ABSTRACT

BARDEN, LEANN MARIE. Textural Properties of Cheddar Cheese. (Under the direction of Edward Allen Foegeding.)

The goal of this research was to study the role of fat in defining the texture of cheddar cheese. From a materials/structure perspective, it is thought that fat functions as a filler particle in cheese, serving to weaken and disrupt the protein network of milk caseins. The filled gel model has been used to understand the texture of cheese, which is essentially a casein network filled with dispersed fat globules. Texture relates to rheological and mechanical properties, which depend upon network composition, structure, and molecular interactions.

In the first part of this research, three brands of commercial cheddar cheese available in both full-fat and reduced/low-fat varieties were purchased from grocery stores. Regardless of brand and variability in age and/or storage conditions at the store, low-fat and reduced-fat cheese were characterized by structural and deformability differences, which resulted in larger critical stress and strain, maximum compliance, percent recoverable energy, and fracture strain; trends were consistent across the linear viscoelastic region, critical points, and non-linear region up to fracture. These results were compared against cheese obtained directly from the manufacturer, which showed similar changes in properties with fat content. Additional fracture testing on the latter group of cheeses showed fracture energy and toughness did not differ by fat content.

Cheeses received from the manufacturer were subjected to descriptive sensory analysis and consumer panels to determine if sample thickness and size affect evaluation of texture. Sample thickness significantly impacted descriptive analysis panelists' perception of texture using both hand and first-bite attributes but did not affect those attributes determined after several chews ("chewdown attributes"), nor did it impact consumers' evaluation of texture. However, consumers consistently preferred thicker samples, regardless of fat content.

The goal of the second half of this research was to understand how alternate fillers function in a cheese matrix. Low-filler (6% particles), reduced-filler (16%), and full-filler (33%) cheeses were produced using either G-50 Sephadex beads of varying sizes (20 – 150 μm diameter) or milkfat. Small and large-strain rheological tests were run on each treatment at 8, 12, and 18 weeks. Age had no significant effect except on large strain testing; treatments recovered less energy with age. Rheological properties were primarily affected by filler volume rather than filler size or type. Both control and experimental cheeses showed a decrease in deformability and an increase in firmness as filler volume increased above 25%, although the more elastic beads exhibited a greater reinforcing effect. The difference in filler elasticity likely explains why control cheese recovered less energy as filler volume increased, whereas bead-filled cheese recovered more energy. This study found that cheese is not a simple system but was well-modeled by the filled gel theory.

The final study expanded upon the filled gel model by adding Sephadex beads of the same size (40-150 μm in diameter) but varying electrostatic charges. Bead charge affected the cheesemaking process, including coagulation time, moisture retention in the curd and whey syneresis, drain-to-salt time, and net yields. The consequent compositional differences affected rheological and mechanical properties such that the highest moisture treatment was less firm, required less work to fracture, and recovered less energy. Again, age affected only recoverable energy. Charged particles may provide a means for manipulating the texture of low-fat cheese.

Overall, this research characterized textural properties of cheddar cheese currently available on the market. Manufacturers must mimic the properties of full-fat cheese to develop palatable low-fat cheese. This research suggests that adding additional filler volume to low-fat cheese may produce a low-fat product with full-fat texture.

Textural Properties of Cheddar Cheese

by
Leann Marie Barden

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, NC

2010

APPROVED BY:

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DEDICATION

To Mom and Dad, whose countless sacrifices for my education made everything else possible. I'm proud, and lucky, to be your daughter.

.

BIOGRAPHY

Leann was born to Douglas and Sue Barden in Milwaukee, Wisconsin, where she grew up embracing her community's German-Polish-Italian roots and, of course, everything dairy. An avid reader with a penchant for writing, Leann interned at *The Milwaukee Journal Sentinel* newspaper in high school and wrote freelance for several publications. Nevertheless, Leann's career aspirations were undecided through much of high school and the first two years of college. Before deciding on food science, Leann considered careers in forensic science (*before* CSI was a TV show), epidemiology, journalism, economics, business, genetic counseling, and various other medical careers, to name a few. And so it was that Leann enrolled at the University of Wisconsin-Madison (UW) for her B.S. in bacteriology.

Her years at UW were undoubtedly the most formative years and adventurous years of Leann's life. Her fondest memories include sleeping outside and brushing her teeth in academic buildings while camping out (i.e. sleeping in a lawn chair) one straight week for hockey tickets, sailing on Lake Mendota, and the time she wore three pairs of pants in order to walk two miles to school in a windchill of -30°F (uphill both ways because of very large snow mounds). While at UW, Leann was an avid Badgers sports fan and enjoyed playing the bassoon in University Band. She was an active member of the UW Ballroom Dance Association, Women in Science and Engineering, and the UW Food Science Club, of which she eventually became president. Leann discovered food science when she heard Dr. Rich Hartel give a lecture on the science of chocolate, and

why chocolate is chemically and physically different from chocolate compound coatings. Needless to say, it was love at first bite.

Leann's science career began her sophomore year with a research assistantship in Dr. Adel Talaat's lab (UW), where she studied the regulatory effects of three genes in determining the pathogenesis of *Mycobacterium tuberculosis*. Leann spent her junior and senior years working in Dr. Rich Hartel's lab (UW) on a joint project with the local Veteran's Affairs hospital; the project goal was to develop palatable, refreshing beverages for people with dysphagia, a type of swallowing disorder. R&D internships at Danisco (Madison, WI) and General Mills (Minneapolis, MN) further cemented her career plans in food science.

These lab and internship experiences inspired Leann to pursue her Master's degree in Dr. E. Allen Foegeding's lab (North Carolina State University, NCSU), whose lab group's innovative work with protein rheology, tribology, and mastication interactions precede them. While at NCSU, Leann grew academically, professionally, and as a scientist. She was also active in the NCSU Food Science Club; her greatest claim to fame involved co-chairing the club's annual Dairy Bar fundraiser—the most prosperous one to date. Leann also co-chaired the Institute of Food Technologists' 2010 Fun Run Committee, which raised over $70,000 for student scholarships. The experience of pursuing a Master's of Science at NCSU has prompted Leann to pursue a PhD in food science. She looks forward to her next adventure in the food science labs at the University of Massachusetts-Amherst.

ACKNOWLEDGEMENTS

If it takes a village to raise a child, then it must take an entire state to raise a successful Master's candidate! I would like to thank Dairy Management, Inc. and the Southeast Dairy Foods Research Center for providing funding and support for the completion of this research. Above all, I would like to thank Dr. E. Allen Foegeding for providing the opportunity to work on this project and for challenging me to grow as a thinker, a scientist, and a better writer. *This manuscript shows* I will henceforth focus on the data and not the tests or methods! Your guidance, explanations, suggestions, and support have been invaluable during my research and will surely serve me for years to come. Thank you so much for encouraging me to develop my leadership skills within the Food Science Club and IFTSA, allowing me to grow as a well-rounded person, not just a researcher.

I am especially grateful to those NCSU faculty, staff, and students who helped with my project over the past two years. To Neil Rogers, Esra Çakir, and especially Sharon Ramsey: thank you for training me in the Rheology Lab and for helping me troubleshoot new protocols. To Drs. Saad Khan, Chris Daubert, and Allen Foegeding: thank you for all of your patience and countless explanations as I learned to make sense of those rheology protocols and understand the actual theory! I am especially grateful to Dr. Chris Daubert for supporting my enthusiasm to pursue a side-experiment on bubble gum rheology and for his feedback on my seminar presentation and abstract. To Tristan Berry and especially Dr. Eva Johannes: thank you for teaching me everything I know

about microscopy and spending hours of your time helping me image my samples, regardless of whatever trouble the old confocal microscope caused for us! And finally, to Dr. MaryAnne Drake: thank you for helping me incorporate sensory science into my rheology-laden project, and for showing me the ropes on publishing my first paper (in conjunction with Foegeding labmates Esra Çakir, Kelsey Ryan, and Nin Leksrisompong).

Of course, my project would have been very different (i.e. greater standard deviations and a much longer timeframe) if I had actually made the Sephadex-filled cheeses for my project. I would like to acknowledge and thank our collaborators and expert cheese-makers at Utah State University for their contributions. Dr. Don McMahon was particularly helpful in answering my questions and explaining the production differences between the Sephadex-filled cheeses and normal cheddar cheese.

I would like to specially acknowledge the entire Foegeding Lab, who made NC feel like home. Your support and friendship has helped me conquer daunting milestones like my departmental seminar presentation and first tubing adventure behind Dr. Foegeding's boat, and your suggestions, questions, and critiques have strengthened my research and confidence. I owe many thanks to Cal for helping to cut 40-pound blocks of cheese for sensory tests. To Paige: thanks for taking this Wisconsinite under your wing and teaching me about the world of grits and biscuits; now let's talk cheese curds and brats.

To Mom, Dad, and Chris: thank you for your endless support. My favorite Christmas gift from you was always the plane ticket home, although I did appreciate that

you paid for a roundtrip ticket! Thank you to Sarah for reminding me to be a *fun* science nerd and try new things…like the Polar Bear Plunge into Lake Michigan on January 1st while wearing cow costumes (even if the video of us doing it didn't land us a spot on *The Amazing Race*). And finally, thank you to Craig for sharing many NC adventures and laughs with me and for reminding me to breathe when I was under a lot of stress (no pun intended).

TABLE OF CONTENTS

LIST OF TABLES xiii

LIST OF FIGURES xvi

CHAPTER 1: REVIEW OF LITERATURE 1
1. Introduction 2
2. A Brief History of Cheese Production and Consumption 2
   2.1. A Market for Low-fat Cheese 2
   2.2. Cheese Manufacture 5
   2.3. Cheddar Cheese Manufacture and Popularity 9
   2.4. Variability in Cheese Manufacture 10
      2.4.1 Effect of pH 11
3. Low-fat Cheese 15
   3.1. Strategies for Making Low-fat Cheese 15
      3.1.1 Modifying the Manufacturing Process 16
      3.1.2 Using Adjunct Cultures 17
      3.1.3. Using Fat Replacers 18
   3.2. Flavor of Low-fat Cheese 20
   3.3. Texture of Low-fat Cheese 22
   3.4. Functionality of Low-fat Cheese 24
4. Filled-Gel Models 25
   4.1. Filled Gel Theory 25
   4.2. Mathematical Modeling of Filled Gels 27
   4.3. Biopolymer Gels Filled with Sephadex Beads 29
   4.4. Food Applications 31
5. Mechanical Methods for Testing Cheese 32
   5.1. Fundamentals 32
   5.2. Common Small Strain Tests 35
   5.3. Common Large Strain Tests 38
   5.4. Common Fracture Tests 39
6. Confocal Scanning Laser Microscopy 40
7. Project Objective 41
8. References 43

CHAPTER 2: RHEOLOGICAL SURVEY OF OFF-THE-SHELF COMMERCIAL CHEDDAR CHEESE AVAILABLE IN VARYING FAT CONTENTS 54
1. Abstract 55
2. Practical Application 55
3. Introduction 56

4. Materials and Methods....61
4.1. Cheese....61
4.2. Small Strain Rheological Tests....62
4.2.1 Stress Sweeps....62
4.2.2 Frequency Sweeps....62
4.2.3 Creep/recovery Tests....63
4.3. Large Strain Rheological Tests....63
4.4. Fracture Analysis....64
4.5. Statistical Analysis....65
5. Results and Discussion....65
5.1. Statistical Analysis....65
5.2. Observations of Treatments....66
5.3. Small Strain Rheological Tests....66
5.3.1 Stress Sweeps....66
5.3.2 Mechanical Spectra....67
5.3.3 Creep/recovery Tests....69
5.4. Large Strain Rheological Tests....70
5.5. Fracture Analysis....71
6. Conclusion....74
7. References....75

CHAPTER 3: EFFECT OF SAMPLE SIZE ON SENSORY TEXTURE OF CHEDDAR CHEESE....95
1. Abstract....96
2. Practical Application....96
3. Introduction....97
4. Materials and Methods....100
4.1. Cheese....100
4.2. Rheological and Mechanical Tests....100
4.2.1 Controlled-stress Tests....100
4.2.1.1 Stress Sweeps....101
4.2.1.2 Creep/recovery Tests....101
4.2.2 Large Strain Rheological Tests....102
4.2.3 Fracture Analysis....103
4.2.3.1 Torsion Gelometry....103
4.2.3.2 Single Edge Notched Bending....103
4.3. Sensory Testing....105
4.3.1. Descriptive Sensory Analysis....105
4.3.2. Consumer Panel....106
4.4. Statistical Analysis....107
5. Results and Discussion....107
5.1. Rheological Tests....107

5.1.1. Controlled-stress Tests 107
5.1.1.1 Stress Sweeps 107
5.1.1.2 Creep/recovery Tests 108
5.1.2. Large Strain Rheological Tests 109
5.1.3. Fracture Analysis 110
5.1.3.1 Torsion Gelometry 110
5.1.3.2 Single Edge Notched Bending (SENB) 112
5.1.4. Summary of Material Properties Elucidated by Rheological and Mechanical Testing 116
5.2. Sensory Testing 116
5.2.1. Descriptive Sensory Analysis Based on Fat Content 116
5.2.2. Descriptive Sensory Analysis Based on Sample Size 117
5.2.3. Consumer Panel 119
6. Conclusion 120
7. References 121

CHAPTER 4: INVESTIGATING THE FILLED GEL MODEL IN CHEDDAR CHEESE THROUGH USE OF SEPHADEX BEADS 139
1. Abstract 140
2. Practical Application 140
3. Introduction 141
3.1. Filled Gel Theory 141
3.2. Filled Gel Models 143
3.3. Sephadex Properties 145
3.4. Objective 146
4. Materials and Methods 146
4.1. Cheese 146
4.1.1 Materials 146
4.1.2 Milk Substrate 147
4.1.3 Cheesemaking 148
4.2. Proximate Analysis 149
4.3. Microscopy 149
4.4. Rheological Tests 150
4.4.1 Controlled-stress Tests 150
4.4.1.1 Stress Sweeps 151
4.4.1.2 Creep/recovery Tests 151
4.4.1.3 Frequency Sweeps 152
4.4.2 Large Strain Rheological Tests 152
4.5 Statistical Analysis 153
5. Results and Discussion 153
5.1. Selection of Filler Particles and Volumes and Time Points 153
5.2. Statistics 155

5.3. Cheese Appearance and Composition (Proximate Analysis) 155
5.4. Microscopy 157
5.5. Rheological Tests.....159
5.5.1 Controlled-stress Tests 159
5.5.1.1 Stress Sweeps 159
5.5.1.2 Creep/Recovery Tests.....161
5.5.1.3 Frequency Sweeps.....162
5.5.2 Large Strain Uniaxial Compression 164
5.6. Effect of Age, Filler Type, and Filler Size 165
6. Conclusion 167
7. References.....168

CHAPTER 5: EFFECT OF CHARGED FILLER PARTICLES ON CHEDDAR CHEESE FILLED-GEL RHEOLOGY.....187
1. Abstract.....188
2. Practical Application.....188
3. Introduction.....189
4. Materials and Methods.....190
4.1. Cheese.....190
4.2. Proximate Analysis.....191
4.3. Microscopy 191
4.4. Centrifugation of Sephadex Beads from Cheese Whey 192
4.5. Rheological and Mechanical Tests 193
4.5.1 Controlled-stress Tests 193
4.5.1.1 Stress Sweeps 193
4.5.1.2 Creep/recovery Tests.....194
4.5.2 Large Strain Rheological Tests 194
4.5.3 Single Edge Notched Bending (SENB).....195
4.6. Statistical Analysis.....196
5. Results and Discussion 197
5.1. Effect of Particle Charge on Cheese Manufacture, Proximate Analysis, and Appearance 197
5.2. Microscopy 200
5.3. Recovery of Beads from Whey.....201
5.4. Statistics.....202
5.5. Rheological and Mechanical Properties 202
6. Conclusion 206
7. References.....206

APPENDICES 223
Appendix A: Raw Data for Chapter 2 (Off-The-Shelf Commercial Cheeses) Figures and Tables 224

Appendix B: Raw Data for Chapter 3 (Retail Cheese Received Directly From Manufacturer) Figures and Tables 230
Appendix C: Raw Data for Chapter 4 (Filled Gel Cheese Model with Uncharged Sephadex Beads) Figures and Tables 242
Appendix D: Raw Data for Chapter 5 (Filled Gel Cheese Model with Charged Sephadex Beads) Figures and Tables 247

LIST OF TABLES

Table 1.1 Rheological terms and symbols common to the filled gel model 53

Table 2.1 Commercial cheddar cheese 81

Table 2.2 Main factor effects (fat content and brand) and interactions (fat*brand) for rheological attributes of commercial cheddar cheese 81

Table 2.3 Difference in storage moduli of commercial cheddar cheeses at a frequency of 62.8 rad/s 85

Table 2.4 Linear regression of storage moduli vs. temperature for commercial cheese of varying fat content 87

Table 3.1 Commercial cheddar cheese composition.....126

Table 3.2 Descriptive analysis sensory attributes 127

Table 3.3 Critical stress and strain and recoverable energy (RE) based on fat content128

Table 3.4 Material properties and parameters from single edge notched bend (SENB) tests.....132

Table 3.5 Main effects (fat and thickness) and interaction (combined fat and thickness) for descriptive panel sensory-texture attributes.....134

Table 3.6 Descriptive analysis of cheddar cheese in varying thicknesses 135

Table 3.7 Main effects (fat and thickness) and interaction (combined fat and thickness) measuring effect of thickness on consumer perception of texture 137

Table 4.1 Cheese manufacture 172

Table 4.2 Target sampling plan and composition 173

Table 4.3 Main factor effects (filler type, filler volume, and treatment age) and interactions for rheological attributes of commercial cheddar cheese.....174

Table 4.4 Proximate analysis of treatments 175

Table 4.5 Range of Sephadex bead diameters in cheese.....178

Table 4.6 Area of Sephadex beads in 2-D confocal images of cheese 178

Table 5.1 Cheese manufacture.....210

Table 5.2 Target sampling plan and composition.....211

Table 5.3 Proximate analysis of treatments 212

Table 5.4 Range of Sephadex bead diameters in cheese.....214

Table 5.5 Dry matter in whey 216

Table 5.6 Select rheological and material properties.....217

Table A.1 Raw data from small strain tests 225

Table A.2 Raw data from large strain and fracture tests.....226

Table A.3 Effect of temperature and fat content on commercial cheese storage moduli at 8.3 rad/s.....228

Table A.4 Effect of temperature and fat content on commercial cheese storage moduli at 62.8 rad/s.....228

Table A.5 Correlation between creep and frequency sweeps 229

Table B.1 Raw data from stress sweeps and uniaxial compression 231

Table B.2 Raw data from creep/recovery tests at different applied loads 232

Table B.3 Raw data from torsion gelometry 233

Table B.4 Raw data from SENB testing.....234

Table B.5 Descriptive analysis scores (15-point scale) of cheese based on fat content and sample size 235

Table B.6 Top line CompuSense results for consumer sensory panel 241

Table C.1 Property averages from Batch 1 and Batch 2.....243

Table C.2 Effect of temperature and phase volume on storage modulus.....245

Table D.1 Raw data from stress sweeps and uniaxial compression 248

Table D.2 Raw data from creep/recovery tests.....250

Table D.3 Raw data from SENB tests 252

LIST OF FIGURES

Figure 2.1 Critical stress of commercial cheddar cheeses.....82

Figure 2.2 Critical strain of commercial cheddar cheeses.....83

Figure 2.3 Typical relationship between frequency, temperature, storage modulus (G') and phase angle.....84

Figure 2.4 Effect of temperature and fat content on commercial cheese storage moduli at two frequencies (8.3 and 62.8 rad/s) from frequency sweeps ...86

Figure 2.5 Maximum compliance (J_{max}) of commercial cheddar cheeses during creep testing at loads of 100 Pa and 500 Pa 88

Figure 2.6 Correlation between creep and frequency sweeps 89

Figure 2.7 Percent creep recovery (crp) of commercial cheddar cheeses during creep testing at loads of 100 Pa and 500 Pa 90

Figure 2.8 Percent recoverable energy of commercial cheddar cheeses compressed to 80% of initial height during one-cycle, uniaxial compression test.....91

Figure 2.9 Typical stress-strain curve resulting from torsional fracture of cheese 92

Figure 2.10 Texture map based on torsion fracture gelometry 93

Figure 2.11 Comparison between production lots of stress-strain curves by brand.....94

Figure 3.1 Single edge notched bend (SENB) test experimental setup.....126

Figure 3.2 Maximum compliance (J_{max}) of commercial cheddar cheeses during creep/recovery testing at loads of 100 Pa and 500 Pa.....128

Figure 3.3 Percent creep recovery (crp) of commercial cheddar cheeses during creep/recovery testing at loads of 100, 500, and 2200 Pa.....129

Figure 3.4 Effect of torsion rate on full-fat, reduced-fat, and low-fat cheddar cheese stress-strain curves.....130

Figure 3.5 Texture map based on torsion fracture gelometry of cheddar cheese at different rates and fat content 131

Figure 3.6 Characteristic stress-strain curve from single edge notched bend testing (SENB) at crosshead speed of 0.2 mm/s.....132

Figure 3.7 Descriptive analysis of 20-mm cheese cubes.....133

Figure 3.8 Principle components from descriptive analysis.....136

Figure 3.9 Effect of sample thickness on consumer evaluation of cheese 138

Figure 4.1 Superfine (SF) Sephadex beads imaged with PL Fluotar 40.0x1.00 oil UV objective.....176

Figure 4.2 Treatments imaged with HC PL Fluotar 10.0x0.30 objective 177

Figure 4.3 Images of overlapping beads taken with HC PL Fluotar 10.0x0.30 objective.....179

Figure 4.4 Critical stress of filled gel cheeses.....180

Figure 4.5 Critical strain of filled gel cheeses.....181

Figure 4.6 Maximum compliance (J_{max}) under 500 Pa load.....182

Figure 4.7 Frequency sweep on control and medium Sephadex bead treatments at FF and 18 weeks of age.....183

Figure 4.8 Effect of temperature and phase volume on storage modulus (G').....184

Figure 4.9 Effect of temperature and phase volume on percent recoverable energy, RE 185

Figure 4.10 Effect of age and filler volume (reduced filler vs. full filler) on percent recoverable energy, RE.....186

Figure 5.1 Images of treatments.....213

Figure 5.2 Treatments imaged with PA 10.0x0.45 confocal objective 215

Figure 5.3 Maximum compliance (J_{max}) of treatments at varying loads 218

Figure 5.4 Percent recoverable energy (RE) of treatments at different ages 219

Figure 5.5 Representative force-deformation curves from single edge notched bend (SENB) testing 220

Figure 5.6 FF+ inset 221

Figure C.1 Correlation between frequency sweep and maximum creep compliance 246

Chapter 1

Review of Literature

1. INTRODUCTION

The goal of this research was to study the role of fat in defining the texture of cheddar cheese. Understanding the function of fat on texture can lead to the development of suitable fat mimetics. From a materials/structure perspective, it is thought that fat functions as a filler particle in cheese, serving to weaken and disrupt the protein network, which is primarily composed of milk caseins. Thus, suitable fat mimetics should impart the same filler effects as the fat particles. The filled gel model has been used to understand the rheology of cheddar cheese, which is essentially a casein network filled with dispersed fat globules (Visser 1991; Walstra 2003). Texture relates to the rheological and mechanical properties of cheese, which depend upon network composition, structure, and molecular interactions (Lucey *et al.* 2003). This research used Sephadex beads as model filler particles in cheddar cheese. The effects of filler particle properties and volume on cheese microstructure and rheological properties were examined based on a filled gel model.

2. A BRIEF HISTORY OF CHEESE PRODUCTION AND CONSUMPTION

2.1. A Market for Low-fat Cheese

Cheese is a term generally used to define any solid-like, fermented, milk-based food product; cheese flavors, textures, and milk sources (e.g. cow, sheep, buffalo, camel, goat, yak, zebu, reindeer, horse, and donkey) vary by location and cultural practices (Fox *et al.* 2004; Scott *et al.* 1998). Cheese is manufactured and consumed all around the

world; over 1400 types of cheeses are listed in a database maintained by the University of Wisconsin Center for Dairy Research (Madison, WI) (www.cdr.wisc.edu). Cheese has been described as "milk's great leap to immortality," and, in one form or another, cheese has long been enjoyed by humans. Archeological evidence suggests humans have been making and consuming cheese since the Sumerian Ages, circa 3000 BC (Scott *et al.* 1998), and its popularity continues today with annual consumption increasing amongst Americans almost every year (2003 and 2009 being the only exceptions) since 1998 (Mintel 2009, 2010). Sales of natural cheese decreased slightly in 2009, but the sector still sold 7.51 billion dollars worth of product (Mintel 2010). Furthermore, Mintel forecasters predict that the cheese sector should grow an additional 18% from 2010-2014 (Mintel 2010). The growth of cheese intake contrasts Americans' declining consumption of milk, perhaps because cheese can be eaten with meals, as a topping, and as a standalone, handable snack (Mintel 2009). What's more, aged cheese does not contain an appreciable amount of lactose and so can be marketed to lactose-intolerant consumers.

Much of this popularity is likely due to the recognized health benefits of cheese, given that cheese manufacture presents a means of preserving the water-insoluble nutritional qualities of milk. The casein proteins from milk—and the primary proteins found in cheese—for example, have a protein digestibility corrected amino acid score (PDCAAS) of 100 (Gropper *et al*. 2005), and bioactive peptides from casein have been widely shown to have positive effects on the nervous, cardiovascular, gastrointestinal, and immune systems (Tidona *et al.* 2009). Cheese is also a source of calcium,

phosphorus, magnesium, conjugated linoleic acid (CLA), folic acid, vitamin B6, and vitamin B12 (O'Brien and O'Connor 2004). Furthermore, the health sections in many newspapers and magazines, as well as marketing claims touted on product packaging, are making more consumers cognizant of recent studies proclaiming the health benefits of cheese on cardiovascular health in women (Houston *et al.* 2008); calcium on weight loss, bone health, and hypertension (Tudor *et al.* 2009); protein on satiety (Westerterp-Plantenga *et al.* 2009); vitamin B6 and B12 on plasma homocysteine levels, which decrease the risk of atherosclerosis (Pfeuffer and Schrezenmeir 2000); and the antioxidant/anticarcinogenic properties of CLA (Pfeuffer and Schrezenmeir 2000).

While cheese consumption carries many health benefits, it also contains cholesterol and a high percentage of saturated fatty acids—components commonly associated with an increased risk for various cardiac conditions and numerous other diseases (Ulbricht and Southgate 1991). It is known, for instance, that milkfat does increase serum cholesterol (Wolk *et al.* 1998, 2001; Smedman *et al.* 1999) although a review of literature has shown that dairy fat alone is not conclusively responsible for cardiac diseases (Kumar and Singhal 1992). Nevertheless, conventional wisdom advises consumers to reduce their intake of fats—especially saturated fats. The recent upsurge in obesity rates also provides stimulus for consumers to reduce their fat intake. Thus, there is a market for low-fat cheeses, and these health benefits are recognized by consumer focus groups (Childs and Drake 2009). However, the health benefits of low and reduced-fat cheeses are countered by consumers' acceptance of the unique texture that

accompanies such cheeses and differentiates them from their full-fat counterparts. These differences shall be elaborated in an upcoming section.

2.2. Cheese Manufacture

Given that humans have been making and consuming cheese for thousands of years, cheese may be viewed as a process that decreases water activity (i.e. water content) and pH in order to stabilize and preserve milk nutrients. Cheese manufacture is essentially a dehydration process in which fat and casein are present at 6-12 times greater concentrations than they are in liquid milk (Fox *et al.* 2004). All cheese varieties share five common processing steps: acidification, coagulation, dehydration, shaping, and salting.

All cheese begins from the same raw material, milk, which may or may not be subjected to pasteurization, microfiltration, and/or bactofugation before cheese manufacture (Fox *et al.* 2004). The milk is then progressively acidified either by adding a chemical acidifier like gluconic acid-δ-lactone or by adding a starter culture blend of selected lactic acid-producing bacteria. This stage is sometimes referred to as "ripening the milk." According to Fox *et al.* (2004), the rate and extent of acidification affects:

- Rate and efficiency of the coagulant during the coagulation step.
- Denaturation and retention of the coagulant in the curd, which subsequently affects the rate of proteolysis during ripening.
- Strength of the coagulum and, consequently, cheese yield.
- Gel syneresis, which affects moisture content of the cheese.

- Dissolution of colloidal calcium phosphate, which affects casein proteolysis, cheese rheology, and curd meltability and strechability.
- The safety and quality of the cheese.

Cheese is produced by destabilizing milk proteins, thereby producing a protein (casein) gel that envelopes milk fat globules. The term "casein" actually refers to a family of four phosphoproteins (α_{s1}-, α_{s2}-, β-, and κ-casein) that account for nearly 80% of all proteins found in cow milk (Walstra and van Vliet 1986); an excellent review of casein literature is found in Lucey *et al.* (2003). The constituents differ by their number and distribution of phosphoseryl residues (α_{s1}-, α_{s2}-, and β-casein have many phosphoseryl residues, whereas κ-casein has only one) and sensitivity to Ca^{2+} (Lucey *et al.* 2003). These four phosphoproteins associate primarily through electrostatic (aided by colloidal calcium phosphate) and hydrophobic interactions to form colloidal casein micelles with κ-casein always positioned at the surface (Lucey *et al.* 2003), much like a polymer brush or frizzy hairs on a human head. The glycosylated κ-casein tail provides steric stabilization, thereby aiding in casein colloid dispersion.

The destabilization of the colloidal casein micelle occurs during the coagulation step and may be achieved via either enzyme addition or acid addition. Most cheeses are made via enzyme addition by adding rennet, which was originally isolated from the stomachs of young animals but can now be made commercially using genetically modified *E. coli, Kbryveromyces lactis,* or *Aspergillus niger* (Fox *et al*. 2004); the isolated enzyme is called chymosin in the case of the latter production method, and it

possesses optimal activity at pH 5.1 - 5.5 (Fox *et al*. 2004). Chymosin hydrolyzes the peptide linkage between Phe_{105} and Met_{106}, effectively cleaving the κ-casein tails from the rest of the colloidal casein micelle (Delfour *et al.* 1965). As mentioned, the fragment, or caseinomacropeptide, of the κ-casein that is cleaved off is hydrophilic and contains the C-terminus. The remaining exposed end of κ-casein that is attached to the larger micelle is hydrophobic. This hydrophobicity—and the loss of the steric stabilization afforded by the κ-casein tail—prompts quick aggregation of the proteins. Many aggregates associate into clusters and chains that span the system and form a three-dimensional network, or coagulum, entrapping fat globules and serum. The hydrolysis of κ-casein, the micellar aggregation, and the gel formation are all subject to different kinetic regulations, with the enzymatic hydrolysis of κ-casein being the rate-limiting step (Carlson *et al*. 1987). Enzymatic hydrolysis of κ-casein initiates the gelation process, but gel formation and strengthening continues through the subsequent steps.

During the dehydration step, the coagulum is cut, cooked, stirred, pressed, and/or salted. The goal of this step is to promote gel syneresis, which refers to the loss of serum (whey, water-soluble constituents like the cleaved κ-casein tails, and excluded fat globules) from the gelled curd. The rate and extent of syneresis varies based on the size of the cut curds, concentration of calcium ions and casein, pH, rate of stirring, and time (Fox *et al*. 2004). If using a starter culture blend, cheese manufacturers must be careful when adding salt to dehydrate and flavor the cheese because too much salt will inhibit culture growth. Typically, manufacturers brine the cheese or add dry salt to the surface;

the slow diffusion of salt into the cheese allows the starter cultures enough time to acidify the cheese and decrease the pH (Fox *et al*. 2004). After draining the expressed serum and sufficiently dehydrating the cheese, it is shaped. Soft cheeses may be shaped into moulds, whereas harder cheeses are typically cut into blocks.

After shaping, the cheese is allowed to ripen. The ripening period varies from two weeks to two years, depending on the type of cheese (Fox *et al*. 2004), and occurs in two stages. Texture changes substantially during the first stage, lasting 7-14 d, as the rubbery curd becomes more homogenous due to proteolytic cleavage of the α_{s1}-casein (Lawrence *et al.* 1987); the second stage lasts anywhere from weeks to years and involves more gradual flavor and texture development. The final product attributes are largely defined by physical, chemical, and microbiological reactions that occur during the ripening process, although these reactions are heavily influenced by composition and the preceding processing steps. Flavor compounds, aroma compounds, and gas arise from the activity of the coagulant, indigenous milk proteases (e.g. plasmin) and lipases (e.g. lipoprotein lipase), starter culture bacteria and their enzymes, and secondary microflora (bacteria, yeasts, and/or fungi) and their enzymes (Fox *et al*. 2004). For instance, the microflora continue to metabolize residual lactose, lactate, and citrate in the cheese (hence why aged cheeses are lactose-free), as well as catabolizing fatty acids and amino acids in cheese. Additionally, dehydration and curd knitting, or compacting, continue throughout the ripening period. Longer ripening times obviously result in more pronounced changes.

For more details on cheese production, ripening, biochemistry, microbiology, technology, history, or recipes for different types of cheese, the interested reader should refer to any number of published references.

2.3. Cheddar Cheese Manufacture and Popularity

One of the most popular cheeses in the United States is cheddar cheese, which is characterized by a "buttery but firm body," a continuous but curdy texture with no eyes (holes) or cracks, a "clean, nutty" flavor, and a shelf life of at least one year (Scott *et al.* 1998). Cheddar is distinguished from other cheeses by its unique dehydration step, a process called "cheddaring." The coagulum that forms after rennetting is cut into pea-sized pieces and slowly stirred for several hours while the temperature and acidity are gradually increased. The whey is then drained from the curds, and the true "cheddaring" process begins. The curds are pressed together into hoops or blocks, which are then stacked upon each other; this stacking process is repeated every 15 minutes (Scott *et al.* 1998). The weight of these stacked blocks helps to express more whey and also squashes, or knits, the individual curd particles together. The resulting cheese texture has been described as looking like "chicken breast skin" because individual curd bumps can still be distinguished (Scott *et al.* 1998). After cheddaring, the large curd blocks are "milled" into silver dollar-sized pieces and sprinkled with salt in order to reduce this particulated, or "curdy," appearance. The curds and salt are stirred for several hours until the salt dissolves, and then the curds are molded, pressed for several hours, "dressed" to remove rough edges, and pressed again for 24-30 hours (Scott *et al.* 1998). Of course,

large-scale commercial manufacturers have developed ways to mechanize this otherwise batch process.

The cheddaring process and accompanying expulsion of serum produces the hard cheese familiar to, and enjoyed by, many Americans. Historical records as old as 1500 A.D. document the production and consumption of Cheddar cheese (Scott *et al.* 1998). According to the USDA National Agricultural Statistics Service, the USA produced 3,170,629,000 lbs (~1,585,315 US tons) of cheddar cheese in 2009 alone (cited in Gould 2010)! Full-fat cheddar cheese must legally contain at least 30.5% (w/w) milkfat (CFR 21 [133.113]), but it is also available in reduced and low-fat versions. The Food and Drug Administration (FDA) regulates the usage of these fat descriptors such that reduced-fat cheese contains at least 25% less fat than the full-fat counterpart, and low-fat cheese contains 3 g of fat or less per reference amount (CFR 21 [101.62b]). Therefore, if the standard cheddar contains ~31% fat (w/w), the counterpart reduced-fat cheese would contain less than 24% fat (w/w), and the counterpart low-fat cheese would contain less than 10% fat (w/w), although 6% fat is the usual US standard (Johnson *et al.* 2009).

2.4. Variability in Cheese Manufacture

Many variables exist in cheesemaking; these variables can lead to new types of cheese or can simply cause difficulties in replicating batches, especially for small-scale, artisan cheesemakers. Raw milk composition, treatment of raw milk, holding times, thermal history, pH, salt and calcium ion concentrations, protein-to-moisture ratio, indigenous and cultured microflora and their metabolic activities, bacteriophage, and

ripening duration all affect the final product. Altering these variables can give rise to new, unique cheeses, but it can be difficult to control these variables when trying to manufacture an already established, defined cheese.

Lawrence *et al.* (1987) identified cheese pH and the ratio of intact (non-hydrolyzed) casein-to-moisture as the two primary determinants of cheese texture and cutting properties in full-fat cheeses (i.e. cheeses with a fixed fat:moisture-to-fat ratio). In the latter case, the protein-to-moisture ratios affect total network density and therefore significantly impact texture. Greater protein concentration equates to denser networks, which are consequently found to be firmer. Creamer and Olson (1982) found that cheddar cheeses containing only 4% more moisture were softer than their low-moisture counterparts at any single point in time. While network density alone accounts for many differences in hardness, the protein-to-moisture ratio also affects enzyme activity due to substrate diffusion (Fennema 1996) and, theoretically, the availability of free moisture due to the association between water molecules and both casein micelles and their hydrolyzed byproducts (Stanley and Emmons 1977; Creamer and Olson 1982; Lawrence *et al.* 1987).

2.4.1. Effect of pH

The natural end pH of the cheese curd post acidification is within the range of 4.6 – 5.1, although it takes 5-15 h to reach that pH depending on the type of cheese (5 h for cheddar versus 15 h for Swiss) (Fox *et al.* 2004). However, relationships exist between pH, water activity, and texture, so similar cheese textures can be achieved, to a certain

extent, by altering any number of factors including pH, salt and calcium content, moisture content, and ripening temperatures (Lawrence *et al.* 1987; Creamer and Olson 1982). Nevertheless, pH at the step of whey draining is an important factor on enzyme activity and cheese texture, while the pH of the cheese after salting will affect the final textural development. Decreases in pH after salting are largely due to microbial activity and can be regulated by controlling the concentration of lactose, the fermentable substrate (Creamer and Olson 1982).

Not only does pH affect the activity of proteolytic and lipolytic enzymes in milk, it also affects the partitioning of enzymes between the curd and serum phases. For instance, researchers have found that greater proportions of calf rennet are sequestered in the curd when the pH at draining is low (pH 5.90 vs. 6.65), which consequently results in greater proteolytic activity on the casein network (Creamer *et al.* 1985). In contrast, the heat-resistant milk protease plasmin dissociates from the casein micelle as pH is decreased, meaning that the plasmin concentration is greatest in cheeses drained at a high pH (Richardson and Elston 1984).

Chemical changes in the protein network of the cheese curd also directly relate to pH and texture. Colloidal calcium phosphate, for example, dissociates from the subcasein micelle as pH decreases, particularly below pH 5.5, and this, in turn, affects the cluster size of the casein micellular aggregates and the mineral content of the cheese (Roefs *et al.* 1985). Similarly, cluster size is affected by electrostatic and hydrophobic interactions that change as the protein nears its isoelectric point, or pI. The major casein

fractions have isoelectric points near 4.5 and will form tighter clusters with strong intra-aggregate forces but weak inter-aggregate forces. This observation led Creamer and Olson (1982) to propose a pH-dependent model for cheddar cheese in which low pH (~4.9) cheeses are like porous masses of casein and fat with unevenly distributed pockets of water, whereas high pH (~5.4) cheeses are more like a "protein emulsion" with little interstitial water. If such a model were proved true, then pH may have a strong effect on the resultant network density of cheese. The range of pH in that study reflects the production range for cheddar cheese.

The effect of pH on casein micelle cluster size impacts overall textural properties. Creamer and Olson (1982) found that cheddar cheese made at pH 5.40 required 1.5 times as much force as cheese made at pH 4.88 to compress the cheese to their respective yield points. And while calcium also affects the size of casein micelle aggregates, Creamer, Gilles, and Lawrence (1988) found that pH had a greater effect on cheddar cheese texture than calcium content. However, that study used real cheddar cheese samples and was confined to a pH range of 4.93–5.38, which is typical of cheddar cheeses. In order to test pH effects over a wider range (pH 5.20-6.22), Watkinson *et al.* (2001) measured rheological and fracture properties of a model cheese that was made by directly acidifying the milk (rather than using a starter culture) and curd. They found that increasing the pH by only 0.2 units caused fracture strain (resistance to crumbling), fracture stress (firmness), and fracture area (toughness) to significantly increase within the range of pH 5.20-5.80, although there were confounding effects with moisture and

age beyond pH 6.0 that caused a decrease in all three values. The modulus of deformability (stiffness) and adhesiveness (stickiness), however, generally decreased as pH increased. Whereas Watkinson *et al.* used compressive tests, Ramkumar *et al.* (1998) used oscillatory tests on pH-controlled (pH 5.45-6.05) cheddar curd models and found similar results. Specifically, the storage modulus was shown to increase for curds of pH 5.45 to 5.90 and decrease for samples at pH 6.05, while the phase angle consistently decreased as pH increased (Ramkumar *et al.* 1998). Both trends suggest the cheese curds become firmer and more solid-like as pH increased.

Watkinson and Ramkumar used model cheese systems largely because of the aforementioned interactions between pH and moisture content, proteolytic activity, and mineral content (e.g. calcium). Thus, model systems present one method of isolating pH as a variable. Other research groups have chosen to high-pressure inject concentrated acidulant into blocks of cheese that have already been manufactured (Pastorino *et al.* 2003a-c). They found similar results as the groups using model systems, namely, cheddar cheese hardness and firmness decreased with pH, and the cheese was more crumbly at low pH (Pastorino *et al.* 2003c). However, the method of injecting acidulant also caused a decrease in cheese cohesiveness after several injections were made. Pastorino *et al.* (2003c) also found that the flow rate, or melting rate, of the cheese changed with pH although the results were parabolic (non-linear), with a maximum flow rate occurring around pH 5.0.

In summary, pH has an important effect on cheese texture and must therefore be carefully controlled. The effects of pH are often confounded since pH affects total and soluble calcium content, moisture content, and proteolytic mechanisms. This section introduced some rheological terminology and the instruments and tests that are commonly used to assess cheese texture, but these will be discussed more thoroughly in an upcoming section.

3. LOW-FAT CHEESE

3.1. Strategies for Making Low-fat Cheese

Three primary strategies for producing low-fat cheese have been identified: (1) modifying the manufacturing process; (2) using adjunct cultures; and (3) using milkfat substitutes (Drake and Swanson 1995; Rodríguez 1998; Mistry 2001). Many commercial cheeses at the store shelves today advertise that they are made with 2% milk. Additionally, the simple substitution of 2% milk for whole milk can reduce fat content by as much as 33%. Drake and Swanson (1995) opined that these strategies are adequate for obtaining reduced-fat cheese (33% fat reduction) with acceptable sensorial properties, and consumer focus groups agreed that cheeses made with 2% milk are acceptable (Childs and Drake 2009). Despite growing consumer interest in low-fat products, however, consumers are not willing to make any compromises on texture or flavor in low or reduced-fat cheddar or mozzarella cheese (Childs and Drake 2009).

The subject of this thesis is to use another strategy to produce low-fat cheese: replace some volume of fat in the cheese with an equal volume of an alternate filler particle. The latter strategy aligns with the filled-gel model for cheese, which purports that cheese is essentially a gelled protein network interrupted by dispersed filler particles. Ordinarily, the filler particle is milkfat, but an alternate filler particle with the same volume and rheological properties of milkfat should produce similar effects on rheological properties of cheese as the milkfat. This concept will be discussed further in an upcoming section on filled gels. Before the filled-gel model can be discussed, however, one must understand the role of fat in cheese and the pitfalls of current efforts to create palatable low-fat cheeses.

3.1.1. Modifying the Manufacturing Process

The goal of most modifications to the standard manufacturing process is done to increase the moisture content of the curd; this practice has been in use since at least 1957 (Drake and Swanson 1995). Tunick *et al*. (1993) found that, to a certain extent, added moisture mimics the lubricity and creamy mouthfeel imparted by fat, thereby making up for a textural short-coming of reduced- and low-fat cheeses. For example, in a study comparing reduced-fat, higher moisture washed curd Viking cheese (a cheese similar to Monterey Jack) to a lower moisture, full-fat control, consumers gave both cheeses equal overall acceptance scores but noted that the higher moisture, reduced fat cheese was softer and creamier than the full-fat control after seven months of age (Drake *et al.* 1995). However, a study by Hort (1997) using trained panelists to evaluate creaminess, defined

as “velvety mouthfeel,” found that creaminess scores significantly increased over the aging period for cheddar, even though moisture remained constant; pH gradually increased by 0.2 units over this time. The authors attributed the increase in creaminess to proteolysis.

Reducing fat content also reduces the final product yield, but increasing the curd moisture content is one way to minimize loss and improve yields. Albeit very economical, the practice of increasing curd moisture content is least practical for low-moisture cheeses like sharp cheddars because additional moisture often causes decreases in firmness and some other textural attributes (Visser 1991). Furthermore, additional moisture negatively affects flavor development, although these effects can be somewhat counteracted through the use of adjunct cultures and controlled acidification (Drake and Swanson 1995).

3.1.2. Using Adjunct Cultures

An adjunct culture is one used in addition to the lactic acid culture(s) typically used to produce a particular cheese; adjunct cultures are typically added to modulate flavor development (Drake and Swanson 1995). For instance, one common modification made when manufacturing low- and reduced-fat cheese is to decrease the cook temperature and/or duration. Doing so, however, allows lactic acid starter bacteria to reach large growth concentrations, which will ultimately lead to an over-production of acid and bitter flavors (unpublished data of Thunnell, cited in Drake and Swanson 1995). Therefore, special slow-growing strains of lactic acid bacteria, reduced inoculant,

reduced ripening time, and curd-washing are often used to decrease and monitor acid-production in low- and reduced-fat cheese. However, proteolysis greatly contributes to flavor in cheese, and strains that produce acid slowly are also likely to have slowed proteolytic activity. Therefore, adjunct cultures are used in tandem to control proteolysis and acid and flavor development.

3.1.3. Using Fat Replacers

Two types of fat replacers exist: fat mimetics and fat substitutes. Fat mimetics mimic the lubricity and creamy mouthfeel of fat and are typically protein or carbohydrate moieties, whereas fat substitutes are structured lipids or synthetic compounds that exhibit the physical properties of fat but are metabolized differently from milkfat and so contribute fewer Calories (Drake and Swanson 1995). Examples of fat mimetics include maltodextrin (Swanson 1996), polysaccharides (Warrand 2006), superheated starch (Steeneken and Woortman 2009), and β-glucan (Sahan *et al.* 2008). Numerous trademarked commercial products exist for use in dairy applications including Simplesse™ (a whey protein derivative sold by CP Kelco) and Avicel Plus™ (a blend of cellulose, cellulose gum, and carrageenan sold by FMC BioPolymer). A review of recent cheese literature shows that many fat mimetics and substitutes are being tested in soft cheeses like Kashar (Sahan *et al.* 2008), Oaxaca (Totosaus and Guemes-Vera 2008), and even mozzarella (Vithanage *et al.* 2008). Unfortunately, the common theme of these studies is that fat substitutes and mimetics may improve product yield, moisture retention, creaminess, and firmness but not other attributes like flavor, meltability, or certain other

textural properties. These differences may be partly attributed to differences in microstructure; Kayanush and Haque (2001) used transmission and scanning electron microscopy on low-fat cheddar cheese made with different commercial protein and starch-derived fat substitutes and found that these substitutes produced cheese with rippled, undulating surfaces and voids around some of the fat substitute particles. Banks (2004) provides a good review of fat substitutes and mimetics in cheese, with a noted emphasis on cheddar cheese.

Polarity is important when designing fat-replacement compounds. For instance, fat substitutes are, by definition, fat-soluble and therefore should be capable of carrying the same non-polar flavor compounds as milkfat, although their differing fatty acid profile will generate different fat-based flavors than those traditionally born of milkfat (Drake and Swanson 1995). In contrast, fat mimetics are polar, water-soluble compounds and are therefore incapable of imparting the exact same mouthfeel, texture, and flavor-carrying capacity as milkfat. However, fat mimetics do represent an easy way to incorporate additional moisture, which, as previously discussed, improves the product yield and may improve some texture attributes such as creaminess.

Sucrose polyesters such as the commercial product Olean™ (Procter & Gamble, Cincinnati, OH) are one type of lipophilic, nonabsorbable, noncaloric fat substitute and are produced by esterifying fatty acids onto the hydroxyl groups of sucrose. Drake *et al.* (1994a,b) synthesized their own sucrose polyesters from milkfat fatty acids and incorporated these into cheddar cheese, substituting the sucrose polyesters for 10-75% of

the natural milkfat. When the sucrose polyesters comprised 25% of the total fat, the resulting cheese varied predictably in melting properties and hardness based on the fatty acid composition. Within this range of melting and hardness properties, some sucrose polyesters yielded cheese with properties similar to full-fat cheddar cheese (Drake *et al.* 1994b). However, trained sensory panelists determined the cheese had significantly different flavor and color when the sucrose polyesters comprised 10-75% of the total fat (Drake *et al.* 1994a). A different study used λ-carrageenan, pectin, alginate, and guar gum to create reduced- and low-fat cheese spreads, but again, researchers concluded that spreads containing the fat mimetics had either undesirable or less flavor than the full-fat counterparts (Brummel and Lee 1990). Favorable texture but unsatisfactory flavor were again obtained when two commercial hydrocolloid fat-replacers (dairy protein-derived Simplesse™ from the Nutrasweet Co. and carbohydrate-derived Novagel™ from the FMC Corp.) that are specifically designed to replace fat in dairy products were used in low-fat, white-brined cheeses to achieve a 60% fat reduction (Romeih *et al.* 2002). More work is needed in this area to develop fat substitutes and/or mimetics that compromise neither texture nor taste and can deliver greater than 33% fat reduction.

3.2. Flavor of Low-fat Cheese

Lactic acid starter cultures influence cheese flavor development in several ways: control of the growth and composition of the microbiota by depleting fermentable carbohydrates and reducing the pH; reduction of the oxidation-reduction potential; protein hydrolysis; and synthesis of flavor compounds and precursors (Olson 1990). As

mentioned, the volatile compounds in cheese often change due to the rate of acidification and starter culture activity, which is why adjunct cultures are often added to reduced- and low-fat cheese to achieve desirable rates of acidification and help round out the flavor profile. Often, adjunct cultures have high enzymatic activity. Specifically, they release aminopeptidases that decrease the bitterness typically associated with reduced- and low-fat cheese (Mistry 2001).

In addition to increased bitterness, low-fat cheeses—particularly the low-moisture varieties—differ from their full-fat counterparts in that they either lack flavor or have atypical, undesirable cheese flavors (Banks *et al*. 1989; Olson 1990; Drake and Swanson 1995; Mistry 2001; Banks 2004; Yates and Drake 2007). For example, Milo and Reineccius (1997) identified a "meaty-brothy" odor defect in low-fat cheddar cheese that they believed was caused by overly high amounts of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol), homofuraneol, and methional. Reduced-fat cheeses, however, are less susceptible to atypical flavors but are more likely to be weak in flavor. Whetstine *et al.* (2006) found that reduced-fat and full-fat cheeses have the same flavor profiles at any given age point, but the flavors are less pronounced in the reduced-fat versions. Similarly, Fenelon *et al.* (2000) found that characteristic cheddar flavor notes like buttery, creamy, and caramel decrease with decreasing fat content, whereas the appearance of uncharacteristic flavors increases with the decreasing fat content. Lipolysis is a major source of flavor constituents, so less fat in a cheese equates with lower levels of lactones and fatty acids and hence, flavor (Banks *et al* 1989).

