products (Fox

and McSweeney, 2004), there has been little progress in creating a low fat Cheddar cheese capable of emulating the full fat product (Johnson *et al.* 2009).

The US Food and Drug Administration regulates that low fat cheese contain no more than 3 g of fat per reference amount. This puts the target at 6% w/w fat, which requires a 75-80% reduction from the full fat product (Narasimmon, 2008). Cheddar cheese production is an inherently complex process which relies on microbiological and enzymatic activity over an extended ageing period to generate its characteristic texture and flavor (Grappin *et al.* 1985). Like most cheeses, Cheddar is produced through a series of steps that remove water, minerals and lactose to concentrate casein and fat into a semi-solid material. Protein composition is dependent upon the volume fraction of incorporated milk fat (Roberts and Vickers, 2006). While full fat Cheddar must contain a minimum of 50% solids (wt/wt), moisture content is limited to 39% (wt/wt) (CFR 133.113), so protein content is concentrated upwards from c.a. 20% as fat is removed (Lynch *et al.* 1996). Ageing is also critical to producing desirable Cheddar cheese flavor and texture (Lawrence *et al.* 2006; Rogers *et al.* 2009). Therefore, a desirable reduced (25% reduction from reference amount) or low (< 3 g per reference amount) fat Cheddar cheese requires substituting for the physical and chemical contribution of fat while taking care not to interfere with the ageing process.

Not surprisingly, fat reduction in cheese is a challenging task which results in a phase volume shift, concentrating the continuous casein network to distinctly alter texture and sensory characteristics (Drake and Swanson, 1995; Milo and Reineccius 1997; Mistry, 2001; Banks, 2004). As fat content is reduced, cheese mechanical properties become harder and more springy (Bryant *et al.* 1995), the storage modulus increases (Ustunol *et al.* 1995), and

the absence of specific free fatty acids dictate an attenuated "Cheddar" flavor development (Banks *et al.* 1989). Rodriguez (1999) then concluded that low fat cheeses made using a conventional method, i.e. using low fat milk, is an insufficient strategy, as these cheeses will be replete with textural and flavor defects. The challenge of producing a desirable low fat cheese must therefore be addressed by consideration of the respective phase volumes of constituent elements and the structural mechanisms which affect finished product texture and flavor development. To apply this modality, cheese is modeled as a composite gel with the textural properties compliant upon the continuous protein phase and discontinuous, or filler, fat phase (Tolstoguzov and Braudo 1983). It then follows that as a large portion of the filler phase is removed, a solution must be found to restore this depleted phase volume if the low fat product is to exhibit similar textural characteristics as its full fat counterpart. Several commercial fat mimetics have been developed for this purpose (reviewed in chapter 1). Ideally, these mimetics would be able to emulate the mechanical properties of fat while using a different component structure.

Several studies have been conducted to assess the efficacy of commercial fat mimetics in cheese (See chapter 1), but have found these particles largely incapable of replicating the functionality of fat. This outcome may be affected by the desire of manufacturers to create mimetics designed for application into a wide variety of products. Building on this effect, the manufacturer's recommended addition level for Dairy-Lo and Simplesse fat replacers are 1% (w/v) and 1.5% (w/v) respectively (Fenelon and Guinee, 1997; Kayanush and Haque, 2001), which even with a concentration factor of 10 (when whey is drained from the cheese), would not be sufficient to replenish the filler volume lost from

fat. The better approach for developing a suitable fat mimetic is therefore to first identify the physio-chemical function of fat in Cheddar cheese, which then allows for the creation of a fat mimetic with a product specific design. But while the relationship between fat content and finished cheese texture is well researched (Emmons *et al.* 1980; Bryant *et al.* 1995; Fenelon *et al.* 1999; Guinee *et al.* 2000; Gwartney *et al.* 2002; Rogers et al 2009; Xin *et al.* 2011), the mechanisms by which fat induces this characteristic texture profile is less understood.

A prevalent approach to understanding the relationship between fat content and texture is to model Cheddar cheese as a particle filled gel. According to theory, texture is dictated by a discontinuous filler phase (fat) and a continuous phase (casein) (Rogers *et al.* 2009). The relationship between these two phases is also important, as the discontinuous phase can function as an active (interacts with the continuous phase) or inactive (no interaction with the continuous phase) filler. The effect of the filler's interaction with the continuous phase is also dependent on the relative strength of the latter (van Villet, 1988). Active fillers will have a strong effect on a gel with a relatively low storage modulus, while their effect will be more palliated if the storage modulus of the gel is already high (Dickinson and Chen, 1999). Yang *et al.* (2011) applied the filled gel theory to model the effects of fat content on Cheddar cheese. While this study found several filled gel derived models an apt predictor of the cheese storage modulus, these properties are only relevant when applied to small strain testing (within the linear viscoelastic region), and our understanding of mechanisms controlling fracture properties remains relatively limited.

Our objective in this study was to create a dynamic range of whey protein gels with known structure-texture relationships (van Vilet, 1998; Chen and Dickenson, 2007; Mor *et*

al., 2009; McClements *et al.* 1993), so that when applied to Cheddar cheese, these gels may be used to better understand the fracture mechanisms responsible for full fat texture. There are currently commercially available whey protein based fat mimetics, most notably Simplesse (CPKelco. Atlanta, GA) and Dairy-Lo (Parmalat SpA. Collecchio, Italy) which are formed using a microparticulation process to provide a tight particle size distribution of essentially a dry, protein aggregate. However, this method is limited in that the mechanical properties of the whey protein particles are process dependent, i.e. the particles must be formulated in such a way so as to allow for microparticulation. As our study first creates a whey protein gel with the desired functional properties and subsequently uses mechanical means to control particle size, our approach is therefore novel in allowing for a process independent functional design of a whey protein based fat mimetic.

3. MATERIALS AND METHODS

3.1. Preparation of Whey Protein Particles

Previous work employed a method for particle particulation which used a peristaltic pump at constant flow rate to add heat-set whey protein soluble aggregates into a salt solution (see appendix A). While this method was able to produce a relatively controlled particle size distribution, gel composition was process dependent (i.e. particles could only be formed at specific salt and protein concentrations). A novel method for the production of whey protein particles was therefore developed in which particles could be designed with the rheological and textural properties necessary to impart a desired functionality tailored to a specific food product (Figure 2-1). This process first involves the creation of a heat-set whey

protein gel with the potential for a wide range in structure and composition, requiring only that it form a cohesive soft-solid. Particulation is achieved using a mechanical mixer. It is important to note that this method results in a relatively broad spectrum of particle sizes, and thus a homogenizer was used on select treatments for further particulation.

3.1.1. Formation of Whey Protein Macrogels.

Whey Protein Isolate (WPI) (94.06% protein, dry basis) was obtained from Davisco Foods International Inc. (Le Suer, Minn. USA) and stored at room temperature (23 ± 2°C). The Deionized water system was obtained from a Dracor Water Systems (Durham, NC, USA) with a resistivity minimum of 18.2MΩ-cm. Cream was obtained from the North Carolina State University Dairy, and fat content was determined using a CEM Smart Trac Rapid Fat Analyzer (Matthews, NC, USA). Sodium Chloride (Iodine free) was purchased from a local grocery.

Gels were heat set (1 hr at 85°C) from solutions containing various protein (% w/w), fat (% w/w), and salt concentrations (mM) to induce differing rheological properties. As one of the major objects of this study we sought to demonstrate the range of functionalities induced by differences in particle composition and structure. While certainly not a comprehensive demonstration of gel compositions, the above components were used to illicit differences in gel structure (Table 2-1). Particulate and stranded particle types were selected to assess differences in gel network structure as dictated by salt concentration. The native fat composite-L was used for comparison to the particulate gel, and the homogenized composite was used to evaluate the role of fat globule structure (native vs. homogenized) and its impact on gel rheology and texture. The native fat composite-S particles were formed by

homogenization of the native fat composite-L particles, after mechanical grinding, and were intended to assess the feasibility of a particle type with a tightly controlled size distribution rage (Figure 2-4).

3.1.2. Whey Protein Microgel Particulation.

Heat-set whey protein gels were created on a macro scale, heretofore referenced macrogels, and were allowed to set for 24 hrs at 8 ± 2°C prior to testing. Macrogels were mechanically ground using a Stephen UMC 5 Mixer (Stephan Machinery gmbH; Hameln, Germany) at 3,000 rpm for 1 minute under continuous stirring. A 1:1 (w/w) addition of cylindrical nugget ice was used to induce flow. Additional particulation of select treatments (Table 2-1) was achieved using a Panda NS1001L2K batch homogenizer (Niro Soavi: Parma, Italy) in which particle suspensions in deionized water with a 1:4 (w/w) dilution were created and homogenized at 400 bar.

3.2. Particle Size Analysis

A Horiba LA 300 (Kyoto, Japan) equipped with a 42 channel photo-diode detector was used for particle size analysis. Particles were diluted in deionized water at a 1/100 (w/w) ratio, and were stirred vigorously before measurement. The light was set at a wavelength of 650nm, and size distribution data (Figure 2-4) represent the average of 5 replications.

3.3 Rheological Analysis

3.3.1. Small Strain Tests

3.3.1.1. Determination of the Linear Viscoelastic Region

Samples were evaluated with a Stress Tech controlled stress rheometer (ATS Rheosystems: Brodentown, NJ, USA). The strain sweeps were conducted from a range of 1

to 3,000 Pa using a 20 mm serrated plate geometry. Samples were shaped to a 4 mm thickness with a commercial deli slicer, trimmed to the size of the plate, and the exposed surface was coated with a synthetic lubricant (SuperLube, Synco Chemical: Bohemia, NY, USA) to prevent moisture loss during sample testing. Strain sweeps were conducted at 25°C at a constant frequency of 10 Hz. Critical stress and strain values were determined as the point at which G* regularly differed by > 1% of the previous value.

3.3.1.2. Controlled Temperature Frequency Sweeps

Frequency sweeps were conducted using a Stress Tech controlled stress rheometer (ATS Rheosystems: Brodentown, NJ) at 25°C. Samples were prepared and shaped using the same procedure as described in the determination of the LVR. As critical stress values varied widely across samples, a strain value of 0.0036, which the rheometer calculated as deformation induced by a known torque, was selected as it was well within the LVR for all samples. The corresponding stress value for each sample treatment was then used for the frequency sweep.

3.3.2. Large Strain Rheological Tests

A compression/decompression test was used to assess the large strain deformation properties of the whey protein gels. The method was adapted from Van Den Berg *et al.* (2008) and Rogers *et al.* (2010). Samples were cut into a cylindrical shape with a diameter of 19mm and a height of 21.5mm using a cork borer. After shaping at 7 ± 2°C, the gels were sealed in a plastic bag to prevent moisture loss while they equilibrated to room temperature (23 ± 2°C). An Instron 5565 rheometer (Instron; Norwood, MA, USA) with a flatplate (5cm diameter) was used to compress the samples to 80% of their original height (17.2mm) at a

rate of 50 mm/min. Simpson's rule, a trapezoidal approximation well adapted to third degree polynomials, was used to calculate the area under the force-deformation curve with an even number of sub-intervals (x) with a width of Δx (Eq. 2.1).

$$S_n = \frac{\Delta x}{3}[f\ x_0\ + 4f\ x_1\ + 2f\ x_2\ + 4f\ x_3\ + 2f\ x_4\ + \cdots + 4f\ x_{n-1}\ + 2f\ x_{n-2} \quad \text{(Eq. 2.1)}$$

The % recoverable energy (not lost to heat or flow) was calculated from the ratio of the area under the second half of the curve to the area under the first.

$$\%RE = \frac{area\ under\ decompression}{area\ under\ compression} \times 100 \quad \text{(Eq. 2.2)}$$

3.3.3. Fracture Analysis

A torsion method adapted from Rogers et al. (2009) was used to determine non-linear fracture analysis. Gel samples were shaped into a cylindrical geometry with a length of 28 mm and diameter of 19 mm using a cork borer. During the proceeding steps, gels were kept in a sealed plastic bag to avoid moisture lost. Notched plastic disks (Gel Consultants; Raleigh, NC, USA) were adhered to both ends of the cylindrical samples using cyanoacrylate adhesive (Loctite 401: Loctite Corp.; Rocky Hill, CT, USA). These served as stabilization points and allowed for the subsequent gel shaping into a capstan geometry using a precision grinding machine (Model GCPM92 US, Gel Consultants. Raleigh, NC, USA). Samples were tested at a strain rate (γ) of 0.45 s^{-1} on a Haake VT-550 rotational viscometer (Gerbruder

Haake GmbH, Karlsruhe, Germany) with an added attachment designed for torsion testing (Truong and Daubert, 2000). Elapsed time (*t)* at maximum torque (*M*) was used to calculate angular deformation (φ) of the sample (φ= γ*t*). True shear strain (γ_{true}) and shear stress (σ) were calculated using the equations derived by Deihl et al. (1980) listed below:

$$\sigma = \frac{2KM}{\pi r_{\min}^3} \qquad \text{(Eq. 2.3)}$$

$$\gamma_t = \frac{2K\phi}{\pi r_{\min}^3 Q} \qquad \text{(Eq. 2.4)}$$

$$\gamma_{true} = \frac{1}{2}\ln\left[1 + \frac{\gamma_t^2}{2} + \gamma_t\left(1 + \frac{\gamma_t^2}{4}\right)^{1/2}\right] \qquad \text{(Eq. 2.5)}$$

K (1.08) is a shape factor constant and Q (8.45×10^6 m^{-3}) a curvature section constant, while r_{min} is the minimum radius in the middle of the capstan-shaped sample.

3.3.4. Post Fracture Analysis

An Instron 5565 rheometer (Instron; Norwood, MA) with parallel plate geometry (5cm diameter) was used to compress samples to 1% of their original height (from 21.5mm to 0.215mm) at a rate of 50 mm/min. The post fracture region was identified as those points on the curve occurring after the incidence of maximum fracture stress. Sample post fracture regions were compared by normalizing values of force and deformation.

3.4. Descriptive Sensory Texture Analysis

A trained Sensory panel using a cheese texture lexicon modified from Brown et al. (2003) by the method as described in Barden et al. (2010) was used (Table 2-3). The descriptive analysis was performed by seven panelist using the Spectrum^{TM} method as reviewed by Drake and Civille (2003). All panelists had over 200 hours of experience in texture-specific evaluations. Each sample was evaluated in duplicate using a randomized balanced design. Gels were shaped (19mm diameter and 15mm height) using a cork borer, stored in a 112 mL soufflé cup, and were brought to room temperature (23 ± 2°C) immediately prior to evaluation so as to prevent variation due to moisture loss. Samples were given a randomized three digit code and evaluated using a 0-15 point (Table 2-3). Panelists expectorated samples after evaluation.

3.5 Confocal Laser Scanning Microscopy

Using a scalpel, gel samples were sliced into sections approximately 5 mm x 5 mm x 5 mm thick and were observed using a Zeiss LSM 710 confocal scanning laser microscope (CSLM) with inverted stage. A deionized water solution of 0.2% w/w Nile Blue (Invitrogen Molecular Probes, Eugene, OR) was used to stain the respective protein (excitation 633nm, emission 650-800nm) and fat phases (excitation 488nm, emission 500-650nm) of the gels. Images were recorded with a 40 x objective lens (LDC-Apochromat 40x/1.1 W Korr M27) in 2048 x 2048 pixel resolution.

3.6 Statistical Analysis

Statistical analyses were conducted using MINI TAB statistical software (Version 16). One way Analysis of Variance with a Tukey post hoc test was used to determine significance at $p < 0.05$.

4. RESULTS AND DISCUSSION

4.1 Small Strain Rheological Tests

Gels were tested for frequency dependence (Figure 2-2) at stress values corresponding to a strain of 0.0036, which was determined to be within the linear viscoelastic region. Evaluations were conducted at 25°C which was reasoned an approximate natural consumption temperature. Gel network structure affected the storage modulus of the samples. Stranded and particulate gels were both formulated at 10% (w/w) protein, yet the former exhibited less frequency dependence and lower storage modulus values. The homogenized fat composite gels exhibited greater storage modulus values than the particulate or stranded types. The composite gels contained a greater protein to water ratio, and thus the difference is likely the result of a diminished phase volume in the fluid water component. Practically, there was little difference incurred by the degree of homogenization and resultant fat globule membrane structure on the G' values of the composite type whey protein gels.

4.2. Large Strain Rheological Tests

Recoverable energy in a viscoelastic gel is a rate dependent property which reflects a material's relative proclivity for energy storage. Gels are compressed to 80% of their original height and then the load is released. Energy loss is assumed to be the result of structural

decomposition and energy dissipation through the viscous component of the gel. Homogenization had no significant effect on fat functionality (table 2-2). Both salt and fat reduced energy recovery with increasing concentration, with the stranded type gels exhibiting the greatest recoverable energy of all the treatments. Whey protein concentration had no significant effect on recoverable energy.

4.3. Fracture Analysis

Torsion was used to measure sample fracture stress and strain as the twisting deformation minimizes syneresis during testing as compared to a uniaxel compression method (Hamman et al. 2006). The fracture stress of the gels was highly dependent upon protein concentration (table 2-2). Increasing protein content resulted in an increased fracture stress, yet also lowered fracture strain. While the stranded gel had the lowest fracture stress of the samples tested, it also had the greatest fracture strain. Thus, while these results are well understood as they relate to gel network structure (Foegeding et al. 1992; Gwartney et al. 2004), they illustrate that adjustments to fracture properties are best understood as a series of tradeoffs, as lowering either fracture stress or strain will often increase the other. Salt concentration demonstrated a significant effect on gel fracture stress and strain. In agreement with Kuhn and Foegeding (1991) fracture stain was lowest at 150mM NaCl concentration. McClements et al. (1993) found a maximum compressive stress at an NaCl concentration of 75mM which was very near what we observed. Notably, degree of fat homogenization in the composite type gels proved to insignificantly affect fracture stress or strain.

4.4 Post Fracture Analysis

Whey protein gels were compressed beyond their initial point of fracture in order to further elucidate their breakdown mechanism (Figure 2-3). The gels were selected to represent a diverse range of compositions, and were normalized to the point of fracture. The initial drop in steepness of the curve is related to the speed of crack propagation in the sample, with a sharp decrease post fracture indicating a free running crack, and a more gradual curve indicating several concurrent fracture sites (Van den Berg et al., 2008). Cakir et al. (2012) related the development of a free running crack to a high elastic energy recovery. This was also observed in our study as the stranded type gel exhibited the greatest recoverable energy (Table 2-2). Additionally, the particulate and composite gels exhibited more gradual curves corresponding to their relative lower amounts of recoverable energy. As the composite type gels were made at identical protein and salt contents (Table 2.1) as the particulate, it is reasonable that the differences, as demonstrated by the slope of the post fracture curve, indicate simultaneous fracture locations and a greater ability to dissipate energy.

4.5 Descriptive Sensory Texture Analysis

Descriptive texture terms were subdivided into groups which reflected two distinct events during gel mastication. The force-deformation terms relate to first bite and initial breakdown of the sample, while the bolus formation terms relate to post fracture oral processing. All gels were similar in hand springiness and hand rate of recovery (Figure 2-5a), but the two composite type gels were differentiated by a low degree of hand firmness and fracturability. These differences are likely more the result of fat volume fraction than fat

particle structure (native vs. homogenized) as the composite gels were similar in all force-deformation terms yet differed from the particulate gel which contained the same concentration of salt and protein as the composites (Table 2-1). The native and homogenized fat composites were again similar in bolus formation terms. The particulate gel was significantly greater in cohesion and adhesion than the stranded gel, and significantly lower in smoothness of mouth coating and smoothness of mass (Table 2-4). These results are in agreement with Gwartney et al. (2004) which demonstrated the degree of texture differentiation as a result of stranded or particulate network structure. Additionally, the composite type gels showed increased values of cohesion and adhesion, which are characteristic of full fat cheese texture.

4.6 Particle Size Analysis

A laser scattering particle size analyzer was used to determine size distribution. Gels were particulated using mechanical shear, and the native fat composite-S particle was sheared mechanically and then homogenized (Table 1). The stranded and particulate type gels exhibited a bimodal distribution (Figure 2-4a, Figure 2-4b) in which a large frequency of the particles were larger than 200μm. This was observed visually, and as a result of the wide spectrum of size distribution, a portion of the particles quickly settled from the water dispersion. The native and homogenized composite gels similarly exhibited a large frequency of particles around 30 μm (Figure 2-4c, Figure 2-4d), but did not have the second modal distribution above 100 μm. The grinding method was identical for all particles, so these results may reflect different fracture paths among the gel treatments. A faster propagating fracture site in the stranded and particulate gels would quickly drop the critical size of the

particles so that they could not be further reduced by mechanical shear, resulting in both medium and large sized particles. The composite gels would undergo a more ubiquitous, gradual fracture path allowing for a more uniform particle size. The homogenized particle treatment (Figure 2-4e) exhibited a very tight size distribution.

4.7 Confocal Laser Scanning Microscope Observations (CLSM)

The mechanical method used for particulation resulted in a large particle size distribution. Particles were diluted with water at a ratio of 1:1 to facilitate visualization. The stranded type gel (figure 2-6a) was characterized with an irregular geometry and well dispersed particles. Particulate type particles (Figure 2-6b) exhibited similar geometric characteristics to those of the stranded gel, but with a greater frequency of smaller particles. Given that the stranded type was the only particle formed with relatively clean fracture edges, the shattering effect evident in all other treatments likely reflects the characteristically brittle structure of the particulate gel network. The native and homogenized fat composite particles (Figure 2-6c, Figure 2-6d) appeared very similar, and the native composite had only a slight degree more fat globule coalescence than the homogenized. Both composite type particles did however exhibit a more spherical geometry with surface aspersions. This could be the result of fracture occurring at the filler site, explaining the relative high density of fat embedded near the particle centers and nearly all fat at the fracture surface liberated. CSLM also allows a 3 dimensional rendering to identify if fat globules are imbedded within the larger protein particles, or whether they are simply liberated globules closer to the lens surface. While this is unlikely given the inverted microscope stage (liberated particles would be more likely to have moved away from the focal plane), a cross sectional analysis reveals

that the globules are embedded within the protein matrix (Figure 2-6f, Figure 2-6g). The composite gel, which was subsequently homogenized after particlulation (Figure 2-6e), had a very tight size distribution of particles (approximated the size of the homogenized fat globules). The homogenization treatment appears to have entirely liberated the fat globules from the protein particles.

5. CONCLUSIONS

The primary objective of our research was to develop a mimetic capable of creating a full fat texture in a low fat cheese. This study demonstrates the versatility of a whey protein based particle to be designed from a dynamic range of textures and particle characteristics to fit a specific functional need. Increasing NaCl concentrations ranging from 50 to 150μm was found to significantly decrease fracture strain and recoverable energy. Fat content significantly reduced gel recoverable energy, while increasing protein content resulted in greater values of fracture stress but had no effect on recoverable energy. Compositional differences also had an effect on particle size and shape as determined using CSLM. Treatments were undifferentiated by degree of fat homogenization. With the potential application of this methodology established, the next chapter will examine the efficacy of these particles as they are applied in Cheddar cheese over an extended ripening period.

6. REFERENCES

Banks, J. M. (2004). The technology of low-fat cheese manufacture. *International Journal of Dairy Technology, 57*(4), 199-207.

Barden, L. M., Çakir, E., Leksrisompong, P. N., Ryan, K. N., Foegeding, E. A., & Drake, M. A. (2010). Effect of Flavor on Perceived Texture of Whey Protein Isolate Gels. *Journal of Sensory Studies, 25*(3), 447-462.

Brown, J.A., Foegeding, E.A., Daubert, C.R., Drake, M.A. and Gumpert, M. (2003). Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. **86**, 3054–3067.

Çakır, E., Daubert, C. R., Drake, M. A., Vinyard, C. J., Essick, G., & Foegeding, E. A. (2012). The effect of microstructure on the sensory perception and textural characteristics of whey protein/κ-carrageenan mixed gels. *Food Hydrocolloids, 26*(1), 33-43.

CFR 21, 2006. <www.fda.gov>.

Chen, J. and Dickenson, E. (1998). Viscoelastic properties of protein-stabilized emulsions: effect of protein−surfactant interactions *J. Agric. Food Chem., 46* (1), pp 91–97.

Chen, J., & Dickenson, E. (1998). Viscoelastic Properties of Heat-Set Whey Protein Emulsion. *Journal of Texture Studies, 29*(3), 285-304.

Chen, J., Dickinson, E., Langton, M., & Hermansson, A. (2000). Mechanical properties and microstructure of heat-set whey protein emulsion gels: Effect of emulsifiers. *LWT - Food Science and Technology, 33*(4), 299-307.

Creamer, L. K., & Olson, N. F. (1982). Rheological evaluation of maturing cheddar cheese. *Journal of Food Science, 47*(2), 631-636.

Dickenson, E. and Chen, J. (1999). Heat-set whey protein emulsion gels: Role of active and inactive filler particles. Journal of Dispersion Science and Technology Vol. 20 (1-2) 197-213.

Diehl, K.C., Hamann, D.D., and Whitfield, J.K. (1979). Structural failure in selected raw fruits and vegetables. J. Texture Studies 10, 371–400.

Drake, M. A., & Civille, G. V. (2003). Flavor lexicons. *Comprehensive Reviews in Food Science and Food Safety, 2*(1), 33-40.

Drake, M. A., & Swanson, B. G. (1995). Reduced- and low-fat cheese technology: A review. *Trends in Food Science & Technology, 6*(11), 366-369.

Fenelon, M. A., O'Connor, P., & Guinee, T. P. (2000). The effect of fat content on the microbiology and proteolysis in cheddar cheese during ripening. *Journal of Dairy Science, 83*(10), 2173-2183.

Foegeding, E. A., Gwartney, E. A., and Errington, A. D. Functional properties of whey proteins in forming networks. *Functional Properties of Proteins and Lipids*. (1998) Chapter 9, pp 145–157.

Fox, P. F., & McSweeney, P. L. H. Cheese: An overview. *Cheese: Chemistry, physics and microbiology* (pp. 1-18) Academic Press.

Grappin, R., Rank, T. C., & Olson, N. F. (1985). Primary proteolysis of cheese proteins during ripening. A review. *Journal of Dairy Science, 68*(3), 531-540.

Gwartney, E. A., Larick, D. K., & Foegeding, E. A. (2004). Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. *Journal of Food Science, 69*(9), S333-S339.

Hamann, D. D., Zhang, J., Daubert, C. R., Foegeding, E. A., & Diehl, K. C. (2006). Analysis of compression, tension, and torsion for testing food gel fracture properties. *Journal of Texture Studies, 37*(6), 620-639.

Ikeda, S. Foegeding, E. A., and Hagiwara, T. (1999). Rheological study on the fractal nature of the protein gel structure. *Langmuir*, 1999, *15* (25), pp 8584–8589.

Johnson, M. E., Kapoor, R., McMahon, D. J., McCoy, D. R., & Narasimmon, R. G. (2009). Reduction of sodium and fat levels in natural and processed cheeses: Scientific and technological aspects. *Comprehensive Reviews in Food Science and Food Safety, 8*(3), 252-268.

Krauss, R. M., Lecerf, J. M., LeGrand, P., Nestel, P., Rise´rus, U. Sanders, T., Sinclair, A. Stender, S., Tholstrup, T. and Willett, W. C. (2011). The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? American Journal of Clinical Nutrition vol. 93 (4) p. 684-688.

Kuhn, P. R., & Foegeding, E. A. (1991). Factors influencing whey protein gel rheology: Dialysis and calcium chelation. *Journal of Food Science, 56*(3), 789-791.

Lalor, F., Kennedy, J., Flynn, M. A. T., Wall, P. G. (2010). A study of nutrition and health claims – a snapshot of what's on the Irish market. Public Health Nutrition 13 (5), p 704-11.

Langey, K. R., & Green, M. L. (1989). Compression strength and fracture properties of model particulate food composites in relation to their microstructure and particle-matrix interaction. *Journal of Texture Studies, 20*(2), 191-207.

Matsumura, Y., Kang, I., Sakamoto, H., Motoki, M., & Mori, T. (1993). Filler effects of oil droplets on the viscoelastic properties of emulsion gels. *Food Hydrocolloids, 7*(3), 227-240.