Furthermore, the approaches for producing lower-fat cheeses may not support flavor development. For instance, one major problem with fat mimetics is that the water-soluble compounds cannot carry key non-polar volatile compounds.

Flavor problems must be solved because conjoint analysis showed consumer groups identify flavor as the most important attribute when deciding to consume cheese (Childs and Drake 2009). In that study, consumers placed more importance on flavor and texture than fat level or price when deciding whether or not to purchase cheddar and mozzarella cheese.

3.3. Texture of Low-fat Cheese

Consumers have identified texture as one of the most important drivers in their acceptance of cheese quality (McEwan *et al.* 1989; Jack *et al.* 1993a,b). Untrained consumer panelists can even discriminate amongst cheddar cheeses on the basis of texture alone (Jack *et al.* 1993a). Furthermore, consumers associate specific textures with specific varieties of cheese, and deviations from the expected texture result in decreased acceptability/liking scores amongst consumer panelists (Carunchia Whetstine *et al.* 2006; Yates and Drake 2007). However, one of the greatest differences between regular and reduced-fat cheese is texture. For example, using a trained sensory panel and torsion testing, Gwartney *et al*. (2002) found that commercial reduced-fat Monterey Jack, reduced-fat mild cheddar, and reduced-fat sharp cheddar all had significantly higher scores than their full-fat counterparts when evaluated for springiness, hardness, fracturability, waxiness, chewiness, and fracture stress. Reduced-fat, processed

American cheese also differed from its full-fat counterpart in fracturability, waxiness, chewiness, and fracture stress. In contrast, all full-fat cheeses were higher in cohesiveness, stickiness, smoothness, and meltability. Thus, both natural and processed cheeses showed similar defects in their reduced-fat versions. Given that fracture stress and elastic recovery increase with protein content (Foegeding 1992; Jack *et al.* 1993b; Bowland and Foegeding 1999; Li *et al.* 1999), these results may simply reflect a higher percent of protein in the reduced-fat cheeses. Furthermore, reducing fat content of cheese means that the protein network is more densely packed, which allows for more interactions between protein molecules and strongly impacts texture (Bryant *et al.* 1995; Mistry 2001).

Differences in texture do not affect only the feel of the cheese in the mouth and hand or the sound the sample makes during mastication. Texture can also impact flavor. For instance, Tournier *et al.* (2009) found that viscosity of custard desserts affected flavor, but not aroma, intensity. In more solid foods, research has shown that increased gel rigidity of gelatin and pectin gels caused decreased perception of added strawberry odor, flavor, and sweetness, as well as higher intensity ratings for thickness amongst trained sensory panelists (Boland *et al.* 2006). Other researchers have investigated texture-aroma interactions in more complicated food matrices than hydrocolloid gels. In one study, model cheeses were made by adding three different flavor compounds and modifying texture in each by changing the fat, salt, and dry matter content of model cheddar cheeses (Saint-Eve *et al.* 2009). Researchers found that textural changes greatly

affected the release of some odor compounds (i.e. blue cheese aroma) but not others (i.e. buttery aroma). Finally, researchers found that textural differences in 8 model cheeses affected consumers' chewing activity, which in turn affected aroma release from the cheese (Tarrega *et al.* 2008). Likewise, another study found that texture of whey protein gels determined panelists' ability to perceive flavor and flavor intensity (Weel *et al.* 2002).

Thus, lower-fat cheeses not only differ from their full-fat counterparts in texture, but these differences can have cross-modal effects on aroma and flavor perception as well.

3.4. Functionality of Low-fat Cheese

In addition to flavor and texture issues, low-fat cheeses have functionality issues. In other words, low-fat cheeses do not give the same results as full-fat cheeses when used as an ingredient. For example, mozzarella cheeses with reduced fat content exhibit reduced melting capacity (Fife *et al.* 1996) but are more likely to brown and darken, even burn, during heating because there is less lipid at the surface of the mozzarella shreds (Rudan and Barbano 1998). In their review, Johnson *et al.* (2009) noted that lower-fat cheeses also tend to be more translucent, gummier, and/or more rubbery.

4. FILLED-GEL MODELS

4.1. Filled Gel Theory

As mentioned, cheese can viewed as a protein network filled with dispersed milkfat particles, making cheese suitable for analysis using the filled gel model. Particle-filled gels, or filled gels, are also known as "gelled emulsions" and "composite gels". Filler particles may be relatively deformable as in the case of oil-filled emulsions, or they may be relatively rigid, e.g. glass beads. Typically, both the filler and network are deformable to a certain extent, which causes the composite gel to exhibit unique, and often complex, mechanical responses to applied stresses and deformations. The filled gel model provides a simplified way of understanding these responses. The model purports that the elastic modulus (G'; see Table 1.1) and fracture properties (fracture stress, σ_f; fracture strain, γ_f; and fracture modulus, $G_f = \sigma_f / \gamma_f$) of composite gels depend on:

1. Properties of the suspending medium (i.e. casein network in cheese),
2. Properties of the dispersed filler particles (i.e. milkfat globules in cheese), and
3. Interactions between the filler particles and protein network.

Specifically, properties of the casein network include both the elastic modulus and fracture modulus of the network. Properties of the dispersed filler particles include both the elastic modulus and the phase volume (φ, v/v), as well as shape and orientation. With regard to the third point: filler particles display a range of interactions with the suspending network and may be considered either "active" or "inactive" (van Vliet

1988); these classifications are further divided into "interacting" and "non-interacting" particles. Particles that interact with the network are always termed "active" because they increase the storage modulus such that the filled gel has larger moduli values than does the unfilled gel. However, non-interacting particles may also cause an increase in the storage modulus, and this is known as a reinforcing effect (Brownsey *et al.* 1987). Furthermore, the reinforcing effect increases as the filler phase volume increases, regardless of whether the particle is interacting or non-interacting (Kerner 1956; Brownsey *et al.* 1987; Loret *et al.* 2007). Non-interacting particles can, however, actually decrease the storage modulus because the particles disrupt and weaken the surrounding gel network. Under these circumstances, the non-interacting particles are called "inactive fillers." Just as the reinforcing effect of active fillers increased with filler phase volume, the effect of inactive fillers increases with phase volume. In other words, increasing the filler phase volume of inactive fillers causes subsequent decreases in the composite network moduli (Chen and Dickinson 1998a). To explain these effects, van Vliet (1988) proposed that inactive particles at small deformations essentially behave like globules of low-viscosity liquid (e.g. water) in the network. Chen and Dickinson (1999) attributed these properties of inactive particles to their surface interactions. The authors theorized that inactive fillers essentially act like holes in the composite gel because the fillers do not interact electrostatically with the suspending network and therefore are not an integrated part of the composite gel. Such a view is only valid for small-strain analyses, however.

In summary, a review of filled gel literature reveals some discontinuity in the lexicon. Some authors use active/interacting and inactive/non-interacting terms interchangeably, whereas other authors make clear distinctions between each term. Such distinctions depend upon the author's focus—chemical interactions versus rheological interactions and rheological methodology (i.e. large or small strain testing). Other authors label fillers as either strengthening (active), low-efficient (inactive), or loosening (strength-reducing) (Lozinsky *et al.* 1992). Nevertheless, it can be said that active fillers may be either interacting or non-interacting particles but always have a storage modulus greater than that of the suspending network ($G'_{filler} > G'_{suspending\ network}$) and, consequently, serve to increase the composite gel moduli. Inactive fillers are always non-interacting particles that cause a decrease in the composite gel moduli because the storage modulus of the filler is less than that of the suspending network ($G'_{filler} < G'_{suspending\ network}$). One can also imagine the rare case in which both the filler and suspending network have equivalent moduli ($G'_{filler} = G'_{suspending\ network}$). In this special case, changing the filler particle volume would have no effect on the composite gel moduli because the network is homogenous in G', albeit heterogeneous in composition.

4.2. Mathematical Modeling of Filled Gels

In order to eliminate the effects of particle orientation, filled gel mathematical models assume spherical filler particles. The Kerner (1956) and van der Poel (1958) are two of the earliest models and were developed to quantitatively predict the impact of a filler particle on the resulting composite gel moduli. While these two models are

commonly referenced, interest in material science and the development of new polymeric materials (e.g. rubbers) has led to the development of other models. Ahmed and Jones (1990) provide a good review and critique of many of these mathematical models, although new models have also been developed since then (Palierne 1990; Pal 2002; Pal 2008).

The Kerner (1956) and van der Poel (1958) models were early forays into filled gel modeling that assumed simple, isotropic systems and synthetic, spherical, mono-dispersed particles that did not aggregate (van Vliet 1988; Ahmed and Jones 1990). For instance, the van der Poel model failed to describe the viscoelastic properties of heat-set whey protein emulsion gels because the filler (oil) tended to flocculate (Chen and Dickinson 1998b). Therefore, the filled gel models needed to be validated in more complex, realistic systems, e.g. biopolymer systems. To this effect, Ross-Murphy and Todd (1983) made composite gels of gelatin (20% w/v) and glass beads, which were available in spherical and cubical shapes and ranged in filler volume from 0-80%. Glass beads were used because they are non-deformable and exhibit perfect adhesion to the network at all strain levels, meaning they are active fillers. Bracketing the effects of filler particle size, shape, and volume in force-extension failure "envelopes," Ross-Murphy and Todd showed that an increase in filler phase volume caused an increase in fracture stress but a decrease in fracture strain. This means the composite gels became tougher but less deformable as phase volume increased. Gwartney *et al.* (2004) found similar results

using a more complicated system of whey protein isolate (12% w/v) gels filled with emulsified sunflower oil (filler volume = 0-20%).

4.3. Biopolymer Gels Filled with Sephadex Beads

Sephadex beads have been used in several studies to study filler effects in filled gel systems. These beads are made from cross-linked polydextrans and are available in different sizes (20-300 μm), rigidities, and charges. The rigidity of the bead relates to the bead porosity and the amount of water it absorbs during hydration. One of the earliest Sephadex studies used uncharged beads of varying rigidities in either a 3 or 6% gelatin system (Brownsey *et al.*1987). The authors called the beads non-interacting fillers because they were uncharged. However, the beads behaved as active fillers because they increased the complex gel modulus; this reinforcing effect increased with both Sephadex phase volume and bead rigidity. Furthermore, the phase volume at which the reinforcing effect was first observed decreased as bead rigidity increased. However, when the gelatin concentration was increased from 3 to 6%, the phase volume at which the reinforcing effect was first observed increased, supporting the tenet that filler effects depend upon both the filler properties and the suspending network properties. The data also suggested there was a critical phase volume requirement necessary to observe a filler effect—results not predicted by the Kerner model. In other words, the authors found that, below the critical phase volume, increasing the phase volume did not impact the complex gel modulus. The authors repeated the experiments using beads of different sizes but did not observe any significant particle-size effects. The experiments were also repeated using

negatively charged Sephadex beads, which were suspected to interact with the positively charged gelatin network, making the beads interacting, active fillers. Indeed, these charged beads caused a significant increase in the reinforcing effect.

While Brownsey *et al.* (1987) used a gelatin-based system, Lozinsky *et al.* (1992) added Sephadex beads of varying rigidities and sizes to poly(vinyl alcohol)-based cryogels, which are highly porous, hydrophilic, thermoreversible gels. The bead volume fraction varied from 0-20% (v/v), although it was noted that the poly(vinyl alcohol) (PVA) macromolecules partially diffused into the Sephadex beads, making it impossible to calculate the filler volume exactly. The results showed that increasing the phase volume of filler caused the gel complex moduli (G*) to increase and creep compliance values to decrease (in agreement since compliance ~ 1/G*). . However, these effects were more pronounced as bead rigidity *decreased*, which is the exact opposite trend expected based on mathematical models (Kerner 1956) and previous Sephadex studies (Brownsey *et al*. 1987). The authors attributed this to diffusion of PVA macromolecules into the beads—as evidenced by scanning electron microscopy—because the less rigid beads are more porous. Finally, this study showed that particle size had no effect on G* values, but increasing particle diameter did cause creep compliance values to decrease, particularly in the least rigid Sephadex samples that had exhibited the greatest reinforcing effects. Given that creep compliance relates to the inverse of the complex modulus, the significance of particle size was most likely due to the difference in time scales between the two tests. Finally, it was observed that filler particles affected melting properties.

Specifically, filled gels melted at higher temperatures than unfilled gels, and the least rigid particles melted at the highest temperatures. Yet, regardless of particle rigidity, increasing the filler volume sequentially increased the melting temperature. Again, the authors attributed these effects to interactions between particles and network and diffusion of PVA macromolecules into the Sephadex beads.

Lozinskii *et al*. (2002) expanded on that work by adding hydrophilic (unmodified beads) and hydrophobic (modified with propylene oxide group) Sephadex beads into PVA cryogels. The beads with the most hydrophobic moieties had little effect on the composite gel; regardless of filler phase volume, the ratio of the unfilled gel modulus to the filled gel modulus was approximately equal to 1. In contrast, the less hydrophobic bead and the two hydrophilic bead types exhibited reinforcing effects, which increased as the degree of hydrophilicity increased. This study, like Brownsey *et al.* (1987), showed that increasing bead rigidity did increase the reinforcing effect. The authors explained that they used larger PVA macromolecules than the study by Lozinsky *et al.* (1992), and these larger molecules were too large to diffuse into the Sephadex pores.

4.4. Food Applications

The filled gel model has been applied to real foods, including surimi (Filipi and Lee 1998) and fruit pulp in jams (Genovese *et al.* 2010). As previously mentioned, the filled gel model was recently used to understand the impact of milkfat on cheese rheology. Research showed that milkfat acted as an active filler when testing temperature was below 20C, dominating the cheese rheology; above 20C, cheddar cheese rheology

was dominated by the suspending network, the casein gel (Rogers *et al.* 2010; Yang *et al.* in press). Thus, previous research has demonstrated that the filled gel model can be used to understand cheddar rheology. The scope of this project, as presented in section 7, is to understand the rheological effects of substituting Sephadex beads for milkfat in cheddar cheese with regard to the filled gel model.

5. MECHANICAL METHODS FOR TESTING CHEESE

5.1. Fundamentals

The texture of cheese may be analyzed through the use of rheology, the study of flow and deformation whereby deformation of a material may occur through shear, extension, or bulk compression, as well as , and fracture mechanics, the study of forces, deformations and crack initiation related to material fracture (Steffe 1996; Anderson 2005). Instrumental testing is used to obtain fundamental rheological measurements of food in order to understand the sample's material properties and relate those properties to theories that relate molecular properties and interactions to bulk rheological properties (Foegeding *et al.* 2003). Ideally, physical properties of food should correlate to sensory terms, such that mechanical tests could be used to determine sensory texture. In general, mechanical tests correlate very well to sensory terms describing firmness and strength (Autio *et al*. 2002; Gwartney *et al.* 2002; Pereira *et al.* 2003; Barrangou *et al*. 2006; van den Berg *et al.* 2007).

Rheological testing may occur under small deformations and forces designed to prevent structural damage or under large deformations and forces that actually disturb and damage the material. Oral chewing obviously causes great destruction to food samples (Luyten *et al.* 1992), so tests that cause samples to fracture are also important tools for understanding food texture. Rheological tests are generally designed with the assumption that samples are homogenous and isotropic, and this assumption must be considered when selecting tests and interpreting the results (Foegeding *et al.* 2003). There are numerous different rheological tests, and their usage depends on the solidity of the food sample, i.e. fluid, semi-solid, or solid. Cheese is a semi-solid material and exhibits both viscous and elastic properties. Properties of such viscoelastic materials are typically elucidated using tests in the small and large strain regions, as well as using fracture tests. The specific tests used in this study, as well as the important terms derived from each test, are presented in Table 1-1.

Small strain rheological studies are performed in the linear viscoelastic region (LVR) where stress is linearly proportional to strain, whereas large strain rheological studies are performed at stresses and strains beyond the linear viscoelastic region—up until fracture. Within the LVR, materials respond instantaneously to stress, and there is no permanent deformation (Steffe 1996); stresses may be applied either in the normal mode (force applied vertically, perpendicular to the sample surface) or shear mode (force applied horizontally across the sample surface). The LVR applies to all materials, but it is more pronounced in some materials than others. For example, a Hookean spring is the

classic, idealized elastic material. It responds to stresses entirely in the LVR—unless, of course, the spring is overextended and breaks! The spring is perfectly elastic because it stretches, releases, stretches, releases, and so on, endlessly; it exhibits no permanent deformation in the LVR. In contrast, fluids represent purely viscous materials. The fluid molecules never return to exactly the same spot (discounting random diffusion) after a stress is applied; the molecules remain permanently displaced.

All rheological measurements are subject to the effects of time, which may manifest itself in instrument frequency or the interval over which measurements are collected. The effect of time is often explained using the Deborah Number, defined as

$$N_{DE} = \frac{t_{material}}{t_{process}} \quad \text{(Eq. 1.1)}$$

where $t_{material}$ is the characteristic time of the material and is estimated to be ∞ for perfectly elastic materials and 0 for perfectly viscous materials (Steffe 1996). Time significantly impacts the behavior of viscoelastic materials, which are like a hybrid of elastic and viscous materials. Silly Putty® is the classic viscoelastic material: pull it slowly, and it stretches like a viscous fluid because $t_{process}$ is large; pull it quickly, and it snaps as though brittle because $t_{process}$ is minute. The viscous deformation under slow frequencies corresponds to a large Deborah Number, whereas the elastic deformation under rapid frequencies corresponds to a small Deborah Number. (The term "Deborah Number" is a biblical reference emphasizing the idea that, with time, even the mountains will flow (Steffe 1996).) As mentioned, the Hookean spring is used to model the behavior of perfectly elastic materials, whereas the dashpot (e.g. a piston) is used to

model the behavior of perfectly viscous materials. Viscoelastic materials are modeled using combinations of springs and dashpots, which are also known as "elements". Certain combinations of these elements relate to common rheological tests like the creep test.

Temperature also affects all rheological tests since most materials soften or melt as temperature increases. Thus, samples tested at higher temperatures will have a greater viscous component than if the same material were tested at lower temperatures. Rheological tests may be categorized as either empirical or fundamental (Foegeding *et al.* 2003). Empirical tests are relatively simple procedures typically meant for quick use in the pilot plant and sometimes for quality control in industrial settings. Such tests typically measure force on a sample and the resultant deformation, and they are subject to the variability in test parameters, including sample volume, shape, and testing speed. True fundamental tests, in contrast, account for sample volume, shape, and testing conditions.

5.2. Common Small Strain Tests

The LVR must be identified before any subsequent tests to allow for proper interpretation of measurements. The end of the LVR corresponds to specific stress and strain values, which are known as the critical stress and critical strain, respectively. These values are commonly found using stress/strain sweeps, although creep tests can also be used to approximate the LVR (Rosalina and Bhattacharya 2001). Tedious and less precise than stress/strain sweeps, creep tests are not commonly used to determine the

LVR because the method requires a series of creep tests at different loads; creep compliance curves should increase proportionately at loads within the LVR (Rosalina and Bhattacharya 2001). Stress sweeps are conducted on stress-controlled rheometers and involve an incremental increase of applied stress, whereas strain sweeps are conducted on strain-controlled rheometers and involve an incremental increase of applied strain (Steffe 1996). Both tests are oscillatory measurements using the cup and bob, cone and plate, or parallel plate attachment, depending upon the nature/fluidity of the sample.; The tests maintain constant frequency and allow for the calculation of the non-controlled parameter via the complex modulus (see Table 1-1). The resulting output is a range of stresses or strains that follow a linear pattern, the LVR, followed by a region that deviates from that linear pattern. Higher critical points (longer linear regions) generally indicate stronger materials as strong gels may remain in the LVR over greater strains than weak gels (Steffe 1996), although other tests (e.g. fracture tests) are more useful for determining actual material strength.

Stress/strain and frequency sweeps hold opposite parameters constant, although all tests use dynamic oscillatory measurements applied in a sinusoidal pattern. In stress/strain sweeps, the frequency is held constant and the stress or strain varied, whereas frequency sweeps hold the applied stress/strain constant and vary the applied frequency. As previously mentioned, all viscoelastic materials are subject to time scales. Therefore, frequency sweeps illuminate the degree of viscoelasticity in a sample by measuring how the viscous and elastic components change with frequency (Steffe 1996;

Foegeding *et al.* 2003). Important calculated parameters obtained from dynamic oscillatory tests, particularly from frequency sweeps, include changes in the storage modulus (G'), loss modulus (G"), complex modulus (G*), and phase angle (δ) with time; these patterns are often called the "mechanical spectra" of a material. The phase angle indicates the degree of in-phase behavior between the stress and strain oscillations. Purely elastic materials store all energy (recall the Hookean spring) and are characterized by a phase angle equal to 0°; purely viscous materials dissipate all inputted energy and so are characterized by a phase angle equal to 90° (Steffe 1996). The storage and loss moduli are calculated from the complex modulus and phase angle; stress, strain, and phase angle are the only values measured directly from oscillatory tests. The relationships between the moduli and phase angle are explained in Table 1-1.

Creep tests are conducted on stress-controlled rheometers; the related step-strain test would be conducted on a strain-controlled rheometer. Creep tests are a type of transient shear flow experiment in which an "instantaneous" load, or stress, is applied to the sample for a given period of time (Steffe 1996). Compliance (J), the amount of deformation per instantaneous load, is measured. Creep tests are often paired with a recovery test, which measures the degree to which a sample "rebounds" after the stress is removed. Perfectly elastic materials deform instantly and constantly under an applied stress and rebound fully and instantaneously once the stress is removed (again, consider the Hookean spring); there is no dependence on time. In contrast, perfectly viscous samples deform according to their viscosity under the stress so that strain increases

linearly with time, and the output is a constant strain rate; perfectly viscous samples do not recover at all. Viscoelastic materials, of course, exhibit intermediate behavior. The applied load and testing duration are important variables in this test. Applied loads may lie within the LVR (applied load < critical stress) or beyond the LVR (applied load > critical stress), and the extent of deformation and recovery are dependent upon how much time is allotted for each.

5.3. Common Large Strain Tests

As mentioned, large strain tests occur beyond the LVR and inflict actual damage—even if only at the microscale level—to the material. Such tests are commonly used to mimic oral chewing, "first bite", and two-finger compression terms of sensory tests. For this thesis, creep tests were conducted at loads within the LVR (small strain test) and beyond the LVR (large strain test). A uniaxial compression test was also conducted; this test has been shown to correlate well with sensory terms and to differentiate cheddar cheese samples on the basis of fat content such that low-fat cheeses were shown to recover more energy—correlating to elasticity or springiness—than full-fat cheeses (Rogers *et al.* 2010; Rogers *et al.* 2009). Uniaxial compression tests and other normal force tests are essentially used to measure the springiness of a cylindrical sample when it is compressed between two flat plates. This test directly measures the stress applied to the sample and the distance the top plate moves over time, which is converted to strain (Bot *et al.* 1996). Uniaxial tests are popular because they are simple to conduct.

5.4. Common Fracture Tests

While large strain tests damage the food sample microstructure, fracture tests deform to the point of complete failure and separation of the material to distinct pieces. Four main types of fracture tests exist: compression, tension, torsion, and bending (Luyten *et al.* 1992; Hamann *et al*. 2006; Truong and Daubert 2000). Uniaxial compression tests were already described and, at large enough stresses and strains, can be used as fracture tests. While uniaxial compression tests compress samples under a normal load, tension tests are used to elongate samples. The advantage to this type of test is that strains are theoretically infinite and fracture will always be observed. However it is difficult to use with food because of problems with attaching the samples to the instrument. Bending tests are easy to use with food and advantageous in that fracture typically begins at the outside of the sample. The three point bend test, also known as the single edge notch bend test, utilizes a small notch in the sample to ensure that fracture occurs at a designated location. This test is useful for determining both fracture toughness and the energy required to propagate fracture through the entire sample (see Table 1-1). The deformation in such bending tests is typically a combination of shear, compression, and extension. Torsion testing produces shear stress and causes the sample to twist on its horizontal axis. In order to minimize normal stress and ensure pure shear conditions, the sample is ground into a capstan shape; this shape also ensures that fracture will occur at the middle portion of the sample. The testing instrument then twists the sample at a constant shear rate, measuring torque and time. Fracture stress and strain are

then calculated from the measured torque, time, and angular deformation at fracture (Hamann 1983; Diehl and Hamann 1979; Brown *et al.* 2003; Rogers *et al.* 2009).

6. CONFOCAL SCANNING LASER MICROSCOPY

Various rheological instruments can be used to probe the structure elements of food, but microscopy can be used to view those elements at some level of detail. Confocal scanning laser microscopy (CSLM) is advantageous over other types of light microscopy in that (1) the image is always in focus due to the two pinhole set-up, and (2) the lasers used in CSLM microscopy are strong enough to penetrate thick samples such that the experimenter need not worry about slicing delicately thin samples. Most importantly, no extreme sample preparation is necessary. One needs only to start with a sample size appropriate for the instrument, apply a fluorescent dye, and collect images. This is a stark contrast to scanning electron microscopy (SEM) and transmission electron microscopy (TEM), both of which require extensive sample preparation and/or analysis in non-native environments that may damage the sample's natural intrinsic structure. Researchers have been using CSLM to image food since the 1980's (Pawley 2006).

In its simplest form, CSLM uses either spinning disks or rotating mirrors to scan a specimen with diffraction-limited pinpoints of light. Raster scans are used to image a single plane of the specimen; computer software is then used to compile these "z-stacks" into a three-dimensional reconstruction—much like a CAT scan in medical technology. The selection of different fluorescent dyes allows researchers to elicit structures of

interest. For example, different dyes have affinities for different chemical moieties. One dye might be used to visualize all carbohydrate compounds in a sample, whereas other dyes adhere to, say, fat or protein. Cellular biologists wanting to track mobile elements or wanting to measure the distance and/or activity between compounds and organelles may make use of more complex fluorescent probes and imaging techniques like green fluorescent protein markers (GFP-tagging) and Förster resonance energy transfer (aka "fluorescence resonance energy transfer" or FRET). The *Handbook of Biological Confocal Microscopy* (Pawley 2006) details the nuances of CSLM and its applications, staining methods, and pitfalls of CSLM. As with any fluorescent imaging, photobleaching, the loss of fluorescence due to photochemical destruction of flurophores, is endemic to CSLM. Precautions must also be taken when imaging and reconstructing three-dimension images from two-dimensional raster scans. For example, imagine cutting an orange and looking at a two-dimensional image of the cross-section. The dimensions of the resultant circle will depend upon where the orange was sliced: sections near the outer edges of the orange will have the smallest diameter, whereas an orange sliced through its exact center will have the greatest diameter. *Image Analysis of Food Microstructure* (Russ 2005) provides guidance for imaging three-dimensional specimens.

7. PROJECT OBJECTIVE

Previous research has shown that milkfat can act as an active or inactive filler in cheese based on its phase volume and temperature (Rogers *et al.* 2008). The objective of

this project is to validate the use of the filled gel model for understanding cheese texture by substituting Sephadex beads for fat. As previously mentioned, Sephadex beads are spherical, deformable particles made from a commercial process that cross-links dextran polysaccharide molecules. They are classically used for filtration (ion-exchange chromatography, size-exclusion chromatography, etc.) and so are *not* available as food-grade materials. Consequently, the scope of this project did not include any sensory evaluations of the Sephadex-filled cheese. Sephadex beads were used as the filler particle in this study in order to minimize variables and simplify an otherwise complex system. Sephadex beads are simpler than milkfat globules because they do not melt within the temperature range tested in these experiments and because the uncharged beads are not suspected to have any chemical interactions with the protein network. Sephadex beads were selected over other potential fillers because they are pre-made and readily available in a variety of rigidities, diameters, and charges (both neutral and salt forms are sold). Furthermore, the commercial manufacturing process of these beads guarantees a uniform, spherical shape, and previous studies have shown that particle size, shape, and orientation are important (Chow 1980; Ross-Murphy and Todd 1983; Langley and Green 1989; Sala *et al.* 2009).

Thus, the substitution of Sephadex beads for milkfat in cheddar cheese will allow us to understand the effect of filler volume, regardless of filler type, on cheese texture and rheology. This understanding will provide a tool for developing more palatable low-fat cheddar cheese. If the filled gel model holds true for cheddar cheese, then

theoretically, acceptable low-fat cheese could be produced by adding filler particles with the appropriate properties. Specifically, full-fat cheese containing 33% filler (all milkfat globules) should have the same rheological properties as low-fat cheese containing 33% filler (6% milkfat + 27% other filler particle). Such a goal assumes, of course, that the developer intimately understands the cheese products currently available to consumers. Therefore, this thesis contains four studies:

1. Rheological analysis of off-the-shelf commercial cheddar cheese;
2. Rheological and sensorial analysis of commercial cheddar cheese received directly from the manufacturer;
3. Effect of filler particle size and volume on rheological properties; and
4. Effect of filler charge on rheological properties.

If Sephadex-filled cheddar cheese behaves as predicted under the filled gel model, then a molecular mechanism and material model will be established. Therefore, this research is important for validating the filled gel model as it applies to cheddar cheese because such research will help lead to the development of more palatable low-fat cheese, which is a large and expanding economic sector.

8. REFERENCES

AUTIO, K., KUUVA, T., ROININEN, K. and LAHTEENMAKI, L. 2002. Rheological properties, microstructure and sensory perception of high-amylose starch-pectin mixed gels. J. Texture Stud. *33*, 473-486.

AHMED, S. and JONES, F.R. 1990. A review of particulate reinforcement theories for polymer composites. J. Mater. Sci. *25*, 4933-4942.

ANDERSON, T.L. 2005. *Fracture Mechanics: Fundamentals and Applications*, 3rd ed. Taylor & Francis, Boca Raton, FL.

BANKS, J.M., BRECHANY, E.Y. and CHRISTIE, W.W. 1989. The production of low fat cheddar-type cheese. J. Soc. Dairy Technol. *42*, 6-9.

BANKS, J.M. 2004. The technology of low-fat cheese manufacture. Int. J. Dairy Technol. *57*, 199-207.

BARRANGOU, L.M., DRAKE, M.A., DAUBERT, C.R. and FOEGEDING, E. A. 2006. Sensory texture related to large-strain rheological properties of agar/glycerol gels as a model food. J. Texture Stud. *37*, 241-262.

BOT, A., VAN AMERONGEN, I.A., GROOT, R.D., HOEKSTRA, N.L. and AGTEROF, W.G.M. 1996. Large deformation rheology of gelatin gels. Polym. Gels Networks *4*, 189-227.

BOWLAND, E.L. and FOEGEDING, E.A. 1999. Factors determining large-strain (fracture) rheological properties of model processed cheese. J. Dairy Sci. *82*, 1851-1859.

BROWNSEY, G.J., ELLIS, H.S., RIDOUT, M.J. and RING, S.G. 1987. Elasticity and failure in composite gels. J. Rheol. *31*, 635-649.

BROWN, J.A., FOEGEDING, E.A., DAUBERT, C.R., DRAKE, M.A. and GUMPERTZ, M. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. *86*, 3054-3067.

BRUMMEL, S.E. and LEE, K. 1990. Soluble hydrocolloids enable fat reduction in process cheese spreads. J. Food Sci. *55*, 1290-1292.

BRYANT, A. USTUNOL, Z. and STEFFE, J. 1995. Texture of cheddar cheese as influenced by fat reduction. J.Food Sci. *60*, 1216-1219.

CARLSON, A., HILL, Jr., C.G. and OLSON, N.F. 1987. Kinetics of milk coagulation: I. The kinetics of kappa casein hydrolysis in the presence of enzyme deactivation. Biotechnol. Bioeng. *29*, 582-589.

CARUNCHIA WHETSTINE, M.E., DRAKE, M.A., NELSON, B.K. and BARBANO, D. 2006. Flavor profiles of full-fat and reduced-fat cheese and cheese fat made from aged cheddar with the fat removed using a novel process. J. Dairy Sci. *89*, 505-517.

CHEN, J. and DICKINSON, E. 1998a. Viscoelastic properties of protein-stabilized emulsions: effect of protein-surfactant interactions. J. Agric. Food Chem. *46*, 91-97.

CHEN, J. and DICKINSON, E. 1998b. Viscoelastic properties of heat-set whey protein emulsion gels. J. Texture Stud. *29*, 285-304.

CHEN, J. and DICKINSON, E. 1999. Effect of surface character of filler particles on rheology of heat-set whey protein emulsion gels. Colloids Surf., B *12*, 373-381.

CHILDS, J.L. and DRAKE, M.A. 2009. Consumer perception of fat reduction in cheese. J. Sens. Stud. *24*, 902-921.

CHOW, T.S. 1980. The effect of particle shape on the mechanical properties of filled polymers. J. Mater. Sci. *15*, 1873-1888.

CREAMER, L.K. and OLSON, N.F. 1982. Rheological evaluation of maturing cheddar cheese. J. Food Sci. *47*, 631-636, 646.

CREAMER, L.K., LAWRENCE, R.C. and GILLES, J. 1985. Effect of acidification on cheese milk on the resultant cheddar cheese. N.Z. J. Dairy Sci. Technol. *20*, 185-203.

CREAMER, L.K., GILLES, J. and LAWRENCE, R.C. 1988. Effect of pH on the texture of cheddar and Colby cheese. N.Z. J. Dairy Sci. Technol. *23*, 23-35.

DELFOUR, A., JOLLES, J., ALAIS, C. and JOLLES, P. 1965. Caseino-glycopeptides: characterization of a methionine residue and of the N-terminal sequence. Biochem. Biophys. Res. Commun. *19*, 452-455.

DIEHL, K.C. and HAMANN, D.D. 1979. Structural failure in selected raw fruits and vegetables. J. Texture Stud. *10*, 371-400.

DRAKE, M.A., BOUTTE, T.T., LUEDECKE, L.O. and SWANSON, B.G. 1994a. Milkfat sucrose polyesters as fat substitutes in cheddar-type cheeses. J. Food Sci. *59*, 326-327, 365.

DRAKE, M.A., BOUTTE, T.T., YOUNCE, F.L., CLEARY, D.A. and SWANSON, B.G. 1994b. Melting characteristics and hardness of milkfat blend sucrose polyesters. J. Food Sci. *59*, 652-654.

DRAKE, M.A. and SWANSON, B.G. 1995. Reduced- and low-fat cheese technology: a review. Trends Food Sci. Technol. *6*, 366-369.

DRAKE, M.A., HERRETT, W., BOYLSTON, T.D. and SWANSON, B.G. 1995. Sensory evaluation of reduced fat cheeses. J. Food Sci. *60*, 898-901, 905.

FENELON, M.A., O'CONNOR, P. and GUINEE, T.P. 2000. The effect of fat content on the microbiology and proteolysis in cheddar cheese during ripening. J. Dairy Sci. *83*, 2173-2183.

FENNEMA, O.R. 1996. *Food Chemistry*, 3rd ed. Marcel Dekker, Inc. New York, NY.

FIFE, R.L., MCMAHON, D.J. and OBERG, C.J. 1996. Functionality of low-fat mozzarella cheese. J. Dairy Sci. *79,* 1903-1910.

FILIPI, I. and LEE, C.M. 1998. Preactivated iota-carrageenan and its rheological effects in composite surimi gel. LWT—Food Sci. Technol. *31*, 129-137.

FOEGEDING, E.A. 1992. Rheological properties of whey protein isolate gels determined by torsional fracture and stress relaxation. J. Texture Stud. *23*, 337-348.

FOEGEDING, E.A., BROWN, J., DRAKE, M.A. and DAUBERT, C.R. 2003. Sensory and mechanical aspects of cheese texture. Int. Dairy J. *13*, 585-591.

FOEGEDING, E.A. and DRAKE, M.A. 2007. Invited review: Sensory and mechanical properties of cheese texture. J. Dairy Sci. *90*, 1611-1624.

FOX, P.F., MCSWEENEY, P.L.H., COGAN, T.M. and GUINEE, T.P. 2004. *Cheese: Chemistry, Physics and Microbiology—Volume 1,* 3rd ed. Elsevier, London, UK.

GENOVESE, D.B., YE, A.Q. and SINGH, H. 2010. High methoxyl pectin/apple particles composite gels: effect of particle size and particle concentration on mechanical properties and gel structure. J. Texture Stud. *41*, 171-189.

GOULD, B. 2010. Understanding dairy markets: your source for market information and price risk management principles. <http://future.aae.wisc.edu/data/monthly_values/by_area/154?tab=production&year=2009>.

GROPPER, S.S., SMITH, J.L. and GROFF, J.L. 2005. *Advanced Nutrition and Human Metabolism*, 4th ed. Wadsworth, Belmont, CA.

GWARTNEY, E.A., FOEGEDING, E.A. and LARICK, D.K. 2002. The texture of commercial full-fat and reduced-fat cheese. J. Food Sci. *67*, 812-816.

GWARTNEY, E.A., LARICK, D.K. and FOEGEDING, E.A. 2004. Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. J. Food Sci. *69*, S333-S339.

HAMANN, D.D. 1983. Structural failure in solid foods. In *Physical Properties of Foods* (M. Peleg and E.B. Bagley, eds.) pp. 351-383, AVI Pub. Co., Westport, CT.

HAMANN, D.D., ZHANG, J.H., DAUBERT, C.R., FOEGEDING, E.A. and DIEHL, K.C. 2006. Analysis of compression, tension and torsion for testing food gel fracture properties. J. Texture Stud. *37*, 620-639.

HOUSTON, D.K., DRIVER, K.E., BUSH, A.J. and KRITCHEVSKY, S.B. 2008. The association between cheese consumption and cardiovascular risk factors among adults. J. Hum. Nutr. Diet. *21*, 129-140.

HORT, J., LE GRYS, G. and WOODMAN, J. 1997. Changes in the perceived textural properties of cheddar cheese during maturation. J. Sens. Stud. *12*, 255-266.JACK, F.R., PIGGOTT, J.R. and PATERSON, A. 1993a. Discrimination of texture and appearance in cheddar cheese using consumer free choice profiling. J. Sens. Stud. *8*, 167-176.

JACK, F.R., PATERSON, A. and PIGGOTT, J.R. 1993b. Relationships between rheology and composition of cheddar cheeses and texture as perceived by consumers. Int. J. Food Sci. Technol. *28*, 293-302.

JOHNSON, M.E., KAPOOR, R., MCMAHON, D.J., MCCOY, D.R. and NARASIMMON, R.G. 2009. Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. Compr. Rev. Food Sci. Food Saf. *8*, 252-268.

KAYANUSH, J.A. and HAQUE, Z.U. 2001. Effect of commercial fat replacers on the microstructure of low-fat cheddar cheese. Int. J. Food Sci. Technol. *36*, 169-177.

KERNER, E.H. 1956. The elastic and thermo-elastic properties of composite media. Proc. Phys. Soc., London, Sect. B. *8*, 808-813.

KUMAR, N. and SINGHAL, O.P. 1992. Atherosclerosis—are dairy-products safe. J. Soc. Dairy Technol. *45*, 49-52.

LANGLEY, K.R. and GREEN, M.L. 1989. Compression strength and fracture properties of model particulate food composites in relation to their microstructure and particle-matrix interaction. J. Texture Stud. *20*, 191-207.

LAWRENCE, R.C., CREAMER, L.K. and GILLES, J. 1987. Texture development during cheese ripening. J. Dairy Sci. *70*, 1748-1760.

LI, H., ERRINGTON, A.D. and FOEGEDING, E.A. 1999. Isostrength comparison of large-strain (fracture) rheological properties of egg white and whey protein gels. J. Food Sci. *64*, 893-898.

LORET, C., FRITH, W.J. and FRYER, P.J. 2007. Mechanical and structural properties of maltodextrin/agarose microgels composites. Appl. Rheol. *17*, 31412.1-31412.13.

LOZINSKY, V.I., ZUBOV, A.I., KULAKOVA, V.K., TITOVA, E.F. and ROCOZHIN, S.V. 1992. Study of cryostrudurization of polymer systems. IX. Poly(vinyl alcohol)

cryogels filled with particles of crosslinked dextran gel. J. Appl. Polym. Sci. *44*, 1423-1435.

LOZINSKII, V.I. and SAVINA, I.N. 2002. Study of cryostructuring polymer systems: 22. Composite poly(vinyl alcohol) cryogels filled with dispersed particles of various degrees of hydrophilicity/hydrophobicity. Colloid J. *3*, 336–343.

LUCEY, J.A., JOHNSON, M.E. and HORNE, D.S. 2003. Invited Review: Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. *86*, 2725-2743.

LUYTEN, H., VAN VLIET, T. and WALSTRA, P. 1992. Comparison of various methods to evaluate fracture phenomena in food materials. J. Texture Stud. *23*, 245-266.

MCEWAN, J.A., MOORE, J.D. and COLWILL, J.S. 1989. The sensory characteristics of cheddar cheese and their relationship with acceptability. J. Soc. Dairy Technol. *42*, 112-117.

MILO, C. and REINECCIUS, G.A. 1997. Identification and quantification of potent odorants in regular fat and low-fat mild cheddar cheeses. J. Agric. Food Chem. *45*, 3590-3594.

MINTEL INTERNATIONAL GROUP LTD. 2009. Mintel Oxygen report on *Cheese – US – May 2009*. Mintel Publications, London.

MINTEL INTERNATIONAL GROUP LTD. 2010. Mintel Oxygen report on *Cheese – US – May 2010.* Mintel Publications, London.

MISTRY, V.V. 2001. Low fat cheese technology. Int. Dairy J. *11*, 413-422.

O'BRIEN, N.M. and O'CONNOR, T.P. 2004. Nutritional aspects of cheese. In *Cheese: Chemistry, Physics, and Microbiology* (P.F. Fox, P.L.H. McSweeney, T.M. Cogan, and T.P. Guinee, eds.) pp. 573-581, Elsevier, London, UK.

OLSON, N.F. 1990. The impact of lactic acid bacteria on cheese flavor. FEMS Microbiol. Rev. *87*, 131-148.

PAL, R. 2002. Complex shear modulus of concentrated suspensions of solid spherical particles. J. Colloid Interface Sci. *245*, 171-177.

PAL, R. 2008. A new linear viscoelastic model for emulsions and suspensions. Polym. Eng. Sci. *48*(7), 1250-1253.

PALIERNE, J.F. 1990. Linear rheology of viscoelastic emulsion with interfacial tension. Rheol. Acta. *29*, 204-214.

PASTORINO, A.J., HANSEN, C.L. and MCMAHON, D.J. 2003a. Effect of salt on structure-function relationships of cheese. J. Dairy Sci. *86*, 60-69.

PASTORINO, A.J., RICKS, N.P., HANSEN, C.L. and MCMAHON, D.J. 2003b. Effect of calcium and water injection on structure-function relationships of cheese. J. Dairy Sci. *86*, 105-113.

PASTORINO, A.J., HANSEN, C.L. and MCMAHON, D.J. 2003c. Effect of pH on the chemical composition and structure-function relationships of cheddar cheese. J. Dairy Sci. *86*, 60-69.

PAWLEY, J.B. 2006. *Handbook of Biological Confocal Microscopy*, 3rd ed. Springer Science, New York, NY.

PFEUFFER, M. and SCHREZENMEIR, J. 2000. Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. Br. J. Nutr. *84*, S155 -S159.

RAMKUMAR, C., CAMPANELLA, O.H., WATKINSON, P.J., BENNETT, R.J. and CREAMER, L.K. 1998. The effects of pH and time on rheological changes during early cheese maturation. J. Texture Stud. *29*, 633-644.

RICHARDSON, B.C. and ELSTON, P.D. 1984. Plasmin activity in commercial caseins and caseinates. New Zeal. J. Dairy Sci. *19*, 63-67.

RODRÍGUEZ, J. 1998. Recent advances in the development of low-fat cheeses. Trends Food Sci. Technol. *9*, 249-254.

ROEFS, S.P.F.M., WALSTRA, P., DALGLEISH, D.G. and HORNE, D.S. 1985. Preliminary note on the change in casein micelles caused by acidification. Neth. Milk Dairy J. *39*, 119-122.

ROGERS, N.R., DRAKE, M.A., DAUBERT, C.R., MCMAHON, D.J., BLETCH, T.K. and FOEGEDING, E.A. 2009. The effect of aging on low-fat, reduced-fat, and full-fat cheddar cheese texture. J. Dairy Sci. *92*, 4756-4772.

ROGERS, N.R., MCMAHON, D.J., DAUBERT, C.R., BERRY, T.K. and FOEGEDING, E.A. 2010. Rheological properties and microstructure of cheddar cheese made with different fat contents. J. Dairy Sci. *in press*.

ROMEIH, E.A., MICHAELIDOU, A., BILIADERIS, C.G. and ZERFIRIDIS, G.K. 2002. Low-fat white-brined cheese made from bovine milk and two commercial fat mimetics: chemical, physical and sensory attributes. Int. Dairy J. *12*, 525-540.

ROSALINA, I. and BHATTACHARYA, M. 2001. Flow curves, stress relaxation and creep measurements of starch gels. J. Texture Stud. *32*, 247-269.

ROSS-MURPHY, S.B. and TODD, S. 1983. Ultimate tensile measurements of filled gelatin gels. Polymer. *24,* 481-486.

RUDAN, M.A. and BARBANO, D.M. 1998. A model of mozzarella cheese melting and browning during pizza baking. J. Dairy Sci. *81,* 2312-2319.

RUSS, J.C. 2005. *Image Analysis of Food Microstructure*. CRC Press, Boca Raton, FL.

SAHAN, N., YASAR, K., HAYALOGLU, A.A., KARACA, O.B. and KAYA, A. 2008. Influence of fat replacers on chemical composition, proteolysis, texture profiles, meltability and sensory properties of low-fat Kashar cheese. J. Dairy Res.

SAINT-EVE, A., LAUVERJAT, C., MAGNAN, C., DÉLÉRIS, I. and SOUCHON, I. 2009. Reducing salt and fat content: impact of composition, texture, and cognitive interactions on the perception of flavoured model cheeses. Food Chem. *116,* 167-175.

SALA, G., VAN VLIET, T., STUART, M.C., VAN DE VELDE, F. and VAN AKEN, G.A. 2009. Deformation and fracture of emulsion-filled gels: effect of gelling agent concentration and oil droplet size. Food Hydrocolloids *23,* 1853-1863.

SCOTT, R., ROBINSON, R.K. and WILBEY, R.A. 1998. *Cheesemaking Practice,* 3rd ed. Aspen Publishers, Inc., Gaithersburg, MD.

SMEDMAN, A., GUSTAFSSON, I.B., BERGLUND, L. and VESSBY, B. 1999. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk and metabolic risk factors. Am. J. Clin. Nutr. *69,* 22-29.

STANLEY, D.W. and EMMONS, D.B. 1977. Cheddar cheese made with bovine pepsin. II. Texture-microstructure-composition relationships. Can. Inst. Food Sci. Technol. J. *10,* 78-84.

STEENEKEN, P.A.M. and WOORTMAN, A.J.J. 2009. Superheated starch: a novel approach towards spreadable particle gels. Food Hydrocolloids. *23,* 394-405.

STEFFE, J.F. 1996. *Rheological Methods in Food Process Engineering*. Freeman Press, East Lansing, MI.

TARREGA, A., YVEN, C. SÉMON, E. and SALLES, C. 2008. Aroma release and chewing activity during eating different model cheeses. Int. Dairy J. *18,* 849-857.

TIDONA, F., CRISCIONE, A., GUASTELLA, A.M., ZUCCARO, A., BORDONARO, S. and MARLETTA, D. 2009. Bioactive peptides in dairy products. Ital. J. Anim. Sci. *8,* 315-340.

TOTOSAUS, A. and GUEMES-VERA, A. 2008. Effect of kappa- and lambda-carrageenans as fat-replacers in low-fat Oaxaca cheese. Int. J. Food Prop. *11*, 656-668.

TOURNIER, C., SULMONT-ROSSÉ, C., SÉMON, E., VIGNON, A., ISSANCHOU, S. and GUICHARD, E. 2009. A study on texture-taste-aroma interactions: physico-chemical and cognitive mechanisms. Int. Dairy J. *19*, 450-458.

TRUONG, V.D. and DAUBERT, C.R. 2000. Comparative study of large strain methods for assessing failure characteristics of selected food gels. J. Texture Stud. *31*, 335-353.

TUDOR, M., HAVRANEK, J. and SERAFINI, M. 2009. Dairy foods and body weight management. Mljekarstvo *59*, 88-95.

TUNICK, M.H., MACKEY, K.L., SHIEH, J.J., SMITH, P.W., COOKE, P., and MALIN, E.L. 1993. Rheology and microstructure of low-fat mozzarella cheese. Int. Dairy J. *3*, 649-662.

ULBRICHT, T.L.V. and SOUTHGATE, D.A.T. 1991. Coronary heart disease: seven dietary factors. Lancet *338*, 985-992.

VAN DEN BERG, L., VAN VLIET, T., VAN DER LINDEN, E., VAN BOEKEL, M. and VAN DE VELDE, F. 2007. Breakdown properties and sensory perception of whey proteins/polysaccharide mixed gels as a function of microstructure. Food Hydrocolloids *21*, 961-976.

VAN DER POEL, C. 1958. On the rheology of concentrated dispersions. Rheol. Acta *1*, 198-205.

VAN VLIET, T. 1988. Rheological properties of filled gels. Influence of filler matrix interaction. Colloid Polym. Sci. *266*, 518-524.

VISSER, J. 1991. Factors affecting the rheological and fracture properties of hard and semi-hard cheese. In *Rheological and Fracture Properties of Cheese* (P. Walstra, ed.) pp. 49-61, Bull. No. 268, International Dairy Federation, Brussels, Belgium.

WALSTRA, P. and VAN VLIET, T. 1986. The physical chemistry of curd making. Neth. Milk Dairy J. *40*, 241-159.

WALSTRA, P. 2003. *Physical Chemistry of Foods*. Marcel Dekker, Inc., New York, NY.

WARRAND, J. 2006. Healthy polysaccharides: the next chapter in food products. Food Technol. Biotechnol. *44*, 355-370.

WATKINSON, P., COKER, C., CRAWFORD, R., DODDS, C., JOHNSTON, K., MCKENNA, A. and WHITE, N. 2001. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. 2001. Int. Dairy J. *11*, 455-464.

WEEL, K.G.C., BOELRIJK, A.E.M., ALTING, A.C., VAN MIL, P.J.J.M., BURGER, J.J., GRUPPEN, H., VORAGEN, A.G.J. and SMIT, G. 2002. Flavor release and perception of flavored whey protein gels: perception is determined by texture rather than by release. J. Agric. Food Chem. *50*, 5149-5155.

WESTERTERP-PLANTENGA, M.S., NIEUWENHUIZEN, A., TOMÉ, D., SOENEN, S. and WESTERTERP, K.R. 2009. Dietary protein, weight loss, and weight maintenance. Annu. Rev. Nutr. *29*, 21-41.

WHETSTINE, M.E.C., DRAKE, M.A., NELSON, B.K. and BARBANO, D. 2006. Flavor profiles of full-fat and reduced-fat cheese and cheese fat made from aged cheddar with the fat removed using a novel process. J. Dairy Sci. *89*, 505-517.

WOLK, A., VESSBY, B., LJUNG, H. and BARREFORS, P. 1998. Evaluation of a biological marker of dairy fat intake. Am. J. Clin. Nutr. *68*, 291-295.

WOLK, A., FURUHEIM, M. and VESSBY, B. 2001. Fatty acid composition of adipose tissue and serum lipids are valid biomarkers of dairy fat intake among men. J. Nutr. *13*, 828-833.

YANG, X., ROGERS, N.R., BERRY, T.K. and FOEGEDING, E.A. In press. Modeling the rheological properties of cheeses of different fat content. J. Texture Stud.

YATES, M.D. and DRAKE, M.A. 2007. Texture properties of gouda cheese. J. Sens. Stud. *22*, 493-506.

Table 1.1. RHEOLOGICAL TERMS AND SYMBOLS COMMON TO THE FILLED GEL MODEL

Symbol	**Term Name(s) (and Units)**	**Description**	**Associated Rheological Tests[1]**
G'	Shear storage modulus, Elastic modulus (Pa)	Represents the degree of elasticity in a viscoelastic sample	Frequency sweeps, Stress sweeps
G"	Shear loss modulus, Viscous modulus (Pa)	Represents the viscous component in a viscoelastic sample	Frequency sweeps, Stress sweeps
G*	Shear complex modulus (Pa)	Relates the storage and loss moduli; $G^* = \sqrt{[(G')^2 + (G'')^2]}$	Frequency sweeps, Stress sweeps
δ	Phase angle (degrees)	Relates the viscous and elastic components; $\delta = 90° \rightarrow$ purely viscous sample $\delta = 0° \rightarrow$ purely elastic sample $\tan(\delta) = G''/G'$	Frequency sweeps, Stress sweeps
σ_f	Fracture stress (Pa)	Stress (Pa) at which sample breaks or ruptures	Torsion test
γ_f	Fracture strain (unitless)	Deformation at which sample breaks or ruptures	Torsion test
G_f	Fracture modulus (Pa)	Ratio of the fracture stress over fracture strain	Torsion test
J_{max}	Shear maximum creep compliance (Pa^{-1})	The maximum deformation under a given load after a specified length of time	Creep test
J_r	Shear maximum creep recovery (Pa^{-1})	The compliance after a specified recovery time post creep test; $J_r = J_{max} - J_{end\ of\ recovery}$	Creep/recovery test
K_c	Fracture toughness ($kPa \cdot m^{0.5}$)	The amount of force needed to propagate fracture through a notched sample of set dimensions	Single edge notch bend test
G_c	Fracture energy (J/m^2)	The amount of energy needed to propagate fracture through a notched sample of set dimensions	Single edge notch bend test

[1]Refers to tests used in this thesis

Chapter 2

Rheological Survey of Off-The-Shelf Commercial Cheddar Cheese Available in Varying Fat Contents

1. ABSTRACT

Cheese is a major economic sector in the US, and manufacturers and researchers trying to develop low-fat cheeses need to understand what range of cheese textures currently confronts consumers. Three brands of commercial cheddar cheese available in both full-fat and reduced/low-fat varieties were purchased from grocery stores. Rheological properties were measured using stress sweeps, frequency sweeps at 10, 15, 20, and 25C, and creep/recovery tests. Large strain and fracture properties were determined with uniaxial compression tests and torsional fracture. Low-fat and reduced-fat cheese were characterized by structural and deformability differences, which resulted in larger critical stress and strain, maximum compliance, percent recoverable energy, and fracture strain across all brands; trends were consistent across the linear viscoelastic region, critical points, and non-linear region up to fracture. Torsional fracture particularly illustrated the variability of off-the-shelf commercial cheese, particularly between lots of the same brand.

Keywords: low/reduced-fat cheddar cheese; texture; rheology

2. PRACTICAL APPLICATION

Cheddar cheese is a widely consumed food product in the U.S. Consumers are increasingly interested in reduced and low-fat products, but reduced and low-fat cheeses have historically been criticized for overly firm, elastic textures. This research serves as an updated rheological survey of full, reduced, and low-fat cheeses that are currently

available on grocery store shelves. Such information will help product developers to understand the products and range of textures that cheddar cheese consumers currently experience, as well as any gains that have been made in reduced and low-fat cheddar cheese products.

3. INTRODUCTION

Cheese is a major economic sector and vital industry within US markets. Cheese consumption has increased amongst Americans almost every year since 1998, with 2003 and 2009 being the only exceptions (Mintel 2009, 2010). Sales of natural cheese decreased slightly in 2009, but the sector still sold 7.51 billion dollars worth of product (Mintel 2010). . Furthermore, Mintel forecasters predict that the cheese sector should grow an additional 18% from 2010-2014 (Mintel 2010). Cheddar cheese is a vital part of this growing cheese market. According to the USDA National Agricultural Statistics Service, the USA produced 3,170,629,000 lbs (~1,585,315 US tons) of cheddar cheese in 2009 alone (cited in Gould 2010)!

Much of this popularity is likely due to the recognized health benefits of cheese, given that cheese manufacture presents a means of preserving the water-insoluble nutritional qualities of milk. Furthermore, the health sections in many newspapers and magazines, as well as marketing claims touted on product packaging, are making more consumers cognizant of recent studies proclaiming the health benefits of cheese on cardiovascular health in women (Houston *et al.* 2008); calcium on weight loss, bone

health, and hypertension (Tudor *et al.* 2009); protein on satiety (Westerterp-Plantenga *et al.* 2009); vitamin B6 and B12 on plasma homocysteine levels, which decrease the risk of atherosclerosis (Pfeuffer and Schrezenmeir 2000); and the antioxidant/anticarcinogenic properties of CLA (Pfeuffer and Schrezenmeir 2000). While cheese consumption carries many health benefits, it also contains cholesterol and a high percentage of saturated fatty acids—components commonly associated with an increased risk for various cardiac conditions and numerous other diseases (Ulbricht and Southgate 1991). Thus, there is a market for low-fat cheeses, and these health benefits are recognized by consumer focus groups (Childs and Drake 2009).

Full-fat cheddar cheese must legally contain at least 30.5% (w/w) milkfat (CFR 21 [101.113]), but it is also available in reduced and low-fat versions. The Food and Drug Administration (FDA) regulates the usage of these fat descriptors such that reduced-fat cheese contains at least 25% less fat than the full-fat counterpart, and low-fat cheese contains 3 g of fat or less per reference amount (CFR 21 [101.62b]). Therefore, if the standard cheddar contains ~31% fat (w/w), the counterpart reduced-fat cheese would contain less than 24% fat (w/w), and the counterpart low-fat cheese would contain less than 10% fat (w/w), although 6% fat is the usual US standard (Johnson *et al.* 2009).

While interest in reduced and low-fat cheese may be on the rise, most consumers are unwilling to sacrifice flavor or texture in reduced-fat cheese, regardless of potential health benefits or price reductions (Childs and Drake 2009). Unfortunately, numerous studies have documented that reduced and low-fat cheeses differ markedly in flavor

(Drake and Swanson 1995; Rodríguez 1998; Mistry 2001; Banks 2004; Johnson *et al.* 2009). For instance, low-fat cheeses—particularly the low-moisture varieties—differ from their full-fat counterparts in that they either lack flavor or have atypical, undesirable, and often bitter, cheese flavors (Banks *et al*. 1989; Olson 1990; Milo and Reineccius 1997; Yates and Drake 2007). Reduced-fat cheeses, however, are less susceptible to atypical flavors but are more likely to be weak in flavor. Whetstine *et al.* (2006) found that reduced-fat and full-fat cheeses have the same flavor profiles at any given age point, but the flavors are less pronounced in the reduced-fat versions. Similarly, Fenelon *et al.* (2000) found that characteristic cheddar flavor notes like buttery, creamy, and caramel decrease with decreasing fat content, whereas the appearance of uncharacteristic flavors increases with the decreasing fat content. Lipolysis is a major source of flavor constituents, so less fat in a cheese equates with lower levels of lactones and fatty acids and hence, flavor (Banks *et al* 1989). Furthermore, the approaches for producing lower-fat cheeses may not support flavor development. For instance, one major problem with fat mimetics is that the water-soluble compounds cannot carry key non-polar volatile compounds (Drake and Swanson 1995).

Likewise, numerous studies have documented that reduced and low-fat cheeses differ in texture from their full-fat counterparts (Drake and Swanson 1995; Rodríguez 1998; Mistry 2001; Banks 2004; Johnson *et al.* 2009). Using sensory and large-strain fracture analysis, Gwartney *et al*. (2002) found that commercial reduced-fat Monterey

Jack, reduced-fat mild cheddar, reduced-fat sharp cheddar, and processed American cheese all had significantly higher scores than their full-fat counterparts when evaluated for springiness, hardness, fracturability, waxiness, chewiness, and fracture stress. In contrast, all full-fat cheeses were higher in cohesiveness, stickiness, smoothness, and meltability. Thus, both natural and processed cheeses showed similar differences in their reduced-fat versions. Similar results were obtained with reduced-fat Gouda (Yates and Drake 2007) and cheddar cheese manufactured in a pilot plant (Rogers *et al.* 2009). These differences are critical because consumers are sensitive to differences in cheese texture and use texture as a primary means of differentiating cheeses (Jack *et al*. 1993a) and determining liking (McEwan *et al.* 1989; Guinard and Mazzucchelli 1996).

In addition to flavor and texture issues, low-fat cheeses have functionality issues and do not yield the same results as full-fat cheeses when used as an ingredient. For example, reduced-fat mozzarella cheeses exhibit reduced melting capacity (Fife *et al.* 1996) but are more likely to brown, darken, or even burn during heating because there is less lipid at the surface of the mozzarella shreds (Rudan and Barbano 1998). Lower-fat cheeses also tend to be more translucent, gummier, and/or more rubbery (Johnson *et al.* 2009).

Nevertheless, Wiersum (2004) claimed changes in cheese production technology and the advent of new adjunct cultures to reduce bitter compounds have improved reduced and low-fat cheeses. Thus, an updated review of commercial cheeses available in both full and reduced/low-fat varieties is warranted. Recent research on cheddar

cheese texture was conducted using "typical" (i.e. production methods commonly used in current cheddar cheese production) cheeses produced under carefully controlled settings in university or industrial pilot plants (Halmos *et al.* 2003; Pollard *et al.* 2003; Everard *et al*. 2006; *Rogers et al. 2010*; Rogers *et al.* 2009), but such research does not reflect what consumers are experiencing from cheeses purchased at local grocery stores. The cheese that consumers purchase off supermarket shelves has been subjected to unknown stresses (such as loads from stacking boxes) and temperature fluctuations during shipping and storage at the supermarket, and the exact age of the cheese is typically unknown. In one of the most recent studies on the texture of store-bought commercial cheeses, Gwartney *et al.* (2002) used descriptive sensory analysis to map perceived textural characteristics of full-fat and low-fat cheeses. However, this study made limited use of rheological methods and only used torsional fracture to measure fracture stress and strain. Adhikari *et al.* (2003) used instrumental and sensory methods to assess the texture of commercial cheddar, Gouda, and Swiss cheeses varying in fat content and smoky flavor, but that study focused only on large-strain rheological testing (i.e. no small strain or fracture tests). Furthermore, the number of variables and treatments being tested meant few varietal or brand comparisons were made. For instance, only two reduced/low-fat cheeses were tested—one Swiss and one cheddar.

Therefore, the objective of this study was to measure textural properties of full and reduced/low-fat cheddar cheeses currently available in supermarket grocery stores by using small-strain, large-strain, and fracture rheological tests. Three different brands

were tested, and multiple lots were compared from each brand in order to assess lot-to-lot variation.

4. MATERIALS AND METHODS

4.1. Cheese

Cheeses for this study were purchased from local (Raleigh, NC) Food Lion and Harris Teeter grocery stores and stored at 4C. Only those commercial cheeses available in both full-fat and reduced-fat or low-fat varieties were tested. Specifically, these cheeses included Food Lion (store brand) sharp cheddar cheese, reduced-fat Food Lion sharp cheddar cheese made from 2% milk, Cracker Barrel (Kraft) Natural extra sharp cheese, reduced-fat Cracker Barrel (Kraft) Natural extra sharp cheese made from 2% milk, "Racers Edge" (Cabot) sharp Vermont-style cheddar cheese, 50% reduced-fat Cabot sharp Vermont-style cheddar cheese, and 75% reduced-fat Cabot sharp Vermont-style cheddar cheese (Table 2.1). Two lots of each treatment were purchased, as determined by the lot number printed on the packaging; for all tests, an equal number of samples were removed from each lot/block and averaged together to report treatment results. The term "block" refers to a single lot throughout the rest of this paper. After opening the package, cheeses were wrapped in aluminum foil, sealed in a Ziploc bag, and refrigerated at 4C; cheeses were tested within five days of opening.

4.2. Small Strain Rheological Tests

A Stresstech controlled-stress rheometer (ATS Rheosystems; Bordentown, NJ) fitted with a 20-mm smooth, parallel plate geometry was used to determine viscoelastic properties through stress sweeps, frequency sweeps, and creep/recovery tests. For each test, cheese samples, 4-mm thick, were trimmed to the size of the upper plate and glued to both plates with Loctite 401 cyanoacrylate glue (Loctite Corp.; Rocky Hill, CT) to prevent sample slip during testing. A thin layer of lubricant (SuperLube, Synco Chemical; Bohemia, NY) was applied to any exposed cheese edges to prevent moisture loss.

4.2.1. Stress Sweeps

Stress sweeps were conducted in order to determine the linear viscoelastic region (LVR). At least two samples were removed from each block, so a total of 4^{+} samples were tested per treatment. Stress sweeps were conducted at 25C from 1 to 2000 Pa at 10 Hz (62.8 rad/s); the temperature was regulated using a clamshell oven that was attached to the rheometer and whose two halves closed around the plate area. The LVR was identified by the plateau region of the dynamic viscoelastic function G*, the complex modulus. The critical stress and strain values were identified as the point when G* values decreased 1% from the constant plateau value.

4.2.2. Frequency Sweeps

Frequency sweeps were conducted from 0.001 to 10 Hz (0.006 to 62.8 rad/s) at 150 Pa, which was determined to be within the LVR for all samples (Fig. 2.1). The

frequency sweep was repeated on a single sample at 10, 15, 20, 25, and again at 10C. Due to time constraints, one frequency sweep was conducted per cheese treatment.

4.2.3. Creep/recovery Tests

Creep/recovery tests were conducted at 100 Pa (within the LVR) and 500 Pa (near the end of the LVR) on different samples. Based on the method by Rogers *et al*. (2009), loads were applied for 200 s and then removed such that the sample was allowed to recover for an additional 200 s. Tests were conducted in duplicate at each load value, but only one lot was tested. The maximum compliance (J_{max}) reached before the load was removed and the maximum recovery (J_r) obtained after the load was removed were recorded from each test. Percent creep recovery (crp) was calculated using the equation from Brown *et al.* (2003).

$$crp = \frac{J_{max} - J_r}{J_{max}} \times 100 \quad \text{(Eq. 2.1)}$$

$$J_r = J_{max} - J_{min} \quad \text{(Eq. 2.2)}$$

where J_{max} was reached after 200 s of creep, and J_{min} was reached after 200 s of recovery.

4.3. Large Strain Rheological Tests

A one-cycle compression test was performed to determine the structural changes of cheese at deformations beyond the linear viscoelastic region and prior to fracture; the method was adapted from that of Rogers *et al.* (2010) and van den Berg *et al.* (2008). Each cheese block was sealed in plastic storage bags to prevent moisture loss and allowed to equilibrate to room temperature (23 ± 2C) for 12 h. Cheese cylinders were removed from each lot/block using a 15.6 mm diameter cork borer and cut to a length of 17 mm; at

least four samples were tested per cheese treatment. Samples were removed from the interior of the block to account for any moisture loss at the block edge. Each cheese cylinder was uniaxially compressed by 20% of the initial height (i.e. from 17 mm to 13.6 mm), corresponding to a true strain (ε_H) of 0.18. Compression was conducted using an Instron 5565 universal testing machine (Norwood, MA) and flat plates coated with mineral oil to prevent friction. Moving at a rate of 50 mm/min, the top plate compressed the cheese cylinder until the target strain was reached and then subsequently reversed direction at the same rate to allow for recovery. The area under the resultant force-deformation curve was calculated using Simpson's Rule. Percent recoverable energy (RE) was then calculated as a ratio of the area under the second half of the curve (a_2, work recovered from decompression) over the first half of the curve (a_1, work to compress).

$$RE = \frac{a_2}{a_1} \times 100 \quad \text{(Eq. 2.3)}$$

4.4. Fracture Analysis

Fracture analysis was conducted using the torsion method adapted from Brown *et al.* (2003) as written by Rogers *et al.* (2009). Briefly, cylinders were removed from each cheese block, which had been equilibrated to room temperature (23 ± 2C). Cylinders were trimmed, glued to plastic, notched disks (Gel Consultants; Raleigh, NC) at each end, and ground into a capstan shape via a precision grinding machine (Model GCPM92 US, Gel Consultants; Raleigh, NC). Each sample (at least 5 per treatment) was then twisted at a strain rate of 0.405 s^{-1} on a Haake VT-550 rotational viscometer (Gerbruder

Haake GmbH; Karlsruhe, Germany) that was fitted with an attachment designed to facilitate torsion testing (Truong and Daubert 2000). True shear fracture stress (fracture stress) and true shear fracture strain (fracture strain) were calculated according to the equations by Nadai (1937), Diehl and Hamann (1979), and Hamann (1983) as written in Rogers *et al*. (2009). "True shear fracture strain" is a nonlinear strain measure (used for large-strain tests) that is dependent upon the final length, curvature, and angle of deformation of the fractured specimen (Nadai 1937; Diehl and Hamann 1979).

4.5. Statistical Analysis

All statistical analysis was conducted using SAS statistical software (v.9.2, SAS Institute Inc., Cary, NC). Sensory data was analyzed using two-way analysis of variance followed by Tukey's test to look at effects of brand, fat content, and combined interactions.

5. RESULTS AND DISCUSSION

5.1. Statistical Analysis

Main effects (fat content and brand) and interactions for rheological results are presented in Table 2.2. Few interactions existed; most differences between treatments were attributed to either fat content or differences in manufacture. Many of the measurements were characterized by large standard error that obscured significant differences in some cases. Such standard error likely reflects variability in the off-the-shelf cheeses.

5.2. Observations of Treatments

Cabot cheese samples were more difficult to prepare for testing. Regardless of fat content or lot, this brand appeared to have more nicks, holes, and hairline fractures throughout the entire block than the other two brands. These defects were probably due to mechanical processing. Samples with visible defects were discarded and replaced, which means that multiple 8-oz. blocks of cheese from the same lot were required to sufficiently sample this brand.

5.3. Small Strain Rheological Tests

5.3.1. Stress Sweeps

Critical stress and strain values were identified from 1% deviations of the LVR. Larger critical stress and strain values signify the sample being tested can withstand greater loads and deformations, respectively, before the material structure is damaged at the nano or micro-scale and linear relationships between applied stress and strain cease to exist. Larger critical stress and strain values also indicate a longer LVR, which is characteristic of polymeric gels with flexible, entropic chains and strain-hardening behavior versus particulate gels (Dickinson and Chen 1999). Particulate gels and filled gels exhibit short linear regions and strain-weakening behavior and are consistent with enthalpic behavior under large deformations. Critical stress (Fig. 2.1) decreased as fat content increased, indicating that FF samples show initial yielding at smaller forces than their RF counterparts. Critical strain tended to decrease as fat content increased—Cabot 75% LF being the only exception—indicating that FF samples are less deformable than

their RF counterparts. Filler particles are known for decreasing critical strain because they serve as stress concentration factors (Sala *et al.* 2009). Values for critical stress and strain trends are consistent with models for filled gels (Rogers *et al.* 2010).

5.3.2. Mechanical Spectra

Fig. 2.3 shows the mechanical spectra from frequency sweep analysis of FF Food Lion cheese at varying 10 to 25C. These trends are representative of those observed in all of the samples. Such frequency sweeps give insight on the network viscoelasticity. All cheese treatments softened as temperature increased, as evidenced by the decreasing storage modulus (G') and increasing phase angle (δ). Such softening is caused by both changes in lipid structure and changes within the protein network. Milk fat crystals melt as temperature increases, thereby decreasing the mass of the solid crystalline phase and making the cheese less solid-like. Hydrogen bonding in the protein network decreases as temperature increases, which contributes to softening, evidenced by the decreasing G'. The contributions of hydrogen bonding and crystalline phase are evidenced by the larger gap between G' values at 15C and 20C (Table 2.3). The size of the gap varied between treatments, but the gap was consistently larger in FF treatments. Milkfat has a wide compositional range (one-third is unsaturated fat; the other two-thirds are fatty acids from C4:0 to C18:0) and consequently melts over a wide range from approximately -30C to +40C (Walstra 2003). The cheese phase angles were consistently below 25° with only mild increases with temperature, indicating that the cheese softened as the temperature increased. A sharp increase in phase angle would imply that the cheese fully melted

(Lucey *et al.* 2003), but that was not observed. The phase angle can vary between 0 and 90° whereby 0° represents full energy recovery and corresponds to a perfectly elastic material, and 90° corresponds to a perfectly viscous material.

The effects of melting milkfat were also observed in the dissimilarities of the mechanical spectra determined initially at 10C then after cooling to 10C. As per the method for the frequency sweeps, temperatures were increased, and samples were allowed to equilibrate at each new temperature for five minutes in a sealed oven chamber on the rheometer; testing ended with a cool-down period and final frequency sweep at 10C. The 10C curves obtained after the cool-down did not overlay the initial 10C obtained as temperatures increased (Fig. 2.3). These results are consistent with melting of the fat crystalline phase; the equilibration period was probably enough time for hydrogen bonds within the protein network to reform given that the energy associated with such bonds is only 5 kJ/mol regardless of environmental polarity (van der Spoel *et al.* 2006), but not enough time for phase changes or re-crystallization of the fat phase. Total fat content affected the storage modulus values (Fig. 2.4). These results are consistent with the filled gel theory if milkfat is considered an active filler that reinforces the rigidity of the protein composite network. Regardless of temperature and frequency, G' increased with fat content, although the degree of the increase lessened with increasing temperature. Some previous studies found that the difference in G' amongst fat levels was negligible at temperatures around 25C (Visser 1991; Rogers *et al.* 2010), but complete liquidation of the fat crystals is not expected at those temperatures based on the

aforementioned melting range of milkfat. Although bovine milk does differ in triglyceride composition depending on seasonal, dietary, and species variability (Pitas *et al.* 1967; Zegarska and Jaworski1981), such variability is minimal. Thus, the difference in decreasing G' to a single value that was observed by Visser (1991) and Rogers *et al.* (2010) was more likely due to differences in the protein network and its surface interactions with the entrapped milkfat. For instance, total calcium, insoluble calcium, nitrogen fractions, water-binding capacity, and manufacture pH were all observed to have significant effects on "melting" (including flow of casein network) of model Raclette cheese (Froehlich-Wyder *et al.* 2009). Nevertheless, the full-fat treatments were expected to decrease in G' with rising temperature more rapidly than their reduced-fat counterparts because of the contributions of melting/softening milkfat. Table 2.4 shows that melting effects appeared to brand-dependant at low frequencies, although it is unknown whether those brand effects were due to processing conditions or storage and thermal history. At high frequencies, however, FF treatments clearly exhibited greater melting effects, as evidenced by consistently steeper slopes.

5.3.3. Creep/recovery Tests

Although numerous parameters can be derived from creep/recovery tests, this study measured J_{max}, J_r, and %crp because these terms have been shown to have at least some weak correlation to sensory terms (Brown *et al.* 2003). The long load time (200 s) in the creep test provided a greater sensitivity to slow relaxing viscous effects (Steffe 1996). The maximum deformation at constant load (J_{max}) tended to increase as fat

content decreased, with Cabot 75% LF being the only exception (Fig. 2.5), although that treatment anomaly was also observed in the critical strain values. This means that RF cheeses deform more under a constant load than their FF counterparts, as was expected based on Fig. 2.4. These results were consistent with those of Rogers *et al.* 2009. Applying a stress of 500 Pa produced greater deformation than a load of 100 Pa (Fig. 2.5), as expected. The positive correlation between G* and $1/J_{max}$ at both loads is depicted in Fig. 2.6. Although the correlation is low—probably due to the difference in test time—there is clearly a trend in increasing firmness. Furthermore, both regression lines have the expected similar slopes, given that testing was within the LVR.

J_r depends on J_{max}, and both terms are accounted for in the %crp calculation (Eq. 2.1). Neither J_r nor %crp (Fig. 2.7) differentiated treatments based on fat content, and these results agree with those of Rogers *et al.* (2009).

5.4. Large Strain Rheological Tests

No sample barreling was observed during compression testing, which indicates that frictional effects were minimal and the application of stress and strain was evenly applied throughout the sample (Culioli and Sherman 1976; Carter and Sherman 1978). Fig. 2.8 shows the recoverable energy of cheeses after being compressed 20% of their initial height. This measurement was highly significant (Table 2.2) and has been shown to differentiate gels of variable texture and microstructure because it elucidates whether any part of the network is not recovering. van den Berg *et al.* (2008) found that percent recoverable energy differentiated whey protein isolate/polysaccharide mixed gels on the

basis of filler volume and type, and that energy loss correlated with sensory crumbliness. Rogers *et al.* (20010) found percent recoverable energy correlated with sensory texture and the fat content of cheese, with full-fat cheeses exhibiting greater energy loss. From a structural standpoint, crumbliness likely relates to energy dissipation through viscous or internal frictional components because fat globules are large inhomogeneities in the casein network (van Vliet and Walstra 1995). Thus, reduced and low-fat commercial cheeses were shown to be more cohesive and elastic than full-fat cheeses.

5.5. Fracture Analysis

The force-deformation curve produced by torsional fracture of one Cabot 75% LF sample is shown in Fig. 2.9 and reflects trends seen in all of the samples. At small strains, the sample responds linearly. After a critical stress and strain, the sample becomes damaged at the nano or micro-level and behaves non-linearly. As structural damage increases, the sample eventually begins to fracture and crack. That fracture point is denoted by the fracture stress and strain, which correspond to the y and x-values, respectively, of the curve maxima. All tested samples exhibited strain-weakening (Fig. 2.11).

Torsion testing reflects the force (stress) and deformation (strain) required to break a sample into two pieces; these critical values can be used to develop a texture map comparing multiple samples. Fig. 2.10 shows that fracture stress did differ amongst brands but not fat content. Fracture strain, however, was statistically different between LF/RF and FF treatments. RF treatments withstood greater strain before fracturing,

indicating that these materials are more rubbery, and these results echo those observed at small strains and published literature values (Gwartney *et al.* 2002; Rogers *et al.* 2009, 2010). The Cabot 75% LF treatment did not follow the trend of the other RF treatments, however. Using torsional fracture and four commercial natural cheeses, Gwartney *et al.* (2002) found that fracture stress and strain tended to be greater in RF treatments, although the results were only significantly different for one-half of the tested cheeses. However, Rogers *et al.* (2009, 2010) found that torsional fracture stress and strain are dependent upon cheese age, which was uncontrolled in this study.

Torsion testing has successfully been used to assess textural properties of many food gels (Hamann *et al.* 2006). Large strain testing revealed the greatest variability within and between treatment lots (Fig. 2.11), although all treatments exhibited strain-weakening. For example, mechanical nicks in the sample may be caused during processing; such nicks will weaken the sample, causing it to fail sooner. If the nick only weakens the sample, then force-deformation up to fracture remains the same and the resultant data will fall along a straight line with constant shear modulus. However, cracks or imperfections at the micro-scale are stress concentration factors and would likely affect the mechanism of fracture and hence, fracture stress and strain. Curd lines also cause variability in cheese fracture, however, because the curd is often as large as the surface area being tested, and so fracture lines typically occur along the curd lines. Some samples exhibited pronounced particulation along the fracture line, whereas fracture lines were characterized by only slight "roughness" or curd bumps. Such particulation may

explain the deviation seen in some of the cheese force-deformation curves (the difference between lots for the same fat content in Fig. 2.11A, for instance). In contrast, Brown *et al.* (2003) did not note such particulation when using torsion gelometry to measure fracture properties of Monterey Jack or mozzarella cheeses, neither of which are made using the cheddaring process of pressing curds.

Therefore, more research is needed to ascertain the mechanisms behind cheddar cheese fracture, including the mechanism for fracture initiation. While most cheese varieties are subject to the effects of age and protein concentration (Brown *et al*. 2003), we hypothesize that fracture in cheddar cheese is also strongly affected by the pressed curd particles that arise from cheddaring. These curd particles presumably vary in height and spacing. As mentioned above, some samples exhibited pronounced particulation along the curd line; fracture was not smooth. Therefore, the fracture and strain values obtained at failure through torsion testing on cheddar cheeses should be related to the macroscopic geometry of the cheese particles in order to better understand cheese texture. Cheese particle geometry could be studied by imaging the fracture line along the broken surface of the capstan samples in order to measure particle height and spacing—essentially mimicking current studies on surface of road asphalt/concrete. Small punch testing, which drives a probe into a plane of sample to record stress-deformation changes up until fracture, could also be used to elucidate the effects of curd particle orientation (Lacalle *et al.* 2008).

6. CONCLUSION

This study captured an overview of cheeses the average grocery shopper might find and consume. As found in previous studies, reduced/low-fat cheeses were shown to be firmer, more deformable, and more elastic. Full-fat cheeses were weaker, withstood less deformation, and recovered less energy. Given that fracture stress and elastic recovery increase with protein content (Foegeding 1992; Jack *et al.* 1993b; Bowland and Foegeding 1999; Li *et al.* 1999), these results may simply reflect a higher percent of protein in the reduced-fat cheeses. Furthermore, reducing fat content of cheese means that the protein network is more densely packed, which allows for more interactions between protein molecules and strongly impacts texture (Bryant *et al.* 1995; Mistry 2001).

The Cabot 75% LF treatment consistently deviated from trends seen amongst the other reduced-fat cheeses. This likely reflects a compositional difference because the LF treatment was known to contain cornstarch and monoglyceride (Table 2.1), ingredients not typically found in natural cheese. The filled gel model was mentioned as a means of describing composite network rigidity. Milkfat serves the role of "filler" in cheese, but swollen cornstarch granules are also filler particles. In order to avoid overly firm cheese from the addition of cornstarch granules and high protein concentration, monoglyceride was probably added to weaken the protein network.This means commercial cheese mechanical spectra cannot simply be attributed to fat content; all processing conditions (pH, age, moisture, etc.) and ingredients must be considered.

Many measurements of this study were associated with large standard errors and a lack of statistical differences, which reflects the lack of control in these off-the-shelf grocery store cheeses since the age, pH, thermal history, stress history, etc. were unknown at the time of testing. It is important for researchers and manufacturers to understand the products currently available to consumers, but testing off-the-shelf cheeses incorporates too many variables to truly understand the mechanics of the cheese system. For this reason, additional testing of commercial cheeses produced under controlled conditions is needed.

7. REFERENCES

ADHIKARI, K., HEYMANN, H. and HUFF, H.E. 2003. Textural characteristics of lowfat, fullfat and smoked cheeses: sensory and instrumental approaches. Food Qual. Prefer. *14*, 211-218.

BANKS, J.M., BRECHANY, E.Y. and CHRISTIE, W.W. 1989. The production of low fat cheddar-type cheese. J. Soc. Dairy Technol. *42*, 6-9.

BANKS, J.M. 2004. The technology of low-fat cheese manufacture. Int. J. Dairy Technol. *57*, 199-207.

BOWLAND, E.L. and FOEGEDING, E.A. 1999. Factors determining large-strain (fracture) rheological properties of model processed cheese. J. Dairy Sci. *82*, 1851-1859.

BROWN, J.A., FOEGEDING, E.A., DAUBERT, C.R., DRAKE, M.A. and GUMPERTZ, M. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. *86*, 3054-3067.

BROWNSEY, G.J., ELLIS, H.S., RIDOUT, M.J. and RING, S.G. 1987. Elasticity and failure in composite gels. J. Rheol. *31*, 635-649.

BRYANT, A. USTUNOL, Z. and STEFFE, J. 1995. Texture of cheddar cheese as influenced by fat reduction. J.Food Sci. *60*, 1216-1219.

CARTER, E.J.V. and SHERMAN, P. 1978. Evaluation of firmness of Leicester cheese by compression tests with Instron universal testing machine. J. Texture Stud. *9*, 311-324.

CHILDS, J.L. and DRAKE, M.A. 2009. Consumer perception of fat reduction in cheese. J. Sens. Stud. *24*, 902-921.

CULIOLI, J. and SHERMAN, P. 1976. Evaluation of gouda cheese firmness by compression tests. J. Texture Stud. *7*, 353-372.

DICKINSON, E. and CHEN, J. 1999. Heat-set whey protein emulsions gels: role of active and inactive filler particles. J. Dispersion Sci. Technol. *20*, 197-213.

DIEHL, K.C. and HAMANN, D.D. 1979. Structural failure in selected raw fruits and vegetables. J. Texture Stud. *10*, 371-400.

DRAKE, M.A. and SWANSON, B.G. 1995. Reduced- and low-fat cheese technology: a review. Trends Food Sci. Technol. *6*, 366-369.

DRAKE, M. A., TRUONG, V.D. and DAUBERT, C.R. 1999. Rheological and sensory properties of reduced-fat processed cheeses containing lecithin. J. Food Sci. *64*, 744–747.

EVERARD, C.D., O'CALLAGHAN, D.J., HOWARD, T.V., O'DONNELL, C.P., SHEEHAN, E.M. and DELAHUNTY, C.M. 2006. Relationships between sensory and rheological measurements of texture in maturing commercial cheddar cheese over a range of moisture and ph at the point of manufacture. J. Texture Stud. *37*, 361-382.

FENELON, M.A., O'CONNOR, P. and GUINEE, T.P. 2000. The effect of fat content on the microbiology and proteolysis in cheddar cheese during ripening. J. Dairy Sci. *83*, 2173-2183.

FIFE, R.L., MCMAHON, D.J. and OBERG, C.J. 1996. Functionality of low-fat mozzarella cheese. J. Dairy Sci. *79*, 1903-1910.

FOEGEDING, E.A. 1992. Rheological properties of whey protein isolate gels determined by torsional fracture and stress relaxation. J. Texture Stud. *23*, 337-348.

FROEHLICH-WYDER, M.T., GUGGISBERG, D. and WECHSLER, D. 2009. Influence of low calcium and low pH on melting characteristics of model Raclette cheese. Dairy Sci. Technol. *89*, 463-483.

GOULD, B. 2010. Understanding dairy markets: your source for market information and price risk management principles.

<http://future.aae.wisc.edu/data/monthly_values/by_area/154?tab=production&year=2009>.

GUINARD, J.X. and MAZZUCCHELLI, R. 1996. The sensory perception of texture and mouthfeel. Trends Food Sci. Technol. *7*, 213-219.

GWARTNEY, E.A., FOEGEDING, E.A. and LARICK, D.K. 2002. The texture of commercial full-fat and reduced-fat cheese. J. Food Sci. *67*, 812-816.

HALMOS, A.L., POLLARD, A., SHERKAT, F. and SEURET, M.G. 2003. Natural cheddar cheese texture variation as a result of milk seasonality. J. Texture Stud. *34*, 21-40.

HAMANN, D.D. 1983. Structural failure in solid foods. In *Physical Properties of Foods* (M. Peleg and E.B. Bagley, eds.) pp. 351-383, AVI Pub. Co., Westport, CT.

HAMANN, D.D., ZHANG, J.H., DAUBERT, C.R., FOEGEDING, E.A. and DIEHL, K.C. 2006. Analysis of compression, tension and torsion for testing food gel fracture properties. J. Texture Stud. *37*, 620-639.

HOUSTON, D.K., DRIVER, K.E., BUSH, A.J. and KRITCHEVSKY, S.B. 2008. The association between cheese consumption and cardiovascular risk factors among adults. J. Hum. Nutr. Diet. *21*, 129-140.

JACK, F.R., PIGGOTT, J.R. and PATERSON, A. 1993a. Discrimination of texture and appearance in cheddar cheese using consumer free choice profiling. J. Sens. Stud. *8*, 167-176.

JACK, F.R., PATERSON, A. and PIGGOTT, J.R. 1993b. Relationships between rheology and composition of cheddar cheeses and texture as perceived by consumers. Int. J. Food Sci. Technol. *28*, 293-302.

JOHNSON, M.E., KAPOOR, R., MCMAHON, D.J., MCCOY, D.R. and NARASIMMON, R.G. 2009. Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. Compr. Rev. Food Sci. Food Saf. *8*, 252-268.

LACALLE, R., ÁLVAREZ, A. and GUTIÉRREZ-SOLANA, F. 2008. Analysis of key factors for the interpretation of small punch test results. Fatigue Fract. Mater. Engng. Struct. *31*, 841-849.

LI, H., ERRINGTON, A.D. and FOEGEDING, E.A. 1999. Isostrength comparison of large-strain (fracture) rheological properties of egg white and whey protein gels. J. Food Sci. *64*, 893-898.

LUCEY, J.A., JOHNSON, M.E. and HORNE, D.S. 2003. Perspectives on the basis of rheology and texture of cheese. J. Dairy Sci. *86*, 2725-2743.

MCEWAN, J.A., MOORE, J.D. and COLWILL, J.S. 1989. The sensory characteristics of cheddar cheese and their relationship with acceptability. J. Soc. Dairy Technol. *42*, 112-117.

MILO, C. and REINECCIUS, G.A. 1997. Identification and quantification of potent odorants in regular fat and low-fat mild cheddar cheeses. J. Agric. Food Chem. *45*, 3590-3594.

MINTEL INTERNATIONAL GROUP LTD. 2009. Mintel Oxygen report on *Cheese – US – May 2009*. Mintel Publications, London.

MINTEL INTERNATIONAL GROUP LTD. 2010. Mintel Oxygen report on *Cheese – US – May 2010*. Mintel Publications, London.

MISTRY, V.V. 2001. Low fat cheese technology. Int. Dairy J. *11*, 413-422.

NADAI, A. 1937. Plastic behavior of metals in the strain-hardening range. Part I. J. Appl. Physics *8*, 205-213.

OLSON, N.F. 1990. The impact of lactic acid bacteria on cheese flavor. FEMS Microbiol. Rev. *87*, 131-148.

PFEUFFER, M. and SCHREZENMEIR, J. 2000. Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. Br. J. Nutr. *84*, S155 -S159.

PITAS, R.E., SAMPUGNA, J. and JENSEN, R.G. 1967. Triglyceride structure of cows' milk fat .I. Preliminary observations on fatty acid composition of positions 1,2, and 3. J. Dairy Sci. *50*, 1332-1336.

POLLARD, A., SHERKAT, F., SEURET, M.G. and HALMOS, A.L. 2003. Textural changes of natural cheddar cheese during the maturation process. J. Food Sci. *68*, 2011-2016.

RODRÍGUEZ, J. 1998. Recent advances in the development of low-fat cheeses. Trends Food Sci. Technol. *9*, 249-254.

ROGERS, N.R., DRAKE, M.A., DAUBERT, C.R., MCMAHON, D.J., BLETCH, T.K. and FOEGEDING, E.A. 2009. The effect of aging on low-fat, reduced-fat, and full-fat cheddar cheese texture. J. Dairy Sci. *92*, 4756-4772.

ROGERS, N.R., MCMAHON, D.J., DAUBERT, C.R., BERRY, T.K. and FOEGEDING, E.A. 2010. Rheological properties and microstructure of cheddar cheese made with different fat contents. J. Dairy Sci. *in press*.

RUDAN, M.A. and BARBANO, D.M. 1998. A model of mozzarella cheese melting and browning during pizza baking. J. Dairy Sci. *81*, 2312-2319.

SALA, G., VAN VLIET, T., STUART, M.C., VAN DE VELDE, F. and VAN AKEN, G.A. 2009. Deformation and fracture of emulsion-filled gels: effect of gelling agent concentration and oil droplet size. Food Hydrocolloids *23*, 1853-1863.

STEFFE, J.F. 1996. *Rheological Methods in Food Process Engineering*. Freeman Press, East Lansing, MI.

TRUONG, V.D. and DAUBERT, C.R. 2000. Comparative study on large strain methods in assessing failure characteristics of selected food gels. J. Texture Stud. *31*, 335-353.

TUDOR, M., HAVRANEK, J. and SERAFINI, M. 2009. Dairy foods and body weight management. Mljekarstvo *59*, 88-95.

ULBRICHT, T.L.V. and SOUTHGATE, D.A.T. 1991. Coronary heart disease: seven dietary factors. Lancet *338*, 985-992.

VAN DEN BERG, L., CAROLAS, A.L., VAN VLIET, T., VAN DER LINDEN, E., VAN BOEKEL, M.A.J.S. and VAN DE VELDE, F. 2008. Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. Food Hydrocolloids *22*, 1404-1417.

VAN DER SPOEL, D., VAN MAAREN, P.J., LARSSON, P. and TIMNEANU, N. 2006. Thermodynamics of hydrogen bonding in hydrophilic and hydrophobic media. J. Phys. Chem. B. *110*, 4393-4398.

VAN VLIET, T. and WALSTRA, P. 1995. Large deformation and fracture behavior of gels. Faraday Discuss. *101*, 359-370.

VISSER, J. 1991. Factors affecting the rheological and fracture properties of hard and semi-hard cheese. In *Rheological and Fracture Properties of Cheese* (P. Walstra, ed.) pp. 49-61, Bull. No. 268, International Dairy Federation, Brussels, Belgium.

WALSTRA, P. 2003. *Physical Chemistry of Foods*. Marcel Dekker, Inc., New York, NY.

WESTERTERP-PLANTENGA, M.S., NIEUWENHUIZEN, A., TOMÉ, D., SOENEN, S. and WESTERTERP, K.R. 2009. Dietary protein, weight loss, and weight maintenance. Annu. Rev. Nutr. *29*, 21-41.

WHETSTINE, M.E.C., DRAKE, M.A., NELSON, B.K. and BARBANO, D. 2006. Flavor profiles of full-fat and reduced-fat cheese and cheese fat made from aged cheddar with the fat removed using a novel process. J. Dairy Sci. *89*, 505-517.

WIERSUM, T. 2004. A culture for low-fat cheese. Dairy Foods *November*, 34.

YATES, M.D. and DRAKE, M.A. 2007. Texture properties of gouda cheese. J. Sens. Stud. *22*, 493-506.

ZEGARSKA, Z. and JAWORSKI, J. 1981. Effect of lactation period and seasonal feeding system on quantitative triglyceride composition of milkfat. Milchwissenschaft *36*, 396-398.

TABLE 2.1. COMMERCIAL CHEDDAR CHEESE

Cheddar Cheese Product	**Code**	**% Fat[1]**	**% Fat Reduction**
Food Lion (store brand) Sharp full-fat	Food Lion FF	32	
Food Lion (store brand) Sharp reduced-fat	Food Lion RF	21	34
Cracker Barrel Natural Extra Sharp full-fat	Cracker Barrel FF	36	
Cracker Barrel Natural Extra Sharp reduced-fat	Cracker Barrel RF	21	42
Cabot "Racers Edge" Sharp Vermont-style	Cabot FF	32	
Cabot Sharp 50% reduced-fat	Cabot 50% RF	16	50
Cabot Sharp 75% reduced-fat[2]	Cabot 75% LF[3]	9	75

[1] The percent fat is the amount listed on the package label
[2] According to package label, the product contains cornstarch and monoglyceride, ingredients not normally found in cheddar cheese
[3] Cheese products containing < 3g of fat per reference amount can legally be called "low fat" (CFR 21 [101.62b])

TABLE 2.2. MAIN FACTOR EFFECTS (FAT CONTENT AND BRAND) AND INTERACTIONS (FAT*BRAND) FOR RHEOLOGICAL ATTRIBUTES OF COMMERCIAL CHEDDAR CHEESE

	Critical stress	Critical Strain	J_{max} (100 Pa)	J_r (100 Pa)	%crp (100 Pa)	J_{max} (500 Pa)	J_r (500 Pa)	%crp (500 Pa)	%RE	Fracture stress	Fracture strain
Fat	**0.011**	**<0.001**	**0.028**	0.053	0.103	**0.004**	**0.023**	0.124	**<0.001**	0.617	**0.026**
Brand	0.253	**0.033**	0.280	**0.023**	0.600	**0.033**	0.246	0.820	**<0.001**	**0.007**	0.077
Fat * Brand	0.747	0.289	0.170	**<0.001**	**0.039**	**0.010**	**0.047**	0.107	0.060	0.940	0.173

P-values < 0.05 were bolded in table and considered significant

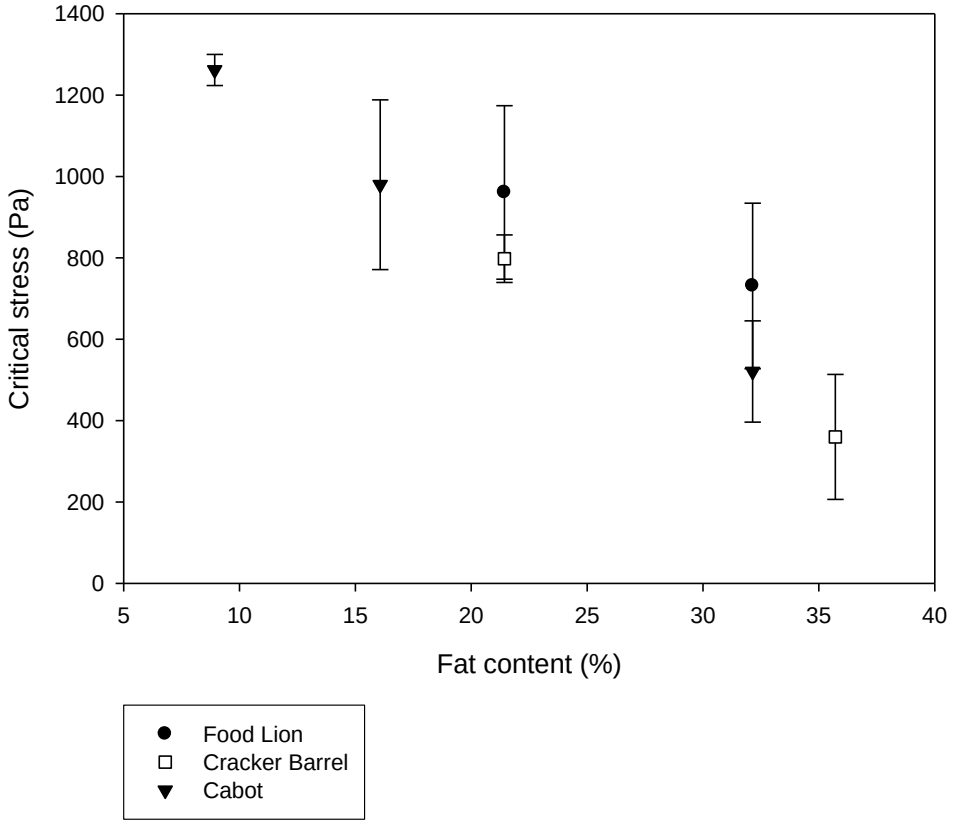


FIG. 2.1. CRITICAL STRESS OF COMMERCIAL CHEDDAR CHEESES
Error bars represent the standard error of the mean.

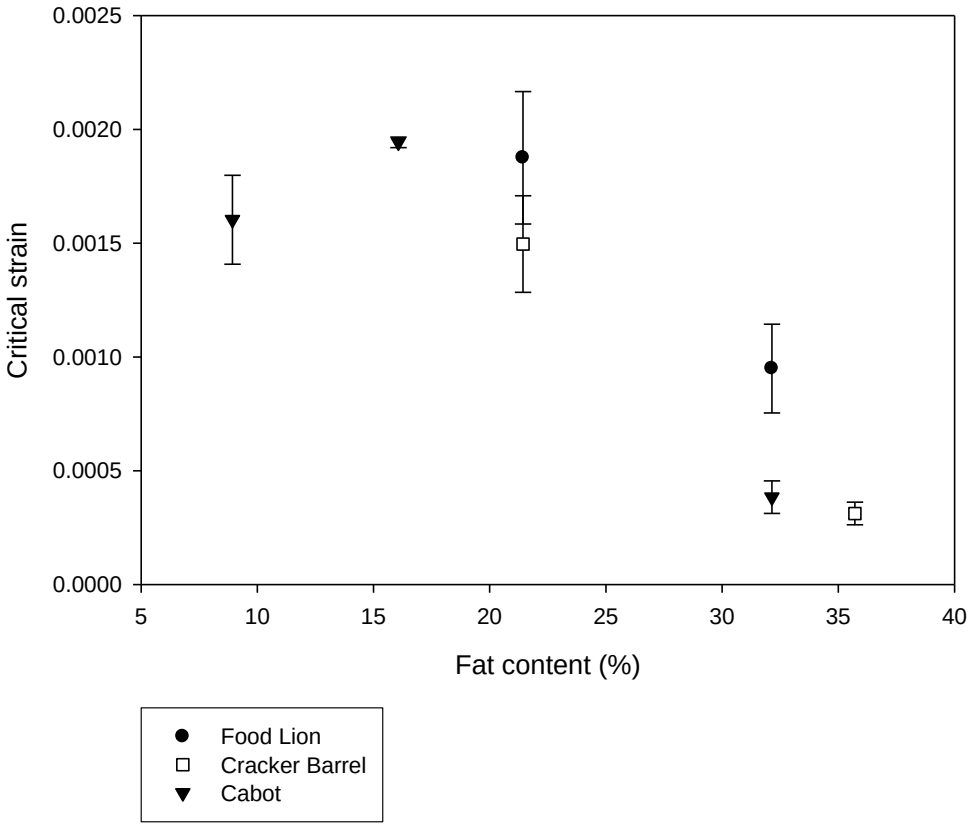


FIG. 2.2. CRITICAL STRAIN OF COMMERCIAL CHEDDAR CHEESES
Error bars represent the standard error of the mean.