McClements, D. J., Monahan, F. J., and Kinsella, J. E. (2007) Effect of Emulsion droplets on the rheology of whey protein isolate gels. Journal of texture studies, 24 (4) p. 411–422.

Milo, C. and Reineccius, G. A. (1997). Identification and Quantification of Potent Odorants in Regular-Fat and Low-Fat Mild Cheddar Cheese. Journal of Agricultural and Food Chemistry 45 (9), 3590-3594.

Mistry, V. V. (2001). Low fat cheese technology. *International Dairy Journal, 11*(4–7), 413-422.

Monahan, F. J., German, J. B., Kinsella, J. E. (1995) Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. Journal of Agricultural and Food Chemistry 43 (1), 46-52.

Mor, Y., Shoemaker, C. F., & Rosenberg, M. (1999). Compressive properties of whey protein composite gels containing fractionated milkfat. *Journal of Food Science, 64*(6), 1078-1083.

Narasimmon, R. G. (2009) Say Low-fat Cheese. Food Product Design. Innovatewithdairy.com

Peleg, M. (1984). A note on the various strain measures at large compressive deformations. *Journal of Texture Studies, 15*(4), 317-326.

Roberts, A. K., & Vickers, Z. M. (1994). Cheddar cheese aging: Changes in sensory attributes and consumer acceptance. *Journal of Food Science, 59*(2), 328-334.

Rodríguez, J. (1998). Recent advances in the development of low-fat cheeses. *Trends in Food Science & Technology, 9*(6), 249-254.

Sala, G., van Aken, G. A., Stuart, M. A. C., & van de Velde, F. (2007). Effect of droplet matrix interactions on large deformation properties of emulsion-filled gels. *Journal of Texture Studies, 38*(4), 511-535.

Tabilo-Munizaga, G., & Barbosa-Cánovas, G. V. (2005). Rheology for the food industry. *Journal of Food Engineering, 67*(1–2), 147-156.

Tidona, F., Criscione, A., Guastella, A. M., Zuccaro, A., Bordonaro, S., Marletta, D. (2009). Bioactive peptides in dairy products. Italian journal of animal science, 8 (3), p. 315-340.

Truong, V. D., & Daubert, C. R. (2000). Comparative study of large strain methods for assessing failure characteristics of selected food gels. *Journal of Texture Studies, 31*(3), 335-353.

Ustunol, Z., Kawachi, K., & Steffe, J. (1995). Rheological properties of cheddar cheese as influenced by fat reduction and ripening time. *Journal of Food Science, 60*(6), 1208-1210.

van den Berg, L., Carolas, A. L., van Vliet, T., van der Linden, E., van Boekel, M. A. J. S., & van de Velde, F. (2008). Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. *Food Hydrocolloids, 22*(7), 1404-1417.

van Vliet, T. (1988) Rheological properties of filled gels. Influence of filler matrix interaction. Colloid and Polymer Science. Volume 266, (6) 518-524.

Vartiainen E., Laatikainen T., Peltonen, M., Juolevi, A., Männistö, S., Sundvall, J., Jousilahti, P., Salomaa, V., Valsta, L., Puska, P. (2010). Thirty-five-year trends in cardiovascular risk factors in Finland. International Journal of Epidemiology, 39 (2) 504–18.

Verheul, M. and Roefs, S. P. F. M. (1998). Structure of Particulate Whey Protein Gels: Effect of NaCl Concentration, pH, Heating Temperature, and Protein Composition. *Journal of Agricultural and Food Chemistry. 46* (12), 4909-4916.

Yost, R. A., & Kinsella, J. E. (1992). Microstructure of whey protein isolate gels containing emulsified butterfat droplets. *Journal of Food Science, 57*(4), 892-897.

Yost, R. A. (1993). Properties of Acidic Whey Protein Gels Containing Emulsified Butterfat. *Journal of food science,* 58 (1), p. 158-163.

Table 2-1. COMPOSITION OF HEAT SET WHEY PROTEIN ISOLATE GELS.

Treatment	Particulate	Stranded	Homogenized Fat Composite	Native Fat Composite-Large	Native Fat Composite-Small
Protein (%w/w)	10%	10%	10%	10%	10%
NaCl (mM)	150	50	150	150	150
Fat (%w/w)	-	-	18%	18%	18%
Fat Globule Condition	-	-	homogenized	native	native
Particulation	mechanical shear	mechanical shear	mechanical shear	mechanical shear	mechanical shear + homogenize

Table 2-2. FRACTURE AND LARGE STRAIN RHEOLOGICAL VALUES OF WHEY PROTEIN GELS (tested as macro gels).

Fat (% w/w)	Protein (%w/w)	NaCl (mM)	Fat Globule Condition	Gel Network	Fracture Stress (kPa)	Fracture Strain	Recoverable Energy (%)
0	15	150	native	particulate	71.9^{a}	0.9^{cde}	47.8^{c}
9	15	150	native	particulate	51.6^{b}	0.81^{de}	40.0^{d}
0	12	150	native	particulate	44.5^{bc}	1.15^{bcd}	49.0^{c}
9	12	150	native	particulate	35.8^{cd}	0.73^{e}	41.1^{d}
18	10	50	native	stranded	32.2^{de}	1.42^{b}	46.1^{c}
18	10	100	native	mixed	28.7^{def}	1.44^{b}	38.7^{d}
18	10	150	homogenized	particulate	23.1^{efg}	1.38^{b}	30.7^{e}
18	10	150	native	particulate	21.4^{fg}	1.48^{b}	32.6^{e}
0	10	100	native	mixed	21.5^{fg}	1.26^{bc}	62.6^{b}
0	10	150	native	particulate	17.4^{gh}	0.92^{cde}	45.5^{c}
0	10	50	native	stranded	10.3^{h}	1.91^{a}	69.3^{a}

Samples with the same letter within a column were not significantly different ($p < 0.05$).

Table 2-3. TEXTURE TERMS USED FOR DESCRIPTIVE SENSORY ANALYSIS.

Descriptive Term	Definition	Technique	Reference
Hand firmness	The amount of force required to completely compress the sample.	Press completely through the sample using the thumb and first two fingers.	3 = Process cheese, 7 = Muenster, 10 = Sharp Cheddar, 14 = Parmesan
Hand springiness	The total amount of recovery of the sample.	Press the sample between the thumb and first two fingers until it is depressed 30%.	1 = Parmesan, 4 = Process cheese, 7 = Sharp Cheddar, 13 = Muenster
Hand rate of recovery	The rate at which the sample recovers (i.e.<comma> the speed at which the sample returns to its original shape).	Press the sample between the thumb and first two fingers until it is depressed 30%.	1 = Feta, 4 = Process cheese, 7 = Muenster
Firmness	The amount of force required to completely bite through the sample.	Completely bite through the sample using the molars.	3 = Process cheese, 7 = Muenster, 10 = Sharp Cheddar, 14 = Parmesan
Fracturability	The amount of fracturability in the sample after biting.	Completely bite through the sample using the molars.	1 = Process cheese, 5 = Sharp Cheddar, 14 = Feta
Degree of breakdown	The amount of breakdown that occurs in the sample as a result of mastication (i.e. the amount of meltability or dissolvability).	Chew the sample 5 times and evaluate the chewed mass.	1 = Parmesan, 10 = Sharp Cheddar, 14 = Process cheese
Cohesiveness	The degree to which the chewed mass holds together.	Chew the sample 5 times and evaluate the chewed mass.	1 = Parmesan, 3 = Feta, 9 = Muenster, 14 = Process cheese
Adhesiveness	The degree to which the chewed mass sticks to mouth surfaces.	Chew the sample 5 times and evaluate the chewed mass.	1 = Parmesan, 7 = Muenster, 12 = Feta, 14 = Process cheese
Smoothness of mass	The degree to which the chewed mass surface is smooth (i.e. evaluation for gritty or grainy particles).	Chew the sample 5 times and evaluate the chewed mass.	1 = Parmesan, 3 = Feta, 8 = Muenster, 14 = Process cheese
Smoothness of mouth coating	The degree of smoothness felt in the mouth after expectorating the sample.	Chew the sample 5 times<comma> expectorate<comma> and evaluate the residual in the mouth.	1 = Parmesan, 5 = Feta, 10 = Muenster, 14 = Process cheese

Figure adapted from Brown et al. (2003).

Table 2-4. DESCRIPTIVE SENSORY TERMS RELATING TO FORCE-DEFORMATION (a) AND BOLUS FORMATION (b) OF WHEY PROTEIN MACROGELS.

Force-Deformation Terms (a)

Treatment	Hand Firmness	Hand Springiness	Hand Rate of Recovery	Firmness	Fracturability
Stranded Gel	9.4 A	14.8 A	15.0 A	4.2 A	11.1 A
Particulate Gel	8.2 B	14.5 A	14.4 AB	4.3 A	11.5 A
Homogenized Fat Composite Gel	6.3 C	14.3 A	13.7 B	3.3 B	5.0 B
Native Fat Composite Gel	6.8 C	14.2 A	14.0 B	3.3 B	3.8 C

Bolus Formation Terms (b)

Treatment	Degree of Break Down	Cohesion	Adhesion	Smoothness of Mass	Smoothness of Mouth Coating
Stranded Gel	0.29 B	0.2 C	0.2 C	13.9 A	13.0 A
Particulate Gel	0.50 AB	1.4 B	3.4 B	8.5 B	7.6 B
Homogenized Fat Composite Gel	0.89 AB	2.9 A	7.6 A	3.8 C	4.0 C
Native Fat Composite Gel	1.0 A	2.7 A	7.7 A	3.7 C	4.5 C

Samples with the same letter within a column were not significantly different ($p < 0.05$).

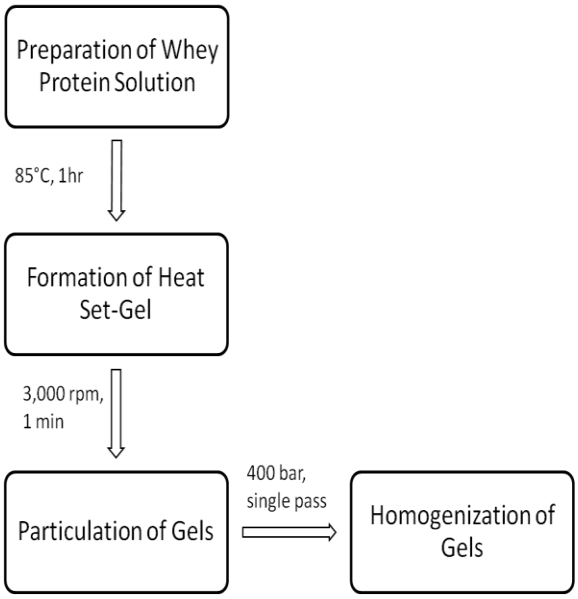


Figure 2-1. Production schematic for the manufacture of whey protein particles.

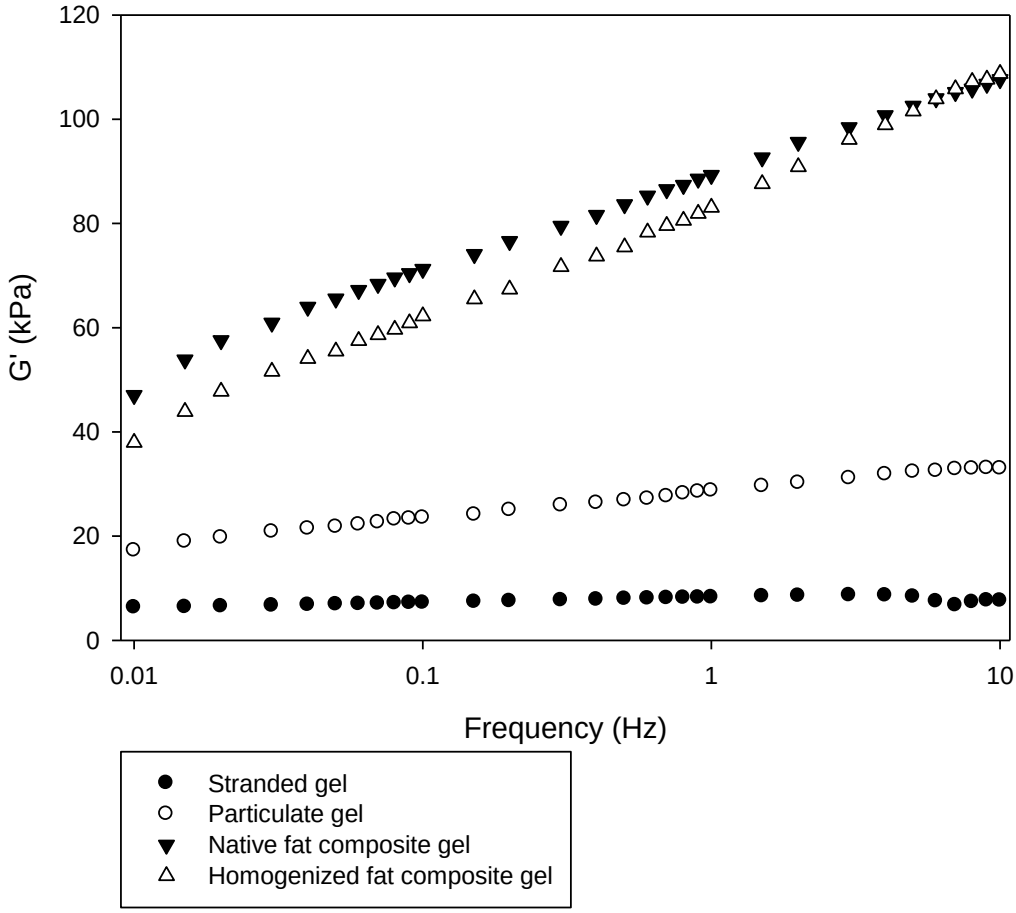


Figure 2-2. Frequency Sweep of whey protein gels at 25°C. Gels were tested at normalized stress values corresponding to a strain value of 3.6E-3 at 25°C , which was well within the LVR for all treatments.

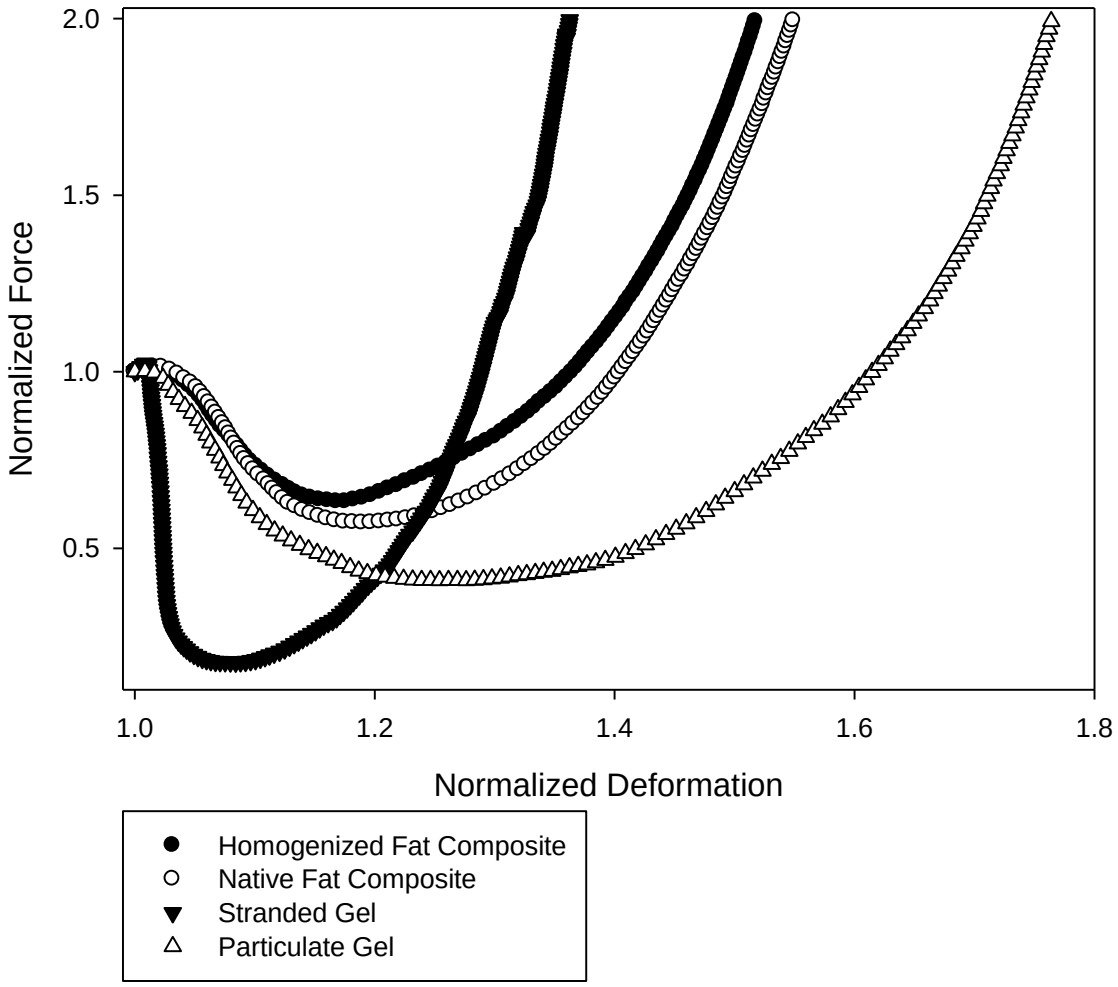
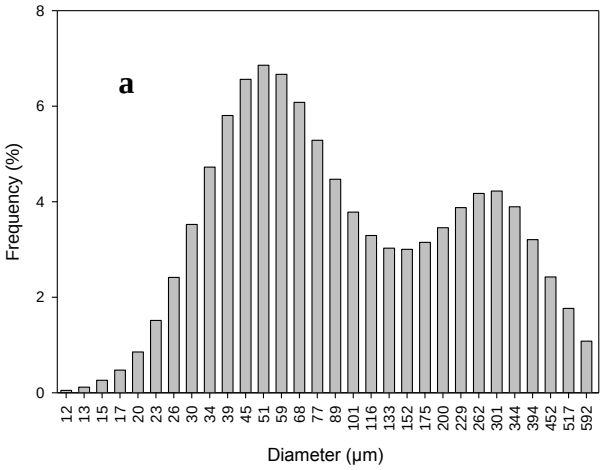
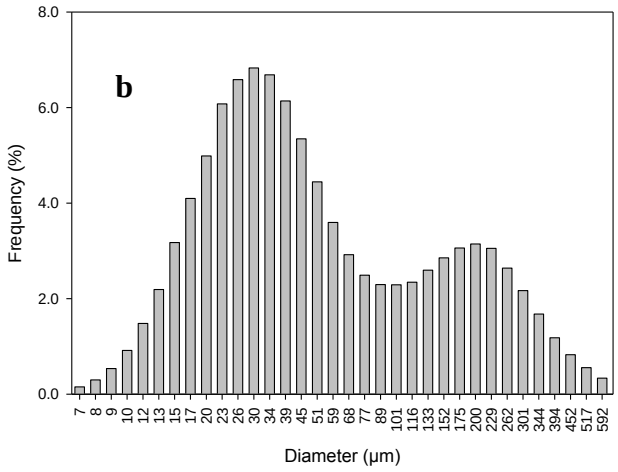
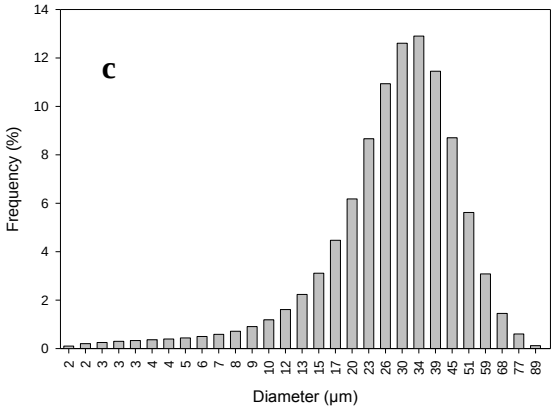


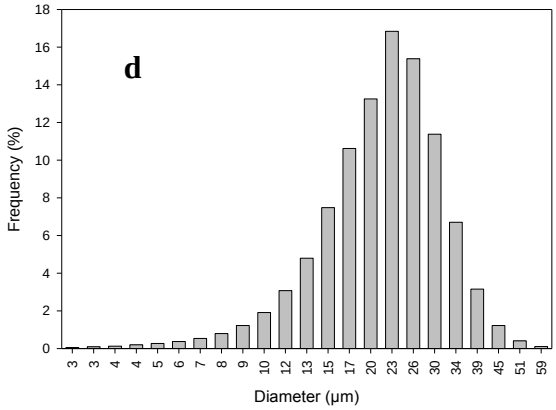
Figure 2-3. Post Fracture Patterns of Gels.

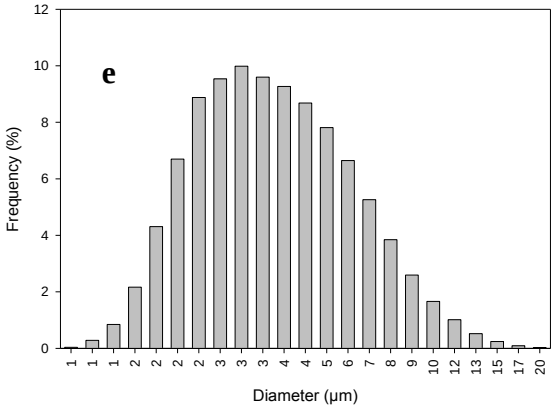
Figure 2-4. Particle size analysis of stranded (a), particulate (b), native fat composite (c), homogenized fat composite (d), and homogenized gel (e). Data are the average of 5 replications.


a
Frequency (%)
0
2
4
6
8
12 13 15 17 20 23 26 30 34 39 45 51 59 68 77 89 101 116 133 152 175 200 229 262 301 344 394 452 517 592
Diameter (µm)


b
Frequency (%)
0.0
2.0
4.0
6.0
8.0
7 8 9 10 12 13 15 17 20 23 26 30 34 39 45 51 59 68 77 89 101 116 133 152 175 200 229 262 301 344 394 452 517 592
Diameter (µm)


c
Frequency (%)
Diameter (µm)


d
Frequency (%)
Diameter (µm)


e
Frequency (%)
Diameter (µm)