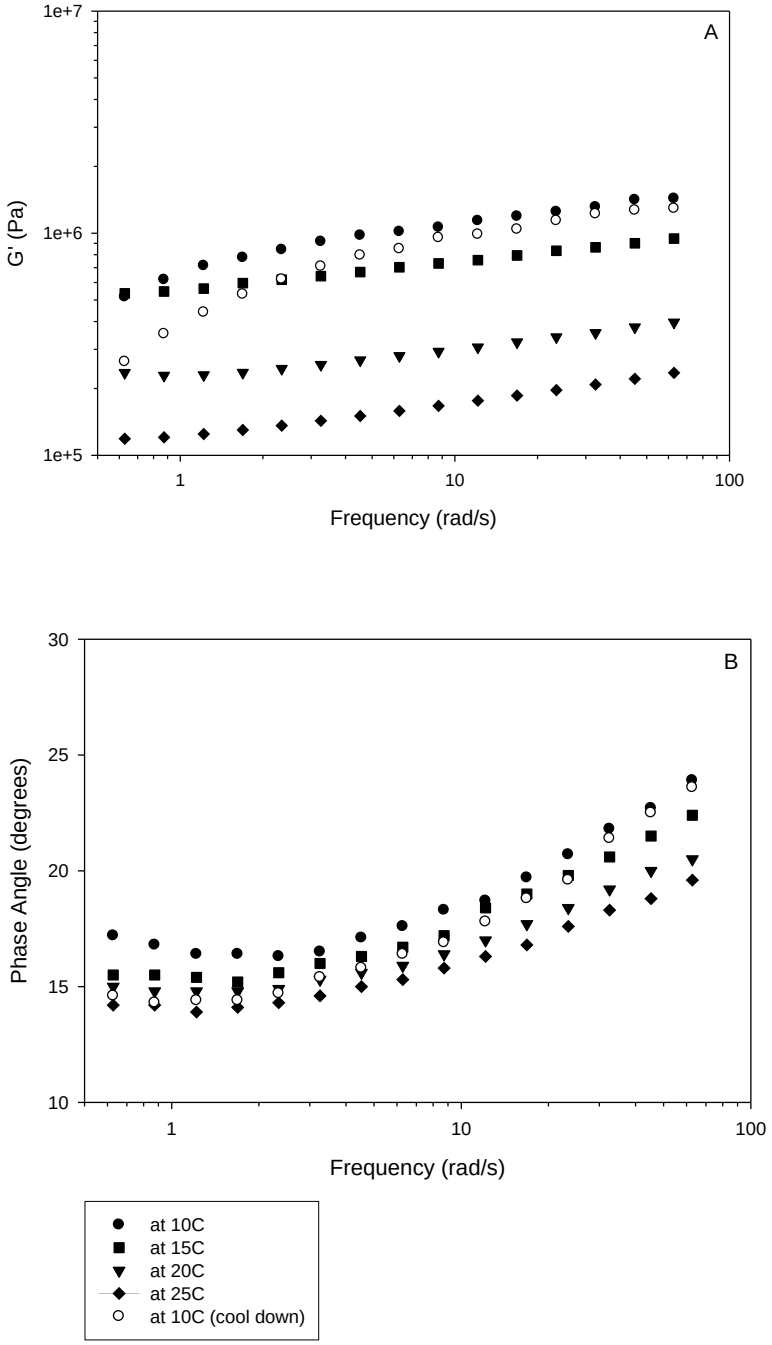


FIG. 2.3. TYPICAL RELATIONSHIP BETWEEN FREQUENCY, TEMPERATURE,STORAGE MODULUS (G') (A), AND PHASE ANGLE (B) Numerical data from testing one sample of full-fat Food Lion cheese.

TABLE 2.3. DIFFERENCE IN STORAGE MODULI OF COMMERCIAL CHEDDAR CHEESES AT A FREQUENCY OF 62.8 RAD/S

Cheese		**$\Delta G'_{15C-20C}$ (kPa)**
Food Lion	FF	548
	RF	226
Cracker Barrel	FF	914
	RF	222
Cabot	FF	797
	50% RF	190
	75% LF	162

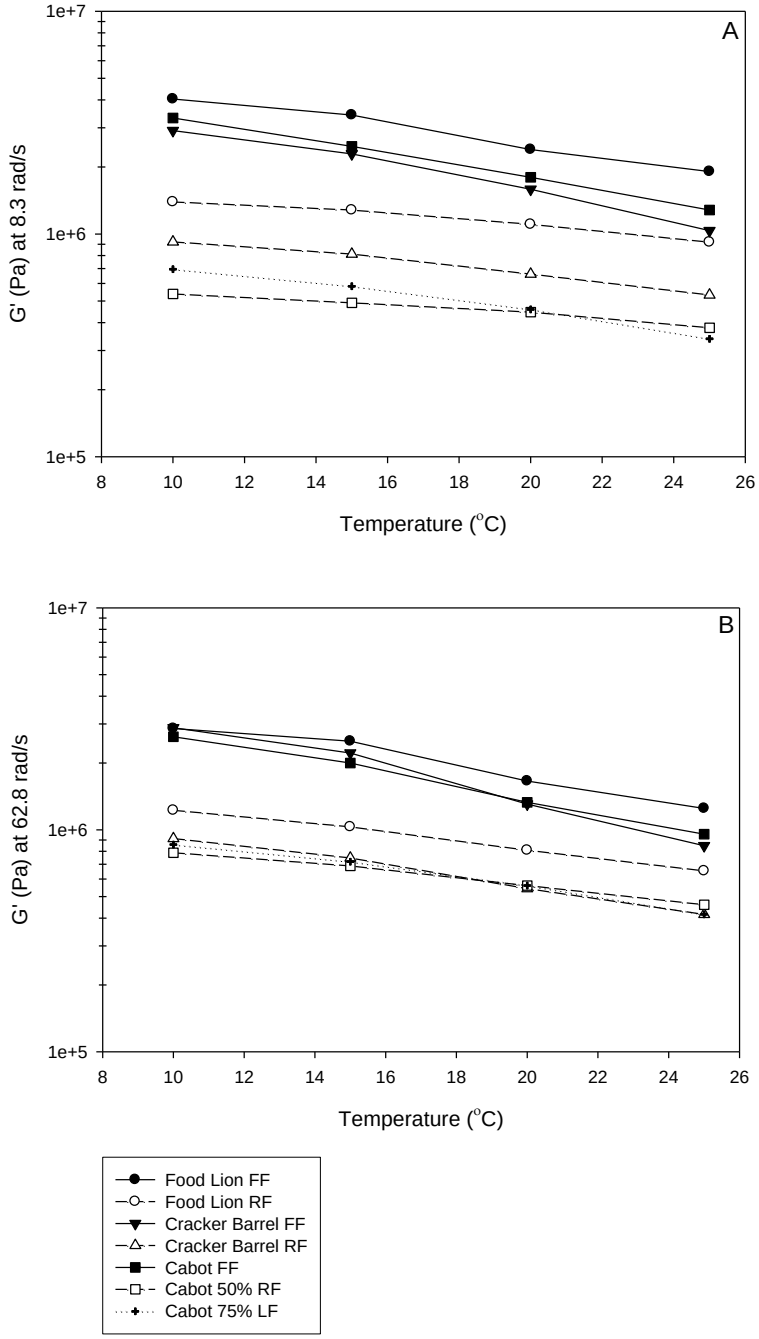


FIG. 2.4. EFFECT OF TEMPERATURE AND FAT CONTENT ON COMMERCIAL CHEESE STORAGE MODULI AT TWO FREQUENCIES (8.3 (A) AND 62.8 (B) RAD/S) FROM FREQUENCY SWEEPS

TABLE 2.4. LINEAR REGRESSION OF STORAGE MODULI VS. TEMPERATURE FOR COMMERCIAL CHEESE OF VARYING FAT CONTENT

Treatment	Slope (kPa/°C) at frequency 8.3 rad/s	Slope (kPa/°C) at frequency 62.8 rad/s
Food Lion FF	-148	-138
Cracker Barrel FF	-127	-140
Cabot FF	-136	-114
Food Lion RF	-32	-39
Cracker Barrel RF	-264	-34
Cabot 50% RF	-10	-22
Cabot 75% LF	-234	-30

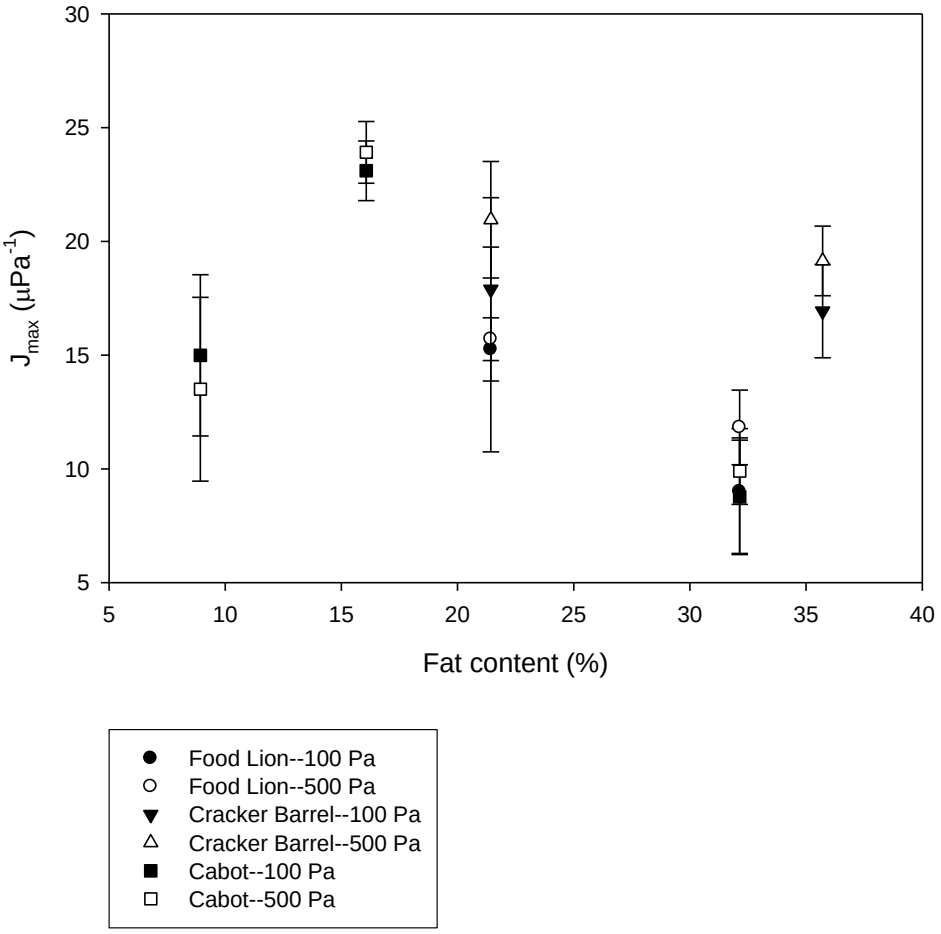


FIG. 2.5. MAXIMUM COMPLIANCE (J_{MAX}) OF COMMERCIAL CHEDDAR CHEESES DURING CREEP TESTING AT LOADS OF 100 Pa AND 500 Pa
Stress loads were within linear viscoelastic region (see Fig. 2.1) and were applied for 200 s. Error bars represent the standard error of the mean.

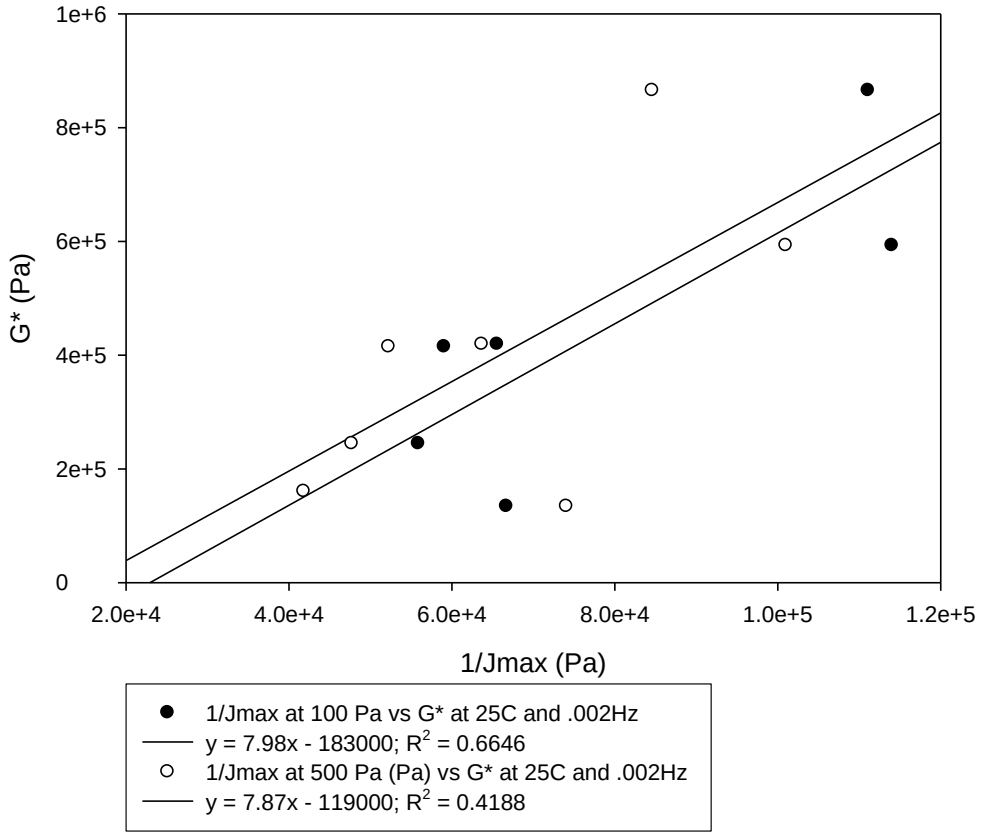


FIG. 2.6. CORRELATION BETWEEN CREEP AND FREQUENCY SWEEPS
G* refers to data point at slowest frequency, 0.013 rad/s.

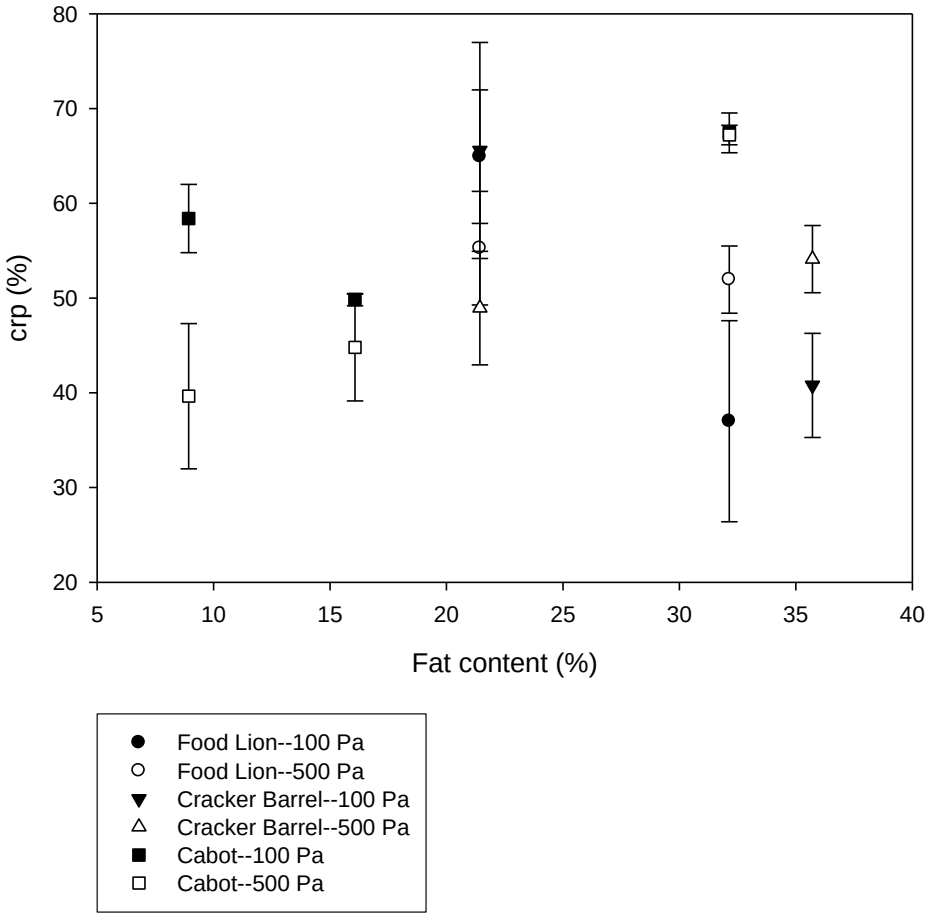


FIG. 2.7. PERCENT CREEP RECOVERY (crp) OF COMMERCIAL CHEDDAR CHEESES DURING CREEP TESTING AT LOADS OF 100 Pa (FILLED SYMBOLS, SOLID LINES) AND 500 Pa (OPEN SYMBOLS, DASHED LINES)
Stress loads were within linear viscoelastic region (see Fig. 2-1) and were applied for 200 s; recovery period lasted 200 s. Error bars represent the standard error of the mean.

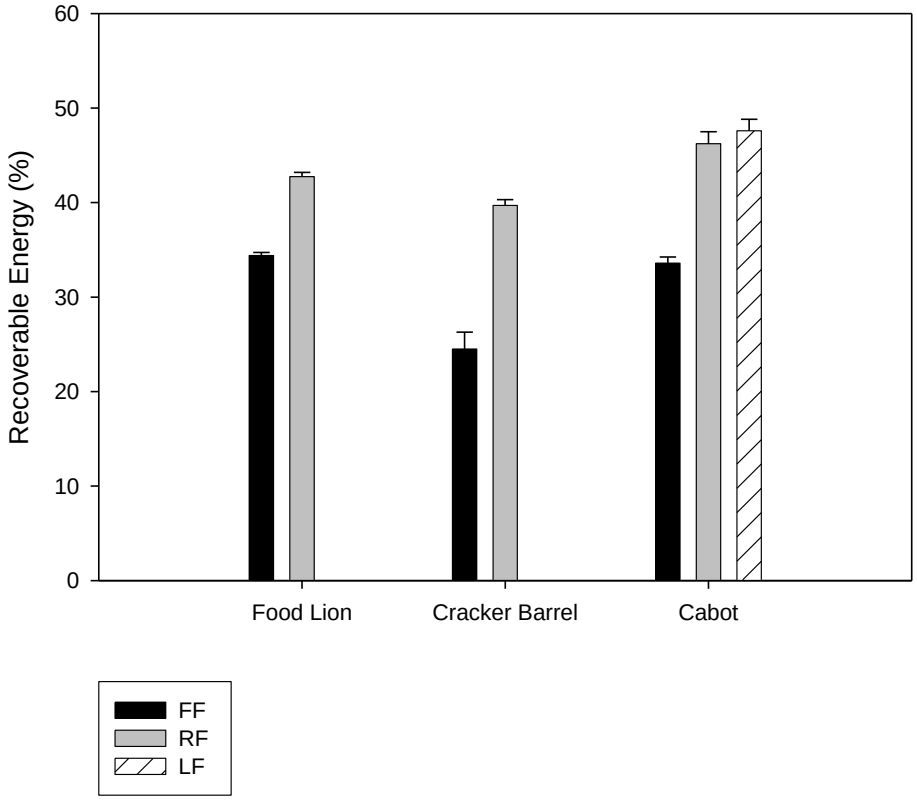


FIG. 2.8. PERCENT RECOVERABLE ENERGY OF COMMERCIAL CHEDDAR CHEESES COMPRESSED TO 80% OF INITIAL HEIGHT DURING ONE-CYCLE, UNIAXIAL COMPRESSION TEST
Error bars represent the standard error of the mean.

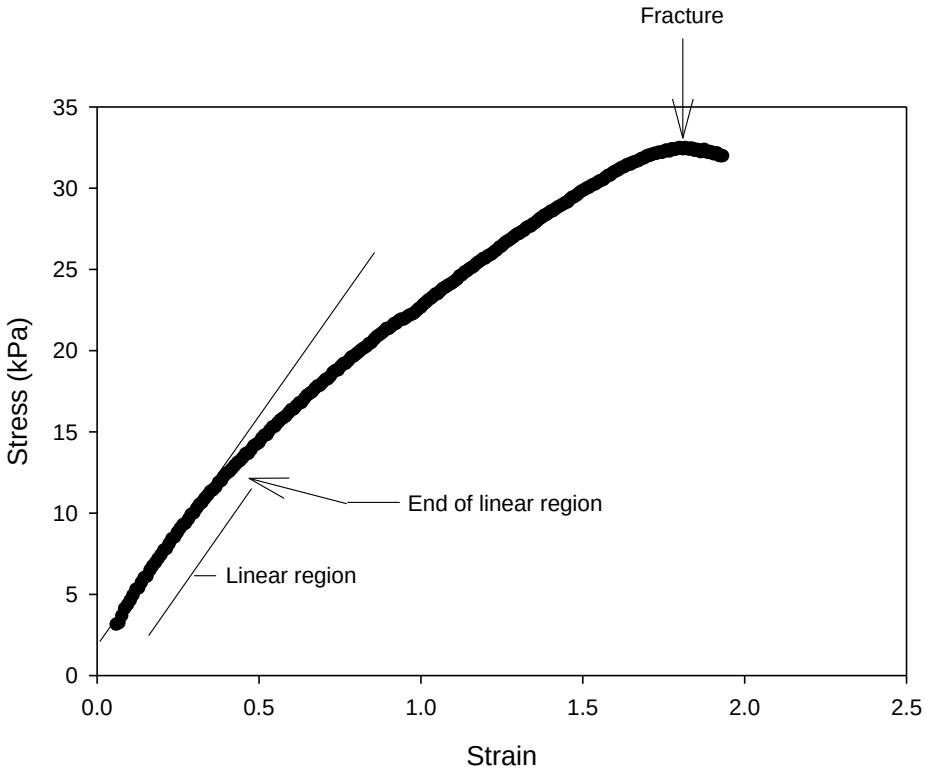


FIG. 2.9. TYPICAL STRESS-STRAIN CURVE RESULTING FROM TORSIONAL FRACTURE OF CHEESE
Numerical data from testing one sample of Cabot 75% low-fat cheese.

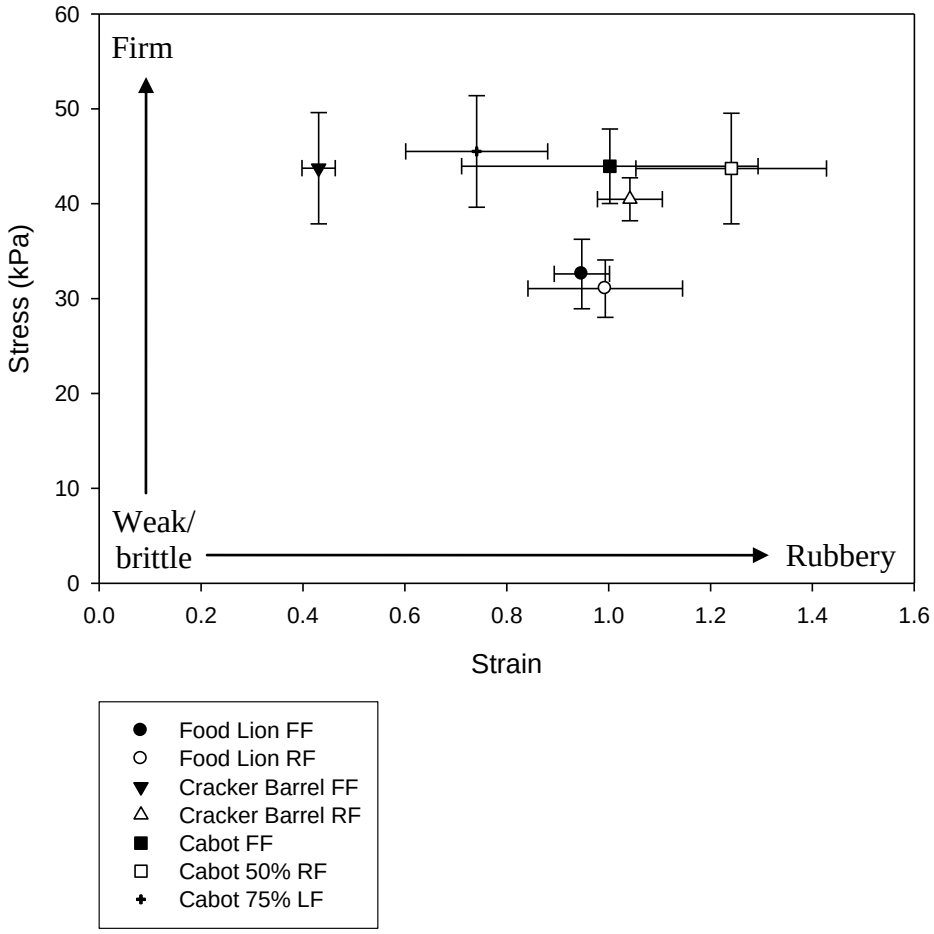


FIG. 2.10. TEXTURE MAP BASED ON TORSION FRACTURE GELOMETRY
Error bars represent the standard error of the mean.

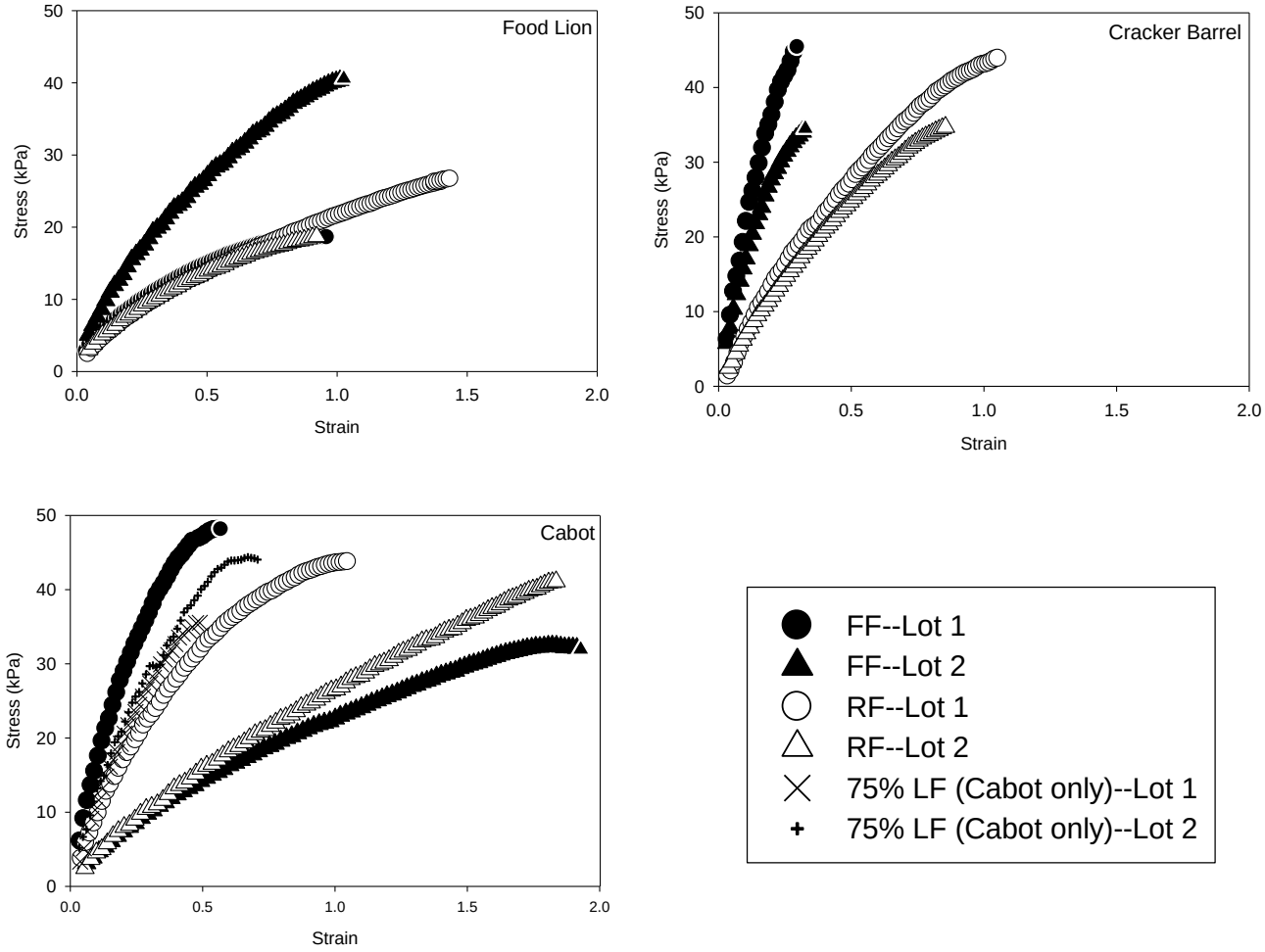


FIG. 2.11. COMPARISON BETWEEN PRODUCTION LOTS OF STRESS-STRAIN CURVES BY BRAND
One of every eight data points shown for simplicity.

Chapter 3

Effect of Sample Size on Sensory Texture of Cheddar Cheese

1. ABSTRACT

The project goal was to test the effect of thickness on textural evaluation of Cheddar cheese at three different fat levels using both descriptive sensory analysis and consumer testing. Rheological and fracture properties of each cheese were obtained using standard rheological methods, including stress sweeps creep/recovery tests, uniaxial compression tests, torsional fracture at three rates, and single edge notched bend (SENB) tests. Rheological tests corroborated those results observed in Chapter 2, and the addition of SENB testing showed fracture energy and toughness did not differ significantly amongst notched treatments. Sample thickness significantly impacted descriptive analysis panelists' perception of texture using both hand and first-bite attributes but did not affect chewdown attributes or consumers' evaluation of texture. Consumers consistently preferred thicker samples, regardless of fat content. The variation in sample mass amongst treatments did not affect descriptive analysis of chewdown attributes but may explain why consumers consistently preferred thicker treatments.

Keywords: reduced-fat cheese; texture; thickness; rheology; fracture

2. PRACTICAL APPLICATION

Cheddar cheese is a widely consumed food product in the U.S. Consumers are increasingly interested in reduced and low-fat products, but reduced/low-fat cheeses have historically been criticized for overly firm, elastic textures. This research characterized

the texture of cheddar cheese at three different fat contents using both rheology and descriptive sensory analysis and investigated whether differences in sample preparation (i.e. thickness) affected consumer liking scores of reduced/low-fat cheese. Such research provides manufacturers with another approach for improving the popularity of reduced/low-fat cheese.

3. INTRODUCTION

Chapter 2 introduced the importance of cheese as a major economic sector within US markets and demonstrated the variability between particular brands and even individual lots of cheddar cheese obtained directly off grocery store shelves. The filled gel model was shown to describe aspects of cheddar cheese rheology because cheese firmness will depend on temperature and this is related to the physical state of the milkfat (Zhou and Mulvaney 1998; Rogers *et al.* 2010). Likewise, a decreased critical strain and fracture strain with increased filler volume is consistent with a filled gel model because filler particles act as stress concentration factors (Sala *et al.* 2009). Deformability, firmness, toughness, springiness, and elasticity generally increased as fat content decreased (Chapter 2). This fat effect was constant across all brands despite the other, numerous, variables in commercial cheese production, including different brand production methods, age, and potential temperature abuse or applied stresses during shipping and storage. Commercial cheese is available in a variety of thicknesses—everything from blocks that allow consumers to choose their own thickness to thin deli

slices to pre-sliced cracker cuts and cubes—and yet few studies have tested for the effects of sample thickness on sensory perception of texture. Numerous studies documented the effect of sample thickness in a variety of foods on processing conditions such as drying rate or extent of Maillard browning. Potato chip thickness has been positively correlated to sensory crispness and crunch acoustics (Salvador *et al.* 2009). For cheese, the effect of thickness has been related to meltability (Wang and Sun 2002) and oiling off (Wang and Sun 2004) of natural cheddar and mozzarella cheese, with thicker slices exhibiting greater meltability and oiling off than thinner slices.

While many studies examined the effects of sample thickness on processing conditions or measured sample thickness/height as an effect of changing some ingredient/processing variable, very few—regardless of food type—have considered the impact of sample dimensions on descriptive and/or consumer sensory panels. Of those that have been published, many relate to meat thickness and the impact on sensory. For example, Boles and Shand (2008) found that thickness of beef stir fry strips did not impact perception of tenderness, and Elsner *et al.* (1999) found that sensory attributes of chicken thigh scaloppini were not affected by thickness, but Liu and Berry (1998) found that thick low-fat beef patties were rated more tender and juicier than patties that were only 0.32 cm thinner. Few sensory studies have been published beyond meat literature, and none on cheese of any variety. A consumer sensory study on oat muesli found that preference for oat thickness is strongly linked to consumer age and cook time (Kalviainen *et al.* 2002), while Herrera-Corredor *et al.* (2004) found that thickness of rolled corn

tortillas was not a predictor of consumer overall acceptance or purchase intent. However, none of these studies considered the effect of thickness on consumer perception of texture.

Consumers are sensitive to differences in cheese texture and use texture as a primary means of differentiating cheeses (Jack *et al*. 1993a) and determining liking (McEwan *et al.* 1989; Guinard and Mazzucchelli 1996). Most consumers are unwilling to sacrifice flavor or texture in reduced-fat cheese, regardless of potential health benefits or price reductions (Childs and Drake 2009). Unfortunately, numerous studies have documented that reduced and low-fat cheeses differ in texture from their full-fat counterparts (Drake and Swanson 1995; Rodríguez 1998; Mistry 2001; Gwartney *et al.* 2002; Banks 2004; Johnson *et al.* 2009).

Therefore, the goal of this project was to test the effect of thickness on one brand of commercial cheese at three different fat levels using both descriptive sensory analysis and consumer testing to determine if sample dimensions affect perception of sample texture. A mechanical texture fingerprint of cheeses was created using rheological and fracture tests. These are material properties that should be invariant with size or shape. Sensory texture was determined on samples with the same surface area but different levels of thickness (therefore also varying sample mass) using both descriptive sensory analysis and consumer panels. Based on published literature documenting consumers' dislike of low-fat cheeses, we hypothesized that decreasing sample thickness would

improve consumers' liking scores of low-fat cheeses but would not affect their liking scores of full-fat cheese.

4. MATERIALS AND METHODS

4.1. Cheese

Low-fat (LF), reduced-fat (RF) and full-fat (FF) Vermont-style white cheddar cheese (Table 3.1) were obtained in 40-lb blocks directly from the manufacturer (Cabot; Montpelier, VT); one lot was tested per fat/treatment level. Cheeses were stored at 4C. All rheological tests and descriptive sensory analysis were conducted at 12 weeks of age; consumer panels were conducted at 14 weeks of age. After opening the package, cheeses were sealed in closeable storage bags to prevent moisture loss.

4.2. Rheological and Mechanical Tests

4.2.1. Controlled-stress Tests

A Stresstech controlled-stress rheometer (ATS Rheosystems; Bordentown, NJ) fitted with a 20-mm smooth, parallel plate geometry was used to determine viscoelastic properties through stress sweeps and creep/recovery tests. For each test, cheese samples, 4-mm thick, were trimmed to the size of the upper plate and glued to both plates with Loctite 401 cyanoacrylate glue (Loctite Corp.; Rocky Hill, CT) to prevent sample slip during testing. A thin layer of lubricant (SuperLube, Synco Chemical; Bohemia, NY) was applied to any exposed cheese edges to prevent moisture loss.

4.2.1.1. Stress Sweeps

Stress sweeps were conducted in order to determine the linear viscoelastic region (LVR). Three samples were tested per treatment. Stress sweeps were conducted at 25C from 1 to 2000 Pa at 10 Hz; the temperature was regulated using a clamshell oven that was attached to the rheometer and whose two halves closed around the plate area. The LVR was identified by the plateau region of the dynamic viscoelastic function G*, the complex modulus. The critical stress and strain values were identified as the point when G* values decreased 1% from the constant plateau value.

4.2.1.2. Creep/recovery Tests

Creep/recovery tests were conducted at 100 Pa (within the LVR), 500 Pa (within the LVR), and 2200 Pa (beyond the LVR) on different samples. Based on the method by Rogers *et al*. (2009), loads were applied for 200 s and then removed such that the sample was allowed to recover for an additional 200 s. Tests were conducted in triplicate at each load value for each treatment. The maximum compliance (J_{max}) reached before the load was removed and the maximum recovery (J_r) obtained after the load was removed were recorded from each test. Percent creep recovery (crp) was calculated using the equation from Brown *et al.* (2003).

$$crp = \frac{J_{max} - J_r}{J_{max}} \times 100 \quad \text{(Eq. 3.1)}$$

$$J_r = J_{max} - J_{min} \quad \text{(Eq. 3.2)}$$

where J_{max} is achieved after 200 s of creep, and J_{min} is achieved after 200 s of recovery.

4.2.2. Large Strain Rheological Tests

A one-cycle compression test was performed to determine the structural changes of cheese at deformations beyond the linear viscoelastic region and prior to fracture; the method was adapted from that of Rogers *et al.* (2010) and van den Berg *et al.* (2008). Cheese was sealed in plastic storage bags to prevent moisture loss and allowed to equilibrate to room temperature (22 ± 2C) for 12 h. Six cheese cylinders were removed per treatment using a 15.6 mm diameter cork borer and cut to a length of 17 mm. Samples were removed from the interior of the block to account for any moisture loss at the block edge. Each cheese cylinder was uniaxially compressed by 20% of the initial height (i.e. from 17 mm to 13.6 mm), corresponding to a true strain (ε_H) of 0.18. Compression was conducted using an Instron 5565 universal testing machine (Norwood, MA) and flat plates coated with mineral oil to prevent friction. Moving at a rate of 50 mm/min, the top plate compressed the cheese cylinder until the target strain was reached and then subsequently reversed direction at the same rate to allow for recovery. The area under the resultant force-deformation curve was calculated using Simpson's Rule. Percent recoverable energy (RE) was then calculated as a ratio of the area under the second half of the curve (a_2, work recovered from decompression) over the first half of the curve (a_1, work to compress).

$$RE = \frac{a_2}{a_1} \times 100 \qquad \text{(Eq. 3.3)}$$

4.2.3. Fracture Analysis

4.2.3.1. Torsion Gelometry

Fracture analysis was conducted using the torsion method adapted from Brown *et al.* (2003) as described by Rogers *et al*. (2009). Briefly, cylinders were removed from each cheese that had been equilibrated to room temperature (22 ± 2C). Cylinders were trimmed, glued to plastic, notched disks (Gel Consultants; Raleigh, NC) at each end, and ground into a capstan shape via a precision grinding machine (Model GCPM92 US, Gel Consultants; Raleigh, NC). Each sample was then twisted at a strain rate of either 4.1, 0.41, or 0.041 s^{-1} on a Haake VT-550 rotational viscometer (Gerbruder Haake GmbH; Karlsruhe, Germany) that was fitted with an attachment designed to facilitate torsion testing (Truong and Daubert 2000); five samples were tested at each rate per treatment. The angle of fracture was determined using a protractor. True shear fracture stress (fracture stress) and true shear fracture strain were calculated according to the equations by Nadai (1937), Diehl and Hamann (1979), and Hamann (1983) as written in Rogers *et al*. (2009). "True shear fracture strain" is a nonlinear strain measure used for large-strain tests that is dependent upon the final length, curvature, and angle of deformation of the fractured specimen (Nadai 1937; Diehl and Hamann 1979); it shall simply be referred to as "fracture strain" throughout the rest of this paper.

4.2.3.2. Single Edge Notched Bending

The fracture energy and fracture toughness were calculated using a single edge notched bend (SENB) test (a.k.a three point bend), modified from that described by

Williams and Cawood (1990) by scaling up all dimensions by 1.5. Sample dimensions and set up are depicted in Fig. 3.1, although the dimensions of every individual sample were measured with calipers before testing. The notch size was kept constant by putting tape on a razor blade such that only 5.4 mm of the blade was exposed. The notch to depth ratio was ~0.45 for each sample. Cheeses were equilibrated to room temperature (22 ± 2C) and tested both with and without notches, six replicates each per treatment. Each sample was deformed using a load cell of 50N and a probe that was 3.12 mm wide at a crosshead speed of 0.2 mm/s until the sample fractured completely. Fracture toughness, K_c, was determined for notched samples based on Williams and Cawood (1990):

$$K_c = f(A) * \frac{P}{BH^{0.5}} \quad \text{(Eq. 3.4)}$$

$$f(A) = 6A^{0.5}[1.99 - A(1-A)(2.15 - 3.93A) + \frac{2.7A^2}{(1+2A)(1-A)}] \quad \text{(Eq. 3.5)}$$

where a = notch depth (m); H = height (m); B = breadth (m); and P = max load (N). "A" is the notch to depth ratio (A = a/H) and is unitless. Note that in these equations, the term "height" is synonymous with what Williams and Cawood (1990) refer to as "width." The terms were switched to better match the visual setup as depicted in Fig. 3.1. Fracture energy, G_c, was determined for notched samples based on Williams and Cawood (1990):

(Eq. 3.6)

where U = area (units of J) under the $G_c = \frac{U}{BH\Phi}$ ıation curve between zero and max load force, P, and Φ = calibration factor for A, the notch-to-depth ratio. In this case, the ratio was ~0.45, and so Φ=0.26 (Williams and Cawood 1990). The value of U was also used

to calculate work to fracture for both notched and non-notched samples (Everard *et al.* 2007).

4.3. Sensory Testing

4.3.1. Descriptive Sensory Analysis

Cheddar cheese was cut into 20 mm x 20 mm x 20 mm cubes, which corresponds to the average commercial cheese cube dimensions. The height of the cubes was sequentially decreased to 14 mm, 8 mm, and 2 mm, with the latter corresponding to the thickness of an average deli slice of cheese. Six samples of each of the 12 treatments were placed in lidded soufflé cups labeled with three-digit codes. Treatments were given to panelists at room temperature (22 ± 2˚C), and each panelist evaluated every treatment in triplicate using a randomized balanced design.

All sensory testing was conducted in accordance with the North Carolina State University Institutional Review Board (IRB) for Human Subjects guidelines. Evaluation of cheese texture was conducted using a descriptive sensory panel (six panelists, all females aged 30-56 y) trained on the Spectrum Method (Meilgaard et al. 1999; Drake and Civille 2003) and an established cheese texture lexicon (Brown et al. 2003) with a few modifications (Table 3.2). Specifically, *hand springiness* was changed from a 30 to 20% compression in order to better match rheological compression. Panelists were given rulers with a mark corresponding to 80% of the original height so the level of compression was similar among panelists (Barrangou *et al.* 2006). Also, *hand hardness* was defined as the amount of force required to fracture the sample, and *fracturability* was

defined as the number of fragments produced. Each term was rated on a 15-point scale as described by Brown *et al.* (2003), and mozzarella cheese (Harris Teeter; Matthews, NC) cubes were used as references, although each attribute scale also included other cheese types as anchors (shown in Fig. 3.7). Each panelist had more than 1000 h of descriptive analysis experience on various products, with 250 h of texture-specific experience.

4.3.2. Consumer Panel

Consumers (n = 107) were recruited through university listserves and paper advertisements to determine how sample thickness affects consumer liking/preference; per IRB guidelines, all participating consumers signed written forms of consent before participating. Consumers were presented with nine cheese samples based on descriptive analysis results suggesting which treatments were most different from each other. Specifically, LF, RF, and FF samples were presented in thicknesses of 2, 8, and 14 mm at room temperature (22 ± 2˚C) in soufflé cups labeled with three-digit codes; two pieces of sample were provided per treatment. Only one treatment (level of fat) was presented at a time. The order of thicknesses within each fat group was randomized, and fat groups themselves were presented in random order. Consumers were forewarned that they would be asked about overall preference after every third sample. Upon receiving a treatment, consumers were first asked about overall liking in order to gauge their initial acceptance of the product before directing them to consider specific attributes like flavor or texture. Consumes were next asked about flavor and texture liking; all questions were based on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). Red grapes

and deionized water at room temperature were provided for palate cleansing between samples (Drake *et al.* 1995), and all participants received a $5 gift card as compensation. Evaluations were conducted in individual sensory booths using Compusense *five* version 4.8 (Compusense Inc.; Guelph, Ontario) software for data collection.

4.4. Statistical Analysis

All statistical analysis was conducted using SAS statistical software (v.9.2, SAS Institute Inc.; Cary, NC), and significant effects were evaluated at the $\alpha = 0.05$ level. Data was analyzed using one-way (stress and uniaxial compression tests) or two-way analysis of variance (all other tests) followed by Tukey's test. Principal component analysis (PCA) was constructed from descriptive sensory analysis data using statistiXL software (v.1.8, StatistiXL; Broadway-Nedlands, Australia).

5. RESULTS AND DISCUSSION

5.1. Rheological Tests

5.1.1. Controlled-stress Tests

5.1.1.1. Stress Sweeps

Critical stress and strain values were identified from 1% deviations of the LVR. In Chapter 2, critical stress decreased as fat increased, indicating that FF treatments show initial yielding at smaller forces. In this study, critical stress (Table 3.3) tended to increase as with fat content, but the increase was not significant. This may suggest that FF, RF, and LF cheeses of this brand all withstand an equal amount of force before

yielding and experiencing damage at the microscale if samples are received directly from the manufacturer (vs. off the grocery store shelves), or it may have simply been a difference in the tested lots. Critical strain also did not differ significantly with fat content (Table 3.3), although there was a trend that strain decreased as fat increased. That trend was the expected result, given that Sala *et al.* (2009) showed filler particles such as fat act as stress concentration factors and decrease treatment deformability and critical strain. Furthermore, that expected trend was observed for RF and FF samples in Chapter 2 as well as published cheese literature (Rogers *et al.* 2010). However, in Chapter 2, the LF treatment was lower in critical strain than the RF cheese, probably because starch granules (Table 3.1) also act as filler particles and stress-concentration factors. Therefore, the lack of statistical differences likely reflects unknown manufacturing parameters for making these commercial cheeses. For instance, it was unknown if treatments differed by final pH or the use of certain adjunct cultures. Thus, the results of this study are better used for capturing a rheological fingerprint of one brand of commercial cheese rather than extrapolating all results to definitively explain the effect of fat on cheese texture.

5.1.1.2. Creep/recovery Tests

If both cornstarch and milkfat are considered fillers in cheese, then "filled" cheeses (LF and FF) reached a significantly lower critical compliance than RF cheese (Fig. 3.2), which means "filled" cheeses were firmer. Maximum compliance (J_{max}) is a measure of deformation under a certain load over a specific period of time. It is also the

reciprocal value of the complex modulus, G*, which is obtained via small strain oscillatory rheology. Large G* values indicate firmer, more rigid samples. Active fillers reinforce the composite network and increase moduli (Brownsey *et al*. 1987). Thus, the smaller J_{max} of LF and FF treatments suggest these "filled" cheeses may exhibit reinforcing effects at low frequencies, which suggests that the filled gel model may be a good tool for understanding cheese rheology. Off-the-shelf cheeses of Chapter 2 also showed that LF and FF cheese were firmer than RF cheese.

Load did not significantly impact maximum compliance (Fig. 3.2) or crp (Fig. 3.3), even though 2200 Pa was beyond the LVR of any treatment (Table 3.3). Furthermore, crp did not differ significantly with fat/filler content, nor did it differ for the off-the-shelf Cabot cheese (Chapter 2). Crp has been used with mixed success for differentiating cheese treatments in previous cheese studies (Brown *et al.* 2003; Rogers *et al.* 2009). The similarity across fat contents suggests that these cheeses contain a balanced ratio of viscous to elastic effects in the small strain region. However, the recoverably energy, determined at strains beyond the LVR did significantly differentiate cheeses based on fat content (Table 3.3). This suggests that structural elements with faster relaxations (approximately 4 s of compression) are different among the samples.

5.1.2. Large Strain Rheological Tests

No sample barreling was observed during compression testing, which indicates that frictional effects were minimal and the application of stress and strain was evenly applied throughout the sample (Culioli and Sherman 1976; Carter and Sherman 1978).

LF and RF treatments clearly recovered more energy than did the FF treatment (Table 3.3) after samples were compressed to 20% of their initial height. As discussed in Chapter 2, this measurement has been shown to differentiate gels of variable texture and microstructure because it elucidates whether any part of the network is not recovering; the addition of fat particles clearly decreases energy recoverability. This elasticity may be due, in part, to greater protein concentration since elastic recovery has been shown to increase with protein content (Foegeding 1992; Jack *et al.* 1993b; Bowland and Foegeding 1999; Li *et al.* 1999).

5.1.3. Fracture Analysis

5.1.3.1. Torsion Gelometry

Force-deformation curves were generated for each cheese treatment tested at all three rates (Fig. 3.4). Testing at multiple rates is important for understanding the degree of viscoelasticity and time-dependency in a material. Fracture behavior of most foods is rate-dependent (Ross-Murphy and Todd 1983; Zoon *et al.* 1989). Larger fracture stress and strains result because the material undergoes fracture propagation and deformation throughout the entire time energy is being inputted to "unzip" the bonds and structural forces (van Vliet and Walstra 1995). The slope of the force-deformation curve is equal to the material property G, the shear modulus. Stiff materials are characterized by greater moduli (steep slopes) (van Vliet and Walstra 1995). Linear elastic materials exhibit the same slope regardless of rate. Fig. 3.4 shows that FF cheese exhibited a similar initial slope regardless of rate but that fracture stress and strain increased with rate; therefore,

FF cheese can be considered a linear viscoelastic material at low strains. However, RF and LF cheeses were more sensitive to rate effects, particularly at the highest strain rate. (Curves at a rate of 4.1 s^{-1} start at larger stress and strain values, i.e. shifted from the origin, due to a limited rate of data collection; the shift away from the origin was an instrumental setting, not a material property of the cheese.) Other work using EMG and jaw-tracking data in our lab has shown that most people move their jaw at a velocity of 68 mm/s and complete about 1.5 chews per s (Çakir *et al.*) when eating cheddar cheese. Given that chewing and other oral deformations to cheese occur at high rates, this difference in torsion curves may explain why consumers are so adept at differentiating cheese on the basis of texture alone (Jack *et al*. 1993a): at high rates, LF and RF samples are stiffer.

Most samples fractured in tensile mode, with the angle of fracture ranging from 35-60°. At the highest strain rate (4.1 s^{-1}), the FF samples fractured in shear, with the angle of fracture ranging from 0-31°. Tunick and van Hekken (2002) reported that a variety of cheese types fractured in shear at 2.3 s^{-1}. However, no mathematical correlations were obvious and/or consistent amongst fat content, shear rate, and angle of fracture (data not shown). The mode of fracture may reflect the mechanism by which bonds and structural elements within the material separate during fracture. FF cheese was the only sample whose angle of fracture changed with the increased strain rate.

Torsion testing reflects the force (stress) and deformation (strain) required to twist a sample into two pieces; these critical values can be used to develop a texture map

comparing multiple samples. The fracture stress and strain of RF and FF cheese increased, almost linearly, with strain rate. LF cheese increased in fracture stress and strain with strain rate, with 0.41 s^{-1} being the only exception. This difference is also reflected in the large variability seen in Fig. 3.4. LF cheese also contained starch, and some researchers have suggested that starch gels are especially susceptible to rate effects because the sliding of starch granules during fracture affects energy dissipation and thus, fracture stress and strain (Luyten and van Vliet 1995); however, that argument has also been met with skepticism from other starch researchers (Gamonpilas *et al.* 2009). Therefore, it is difficult to conclude whether the results of LF cheese fractured at strain rate 0.41 s^{-1} are merely outliers or reflective of true material differences. Fig. 3.5 also shows that FF and RF cheese exhibit the same stress at the two lowest strain rates, but RF is more deformable and rubbery. Deformability is most likely affected by the dispersal of fat globules, which effectively disrupt the protein network and decrease cohesiveness. Testing of off-the-shelf cheeses (Chapter 2) showed no statistical difference in fracture stress amongst fat treatments, but this study showed that fracture stress of LF cheese was consistently greater than that of FF or RF cheese. These findings support the research of Rogers *et al.* (2009), who found that torsional fracture stress is dependent upon cheese age, which was uncontrolled and unknown in the off-the-shelf study.

5.1.3.2. Single Edge Notched Bending (SENB)

SENB can be used to calculate K_c, critical stress intensity factor at fracture initiation, a.k.a. fracture toughness, and G_c, critical strain energy release rate, a.k.a.

fracture energy per unit area of crack. Large K_c and G_c values imply greater material resistance to crack propagation (Kinloch and Young 1983). SENB assumes that samples exhibit linear elastic deformation. Charalambides *et al*. (1995) used SENB testing to measure fracture properties of cheddar cheese, which exhibited viscoelastic deformation. The authors argued that the test results were still valid for determining fracture energy, G_c, because the calculations depend on the area underneath the force-deformation curves, and they argued that area would differ only slightly for cheese versus a true linear elastic material. In that study, G_c was used to successfully differentiate cheddar cheese on the basis of age such that G_c decreased in sharp cheddar as cheeses aged. Those authors also argued that SENB is advantageous over fracture tests using uniaxial compression, wire-cutting, or wedge propagation tests because SENB is less sensitive to frictional effects between the material and instrument surfaces. Values of G_c in this study were similar to those of published literature. Specifically, Charalambides *et al.* (1995) found that mild cheddar ranged in G_c from 18 to 41 J/m^2, and sharp cheddar ranged in G_c from 13 to 31 J/m^2, although treatments showed significant batch effects.

Single force-deformation curves produced during SENB for one sample of each cheese treatment are depicted in Fig. 3.6. Fracture always initiates at the weakest point in a material, which explains why the notched samples fractured at much lower loads than their non-notched counterparts. The maximum load represents the amount of force required to initiate fracture in the material in notched samples. Five of the six non-notched LF samples, including the sample depicted in Fig. 3.6, bent into a perfect U-

shape without showing any visible fracture. Nevertheless, it is clear from Fig. 3.6 that the samples were subject to some maximum force, which suggests that SENB may be sensitive to weakening or fracture at the micro-scale and/or internal crack growth and deformation.

Notched samples were not significantly different when maximum load and strain were used to compute K_c and G_c (Table 3.4), which indicates that fracture toughness and energy did not differ significantly by fat content. Notches are a weak point in the material. Said weak points facilitate testing and measurements because fracture and crack growth occur in a known location. Fracture initiation in non-notched samples is therefore more variable. Fracture in the non-notched samples of this study typically occurred off-center, just to the side of where the probe was pushing the cheese. Also, non-notched samples were more likely to flip or twist under the probe, and these samples were excluded from the study.

As expected, non-notched samples behaved similarly under SENB and torsion testing: as fat content decreased, the maximum stress and strain increased, which was reflected by an increase in work required to reach the maximum load (Table 3.4). Work was calculated from the area under the force-deformation curve up until the peak load, and it significantly differentiated non-notched samples on the basis of fat content. As predicted by Fig. 3.6, non-notched LF treatments were tougher, requiring more work to reach the max load. Nevertheless, it is important to differentiate the energy required to fracture a sample and create new surfaces from the energy required to simply deform the

sample (Williams and Cawood 1990). This distinction was made by calculating the slope of the force-deformation curves for notched and non-notched samples up until a load of 0.1 N because both curves were relatively linear up until that point. These slope values represent the modulus that characterizes the compressive strain loading on the top of the beam and the tensile strain at the bottom of the sample by the notch. The modulus did not differ significantly between notched and non-notched treatments (Table 3.4), implying that there was an initial deformation of material prior to crack propagation.

It has been noted that the instrumental setup used for SENB in this study differs from that of other studies. Specifically, several SENB studies—albeit three from the same lab group—used rolling cylindrical supports (Williams and Cawood 1990; Charalambides *et al.* 1995; Alvarez *et al.* 2000; Gamonpilas *et al.* 2009) versus the rectangular supports in this study. The samples in this study all pivoted around the inner edge of the rectangular supports as the probe moved downward, essentially shortening the length of the beam being tested. Each rectangular support in this study was 12 mm wide; thus, the effective length of each sample beam was really only 36 mm long, not 60. The beam length only affects the calibration factor, Φ, in Eqs. 3.3-3.6. The calibration factor used to calculate G_c assumes that the distance between midpoints of the rolling, cylindrical supporting units equals four times the height of the beam. In other words, the length between pivot points is 4H. That ratio and distance was maintained when using the rectangular supports of this study. Fig. 3.1 shows that S, the distance between pivot points of the rectangular supports, equals 48 mm or 4H. Thus, the use of rectangular

supports in this study did not violate any mathematical assumptions or affect any calculations but may have affected material responses due to unaccounted frictional effects since the rectangular supports did not move like the cylindrical rollers.

5.1.4. Summary of Material Properties Elucidated by Rheological and Mechanical Testing

In summary, the main material property differences among the cheeses were RE and toughness. Both properties are measured using tests that probe beyond the LVR and at faster rates (shorter time frames) than, say, small strain creep tests. While properties measured with small strain testing appeared to be sensitive to the addition of starch granules in the LF treatment, RE and toughness varied directly with content. Since these material properties also scale with sensory attributes, product developers and researchers should ensure new cheeses are subjected to large strain testing and fracture tests at fast rates.

5.2. Sensory Testing

5.2.1. Descriptive Sensory Analysis Based on Fat Content

Descriptive analysis is an analytical tool where trained panelists (usually 6-12) perform analogously to an instrument to identify and quantify attributes of a product (Drake 2007). Panelists evaluating 20-mm cubes found LF treatments to be significantly higher in first bite and hand-compression terms but lower in chewdown terms (Fig. 3.7). Thus, the greater firmness of LF treatments was demonstrated by both sensory (first bite and hand compression) and instrumental (Jmax, G, and fracture stress) measurements,

depending on temperature. Chewdown terms and percent RE differentiated treatments by the greatest percentage. Percent RE has been shown to strongly differentiate treatments in non-cheese systems, too. Using model foods of mixed whey protein isolate and various polymers at different concentrations, van den Berg *et al.* (2008) found that RE statistically differentiated samples on the basis of microstructure and correlated to sensory assessments of crumbliness—a chewdown term—more strongly than did several fracture properties.

The effect of fat agreed with previous descriptive panels on cheese (Gwartney *et al.* 2002; Brown *et al.* 2003; Rogers *et al.* 2009). Specifically, hand firmness (force to compress 20%), hand springiness, hand hardness (force to fracture), and degree of breakdown during mastication all decreased as fat content increased (Fig. 3.7). Cohesiveness (degree to which chewed pieces stuck together), adhesiveness (degree to which chewed mass stuck to oral surfaces), smoothness of mass (lack of grit or grains), and smoothness of mouth coating (smoothness of oral surface after expectorating) all increased as fat content increased. Fracturability did not differ much amongst samples, which probably indicates that all cheddar cheese shares a similar fracture mechanism. Fracturability would better differentiate cheese types, as one can imagine that blue cheese and mozzarella fracture differently than cheddar, regardless of fat content.

5.2.2. Descriptive Sensory Analysis Based on Sample Size

After evaluating fat differences in cubes, descriptive analysis was used to determine the effect of sample size. Both fat content, sample thickness, and the

interaction of these two factors significantly affected sensory results for most attributes (Table 3.5). Sample mass was not constant in this study, but most terms are measured based on the amount of sample directly under a molar or finger, and the surface area of the top and bottom portions of each sample was held constant at 20 mm x 20 mm. Hence, chewing terms are the only terms that could vary with mass chewed, but Table 3.6 shows that degree of breakdown, cohesiveness, adhesiveness, smoothness of mass, and smoothness of mouth did not vary consistently with sample size within a single fat content. These results suggest that keeping sample mass constant would not change these DA results. It also indicates that a 20 mm x 20 mm x 2mm sample was sufficient mass to evaluate.

While thickness had little impact on chewing terms, it did significantly impact first bite and hand compression terms (Table 3.6). When differences were found, the general trend was for the smaller samples (2 and 8 mm) to be judged lower in the mechanical term (although there were exceptions). That trained panelists evaluated cheese texture differently on the basis of sample thickness lends validity to the hypothesis that consumer acceptability of LF cheeses may improve if sample thickness is altered. Therefore, a consumer study was warranted. In order to decrease the number of samples for consumer testing, to better to observe correlation among attributes, and to assess the ability of attributes to differentiate samples, a principle component analysis (PCA) product-attribute biplot was generated (Fig. 3.8).

PCA is an analytical technique used to reduce the number of variables—known or unknown—in a pool of data. Ideally, two uncorrelated (i.e. perpendicular axes) principle components describe a large percentage of the data. Treatments loading near attributes are most strongly described by those attributes and correlate positively. Principle Component 1 (y-axis) explained 83% of the variability in the data, and fat content was the primary loading factor since all FF samples are in the upper quadrants while RF and LF samples are in the lower quadrants. Thickness is probably the primary factor loading on Principle Component 2 (x-axis), which explained an additional 12% of the variability, since the thinnest samples all plotted on the left half of the graph. The thinnest samples (treatments 1, 5, 6, and 9) clearly exhibit different textures than their thicker counterparts. The 20-mm treatments all loaded similarly to the 14 mm treatments and so were excluded from the consumer study.

5.2.3. Consumer Panel

Consumers were less sensitive to differences in thickness than were trained panelists. Differences amongst samples were primarily due to fat content, not thickness, and no interactions existed between the factors (Table 3.7). Thickness within a given fat content did not influence consumer overall liking or texture liking at any fat level (Fig. 3.9). Flavor of the thinnest LF treatment was liked significantly less than for the thickest LF treatment, which contradicts our hypothesis and is an interesting result given consumers' previously noted dislike of LF cheese. RF and FF treatments showed no differences in flavor liking based on sample thickness. After consumers evaluated the

three thickness treatments within a given fat bracket, they were asked which treatment they preferred overall; consumers were unaware that the treatments being evaluated differed only by thickness and not fat. Consumers consistently liked the thinnest treatment the least; 14 mm was most preferred for RF and LF cheese, and 8 mm was most preferred for FF. These preference results also contradicted our hypothesis.

6. CONCLUSION

Although thickness significantly impacted trained panelists' perception of texture, it did not influence consumer perception of texture, nor did it change their overall liking within a given fat bracket. For the most part, thickness did not influence liking of flavor, either. Even though FF cheese was favored over LF cheese, consumers always preferred thicker samples, suggesting that they prefer larger sample masses or sizes regardless of fat content or texture. On the basis of this study, decreasing thickness would not improve consumer liking of LF cheese. .

Chapters 2 and 3 of this thesis generated a rheological fingerprint for commercial cheddar cheese available at different fat contents. An understanding of commercial cheese is important when making model cheeses to understand the contributions of certain variables, particularly fat content. The next chapter discusses the use of Sephadex beads to model the effect of fat content, i.e. filler, in cheddar cheese.

7. REFERENCES

ALVAREZ, M.D., SAUNDERS, D.E.J., VINCENT, J.F.V. and JERONIMIDIS, G. 2000. An engineering method to evaluate the crisp texture of fruit and vegetables. J. Texture Stud. *31*, 457-473.

BANKS, J.M. 2004. The technology of low-fat cheese manufacture. Int. J. Dairy Technol. *57*, 199-207.

BARRANGOU, L.M., DRAKE, M.A., DAUBERT, C.R. and FOEGEDING, E.A. 2006. Textural properties of agarose gels. II. Relationships between rheological properties and sensory texture. Food Hydrocolloids *20*, 196-203.

BOLES, J.A. and SHAND, P.J. 2008. Effect of muscle location, fiber direction, and slice thickness on the processing characteristics and tenderness of beef stir-fry strips from the round and chuck. Meat Sci. *78*, 369-374.

BOWLAND, E.L. and FOEGEDING, E.A. 1999. Factors determining large-strain (fracture) rheological properties of model processed cheese. J. Dairy Sci. *82*, 1851-1859.

BROWN, J.A., FOEGEDING, E.A., DAUBERT, C.R., DRAKE, M.A. and GUMPERTZ, M. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. *86*, 3054-3067.

BROWNSEY, G.J., ELLIS, H.S., RIDOUT, M.J. and RING, S.G. 1987. Elasticity and failure in composite gels. J. Rheol. *31*, 635-649.

CARTER, E.J.V. and SHERMAN, P. 1978. Evaluation of firmness of Leicester cheese by compression tests with Instron universal testing machine. J. Texture Stud. *9*, 311-324.

CHARALAMBIDES, M.N., WILLIAMS, J.G. and CHAKRABARTI, S. 1995. A study of the influence of ageing on the mechanical properties of cheddar cheese. J. Mater. Sci. *30*, 3959-3967.

CHILDS, J.L. and DRAKE, M.A. 2009. Consumer perception of fat reduction in cheese. J. Sens. Stud. *24*, 902-921.

CULIOLI, J. and SHERMAN, P. 1976. Evaluation of Gouda cheese firmness by compression tests. J. Texture Stud. *7*, 353-372.

DIEHL, K.C. and HAMANN, D.D. 1979. Structural failure in selected raw fruits and vegetables. J. Texture Stud. *10*, 371-400.

DRAKE, M.A. and SWANSON, B.G. 1995. Reduced- and low-fat cheese technology: a review. Trends Food Sci. Technol. *6*, 366-369.

DRAKE, M.A., HERRETT, W., BOYLSTON, T.D. and SWANSON, B.G. 1995. Sensory evaluation of reduced fat cheeses. J. Food Sci. *60*, 898-901, 905.

DRAKE, M.A. and CIVILLE, G.V. 2003. Flavor Lexicons. Compr. Rev. Food Sci. Food Saf. *2*, 33-40.

DRAKE, M.A. 2007. Invited review: sensory analysis of dairy foods. J. Dairy Sci. *90*, 4925-4937.

ELSNER, R.J.F., MCWATTERS, K.H. and RESURRECCION, A.V.A. 1998. Consumer preference optimization of scallopini made from boneless, skinless chicken thighs. Int. J. Food Sci. Nutr. *50*, 399-405.

EVERARD, O'CALLAGHAN, D.J., O'KENNEDY, B.T., O'DONNELL, C.P., SHEEHAN, E.M. and DELAHUNTY, C.M. 2007. A three-point bending test for prediction of sensory texture in processed cheese. J. Texture Stud. *38*, 438-456.

FOEGEDING, E.A. 1992. Rheological properties of whey protein isolate gels determined by torsional fracture and stress relaxation. J. Texture Stud. *23*, 337-348.

GAMONPILAS, C., CHARALAMBIDES, M.N. and WILLIAMS, J.G. 2009. Determination of large deformation and fracture behaviour of starch gels from conventional and wire cutting experiments. J. Mater. Sci. *44*, 4976-4986.

GUINARD, J.X. and MAZZUCCHELLI, R. 1996. The sensory perception of texture and mouthfeel. Trends Food Sci. Technol. *7*, 213-219.

GWARTNEY, E.A., FOEGEDING, E.A. and LARICK, D.K. 2002. The texture of commercial full-fat and reduced-fat cheese. J. Food Sci. *67*, 812-816.

HAMANN, D.D. 1983. Structural failure in solid foods. In *Physical Properties of Foods* (M. Peleg and E.B. Bagley, eds.) pp. 351-383, AVI Pub. Co., Westport, CT.

HERRERA-CORREDOR, J.A., SAIDU, J.E.P., KHACHATRYAN, A., PRINYAWIWATKUL, W., CARBALLO-CARBALLO, A. AND ZEPEDA-BAUTISTA, R. 2004. Identifying drivers for consumer acceptance and purchase intent of corn tortilla. J. Food Sci. *72*, S727-S731.

KINLOCH, A.J. and YOUNG, R.J. 1983. *Fracture Behavior of Polymers.* Applied Science Publishers, Ltd., London, UK.

JACK, F.R., PIGGOTT, J.R. and PATERSON, A. 1993a. Discrimination of texture and appearance in cheddar cheese using consumer free choice profiling. J. Sens. Stud. *8*, 167-176.

JACK, F.R., PATERSON, A. and PIGGOTT, J.R. 1993b. Relationships between rheology and composition of cheddar cheeses and texture as perceived by consumers. Int. J. Food Sci. Technol. *28*, 293-302.

JOHNSON, M.E., KAPOOR, R., MCMAHON, D.J., MCCOY, D.R. and NARASIMMON, R.G. 2009. Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. Compr. Rev. Food Sci. Food Saf. *8*, 252-268.

KALVIAINEN, N., SALOVAARA, H. and TUORILA, H. 2002. Sensory attributes and preference mapping of muesli oat flakes. J. Food Sci. *67*, 455-460.

LI, H., ERRINGTON, A.D. and FOEGEDING, E.A. 1999. Isostrength comparison of large-strain (fracture) rheological properties of egg white and whey protein gels. J. Food Sci. *64*, 893-898.

LIU, M.N. and BERRY, B.W. 1998. Patty formation method, thickness and weight effects on properties of cooked low-fat beef patties. J. Muscle Foods *9*, 243-256.

LUYTEN, H. and VAN VLIET, T. 1995. Fracture properties of starch gels and their rate dependency. J. Texture Stud. *26*, 281-298.

MCEWAN, J.A., MOORE, J.D. and COLWILL, J.S. 1989. The sensory characteristics of cheddar cheese and their relationship with acceptability. J. Soc. Dairy Technol. *42*, 112-117.

MEILGAARD, M., CIVILLE, G.V. and CARR, B.T. 1999. *Sensory Evaluation Techniques*. CRC Press, Boca Raton, FL.

MISTRY, V.V. 2001. Low fat cheese technology. Int. Dairy J. *11*, 413-422.

NADAI, A. 1937. Plastic behavior of metals in the strain-hardening range. Part I. J. Appl. Physics *8*, 205-213.

RODRÍGUEZ, J. 1998. Recent advances in the development of low-fat cheeses. Trends Food Sci. Technol. *9*, 249-254.

ROGERS, N.R., DRAKE, M.A., DAUBERT, C.R., MCMAHON, D.J., BLETCH, T.K. and FOEGEDING, E.A. 2009. The effect of aging on low-fat, reduced-fat, and full-fat cheddar cheese texture. J. Dairy Sci. *92*, 4756-4772.

ROGERS, N.R., MCMAHON, D.J., DAUBERT, C.R., BERRY, T.K. and FOEGEDING, E.A. 2010. Rheological properties and microstructure of cheddar cheese made with different fat contents. J. Dairy Sci. *in press*.

ROSS-MURPHY, S.B. and TODD, S. 1983. Ultimate tensile measurements of filled gelatin gels. Polymer *24*, 481-486.

SALA, G., VAN VLIET, T., STUART, M.C., VAN DE VELDE, F. and VAN AKEN, G.A. 2009. Deformation and fracture of emulsion-filled gels: effect of gelling agent concentration and oil droplet size. Food Hydrocolloids *23*, 1853-1863.

SALVADOR, A., VARELA, P., SANZ, T. and FISZMAN, S.M. 2009. Understanding potato chips crispy texture by simultaneous fracture and acoustic measurements, and sensory analysis. LWT Food Sci. Technol. *42*, 763-767.

TRUONG, V.D. and DAUBERT, C.R. 2000. Comparative study on large strain methods in assessing failure characteristics of selected food gels. J. Texture Stud. *31*, 335-353.

TUNICK, M.H. and VAN HEKKEN, D.L. 2002. Torsion gelometry of cheese. J. Dairy Sci. *85*, 2743-2749.

VAN DEN BERG, L., CAROLAS, A.L., VAN VLIET, T., VAN DER LINDEN, E., VAN BOEKEL, M.A.J.S. and VAN DE VELDE, F. 2008. Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. Food Hydrocolloids *22*, 1404-1417.

VAN VLIET, T. and WALSTRA, P. 1995. Large deformation and fracture behavior of gels. Faraday Discuss. *101*, 359-370.

WANG, H.H. and SUN, D.W. 2002. Melting characteristics of cheese: analysis of effect of cheese dimensions using computer vision techniques. J. Food Eng. *52*, 279-284.

WANG, H.H. and SUN, D.W. 2004. Evaluation of the oiling off property of cheese with computer vision: influence of cooking conditions and sample dimensions. J. Food Eng. *61*, 57-66.

WILLIAMS, J.G. and CAWOOD, M.J. 1990. European Group on Fracture: K_c and G_c methods for polymers. Polym Test. *9*, 15-26.

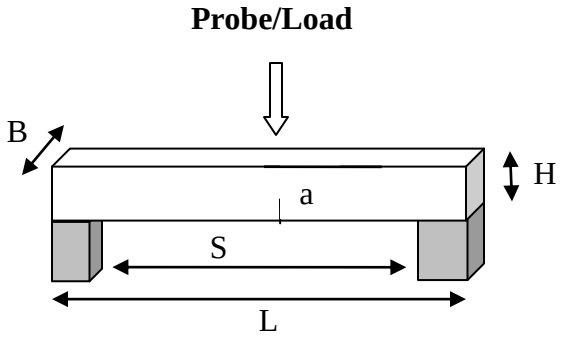
ZHOU, N. and MULVANEY, S.J. 1998. The effect of milk fat, the ratio of casein to water, and temperature on the viscoelastic properties of rennet casein gels. J. Dairy Sci. *81*, 2561-2571.

ZOON, P., VAN VLIET, T. and WALSTRA, P. 1989. Rheological properties of rennet-induced skim milk gels .5. Behavior at large deformation. Neth. Milk Dairy J. *43*, 35-52.

TABLE 3.1. COMMERCIAL CHEDDAR CHEESE COMPOSITION

Cheddar Cheese Product	**Code**	**% Fat[1]**
Cabot full-fat	FF	32
Cabot 50% reduced-fat	RF	16
Cabot 75% reduced-fat[2]	LF[3]	9

[1] The percent fat is the amount listed on the package label
[2] According to package label, the product contains cornstarch and monoglyceride, ingredients not normally found in cheddar cheese
[3] Cheese products containing < 3g of fat per reference amount can legally be called "low fat" (CFR 21 [101.62b])



L, Length = 60mm
H, Height = 12 mm
B, Breadth = 6 mm
a (notch height) = 5.4mm
S (distance between supporting units) = 48mm

FIG. 3.1. SINGLE EDGE NOTCHED BEND (SENB) TEST EXPERIMENTAL SETUP

TABLE 3.2. DESCRIPTIVE ANALYSIS SENSORY ATTRIBUTES
Ballot based on that by Brown *et al.* (2003). Definition or technique of bolded terms was altered from that originally described by Brown *et al.*

	Term	Definition	Technique
Hand Evaluation Terms	Firmness (HFirm)	Amount of force required to completely compress the sample	Press completely through the sample using the thumb and first two fingers
	Springiness (HSpring)	Total amount of recovery of the sample	Press the sample between the thumb and first two fingers until it is depressed 20%. Samples that fracture are *not* springy
	Hardness (HHard)	Amount of force required to fracture the sample	Press completely through the sample using the thumb and first two fingers
First-bite Terms	Firmness (Firm)	Amount of force required to completely bite through the sample	Completely bite through sample using molars
	Fracturability (Frac)	Number of fragments produced after biting	Completely bite through sample using molars
Oral Chewdown Terms	Degree of Breakdown (Deg Break)	Amount of breakdown that occurs in the sample as a result of mastication (i.e. the amount of meltability or dissolvability)	Chew sample five times and evaluate chewed mass
	Cohesiveness (Cohes)	Degree to which the chewed mass holds together	Chew sample five times and evaluate chewed mass
	Adhesiveness (Adhes)	Degree to which the chewed mass sticks to mouth surfaces	Chew sample five times and evaluate chewed mass
	Smoothness of Mass (Smth of Mass)	Degree to which the chewed mass surface is smooth (i.e. evaluation for gritty or grainy particles)	Chew sample five times and evaluate chewed mass
	Smoothness of Mouth (Smth of Mouth)	Degree of smoothness felt in the mouth after expectorating the sample	Chew sample five times, expectorate, and evaluate the residual in mouth

TABLE 3.3. CRITICAL STRESS AND STRAIN AND RECOVERABLE ENERGY (RE) BASED ON FAT CONTENT

	Critical Stress Pa	Critical Strain	RE %
LF	892 A	2.17E-03 A	47.6 A
RF	1270 A	1.28E-03 A	46.2 B
FF	1230 A	7.92E-04 A	33.6 C

Different letters within a column denote treatments that were significantly different at $p < 0.05$

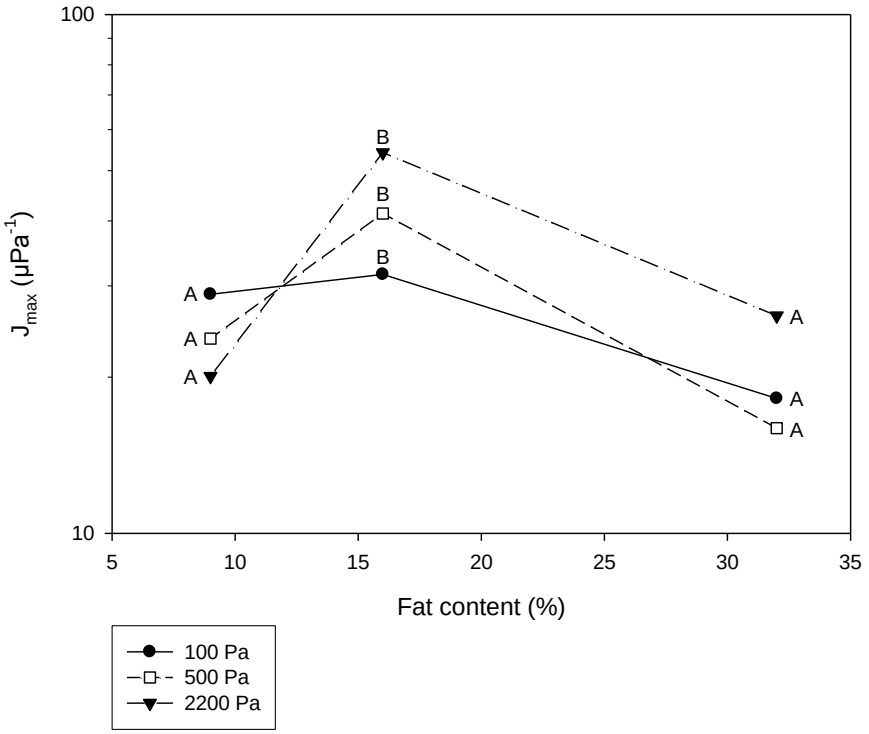


FIG. 3.2. MAXIMUM COMPLIANCE (J_{MAX}) OF COMMERCIAL CHEDDAR CHEESES DURING CREEP/RECOVERY TESTING AT LOADS OF 100 Pa AND 500 Pa

Stress loads were applied for 200 s. Data points with different letters were significantly different at $p < 0.05$.

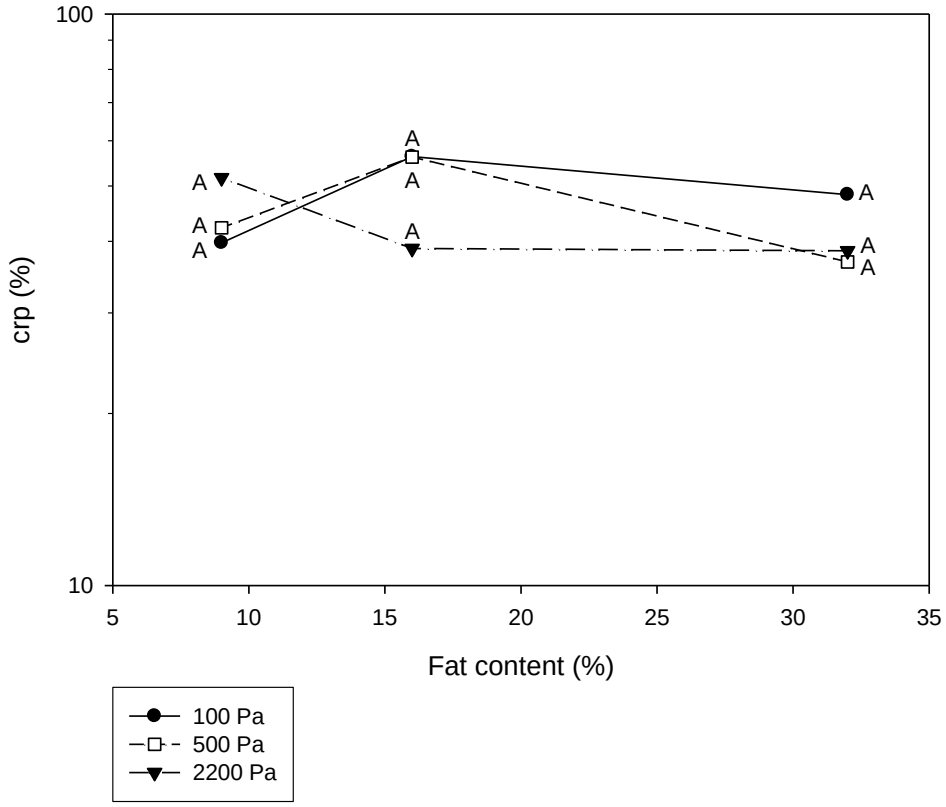


FIG. 3.3. PERCENT CREEP RECOVERY (crp) OF COMMERCIAL CHEDDAR CHEESES DURING CREEP/RECOVERY TESTING AT LOADS OF 100, 500, AND 2200 Pa

Stress loads were applied for 200 s; recovery period lasted 200 s. Data points with different letters were significantly different at $p < 0.05$, although no differences existed.

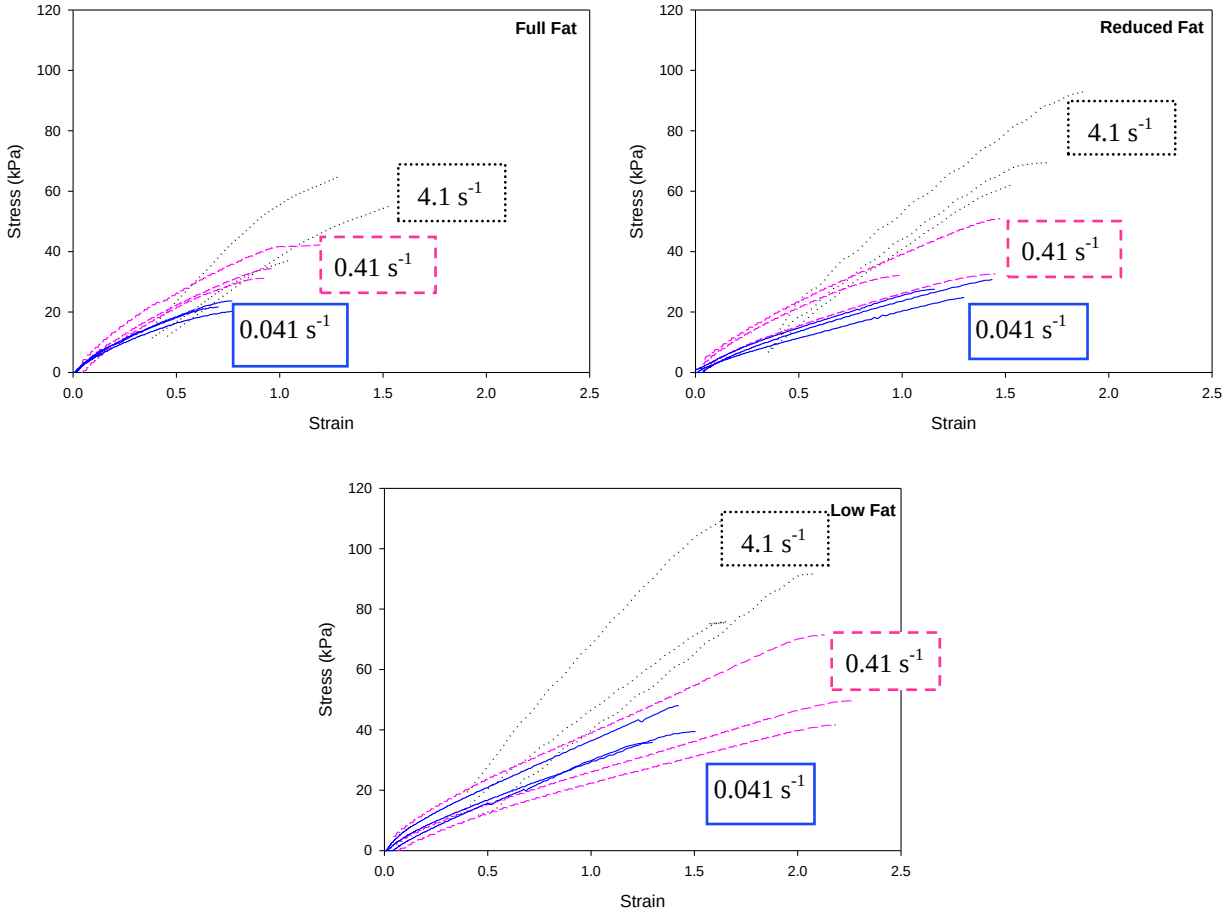


FIG. 3.4. EFFECT OF TORSION RATE ON FULL-FAT, REDUCED-FAT, AND LOW-FAT CHEDDAR CHEESE STRESS-STRAIN CURVES

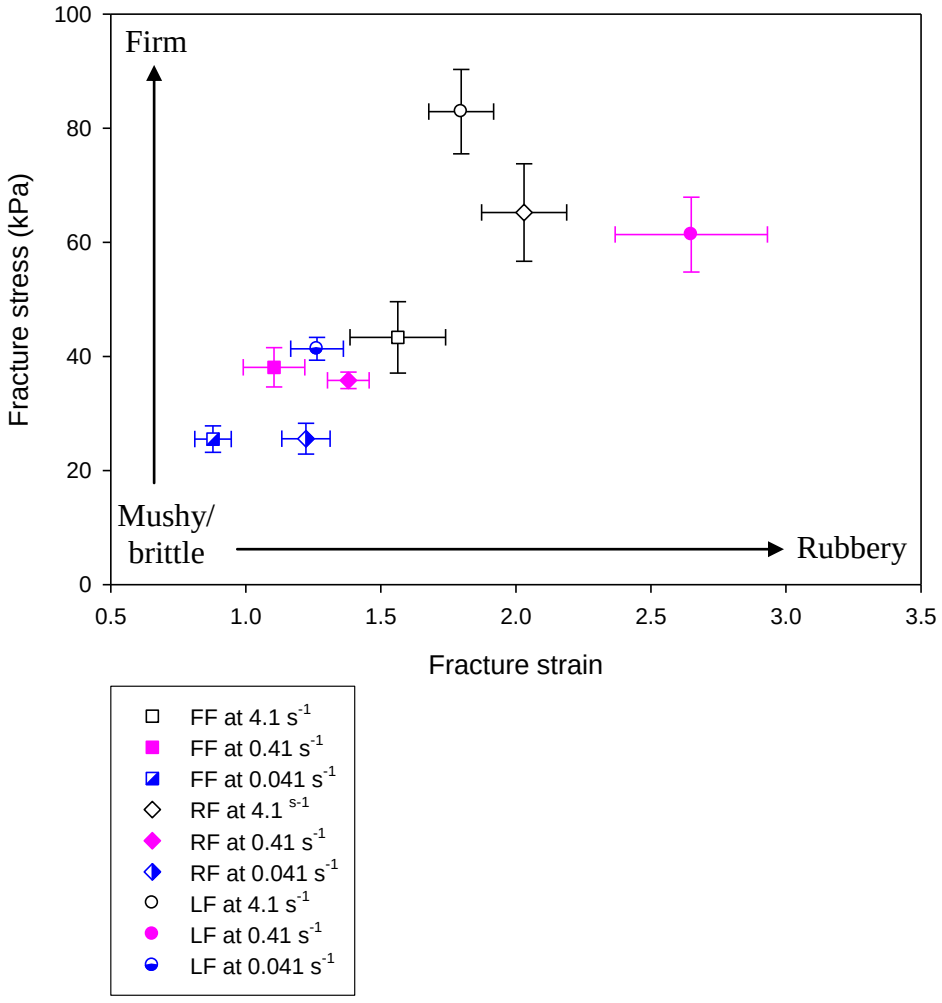


FIG. 3.5. TEXTURE MAP BASED ON TORSION FRACTURE GELOMETRY OF CHEDDAR CHEESE AT DIFFERENT RATES AND FAT CONTENT
Error bars represent the standard error of the mean.

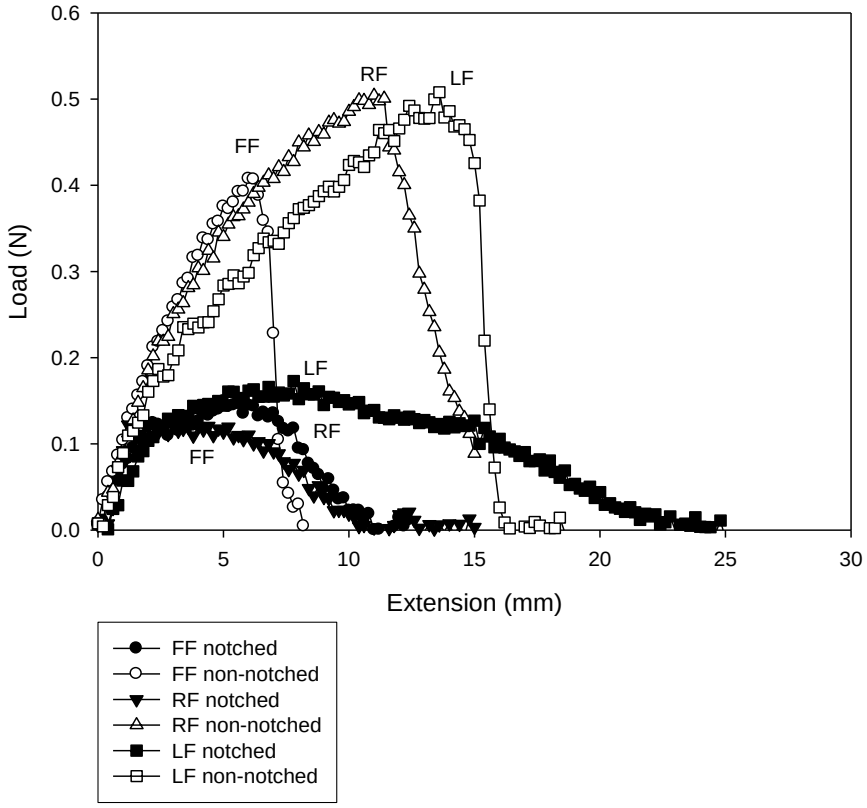


FIG. 3.6. CHARACTERISTIC STRESS-STRAIN CURVE FROM SINGLE EDGE NOTCHED BEND TESTING (SENB) AT CROSSHEAD SPEED OF 0.2 mm/s. For simplicity, every other point was omitted.

TABLE 3.4. MATERIAL PROPERTIES AND PARAMETERS FROM SINGLE EDGE NOTCHED BEND (SENB) TESTS

	K_c kPa·m$^{1/2}$	G_c J/m^2	Work to max load, P J		E (slope up to load of 0.1N) N/mm	
	Notched	Notched	Non-notched	Notched	Non-notched	Notched
LF	2.74 A	22.2 A	6.02E-03 A	4.29E-04 D	0.12 A	0.11 A
RF	2.26 A	11.2 A	3.63E-03 B	2.14E-04 D	0.10 A	0.11 A
FF	2.66 A	16.4 A	1.76E-03 C	3.34E-04 D	0.16 A	0.13 A

Different letters within a column denote treatments that were significantly different at $p < 0.05$

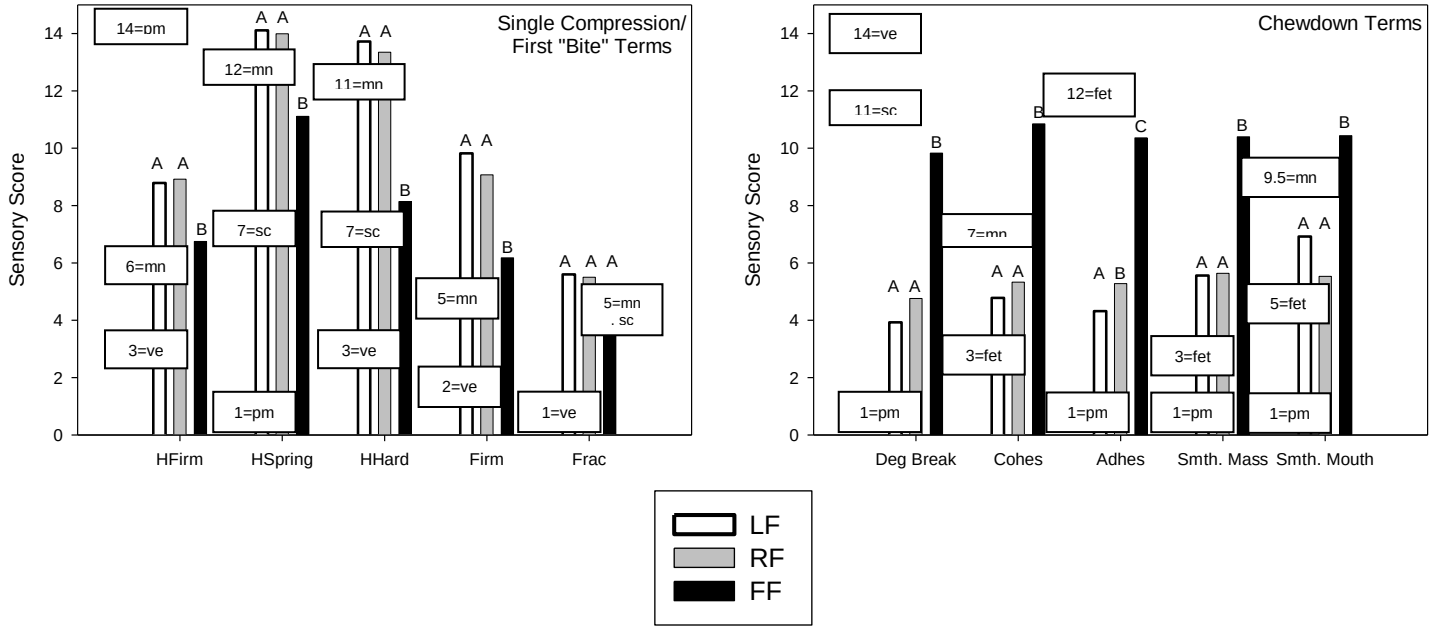


FIG. 3.7. DESCRIPTIVE ANALYSIS OF 20-MM CHEESE CUBES

Attributes included hand firmness (HFirm), hand springiness (HSpring), hand hardness (HHard), firmness (Firm), fracturability (Frac), degree of breakdown (Deg Break), cohesiveness (Cohes), adhesiveness (Adhes), smoothness of mass (Smth. Mass), and smoothness of mouth coating (Smth. Mouth). Anchor points, with corresponding score, from the ballot are also given on scale for parmesan (pm), Munster (mn), sharp Cheddar (sc), Velveeta® (ve), and Feta (fet) cheese. Bars with different letters within a single attribute (e.g. HFirm) were significantly different at $p < 0.05$.

TABLE 3.5. MAIN EFFECTS (FAT AND THICKNESS) AND INTERACTION (COMBINED FAT AND THICKNESS) FOR DESCRIPTIVE PANEL SENSORY-TEXTURE ATTRIBUTES

Attributes included hand firmness (HFirm), hand springiness (HSpring), hand hardness (HHard), first-bite firmness (Firm), first-bite fracturability (Frac), degree of breakdown (Deg Break), cohesiveness (Cohes), adhesiveness (Adhes), smoothness of mass (Smth Mass), and residual smoothness of mouth coating (Smth Mouth).

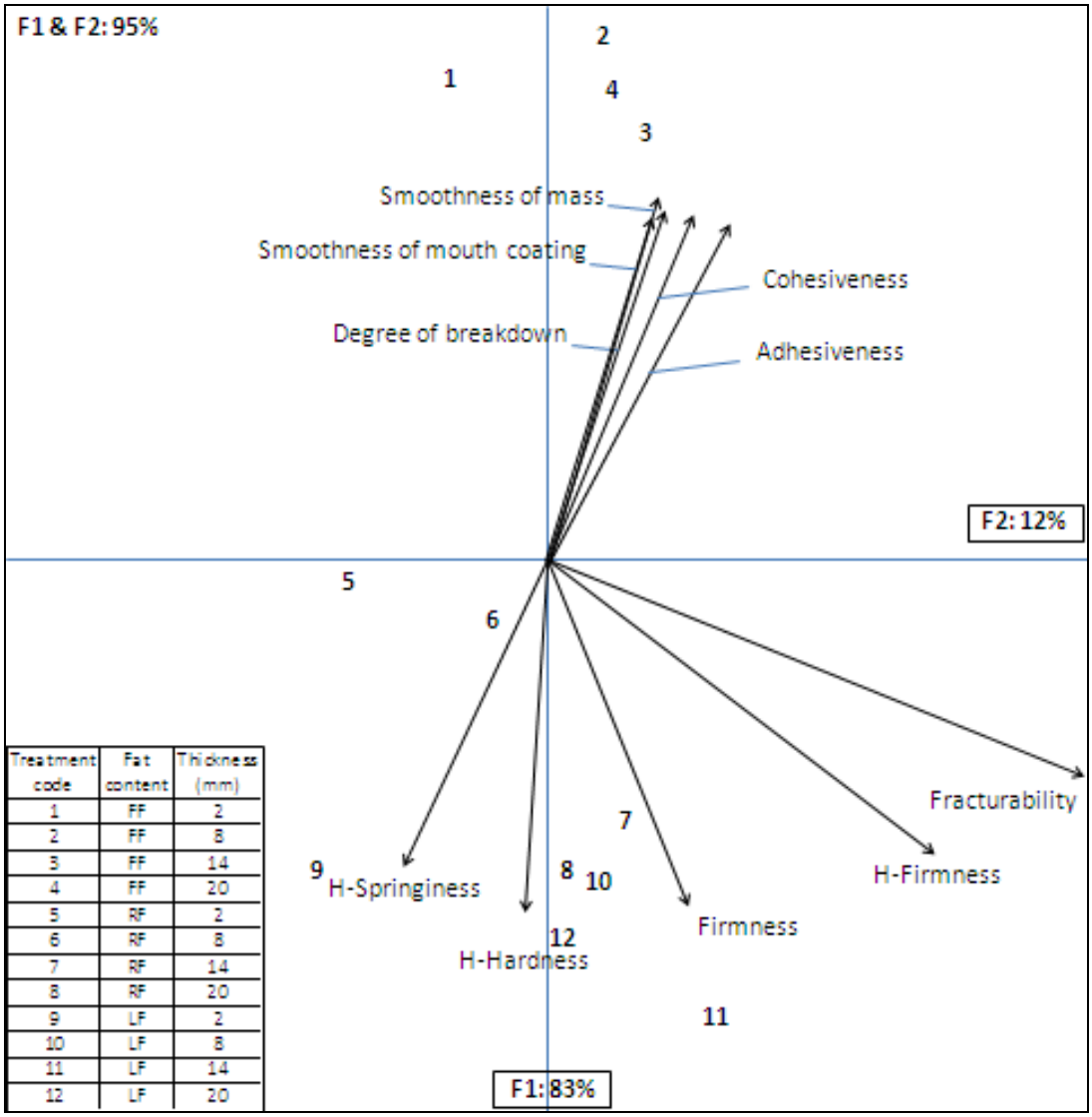
	HFirm	HSpring	HHard	Firm	Frac	Deg Break	Cohes	Adhes	Smth Mass	Smth Mouth
Fat	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Thickness	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.173	**<0.001**	0.093	**0.025**
Interaction	**<0.0001**	**<0.0001**	**<0.0001**	0.024	0.008	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Chart contains p-values; bolded values denote presence of a significant effect at $p < 0.05$.

TABLE 3.6. DESCRIPTIVE ANALYSIS OF CHEDDAR CHEESE IN VARYING THICKNESSES
Attributes included hand firmness (HFirm), hand springiness (HSpring), hand hardness (HHard), first-bite firmness (Firm), first-bite fracturability (Frac), degree of breakdown (Deg Break), cohesiveness (Cohes), adhesiveness (Adhes), smoothness of mass (Smth Mass), and residual smoothness of mouth coating (Smth Mouth).

		HFirm	HSpring	HHard	Firm	Frac	Deg Break	Cohes	Adhes	Smth Mass	Smth Mouth
FF	2 mm	5.00 A	7.64 A	5.35 A	5.01 A	4.36 A	10.75 A	11.19 A	9.61 A	10.08 A	10.25 A
	8 mm	6.54 B	4.72 B	9.07 B	5.58 AB	4.99 ABCD	10.28 A	10.76 A	9.43 A	10.11 A	10.31 A
	14 mm	7.26 B	2.19 C	7.81 C	6.13 B	4.83 ABC	10.75 A	10.90 A	10.31 A	10.69 A	10.25 A
	20 mm	6.75 B	11.11 D	8.14 BC	6.18 B	5.11 BCDE	9.83 A	10.85 A	10.36 A	10.40 A	10.44 A
RF	2 mm	6.64 B	13.35 EF	11.93 D	7.31 C	4.47 AB	5.69 B	6.04 B	5.06 BCD	6.58 BC	8.06 B
	8 mm	6.89 B	12.44 E	11.88 D	8.83 D	5.19 BCDE	7.08 C	5.29 BC	5.08 BCD	7.33 B	8.40 B
	14 mm	8.97 C	13.76 F	13.18 E	9.50 DE	5.75 EF	5.00 BE	5.61 BC	5.22 BD	6.11 CD	7.83 BC
	20 mm	8.92 C	13.99 F	13.35 E	9.07 DE	5.50 CDEF	4.76 BEF	5.33 BC	5.28 B	5.64 DE	5.53 DE
LF	2 mm	7.32 B	13.88 F	14.08 E	8.64 D	4.46 AB	3.28 D	4.08 D	3.64 E	5.11 E	6.31 DE
	8 mm	9.15 C	13.11 EF	13.47 E	9.82 E	5.57 CDEF	5.06 BE	5.96 B	5.61 B	5.28 DE	6.22 E
	14 mm	10.36 D	14.04 F	13.65 E	10.70 F	6.19 F	4.25 EF	4.90 CD	4.18 CE	5.43 DE	7.06 CD
	20 mm	8.79 C	14.11 F	13.72 E	9.82 E	5.60 DEF	3.93 DF	4.78 CD	4.32 DE	5.56 DE	6.92 DE

Different letters within a column denote treatments that were significantly different at $p < 0.05$.



Treatment code	Fat content	Thickness (mm)
1	FF	2
2	FF	8
3	FF	14
4	FF	20
5	RF	2
6	RF	8
7	RF	14
8	RF	20
9	LF	2
10	LF	8
11	LF	14
12	LF	20

FIG. 3.8. PRINCIPLE COMPONENTS FROM DESCRIPTIVE ANALYSIS
Numbers correspond to treatments; arrows correspond to factors/attributes. "H-" denotes a "hand" term (e.g. Hand Firmness).

TABLE 3.7. MAIN EFFECTS (FAT AND THICKNESS) AND INTERACTION (COMBINED FAT AND THICKNESS) MEASURING EFFECT OF THICKNESS ON CONSUMER PERCEPTION OF TEXTURE

	Overall Liking	Flavor Liking	Texture	Preferred Sample
Fat	**<0.0001**	**<0.0001**	**<0.0001**	**0.001**
Thickness	0.063	**0.037**	0.099	1.000
Fat*Thickness	0.867	0.553	0.934	1.000

Chart contains p-values; bolded values denote presence of a significant effect at $p < 0.05$.