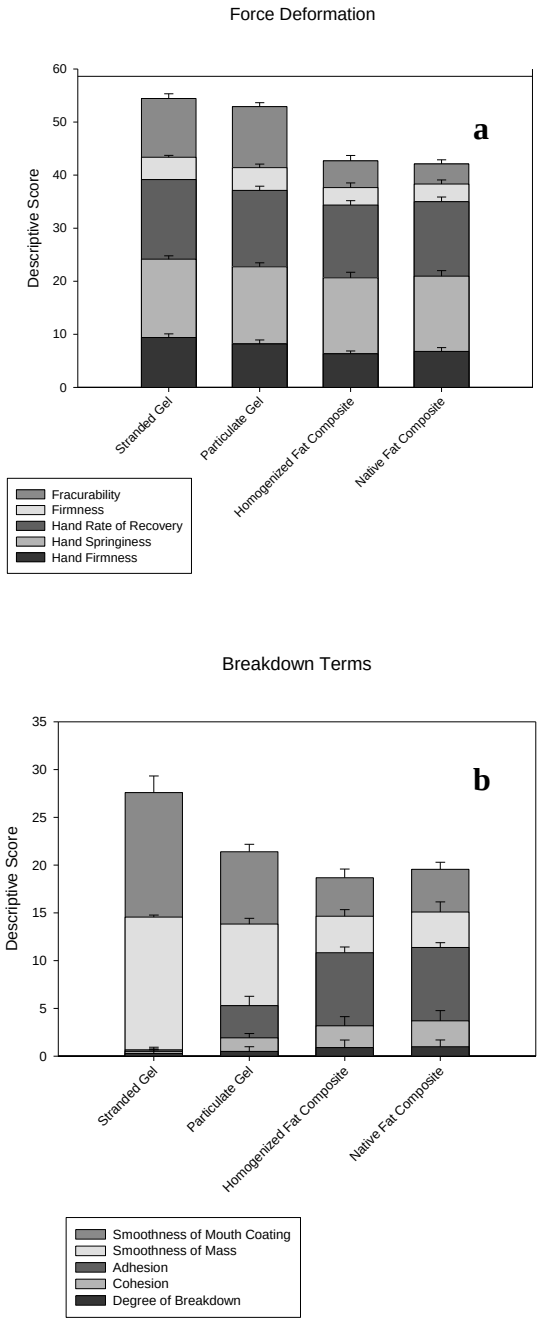
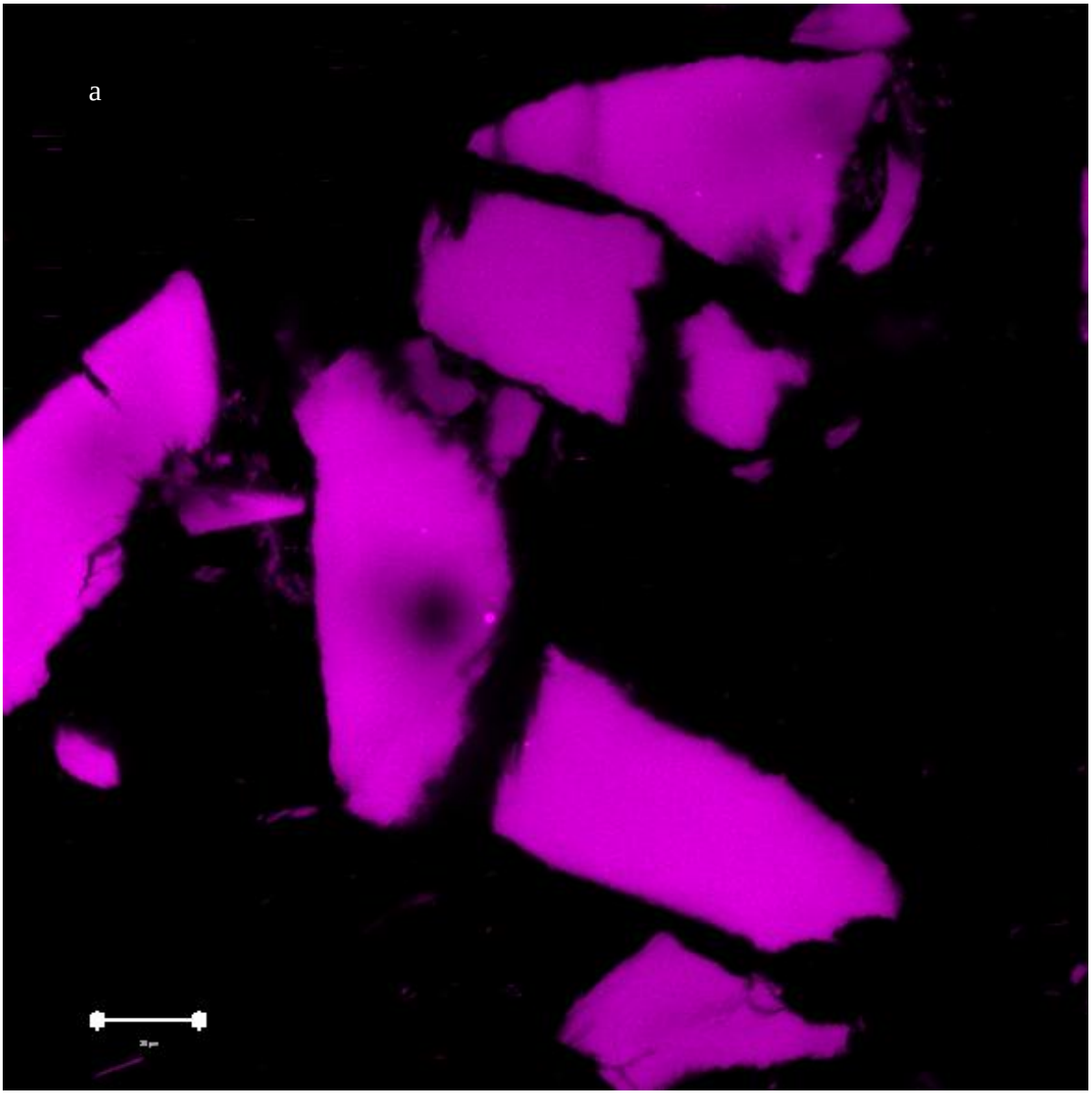
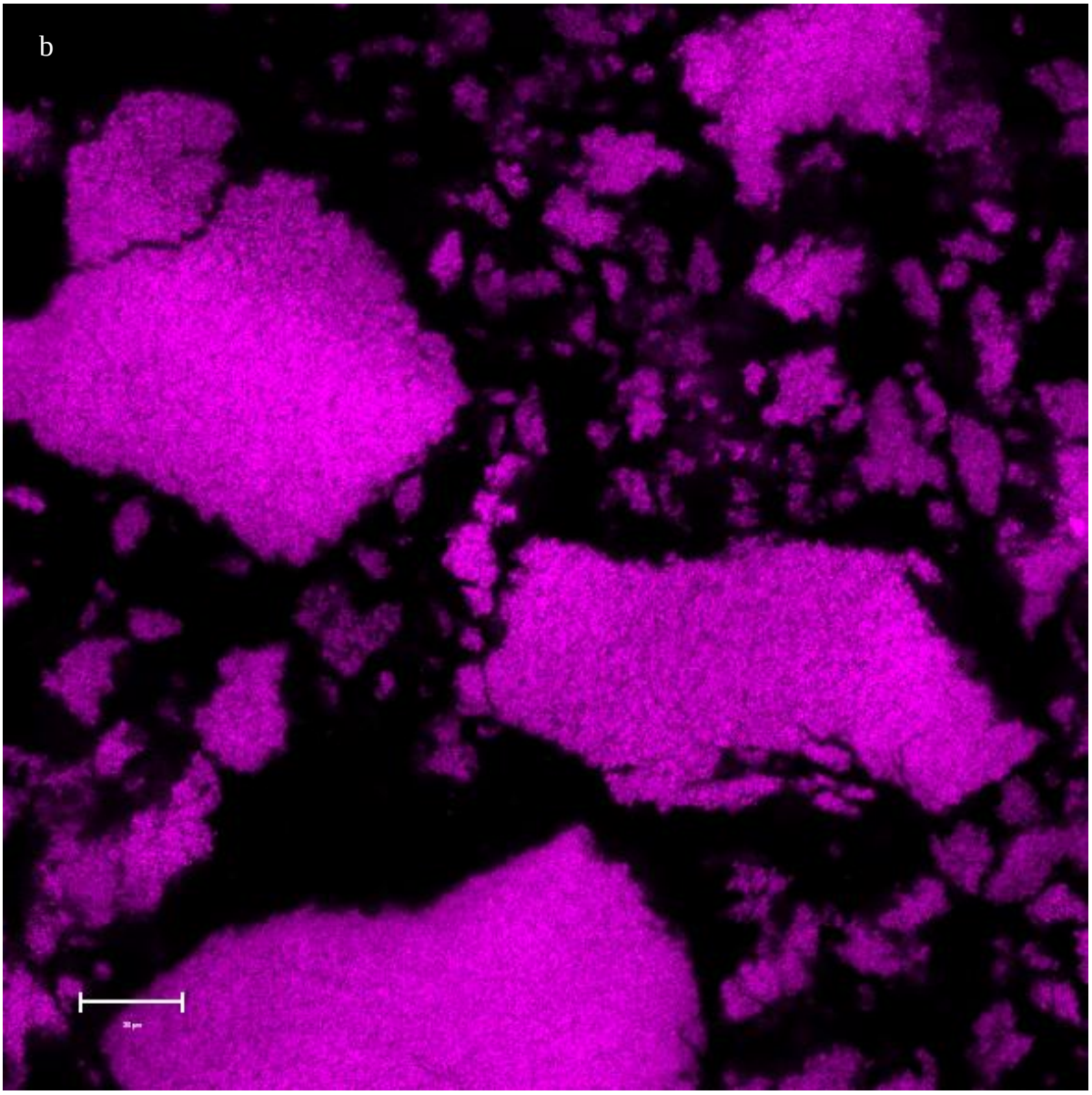
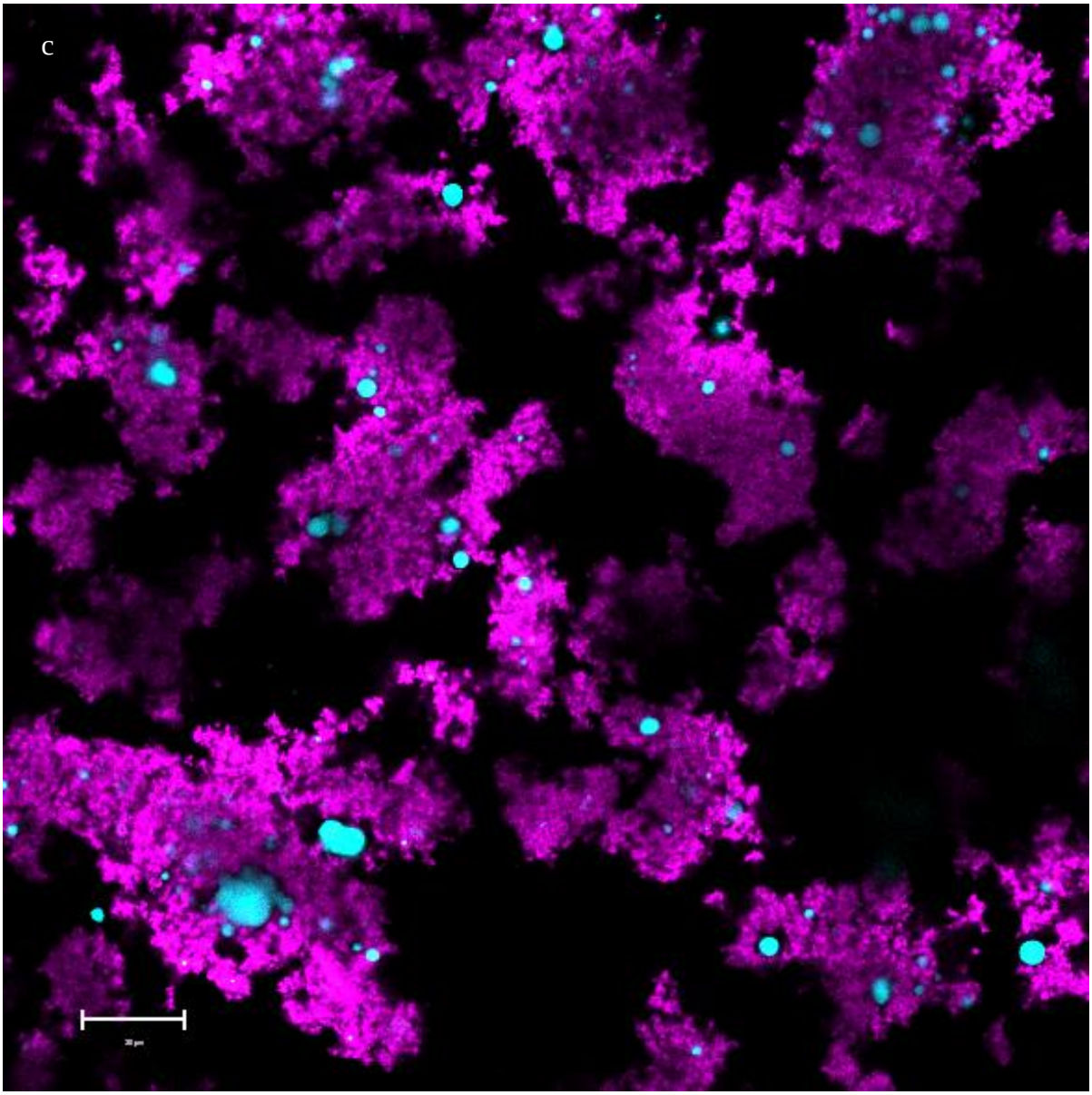


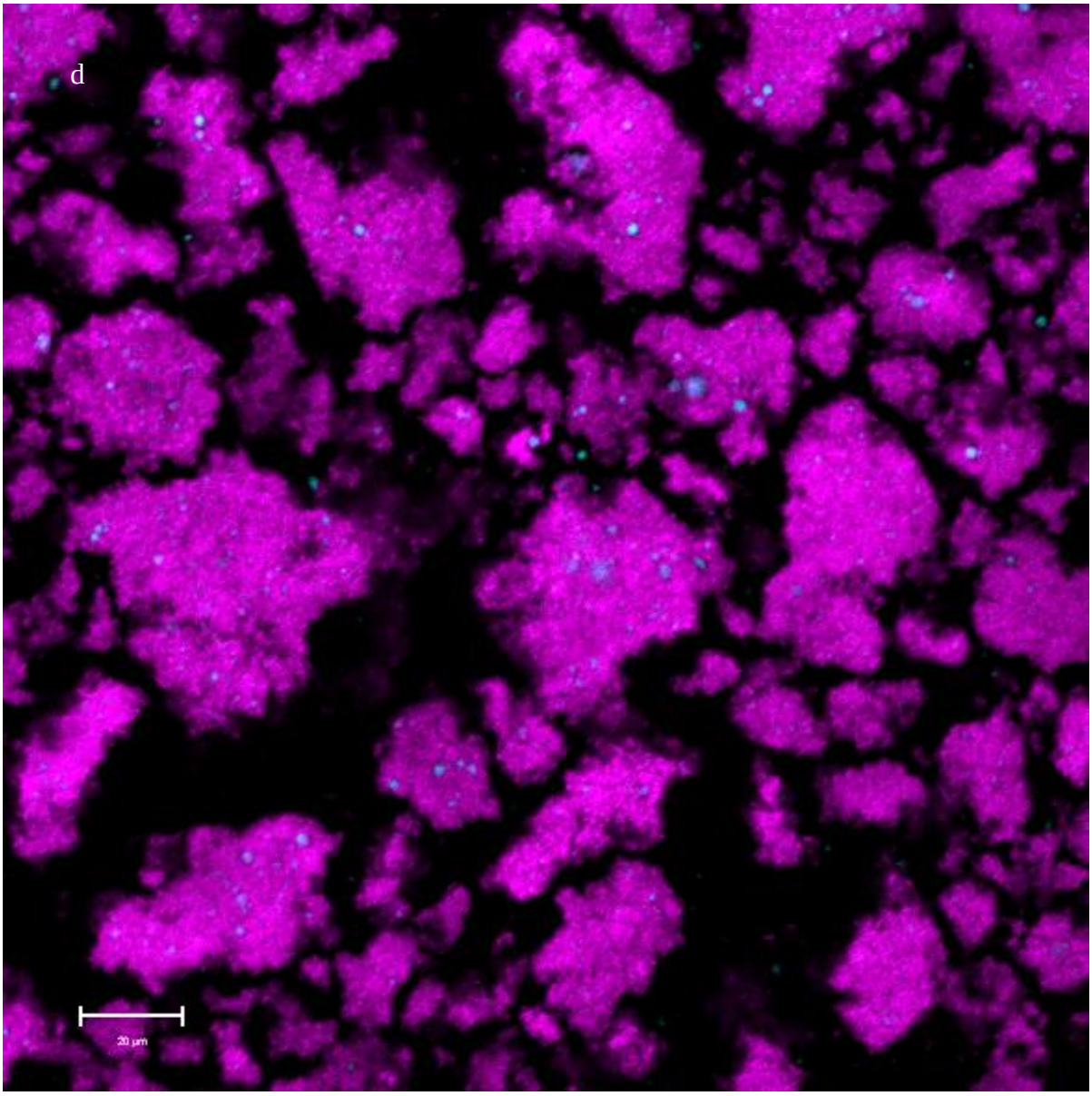
Figure 2-5. Descriptive Sensory Texture of Whey Protein Macrogels. Force-Deformation (a) terms correspond to initial gel fracture properties, and Bolus Formation (b) terms relate to oral processing.

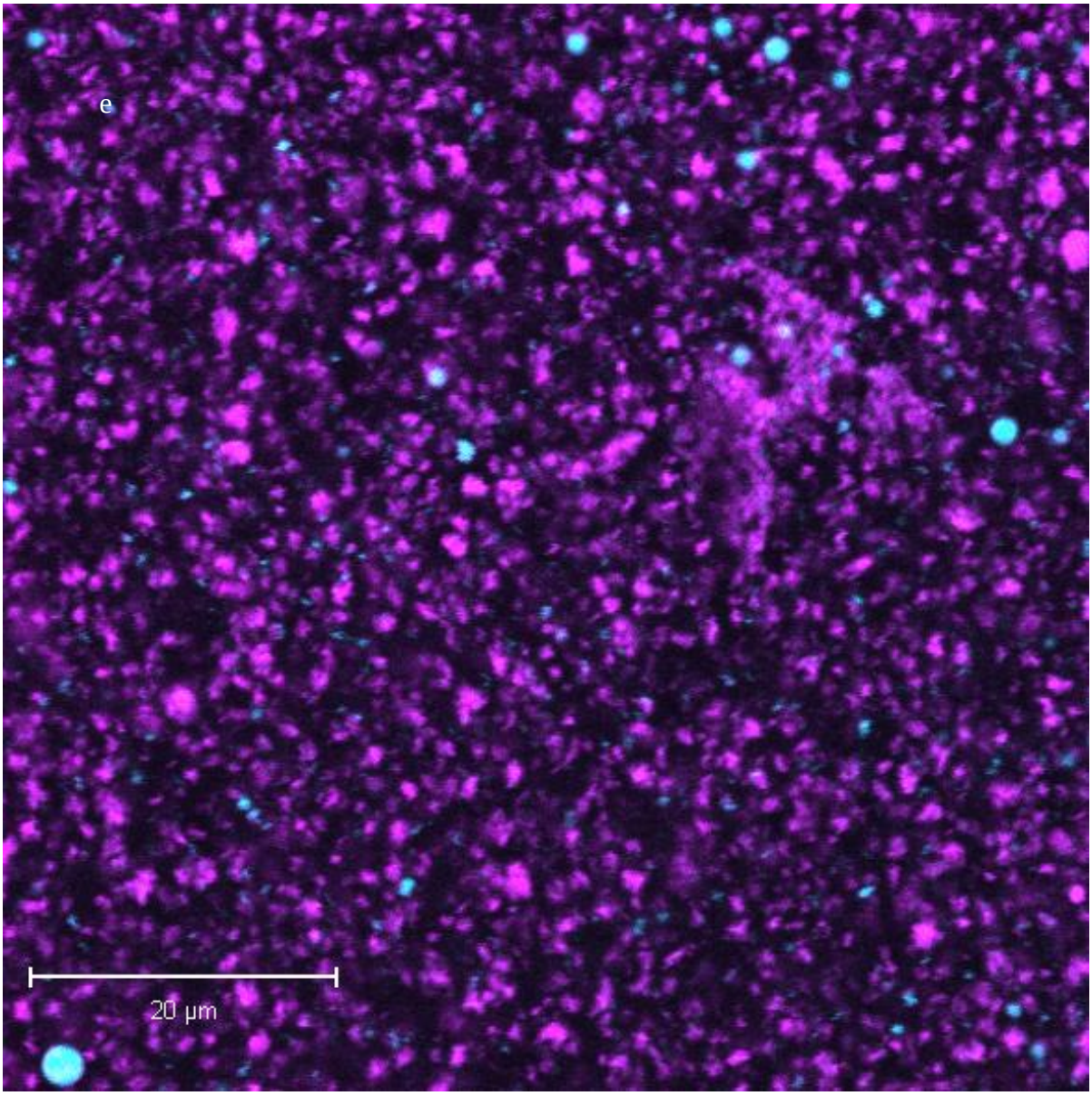
Figure 2-6. (a) Stranded gel particles, (b) Particulate gel particles, (c) Native fat composite gel particles, (d) Homogenized fat composite particles, (e) Homogenized gel particles, (f) Native fat composite particles - cross sectional analysis, (g) Native fat composite particles - cross sectional analysis.

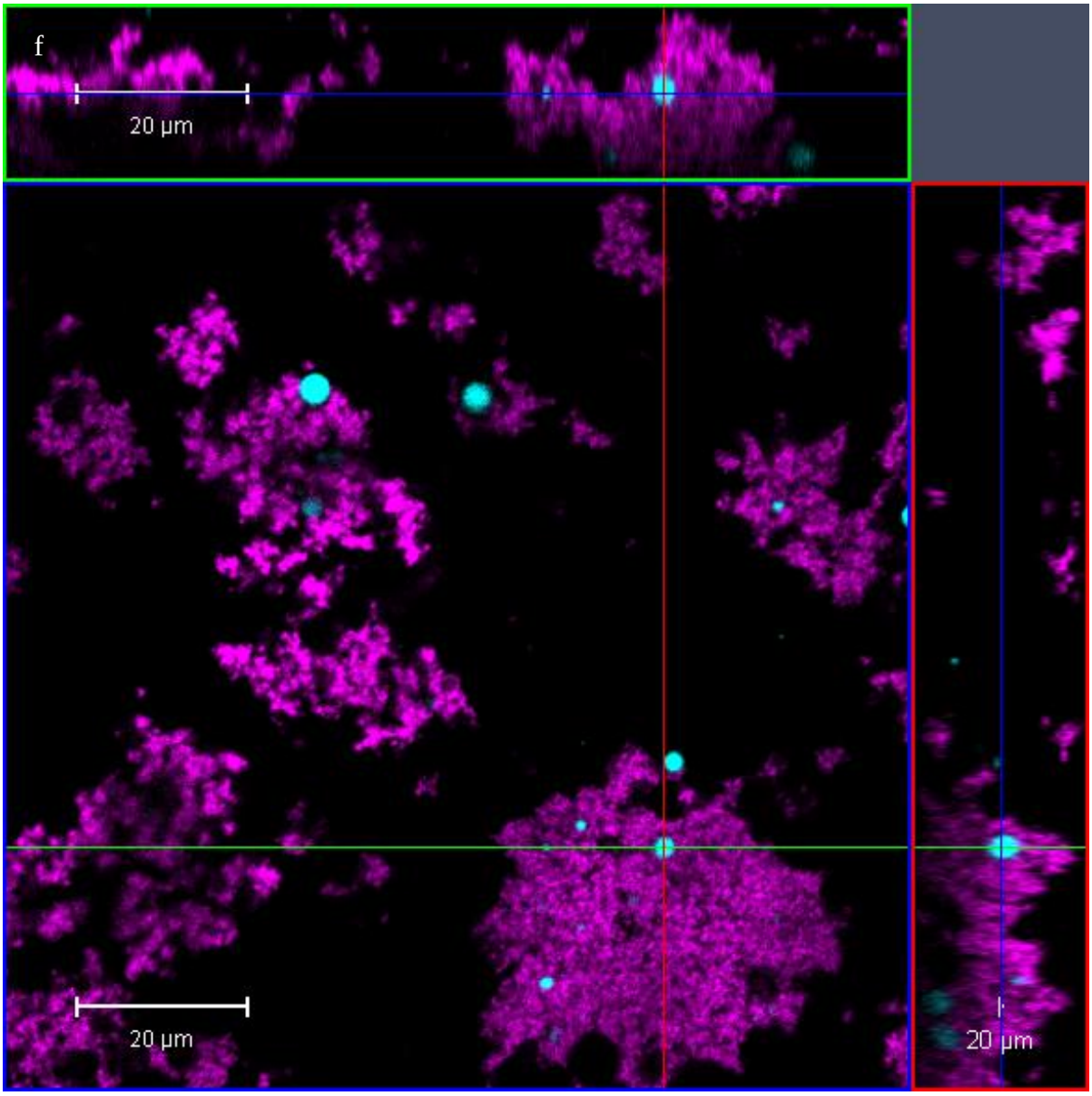

a

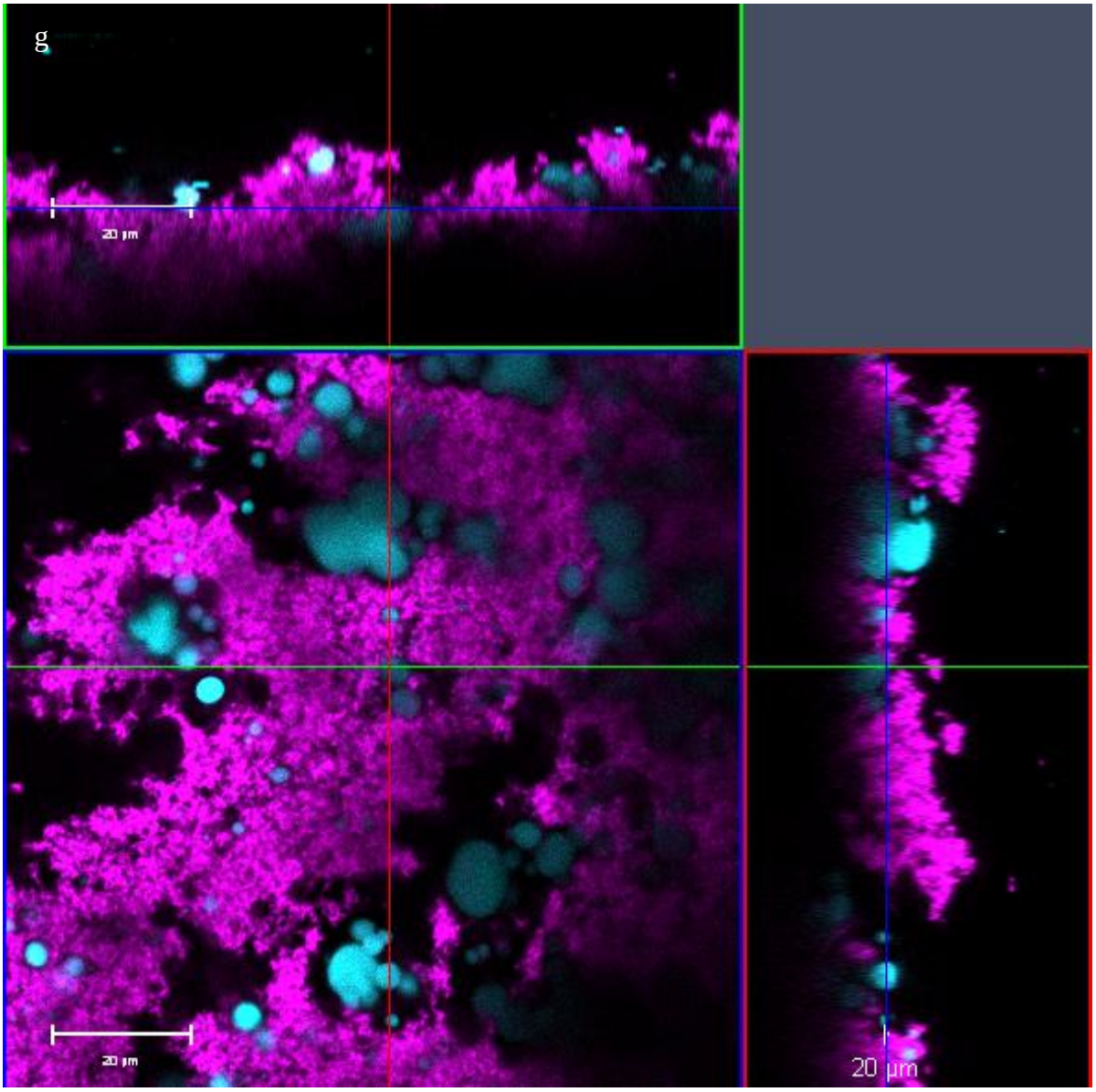

b


c


d
20 µm


e
20 µm


f
20 µm
20 µm
20 µm


g
20 µm
20 µm
20 µm

CHAPTER 3. EVALUATION OF LOW FAT CHEDDAR CHEESE CONTAINING WHEY PROTEIN PARTICLE PARTICLES.

Department of Food, Bioprocessing, and Nutritional Sciences

North Carolina State University

1. ABSTRACT

Whey protein particles (developed in Chapter 2) were incorporated into Cheddar cheese to assess their efficacy as a fat mimetic in a low fat product. Confocal microscopy images confirmed particle retention in cheese, and the phase volume of composite particles was estimated to be 93%, 62%, 67%, and 100% in 4 respective replications. The fracture stress of particle filled gels was, with two exceptions, lower than the low fat control cheese and equal to or greater than the full fat control. This confirmed the desired "soft filler" effect of the added particles. Some, but not all, of the particle treatment cheeses had values for recoverable energy that were similar to the full fat control, although efficacy was not consistent across replications. Similarly, specific replications of cheeses showed improved sensory texture with greater values for cohesion and smoothness of mouth coating. Particle treatments suggest potential for use as a fat mimetic in low fat Cheddar cheese.

2. INTRODUCTION

Over the past decade cheese consumption has been growing to the extent that the average American now eats over 33(lbs) per annum (USDA, 2012). Along with this growth there has developed a concurrent desire for cheeses with reduced levels of fat without loss of sensory quality. However, cheese is a complex heterogeneous system, and the removal of fat frequently results in flavor and textural differences rendering the low fat version less appealing than the full fat original (Tunick, 2000). Manufacturers have therefore adopted strategies to account for the functionality of fat (reviewed in Chapter 1), largely consenting that low fat cheese should 'taste as close to the original as possible' (Archwamety, 2007).

The use of fat mimetics is a common approach for producing low fat products. In cheese, this strategy is especially applicable, as the removal of the fat phase concentrates the continuous protein matrix creating textural defects such as increased firmness and springiness (DMI, 2006). Cheddar cheese is well understood as a 'filled gel,' and thus this model (reviewed in detail in Chapter 1) suggests the potential of methodology targeting an emulation of the structural breakdown pathway to produce the desired texture, rather than the physio-chemical properties of fat. In essence this would improve low fat Cheddar cheese texture by inducing disruptions in the casein matrix that are similar to those caused by fat particles.

As noted by Lucey et al.(2003), descriptive terms used by cheese graders or product judges, such as body and texture, can be misleading as they are often rooted in traditional connotations. Thus texture, as it pertains to this discussion, is 'all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors' (Lawless and Heymann, 2010). Therefore, success of a low fat product will be measured by how close the sensory texture and flavor terms are to a full fat cheese, or their improvement from the low fat control.

Cheddar cheese can be characterized as possessing an intermediate texture which neither crumbles nor shatters when deformed (Fox, 1987). This texture is determined in the initial formation by the packing of the curd particles, and with aging, by equilibration of ions from casein micelles and proteolytic activity (Lucey et al. 2003). The most marked changes occur during the first few weeks of ageing with a steady breakdown continuing throughout

subsequent cheese ripening (Lawrence et al., 1987; Rogers et al. 2009). Proteolysis, as it pertains to cheese, is a series of processes induced by a combination of the cheese coagulant (i.e, rennet), enzymes from the milk, as well as enzymes from starter and non-starter bacteria (Fox, 1988).

Further complicating the evaluation of cheese texture is its inherent variation, even among batches (Lawrence et al., 1987). Thus, a series of sensory, chemical, and physical methods have been developed to assess and monitor cheese texture quality. This need is compounded by the fact that industrial sized batches of cheese often experience a degree of texture variation even within the same block of product. Carunchia Whetstine et al. (2007) reported differences within the same 291kg block of cheese, with the inner portion exhibiting a more intense flavor, while the outermost sections had a more cohesive sensory texture.

There exists no clear method to visually determine which blocks of Cheddar have acceptable textural properties (Lawrence et al. 2004), and thus a variety of mechanical and sensory tests are often used for this purpose. The most basic tests include production observations, such as a hand evaluation of curd strength, for discernment of defects and quality control, but these do not provide an analytical parameter to compare differences among products. Other methods include empirical instrumental testing, but again, are highly specific to the process and application. One such test, the instrumental texture profile analysis method (TPA) (Bourne, 1968), uses a 2 cycle compression at constant deformation rate to generate texture terms. These measures have often been found to correlate well to sensory terms, and thus TPA is most effective in systems in which these relationships are well understood. Although these empirical tests allow the generation of product specific

attributes and comparisons across batches, they are not designed to reflect any fundamental understanding of the structural elements generating the properties. In contrast, fundamental tests are used to assess the specific structural and rheological properties of a food system. Small-strain tests are used to measure rheological properties under conditions that do not cause damage to the structure of material being tested. Properties such as the storage (G') and loss (G") modulus, determined within the linear range, characterize the viscoelasticity of a material (Steffe, 1996). Other tests such as torsion (Diehl et al., 1979) and uni-axial compression are used to assess non-linear and fracture properties. These large-strain deformation tests often generate multiple forces acting upon the sample. While this may complicate measurement of a precise rheological property, it also allows for a greater correlation with oral processing terms, where a variety of forces are enacted upon a system during mastication (Everard et al. 2006).

Although instrumental measurements are important for discerning specific rheological properties and linking properties with food structure, sensory tests provide the human assessment of texture as related to the consumer experience incurred during cheese consumption. This includes oral processing parameters such as saliva and the multiple physical forces incurred during mastication, as well as the cognitive aspects of integrating sensory inputs into a mental representation of texture. These sensory tests can be divided into two major categories; affective tests, which utilize an untrained consumer panel, and descriptive, which employ a panel highly trained in assessing specific attributes, the latter of which is most useful for an analytical approach to understanding texture (Lawless and Heymann, 2010).

A conceivable drawback to sensory analysis is that it is both expensive and laborious (Xiong et al. 2003), and thus efforts to create mechanical tests capable of measuring sensory properties have been actively perused. Several studies have attempted to relate fundamental rheological tests, which provide information on material properties, to their resultant sensory attributes (Drake et al. 1999; Gwartney et al. 2002; Rogers et al. 2009). However, as cheese is a viscoelastic material, it is both rate and temperature dependent. In order to make stronger correlations between physical and sensory data, as suggested by Foegeding and Drake (2007), mechanical tests are generally conducted under conditions which best approximate those during mastication.

The primary objective of this study was to evaluate the efficacy of whey protein particles (Chapter 2) for improving the rheological and sensory texture of low fat Cheddar cheese. Our approach therefore was to first assess the ability of the mircogel particles to affect cheese texture. Drawing on this data, we then sought to employ a particle design type with the specific rheological properties most effective for emulating the breakdown pathway of a full-fat Cheddar cheese. Chapter 2 demonstrated the wide spectrum of particle types, with differing rheological and sensory texture, which may be achieved using our method of gel production. In this experiment, we produced Cheddar cheeses containing various particle types to test our hypothesis that whey particles, if added to a low fat cheese at an equal volume fraction as fat in a full fat Cheddar, would be able to mimic the breakdown pattern of fat and induce a full fat like texture.

3. Materials and Methods

3.1. Particle Filled Cheese Production

The procedure for making particles is outlined in Chapter 2. Particle particles were produced at North Carolina State University (Raleigh, NC) and shipped overnight in a temperature controlled environment to the Gary Haight Richardson Dairy Products Laboratory at Utah State University (Logan, Utah) where cheeses were produced. Milk was pasteurized at 73 °C for 15 sec and adjusted to a pH of 6.65 using distilled white vinegar. Skim (0.2% fat) was used for treatment cheese. After a set temperature of 31 °C was achieved, a DVS 850 *Lactococcus lactis* starter (Chr. Hansen Inc.: Milwaukee, WI, USA) was added. Particles, suspended in deionized water at a dilution of either 1:2 (w/w) or 1:4 (w/w) were stirred vigorously to ensure dispersion before incorporation into milk. Cheeses were coagulated using double strength rennet at a concentration of 34 ml/1000 lbs of milk. Cheeses were aged at 7 °C and were periodically evaluated for rheological and sensory parameters. Experiments 1 and 2 were conducted in replication (experiment 3 was not). Cheeses were evaluated for rheological and sensory values with at least 5 sub samples per measurement.

3.2 Proximate Analysis

The proximate analysis was conducted on cheeses 5 days after production by collaborators at Utah State University. Cheese was grated and mixed with deionized water before stomaching to achieve a homogenized slurry. This was filtered using a Whatman #1 filter (Maidstone: Kent, UK) and then analyzed for salt using a Corning 926 chloride analyzer (Medfield, MA, USA). Fat analysis was determined using the Babcock method,

while moisture was determined by weight difference using a CEM microwave oven (Indian trail, NC, USA).

3.3 Small Strain Analysis

3.3.1. Determination of the Linear Viscoelastic Region

The method was followed as described in Chapter 2.

3.3.2. Controlled Temperature Frequency Sweeps

The method was followed as described in Chapter 2.

3.4. Large Strain Rheological Tests

The method was followed as described in Chapter 2.

3.5. Fracture Analysis

The method was followed as described in Chapter 2.

3.6. Post Fracture Analysis

The method was followed as described in Chapter 2.

3.7 Single Edge Notch Bend Testing

The Single Edge Notch Bend is designed to measure the fracture toughness and the energy at break of brittle materials. The method followed was modified from that as described by Williams and Cawood (1990). Rectangular blocks of cheese were cut with dimensions of 60 mm length, 12 mm width, and 6 mm breadth. A notch 5.5 mm deep was placed into the center of each sample, in the direction of the width, using a calibrated razor blade. Samples were analyzed at room temperature (22 ±2 °C), and an Instron 5565 rheometer with a max load cell of 50N (Instron; Norwood, MA) was used to measure samples at a constant deformation rate of 0.2 mm/s. Cheese fracture toughness (K_c) and

Fracture Energy (G_c) were calculated using equations 5 and 6 respectively (Williams and Cawood, 1990). The variables are defined as follows: a = notch depth (m), H = height (m), A = notch to depth ratio (calculated as $A = a/H$), B = breadth (m), P = max load (N), U = area, and φ is a calibration factor for A.

$$K_c = f\ A\ * \frac{P}{BH^{0.5}} \quad \text{(Eq. 3.1)}$$

$$G_c = \frac{U}{BH\varphi} \quad \text{(Eq. 3.2)}$$

3.7. Descriptive Sensory Texture Analysis

The method was followed as described in Chapter 2.

3.8. Confocal Scanning Laser Microscopy

The method was followed as described in Chapter 2.

3.9. Particle Retention Calculations

The formulated fat percentage in the composite type particles was used to estimate the particle retention in the experiment 2 cheeses. Composite gels were formulated to 18% fat (w/w), and were subjected to the particulation method as described in Chapter 2. In order to account for fat loss during particulation, the composite particles were suspended in deionized water and centrifuged at 10,000 x gravity for 5 minutes. The serum (which contained the liberated fat) was decanted, and the remaining fat content of the particles was determined using a modified Mojonnier method (AOAC 974.09). Thus, using the ratio of fat

retained in the particles after milling, an estimate for particle retention in the cheese could be established.

$$Ratio\ Retained\ in\ Microgels = \frac{Fat\ \%\ of\ microgels\ after\ particulation}{18} \quad \text{(Eq.3.3)}$$

3.11 Statistical Analysis

For experiment 2, separate linear models with mixed effects (PROC MIXED) were fit to each of the rheological and sensory responses. These models included fixed effects for treatment, age and their interaction, as well as random effects for replication and replication-treatment-age interactions to accommodate the subsamples made on each experimental unit. All analyses were conducted using SAS statistical software (version 9.2, SAS Institute Inc., Cary, NC).

4. RESULTS AND DISCUSSION

The objective of this experiment was to determine the efficacy of the whey particles (developed in chapter 2) for improving the texture of low fat Cheddar cheese. Thus, particle filled cheeses were manufactured in 3 subsequent intervals (labeled experiments 1-3) with modifications made after assessing the results of the previous experiment(s). The goals of each session of cheese making are listed below:

Experiment 1: Assess whether cheeses containing particles could be successfully manufactured (i.e. containing no make defects), and secondly, how each particle type

impacted cheese texture. Cheeses were produced in replicate and tested after 34 weeks ageing.

Experiment 2: Determine particle retention fraction in cheeses and evaluate efficacy of additional particle types. Cheeses were produced in replicate and tested at 8 and 24 weeks ageing.

Experiment 3: Evaluate effects of different particle volume fractions on cheese texture. Additionally, the homogenization pressure used for gel particulation was examined to explore its role as a potential source of variation across treatment cheese replications. Cheeses were produced and tested after 8 weeks ageing.

4.1. Experiment 1 Cheeses

Particulate, stranded, and composite type particles were selected to provide a broad spectrum of gel types with differing textural properties (Table 3-1). Due to an inherent limitation in this approach, which will be discussed, an analyzed qualitative analysis of results will be presented.

4.1.1 Cheese Composition

Particles were added to the milk so as to produce a 33% total filler phase volume in the finished cheese. Notably, this volume assumed 100% particle retention. However, cheese makers observed that particles were visible in whey and in the cheese troughs after draining. It is therefore highly probable that many of the cheeses experienced sub-optimal retention.

This effect may account for the lack of differences among treatments. It was not possible to measure particle retention in these treatments. Fat content of particle treatments and the low fat control were similar (Table 3-2), but since particle retention was not measurable, it was an un-controlled variable.

4.1.2. Confocal Microscopy

Particle filled cheeses were labeled using a Nile Blue dye. Fat is represented in green, the continuous casein phase in purple, and the whey particles as a lighter pink (Figure 3-1). All scale bars are 20 μm. The stranded and particulate gels encompassed a wide range of particle sizes after mechanical milling, yet the particles incorporated into the cheeses appear to be more homogenous in size and very near that of the coalesced fat globules (Figures 3-1a-d). This suggests the large particles were settling too quickly after renneting and that particle retention may be lower than the targeted volume fraction. Composite gels were made using a commercial half and half to get an indication if fat would have an effect (Table 3-1). The homogenized fat structure resulted in a smaller globule size within the particles as compared to the native fat contained in the casein matrix (Figure 3-1e; Figure 3-1f). However, as many of the particles incorporated into the cheese were at the smaller end of the distribution, much of the fat embedded within the composite particles appeared liberated. Due to their high volume fraction, full and homogenized fat controls exhibited a large degree of fat globule coalescence and thus were relatively difficult to differentiate on the basis of fat globule structure (Figure 3-1h; Figure 3-1i).

4.1.3. Large Strain and Fracture Rheology

Fracture stress (strength) and fracture strain (deformability) and recoverable energy of the cheeses are reported in Table 3-3. The cheeses were not well differentiated by fracture strain, which has been previously observed in Cheddar cheese (Lane et al. 1997; Rogers et al. 2009). In terms of fracture stress, the composite gel treatment-1 exhibited values similar to the full fat control. The composite treatment-2 was not as effective. In values of recoverable energy, which is a measurement of energy which is not lost to structural damage or flow during deformation within the non-linear region, all the treatment cheeses exhibited slightly lower values than the low fat control.

4.1.4. Descriptive Sensory Analysis

While the composite gel-1 exhibited rheological improvements from the low fat control, these results are largely subjugated by those of sensory analysis. Treatments were largely similar to the low fat control in force-deformation terms, with only the stranded-2 and composite-1 particles exhibiting some improvement in hand firmness (Table 3-4). In terms relating to bolus formation, the composite-2, stranded-2, and particulate-2 treatments were all improved from the low fat control in adhesion, and the composite-1 was improved in smoothness of mouth coating. However, while these treatments did elicit some slight nominal improvements, the general texture characterization of the particle filled cheeses was that there was little or no improvement from the low fat control.

4.1.5. Conclusions

While particle filled cheeses did not exhibit a marked improvement from the low fat control in experiment 1, there was some evidence that specific treatments could improve

cheese texture. Confocal images confirmed some degree of particle retention in the cheese, although notably, the retention fraction could not be quantified.

It was clear that a way to determine the percent particle retention was needed for proper evaluation of results. Also, the composite treatment-1 cheese was found to be the most effective with a reduction in fracture stress, as well as some slight improvements in sensory texture. This suggested that adding fat to the particles may improve the resultant cheese texture. For experiment 2, the composite treatment was altered to include a larger fat percentage within the particle (from 9% to 18%), and a method was developed to estimate its retention fraction in the cheese.

4.2. Experiment 2 Cheeses

Experiment 2 cheeses were manufactured using four different particles types: particulate, homogenized fat composite, native fat composite and homogenized gel (Table 3-1). All particles contained 150 mM NaCl which resulted in a particulate whey protein gel structure. The particulate particle treatment was the gel without fat. Composite gels contained either homogenized or native (un-homogenized) fat. Finally, the a native fat composite gel was subject to homogenization to produce homogenized gel particles. Results were first analyzed using a mixed model statistical analysis and this found little evidence of an age-treatment interaction (Table 3-5). Thus, to gain statistical power, the cheeses were pooled across age and replication. However, it should be noted that as in experiment 1, treatment cheeses exhibited a large degree of variation across replications. Differences among treatments within one replication can be viewed as an experiment and thus the individual values will also be included in the discussion

4.2.1. Particle Retention Calculations

One of the major challenges in adding particles to cheese was assuring their retention in the cheese curd and preventing their loss in the whey. To that end, a method was developed for particle quantification based on fat content. The fat content of the initial milk, particles, and finished cheeses were used to calculate the fat incorporated from the composite particles. The process of milling liberated fat from the particles. This fraction was calculated as the ratio of fat retained in the particles before and after particulation (Eq. 1).

$$Ratio\ Retained\ in\ Particles = \frac{8.9}{18} = 49.4\% \quad \text{(Eq. 3.4)}$$

While this methodological approach is valuable, it is limited to the assessment of only composite type particles containing fat (i.e. the homogenized gel and particulate particles are excluded). The method also assumes that the entire size range of particles created after milling is incorporated into cheese with no selectivity for the size incorporated. Particles must be towards the larger end of the size distribution (see chapter 2) to contain embedded fat globules. However, previous observations have indicated a preference for smaller particles to be incorporated into the cheese as a result of a more rapid settling rate of the larger particles. This evaluation method therefore might overestimate particle inclusion, as roughly half of the particles (Eq. 3-1) were particulated to a size where they no longer contain any fat, and should be understood as an approximate indicator.

The particles were added to the cheese serum to achieve a total filler volume of 33% (this assumed a 10% concentration factor to account for the loss of mass from the whey). The

composite particles were formulated to contain 18% fat. Thus, if composite treatment cheeses exhibited 100% particle retention, the fat content would be predicted to be 5.9% (Eq. 3.5).

$$Maximum\ Fat\ Added\ to\ Cheese\ if\ 100\%\ Retention\ is\ Achieved$$
$$= 33_{\ Targeted\ Filler\ Volume} * 18\%_{\ Fat\ From\ Composite\ Particle} = 5.9\% \qquad \text{(Eq. 3.5)}$$

The fat content of the milk used to make treatment cheeses was 0.2%, which when concentrated, would account for a portion of fat in the cheese (c.a. 2%). In order to determine this concentration factor for each replication, we referenced the particulate gel treatment, which contained no added fat from the particles. As 50.6% of the fat contained within the particles was liberated during the particulation process but still incorporated into the cheese, this fat content along with the fat indigenous to the milk would result in cheeses with 5% fat (replication 1) and 4.2% (replication 2) even if no particles were retained (Eq. 3.6; Eq. 3.7).

$$Fat\ Content\ of\ Cheese\ if\ no\ Paritcles\ Retained\ \ rep1$$
$$= 2.0 + 5.9 * 50.6\%_{\ Fat\ Liberated\ from\ Particles} = 5.0\% \qquad \text{(Eq. 3.6)}$$

$$Fat\ Content\ of\ Cheese\ if\ no\ Paritcles\ Retained\ \ rep2$$
$$= 1.2 + 5.9 * 50.6\%_{\ Fat\ Liberated\ from\ Particles} = 4.2\% \qquad \text{(Eq. 3.7)}$$

If all particles were incorporated the fat content would then be 8% and 7.1% in replications 1 and 2 respectively (Eq.3.8; Eq.3.9).

$$Fat\ Content\ of\ Cheese\ if\ all\ Paritcles\ Retained\ \ rep1$$

$$= 2.0 + 5.9 = 7.9\% \quad \text{(Eq. 3.8)}$$

$$Fat\ Content\ of\ Cheese\ if\ all\ Paritcles\ Retained\ \ rep2$$

$$= 1.2 + 5.9 = 7.1\% \quad \text{(Eq. 3.9)}$$

Particle retention was estimated to be at or near maximum retention in the native fat composite replication 1 cheese (93%) and replication 2 homogenized fat (100%) (Table 3-6). However, for the other replications of these treatment types the retention fraction was lower (67% and 62% respectively). This variation could likely result in differing fracture and sensory properties. Furthermore, as our hypothesis requires an equal filler volume of particles as that of the fat in full fat cheese, retention values of less than 100% may not be utilizing the maximum utility of this approach. Retention was also confirmed using CSLM (not used to quantify volume fraction).

The homogenized gel treatment had an exceptionally low fat content. This treatment was homogenized to obtain a low particle size, and in the process, much of the fat coalesced and creamed to the surface. This layer was skimmed from the top before incorporation into the cheese milk. It is therefore likely that nearly all the fat contained within the particles was removed, and those fat globules present in the cheese were native in the milk.

4.2.2 Cheese Composition

While it was expected that the low fat cheese would exhibit greater moisture values than the full fat or homogenized controls as a result of phase concentration (loss of the fat filler), the treatment cheeses all exhibited greater moisture than the low fat control, which indicates this moisture likely came from the particles. Of the treatments for which retention values could be estimated, particles delivery was variable. Both the native and homogenized fat composite treatments experienced particle delivery at or very near optimal in a single replication, and roughly 2/3 of the target in the other (Table 3-6).

4.2.3. Confocal Laser Scanning Microscope Observations (CSLM)

While the particulation method and cheese making procedures between experiments were identical, the average particle size of the composite type gel retained in the cheeses in experiment 2 appeared relatively larger than in experiment 1 (Figure 3-2 and 3-3). This allowed for closer observation of fat structure within the particles. In order to confirm that fat globules were embedded within the whey particles and not an artifact overlaying the surface from sample preparation, three dimensional renderings of the cheeses were taken by layering images of increasing sample penetration depth. These are referenced as Z-stack images and depict depth from the vertical and horizontal corsairs at the top and right cutaways respectively. The Z-stack images confirm fat globule inclusion in the native fat composite particles (Figure 3-2b; 3-2d) and also illustrate that globule shape remains relatively spherical with increasing sample depth. Globule retention is also apparent in the homogenized fat particles (Figure 3-3b), where fat appears similar to the native fat composite in terms of globule size and exhibits a clear spectrum of small and coalesced globules.

The particulate gel particles were much larger than those contained in experiment 1 and exhibited a degree of fat adherence at the particle edges (Figure 3-4b-d). These particles were set and milled in the absence of fat, so any protein-fat interactions must have occurred after particle addition to the cheese milk. Replication 2 of the particulate gel treatment appeared to have a greater frequency of smaller particles than the first replication. While this is qualitative observation, it may bolster the plausibility of variable retention, as replication 2 of the particulate gel proved far more efficacious in rheological and textural values than replication 1. This observation was also the case for the homogenized gel particles in which the first replication appeared to have greater particle retention than the second (Figure 3-5). Particle size in the homogenized gel particles was very near that of the coalesced fat globules, and the z-stack images illustrate that at this size, virtually all the fat has been liberated from the particles (Figure 3-5b; 3-5d). Notably, the homogenized particle is compositionally identical to the particulate gel once the fat is liberated. This perspective invites a comparison of these particle types and further supports retention variability as an explanation of differing effectiveness across replications. The first replication of the full fat control cheese is pictured at a curd junction, identified by a region characteristically void of fat (3-6a). This feature is formed when the curd is cut and the exposed fat is washed away leaving a distinctly dense protein area when the curd re-knits (Kalab et al. 1982). The full fat control Z-stack images reveal a large degree of globule coalescence and irregular shape even at depths far from the sample surface (Figure 3-6b; Figure 3-6d). Only the first replications of the low and homogenized fat controls are depicted as their structure is well studied (Guinee et al. 2000**)** and exhibited little if any discernible differences between replications. The low

fat control exhibited highly spherical fat globule shape and minimal coalescence (3-6e). The homogenized control appeared very similar to the full fat control, and it is likely that any structural differences are masked by particle coalescence as globule size appeared to be relatively similar for native and homogenized control treatments.

4.2.4. Small Strain Analysis

A frequency sweep of the particle filled cheese at 24 weeks ageing was conducted to differentiate the effects of the particle treatments on the viscoelastic properties of the cheeses. Since the low fat control had a higher gel phase volume, it was expected to have a higher G' than the full fat control, which was observed in both replications. The homogenized gel and native fat composite exhibited very similar curves to the full and homogenized fat controls in replication 1 (Figure 3-7). For replication 2, the particulate and native fat composite treatments were most similar to the full fat and homogenized fat controls. These results may suggest the mixed influence of particle retention volume and compositional type at small strains. It has been observed that an increase in fat content at room temperature had little bearing on the G' of cheese, and thus it is plausible that the storage moduli of the casein and fat phases are similar at this temperature (Ustunol, et al. 1995; Rogers et al. 2010). Given the range of storage moduli values (50-300 kPa) induced from fat content differences at 10°C in the study by Rogers et al. (2010), it is questionable whether the observed values at room temperature denote significant differences.

4.2.5. Fracture Analysis

Using pooled values across both replication and age (Table 3-6), the native fat composite and homogenized gel treatments exhibited fracture stress values which were not

statistically different than the full fat control (Table 3-7). In observing the individual treatment replications, the homogenized gel treatment proved highly effective in replication 1 at both 8 and 24 weeks ageing in reducing both fracture stress and modulus (Table B-1; B-2). This was not the case for the second replication of the homogenized gel treatment, which exhibited values more similar to the low fat control. Replication 2 of the particulate type gel was also found to be effective and exhibited fracture stress and modulus values similar to the full fat control. Fracture strain was not found to be a good predictor of texture in Cheddar cheese. After 24 weeks ageing, both the native fat composite and homogenized gel type particles were similar to the full fat control in values of fracture stress for replication 1. In replication 2, the native fat composite and particulate treatments were again much improved from the low fat control. Interestingly, the native fat composite proved to be more efficacious than the homogenized composite in reducing fracture stress across both replications (Table B-2), even though these particle type swere found to be very similar in terms of gel rheology and texture in chapter 2.

4.2.6. Single Edge Notch Bend

The single edge notch bend test uses 3 points of loading on a rectangular parallelepiped sample geometry where maximum tensile strain occurs at the bottom notch of the sample and peak compressive strain is exerted at the top (Everard et al. 2007). The degree of interaction between the casein matrix and the whey particles in this experiment was not fully understood, but the extent of this interaction will likely dictate the speed and direction of crack propagation (Cakir et al. 2012) as well as the orientation of the sample fracture path through either the whey particle or phase interface. However, fracture property dependence

on filler phase volume has been noted (Langley et al. 1994; Cakir et al. 2012) and must be considered along with effects imparted by treatment particle functionality. No differences were found using the pooled data and so treatments were evaluated for individual differences across replications. After 8 weeks ageing, the native fat composite and particulate gel treatments were similar to the full fat control in replication 2 (Table B-1). At 24 weeks, the native fat composite and homogenized gel in replication 1 and particulate gel in replication 2 were similar to the full fat control (Table B-2). These results largely echo torsional fracture data, and illustrate a persistent rheological effect elicited by the homogenized gel and particulate particles in replications 1 and 2 respectively. Importantly, while not able to fully match the full fat control, the native fat composite particles consistently decreased fracture toughness and energy in both replications.

4.2.7. Large Strain Rheology

The particulate and homogenized gels were more similar to the full fat control in terms of recoverable energy. The cheeses exhibited a notable drop in recoverable energy between the 8 and 24 week evaluations (Table B-1; B-2). This was expected, as proteolytic changes to the casein matrix during ripening weaken the structure and allow for energy dissipation. A study by Van den Berg et al. (2008) correlated recoverable energy to crumbliness and observed that systems with lower percentages of recoverable energy exhibited a more yielding breakdown pattern, while those with higher energy recovery were more related to exhibiting a free running crack during breakdown. After 24 weeks aging, none of the treatment cheeses were able to fully match the values of the full fat control (Table B-2). However, the homogenized gel in replication 1, and the particulate gel in

replication 2 both exhibited lower amounts of recoverable energy than the low fat controls. This data indicates that particles may be able to impart a means of energy dissipation.

4.2.8. Descriptive Sensory Analysis

In force deformation terms, the homogenized gel was found to not be statistically different than full fat in hand firmness and firmness (Table 3-8). Treatment cheeses were all similar to the low fat control in Bolus formation terms. However, distinct improvements were observed in specific replications of cheeses at 8 and 24 weeks. While cheese texture continues to develop throughout the ripening process, the greatest changes occur during the first three months of ageing (Rogers et al. 2009), and thus texture at 6 months was considered largely stable with only gradual shifts with further ageing. At 8 weeks, the treatment cheeses exhibited some slight improvements from the low fat control in force-deformation terms (Table B-3a). In respect to the bolus formation terms, the homogenized gel treatment-1 was similar to the full fat control in degree of breakdown and cohesion, and was greater than the low fat control in adhesion, smoothness of mass, and smoothness of mouth coating (Table B-3b). This suggests that the particles are capable of not only rendering a desirable initial fracture pattern, but also of emulating the functionality of fat as the cheese is orally processed in preparation for swallowing. The particulate gel treatment also exhibited greater values than the low fat control in terms of degree of breakdown and cohesion, as well as a reduction in hand firmness and firmness in force-deformation terms. At 24 weeks the homogenized gel in replication 1 was again improved in hand firmness and firmness, as well as in degree of breakdown, cohesion, adhesion, and smoothness of mass (Table B-4a; B-4b). In replication 2, the particulate treatment exhibited reduced firmness, hand firmness, and hand rate of

recovery. Both the particulate gel and native fat composite had greater values in nearly all bolus formation terms in replication two (Table B-4b).

As is evident from the principal component biplot, cheeses were largely distinguished by two distinct sets of sensory terms (Table 3-9; Table 3-10; Figure 3-8). The full fat controls exhibited a highly positive loading in bolus formation terms, and a negative loading in force deformation, most notably firmness. Thus, textural improvements to Cheddar cheese can be generally understood as a decrease in force-deformation and an increase in bolus formation values.

4.2.9. Post Fracture Analysis

Several mechanical tests have been designed to assess large strain and fracture rheological behavior of food materials. However, a substantial component of mastication involves oral processing beyond the initial fracture of a food system, i.e. everything which occurs beyond the first bite. In order to gain a better understanding of mechanical values relating to the later stages of oral processing, force and deformation values were normalized from the point of fracture to illustrate sample behavior in this region. As discussed in chapter 2, a steep initial decreasing curve represents a fast running crack. Decreasing curves were not observed with any cheeses. An initial plateau indicates a ductile yielding of structure without a clean fracture into two or more pieces. A more or less continued increase in normalized force indicates an overall flow with minimal abrupt changes. At 24 weeks, the homogenized gel and particulate treatments exhibited breakdown patterns very similar to the full fat control in replications 1 and 2 respectively (Figure 3-10). These results correspond with those of the sensory analysis in that treatments exhibiting the most gradual post fracture

slopes also most resemble the full fat controls in bolus formation terms. This observation is of particular interest in that a large hurdle in the production of fat mimetics is the difficulty of emulating the specific tribological functionality of milk fat with a non lipid material. A conventional strategy in soft solid foods has used particle size and shape to impart a desired mouth feel (Guinard and Rossella, 1996). However, this approach is likely not suitable for a system such as cheese in which fat is well dispersed within a protein matrix. Thus, the treatments exhibiting gradual post fracture breakdown curves and bolus formation terms similar to the full fat control imply a breakdown pattern partially independent of molecular identity i.e. protein vs. fat, and potentially validate a structural approach to understanding texture in Cheddar cheese.

4.2.10. Conclusions

Treatment cheeses were produced in replication and tested after 8 and 24 weeks ageing. Data was pooled across replications and age to improve statistical resolution. Limited improvements were found in both rheological and sensory properties using this approach, but it was noted that large variations existed between replications. Thus individual replications of cheeses were also included in the discussion so as to gain a better understanding of the effect of the particles in the cheese. The homogenized particle treatment in replication 1 and the particulate treatment cheese in replication 2 exhibited lower values of fracture stress than the low fat control. The native fat composite treatments, although to a lesser extent, also exhibited lower values of fracture stress, and in both replications. Sensory values for these cheeses were in accordance with the results from fracture rheology, as the homogenized gel (rep 1) and particulate gel (rep 2) treatments both exhibited decreased values of firmness and

hand firmness. Additionally, and although not equal to the full fat control, these two treatments also had greater values of cohesion and smoothness of mouth coating than low fat control. Overall, cheeses demonstrated a large degree of variance within treatment types, but specific replications of several treatment types were found to exhibit a more fat like cheese texture than the low fat control. Thus, experiment 3 was primarily conducted to detect the source of variation between replications for the homogenized gel treatment type.