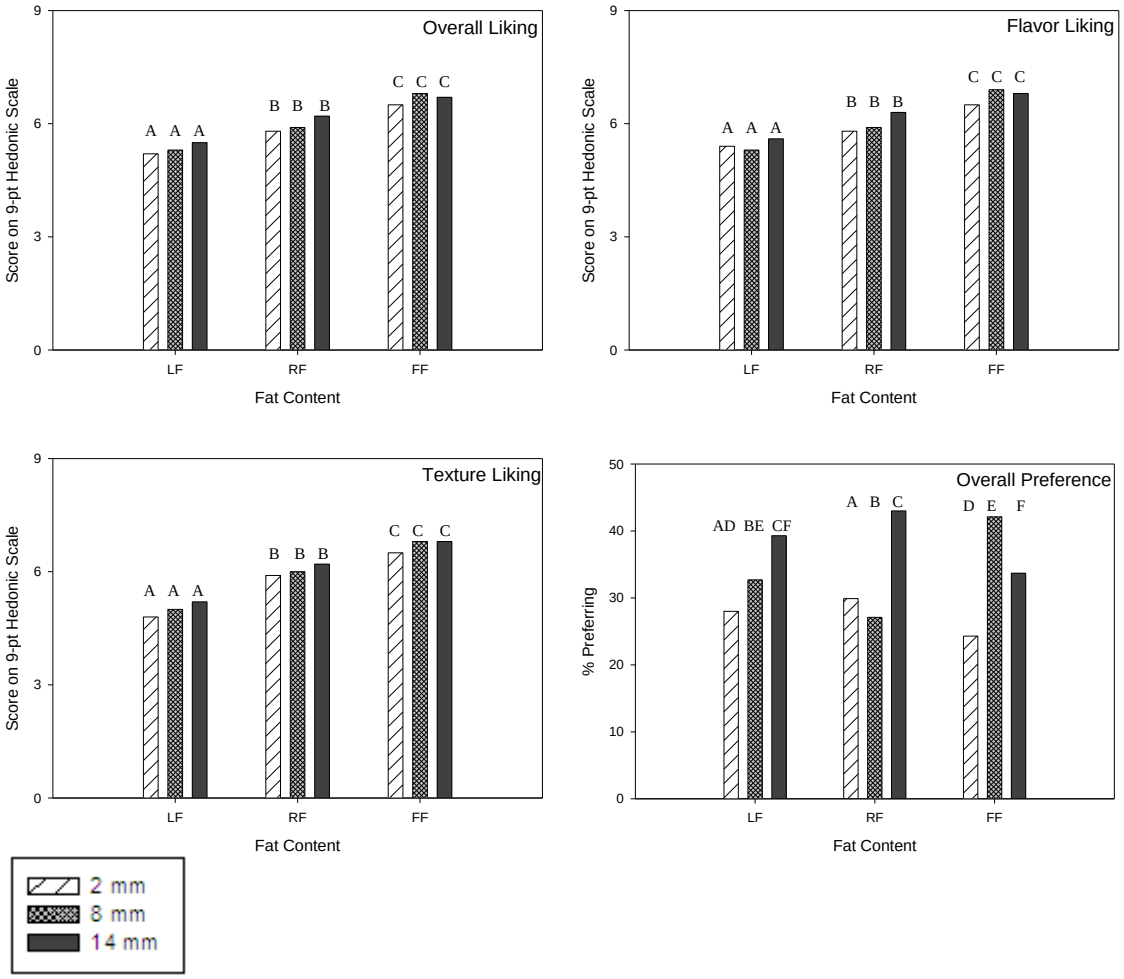


FIG. 3.9. EFFECT OF SAMPLE THICKNESS ON CONSUMER EVALUATION OF CHEESE

Overall liking, flavor liking, texture liking, and overall preference were evaluated. Bars with different letters/numbers were significantly different at $p < 0.05$.

Chapter 4

Investigating the Filled Gel Model in Cheddar Cheese Through Use of Sephadex Beads

1. ABSTRACT

Cheese can be modeled as a filled gel whereby milkfat globules are dispersed in a casein network. The goal was to understand how alternate fillers function in a cheese matrix. Low-filler (6% particles), reduced-filler (16%), and full-filler (33%) cheeses were produced using either G-50 Sephadex beads of varying sizes (20 – 150 μm diameter) or milkfat. Small and large-strain rheological tests were run on each treatment at 8, 12, and 18 weeks. Age had no significant effect except on large strain testing; treatments recovered less energy with age. Rheological properties were most affected by filler volume rather than filler size. Both control and experimental cheeses showed a decrease in deformability and an increase in firmness as filler volume increased above 25%, although the more elastic beads exhibited a greater reinforcing effect. The difference in filler elasticity likely explains why control cheese recovered less energy as filler volume increased, whereas bead-filled cheese recovered more energy. Cheese is not a simple system but was well-modeled by the filled gel theory.

Keywords: cheese, filled gel, Sephadex beads, particle size

2. PRACTICAL APPLICATION

Previous research shows that the rheological properties of cheese closely correlate to the filler volume, or amount of fat, present. Low-fat cheese contains 6% fat as compared to ~33% fat in normal cheddar cheese. Low-fat cheese is known to differ markedly—and often, undesirably—from its full-fat counterpart in gel firmness and

properties experienced during chewing. Understanding how milkfat affects structural mechanics in cheese allows researchers and developers to find acceptable substitutes to replace milkfat. The filled gel model suggests that an acceptable low-fat cheese might be obtained by substituting low-Calorie filler particles for fat, resulting in a cheese that has only 6% milkfat but contains 33% filler particle overall. Thus, this research is important for the future development of more palatable low-fat cheese.

3. INTRODUCTION

3.1. Filled Gel Theory

Cheese can be viewed as a protein network filled with dispersed milkfat particles (Visser 1991; Walstra 2003), making cheese suitable for analysis using the filled gel model. Particle-filled gels, or filled gels, are also known as "gelled emulsions" and "composite gels". Filler particles may be relatively deformable as in the case of oil-filled emulsions, or they may be relatively rigid, e.g. glass beads. Typically, both the filler and network are deformable to a certain extent, which causes the composite gel to exhibit unique, and often complex, mechanical responses to applied stresses and deformations. The filled gel model provides a simplified way of understanding these responses. The model purports that mechanical properties of the composite gel depends on:

1. Properties of the suspending medium (i.e. casein network in cheese),
2. Properties of the dispersed filler particles (i.e. milkfat globules in cheese), and

3. Interactions between the filler particles and protein network.

Specifically, properties of the casein network include both the elastic modulus (G') and fracture stress and strain of the network. Properties of the dispersed filler particles include both the elastic modulus and the phase volume (φ, v/v), as well as shape and orientation. With regard to the third point: filler particles display a range of interactions with the suspending network and may be considered either "active" or "inactive" (van Vliet 1988); these classifications are further divided into "interacting" and "non-interacting" particles. Particles that interact with the network are always termed "active" because they increase the storage modulus such that the filled gel has larger moduli values than does the unfilled gel. However, non-interacting particles may also cause an increase in the storage modulus, and this is known as a reinforcing effect (Brownsey *et al.* 1987). Furthermore, the reinforcing effect increases as the filler phase volume increases, regardless of whether the particle is interacting or non-interacting (Kerner 1956; Brownsey *et al.* 1987; Loret *et al.* 2007). Non-interacting particles can, however, actually decrease the storage modulus because the particles disrupt and weaken the surrounding gel network. Under these circumstances, the non-interacting particles are called "inactive fillers." Just as the reinforcing effect of active fillers increased with filler phase volume, the effect of inactive fillers increases with phase volume. In other words, increasing the filler phase volume of inactive fillers causes subsequent decreases in the composite network moduli (Chen and Dickinson 1998). To explain these effects, van Vliet (1988) proposed that inactive particles at small deformations essentially behave like

globules of low-viscosity liquid (e.g. water) in the network. Chen and Dickinson (1999) attributed these properties of inactive particles to their surface interactions. The authors theorized that inactive fillers essentially act like holes in the composite gel because the fillers do not interact electrostatically with the suspending network and therefore are not an integrated part of the composite gel. These theories have been developed based on linear elastic models and do not account for viscoelastic effects seen in most foods.

In summary, it can be said that active fillers may be either interacting or non-interacting particles but always have a storage modulus greater than that of the suspending network ($G'_{filler} > G'_{suspending\ network}$) and, consequently, serve to increase the composite gel moduli. Inactive fillers are always non-interacting particles that cause a decrease in the composite gel moduli because the storage modulus of the filler is less than that of the suspending network ($G'_{filler} < G'_{suspending\ network}$). Theoretically, fillers, regardless of interactions, may have an equivalent storage modulus to that of the suspending network ($G'_{filler} = G'_{suspending\ network}$), in which case the material would be homogenous in G', and the relative volumes of filler to network would not matter.

3.2. Filled Gel Models

The Kerner (1956) and van der Poel (1958) are two of the earliest models and were developed to quantitatively predict the impact of a filler particle on the resulting composite gel moduli, assuming simple, isotropic systems and synthetic, spherical, mono-dispersed particles that did not aggregate (van Vliet 1988; Ahmed and Jones 1990). Therefore, the filled gel models needed to be validated in more complex, realistic

systems, e.g. biopolymer systems. To that effect, Ross-Murphy and Todd (1983) made composite gels of gelatin (20% w/v) and glass beads, which were available in spherical and cubical shapes, and ranged in filler volume from 0-80%. Glass beads were used because they are non-deformable and exhibit perfect adhesion to the network at all strain levels, meaning they are active fillers. Bracketing the effects of filler particle size, shape, and volume in force-extension failure "envelopes," Ross-Murphy and Todd showed that an increase in filler phase volume caused an increase in fracture stress but a decrease in fracture strain. This means the composite gels became firmer but less deformable as phase volume increased. Gwartney *et al.* (2004) found similar results using a more complicated system of whey protein isolate (12% w/v) gels filled with emulsified sunflower oil (filler volume = 0-20%).

Sephadex beads have been used in several studies to study filler effects in gels. One of the earliest Sephadex studies used uncharged beads of varying rigidities in either a 3 or 6% gelatin system (Brownsey *et al.*1987). The authors called the beads non-interacting fillers because they were uncharged. However, the beads behaved as active fillers because they increased the complex gel modulus; this reinforcing effect increased with both Sephadex phase volume and bead rigidity. Furthermore, the phase volume at which the reinforcing effect was first observed decreased as bead rigidity increased. However, when the gelatin concentration was increased from 3 to 6%, the phase volume at which the reinforcing effect was first observed increased, supporting the tenet that filler effects depend upon both the filler properties and the suspending network

properties. The data also suggested there was a critical phase volume requirement necessary to observe a filler effect—results not predicted by the Kerner model. In other words, the authors found that, below the critical phase volume, increasing the phase volume did not impact the complex gel modulus. The authors repeated the experiments using beads of different sizes but did not observe any significant particle-size effects. The experiments were also repeated using negatively charged Sephadex beads, which were suspected to interact with the positively charged gelatin network, making the beads interacting, active fillers. Indeed, these charged beads caused a significant increase in the reinforcing effect.

3.3. Sephadex Properties

Sephadex beads were designed for use in chromatography and separation experiments. The beads are made from cross-linked polydextrans and are available in different sizes (20-300 μm), rigidities, and charges. The relative rigidity of the bead relates to the bead porosity and the amount of water it absorbs during hydration. Because Sephadex is traditionally used for filtering, it may adsorb other compounds from the complex matrix, presumably before gelation is complete. Brownsey *et al.* (1987) observed that gelatin was excluded from all grades of Sephadex, but Langley *et al.* (1990) found that whey protein, the suspending network, adsorbed into the porous particles. Langley *et al.* further noted that Sephadex-filled whey protein gels failed at or near the particle-protein interface, unlike whey protein gels filled with hydrophobic particles,

which failed adjacent to the particle surface. The authors interpreted these results to mean that adsorption produced a strong interaction between filler and matrix.

3.4. Objective

Cheese is a complex system and, as such, interactive mechanisms between its constituent parts and structural elements are difficult to understand. Previous work suggested that milkfat acts as active or inactive filler in cheese depending upon the temperature and phase volume (Zhou and Mulvaney 1998; Rogers *et al.* 2010). In this investigation, we used a model cheese system in which Sephadex beads were substituted for milkfat in order to more clearly understand the role of filler particles in cheese by adding non-melting filler particles. Results were related to the filled gel model. If Sephadex-filled cheddar cheese behaves as predicted under the filled gel model, then a material model will be established.

4. MATERIALS AND METHODS

4.1. Cheese

4.1.1. Materials

Collaborators at Utah State University made all cheese. Starter culture DVS850, a blend of *Lactococcus lactis* species as frozen pellets of cell concentrate was from Chr. Hansen Inc. (Milwaukee, WI). Double strength chymosin (Maxiren) with nominal 650 International milk clotting units/ml activity was from DSM Food Specialties USA Inc. (Eagleville, PA). White distilled vinegar with 5% acidity was from Sysco Corporation

(Houston, TX). Sephadex G-50 (Sigma-Aldrich Co., St. Louis, MO) was obtained as medium, fine, and superfine (SF) powders with nominal dry bead sizes of 50 to 150μm, 20 to 80 μm, and 20 to 50 μm, respectively, and mixed with distilled water in a 1:9 ratio and allowed to hydrate overnight at 4C. Cow's milk (pH 6.6 to 6.7) was from Utah State University's Caine Dairy Research and Teaching Center (Wellsville, UT). Milk was processed and cheesemaking performed in the Gary Haight Richardson Dairy Products Laboratory (Utah State University). After cream separation, the skim milk (~0.2% fat) was pasteurized at 73C for 15 s, and the cream (~32% fat) was pasteurized at 68C for 30 min; both were stored overnight at 4C.

4.1.2. Milk Substrate

Sixteen kilograms of milk substrate was prepared using skim milk plus cream for the control cheeses and skim milk plus hydrated Sephadex beads (a slight excess of water was used in hydrating the beads to form a slurry and allow easier mixing) for the experimental cheeses. Control cheeses at three nominal fat levels cheese were manufactured representing full fat (FF), 50% reduced fat (RF), and low fat (LF) cheddar cheeses, for which skim milk was standardized to protein to fat ratios of 0.83, 1.9, and 4.5, respectively.

Milk substrate for the experimental cheeses corresponding to FF, RF and LF were prepared by adding 80, 45, and 19 g (dry bead weight) of hydrated Sephadex beads to 15.3, 15.6, and 15.8 kg of skim milk, respectively. Assuming a 9:1 weight ratio of hydrated beads to dry beads, this corresponds to milk substrate containing 4.5%, 2.5%

and 1.1% (wt/wt) of hydrated Sephadex beads. Assuming a loss of ~20% beads into the whey during cheesemaking and the usual loss of ~87% starting liquid to cheese whey (Fox *et al.* 2004), this was expected to produce cheeses in which the hydrated Sephadex beads would occupy ~33%, 16%, and 6% of the final cheese volume, respectively.

4.1.3. Cheesemaking

Parameters for the various cheese manufacturing procedures are shown in Table 4.1. Skim or standardized milk was warmed to the set temperature and 7 g of starter culture and 1.8 mL of annatto were added. Forty minutes after adding the starter, the Sephadex slurry was added with stirring, followed immediately by addition of 3.5 mL of chymosin. Then the milk was allowed to coagulate. The curd was cut when firm (~15 min), allowed to heal, then stirred and cooked, and then the whey was drained when the target curd pH was reached (Table 4.1). The curds for making the LF and RF cheeses were washed using ~2 kg of cold water for 10 min, and then the curd was dry stirred until the target salting pH was reached (Table 4.1). After salting, the cheese was filled into round plastic hoops and pressed at 60 kPa overnight (~18 h) at room temperature (20±3C) into nominal blocks. Cheeses were vacuumed packaged and stored at 6C for aging. Sample blocks were shipped overnight (with ice packs) to North Carolina State University for rheological testing. Target treatments and sampling plan are shown in Table 4.2.

4.2. Proximate Analysis

Collaborators at Utah State performed all proximate analysis. Sodium chloride content was measured using a chloride analyzer (model 926, Corning; Medfield, MA). Grated cheese was mixed with distilled water for 4 min and homogenized at 260 rpm in a Stomacher 400 (Seward, England). The slurry was filtered through Whatman #1 filter paper (Maidstone, Kent UK), and the filtrate was analyzed for salt. Moisture content was determined in triplicate by weight loss using a CEM microwave oven (CEM Corp.; Indian trail, NC). Fat content was determined using the Babcock method (15.8.A; Marshall 1992). All proximate analyses were completed five d after the cheese was manufactured.

4.3. Microscopy

Cheese samples were imaged using confocal scanning laser microscopy (CSLM). The method for imaging samples was similar to one used by Guinee *et al.* (2000). Cheese samples were held at 4C until sliced into sections approximately 5 mm x 5 mm x 1 mm thick using a razor blade; samples were taken from near the edge of the cheese and from the interior. A 0.2% solution of Nile Blue A Sulfate (MP Biomedicals, LLC; Solon, OH) fluorescent dye in deionized water was filtered twice using Whatman No. 3 (Maidstone, Kent UK) filter paper, and 20 µL of the solution was pipetted onto the cut surface of each cheese slice. The dye was allowed to absorb into the cheese at room temperature for 10 min. Cheese samples were then turned over (dyed cheese surface against the glass slide) onto a single-welled slide with a #1.5 coverslip adhered to the

bottom of the slide via silicone grease. Samples were imaged on an inverted Leica TCS SP1 CSLM (Leica Inc.; Bannockburn, IL) using both PL FLUOTAR 40.0x1.00 OIL UV and HC PL FLUOTAR 10.0x0.30 objectives. A 488 nm laser (to excite Nile Blue in the fat phase) and a 633 nm laser (to excite Nile Blue in the protein phase) were used sequentially to image the samples. Emission spectra were collected from 500-650 nm for the fat phase and 650-800 nm for the protein phase, and the resulting images were overlaid. For each cheese treatment, at least 4 samples were prepared; 2 images were taken per sample at the 10x objective, and 3 images were taken per sample at the 40x (whenever possible, one of these images was taken with no Sephadex beads in view), resulting in a total of 20+ images per treatment. MetaMorph software (v. 7.5, Molecular Devices; Downingtown, PA) was used to analyze the area of Sephadex beads in the protein phase and the bead diameters.

4.4. Rheological Tests

All tests were conducted at 8, 12, and 18 weeks of age. After opening the package, cheeses were sealed in closeable storage bags to prevent moisture loss. Tests were completed within 3 days of opening a package.

4.4.1. Controlled-stress Tests

A Stresstech controlled-stress rheometer (ATS Rheosystems; Bordentown, NJ) fitted with a 20-mm smooth, parallel plate geometry was used to determine viscoelastic properties through stress sweeps, creep/recovery tests, and frequency sweeps. For each test, cheese samples, 4-mm thick, were trimmed to the size of the upper plate and glued

to both plates with Loctite 401 cyanoacrylate glue (Loctite Corp.; Rocky Hill, CT) to prevent sample slip during testing. A thin layer of lubricant (SuperLube, Synco Chemical; Bohemia, NY) was applied to any exposed cheese edges to prevent moisture loss.

4.4.1.1. Stress Sweeps

Stress sweeps were conducted in order to determine the linear viscoelastic region (LVR). Three samples were tested per treatment per batch. Stress sweeps were conducted at 25C from 1 to 2000 Pa at 10 Hz; the temperature was regulated using a clamshell oven that was attached to the rheometer and whose two halves closed around the plate area. The LVR was identified by the plateau region of the dynamic viscoelastic function G*, the complex modulus. The critical stress and strain values were identified as the point when G* values decreased 1% from the constant plateau value.

4.4.1.2. Creep/recovery Tests

Creep/recovery tests were conducted at 100 Pa and 500 Pa on different samples. Based on the method by Rogers *et al*. (2009), loads were applied for 200 s and then removed such that the sample was allowed to recover for an additional 200 s. Tests were conducted in triplicate per batch at each load value for each treatment. The maximum compliance (J_{max}) reached before the load was removed and the maximum recovery (J_r) obtained after the load was removed were recorded from each test. Percent creep recovery (crp) was calculated using the equation from Brown *et al.* (2003).

$$crp = \frac{J_{max} - J_r}{J_{max}} \times 100 \qquad \text{(Eq. 4.1)}$$

$$J_r = J_{max} - J_{min} \quad \text{(Eq. 4.2)}$$

where J_{max} was achieved after 200 s of creep, and J_{min} was achieved after 200 s of recovery.

4.4.1.3. Frequency Sweeps

Frequency sweeps were conducted on Batch 1 treatments from 0.01 to 10 Hz. at 150 Pa. The frequency sweep was repeated on a single sample at 10, 15, 20, 25, and again at 10C. For Batch 2 treatments, frequency sweeps were prolonged and run from 0.001 to 10 Hz. Due to time constraints, one frequency sweep was conducted per cheese treatment per batch.

4.4.2. Large Strain Rheological Tests

A one-cycle compression test was performed to determine the structural changes of cheese at deformations beyond the linear viscoelastic region and prior to fracture; the method was adapted from that of Rogers *et al.* (2010) and van den Berg *et al.* (2008). Cheese was sealed in plastic storage bags to prevent moisture loss and allowed to equilibrate to room temperature (22 ± 2C) for 12 h. Six cheese cylinders were removed per treatment using a 15.6 mm diameter cork borer and cut to a length of 17 mm. Samples were removed from the interior of the block to account for any moisture loss at the block edge. Each cheese cylinder was uniaxially compressed by 20% of the initial height (i.e. from 17 mm to 13.6 mm), corresponding to a true strain (ε_H) of 0.18. Compression was conducted using an Instron 5565 universal testing machine (Instron; Norwood, MA) and flat plates coated with mineral oil to prevent friction. Moving at a

rate of 50 mm/min, the top plate compressed the cheese cylinder until the target strain was reached and then subsequently reversed direction at the same rate to allow for recovery. The area under the resultant force-deformation curve was calculated using Simpson's Rule. Percent recoverable energy (RE) was then calculated as a ratio of the area under the second half of the curve (a_2, work recovered from decompression) over the first half of the curve (a_1, work to compress).

$$RE = \frac{a_2}{a_1} \times 100 \qquad \text{(Eq. 4.3)}$$

4.5. Statistical Analysis

All statistical analysis was conducted using SAS statistical software (v.9.2, SAS Institute Inc.; Cary, NC). Internal replicates were averaged, and the averages from each batch were analyzed using a univariate linear model in which treatment and batch effects were additive. Data were fit for each response separately using a randomized complete block design of the following form:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + (\alpha\beta\gamma)_{ijk} + B_l + E_{ijkl}$$

Term = overall mean + $(\text{filler type})_i$ + $(\text{filler volume})_j$ + $(\text{age})_k$ + $(\text{filler type x volume})_{ij}$ + $(\text{filler volume x age})_{jk}$ + $(\text{filler type x volume x age})_{ijk}$ + $(\text{batch})_l$ + error_{ijkl}.

Tukey's test was used to determine which means were significantly different at $\alpha = 0.05$.

5. RESULTS AND DISCUSSION

5.1. Selection of Filler Particles and Volumes and Time Points

In order to eliminate the effects of particle orientation, filled gel mathematical models assume spherical filler particles (Kerner 1956; van der Poel 1958). Therefore,

Sephadex beads were selected because they are perfectly spherical and stable in water, salt solutions, organic, and denaturing solvents, according to the manufacturer. Furthermore, Sephadex is available in controlled size brackets and varying rigidities, which is determined by the density of cross-linking within the bead matrix. G-50 Sephadex was chosen because it was an intermediate of the "stiff" (G-25, G-50, and G-75) beads tested by Brownsey *et al.* (1987) and was sure to exhibit reinforcing effects. According to the manufacturer, the G-50 bead regains 5.0 g water per g dry gel.

Filler volumes were chosen to correspond to commercial full-fat (FF), reduced-fat (RF), and low-fat (LF) cheese. According to FDA regulation, full-fat cheddar cheese must legally contain at least 30.5% (w/w) milkfat (CFR 21 [101.113]). Reduced-fat cheese contains at least 25% less fat than the full-fat counterpart, and low-fat cheese contains 3 g of fat or less per reference amount (CFR 21 [101.62b]). Therefore, if the standard cheddar contains ~31% fat (w/w), the counterpart reduced-fat cheese would contain less than 24% fat (w/w), and the counterpart low-fat cheese would contain less than 10% fat (w/w), although 6% fat is the usual US standard (Johnson *et al.* 2009). Therefore, filler volumes of 33, 16, and 6% were selected for this project to mimic commercial cheeses and ensure a reinforcing effect amongst the control cheeses.

Finally, cheese was tested at 8, 12, and 18 weeks of age because some material properties have been shown to be sensitive to aging at or near these time points (Rogers *et al.* 2010). Moreover, these time points are reflective of younger cheeses that consumers might purchase off grocery store shelves if one assumes that mild cheddar

cheese is aged at least 100 d (Amantea *et al.* 2006). Furthermore, it is assumed that, at these ages, the curds have "knit" into a homogenous cheese mass because it takes only 14 d of ripening and hydrolysis of one bond in 20% of the α_{s1}-caseins to form a smooth mass (Creamer and Olson 1982), and homogeneity is an important criterion for rheological and mechanical testing.

5.2. Statistics

Table 4.3 shows the main effects and interactions that explained the most variability amongst treatment means. Filler type and volume were significant across most parameters, but age was only significant for RE. Therefore, values from 8, 12, and 18 weeks of ages were averaged together for all parameters except RE. Under this statistical model, a significant difference did exist between batches, but it was assumed that these differences were indicative of the innate variability of cheese manufacture and any errors associated with sampling and mechanical testing. Therefore, both batches were still viewed as "like" treatments, and data from each batch was averaged. Such averaging is justified because t-tests run on internal replicates did not reveal significant differences between batches. The statistical model revealed batch interactions because it was based on averages of those internal replicates.

5.3. Cheese Appearance and Composition (Proximate Analysis)

All treatments looked uniform, but Sephadex-filled cheeses were characterized by a much darker, deeper orange color than the control cheese due to the difference in light-scattering properties of Sephadex beads versus milkfat. The size of the Sephadex particle

did not affect the observed color, however. Cheesemakers also observed that Sephadex-filled cheeses were taller in height, either because they retained more moisture (perhaps in the beads) or because they compressed less.

The actual composition of each treatment is reported in Table 4.4. The salt content and pH were relatively constant across all treatments; consistency in the latter is particularly important because pH significantly impacts cheese structure and breakdown. For instance, Creamer and Olson (1982) found that cheddar cheese made at pH 5.40 required 1.5 times as much force as cheese made at pH 4.88 to compress the cheese to their respective yield points, and they attributed this to the difference in casein micelle cluster size. Watkinson *et al.* (2001) measured rheological and fracture properties of a model cheese that was made by directly acidifying the milk (rather than using a starter culture) and curd. They found that increasing the pH by only 0.2 units caused fracture strain (resistance to crumbling), fracture stress (firmness), and fracture area (toughness) to significantly increase within the range of pH 5.20-5.80.

The control cheeses matched the target filler values reasonably closely. Although the FF control contained only 29% as compared to the targeted 33%, work by Rogers *et al.* (2010) showed the two fat contents exhibited similar rheology and similar degrees of reinforcement to the composite network. As indicated by the standard deviations, the fat content varied somewhat between batches, particularly for the RF Sephadex treatments, but the fat content amongst treatments in each batch was similar. The goal when making particle-filled cheeses was to minimize fat content as much as possible such that the

Sephadex beads would be the only filler particle and the fat would have a negligible filler effect; this goal was largely achieved.

Protein content was not measured directly, but Table 4.4 contains a rough estimate of protein content that was calculated by mass balance (i.e. 100 - salt - moisture - fat - dry Sephadex = %protein). Note that this estimate for protein may also include the mass of ash, breakdown compounds, etc., and this estimate also includes errors associated with measuring the other proximates. Moisture was located in both the hydrated Sephadex beads and the protein network, which is why the moisture-to-protein ratio was greater for Sephadex treatments. Even with the variability associated with the estimated protein content and moisture distribution, the moisture-to-protein content was relatively constant, which is important because it implies that the protein density was constant throughout each treatment.

5.4. Microscopy

Sephadex beads neither absorbed dye nor reflected light and were therefore easily viewed via CSLM. No differences were observed in the fat or protein phase on the basis of age on position from cheese (outside edge versus inside core). Magnification of 40X (Fig. 4.1) was used to search for any abnormalities or potential interactions between the fat, protein, and bead, but none were observed. Treatments were better viewed at 10X magnification in order to view the spread of beads over a larger field. The bright line in the protein phase of the FF treatment (indicated by white arrow in Fig. 4.2) has previously been associated with the knitting of adjacent curd particles (Lopez *et al.* 2007;

Rogers *et al.* 2010), a process that largely excludes milkfat (Kaleb *et al.* 1982). Sephadex beads were identified in images on the basis of their size, perfect spherical dimensions, lack of color (did not absorb dye), and absence from control images (Fig. 4.2). Pre-hydration bead diameters were obtained from the manufacturer, but CSLM images were used to determine an average bead diameter post-hydration (Table 4.5). The change in average diameter after hydration indicates that the beads obviously swelled, but it is important to note the large range of diameters observed. These measurements are merely the diameters of the two-dimensional images and not the true diameter of the sphere at its largest girth. During both sample preparation and imaging, the spherical beads were sliced. If the sphere were sliced near one of its ends (imagine cutting an orange from the outside and working towards the center), the resultant 2D circle would have a much smaller diameter than the sphere. Hence, the large range in diameters was due to both variation in sphere sizes and variation in "slice" position.

The percent area occupied by a 2D Sephadex slice is proportional to the percent volume occupied by a sphere of equivalent diameter (Russ 2005). Table 4.6 shows that volume of filler calculated from microscopy images matched target volumes for LF treatments but probably under-estimated the percentage of beads in the cheese for FF treatments. It was assumed this was an underestimation because a low concentration of dry matter was measured in the cheese whey (see Chapter 5 for discussion of an alternate method that was used to determine bead volume in the cheese by which the beads in the excluded whey fraction were measured by centrifugation, drying, and mass balance). If a

large volume of Sephadex beads had been lost from the cheese to the whey, then the dry matter content of the whey would have been greater than that of "typical" cheddar cheese, but that effect was not observed (Chapter 5). Thus, the underestimation resulting from image analysis of FF treatments is likely due to both the bead slice position issue that was already mentioned and the large degree of bead overlap seen in many images; Fig. 4.3 shows some characteristic examples of such overlap.

Given (1) the good agreement between target/predicted filler volume and microscopy for LF and, to some extent, RF treatments and (2) the minimal dry matter content of the whey, it was assumed that the target Sephadex filler volumes were achieved. This assumption is similar to that by Madziva *et al.* (2006), who used light microscopy to approximate recovery of alginate-pectin capsules in cheddar cheese. However, both Sephadex beads and milkfat act as filler particles. Therefore, all figures and analysis were based on *total filler volume*, the sum of Sephadex bead and milkfat volumes as determined by target values and proximate analysis (Table 4.4), respectively.

5.5. Rheological Tests

5.5.1. Controlled-stress Tests

5.5.1.1. Stress Sweeps

The critical stress (Fig. 4.4) and strain values (4.5) were determined from stress sweeps and mark the end of the linear viscoelastic region (LVR) for rheological testing. This critical point can be considered the level of stress and strain where damage or long term relaxations take place in the network; larger critical strain values signify a longer

LVR. As previously mentioned, age had no significant effect, so critical points from each age were averaged together. Filler type did have a significant effect on critical stress, however. Specifically, the control cheeses yielded at smaller forces, as evidenced by the lower critical stress when compared against any Sephadex treatment. This suggests that Sephadex beads and milkfat differ in elasticity, which was expected since the G-50 Sephadex series was considered "stiff" amongst the other Sephadex treatments (Brownsey *et al.* 1987). Similar trends were observed regardless of filler type, however, highlighting the importance of filler volume as a primary effect. As filler volume increased above 25% within each treatment, the critical stress increased. No statistical difference was observed between LF and RF volumes for any filler type, and little difference was seen as filler volume increased above 25%. The lack of significant trends between critical stress and filler volume agrees with previous observations (Chapter 3). Rogers *et al.* (2010) found that critical stress tended to decrease with increasing milkfat volume, but however, the pH of the cheese was greater than the pH of cheeses in this study, and the importance of pH on cheese microstructure and rheology has already been mentioned. Also, the LF cheese in that study was made from milk that was preacidified to pH 6.3 to reduce the calcium content and soften the cheese by holding more moisture, which is the standard strategy for making LF cheese. In contrast, this study did not use pre-acidification in order to maintain similar protein-protein interactions at each fat level. The pH of the commercial cheese used in Chapter 3 during manufacturing was, of course,

unknown. More importantly, this study showed that when pH was maintained at 5.0-5.2, the type of filler did not impact critical stress.

Critical strain (Fig. 4.5) also did not vary with filler type; milkfat and Sephadex beads performed similarly. Again, LF and RF treatments were statistically similar; FF was significantly lower in critical strain, implying that deformability decreased as filler volume increased. That suggests that filler particles—regardless of type—disrupt the protein network, disrupting its continuity and hindering its ability to linearly deform. These results agreed with those of previous filled gel work that showed filler particles decrease critical strain because the particles cause stress concentration (Sala *et al.* 2009).

5.5.1.2. Creep/recovery Tests

Stress tests are useful for probing material responses at an instantaneous time scale, whereas creep tests probe material responses at a longer time scale—200 seconds of applied load and 200 seconds for recovery, in this case. As in the previous chapters, J_r and %crp showed no consistent trends with either filler type or volume (data not shown). Maximum compliance (J_{max}) is the deformation over a certain load after 200 s of creep, and it did differentiate samples on the basis of filler type and phase volume, although, like the previous chapters, load had no statistical difference. Therefore, results are only shown for J_{max} at 500 Pa (Fig. 4.6). As seen with critical stress, the RF and LF treatments did not differ significantly within a single filler type. However, as phase volume increased above 25%, J_{max} decreased, indicating the FF samples were firmer. Decreasing J_{max} has been significantly related to increases in sensory firmness (Brown *et al*. 2003).

Lozinsky *et al.* (1992) also observed the same volume-dependent results upon adding Sephadex beads of varying rigidities and sizes to poly(vinyl alcohol)-based cryogels, which are highly porous, hydrophilic, thermoreversible gels. Creep compliance is inversely related to the complex modulus, G*. Therefore, the decreasing J_{max} values as phase volume increased implies a reinforcing effect as the volume of active filler increases; such results are described well by the filled gel model. Again, the extent of this reinforcing effect was influenced by the filler material.

5.5.1.3. Frequency Sweeps

Frequency sweeps were performed on every cheese treatment, but the trends seen in Fig. 4.7 are representative of those observed in all samples. Frequency sweeps give insight on the network viscoelasticity and structure. As per the method for the frequency sweeps, temperatures were increased, and samples were allowed to equilibrate at each new temperature for five minutes in a sealed oven chamber on the rheometer; testing ended with a cool-down period and final frequency sweep at 10C. In the control samples, the 10C curves obtained after the cool-down do not overlay the initial 10C obtained as temperatures increased. The difference between these curves is known as hysteresis, and it was greatest at low frequencies. These results are consistent with melting of the fat crystalline phase. Milkfat has a wide compositional range (one-third is unsaturated fat; the other two-thirds are fatty acids from C4:0 to C18:0) and consequently melts over a wide range from approximately -30C to +40C (Walstra 2003). The melting or softening of milkfat crystals decreases the mass of the solid crystalline phase, thereby making the

cheese less solid-like. This perceived "softening" is reflected by a decrease in the storage modulus (G') and produces hysteresis between the two 10C curves. Such hysteresis was also observed in the off-the-shelf commercial cheeses of Chapter 2. Unlike milkfat, however, Sephadex beads do not melt. Therefore, there is no perceptible hysteresis between the two 10C curves on the Sephadex graphs. The storage modulus decreased as temperature increased because hydrogen bonding weakens as temperature increases, thereby allowing the protein matrix to relax somewhat. All samples were characterized by a U-shaped change in phase angle with frequency. This has been observed in other protein gels such as bovine serum albumin (Clark and Lee-Tuffnell 1986).

The reinforcing effect characteristic of active fillers is clearly demonstrated in Fig. 4.8. Regardless of filler type, the FF treatments exhibit the greatest storage modulus; both milkfat and Sephadex beads are active fillers in a cheese system. This is most apparent at 10C. For fine and medium-sized beads, there is little differentiation between the LF and RF treatments. The FF control shows the greatest decline in modulus with increasing temperature, which likely reflects the melting/softening fat phase. Although maximum compliance and critical stress values suggested that Sephadex beads may be more rigid than milkfat and capable of exerting a larger reinforcing effect, Fig. 4.8 shows that milkfat actually exerted a greater reinforcing effect at 10 Hz when the temperature was low and the phase volume high (e.g. 25%). However, that was not the case at lower frequencies (Fig. 4.7). Such frequency-dependency is also illustrated by the U-shaped phase angle seen in Fig. 4.7. Thus, the reinforcing effect, which is consistent with the

filled gel model, was apparent for both types of filler particles, but the exact magnitude of reinforcement varied with temperature, phase volume, and frequency of test.

5.5.2. Large Strain Uniaxial Compression

No sample barreling was observed during compression testing, indicating that frictional effects were minimal and the application of stress and strain was evenly applied throughout the sample (Culioli and Sherman 1976; Carter and Sherman 1978). Percent recoverable energy (RE) has been shown to differentiate gels of variable texture and microstructure because it elucidates whether any part of the network is losing energy due to frictional effects or viscous flow (van Vliet and Walstra 1995). In Chapters 2 and 3, this test was shown to highly differentiate commercial cheddar cheeses of varying fat contents. Here, we see that the test again differentiated samples on the basis of filler volume (Fig. 4.9), but the Sephadex beads exhibited the exact opposite trend of the milkfat control. Whereas control cheeses decreased in RE with increasing phase volume, Sephadex treatments increased in RE with increasing phase volume. This is logical considering that Sephadex beads are an elastic polymer network while milk fat is in various degrees of liquid and crystalline states, and the liquid state is very inelastic. Energy recovery can be modeled (van Vliet and Walstra 1995) as:

$$W = W_e + W_{d,v} + W_{d,e} + W_f$$

where W = total deformation energy

W_e = elastic storage

$W_{d,v}$ = viscoelastic dissipation

$W_{d,e}$ = frictional dissipation, and

W_f = fracture.

Energy storage in the protein network should be consistent for all filler types at a single filler volume assuming there is no difference in the nature of the gel network. Therefore, W_e would vary most with filler viscoelasticity. Since Sephadex-filled cheeses recovered more energy, we conclude that Sephadex beads are more elastic than milkfat. Increasing the volume of elastic fillers clearly increased percent RE. Interestingly, Sephadex treatments were statistically different from each other, and these differences seemed to correlate with bead size: treatments increased in recoverable energy as filler particle size increased.

As mentioned, RE has been highly correlated to sensory properties; particles with too much elasticity have unfavorable sensory scores with regards to cheese (Rogers *et al.* 2010). Even though Sephadex and control treatments exhibited similar trends in small strain rheology, the results depicted in Fig. 4.9 show that elasticity determined at strains greater than the LVR but less than fracture is a key property for developing cheese of acceptable texture. RE must be consistent for LF and FF treatments in order to optimize texture.

5.6. Effect of Age, Filler Type, and Filler Size

Age had no significant effect on any of the properties determined within the LVR; however, testing beyond the LVR showed that RE decreased with age; this decrease was more evident in LF and RF treatments than FF treatments (Fig. 4.10), which implies that

the protein network was changing with age. Proteolyis is caused by microbial and enzymatic activity in the cheese (Fox *et al*. 2004). Age probably did not affect the other tests—which were all small strain in comparison to the large strain uniaxial test—because large and small strain tests measure different responses in the material and may therefore have different sensitivities to the effects of aging.

In summary, the Sephadex beads were more rigid or firmer than the milkfat globules. That caused the control to be significantly different from all of the Sephadex treatments in the case of critical stress and RE. However, effects from the type of filler were confounded with filler size for J_{max} and RE. Milkfat globules are orders of magnitude smaller than the Sephadex beads (Table 4.5). Nevertheless, controls were significantly similar to the Fine and SF beads in the case of critical strain, J_{max} at both 100 and 500 Pa, J_r at both 100 and 500 Pa (data not shown), and percent creep recovery at both 100 and 500 Pa (data not shown). However, the largest beads, the Medium Sephadex treatment, were often either significantly different from all other treatments or similar to only the next closes bead in size, the Fine treatment. This suggests that bead size may affect the rheology of filled gels, although there appears to be some kind of threshold size at which these effects are noticeable. Previously studies have published mixed results on filler particle size. For instance, Brownsey *et al.* (1987) found no size effects from Sephadex beads, but Lozinsky *et al.* (1982) found that creep compliance decreased as Sephadex bead diameter increased, although bead diameter did not affect the compliance modulus. Lozinsky's results match those of this study in that respect.

Ross-Murphy and Todd (1983) studied gelatin gels filled with glass particles of different shapes and found that both shape and volume impact rheology. Using a fruit jam matrix, however, Genovese *et al.* (2010) found that the size of pectin particles did not affect the rheology of that filled gel. As previously mentioned, the filled gel model assumes filler particles are spherical, and it accounts only for filler volume, not shape or size. The mixed results of published filled gel studies make it difficult to reach any definite conclusions about the effect of particle size on filled gel rheology.

6. CONCLUSION

Within and beyond the linear viscoelastic region, milkfat controls and Sephadex-filled cheeses exhibited similar behavior that closely corresponded to the results predicted by the filled gel model. Thus, we conclude that cheddar cheese, much like simpler biopolymer systems, can accurately be modeled as a filled gel. This mechanistic understanding of cheese rheology gives researchers and product developers an important tool for manipulating and developing new cheeses. Many commercial cheeses today are available in 33% reduced fat varieties, and research indicates consumer groups find these RF cheeses acceptable (Drake and Swanson 1995; Childs and Drake 2009). However, commercial LF cheeses are disliked for their different flavors, odors, and textures, and consumer groups are unwilling to compromise on those attributes despite potential health benefits (Childs and Drake 2009). This filled gel research suggests that a LF cheese with texture very similar to its FF counterpart could be developed by reducing the fat one-third

and substituting alternate filler particles. The ideal substitute would melt or soften at body temperature, much like a full-fat cheese. We recommend further research into food materials that melt and match the rigidity of milkfat.

However, pH and ionic strength are other variables in cheese manufacture with which developers must contend. The casein network, like all proteins, is susceptible to changes in pH and other charged particles. The Sephadex beads in this study had no charge since milkfat also carries no ionic charge. Therefore, more work is needed using charged particles to further investigate the filler effects.

7. REFERENCES

AHMED, S. and JONES, F.R. 1990. A review of particulate reinforcement theories for polymer composites. J. Mater. Sci. *25*, 4933-4942.

AMANTEA, G.F., SKURA, B.J., NAKAI, S. 1986. Culture effect on ripening characteristics and rheological behavior of cheddar cheese. J. Food Sci. *51*, 912-918.

BROWN, J.A., FOEGEDING, E.A., DAUBERT, C.R., DRAKE, M.A. and GUMPERTZ, M. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. *86*, 3054-3067.

BROWNSEY, G.J., ELLIS, H.S., RIDOUT, M.J. and RING, S.G. 1987. Elasticity and failure in composite gels. J. Rheol. *31*, 635-649.

CARTER, E.J.V. and SHERMAN, P. 1978. Evaluation of firmness of Leicester cheese by compression tests with Instron universal testing machine. J. Texture Stud. *9*, 311-324.

CHEN, J. and DICKINSON, E. 1998a. Viscoelastic properties of protein-stabilized emulsions: effect of protein-surfactant interactions. J. Agric. Food Chem. *46*, 91-97.

CHEN, J. and DICKINSON, E. 1999. Effect of surface character of filler particles on rheology of heat-set whey protein emulsion gels. Colloids Surf., B *12*, 373-381.

CHILDS, J.L. and DRAKE, M.A. 2009. Consumer perception of fat reduction in cheese. J. Sens. Stud. *24*, 902-921.

CLARK, A.H. and LEE-TUFFNELL, C.D. 1986. Gelation of globular proteins. In *Functional Properties of Food Macromolecules* (J.R. Mitchell and D.A. Ledward, eds.) pp 216-224, Elsevier Applied Science, London, UK.

CREAMER, L.K. and OLSON, N.F. 1982. Rheological evaluation of maturing cheddar cheese. J. Food Sci. *47*, 631-636, 646.

CULIOLI, J. and SHERMAN, P. 1976. Evaluation of gouda cheese firmness by compression tests. J. Texture Stud. *7*, 353-372.

DRAKE, M.A. and SWANSON, B.G. 1995. Reduced- and low-fat cheese technology: a review. Trends Food Sci. Technol. *6*, 366-369.

FOX, P.F., MCSWEENEY, P.L.H., COGAN, T.M. and GUINEE, T.P. 2004. *Cheese: Chemistry, Physics and Microbiology—Volume 1*, 3rd ed. Elsevier, London, UK.

GENOVESE, D.B., YE, A.Q. and SINGH, H. 2010. High methoxyl pectin/apple particles composite gels: effect of particle size and particle concentration on mechanical properties and gel structure. J. Texture Stud. 41, 171-189.

GUINEE, T.P., AUTY, M.A.E., MULLINS, C., CORCORAN, M.O. and MULHOLLAND, E.O. 2000. Preliminary observations on effects of fat content and degree of fat emulsification on the structure-functional relationship of Cheddar-type cheese. J. Texture Stud. *31*, 645-663.

GWARTNEY, E.A., LARICK, D.K. and FOEGEDING, E.A. 2004. Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. J. Food Sci. *69*, S333-S339.

JOHNSON, M.E., KAPOOR, R., MCMAHON, D.J., MCCOY, D.R. and NARASIMMON, R.G. 2009. Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. Compr. Rev. Food Sci. Food Saf. *8*, 252-268.

KALAB, M., LOWRIE, R.J. and NICHOLS, D. 1982. Detection of curd granule and milled curd junctions in Cheddar cheese. J. Dairy Sci. *65*, 1117-1121.

KERNER, E.H. 1956. The elastic and thermo-elastic properties of composite media. Proc. Phys. Soc., London, Sect. B. *8*, 808-813.

LANGLEY, K.R., GREEN, M.L. and MARTIN, A. 1990. Rheological and structural properties of model composite foods. In *Third European Rheology Conference and Golden Jubilee Meeting of the British Society of Rheology* (D.R. Oliver, ed.) pp 301-303, Elsevier Applied Science, London, UK.

LAWRENCE, R.C., CREAMER, L.K. and GILLES, J. 1987. Texture development during cheese ripening. J. Dairy Sci. *70*, 1748-1760.

LOPEZ, C., CAMIER, B. and GASSI, J.Y. 2007. Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. Int. Dairy J. *17*, 235-247.

LORET, C., FRITH, W.J. and FRYER, P.J. 2007. Mechanical and structural properties of maltodextrin/agarose microgels composites. Appl. Rheol. *17*, 31412.1-31412.13.

LOZINSKY, V.I., ZUBOV, A.I., KULAKOVA, V.K., TITOVA, E.F. and ROCOZHIN, S.V. 1992. Study of cryostrudurization of polymer systems. IX. Poly(vinyl alcohol) cryogels filled with particles of crosslinked dextran gel. J. Appl. Polym. Sci. *44*, 1423-1435.

MADZIVA, H., KAILASAPATHY, K. and PHILLIPS, M. 2006. Evaluation of alginate-pectin capsules in cheddar cheese as a food carrier for the delivery of folic acid. LWT—Food Sci. Technol. *39*, 146-151.

MARSHALL, R.T. 1992. *Standard Methods for the Examination of Dairy Products*. American Public Health Association, New York, NY.

ROGERS, N.R., DRAKE, M.A., DAUBERT, C.R., MCMAHON, D.J., BLETCH, T.K. and FOEGEDING, E.A. 2009. The effect of aging on low-fat, reduced-fat, and full-fat cheddar cheese texture. J. Dairy Sci. *92*, 4756-4772.

ROGERS, N.R., MCMAHON, D.J., DAUBERT, C.R., BERRY, T.K. and FOEGEDING, E.A. 2010. Rheological properties and microstructure of cheddar cheese made with different fat contents. J. Dairy Sci. *in press*.

ROSS-MURPHY, S.B. and TODD, S. 1983. Ultimate tensile measurements of filled gelatin gels. Polymer. *24*, 481-486.

RUSS, J.C. 2005. *Image Analysis of Food Microstructure*. CRC Press, Boca Raton, FL.

SALA, G., VAN VLIET, T., STUART, M.C., VAN DE VELDE, F. and VAN AKEN, G.A. 2009. Deformation and fracture of emulsion-filled gels: effect of gelling agent concentration and oil droplet size. Food Hydrocolloids *23*, 1853-1863.

VAN DEN BERG, L., CAROLAS, A.L., VAN VLIET, T., VAN DER LINDEN, E., VAN BOEKEL, M.A.J.S. and VAN DE VELDE, F. 2008. Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. Food Hydrocolloids *22*, 1404-1417.

VAN DER POEL, C. 1958. On the rheology of concentrated dispersions. Rheol. Acta *1*, 198-205.

VAN VLIET, T. 1988. Rheological properties of filled gels. Influence of filler matrix interaction. Colloid Polym. Sci. *266*, 518-524.

VAN VLIET, T. and WALSTRA, P. 1995. Large deformation and fracture behavior of gels. Faraday Discuss. *101*, 359-370.

VISSER, J. 1991. Factors affecting the rheological and fracture properties of hard and semi-hard cheese. In *Rheological and Fracture Properties of Cheese* (P. Walstra, ed.) pp. 49-61, Bull. No. 268, International Dairy Federation, Brussels, Belgium.

WALSTRA, P. 1999. Dairy Technology: principles of milk properties and processes. Marcel Dekker, Inc., New York, NY.

WALSTRA, P. 2003. *Physical Chemistry of Foods*. Marcel Dekker, Inc., New York, NY.

WATKINSON, P., COKER, C., CRAWFORD, R., DODDS, C., JOHNSTON, K., MCKENNA, A. and WHITE, N. 2001. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. 2001. Int. Dairy J. *11*, 455-464.

ZHOU, N. and MULVANEY, S.J. 1998. The effect of milk fat, the ratio of casein to water, and temperature on the viscoelastic properties of rennet casein gels. J. Dairy Sci. *81*, 2561-2571.

TABLE 4.1. CHEESE MANUFACTURE

Parameters used converting 16 kg of milk substrate[1] into full fat (FF), reduced fat (RF) and low fat (LF) cheddar cheeses, and their Sephadex-containing counterparts S-FF, S-RF, and S-LF, respectively.

	Cheese					
Parameter	**FF**	**S-FF**	**RF**	**S-RF**	**LF**	**S-LF**
Milk fat, % (wt/wt)	3.5	0.2	1.6	0.2	0.7	0.2
Dry Sephadex added, g/kg milk	-	80	-	45	-	19
Set temperature, ^{o}C	31	31	31	31	32	32
Cooking temperature, ^{o}C	38	38	36	36	36	36
Cooking time, min	25	25	15	15	15	15
Drain pH	6.10	6.30	6.20	6.30	6.20	6.20
Wash water temperature, ^{o}C	-	-	17	19	16	16
Curd temperature, ^{o}C	-	-	27	27	26	26
Set-to-Drain time, min	150	120	135	135	130	130
Salt pH[2]	5.45	5.75	5.80	5.90	5.95	5.95
Drain-to-Salt time, min	70	70	40	55	50	50
Salting, g/kg curd	22	26	25	28	22	26

[1]Milk substrate was standardized milk for the control (FF, RF and LF) cheeses while for the experimental cheeses (S-FF, S-RF and S-LF) it included the skim milk and the Sephadex slurry.