4.3 Experiment 3 Cheeses

Drawing from the success of the homogenized gel particle in replication 1 of experiment 2, the third experiment of cheese was made using only this type particle. The replication 1 homogenized gel particles in experiment 2 were homogenized at both USU and NCSU, while the second replication was homogenized only at NCSU (Table 3-12). Cheeses A-C in experiment 3 were therefore designed to assess any effects from these differing homogenization treatments. Cheeses D and E were made with particle volumes at a greater percentage than the filler phase in the full fat control (c.a. 33%) to examine the effect of retention. Fat percentages of cheeses (Table 3-12) provide some indication of particle retention (cheeses were all made with milk with the same fat content), with higher fat percentages likely related to higher particle retention. However, as it was determined that fat was completely liberated from the particles in this treatment type (Chapter 2), treatments with similar fat content could have differences in particle retention volume.

4.3.1. Large Strain and Fracture Analysis

Treatments A-D were relatively high in fracture stress at 8 weeks (Table 3-13) as compared with experiment 2 and the results reported from Gwartney et al. (2002). Treatment

E however exhibited values which were much lower than any of the other treatments in experiment 3, and similar to the full fat controls in experiment 2 at the same age. Using the single edge notch bend, treatments B, C, and E exhibited the lowest values in fracture energy and toughness (Table 3-13). Rogers et al. (2009) hypothesized that fat served to impart strain weakening points and a higher degree of breakdown at higher contents. As the casein matrix of the cheese undergoes proteolysis and breaks down over time, fracture stress, which is correlated with the degree of intact casein, also decreases (Tunick and van Hekken, 2003). This was observed in experiment 2 and thus fracture values will likely continue to decrease with further ageing. Experiment 3 cheeses did not exhibit large differences in recoverable energy among treatments at 8 weeks ageing.

4.3.2. Descriptive Sensory Analysis

Experiment 3 cheeses were similar in force-deformation terms, but treatment E proved highly effective in improving texture in all bolus formation terms (Table 3-14). Particles in treatments D and E were added into the cheese milk at volumes designed to impart greater than 33% filler volume in the cheese (Table 3-5), which may have imparted a greater filler phase volume than in the full fat control if optimum particle delivery was achieved. Thus, the relatively large difference in efficacy between these treatments may suggest a critical phase volume necessary to impart this functionality.

4.3.3. Post Fracture Analysis

Treatment E also displayed a gradual breakdown pattern at 8 weeks ageing (Figure 3-1) which was very similar to that of the full fat control in experiment 2. This finding is in

agreement with the sensory evaluation which found treatment E to exhibit bolus formation terms similar to that of a full fat Cheddar.

4.3.4. Confocal Laser Scanning Microscope Observations (CSLM)

The primary objective of experiment 3 was to discern the potential cause of differences between replications of the homogenized gel particle. Treatments A and B had similar fat globule size distributions (Figure 3-12a; 3-12b). These particles were differentiated only by an additional homogenization application at USU for treatment B. The NCSU homogenization pressure (400 bar) was far greater than that of USU (172 bar), and thus it is unlikely that the additional homogenization at USU had a significant impact on fat globule shape or size. This is further evident in that treatment C cheese (Figure 3-12c), homogenized only at USU, had a greater degree of globule coalescence and appeared to have a larger globule size distribution. Treatment D (Figure 3-12d), which was also homogenized at NCSU and USU, resembles A and B. Sample E (Figure 3-12e) notably displays greater particle retention than the other treatments. Fat globule shape appears more irregular in treatment E, and may be the result of interactions between the particles and disrupted fat globule membrane.

4.3.5 Conclusions

Differences as a result of homogenization pressure were identified using CSLM, but were not observed with rheological or sensory data. Thus, it is unlikely that this parameter was responsible for the difference in efficacy observed between replications 1 and 2 in the experiment 2 cheeses. Treatment E was formulated with a higher particle addition than the

other treatments. It is therefore plausible that the high degree of effectiveness in treatment E is related to a greater particle retention fraction in the cheese.

5. CONCLUSIONS

Experiment 1 demonstrated that low fat Cheddar cheeses containing whey protein particles could be produced without significant make defects. Conceptually, this experiment validated our scaling approach and inclusion method, with some particle retention in cheese confirmed using CSLM. Experiment 2 cheeses were made in 20 kg replicates and sampled at 8 and 24 weeks ageing. Individual cheese replications were found highly efficacious in improving both rheological and sensory values but not repeated between replications. Experiment 3 was targeted at discerning this source of variation. No differences were observed when particulation methods were modified, but when a greater initial volume of particles were added, (which would theoretically result in a higher retention volume), the treatment cheese again proved highly effective in improving the low fat texture. Particle retention volume could not be directly measured, but our estimation method found a degree of variations across replications of the composite type particles. This could plausibly be the case for other treatments as well. Furthermore, favorable results were found from the native fat composite, homogenized particle, and particulate gel treatment types. It is therefore plausible that the effective treatments achieved a critical volume fraction necessary to produce a breakdown structure similar to full fat in a low fat Cheddar cheese.

Future work should first be directed at quantification of particle retention in cheese. With a consistent volume fraction achieved, the efficacy of individual gel types can then be

evaluated. Although the particles in this study were selected for the effectiveness in Cheddar cheese, the broad spectrum of gel types achieved using our method for particle production would facilitate their potential application into other low fat products. In order to gain a more fundamental knowledge of the mechanisms governing Cheddar cheese breakdown, as outlined by Foegeding and Drake (2007), work should be related towards predicting mechanical and sensory properties from expected functionality of particle treatments. While much work still remains to fully evaluate the effectiveness of the particle particles, the success of specific cheese replications strongly suggests a potential for this strategy in the improvement of low fat texture.

6. REFERENCES

Archwamety, R. Companies Work to Improve, Expand Reduced-Fat Cheeses. Cheese Market News. November 30th, 2007. http://www.cheesemarketnews.com.

Charalambides, M. N., J. G. Williams, and S. Chakrabarti. (1995). A study of the influence of ageing on the mechanical properties of Cheddar cheese. *Journal of material science* 30(16), 3959-3967.

Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2010). Comparison of the effects of high-pressure microfluidization and conventional homogenization of milk on particle size, water retention and texture of non-fat and low-fat yoghurts. *International Dairy Journal, 20*(5), 314-320.

Dickinson, Eric, and Jianshe Chen. (1999). Heat-set whey protein emulsion gels: role of active and inactive filler particles. *Journal of dispersion science and technology* 20.1-2: 197-213.

Drake, M. A., Miracle, R. E., & McMahon, D. J. (2010). Impact of fat reduction on flavor and flavor chemistry of cheddar cheeses. *Journal of Dairy Science, 93*(11), 5069-5081.

Everard, C. D., O'Callaghan, D. J., O'Kennedy, B. T., O'Donnell, C. P., Sheehan, E. M., & Delahunty, C. M. (2007). A three-point bending test for prediction of sensory texture in processed cheese. *Journal of Texture Studies, 38*(4), 438-456.

Everett, D. W., and N. F. Olson. "Free oil and rheology of Cheddar cheese containing fat globules stabilized with different proteins." *Journal of dairy science* 86.3 (2003): 755-763.

Foegeding, E. A. (1992). Rheological properties of whey protein isolate gels determined by torsional fracture and stress relaxation 1. *Journal of Texture Studies, 23*(3), 337-348.

Foegeding, E. A., & Drake, M. A. (2007). Invited review: Sensory and mechanical properties of cheese texture. *Journal of Dairy Science, 90*(4), 1611-1624.

Foegeding, E. A., Brown, J., Drake, M., & Daubert, C. R. (2003). Sensory and mechanical aspects of cheese texture. *International Dairy Journal, 13*(8), 585-591.

Guinard, J., & Mazzucchelli, R. (1996). The sensory perception of texture and mouthfeel. *Trends in Food Science & Technology, 7*(7), 213-219.

Guinee, T. P., Auty, M. A. E., & Fenelon, M. A. (2000). The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of cheddar cheese. *International Dairy Journal, 10*(4), 277-288.

Gunasekaran, S., & Ding, K. (1999). Three-dimensional characteristics of fat globules in cheddar cheese. *Journal of dairy science, 82*(9), 1890-1896.

Gwartney, E. A., Foegeding, E. A., & Larick, D. K. (2002). The texture of commercial full-fat and reduced-fat cheese. *Journal of Food Science, 67*(2), 812-816.

Harper, J., Iyer, M., Knighton, D., & Lelievre, J. (1989). Effects of whey proteins on the proteolysis of Cheddar cheese slurries (a model for the maturation of cheeses made from ultrafiltered milk). *Journal of Dairy Science, 72*(2), 333-341.

Johnson, M. E., Kapoor, R., McMahon, D. J., McCoy, D. R., & Narasimmon, R. G. (2009). Reduction of sodium and fat levels in natural and processed cheeses: Scientific and technological aspects. *Comprehensive Reviews in Food Science and Food Safety, 8*(3), 252-268.

Kalab, M., Lowrie, R. J., & Nichols, D. (1982). Detection of curd granule and milled curd junctions in cheddar cheese. *Journal of Dairy Science, 65*(7), 1117-1121.

Lane, C. N., Fox, P. F., Johnston, D. E., & McSweeney, P. L. H. (1997). Contribution of coagulant to proteolysis and textural changes in cheddar cheese during ripening. *International Dairy Journal, 7*(6–7), 453-464.

Langley, K. R., Martin, A., & Ogin, S. L. (1994). The effect of filler volume fraction on the fracture toughness of a model food composite. *Composites Science and Technology, 50*(2), 259-264.

Lawless, H. T. and Heymann, H. Sensory Evaluation of Food: Principles and Practices 2nd ed. Springer. New York, 2010.

Lawrence, R. C., Creamer, L. K., & Gilles, J. (1987). Texture development during cheese ripening. *Journal of Dairy Science, 70*(8), 1748-1760.

Low Fat Cheese Research: The latest Product Research, Trends, and Applications. (2006). Dairy Management Inc. < http://www.innovatewithdairy.com>.

Lucca, P. A., & Tepper, B. J. (1994). Fat replacers and the functionality of fat in foods. *Trends in Food Science & Technology, 5*(1), 12-19.

Lucey, J.A. Johnson, M.E. Horne, D.S. (2003) Perspectives on the basis of the rheology and texture properties of cheese J. Dairy Sci., (86), 2725–2743.

McMahon, Donald J., Alleyne, M. C., Fife, R.L., Oberg, C. J.. Use of fat replacers in low fat mozzarella cheese. *Journal of Dairy Science* 79.11 (1996): 1911-1921.

Meilgaard, Morten C., Gail Vance Civille, and B. Thomas Carr. *Sensory evaluation techniques*. CRC, 2006.

Olson, N. F., S. Gunasekaran, and D. D. Bogenrief. (1996). Chemical and physical properties of cheese and their interactions. *Nederlands melk en Zuiveltijdschrift* 50(2): 279-294.

Roberts, A. K., & Vickers, Z. M. (1994). Cheddar cheese aging: Changes in sensory attributes and consumer acceptance. *Journal of Food Science, 59*(2), 328-334.

Rogers, Neal Robert. "The Effect of Fat Content and Aging on the Texture of Cheddar Cheese." (2009).

Tunick, M. H. (2000). Rheology of dairy foods that gel, stretch, and fracture. *Journal of Dairy Science, 83*(8), 1892-1898.

Tunick, M. H., & van Hekken, D. (2003). Torsional analysis of fresh and aged cheeses. *Journal of Texture Studies, 34*(2), 219-229.

"U.S. Per Capita Cheese Consumption- 1985-2010". Cheese Statistics. USDA, 2012. <http://media.eatwisconsincheese.com>.

Ustunal, Z., Kawachi, K., & Steffe, J. (1995). Rheological properties of cheddar cheese as influenced by fat reduction and ripening time. *Journal of Food Science, 60*(6), 1208-1210.

van den Berg, L., Carolas, A. L., van Vliet, T., van der Linden, E., van Boekel, M. A. J. S., & van de Velde, F. (2008). Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. *Food Hydrocolloids, 22*(7), 1404-1417.

Williams, J. G., & Cawood, M. J. (1990). European group on fracture: Kc and gc methods for polymers. *Polymer Testing, 9*(1), 15-26.

Table 3-1. COMPOSITION OF HEAT SET WHEY PROTEIN ISOLATE GELS

Treatment	Particulate	Stranded	Composite Particle	Homogenized Fat Composite	Native Fat Composite	Homogenized Gel
Protein (%w/w)	10%	10%	10%	10%	10%	10%
NaCl (mM)	150	50	150	150	150	150
Fat (%w/w)	-	-	9%	18%	18%	18%
Fat Structure	-	-	homogenized	homogenized	native	native
Particulation Method	mill	mill	mill	mill	mill	homogenize
Experiment in which Particle Type Was Incorporated	1,2	1	1	2	2	2,3

Table 3-2. PROXIMATE ANALYSIS OF EXPERIMENT 1 PARTICLE FILLED CHEESE.

Treatment	Fat (%)	Moisture (%)	pH	% S/(W+S)
Particulate-1	7.0	48.66	5.39	2.27
Particulate-2	7.0	50.7	5.17	1.73
Stranded-1	7.0	50.74	5.26	1.85
Stranded-2	7.0	51.7	5.14	1.96
Composite-1	8.0	51.9	5.37	1.95
Composite-2	7.2	50.6	5.18	1.75
Low Fat Control	7.0	49.71	5.36	1.12
Homogenized Fat Control	29.5	41.2	5.11	1.53
Full Fat Control	31.0	39.82	5.34	1.74

Table 3-3. FRACTURE AND LARGE STRAIN RHEOLOGY OF EXPERIMENT 1 PARTICLE FILLED CHEESE EVALUATED AFTER 34 WEEKS AGEING..

Treatment	Fracture Stress (kPa)	Fracture Strain	Recoverable Energy (%)
Particulate-1	70.3 ± 5.0	1.41 ± 0.16	30.4 ± 1.5
Particulate-2	53.1 ± 6.1	1.1 ± 0.17	27.1 ± 0.7
Stranded-1	50.3 ± 9.9	1.5 ± 0.15	30.8 ± 0.9
Stranded-2	64.6 ± 6.0	0.93 ± 0.03	26.9 ± 0.4
Composite-1	30.4 ± 2.3	1.3 ± 0.19	27.6 ± 0.7
Composite-2	67.3 ± 11.8	0.94 ± 0.20	25.5 ± 0.8
Full Fat Control	20.5 ± 8.6	1.03 ± 0.12	22.4 ± 1.0
Homogenized Fat Control	21.9 ± 32.0	1.16 ± 0.20	16.6 ± 0.6
Low Fat Control	66.3 ±11.1	1.34 ± 0.27	31.45 ± 0.6

Numbers after treatment names indicate replication (e.g. Particulate-1 is the first replication of cheese made using the particulate type particle).

Table 3-4. SENSORY TEXURE OF EXPERIMENT 1 PARTICLE FILLED CHEESE EVALUATED AFTER 34 WEEKS AGEING.

FORCE-DEFORMATION TERMS

Treatment	Hand Firmness	Hand Springiness	Hand Rate of Recovery	Firmness	Fracturability
Particulate-1	14.2 ± 0.4	14.0 ± 1.0	13.1 ± 1.5	11.0 ±1.0	7.0 ± 0.9
Particulate-2	13.5 ± 0.3	14.6 ± 0.5	14.3 ± 0.6	10.6 ± 0.4	6.8 ± 0.5
Stranded-1	14.1 ± 0.5	14.0 ± 0.8	13.8 ± 0.8	10.8 ± 1.0	6.6 ± 0.6
Stranded-2	12.7 ± 0.5	14.1 ± 0.8	14.0 ± 0.6	9.7 ± 0.3	5.9 ± 0.6
Composite-1	13.2 ± 0.6	13.5 ± 1.1	12.7 ± 1.7	10.5 ± 1.1	6.3 ± 1.6
Composite-2	13.9 ± 0	14.6 ± 0.3	14.5 ± 0.2	10.5 ± 0.5	6.3 ± 0.6
Full Fat Control	6.6 ± 0.4	10.5 ± 0.8	8.9 ± 1.9	4.2 ± 0.8	4.1 ± 0.6
Low Fat Control	14.1 ± 0.7	14.1 ± 0.8	13.7 ± 1.0	10.6 ± 0.7	6.7 ± 0.5
Homogenized Fat Control	4.5 ± 0.8	4.5 ± 2.1	4.6 ± 2.3	3.6 ± 0.4	3.8 ± 0.8

BOLUS FORMATION TERMS

Treatment	Degree of Breakdown	Cohesion	Adhesion	Smoothness of Mass	Smoothness of Mouth Coating
Particulate-1	4.0 ± 0.8	4.9 ± 1.0	4.5 ± 0.9	6.3 ± 1.3	7.0 ± 0.9
Particulate-2	4.0 ± 0.8	8.7 ± 1.1	7.0 ± 1.2	6.9 ± 0.8	7.4 ± 0.4
Stranded-1	5.2 ± 1.3	5.9 ± 1.0	4.9 ± 0.9	6.2 ± 0.7	7.0 ± 0.7
Stranded-2	4.6 ± 1.0	7.6 ± 0.7	6.2 ± 0.7	6.3 ± 0.6	7.6 ± 0.6
Composite-1	5.6 ± 0.7	6.0 ± 0.8	5.2 ± 0.5	6.6 ± 0.7	8.1 ± 1.0
Composite-2	4.6 ± 0.6	9.5 ± 0.4	6.5 ± 0.6	5.9 ± 0.5	7.4 ± 0.5
Full Fat Control	11.0 ± 1.1	11.4 ± 0.6	10.1 ± 0.6	11.5 ± 0.9	10.8 ± 0.6
Low Fat Control	4.8 ± 0.7	6.4 ± 0.7	5.2 ± 0.4	6.0 ± 0.5	6.8 ± 0.7
Homogenized Fat Control	12.0 ± 0.5	11.4 ± 0.4	10.4 ± 0.4	11.5 ± 0.5	10.9 ± 0.7

Numbers after treatment names indicate replication (e.g. Particulate-1 is the first replication of cheese made using the particulate type particle).

Table 3-5. TABLE OF MAIN EFFECTS AND INTERACTIONS FOR RHEOLOGICAL TERMS IN EXPERIMENT 2.

Parameter	Fracture Stress	Fracture Strain	Modulus	Recoverable Energy	Fracture Toughness	Fracture Energy
Treatment	*	*	*	*	-	-
Age	*	-	*	*	-	-
Replication	-	-	-	-	-	-
Treatment-Age Interaction	-	-	-	-	-	-

Star (*) indicates an effect at $P < 0.05$, while a dash (-) represents a non significant effect.

Table 3-6. PROXIMATE ANALYSIS OF EXPERIMENT 2 PARTICLE FILLED CHEESE.

Treatment	% Moisture		% Salt		% Fat		% Estimated Particle Retention	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Low Fat Control	50.2	48.7	1.53	1.53	8	6	-	-
Full Fat Control	37	37.3	1.6	1.4	28	27.2	-	-
Homogenized Fat Control	42.2	41.1	1.63	1.51	30	28.2	-	-
Native Fat Composite	53.9	51.6	2.45	2.29	7.8	6	93	62
Homogenized Fat Composite	53.8	51.4	2.51	2.57	7	7.2	67	100
Particulate Gel	53.9	58.9	2.6	2.16	2	1.2	-	-
Homogenized Gel	53.7	49.2	1.85	1.85	2	1.8	-	-

Treatment	pH at 8 days		% S/(W+S)	
	Rep1	Rep2	Rep1	Rep2
Low Fat Control	4.91	5.05	2.96	3.04
Full Fat Control	4.96	5.02	4.15	3.62
Homogenized Fat Control	4.88	4.97	3.72	3.54
Native Fat Composite	5.06	5.36	4.35	4.25
Homogenized Fat Composite	5.12	5.26	4.46	4.76
Particulate Gel	5.05	5.08	4.6	3.54
Homogenized Gel	4.89	5.28	3.33	3.62

Table 3-7. LARGE STRAIN AND FRACTURE RHEOLOGY OF EXPERIMENT 2 PARTICLE FILLED CHEESE.

Treatment	Fracture Stress (kPa)	Fracture Strain	Recoverable Energy (%)	Fracture Toughness ($Pa \cdot m^{1/2}$)	Fracture Energy (J/m^2)
Low Fat Control	63.4 a	1.17 abc	28.8 a	1160 a	16.8 a
Full Fat Control	30.0 bc	0.94 bc	19.4 b	547 a	6.82 a
Homogenized Fat Control	25.2 c	0.89 c	22.3 ab	455 a	3.97 a
Native Fat Composite	47.3 abc	1.51 a	27.7 a	654 a	8.11 a
Homogenized Fat Composite	51.8 ab	1.27 ab	29.1 a	994 a	17.0 a
Particulate Gel	56.1 a	1.19 abc	27.0 ab	991 a	18.0 a
Homogenized Gel	43.3 abc	1.20 abc	26.7 ab	717 a	11.6 a

Values incorporate pooled data of two replications taken at both 8 and 34 weeks ageing. Different letters in the same column are significantly different ($P < 0.05$).

Table 3-8. SENSORY TEXTURE OF EXPERIMENT 2 PARTICLE FILLED CHEESE.

FORCE-DEFORMATION TERMS

Treatment	Hand Firmness	Hand Springiness	Hand Rate of Recovery	Firmness	Fracturability
Full Fat Control	9.1 bc	11.8 ab	11.4 ab	7.7 bc	5.1 b
Low Fat Control	14.1 a	14.6 a	14.4 a	11.8 a	6.6 a
Homogenized Fat Control	8.5 c	9.4 b	9.0 b	6.8 c	4.9 b
Native Fat Composite	13.2 a	14.4 a	14.1 a	11.0 ab	7.0 a
Homogenized Fat Composite	13.0 ab	14.2 a	14.2 a	11.5 a	7.0 a
Particulate Gel	13.6 a	14.2 a	14.1 a	11.3 a	6.6 a
Homogenized Gel	11.5 abc	13.4 ab	13.9 a	10.8 ab	6.7 a

BOLUS FORMATION TERMS

Treatment	Degree of Breakdown	Cohesion	Adhesion	Smoothness of Mass	Smoothness of Mouth Coating
Full Fat Control	8.6 a	9.1 ab	8.6 a	9.8 a	9.6 a
Low Fat Control	2.5 b	3.1 c	2.8 b	3.9 b	4.3 b
Homogenized Fat Control	9.4 a	9.7 a	8.6 a	9.8 a	9.6 a
Native Fat Composite	3.0 b	3.6 c	3.7 b	4.2 b	4.9 b
Homogenized Fat Composite	2.6 b	2.7 c	3.3 b	3.3 b	4.3 b
Particulate Gel	3.1 b	4.0 c	3.5 b	3.8 b	4.6 b
Homogenized Gel	3.3 b	5.0 bc	4.2 b	4.6 b	5.0 b

Values reflect pooled data of replications taken at both 8 and 24 weeks ageing. Different letters in the same column are significantly different ($P < 0.05$).

Table 3-9. ABBREVIATIONS FOR PARTICLE FILLED CHEESE TREATMENTS.

Abbreviation	Treatment	Age at Evaluation (weeks)	Replication
A1	Native Fat Composite	24	1
A2	Native Fat Composite	24	2
B1	Homogenized Fat Composite	24	1
B2	Homogenized Fat Composite	24	2
C1	Particulate Gel	24	1
C2	Particulate Gel	24	2
D1	Homogenized Gel	24	1
D2	Homogenized Gel	24	2
FFC1	Full Fat Control	24	1
FFC2	Full Fat Control	24	2
HC1	Homogenized Fat Control	24	1
HC2	Homogenized Fat Control	24	2
LFC1	Low Fat Control	24	1
LFC2	Low Fat Control	24	2
a1	Native Fat Composite	8	1
a2	Native Fat Composite	8	2
b1	Homogenized Fat Composite	8	1
b2	Homogenized Fat Composite	8	2
c1	Particulate Gel	8	1
c2	Particulate Gel	8	2
d1	Homogenized Gel	8	1
d2	Homogenized Gel	8	2
ffc1	Full Fat Control	8	1
ffc2	Full Fat Control	8	2
hc1	Homogenized Fat Control	8	1
hc2	Homogenized Fat Control	8	2
lfc1	Low Fat Control	8	1
lfc2	Low Fat Control	8	2

Table 3-10. ABBREVIATIONS FOR DESCRIPTIVE SENSORY TEXTURE TERMS

Abbreviation	Treatment
hfirm	hand firmness
hspring	hand springiness
hrr	hand rate of recovery
firm	firmness
frac	fracturability
brkdwn	degree of break down
cohes	cohesion
adhes	adhesion
smass	smoothness of mass
smct	smoothness of mouth coating

Table 3-11. TREATMENT SUMMARY OF EXPERIMENT 3 PARTICLE FILLED CHEESES.

Treatment	Treatment Description	Targeted Addition (%)
A	Homogenized at NCSU	33.0
B	Homogenized at NCSU + USU	33.0
C	Homogenized at USU	33.0
D	Homogenized at NCSU + USU	44.0
E	Homogenized at USU	53.0

Particles were subjected to differing homogenization treatments at North Carolina State University (NCSU) and Utah State University (USU).

Table 3-12. PROXIMATE ANALYSIS OF EXPERIMENT 3 PARTICLE FILLED CHEESE.