[2]For the first replicate the salting pH of these cheeses was ~ 5.85

TABLE 4.2. TARGET SAMPLING PLAN AND COMPOSITION

Treatment name (and abbreviation)		Primary filler particle[a]	Size of filler particle[b]	Target filler volume (wt/wt)[c]	Target fat volume (wt/wt)[d]	Tested time points (weeks)
Low-fat/filler (LF)	control	milk fat globule	0.1 - 10 µm	6%	6%	8, 12, and 18
	superfine	Sephadex bead	20 - 50 µm	6%	NA	8, 12, and 18
	fine	Sephadex bead	20 - 80 µm	6%	NA	8, 12, and 18
	medium	Sephadex bead	50 - 150 µm	6%	NA	8, 12, and 18
Reduced-fat/filler (RF)	control	milk fat globule	0.1 - 10 µm	16%	16%	8, 12, and 18
	superfine	Sephadex bead	20 - 50 µm	16%	NA	8, 12, and 18
	fine	Sephadex bead	20 - 80 µm	16%	NA	8, 12, and 18
	medium	Sephadex bead	50 - 150 µm	16%	NA	8, 12, and 18
Full-fat/filler (FF)	control	milk fat globule	0.1 - 10 µm	33%	33%	8, 12, and 18
	superfine	Sephadex bead	20 - 50 µm	33%	NA	8, 12, and 18
	fine	Sephadex bead	20 - 80 µm	33%	NA	8, 12, and 18
	medium	Sephadex bead	50 - 150 µm	33%	NA	8, 12, and 18

[a] The source of all milk fat was either skim milk or skim milk + cream. All Sephadex beads were of the G-50 series.
[b] Milk fat size range from Walstra (1999). Sephadex size refers to dry bead (pre-hydration) size as identified on the Sigma-Aldrich website <www.sigmaaldrich.com>.
[c] Sephadex volume is an approximation after accounting for bead hydration and loss of beads to whey stream during cheddaring.
[d] Skim milk provided the only source of fat (~0.2% fat pre-cheddaring) in the Sephadex cheeses.

TABLE 4.3. MAIN FACTOR EFFECTS (FILLER TYPE, FILLER VOLUME, AND TREATMENT AGE) AND INTERACTIONS FOR RHEOLOGICAL ATTRIBUTES OF COMMERCIAL CHEDDAR CHEESE

	Critical stress	Critical strain	J_{max} (100 Pa)	J_r (100 Pa)	crp (100 Pa)	J_{max} (500 Pa)	J_r (100 Pa)	crp (500 Pa)	RE
Filler type	**0.0003**	0.1696	**0.0009**	**0.0385**	0.0643	**<.0001**	**0.0101**	**0.0049**	**<.0001**
Volume	**0.0053**	**<.0001**	**<.0001**	**<.0001**	0.8301	**<.0001**	**<.0001**	0.7457	**0.0124**
Age	0.5909	0.3179	0.3110	0.8847	0.4092	0.9438	0.9060	0.1367	**<.0001**
Filler type*Volume	0.0825	0.9980	**0.0368**	0.1306	0.2503	0.1660	0.6352	0.1741	**<.0001**
Filler type*Age	0.8062	0.8550	0.9591	0.8302	0.7632	0.8824	0.7971	0.5393	0.8039
Volume*Age	0.7274	0.5408	0.1246	0.0634	**0.0360**	0.1376	0.2767	0.8578	0.3483
Filler type*Volume*Age	0.4766	0.9964	0.9812	0.5425	0.5187	0.9988	0.8869	0.0822	0.9829
Batch	**<.0001**	**0.0003**	**0.0022**	0.9001	**<.0001**	**0.0013**	0.7470	**<.0001**	**0.0004**

Bolded terms were significant at $p<0.05$.

TABLE 4.4. PROXIMATE ANALYSIS OF TREATMENTS

Treatment name		% Moisture[a] (w/w)	% Salt[a] (w/w)	% Fat[a,b] (w/w)	pH[a]	% Protein[c] (w/w)	Moisture: Protein Ratio
LF	control	53.6 ± 0.8	1.9 ± 0.3	6.3 ± 1.1	5.0 ± 0.1	38.3 ± 1.6	1.38 ± 0.0
	superfine	58.9 ± 0.8	2.0 ± 0.3	1.5 ± 0.7	5.0 ± 0.2	36.7 ± 1.3	1.59 ± 0.0
	fine	57.5 ± 0.7	1.9 ± 0.0	1.8 ± 1.1	5.1 ± 0.2	38.0 ± 1.5	1.50 ± 0.0
	medium	56.5 ± 0.1	1.9 ± 0.3	1.5 ± 0.7	5.1 ± 0.1	39.2 ± 0.3	1.44 ± 0.0
RF	control	49.5 ± 2.0	1.9 ± 0.0	16.0 ± 0.7	5.2 ± 0.1	32.6 ± 2.7	1.47 ± 0.1
	superfine	57.3 ± 3.9	2.0 ± 0.3	4.0 ±2.8	5.1 ± 0.2	34.6 ± 1.3	1.58 ± 0.1
	fine	57.8 ± 0.9	2.1 ± 0.1	3.8 ± 2.5	5.1 ± 0.2	34.3 ± 1.6	1.67 ± 0.0
	medium	55.4 ± 2.2	2.0 ± 0.2	4.8 ± 2.5	5.1 ± 0.2	35.8 ± 0.1	1.51 ± 0.0
FF	control	41.2 ± 0.5	1.9 ± 0.3	29.3 ± 1.1	5.2 ± 0.1	27.6 ± 1.0	1.48 ± 0.0
	superfine	58.7 ± 0.3	2.3 ± 0.0	2.3 ± 1.1	5.0 ± 0.3	33.1 ± 0.7	1.77 ± 0.0
	fine	55.5 ± 0.2	2.3 ± 0.3	2.8 ± 1.1	5.1 ± 0.2	35.8 ± 1.6	1.55 ± 0.0
	medium	53.8 ± 0.4	2.2 ± 0.1	2.8 ± 0.4	5.1 ± 0.4	37.6 ± 0.1	1.42 ± 0.0

[a] Entries represent the average value from two batches ± standard deviation between batches

[b] See Table 4.2 for target fat values

[c] Calculated on a mass balance basis assuming moisture + salt + fat + protein + dry Sephadex = 100%, where dry Sephadex was calculated as 20% of the initial dry weight added divided by a cheese mass of 1746 g (assumes a loss of 87% initial milk mass to whey)

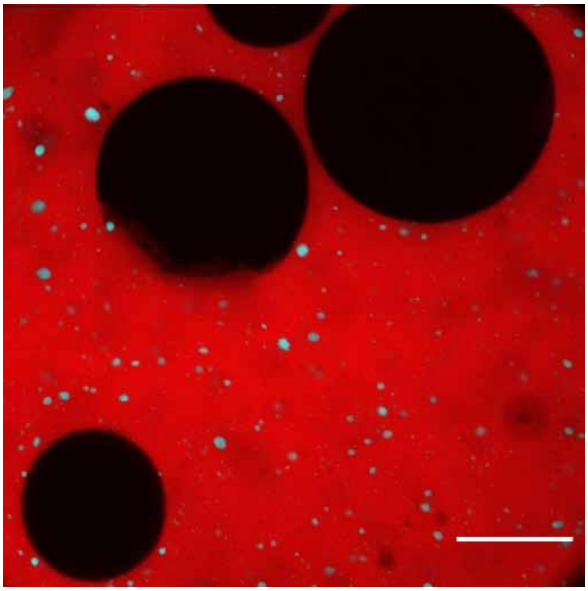


FIG. 4.1. SUPERFINE (SF) SEPHADEX BEADS IMAGED WITH PL FLUOTAR 40.0x1.00 OIL UV OBJECTIVE
Protein phase in red; fat phase in blue; Sephadex beads in black (do not absorb dye). Image (512 x 512 pixels) was taken of a LF treatment at 18 weeks of age. Scale bar is 50 μm.

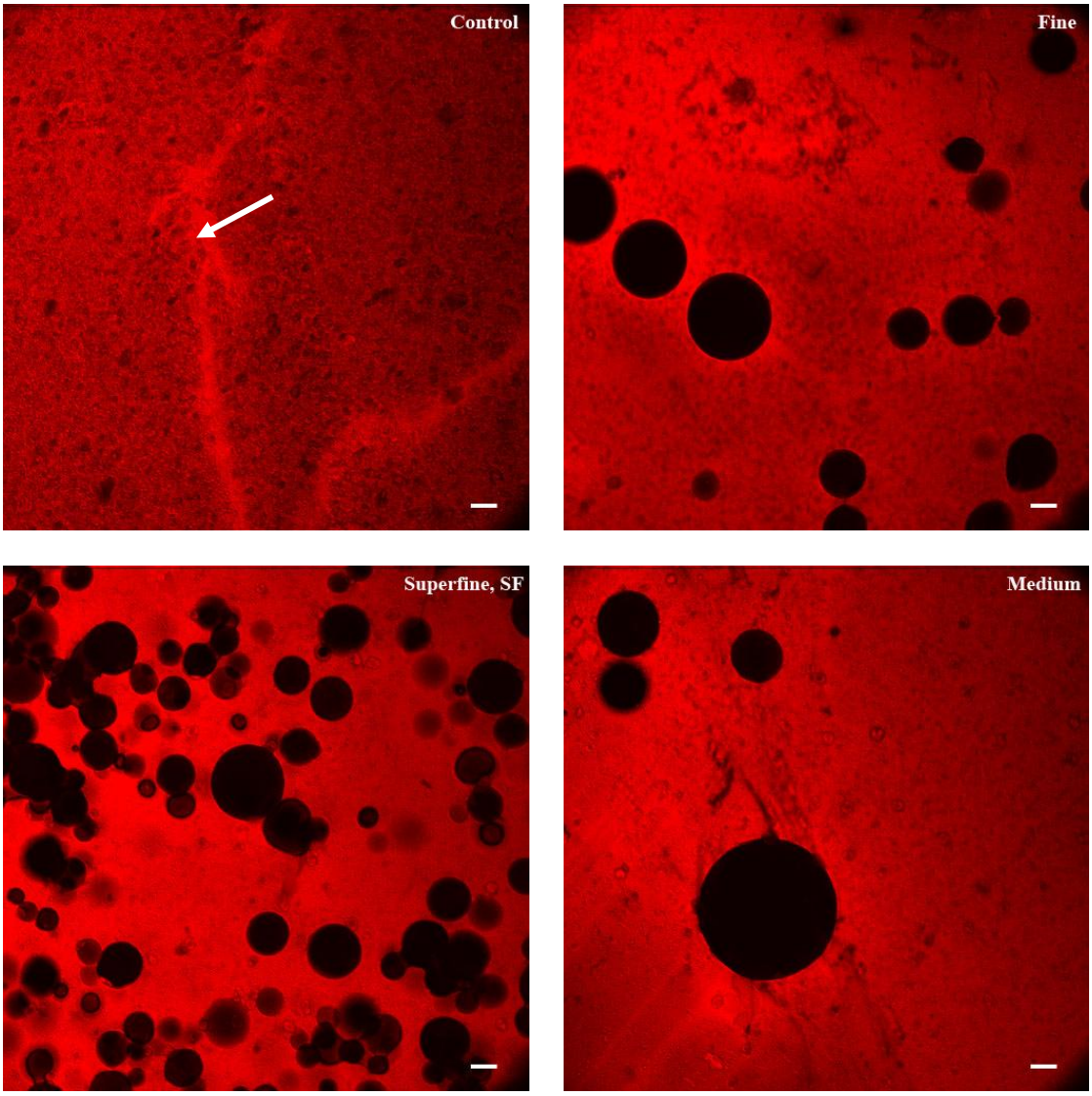


FIG. 4.2. TREATMENTS IMAGED WITH HC PL FLUOTAR 10.0x0.30 OBJECTIVE Protein phase in red; Sephadex beads in black (do not absorb dye). Images (512 x 512 pixels) were taken of FF treatments at 8 weeks of age. Scale bar is 50 μm. White arrow points to an assumed curd line.

TABLE 4.5. RANGE OF SEPHADEX BEAD DIAMETERS IN CHEESE

	SF	Fine	Medium	Milkfat[c]
Dry size[a] (µm	20-50	20-80	50-150	
Range (µm)	25 -98	49 - 186	45 - 311	0.1-10
Mean (µm)	60	103	175	
n[b]	78	79	95	

Bead diameters measured from confocal images (10X magnification) using MetaMorph software

[a] According to manufacturer's literature

[b] Number of individual beads measured from images

[c] Range from Walstra (1999)

TABLE 4.6. AREA OF SEPHADEX BEADS IN 2-D CONFOCAL IMAGES OF CHEESE

	FF		RF		LF	
target filler volume	33%		16%		6%	
	Average bead area[a] (%)	Area overlapping[b] (%)	Average bead area (%)	Area overlapping (%)	Average bead area (%)	Area overlapping (%)
SF	19 ± 1	39	9 ± 0	13	7 ± 1	6
Fine	14 ± 2	12	7 ± 1	1	6 ± 0	3
Medium	16 ± 1	51	15 ± 2	14	9 ± 4	9

[a] Bead area determined using MetaMorph imaging software on images at 10X magnification

[b] % of area in first column that was overlapping and could not be counted as isolated beads (see Figure 4.3)

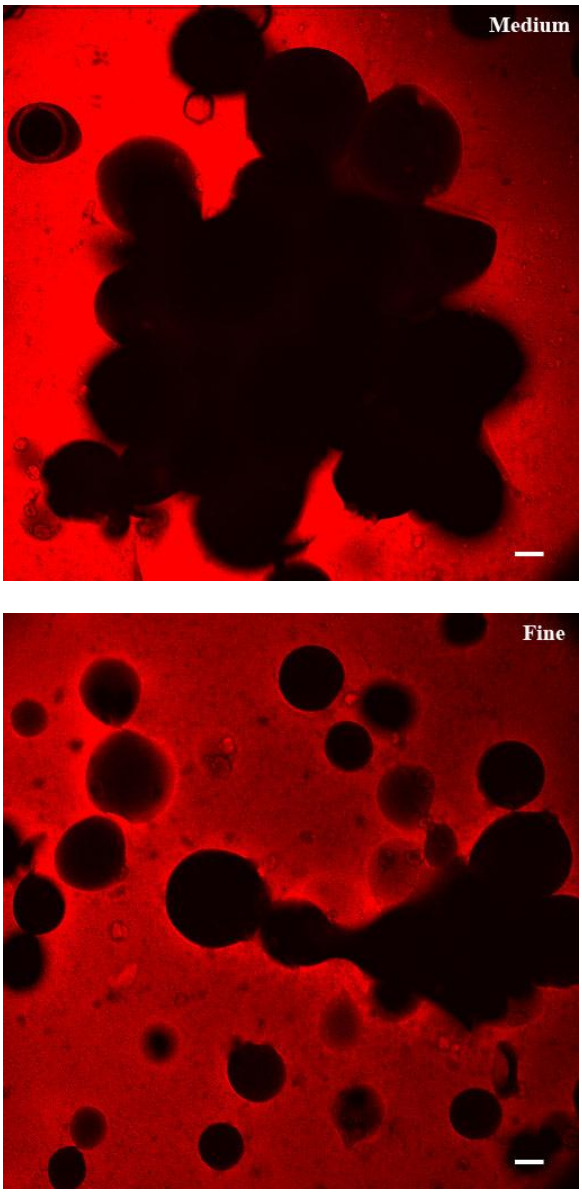


FIG. 4.3. IMAGES OF OVERLAPPING BEADS TAKEN WITH HC PL FLUOTAR 10.0x0.30 OBJECTIVE
Protein phase in red; Sephadex beads in black (do not absorb dye). Image (512 x 512 pixels) was taken of LF treatments at 8 weeks of age. Scale bar is 50 μm.

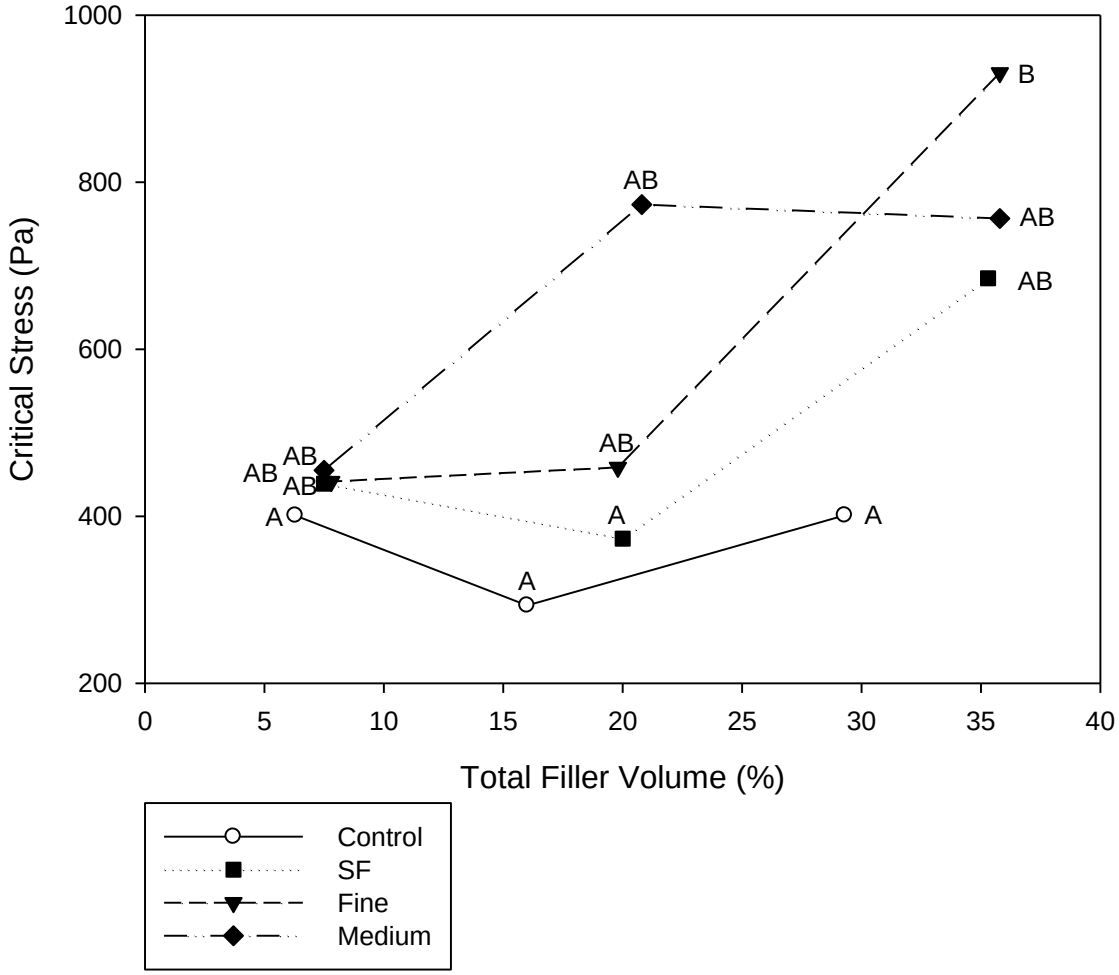


FIG. 4.4. CRITICAL STRESS OF FILLED GEL CHEESES
Data points with different letters were significantly different at $p < 0.05$.

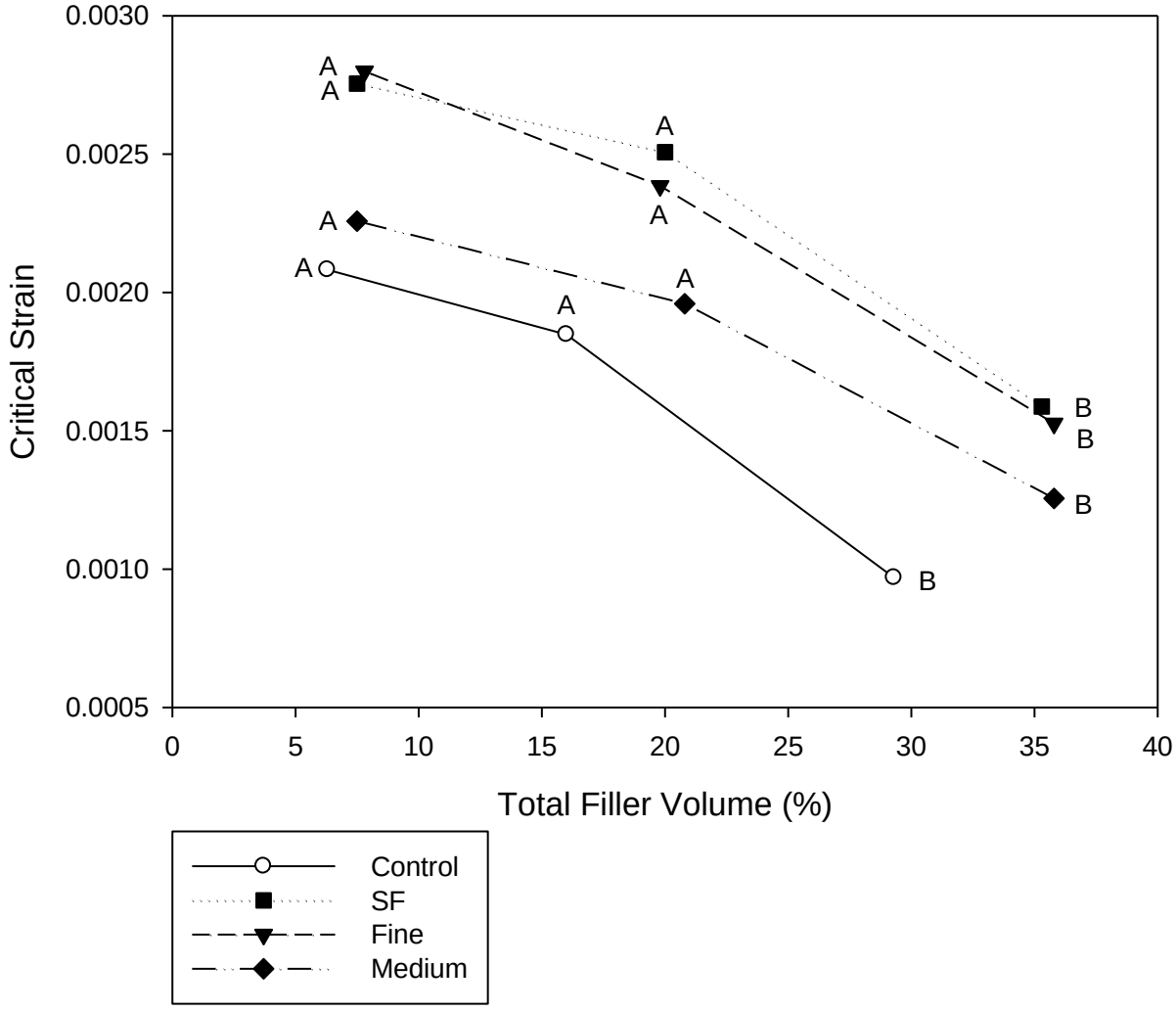


FIG. 4.5. CRITICAL STRAIN OF FILLED GEL CHEESES
Data points with different letters were significantly different at $p < 0.05$.

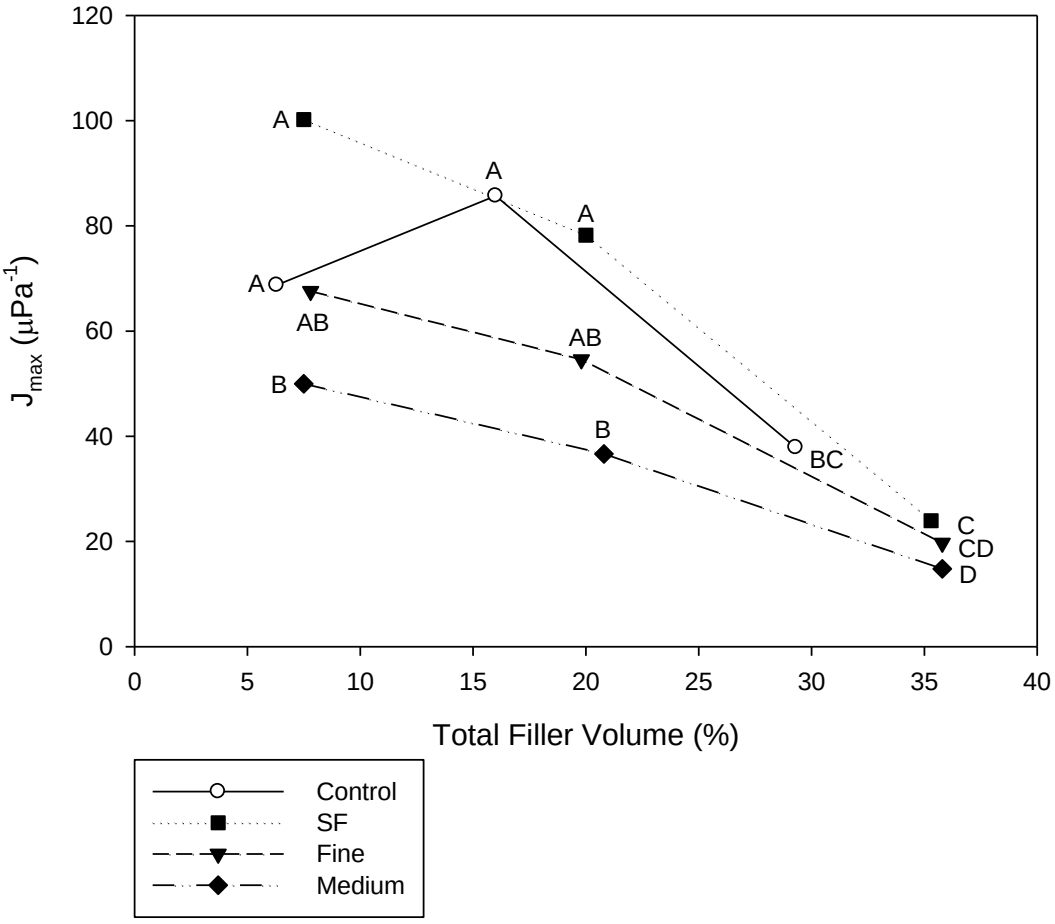


FIG. 4.6. MAXIMUM COMPLIANCE (J_{max}) UNDER 500 Pa LOAD
Maximum compliance determined after 200 s of creep. Data points with different letters were significantly different at $p < 0.05$.

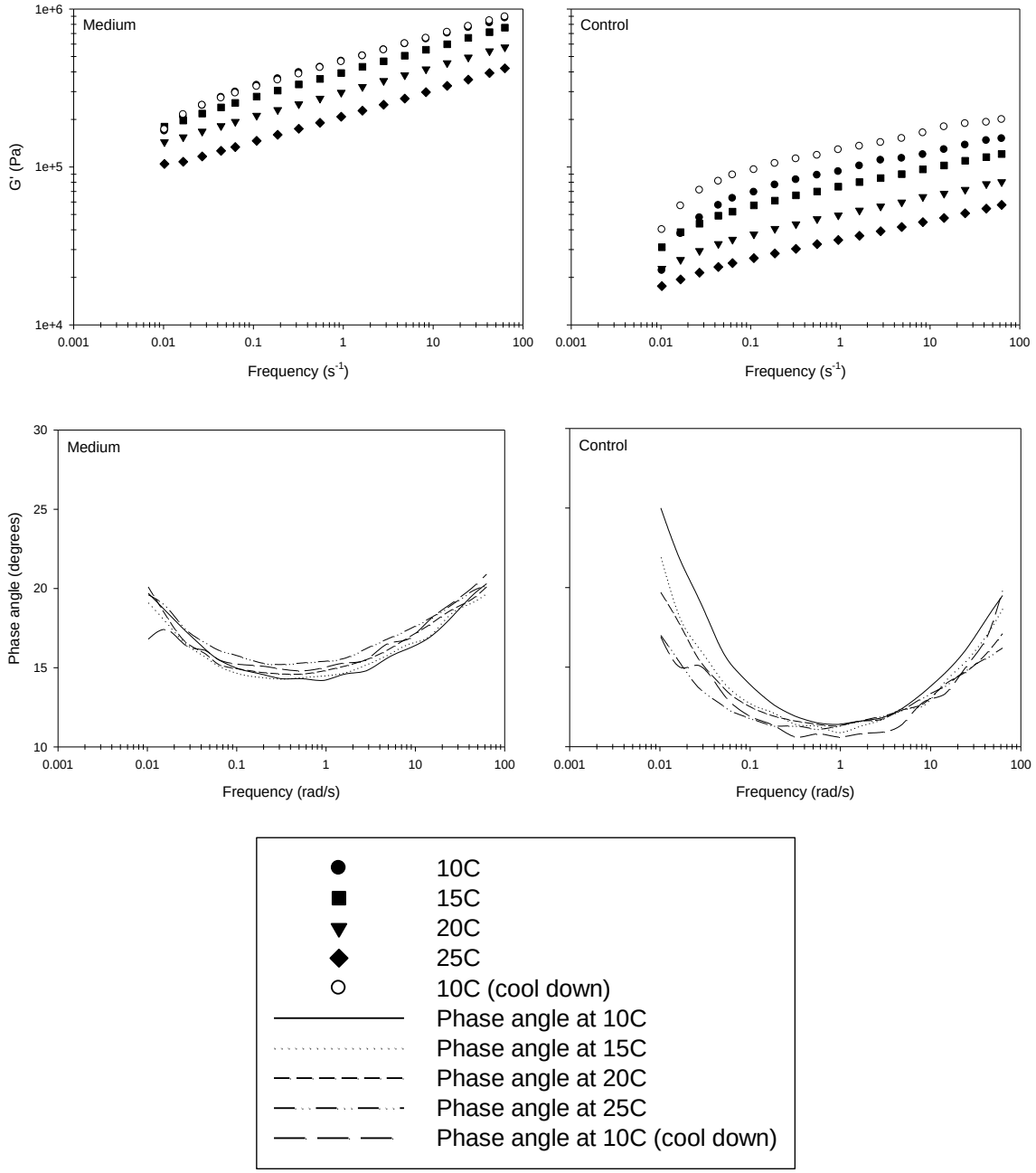


FIG. 4.7. FREQUENCY SWEEP ON CONTROL AND MEDIUM SEPHADEX BEAD TREATMENTS AT FF AND 18 WEEKS OF AGE
Load of 150 Pa.

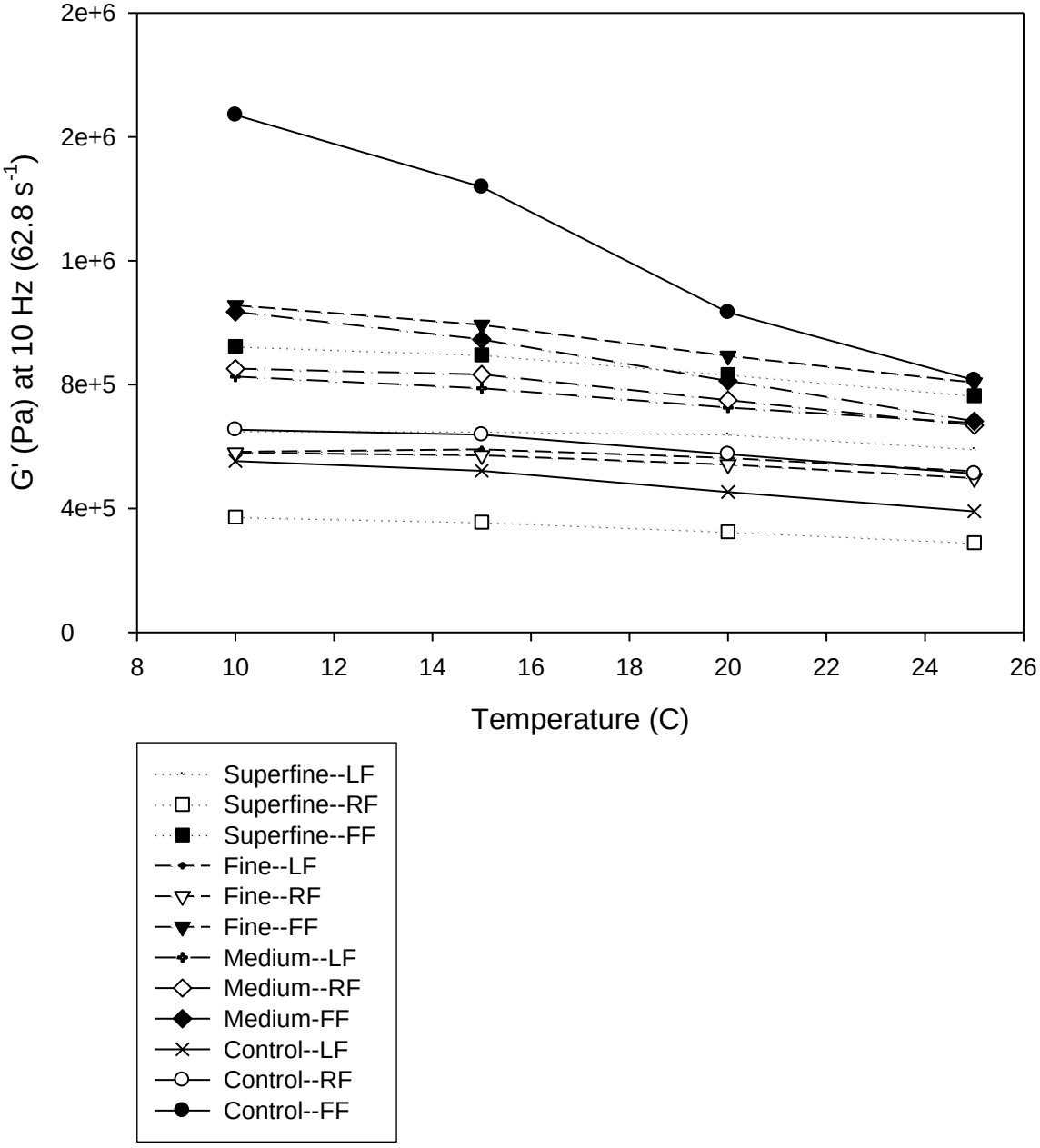


FIG. 4.8. EFFECT OF TEMPERATURE AND PHASE VOLUME ON STORAGE MODULUS (G')

Values, averaged across all ages and both batches, were obtained from frequency sweeps at a frequency of 10 Hz and load of 150 Pa.

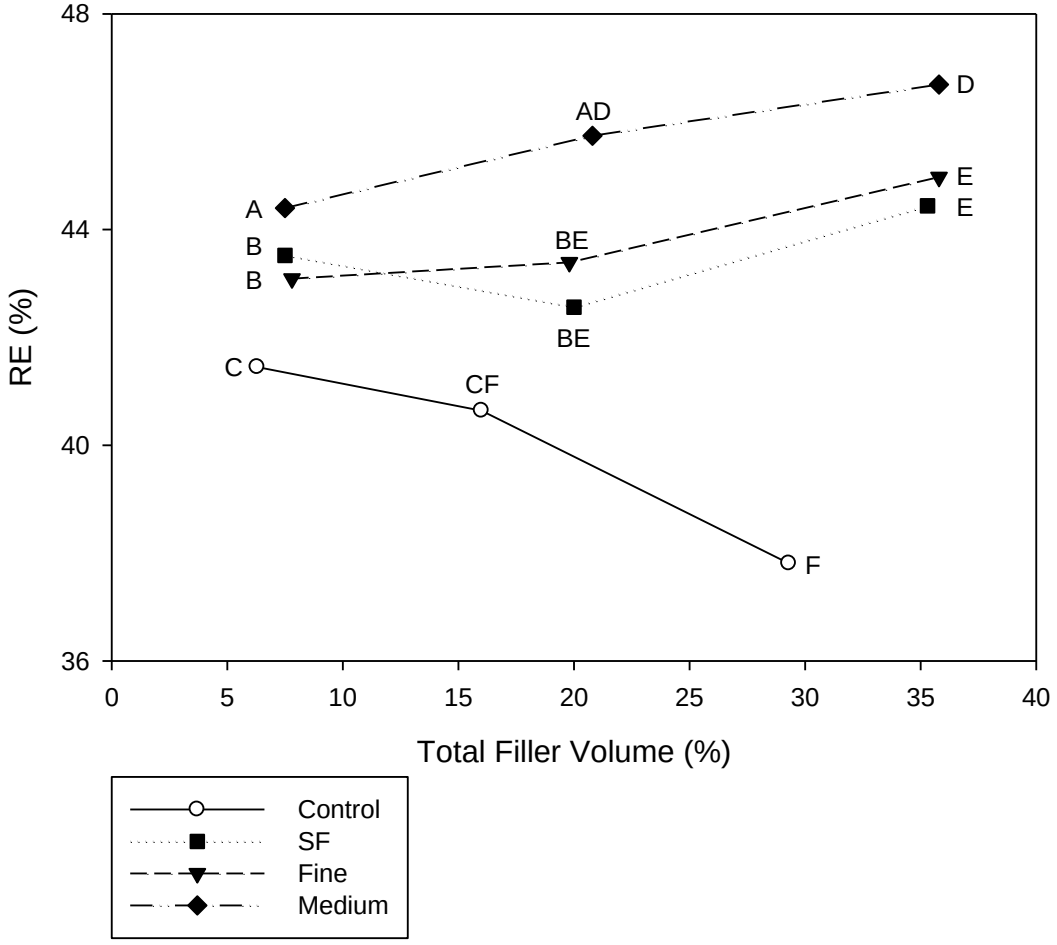


FIG. 4.9. EFFECT OF FILLER TYPE AND PHASE VOLUME ON PERCENT RECOVERABLE ENERGY, RE
Results at 12 weeks of age.

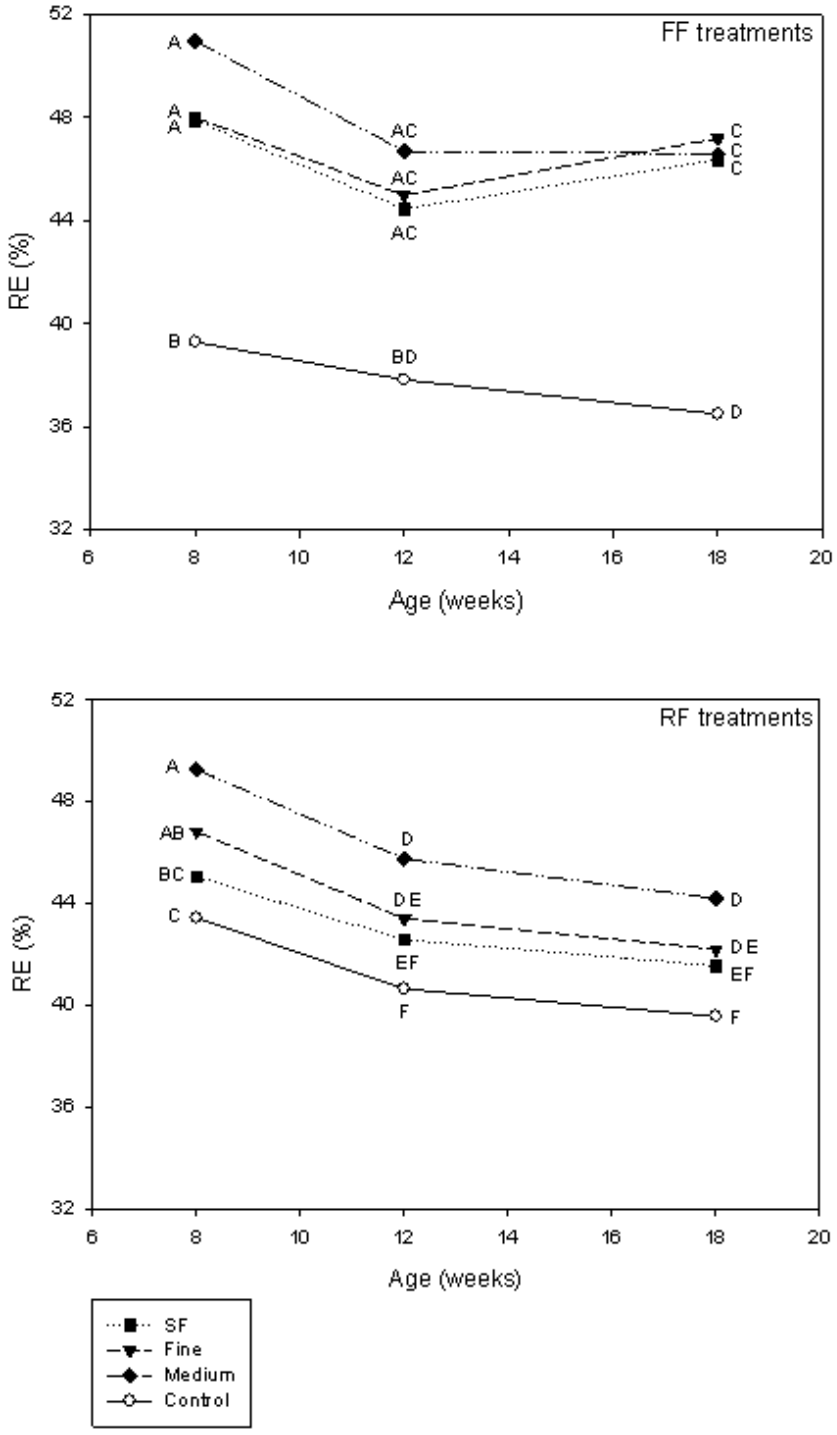


FIG. 4.10. EFFECT OF AGE AND FILLER VOLUME (REDUCED FILLER vs. FULL FILLER) ON PERCENT RECOVERABLE ENERGY, RE

Chapter 5

Effect of Charged Filler Particles on Cheddar Cheese Filled-Gel Rheology

1. ABSTRACT

The goal was to understand how charged fillers function in cheese, a protein network filled with dispersed milkfat globules. Low-fat and full-fat control cheeses were compared against full-filler cheeses containing neutral (G-50 Sephadex), positively (QAE A-50 Sephadex), or negatively (SP C-50 Sephadex) charged beads of the same size in diameter (40-150 μm). Bead charge affected the cheesemaking process, including coagulation time, moisture retention in the curd and whey syneresis, drain-to-salt time, and net yields. The consequent compositional differences affected rheological and mechanical properties such that the highest moisture treatment was less firm, required less work to fracture, and recovered less energy. Cheese was tested at 8 and 12 weeks of age. Age was only significant beyond the linear viscoelastic region but before fracture, where treatments recovered less energy with age. Charged particles may provide a means for manipulating the texture of low-fat cheese.

Keywords: cheese, filled gel, Sephadex beads, particle size, charged particles

2. PRACTICAL APPLICATION

Previous research shows that the rheological properties of cheese closely correlate to the filler volume, or amount of fat, present. Understanding how milkfat affects structural mechanics in cheese allows researchers and developers to find acceptable substitutes to replace milkfat. The research presented in this chapter expands upon previous filled gel research by using charged filler particles. Results show that charged

particles impact the resulting cheese yields, composition, and rheological/mechanical properties, which means charged particles may provide a means for manipulating and optimizing low-fat cheese texture.

3. INTRODUCTION

The benefits of studying real foods in light of the filled gel model were explained in Chapter 4. Research from that chapter showed that cheddar cheese was well described by the filled gel model when uncharged particles were used. Cheese can be described as a protein network filled with dispersed milkfat particles (Visser 1991; Walstra 2003). Like any protein, casein is susceptible to changes in pH and charge. For instance, Lee *et al* (1996) found that the pH at which model processed cheese was produced and the use of charged emulsifiers significantly impacted the hardness and elasticity of the final product. The addition of charged components may affect product texture by inducing an alternate arrangement of molecules such as stranded versus particulate whey protein gels (Langton and Hermansson 1992; Gwartney *et al*. 2000, 2004). In cheese, the ionic charge, or electrostatic repulsion, on casein molecules strongly affects protein-protein interactions (Roefs *et al.* 1990).

Previous work showed that charged filler particles exhibit even greater "active filler" properties, or reinforcing effects, when the filler particle and protein network have opposite charges (Brownsey *et al.* 1987). Therefore, the purpose of this research was to better understand the filler effects in cheddar cheese through use of charged Sephadex

beads; all particles were approximately the same size. The goal was to make low-fat (LF) cheeses with full-fat (FF) texture. We hypothesized that FF texture could be obtained by producing cheese with 6% milkfat and 33% Sephadex beads, yielding a cheese with 39% total filler particle. This would mimic traditional full-fat cheddar cheese, which must legally contain at least 30.5% (w/w) milkfat (CFR 21 [101.113]).

4. MATERIALS AND METHODS

4.1. Cheese

Cheese was prepared as described in Chapter 4 with the following modifications. Except for the low-fat (LF) control, all cheeses were full-fat/filler (FF), using 80 g dry beads in 15.3 kg skim milk. Three different Sephadex-filled treatments were made, each using a different type of Sephadex (Sigma-Aldrich Co., St. Louis, MO): treatment FF0 filled with medium G-50 (50-150 μm dry bead size, no charge) Sephadex; treatment FF+ filled with QAE A-50 chloride form (40-125 μm dry bead size, positively charged) Sephadex; and treatment FF- filled with SP C-50 sodium form (40-125 μm dry bead size, negatively charged) Sephadex. Additional water was required to form a slurry of the charged beads. The coagulation time, pH at draining, and drain-to-salt time varied by filler (Table 5.1). One batch of each treatment was made. Target treatments and sampling plan are shown in Table 5.2.

4.2. Proximate Analysis

Collaborators at Utah State performed all proximate analysis. Sodium chloride content was measured using a chloride analyzer (model 926, Corning; Medfield, MA). Grated cheese was mixed with distilled water for 4 min and homogenized at 260 rpm in a Stomacher 400 (Seward, England). The slurry was filtered through Whatman #1 filter paper (Maidstone, Kent UK), and the filtrate was analyzed for salt. Moisture content was determined in triplicate by weight loss using a CEM microwave oven (CEM Corp.; Indian trail, NC). Fat content was determined using the Babcock method (15.8.A; Marshall 1992). All proximate analyses were completed five d after the cheese was manufactured.

4.3. Microscopy

Cheese samples were imaged using confocal scanning laser microscopy (CSLM). The method for imaging samples was similar to one used by Guinee *et al.* (2000). Cheese samples were held at 4C until sliced into sections approximately 5 mm x 5 mm x 1 mm thick using a razor blade; samples were taken from near the edge of the cheese and from the interior. A 0.2% solution of Nile Blue A Sulfate (MP Biomedicals, LLC; Solon, OH) fluorescent dye in deionized water was filtered twice using Whatman No. 3 (Maidstone, Kent UK) filter paper, and 20 µL of the solution was pipetted onto the cut surface of each cheese slice. The dye was allowed to absorb into the cheese at room temperature for 10 min. Cheese samples were then turned over (dyed cheese surface against the glass slide) onto a single-welled slide with a #1.5 coverslip adhered to the

bottom of the slide via silicone grease. Samples were imaged on a Zeiss LSM 710 confocal laser scanning microscope (CSLM) (Carl Zeiss MicroImaging, Inc.; Thornwood, NY) using a PA 10.0x0.45 objective. A 488 nm laser (to excite Nile Blue in the fat phase) and a 633 nm laser (to excite Nile Blue in the protein phase) were used to image the samples. Emission spectra were collected from 500-650 nm for the fat phase and 650-800 nm for the protein phase, and the resulting images were overlaid. For each cheese treatment, at least 4 samples were prepared, and 4 images were taken per sample at the 10x objective; all images were taken at 8 w of age. MetaMorph software (v. 7.5, Molecular Devices; Downingtown, PA) was used to measure the bead diameters.

4.4. Centrifugation of Sephadex Beads from Cheese Whey

Whey (~17 L from each of treatment) was collected from FF0, FF+, and FF- cheese production, frozen, and shipped from Utah State University to North Carolina State University for analysis; bags of whey were still frozen upon arrival. Whey was thawed and vigorously shaken for 2 min immediately before sampling in order to re-suspend any settled matter, particularly Sephadex beads. Samples (800 mL) were removed from each treatment and divided into 4, 250-mL bottles, which were then centrifuged at 2661 x *g* for 45 min. After centrifuging, the supernatant was separated from the resulting pellet-like gel. The pellet and portions of the supernatant were dried at 65C for six d until constant readings were obtained.

4.5. Rheological and Mechanical Tests

All tests were conducted at 8 and 12 w of age. After opening the package, cheeses were sealed in closeable storage bags to prevent moisture loss. Tests were completed within 3 d of opening a package.

4.5.1. Controlled-stress Tests

A Stresstech controlled-stress rheometer (ATS Rheosystems; Bordentown, NJ) fitted with a 20-mm smooth, parallel plate geometry was used to determine viscoelastic properties through stress sweeps, creep/recovery tests, and frequency sweeps. For each test, cheese samples, 4-mm thick, were trimmed to the size of the upper plate and glued to both plates with Loctite 401 cyanoacrylate glue (Loctite Corp.; Rocky Hill, CT) to prevent sample slip during testing. A thin layer of lubricant (SuperLube, Synco Chemical; Bohemia, NY) was applied to any exposed cheese edges to prevent moisture loss.

4.5.1.1. Stress Sweeps

Stress sweeps were conducted on three samples per treatment in order to determine the linear viscoelastic region (LVR). Stress sweeps were conducted at 25C from 1 to 2000 Pa at 10 Hz; the temperature was regulated using a clamshell oven that was attached to the rheometer and whose two halves closed around the plate area. The LVR was identified by the plateau region of the dynamic viscoelastic function G*, the complex modulus. The critical stress and strain values were identified as the point when G* values decreased 1% from the constant plateau value.

4.5.1.2. Creep/recovery Tests

Creep/recovery tests were conducted at 100, 500, and 2200 Pa on different samples. Based on the method by Rogers *et al*. (2009), loads were applied for 200 s and then removed such that the sample was allowed to recover for an additional 200 s. Tests were conducted in duplicate at each load value for each treatment. The maximum compliance (J_{max}) reached before the load was removed and the maximum recovery (J_r) obtained after the load was removed were recorded from each test. Percent creep recovery (crp) was calculated using the equation from Brown *et al.* (2003).

$$crp = \frac{J_{max} - J_r}{J_{max}} \times 100 \quad \text{(Eq. 5.1)}$$

$$J_r = J_{max} - J_{min} \quad \text{(Eq. 5.2)}$$

where J_{max} was determined after 200 s of creep, and J_{min} was determined after 200 s of recovery.