Cheese	% Moisture	% Salt	% Fat	pH at 8 days	% S/(W+S)
Treatment A	49.37	2.36	5.5	5.72	4.56
Treatment B	49.74	1.80	6.5	5.59	3.49
Treatment C	49.83	1.60	8.0	5.58	3.11
Treatment D	49.06	1.80	7.0	5.61	3.54
Treatment E	52.29	1.60	9.0	5.48	2.97

Table 3-13. LARGE STRAIN AND FRACTURE RHEOLOGY OF EXPERIMENT 3 PARTICLE FILLED CHEESES AT 8 WEEKS AGEING

Treatment	Fracture Stress (kPa)	Fracture Strain	Recoverable Energy (%)	Fracture Toughness ($Pam^{1/2}$)	Fracture Energy (J/m^2)
A	107.1 ± 17.3	1.43 ± 0.06	30.4 ± 2.3	2169.6 ± 227.9	79.5 ± 29.3
B	79.6 ± 11.0	1.37 ± 0.16	28.3 ± 0.8	1009.9 ± 134.0	29.4 ± 5.5
C	72.9 ± 11.3	1.52 ± 0.16	27.2 ± 0.8	1011.3 ± 220.8	23.2 ± 3.5
D	71.5 ± 8.9	1.40 ± 0.24	28.7 ± 1.1	1516.1 ± 345.0	62.2 ± 15.6
E	40.7 ± 4.5	1.18 ± 0.06	27.1 ± 1.4	648.4 ± 93.7	10.2 ± 2.8

Table 3-14. SENSORY TEXTURE OF EXPERIMENT 3 PARTICLE FILLED CHEESES AFTER 8 WEEKS AGEING

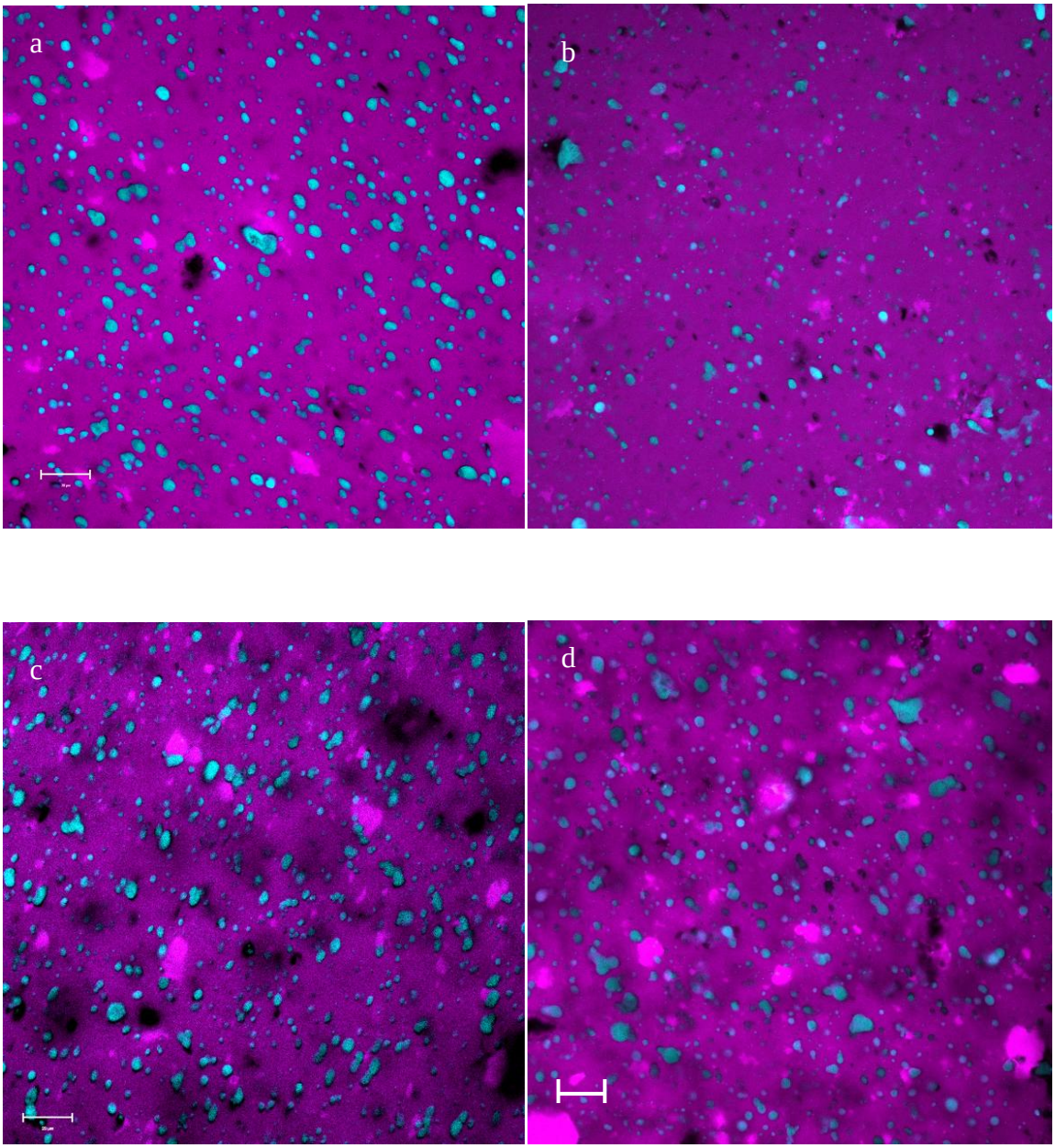
FORCE-DEFORMATION TERMS

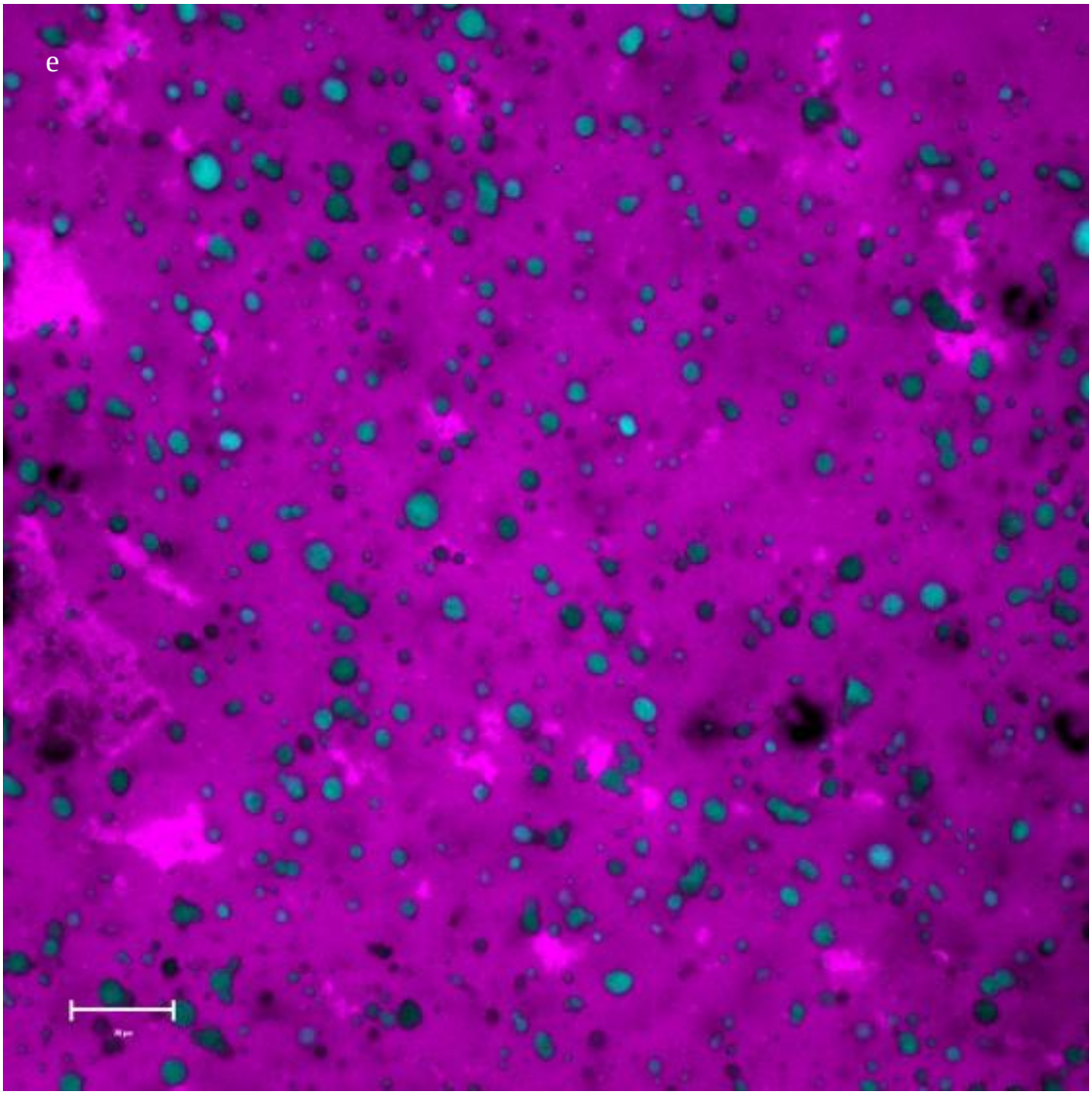
Treatment	Hand Firmness	Hand Springiness	Hand Rate of Recovery	Firmness	Fracturability
A	15.0 ± 0.1	14.8 ± 0.4	14.9 ± 0.4	13.3 ± 0.7	6.9 ± 0.4
B	14.5 ± 0.4	14.6 ± 0.7	14.5 ± 0.9	11.6 ± 0.8	6.3 ± 0.8
C	14.4 ± 0.4	14.7 ± 0.4	14.5 ± 0.6	10.9 ± 0.8	6.4 ± 0.4
D	14.7 ± 0.2	15.0 ± 0.0	15.0 ± 0.1	12.7 ± 0.4	6.4 ± 0.5
E	12.7 ± 0.4	14.3 ± 0.3	14.1 ± 0.7	9.6 ± 0.4	5.3 ± 0.4

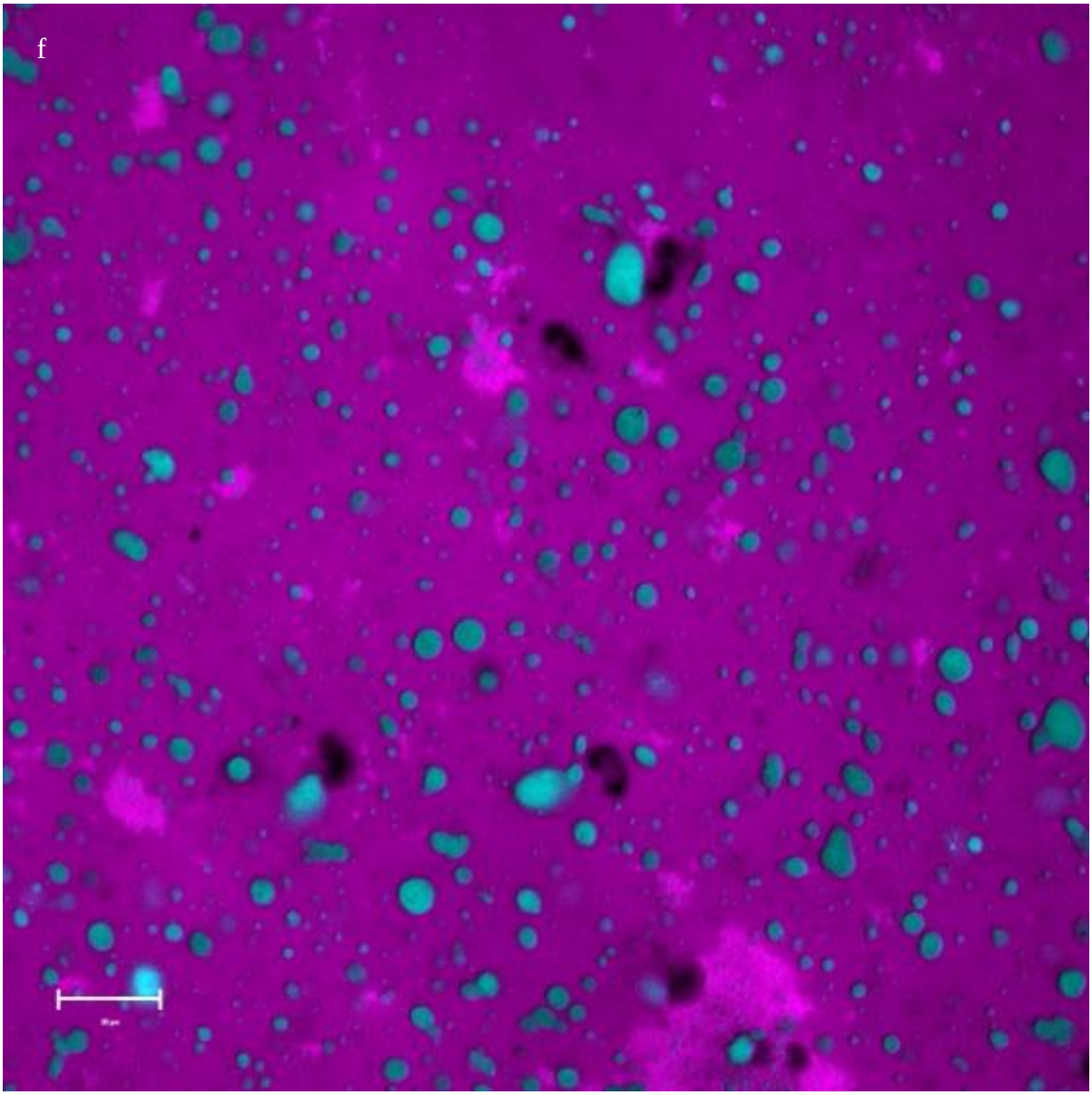
BOLUS FORMATION TERMS

Treatment	Degree of Breakdown	Cohesion	Adhesion	Smoothness of Mass	Smoothness of Mouth Coating
A	0.4 ± 0.4	0.7 ± 0.4	1.3 ± 0.5	1.8 ± 0.8	1.9 ± 0.8
B	1.1 ± 0.8	1.5 ± 0.8	2.2 ± 0.7	3.4 ± 1.0	3.6 ± 0.6
C	1.1 ± 0.8	1.8 ± 0.9	2.4 ± 0.7	4.1 ± 1.0	4.2 ± 0.4
D	0.8 ± 0.3	1.2 ± 0.3	2.6 ± 0.4	2.4 ± 0.5	2.3 ± 0.5
E	7.6 ± 0.5	8.5 ± 0.5	7.9 ± 0.7	7.0 ± 0.5	8.0 ± 0.9

Figure 3-1. CSLM images of experiment 1 particulate gel-1 (a) particulate gel-2 (b), stranded gel-1 (c), stranded gel-2 (d), composite gel-1 (e), composite gel-2 (f), low fat control (g), full fat control (h), and homogenized control (i) particle filled cheeses imaged after 34 weeks ageing. Scale bars are 20 μm.


a
b
c
d


e


f

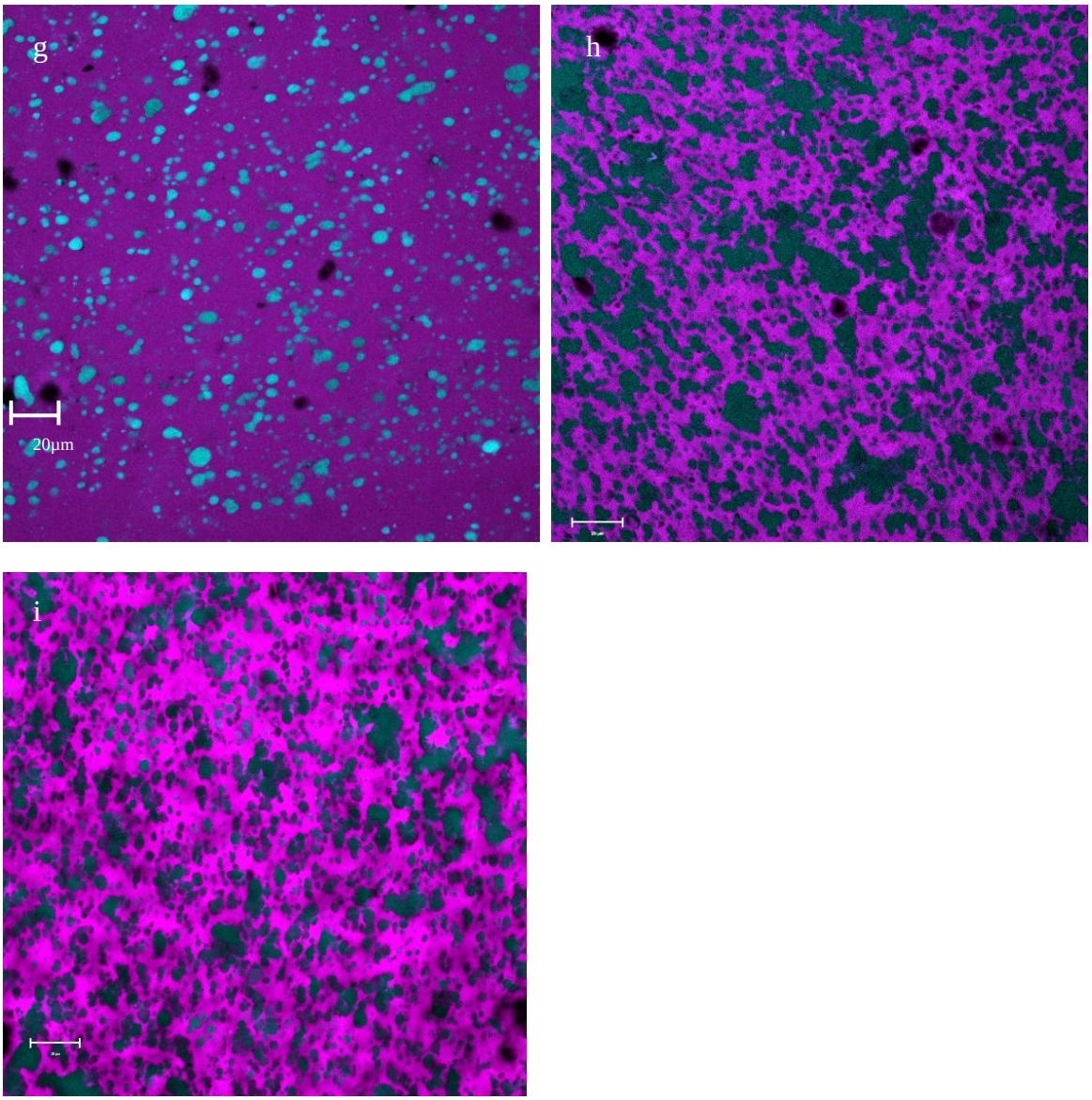
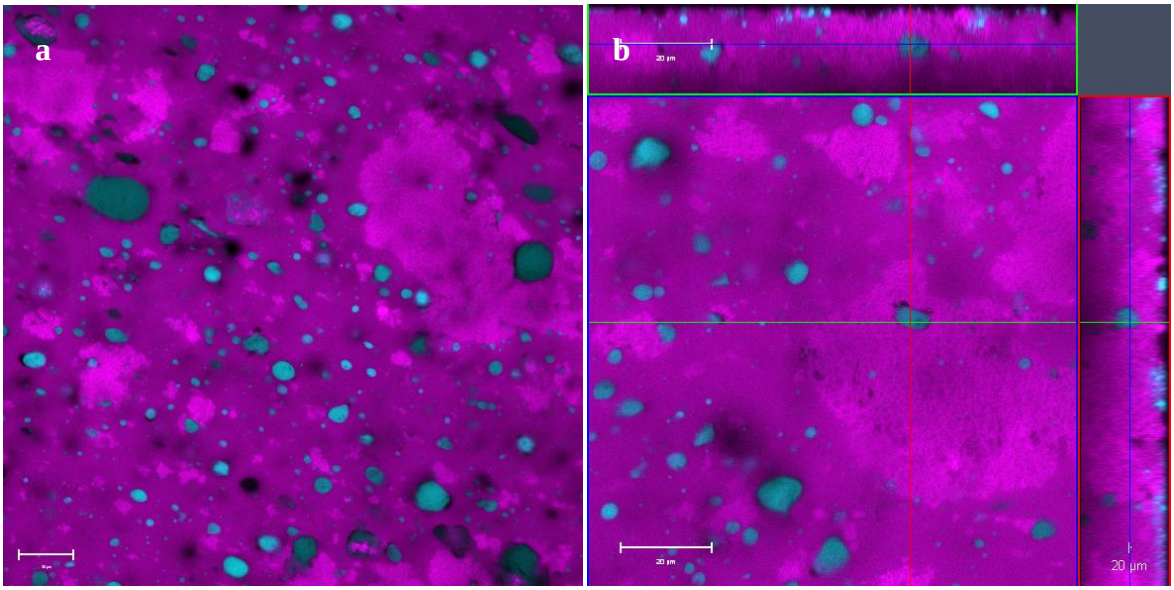
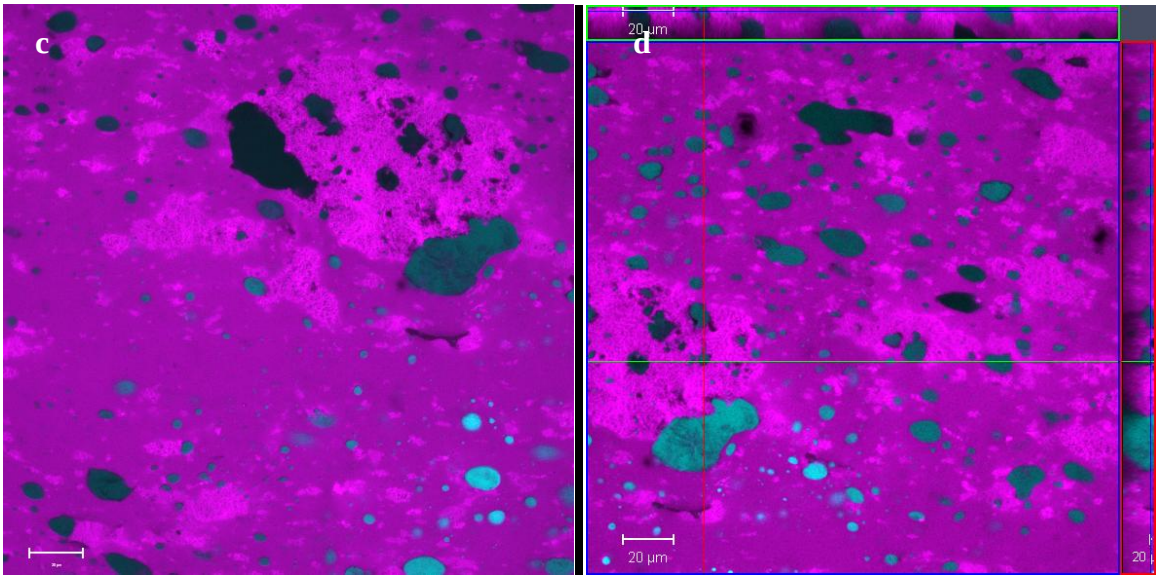

g
20µm
h
i

Figure 3-2. CSLM of experiment 2 replication 1 native fat composite (a) experiment 2 replication 1 native fat composite, Z-stack (b), experiment 2 replication 2 native fat composite (c), and experiment 2 replication 2 native fat composite, Z-stack (d), particle filled cheeses. Scale bars are 20 μm**.**


a
b
20 µm
20 µm


c
d
20 µm
20 µm

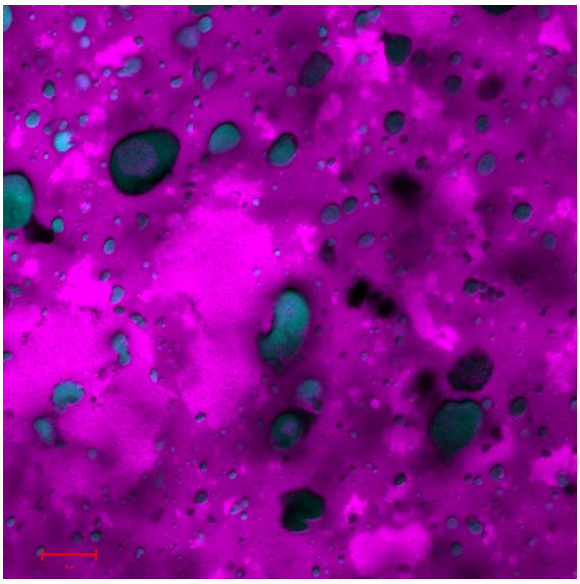


Figure 3-3 (a)

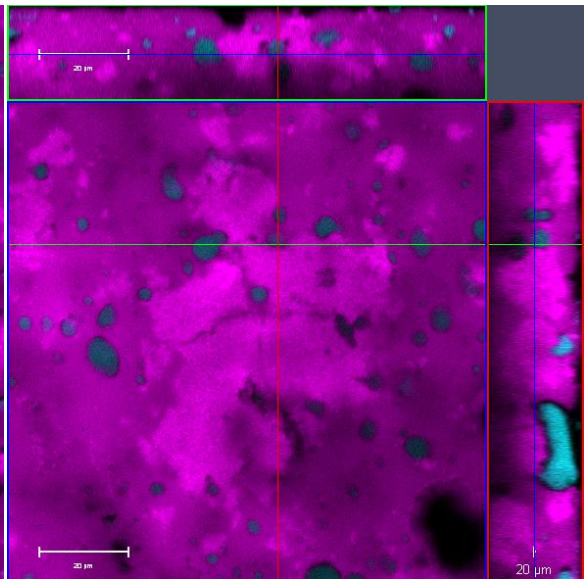


Figure 3-3 (b)

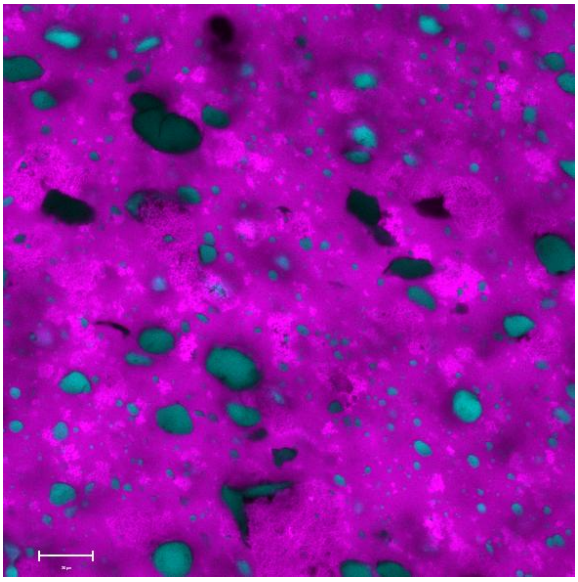


Figure 3-3 (c)

Figure 3-3. CSLM of experiment 2 replication 1 homogenized fat composite (a) experiment 2 replication 1 homogenized fat composite, Z-stack (b), and experiment 2 replication 2 homogenized fat composite (c) particle filled cheeses. Scale bars are 20 µm.

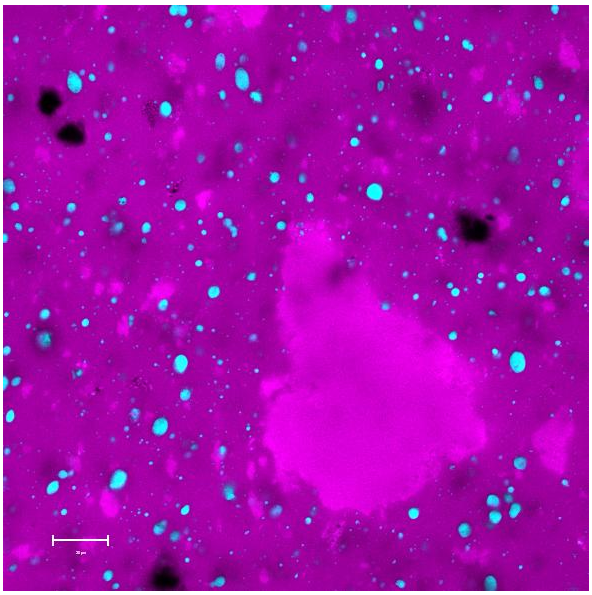


Figure 3-4 (a)

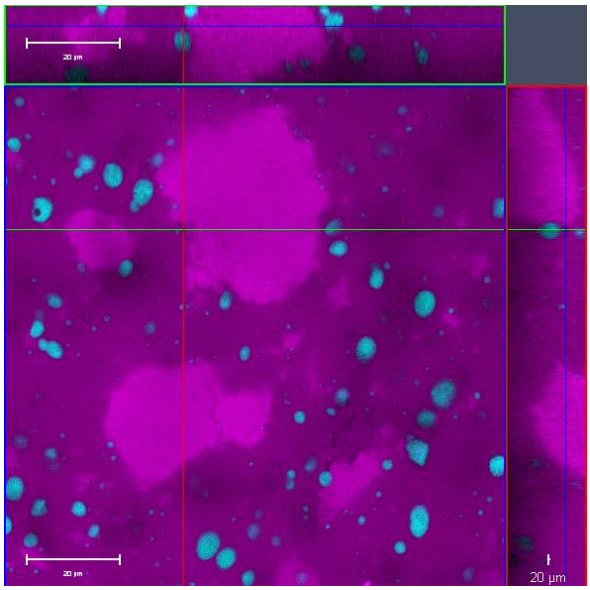


Figure 3-4 (b)

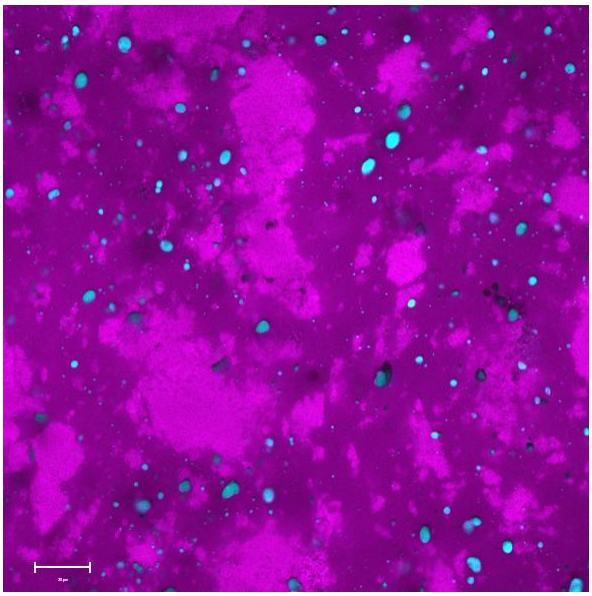


Figure 3-4 (c)

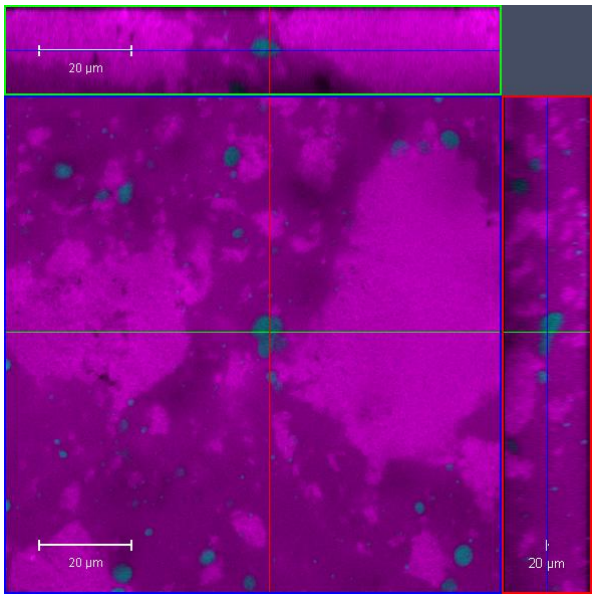


Figure 3-4 (d)

Figure 3-4. CSLM of experiment 2 replication 1 particulate gel (a) experiment 2 replication 1 particulate gel, Z-stack (b), experiment 2 replication 2 particulate gel (c), and experiment 2 replication 2, Z-stack particulate gel (d) particle filled cheeses imaged. Scale bars are 20 µm.

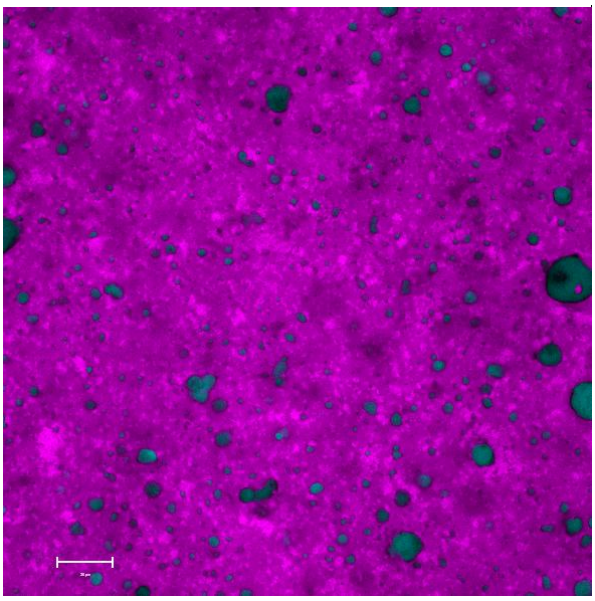


Figure 3-5 (a)

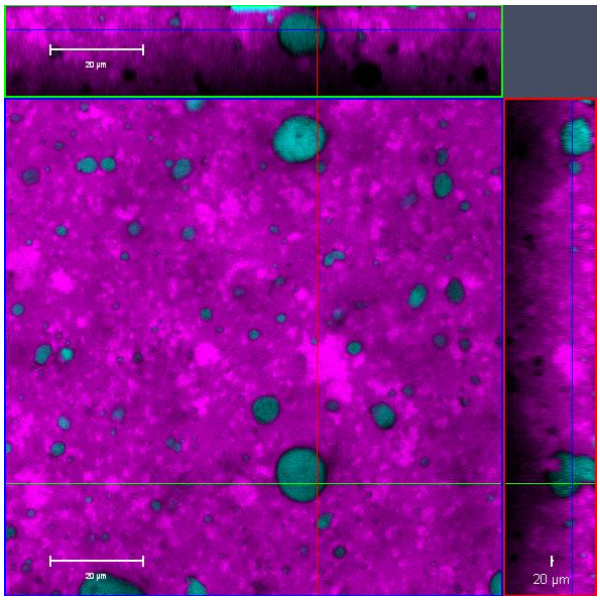


Figure 3-5 (b)

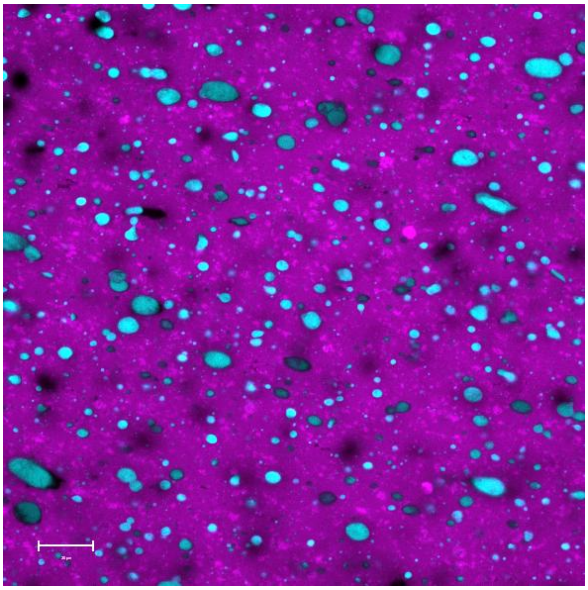


Figure 3-5 (c)

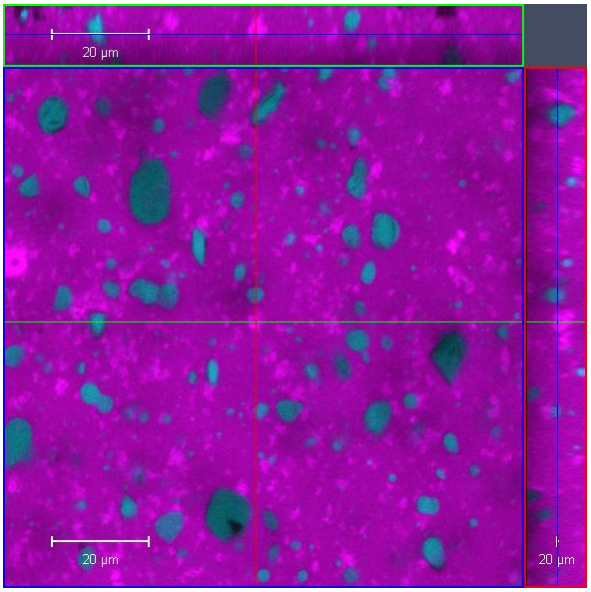
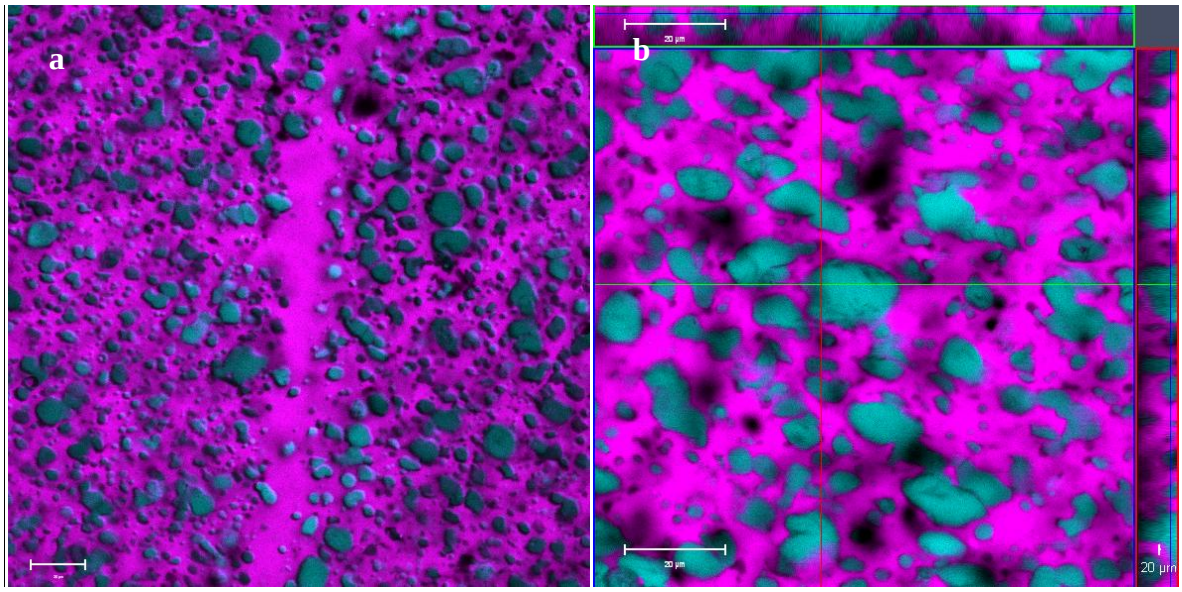
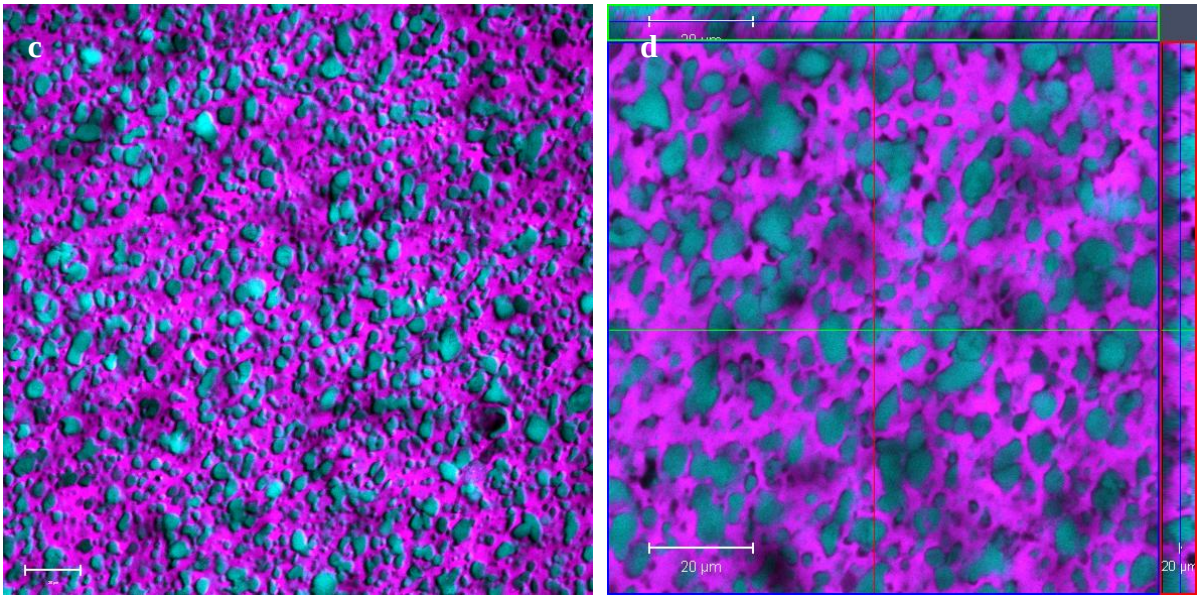


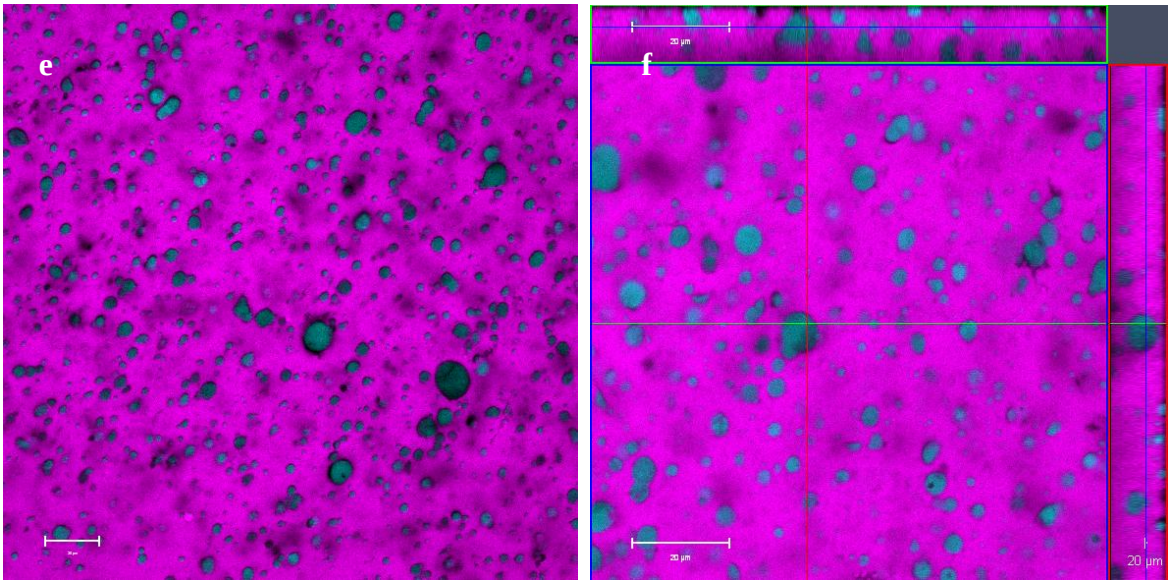
Figure 3-5 (d)

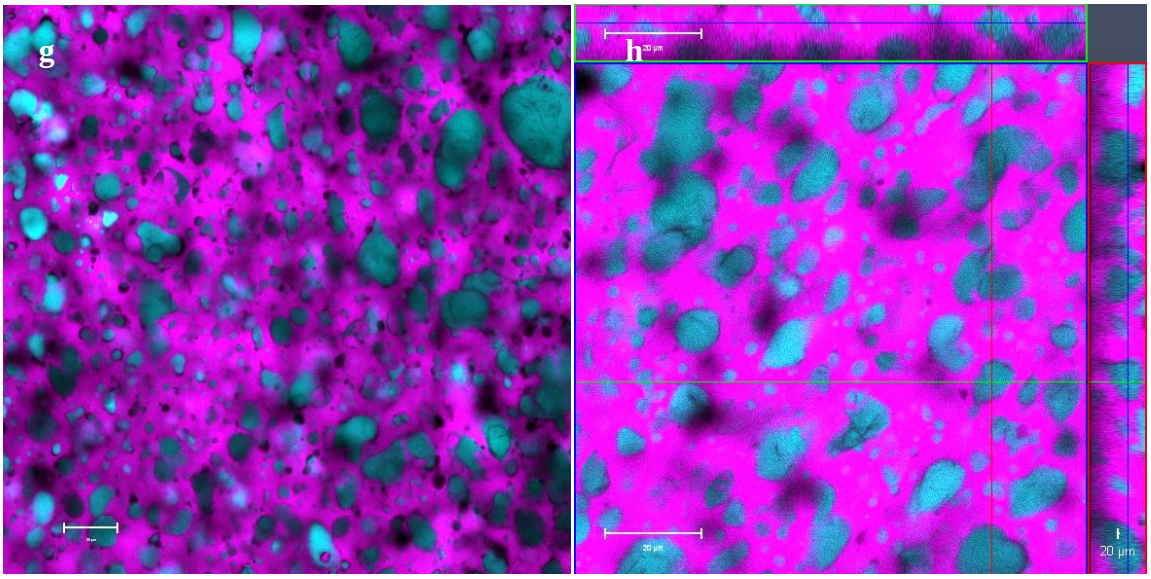
Figure 3-5. CSLM of experiment 2 replication 1 native fat composite (a) experiment 2 replication 1 native fat composite, Z-stack (b), experiment 2 replication 2 native fat composite (c), and experiment 2 replication 2 native fat composite, Z-stack (d), particle filled cheeses. Scale bars are 20 µm.

Figure 3-6. CSLM of experiment 2 replication 1 full fat control (a) replication 1 full fat control, Z-stack (b), replication 1 full fat control (c), replication 1 full fat control, Z-stack (d), replication 1 low fat control (e), replication 1 low fat control, Z-stack (f), replication 1 homogenized fat control (g), and replication 1 homogenized fat control, Z-stack (h) particle filled cheeses. Scale bars are 20 µm.


a
b
20 µm


c
d
20 µm
20 µm


e
f
20 µm
20 µm


g
h
20 µm
20 µm

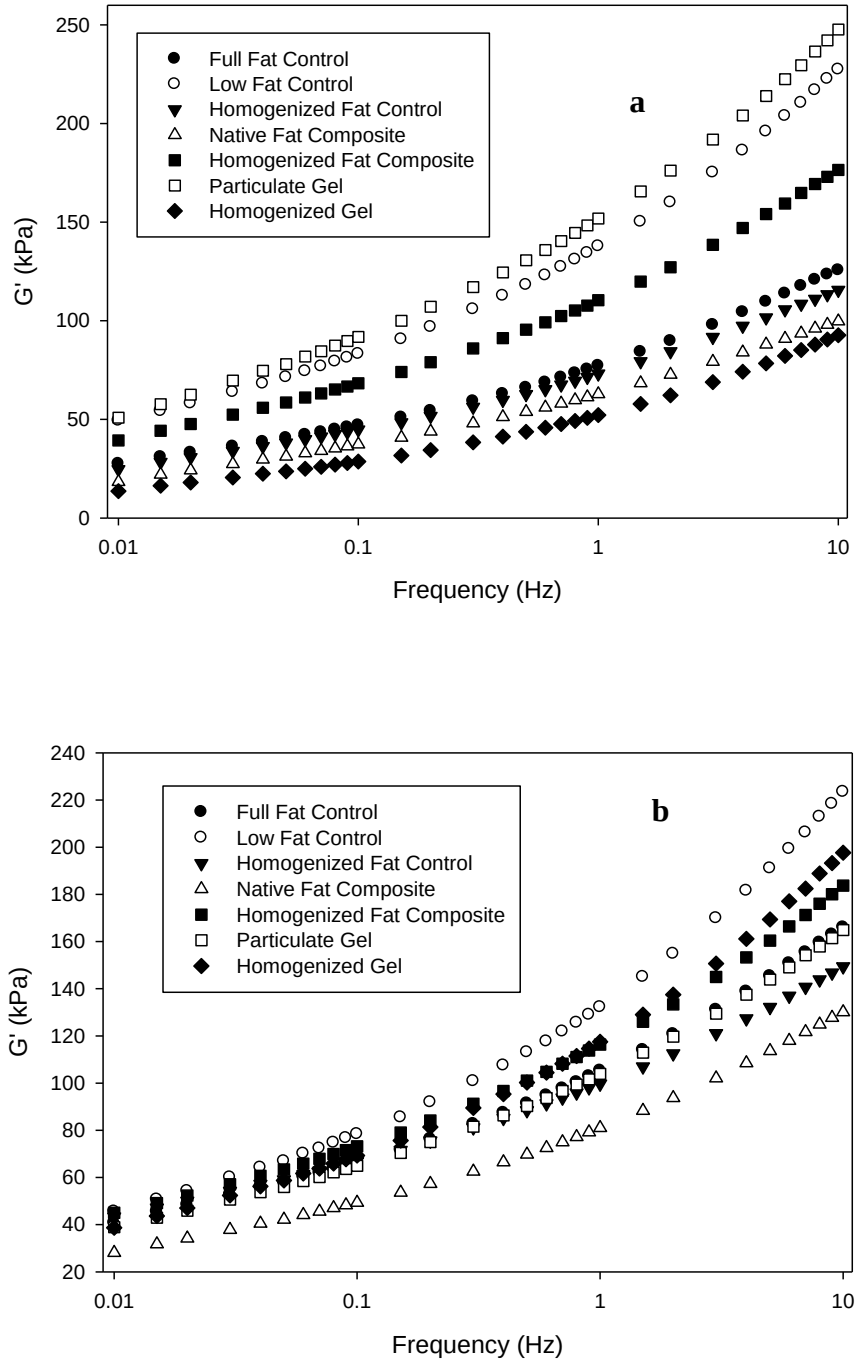


Figure 3-7. Frequency Sweep of experiment 2 replication 1(a) and replication 2 (b) particle filled cheese at 24 weeks ageing. Cheeses were tested at normalized stress values corresponding to a strain of 3.6E-3 at 25°C.

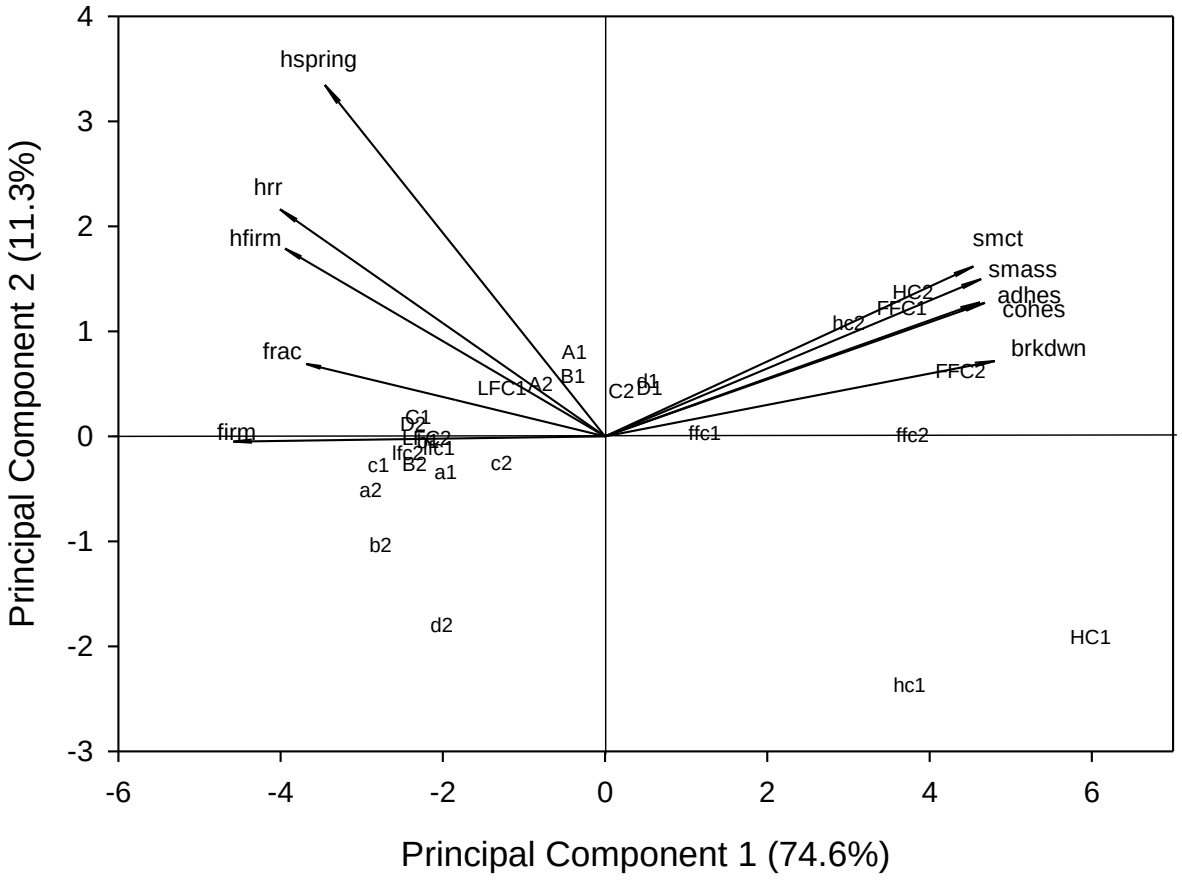


Figure 3-8. Biplot of principal component analysis of Experiment 2 Particle Filled Cheeses. Abbreviations are summarized in Table 3-15 and Table 3-16

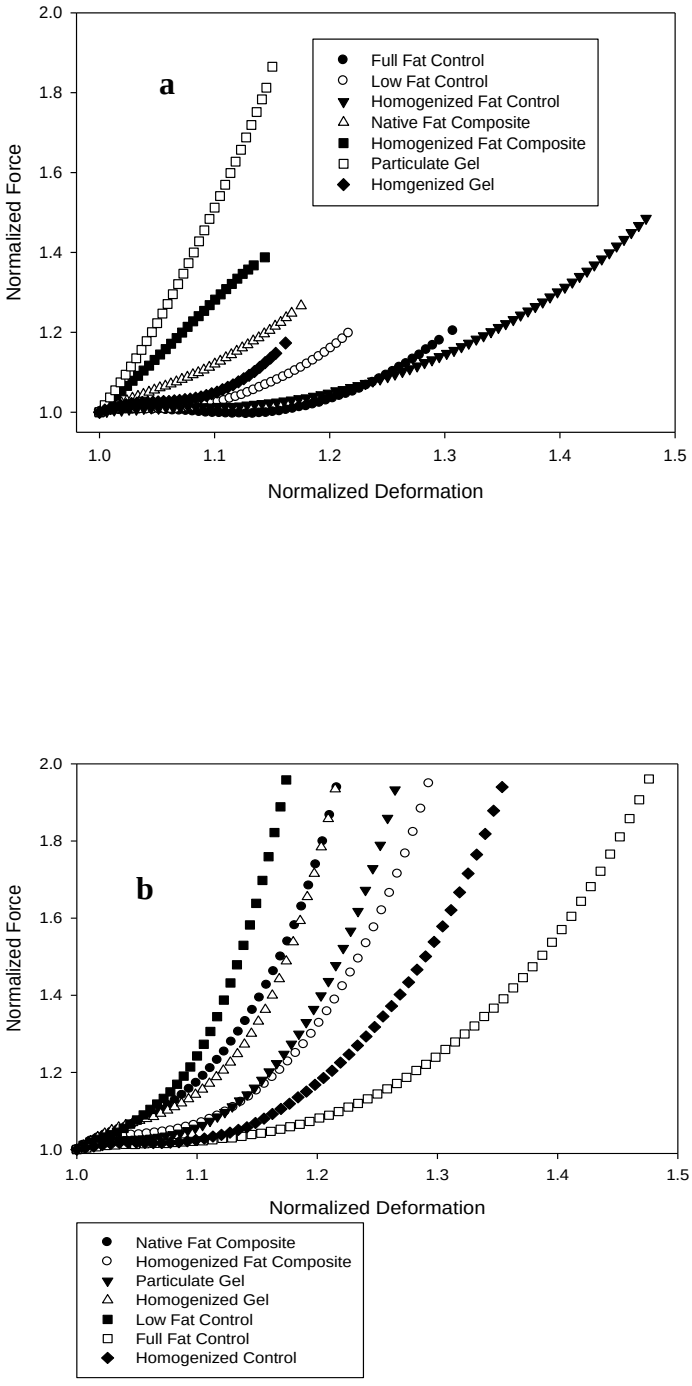
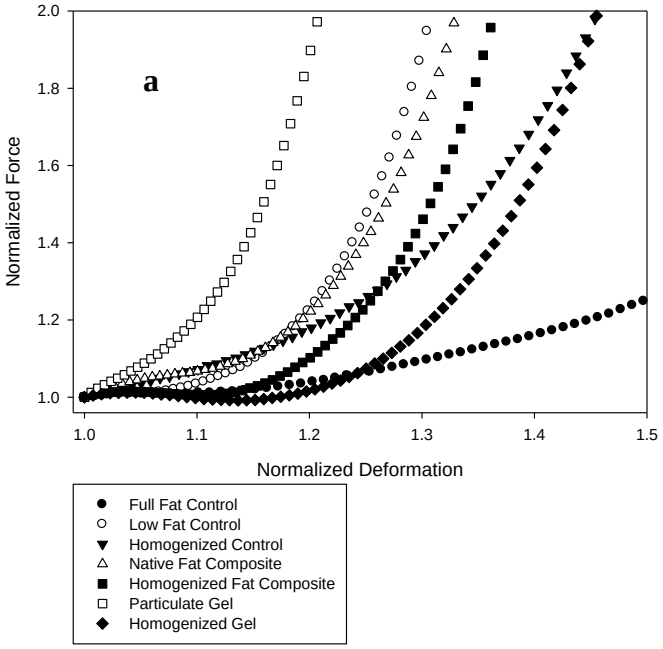


Figure 3-9. Post fracture analysis of experiment 2 replication 1 (a) and replication 2 (b) particle filled cheese at 8 weeks ageing.



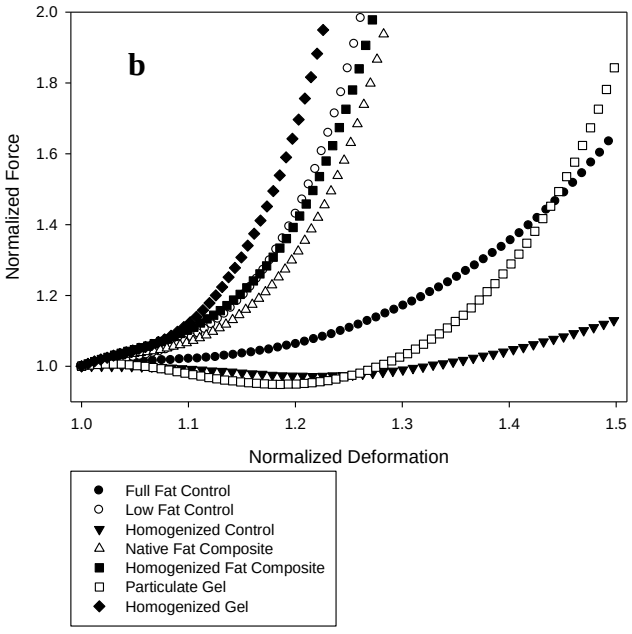


Figure 3-10. Post fracture analysis of experiment 2 replication 1 (a) and replication 2 (b) particle filled cheese after 24 weeks ageing.

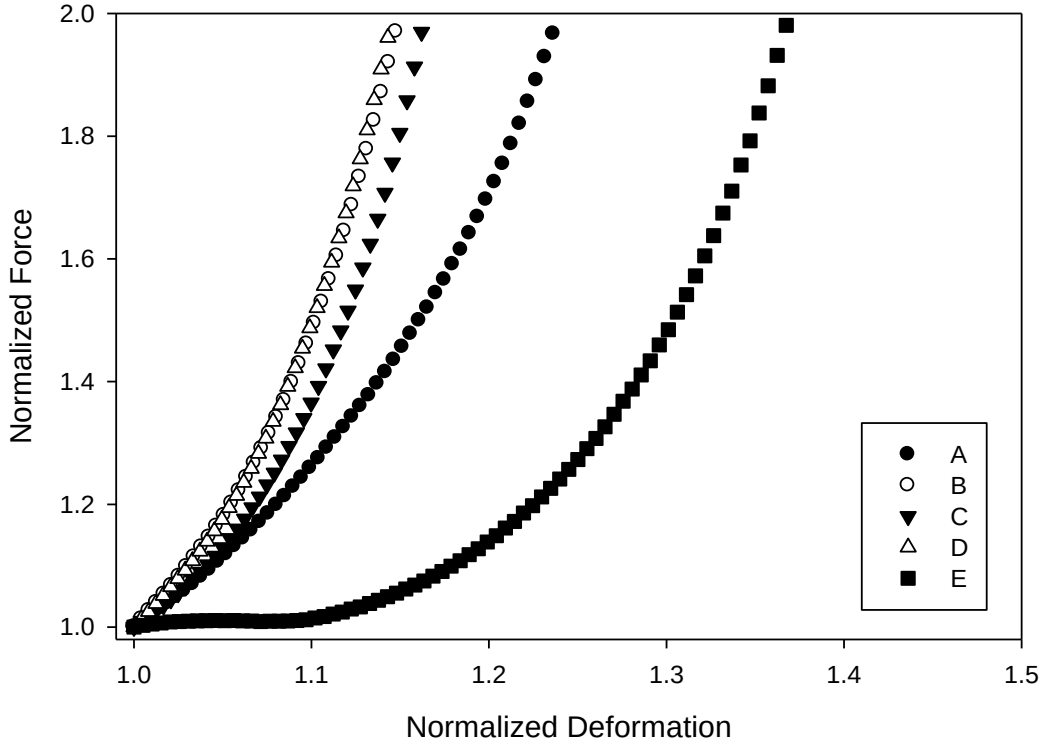
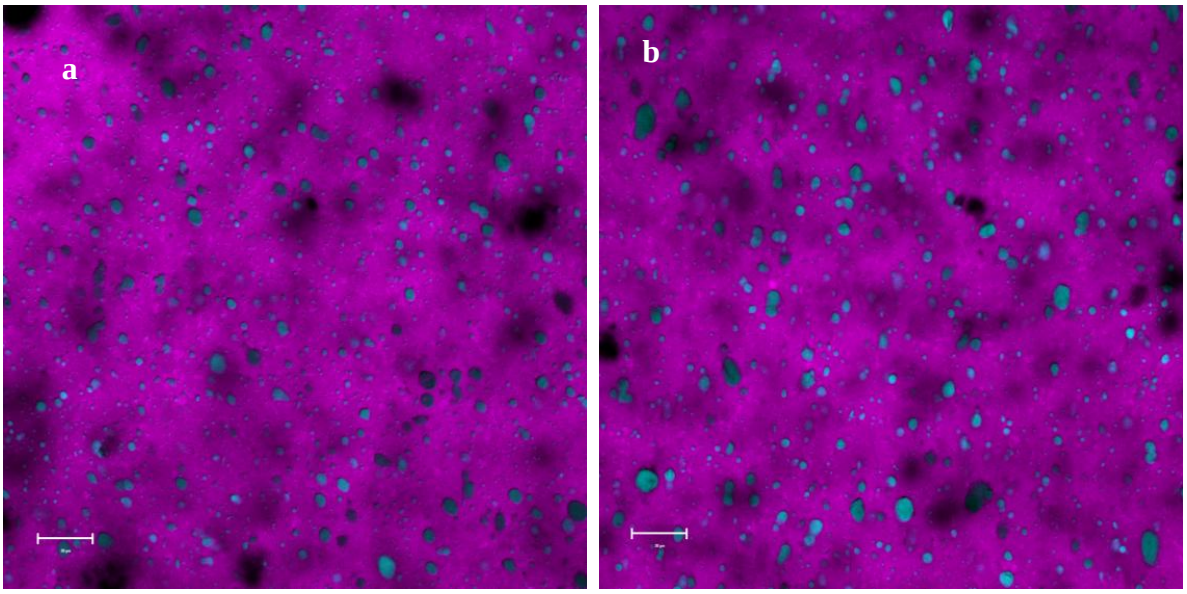
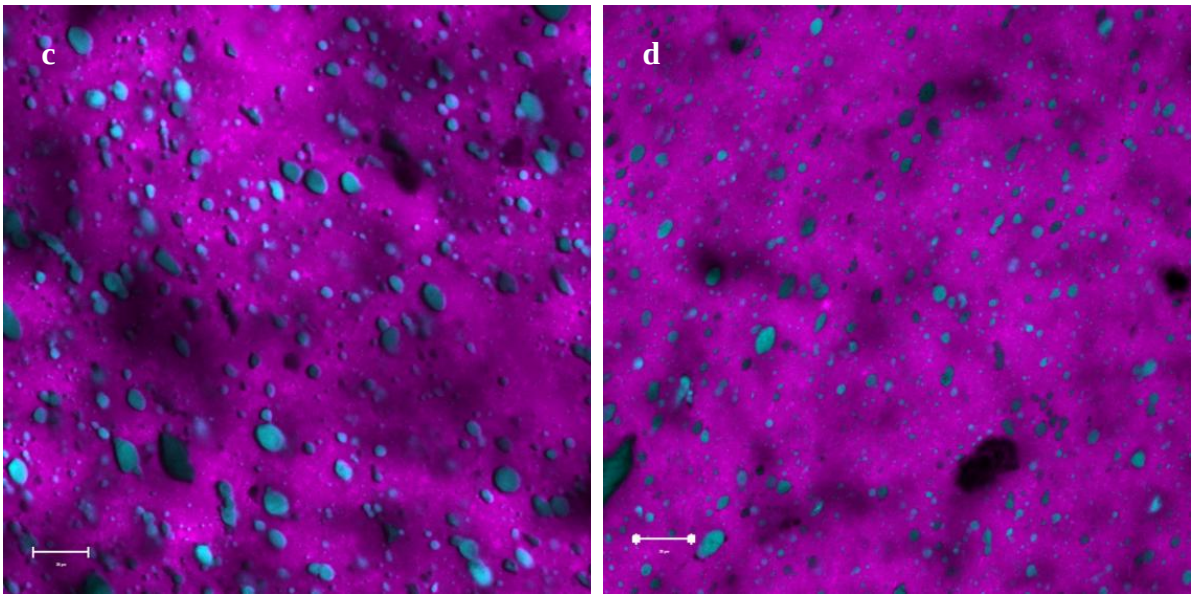


Figure 3-11. Post fracture analysis of experiment 3 particle filled cheese at 8 weeks ageing.

Figure 3-12. CSLM of experiment 3 treatment A (a), treatment B (b), treatment C (c), treatment D (d), and treatment E (e), experiment particle filled cheeses imaged after 8 weeks ageing. Scale bars are 20 μm.


a
b

.


c
d

.

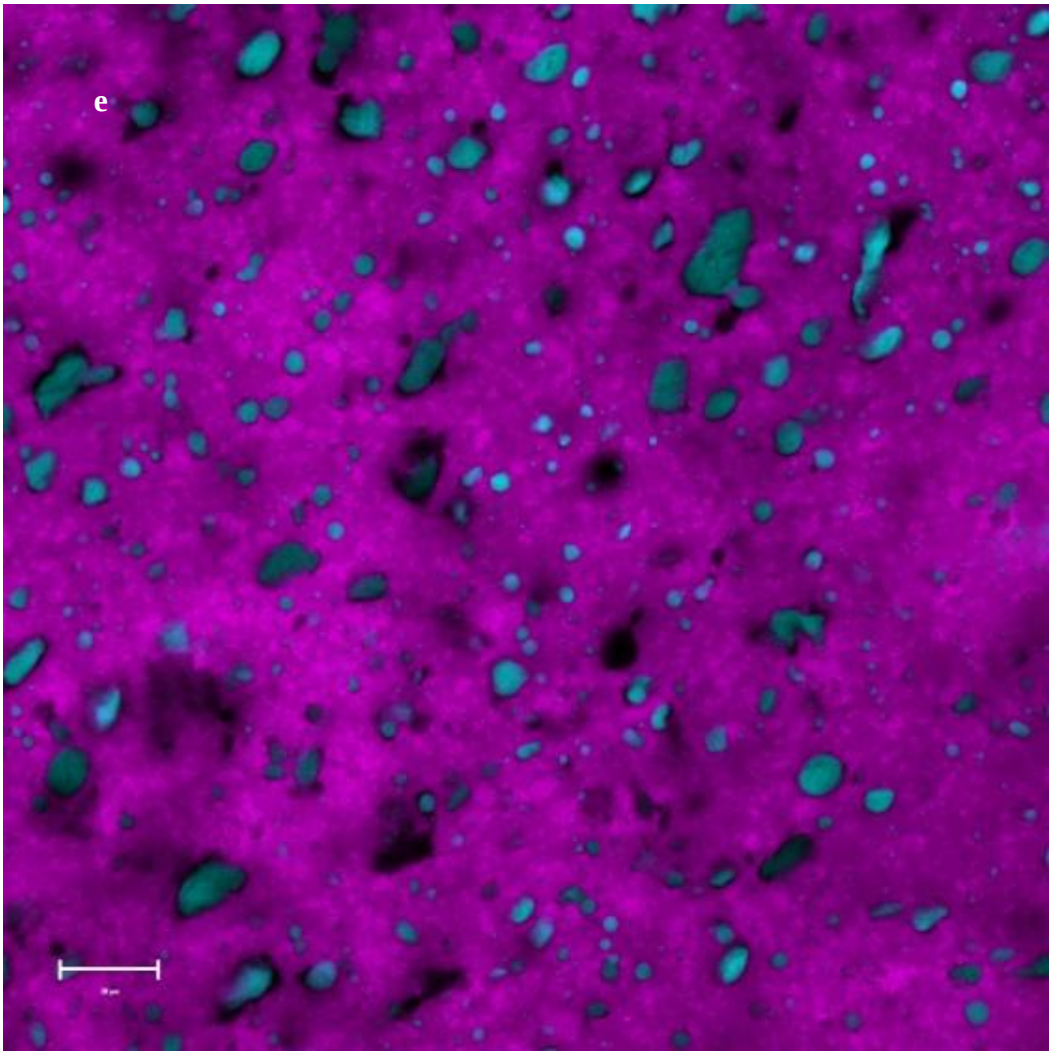


Figure 3-12. (e) Experiment 3 treatment E.

APPENDIX

Table A-1. LARGE STRAIN AND FRACTURE RHEOLOGY OF EXPERIMENT 2 PARTICLE FILLED CHEESE AFTER 8 WEEKS AGEING.

Treatment	Fracture Stress (kPa)	Fracture Strain	Recoverable Energy (%)	Fracture Toughness ($Pam^{1/2}$)	Fracture Energy (J/m^2)
Full Fat Control-1	29.1 ± 9.2	1.01 ± 0.11	23.8 ± 0.8	938.9 ± 44.1	15.9 ± 4.0
Full Fat Control-2	33.6 ± 9.5	1.00 ± 0.16	20.1 ± 0.7	321.0 ± 57.0	3.3 ± 0.8
Homogenized Fat Control-1	24.2 ± 10.3	0.93 ± 0.13	22.6 ± 0.4	808.2 ± 77.3	9.6 ± 1.1
Homogenized Fat Control-2	28.8 ± 5.6	0.86 ± 0.15	28.6 ± 1.5	281.0 ± 71.9	1.6 ± 0.4
Low Fat Control-1	63.9 ± 12.9	1.11 ± 0.19	34.6 ± 1.0	1276.5 ± 507.5	20.9 ± 10.3
Low Fat Control-2	76.3 ± 8.2	1.17 ± 0.19	29.0 ± 1.3	1746.9 ± 340.7	24.5 ± 7.1
Native Fat Composite-1	55.1 ± 20.6	1.24 ± 0.18	29.2 ± 1.9	1178.2 ± 207.6	11.7 ± 6.08
Native Fat Composite-2	57.2 ± 4.0	1.53 ± 0.17	31.4 ± 1.7	551.7 ± 154.1	7.7 ± 3.9
Homogenized Fat Composite-1	60.0 ± 15.9	1.31 ± 0.14	30.0 ± 1.4	1220.6 ± 269.1	21.8 ± 5.9
Homogenized Fat Composite-2	55.0 ± 1.5	1.20 ± 0.05	33.9 ± 1.0	1167.0 ± 154.1	20.2 ± 5.5
Particulate Gel-1	71.3	1.34 ± 0.09	31.5 ± 2.0	2392.4 ± 260.4	51.5 ± 10.2
Particulate Gel-2	54.7 ± 6.3	1.19 ± 0.18	28.1 ± 1.3	329.1 ± 30.4	3.7 ± 0.6
Homogenized Gel-1	34.1 ± 9.0	1.12 ± 0.11	27.6 ± 0.9	910.8 ± 242.2	9.6 ± 2.1
Homogenized Gel-2	56.4 ± 4.7	1.4 ± 0.10	29.1 ± 1.2	703.7 ± 132.9	15.45 ± 6.0

Table A-2. LARGE STRAIN AND FRACTURE RHEOLOGY OF EXPERIMENT 2 PARTICLE FILLED CHEESE AFTER 24 WEEKS AGEING.

Treatment	Fracture Stress (kPa)	Fracture Strain	Recoverable Energy (%)	Fracture Toughness ($Pam^{1/2}$)	Fracture Energy (J/m^2)
Full Fat Control-1	31.0 ± 12.1	0.79 ± 0.30	16.7 ± 1.3	432.7 ± 80.3	3.7 ± 1.8
Full Fat Control-2	26.2 ± 7.5	0.96 ± 0.15	17.1 ± 0.8	495.4 ± 115.2	4.4 ± 1.3
Homogenized Fat Control-1	22.0 ± 6.9	0.91 ± .28	15.4 ± 0.4	318.0 ± 19.3	2.0 ± 0.5
Homogenized Fat Control-2	25.9 ± 9.5	0.86 ± 0.15	22.8 ± 1.3	412.7 ± 111.4	2.6 ± 0.6
Low Fat Control-1	57.4 ± 7.2	1.1 ± 0.15	24.0 ± 0.3	494.0 ± 71.4	5.5 ± 1.2
Low Fat Control-2	56.0 ± 3.9	1.30 ± .24	27.3 ± 0.4	1106.9 ± 322.2	16.3 ± 4.8
Native Fat Composite-1	38.0 ± 5.0	1.60 ± 0.24	23.3 ± 1.3	369.9 ± 94.4	5.0 ± 1.7
Native Fat Composite-2	38.9 ± 10.9	1.70 ± 0.24	26.9 ± 1.2	513.5 ± 122.3	8.0 ± 1.8
Homogenized Fat Composite-1	46.5 ± 7.5	1.15 ± 0.10	23.4 ± 0.5	561.2 ± 110.3	9.4 ± 3.3
Homogenized Fat Composite-2	46.1 ± 5.2	1.42 ± 0.08	29.3 ± 2.0	1025.4 ± 128.1	16.5 ± 2.3
Particulate Gel-1	65.9 ± 12.1	1.25 ± 0.24	24.6 ± 1.0	670.4 ± 109.7	9.7 ± 2.1
Particulate Gel-2	32.6 ± 8.1	1.00 ± 0.12	23.8 ± 1.3	560.5 ± 86.4	6.8 ± 0.9
Homogenized Gel-1	30.4 ± 6.6	0.95 ± 0.15	21.1 ± 1.3	222.3 ± 49.6	2.0 ± 0.9
Homogenized Gel-2	52.2 ± 11.9	1.32 ± 0.12	29.2 ± 0.6	1030.2 ± 196.2	19.5 ± 5.1

Table A-3a. SENSORY TEXTURE OF EXPERIMENT 2 PARTICLE FILLED CHEESES AT 8 WEEKS AGEING

FORCE-DEFORMATION TERMS

Treatment	Hand Firmness	Hand Springiness	Hand Rate of Recovery	Firmness	Fracturability
Full Fat Control-1	11.4 ± 0.5	12.1 ± 1.6	12.1 ± 1.3	10.1 ± 0.7	5.3 ± 0.5
Full Fat Control-2	6.7 ± 0.7	11.6 ± 1.0	10.8 ± 0.9	6.6 ± 0.4	4.8 ± 0.4
Homogenized Fat Control-1	7.3 ± 0.3	6.9 ± 1.4	6.5 ± 1.8	6.4 ± 0.6	4.8 ± 0.4
Homogenized Fat Control-2	9.8 ± 0.8	13.1 ± 1.0	13.3 ± 0.8	7.5 ± 0.9	4.6 ± 0.6
Low Fat Control-1	13.8 ± 0.5	14.6 ± 0.4	14.7 ± 0.3	11.5 ± 0.7	6.3 ± 0.5
Low Fat Control-2	14.6 ± 0.3	14.8 ± 0.4	14.3 ± 0.7	12.0 ± 0.4	6.5 ± 0.4
Native Fat Composite-1	13.1 ± 0.8	14.2 ± 0.8	14.2 ± 0.5	11.1 ± 0.6	6.6 ± 0.5
Native Fat Composite-2	14.1 ± 0.5	14.5 ± 0.5	14.0 ± 0.7	11.9 ± 0.7	7.4 ± 0.9
Homogenized Fat Composite-1	13.6 ± 0.3	14.7 ± 0.4	14.6 ± 0.6	12.1 ± 0.5	6.7 ± 0.5
Homogenized Fat Composite-2	14.1 ± 0.4	14.5 ± 0.5	14.2 ± 0.7	12.1 ± 0.7	7.4 ± 0.6
Particulate Gel-1	14.6 ± 0.4	14.8 ± 0.3	14.9 ± 0.3	12.8 ± 0.6	6.3 ± 0.6
Particulate Gel-2	13.3 ± 0.6	14.4 ± 0.7	14.0 ± 0.8	10.4 ± 0.6	6.3 ± 0.7
Homogenized Gel-1	11.4 ± 0.6	14.1 ± 0.7	13.9 ±0.5	8.8 ± 0.7	6.0 ± 0.5
Homogenized Gel-2	14.6 ± 0.4	14.7 ± 0.4	14.2 ± 0.4	12.0 ± 0.3	7.2 ± 1.0

Table A-3b. SENSORY TEXTURE OF EXPERIMENT 2 PARTICLE FILLED CHEESES AT 8 WEEKS AGEING

BOLUS FORMATION TERMS

Treatment	Degree of Breakdown	Cohesion	Adhesion	Smoothness of Mass	Smoothness of Mouth Coating
Full Fat Control-1	6.0 ± 0.8	6.5 ± 0.7	6.6 ± 0.7	7.2 ± 0.9	7.7 ± 0.7
Full Fat Control-2	8.7 ± 1.0	8.9 ± 1.4	8.0 ± 0.8	10.3 ± 1.2	9.6 ± 0.5
Homogenized Fat Control-1	8.7 ± 1.6	7.7 ± 1.4	6.2 ± 0.6	7.1 ± 0.9	7.6 ± 0.5
Homogenized Fat Control-2	8.6 ± 0.9	9.4 ± 0.9	8.0 ± 1.2	10.0 ± 0.8	9.6 ± 1.3
Low Fat Control-1	2.5 ± 0.5	2.8 ± 1.1	2.5 ± 0.7	3.6 ± 0.6	3.8 ± 0.8
Low Fat Control-2	2.0 ± 0.8	2.3 ± 0.7	2.7 ± 0.8	2.9 ± 1.0	3.4 ± 0.9
Native Fat Composite-1	2.1 ± 0.9	2.4 ± 1.2	2.6 ± 0.5	3.4 ± 0.5	4.1 ± 0.8
Native Fat Composite-2	1.2 ± 0.3	1.5 ± 0.5	2.7 ± 0.7	1.9 ± 0.5	2.5 ± 0.8
Homogenized Fat Composite-1	2.5 ± 0.5	2.5 ± 0.5	2.3 ± 0.9	3.0 ± 0.9	5.0 ± 0.7
Homogenized Fat Composite-2	1.2 ± 0.5	1.1 ± 0.4	2.5 ± 0.8	1.5 ± 0.4	2.5 ± 0.6
Particulate Gel-1	1.7 ± 0.5	2.2 ± 0.9	1.8 ± 0.7	2.2 ± 0.7	3.3 ± 0.4
Particulate Gel-2	3.2 ± 0.9	4.1 ± 1.2	3.8 ± 0.8	3.4 ± 1.0	4.2 ± 1.2
Homogenized Gel-1	5.6 ± 2.0	7.7 ± 1.0	5.2 ± 1.1	5.6 ± 0.9	6.7 ± 0.5
Homogenized Gel-2	1.2 ± 0.4	1.5 ± 0.7	2.0 ± 0.7	2.4 ± 0.6	2.9 ± 0.7

Table A-4a. SENSORY TEXTURE OF EXPERIMENT 2 PARTICLE FILLED CHEESES AT 24 WEEKS AGEING

FORCE-DEFORMATION TERMS

Treatment	Hand Firmness	Hand Springiness	Hand Rate of Recovery	Firmness	Fracturability
Full Fat Control-1	10.6 ± 0.6	12.0 ± 1.4	11.4 ± 1.1	8.3 ± 0.9	5.3 ± 0.5
Full Fat Control-2	7.6 ± 0.7	11.6 ± 2.0	11.1 ± 1.0	5.7 ± 0.6	5.1 ± 0.7
Low Fat Cotrol-1	13.2 ± 0.5	14.3 ± 0.5	14.3 ± 0.4	11.4 ± 0.4	7.3 ± 0.9
Low Fat Control-2	14.6 ± 0.3	14.8 ± 0.2	14.3 ± 0.7	12.5 ± 0.5	6.2 ± 0.8
Homogenized Fat Control-1	7.0 ± 2.6	5.0 ± 4.5	5.0 ± 4.0	5.9 ± 1.5	4.2 ± 1.3
Homogenized Fat Control-2	9.8 ± 0.6	12.5 ± 0.7	11.2 ± 1.3	7.5 ± 0.8	5.9 ± 0.6
Native Fat Composite-1	12.5 ± 0.9	14.2 ± 0.5	14.0 ± 0.7	10.9 ± 0.9	7.2 ± 0.8
Native Fat Composite-2	13.2 ± 0.5	14.5 ± 0.	14.1 ± 0.4	10 ± 0.9	6.8 ± 0.7
Homogenized Fat Composite-1	12.5 ± 1.1	14.3 ± 0.6	13.6 ± 1.0	10.7 ± 0.6	6.8 ± 0.8
Homogenized Fat Composite-2	13.6 ± 0.7	14.5 ± 0.5	14.5 ± 0.6	11.0 ± 1.4	7.2 ± 0.6
Particulate Gel-1	14.3 ± 0.5	14.7 ± 0.4	14.4 ± 0.6	12.5 ± 0.6	7.4 ± 0.9
Particulate Gel-2	12.3 ± 0.5	13.8 ± 0.9	13.1 ± 0.9	9.6 ± 0.7	6.4 ± 0.3
Homogenized Gel-1	11.7 ± 0.7	13.1 ± 0.7	13.0 ± 0.7	9.5 ± 0.6	6.8 ± .5
Homogenized Gel-2	14.6 ± 0.3	14.8 ± 0.2	14.5 ± 0.4	13 ± 0.7	6.6 ± 0.5

Table A-4b. SENSORY TEXTURE OF EXPERIMENT 2 PARTICLE FILLED CHEESES AT 24 WEEKS AGEING

BOLUS FORMATION TERMS

Treatment	Degree of Breakdown	Cohesion	Adhesion	Smoothness of Mass	Smoothness of Mouth Coating
Full Fat Control-1	9.6 ± 1.3	10.4 ± 0.7	9.5 ± 0.6	11.1 ± 0.6	11.2 ± 0.5
Full Fat Control-2	10.0 ± 1.3	10.8 ± 0.7	10.2 ± 0.8	10.6 ± 0.6	9.8 ± 0.7
Low Fat Cotrol-1	3.1 ± 0.6	4.7 ± 0.5	4.1 ± 0.8	5.0 ± 0.4	5.9 ± 0.6
Low Fat Control-2	2.3 ± 0.5	2.7 ± 0.5	2.0 ± 0.6	4.0 ± 0.5	4.3 ± 0.8
Homogenized Fat Control-1	10.7 ± 1.3	11.1 ± 1.1	9.8 ± 1.6	10.6 ± 1.4	10.1 ± 1.8
Homogenized Fat Control-2	9.5 ± 0.7	10.8 ± 0.9	10.3 ± 0.8	11.4 ± 1.4	11.0 ± 1.0
Native Fat Composite-1	4.5 ± 0.6	5.6 ± 0.8	5.2 ± 1.1	6.2 ± 0.8	7.5 ± 0.7
Native Fat Composite-2	3.9 ± 1.5	5.1 ± 2.4	4.4 ± 0.6	5.5 ± 1.2	5.4 ± 0.6
Homogenized Fat Composite-1	4.3 ± 0.6	5.1 ± 0.7	5.6 ± 0.6	5.8 ± 0.7	6.7 ± 0.7
Homogenized Fat Composite-2	2.5 ± 1.1	2.0 ± 0.6	2.7 ± 0.7	2.9 ± 0.9	3.0 ± 0.6
Particulate Gel-1	2.3 ± 1.0	3.4 ± 1.4	3.2 ± 1.1	3.6 ± 0.9	4.1 ± 1.2
Particulate Gel-2	5.2 ± 0.8	6.5 ± 1.3	5.1 ± 0.9	5.8 ± 0.7	6.8 ± 0.6
Homogenized Gel-1	4.8 ± 0.8	7.6 ± 0.9	7.1 ± 0.7	6.4 ± 1.0	6.3 ± 0.5
Homogenized Gel-2	1.7 ± 0.9	3.1 ± 1.1	2.6 ± 0.8	4.2 ± 0.7	4.1 ± 0.7