4.5.2. Large Strain Rheological Tests

A one-cycle compression test was performed to determine the structural changes of cheese at deformations beyond the linear viscoelastic region and prior to fracture; the method was adapted from that of Rogers *et al.* (2010) and van den Berg *et al.* (2008). Cheese was sealed in plastic storage bags to prevent moisture loss and allowed to equilibrate to room temperature (22 ± 2C) for 12 h. Six cheese cylinders were removed per treatment using a 15.6 mm diameter cork borer and cut to a length of 17 mm. Samples were removed from the interior of the block to account for any moisture loss at the block edge. Each cheese cylinder was uniaxially compressed by 20% of the initial

height (i.e. from 17 mm to 13.6 mm), corresponding to a true strain (ε_H) of 0.18. Compression was conducted using an Instron 5565 universal testing machine (Instron; Norwood, MA) and flat plates coated with mineral oil to prevent friction. Moving at a rate of 50 mm/min, the top plate compressed the cheese cylinder until the target strain was reached and then subsequently reversed direction at the same rate to allow for recovery. The area under the resultant force-deformation curve was calculated using Simpson's Rule. Percent recoverable energy (RE) was then calculated as a ratio of the area under the second half of the curve (a_2, work recovered from decompression) over the first half of the curve (a_1, work to compress).

$$RE = \frac{a_2}{a_1} \times 100 \qquad \text{(Eq. 5.3)}$$

4.5.3. Single Edge Notched Bending (SENB)

The fracture energy and fracture toughness were calculated using a SENB test (a.k.a three point bend), modified from that described by Williams and Cawood (1990) by scaling up all dimensions by 1.5. Sample beams were 60 long, 12 mm tall, and 6 mm in breadth. The notch size was kept constant by putting taping a razor blade such that only 5.4 mm of the blade was exposed, and the notch to depth ratio was ~0.45 for each sample. Cheeses were equilibrated to room temperature (22 ± 2C) and tested both with and without notches, six replicates each per treatment. Each sample was deformed using a load cell of 50N and a probe that was 3.12 mm wide at a crosshead speed of 0.2 mm/s until the sample fractured completely. Fracture toughness, K_c, was determined for notched samples based on Williams and Cawood (1990):

$$K_c = f(A) * \frac{P}{BH^{0.5}} \quad \text{(Eq. 5.4)}$$

$$f(A) = 6A^{0.5}[1.99 - A(1-A)(2.15 - 3.93A) + \frac{2.7A^2}{(1+2A)(1-A)}] \quad \text{(Eq. 5.5)}$$

where a = notch depth (m); H = height (m); B = breadth (m); and P = max load (N). "A" is the notch to depth ratio (A = a/H) and is unitless. Note that in these equations, the term "height" is synonymous with what Williams and Cawood (1990) refer to as "width." The terms were switched to be more intuitive. Fracture energy, G_c, was determined for notched and non-notched samples based on Williams and Cawood (1990):

(Eq. 5.6.)

where U = area (units of J) under the f$G_c = \frac{U}{BH\Phi}$tion curve between zero and max load force, P, and Φ = calibration factor for A, the notch-to-depth ratio. In this case, the ratio was ~0.45, and so Φ=0.26 (Williams and Cawood 1990). The value of U was also used to calculate work to fracture for both notched and non-notched samples (Everard *et al.* 2007).

4.6. Statistical Analysis

All statistical analysis was conducted using SAS statistical software (v.9.2, SAS Institute Inc., Cary, NC). Data was analyzed using one-way analysis of variance (ANOVA) to determine statistical differences between filler types. Filler types at different ages (e.g. LFC at 8 weeks and LFC at 12 weeks) were considered different treatments. Tukey's test was used post-hoc to determine significant differences at the α = 0.05 level. All statistics was based on subsampling since treatments were made only once (i.e. one batch/replication).

5. RESULTS AND DISCUSSION

5.1. Effect of Particle Charge on Cheese Manufacture, Proximate Analysis, and Appearance

Adding the charged Sephadex beads impacted milk coagulation. Cheesemakers observed that the SPC Sephadex (treatment FF- in Table 5.2) slowed rennet coagulation but produced a block of cheese similar in weight to the Sephadex G-50 (treatment FF0) cheese. Control treatments (LFC and FFC) and FF0 coagulated at similar rates. The QAE Sephadex (FF+) accelerated rennet coagulation but was observed to have produced almost double the mass of other FF cheeses due to the high moisture content (Table 5.3). Cheesemakers also found that ~4X as much water was required to disperse and hydrate the two ionic forms of Sephadex. One possible explanation for these results is that SPC Sephadex (FF-), a cation exchanger, absorbed Ca^{2+} ions, thereby reducing the casein micelle aggregation rate. In turn, QAE Sephadex (FF+) beads themselves may have acted like calcium ions by contributing positive charge to the system and bridging between casein micelles, which carry a net negative charge. Calcium is known to increase coagulation rates for presumably the same reason (McMahon *et al.* 2005).

The effect of charge—particularly the positively charged QAE beads—was evident in the final treatment composition (Table 5.3). As mentioned, FF+ was higher in moisture than the other treatments. Getting a high moisture block of cheese implies either 1) that the particles interfere with protein network contraction and strand fusion, or 2) the bonds between proteins are weakened. The latter sometimes occurs when calcium

content is reduced (McMahon *et al.* 2005) but is inconsistent with the faster rate of rennet coagulation. It is more probable that the positively-charged particles interfere with protein network contraction and strand fusion. The high moisture of the FF+ treatment also affected the protein-to-moisture ratio, which was expected to affect rheology by changing the protein concentration. The low pH of FF+ was also expected to affect the cheese rheology because pH significantly impacts cheese structure and breakdown. For instance, Creamer and Olson (1982) found that cheddar cheese made at pH 5.40 required 1.5 times as much force as cheese made at pH 4.88 to compress the cheese to their respective yield points, and they attributed this to the difference in casein micelle cluster size. Watkinson *et al.* (2001) measured rheological and fracture properties of a model cheese that was made by directly acidifying the milk (rather than using a starter culture) and curd. They found that increasing the pH by only 0.2 units caused fracture strain (resistance to crumbling), fracture stress (firmness), and fracture area (toughness) to significantly increase within the range of pH 5.20-5.80. Proximate analysis also showed that FF+ was lowest in fat content, although all treatments were close to their total target filler volumes.

All treatments were pressed in hoops of the same dimensions. Thus, differences in whey syneresis during pressing were evident in the final thicknesses, or heights, of the treatments. Cheesemakers noticed that FF0 and FF- treatments were thinner than FFC but comparable in thickness to LFC, indicating the G-50 and SPC beads had the same shrinkage and syneresis as LFC. That implies (1) those Sephadex beads did not have the

same effect on restricting curd shrinkage as fat globules, (2) those Sephadex beads were compressed, or (3) there was less volume of Sephadex than fat. The latter explanation is not likely since confocal microscopy (Chapter 4) and mass balance (see Chapter 5, section 5.2) indicated that target Sephadex volumes were achieved. As mentioned, treatment FF+ was thicker than all other treatments. One possible explanation is that the QAE physically blocked the protein strands from coalescing during the cheese making process. Spacing out adjacent protein strands would therefore result in a thicker cheese block, since all other hoop dimensions were held constant during pressing.

Not only did treatments differ in their rate of rennet coagulation and resultant compositions, they also differed visually (Fig. 5.1). As noted in Chapter 4, Sephadex cheeses were a darker orange color because Sephadex beads scatter light differently than milkfat. However, treatment FF+ was distinctly non-uniform, possessing a mottled appearance with light orange "islands" interspersed through the same darker orange coloring seen in the other Sephadex treatments. This mottling is indicative of phase separation. The darker orange areas of this mottled treatment felt firmer to the touch than the lighter areas and was similar in darkness to the other Sephadex treatments. The color scattering suggests that Sephadex beads and protein-dense areas may have segregated from each other. Furthermore, the cheese crumbled very readily between the different color zones and barely supported its own weight.

Sephadex beads were visible with the naked eye on cut surfaces of all Sephadex treatments, even the FF0 treatment containing G-50 Sephadex beads; G-50 beads were

not noticeable in the previous study (Chapter 4). More beads were visible on the FF+ treatment than either of the other two Sephadex treatments. Touching the FF+ treatment left the hand feeling wet, even though drying on a paper towel showed no moisture transfer. This probably relates to the high moisture content of the FF+ treatment, suggesting the QAE beads were holding or attracting more water.

5.2. Microscopy

Just like the uncharged beads (Chapter 4), charged beads did not absorb Nile Blue dye, although both uncharged and charged beads did scatter light when the Zeiss LSM 710 microscope was used (versus the inverted Leica IMBE microscope used in Chapter 4). Beads were identified from their perfect spherical shape, proper diameters (Table 5.4), and absence from control images (Fig 5.2). Chapter 4 showed that image analysis of the bead areas could be used to approximate filler volume occupied by the beads at low volumes (LF and RF treatments). Here, CSLM was used to view the dispersal of beads in each sample. As noted above, the beads were visible with the naked eye, and there appeared to be more QAE beads along the cut surfaces of FF+ cheese than any of the other treatments. Microscopy confirmed those observations. Most images of FF+ were almost completely dark, covered by a veritable minefield of beads. For purposes of being able to see anything in the printed image of FF+, Fig. 5.2 shows both that heavy covering of beads and the less frequent open space. The small blue dots in the images of Fig. 5.2 are fat globules; they are obviously much smaller than the Sephadex beads. Individual fat globules are distinct in most in the images, but the higher percentage of fat

in the FFC treatment gave that image an overall darker wash or tint. In other words, the FFC treatment appears to be a deeper shade of purple because the viewer is seeing the protein phase overlayed with a higher proportion of milkfat than in the other treatment images. Treatment FF- was characterized by pinprick-sized dark spots across the protein phase. These dark spots most likely represent nano-sized holes or defects in the protein network. The reason for their appearance was unknown but may relate to the slow rate of rennet coagulation observed for that treatment. The brighter protein lines (indicated by white arrows in the images) were observed previously (Lopez *et al.* 2007; Rogers *et al.* 2010; Chapter 4) and are associated with curd lines, the place where cheddared curds knit together in a process that largely excludes milkfat (Kaleb *et al.* 1982).

5.3. Recovery of Beads from Whey

Chapter 4 showed that analysis of CSLM images could be used for low-filler and reduced-filler treatments to estimate the volume of beads in each treatment, and that estimates were close to the target filler values. However, a centrifugation technique was used in this section to quantify the volume of beads in full-filler treatments. Centrifugation did not produce distinct pellets; instead, clumps of centrifuged Sephadex beads formed a sort of floating gel near the bottom of each container. Supernatant was carefully decanted away from these so-called "pellets" and dried; the pellets—with a fair amount of residual supernatant that was left behind so as not to disturb the pellet—were also dried. Pellets and supernatant were dried slowly to ensure that water was fully removed from the Sephadex without forming any type of crust that would hinder

evaporation. Dry matter in the supernatant fraction was attributed to typical whey solids like salt and lactose, and dry matter in the pellet was attributed to Sephadex beads. Typically, 87% of the starting milk material is lost to whey (Fox, *et al.* 2004). Thus, the mass of dry matter in the pellet from the whey (Table 5.5) and the confocal image analysis (Chapter 4) both suggest the target volumes were met. Treatments were assumed to contain the total filler volumes outlined in Table 5.3.

5.4. Statistics

Filler types at different ages (e.g. LFC at 8 weeks and LFC at 12 weeks) were considered different treatments while using ANOVA in order to infer if age had a significant effect. Since testing was only conducted at two different time points, interactions between age and filler type could not be calculated. Age had no significant effect on critical stress, critical strain, J_{max}, J_r, crp, G_c, K_c, or SENB work to peak load. Therefore, means from weeks 8 and 12 were averaged together and reported for the rest of this study. However, RE was sensitive to age effects. The effect of age on RE has been observed previously (Rogers *et al.* 2010; Chapter 4) and attributed to proteolysis. As observed in the previous chapters, creep/recovery term J_{max} differentiated treatments but crp did not.

5.5. Rheological and Mechanical Properties

Treatments differed in rheological and mechanical properties (Table 5.6), but these differences could not be directly attributed to filler charge since treatments also differed in composition. Removal of fat increases the density of the protein network and

has been associated with an increase in firmness, whereas addition of moisture decreases the density of the protein network and firmness (Johnson and Chen 1995). Therefore, the high moisture treatment (FF+) was expected to have the largest J_{max} since J_{max} is proportional to the inverse of the storage modulus, G', and firmer, more rigid materials have larger G'. Indeed, treatment FF+ was the least firm sample (Fig. 5.3.) It also was the least tough (K_c, Table 5.6) and required little fracture energy (G_c, Table 5.6) or work (Table 5.6) to break. It took as little work to fracture the non-notched sample of FF+ as it did to fracture notched samples of all other treatments! These measured properties are consistent with the observed crumbliness of the treatment that was noted above. Percent recoverable energy, RE, correlates strongly with sensory texture terms evaluated after several chews and has been shown to differentiate gels of different texture and fat contents (van den Berg 2008; Rogers *et al.* 2010; Chapters 2-4). Here, the treatment perceived to be the most brittle (FF+) also showed the lowest RE (Fig. 5.4). The proposed model for energy recovery by van Vliet and Walstra (1995) was introduced in Chapter 4. In that chapter, the elasticity of filler particles was responsible for differences in RE, but here, compositional differences in the cheese and the elasticity of filler properties likely affected RE in terms of particle elasticity, viscous dissipation, and frictional dissipation. Finally, it was noted that treatment FF+ was associated with the largest standard deviations, probably because it was inhomogeneous. Rheological and mechanical tests assume homogeneous materials (Steffe 1996); instrumental measurements probably varied depending upon which "phase" (dark versus light orange

areas) was being tested in the cheese, thereby giving rise to inconsistent measurements and large standard deviations.

Control treatments followed the same trends observed in previous chapters, even though the pH of this batch of cheese was higher than the batches made in Chapter 4. As discussed above, cheese structure and breakdown depend upon pH. As seen in the testing of commercial cheese received directly from the manufacturer (Chapter 3), neither critical stress nor critical strain differentiated treatments on the basis of fat content. However, previous filled gel research (Sala *et al.* 2009; Chapter 4) showed that critical strain does differentiate LF and FF treatments because increasing the fat or filler content increases the number of stress concentration factors. The difference between control cheeses in this study and Chapter 2 versus those in Chapter 4 was likely due to pH effects. Control treatments also did not differ much in fracture properties, given that LFC and FFC were equally tough and required similar amounts of work to reach the peak load. Those results were surprising given that torsion fracture has been shown to differentiate cheeses of different fat contents (Chapter 3). However, Chapter 3 also showed variable differentiation of control cheeses. In this chapter, G_c was the only SENB parameter to differentiate treatments on the basis of fat, but work to peak load was the only differentiating term in Chapter 3. This suggests that parameters and sample dimensions may need to be better optimized for SENB testing with these particular treatments; test parameters need to be validated to obtain the most accurate results. As in all previous chapters, however, treatments were best differentiated on the basis of filler volume by

RE. LF cheeses are continuously shown to recover more energy, and this observation has held true against all variation in filler type and processing conditions (i.e. pH).

Sample force-deformation curves from SENB testing are shown in Fig. 5.5 and a "zoomed in" inset of FF+ samples in Fig. 5.6. The short drop off of notched samples (versus the longer plateau of some non-notched samples) is characteristic of samples that fail rapidly due to the presence of a large weak spot, the notch. Treatment FF+ was plotted separately to maximize visibility and showcase the variability indicative of that treatment, particularly since it barely supported its own weight. The force-deformation curves for FF+ were choppy, reflecting the probe's transition through the different phases, which apparently offered different degrees of resistance. Fracture energy for these treatments was similar to published literature. Charalambides *et al.* (1995) found that mild cheddar ranged in G_c from 18 to 41 J/m^2, and sharp cheddar ranged in G_c from 13 to 31 J/m^2, although treatments showed significant batch effects. It is important to differentiate the energy required to fracture a sample and create new surfaces from the energy required to simply deform the sample (Williams and Cawood 1990). This distinction was made by calculating the slope of the force-deformation curves for notched and non-notched samples up until a load of 0.1 N for all treatments except FF+ notched, which was only linear up until 0.04 N (Table 5.6). These slope values represent the modulus that characterizes the compressive strain loading on the top of the beam and the tensile strain at the bottom of the sample by the notch. The modulus did not differ

significantly between notched and non-notched treatments, implying that there was an initial deformation of material prior to crack propagation.

Despite compositional differences, this study suggests an LF cheese with FF texture could be obtained by adding additional filler particles to reach a total filler volume >30%, although more research and sensory testing are needed since only one replication was tested. Treatment FF0 was statistically similar to FFC in every measured parameter, even RE. As mentioned, Sephadex beads were assumed to be more elastic based on greater RE in Chapter 4, but control cheeses were produced at a lower pH in that study. The results of this study suggest that even RE, a parameter most critical to matching sensory chewdown properties, can be matched by adjusting pH and filler volumes.

6. CONCLUSION

A low-fat cheese with full-fat texture can be produced by combining some milkfat with additional filler particle. The charge on that particle will affect cheese manufacture, composition, and rheological/mechanical properties, however.

7. REFERENCES

BROWN, J.A., FOEGEDING, E.A., DAUBERT, C.R., DRAKE, M.A. and GUMPERTZ, M. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. *86*, 3054-3067.

CHARALAMBIDES, M.N., WILLIAMS, J.G. and CHAKRABARTI, S. 1995. A study of the influence of ageing on the mechanical properties of cheddar cheese. J. Mater. Sci. *30*, 3959-3967.

CREAMER, L.K. and OLSON, N.F. 1982. Rheological evaluation of maturing cheddar cheese. J. Food Sci. *47*, 631-636, 646.

EVERARD, O'CALLAGHAN, D.J., O'KENNEDY, B.T., O'DONNELL, C.P., SHEEHAN, E.M. and DELAHUNTY, C.M. 2007. A three-point bending test for prediction of sensory texture in processed cheese. J. Texture Stud. *38*, 438-456.

FOX, P.F., MCSWEENEY, P.L.H., COGAN, T.M. and GUINEE, T.P. 2004. *Cheese: Chemistry, Physics and Microbiology—Volume 1,* 3rd ed. Elsevier, London, UK.

GUINEE, T.P., AUTY, M.A.E., MULLINS, C., CORCORAN, M.O. and MULHOLLAND, E.O. 2000. Preliminary observations on effects of fat content and degree of fat emulsification on the structure-functional relationship of Cheddar-type cheese. J. Texture Stud. *31*, 645-663.

GWARTNEY, E.A., FOEGEDING, E.A. and LARICK, D.K. 2000. The role of texture and fat on flavor release from whey protein isolate gels. In *Flavor Release* (D.D. Roberts and A.J. Taylor, eds.) pp. 355–367, American Chemical Society, Washington, D.C.

GWARTNEY, E.A., LARICK, D.K. and FOEGEDING, E.A. 2004. Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. J. Food Sci.

JOHNSON, M.E. AND CHEN, C.M. 1995. Technology of manufacturing reduced-fat cheddar cheese. In *Chemistry of Structure-Function Relationships in Chees*. (E.L. Malin and M.H. Tunick, eds.) pp 331–337, Plenum Press, New York, NY.

KALAB, M., LOWRIE, R.J. and NICHOLS, D. 1982. Detection of curd granule and milled curd junctions in Cheddar cheese. J. Dairy Sci. *65*, 1117-1121.

LANGTON, M. and HERMANSSON, A.M. 1992. Fine-stranded and particulate gels of β-lactoglobulin and whey protein at varying pH. Food Hydrocolloids *5*, 523-539.

LEE, S.K., KLOSTERMEYER, H., SCHRADER, K. and BUCHHEIM, W. 1996. Rheological properties and microstructure of model processed cheese containing low molecular weight Lopez, C., B. Camier, and J. Y. Gassi. 2007. Development of the

LOPEZ, C., CAMIER, B. and GASSI, J.Y. 2007. Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. Int. Dairy J. *17*, 235-247.

MARSHALL, R.T. 1992. *Standard Methods for the Examination of Dairy Products*. American Public Health Association, New York, NY.

MCMAHON, D.J., PAULSON, B. and OBERG, C.J. 2005. Influence of calcium, pH, and moisture on protein network structure and functionality in direct-acidified nonfat Mozzarella cheese. J. Dairy Sci. *88*, 3754-3763.

ROEFS, S.P.F.M., VAN VLIET, T., VAN DEN BIJGAART, H.J.C.M., DE GROOT-MOSTERT, A.E.A. and WALSTRA, P. 1990. Structure of casein gels made by combined acidification and rennet action. Neth. Milk Dairy J. *44*, 159–188.

ROGERS, N.R., DRAKE, M.A., DAUBERT, C.R., MCMAHON, D.J., BLETCH, T.K. and FOEGEDING, E.A. 2009. The effect of aging on low-fat, reduced-fat, and full-fat cheddar cheese texture. J. Dairy Sci. *92*, 4756-4772.

ROGERS, N.R., MCMAHON, D.J., DAUBERT, C.R., BERRY, T.K. and FOEGEDING, E.A. 2010. Rheological properties and microstructure of cheddar cheese made with different fat contents. J. Dairy Sci. *in press*.

SALA, G., VAN VLIET, T., STUART, M.C., VAN DE VELDE, F. and VAN AKEN, G.A. 2009. Deformation and fracture of emulsion-filled gels: effect of gelling agent concentration and oil droplet size. Food Hydrocolloids *23*, 1853-1863.

STEFFE, J.F. 1996. *Rheological Methods in Food Process Engineering*. Freeman Press, East Lansing, MI.

VAN DEN BERG, L., CAROLAS, A.L., VAN VLIET, T., VAN DER LINDEN, E., VAN BOEKEL, M.A.J.S. and VAN DE VELDE, F. 2008. Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. Food Hydrocolloids *22*, 1404-1417.

VAN VLIET, T. and WALSTRA, P. 1995. Large deformation and fracture behavior of gels. Faraday Discuss. *101*, 359-370.

VISSER, J. 1991. Factors affecting the rheological and fracture properties of hard and semi-hard cheese. In *Rheological and Fracture Properties of Cheese* (P. Walstra, ed.) pp. 49-61, Bull. No. 268, International Dairy Federation, Brussels, Belgium.

WALSTRA, P. 1999. Dairy Technology: principles of milk properties and processes. Marcel Dekker, Inc., New York, NY.

WALSTRA, P. 2003. *Physical Chemistry of Foods*. Marcel Dekker, Inc., New York, NY.

WATKINSON, P., COKER, C., CRAWFORD, R., DODDS, C., JOHNSTON, K., MCKENNA, A. and WHITE, N. 2001. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. 2001. Int. Dairy J. *11*, 455-464.

WILLIAMS, J.G. and CAWOOD, M.J. 1990. European Group on Fracture: K_c and G_c methods for polymers. Polym Test. *9*, 15-26.

TABLE 5.1. CHEESE MANUFACTURE

Parameters used converting 16 kg of milk substrate[1] into low-fat (LF) and full-fat/filler (FF) cheddar cheeses.

	Cheese				
Parameter	**FF**	**FF0**	**FF+**	**FF-**	**LF**
Milk fat, % (wt/wt)	3.5	0.2	0.2	0.2	0.7
Dry Sephadex added, g/kg milk	-	80	80	80	-
Set temperature, ^{o}C	31	31	31	31	32
Coagulation time, min	15	60	30	95	15
Cooking temperature, ^{o}C	38	38	38	38	36
Cooking time, min	25	25	25	25	15
Drain pH	6.10	6.26	6.28	6.15	6.20
Wash water temperature, ^{o}C	-	-	-	-	16
Curd temperature, ^{o}C	-	-	-	-	26
Set-to-Drain time, min	150	120	120	120	130
Salt pH	5.45	5.75	5.75	5.75	5.95
Drain-to-Salt time, min	70	65	70	45	50
Salting, g/kg curd	22	26	26	26	22

[1]Milk substrate was standardized milk for the control (FF and LF) cheeses while for the experimental cheeses (FF0, FF+, and FF- for the G-50, QAE A-50, and SP C-50 treatments, respectively), it included the skim milk and the Sephadex slurry.

TABLE 5.2. TARGET SAMPLING PLAN AND COMPOSITION

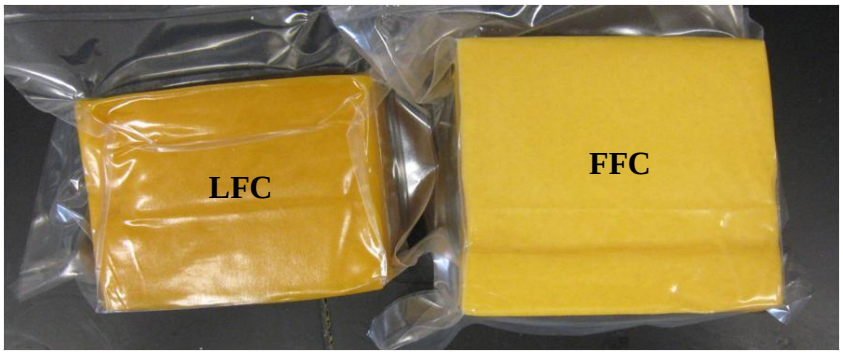
Filler		Particle charge	Treatment abbreviation	Size of filler particle[a]	Target Sephadex volume	Target fat volume	Total filler volume	Tested time points (weeks)
Low fat/filler	milkfat	0	LFC	0.1 - 10 µm	0%	6%	6%	8, 12
Full fat/filler	milkfat	0	FFC	0.1 - 10 µm	0%	33%	33%	8, 12
	G-50 Sephadex	0	FF0	50 - 150 µm	33%	6%	39%	8, 12
	QAE Sephadex	+	FF+	40 - 125 µm	33%	6%	39%	8, 12
	SPC Sephadex	-	FF-	40 - 125 µm	33%	6%	39%	8, 12

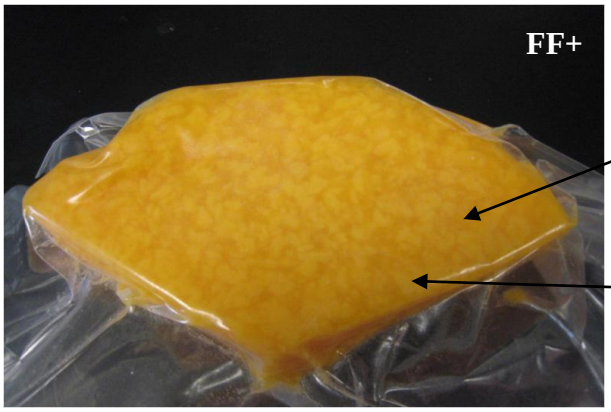
[a] Milk fat size range from Walstra (1999). Sephadex size refers to dry bead (pre-hydration) size as identified on the Sigma-Aldrich website <www.sigmaaldrich.com>.

TABLE 5.3. PROXIMATE ANALYSIS OF TREATMENTS

Treatment abbreviation	pH	%Moisture[a] (w/w)	%Protein[b] (w/w)	Ratio of Moisture to Protein[b]	%Salt[a] (w/w)	%Fat[a] (w/w)	Estimated volume of Sephadex (%)	Total filler volume[c] (%)
LFC	5.59	53.96	35.06	1.54	2.31	5.00	0	5.00
FFC	5.56	38.89	25.94	1.50	2.00	29.50	0	29.50
FF0	5.30	54.74	35.56	1.54	2.03	4.00	33	37.00
FF+	4.87	65.84	25.79	2.55	2.20	2.50	33	35.50
FF-	5.28	55.74	34.98	1.59	1.93	4.00	33	37.00

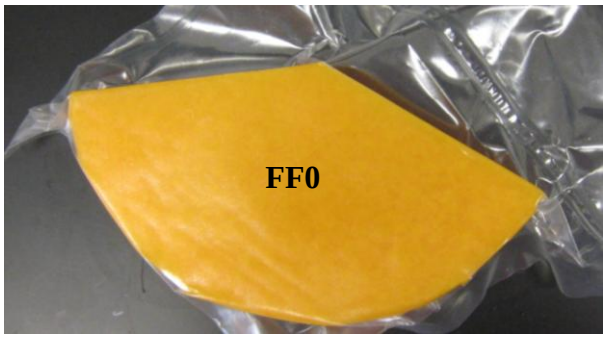
[a] Averaged from three samples.
[b] Assuming moisture + protein + salt + fat + dry Sephadex beads = 100%. Does not account for ash or error in the other measurements. Dry Sephadex was calculated as 20% of the initial dry weight added divided by a cheese mass of 1746 g (assumes a loss of 87% initial milk mass to whey)
[c] Sephadex + fat = total filler.





Dark orange, similar to the color of the other Sephadex treatments

Lighter orange "islands"



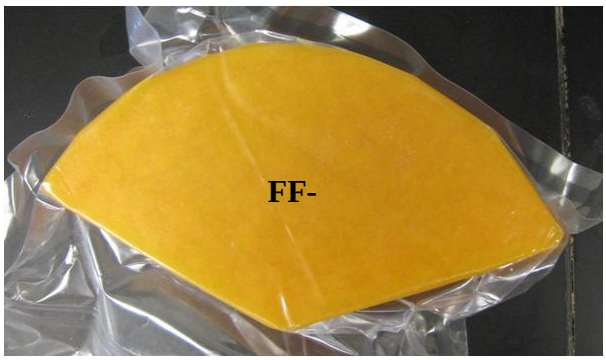


FIG. 5.1. IMAGES OF TREATMENTS

TABLE 5.4. RANGE OF SEPHADEX BEAD DIAMETERS IN CHEESE

	SPC (-)	QAE (+)	G-50 (0)	Milkfat[c]
Dry size[a] (µm	40-125	40-125	50-150	
Range (µm)	62-232	87-276	43-311	0.1-10
Mean (µm)	150	172	175	
n[b]	88	63	95	

Bead diameters measured from confocal images (10X magnification) using MetaMorph software

[a] According to manufacturer's literature

[b] Number of individual beads measured from images

[c] Range from Walstra (1999)

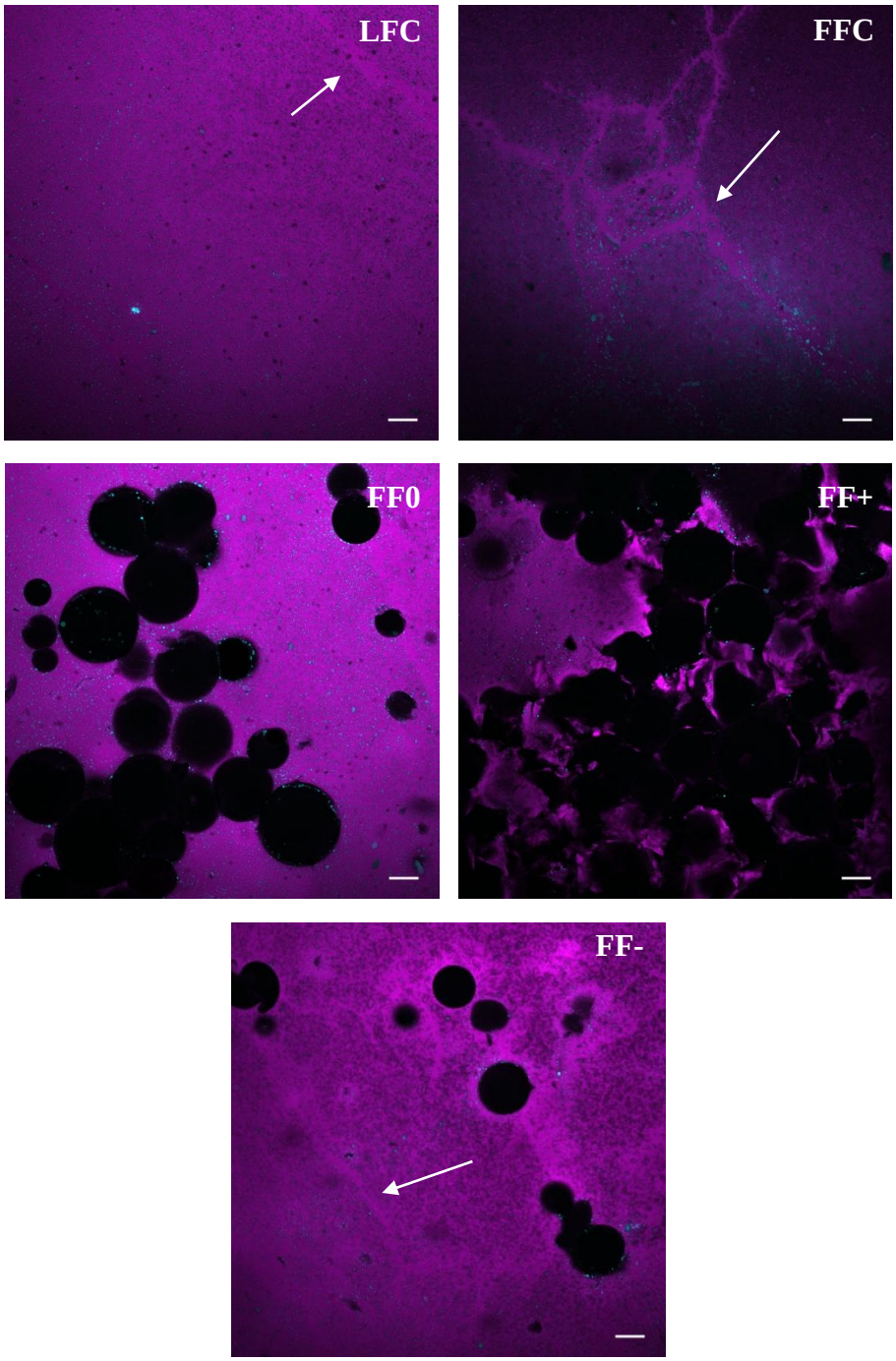


FIG. 5.2. TREATMENTS IMAGED WITH PA 10.0x0.45 CONFOCAL OBJECTIVE Protein phase in purple; fat phase in blue; Sephadex beads did not absorb dye. All scale bars are 50 μm in length. White arrows point to assumed curd lines.

TABLE 5.5. DRY MATTER IN WHEY

	Total amount of whey sampled	Pellet		Supernatant	
		Water (g)	Dry matter (g)	Water (g)	Dry matter (g)
FF0	1265.75	42.66	5.96	1203.00	13.29
FF-	1191.26	10.06	2.23	1164.34	14.61
FF+	1167.71	21.84	2.06	1132.42	11.36

TABLE 5.6. SELECT RHEOLOGICAL AND MATERIAL PROPERTIES

	Critical stress (Pa)		Critical strain		K_c (kPa•m$^{0.5}$)		G_c (J/m^2)		SENB work to peak load (J)				E, initial slope[1] (mm/N)			
									Non-notched		Notched		Non-notched		Notched	
LFC	1270	A	2.52E-03	AB	3.10	A	11.93	A	3.63E-03	AB	2.44E-04	C	0.105	AB	0.083	ADE
FFC	1020	AB	1.83E-03	A	2.85	A	31.13	B	2.37E-03	AB	6.13E-04	C	0.118	B	0.088	BDE
FF0	788	AB	3.08E-03	ABC	2.75	A	34.78	B	4.20E-03	B	6.39E-04	C	0.074	ACDE	0.060	EF
FF-	689	B	3.40E-03	BC	1.58	AB	34.40	B	2.79E-03	AB	4.98E-04	C	0.044	CEFG	0.042	FG
FF+	535	B	4.09E-03	C	0.40	B	13.35	A	3.86E-04	C	2.72E-04	C	0.044	CG	0.023	G

[1] E refers to the modulus from the SENB force-deformation curves. It was calculated up to a load of 0.1 N for all treatments except FF+ notched, which was only linear up until 0.04 N.

Letters differentiate treatment means within a single column heading (e.g. critical stress or SENB work) that were significantly different at $p<0.05$.

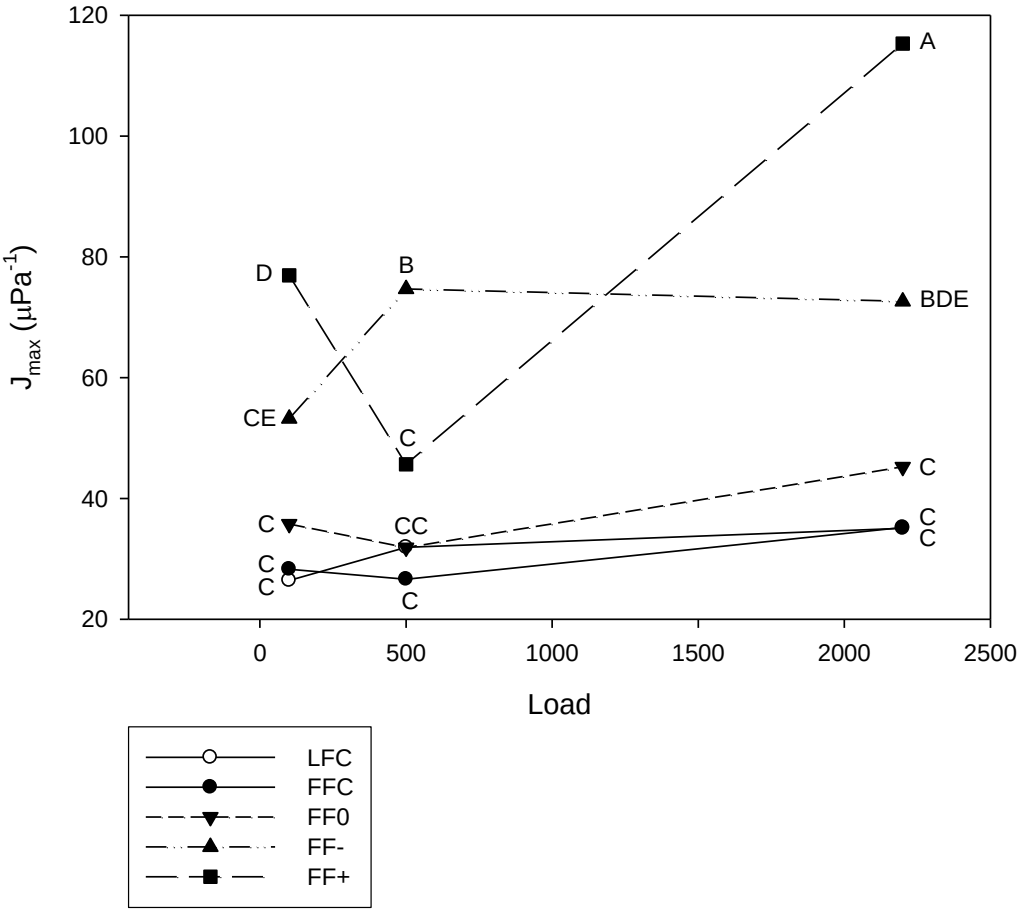


FIG. 5.3. MAXIMUM COMPLIANCE (J_{MAX}) OF TREATMENTS AT VARYING LOADS

J_{max} was determined after applying the load for 200 s. Letters differentiate treatment means that were significantly different at $p<0.05$.

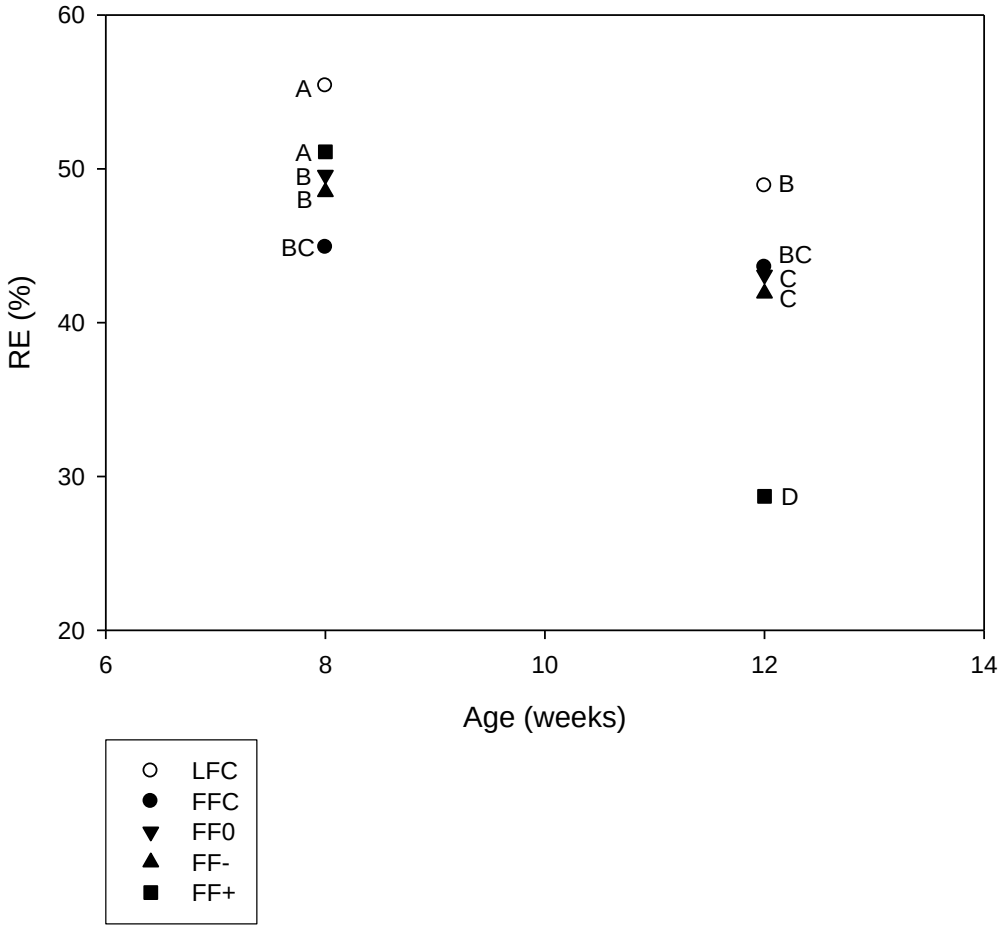


FIG. 5.4. PERCENT RECOVERABLE ENERGY (RE) OF TREATMENTS AT DIFFERENT AGES

Letters differentiate treatment means that were significantly different at $p<0.05$.

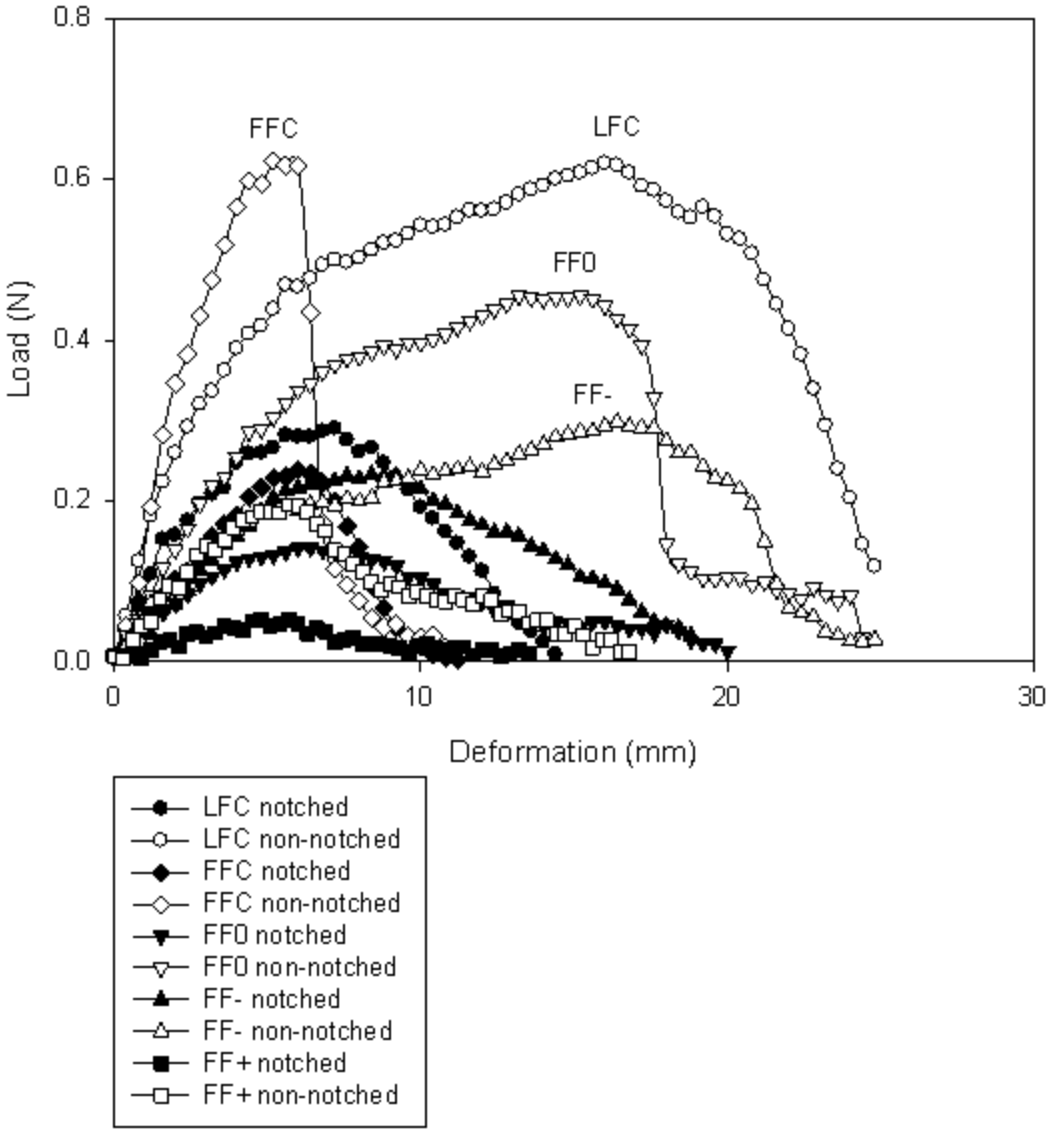


FIG. 5.5 REPRESENTATIVE FORCE-DEFORMATION CURVES FROM SINGLE EDGE NOTCHED BEND (SENB) TESTING
For clarity, one-fourth of the points were plotted.

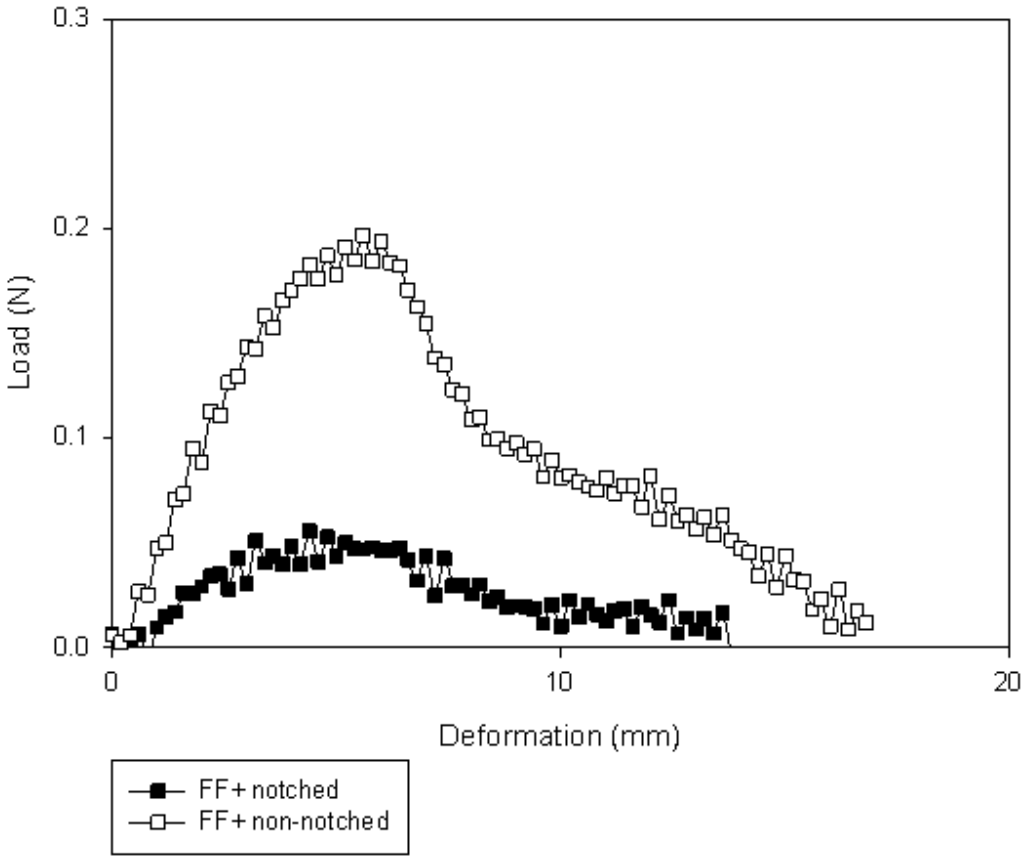


FIG. 5.6. FF+ INSET
For clarity, one-half of the points were plotted

APPENDICES

Appendix A

Raw Data for Chapter 2 (Off-the-Shelf Commercial Cheeses) Figures and Tables

TABLE A.1. RAW DATA FROM SMALL STRAIN TESTS
Brands: FL (Food Lion), CB (Cracker Barrel), and Cabot. The number of samples tested varied depending upon mechanical nicks and breakage during testing.

Brand	Fat content	Critical stress (Pa)	Critical strain	$J_{max,\ 100\ Pa}$ (μPa^{-1})	$J_{r,\ 100\ Pa}$ (μPa^{-1})	$crp_{100\ Pa}$	$J_{max,\ 500\ Pa}$ (μPa^{-1})	$J_{r,\ 500\ Pa}$ (μPa^{-1})	$crp5_{00\ Pa}$
FL	FF	789	1.53E-03	11.76	6.12	47.93%	13.45	6.28	53.32%
FL	FF	1012	1.04E-03	6.23	4.59	26.37%	10.18	5.03	50.58%
FL	FF	1300	1.15E-03	.	.	.	.	.	.
FL	FF	342	5.31E-04	.	.	.	.	.	.
FL	FF	211	4.96E-04	.	.	.	.	.	.
FL	RF	479	1.73E-03	19.75	5.54	71.96%	16.64	7.34	55.91%
FL	RF	1012	3.03E-03	10.75	4.53	57.87%	14.76	6.70	54.61%
FL	RF	479	1.49E-03	.	.	.	.	.	.
FL	RF	1530	1.59E-03	.	.	.	.	.	.
FL	RF	1303	1.54E-03	.	.	.	.	.	.
CB	FF	121	2.73E-04	18.97	10.19	46.28%	20.67	10.22	50.56%
CB	FF	200	3.84E-04	14.88	9.63	35.28%	17.61	7.46	57.66%
CB	FF	45	1.38E-04	.	.	.	.	.	.
CB	FF	850	4.13E-04	.	.	.	.	.	.
CB	FF	584	3.52E-04	.	.	.	.	.	.
CB	RF	789	1.64E-03	13.86	6.35	54.17%	16.97	6.70	60.54%
CB	RF	615	1.90E-03	21.92	5.05	76.98%	25.73	13.96	45.74%
CB	RF	1012	1.92E-03	.	.	.	20.16	11.99	40.53%
CB	RF	896	8.64E-04	.	.	.	.	.	.
CB	RF	686	8.12E-04	.	.	.	.	.	.
CB	RF	789	1.85E-03	.	.	.	.	.	.
Cabot	FF	615	4.33E-04	6.29	1.92	69.54%	9.34	3.16	66.20%
Cabot	FF	200	2.06E-04	11.26	3.90	65.35%	12.66	4.29	66.15%
Cabot	FF	479	3.53E-04	.	.	.	7.70	2.37	69.25%
Cabot	FF	789	5.45E-04	.	.	.	.	.	.
Cabot	RF	423	1.98E-03	21.79	10.79	50.48%	25.27	15.38	39.14%
Cabot	RF	1300	1.95E-03	24.40	12.40	49.18%	22.55	11.18	50.42%
Cabot	RF	1300	1.97E-03	.	.	.	.	.	.
Cabot	RF	896	1.88E-03	.	.	.	.	.	.
Cabot	LF	1300	1.16E-03	18.52	8.37	54.78%	17.54	9.24	47.30%
Cabot	LF	1147	1.40E-03	11.45	4.35	61.99%	9.45	6.43	31.99%
Cabot	LF	1300	1.95E-03	.	.	.	.	.	.
Cabot	LF	1300	1.91E-03	.	.	.	.	.	.

TABLE A.2. RAW DATA FROM LARGE STRAIN AND FRACTURE TESTS
Brands: FL (Food Lion), CB (Cracker Barrel), and Cabot. The number of samples tested varied depending upon mechanical nicks and breakage during testing.

Brand	Fat content	RE	Fracture stress (Pa)	Fracture strain
FL	FF	34.53%	32035	1.0113
FL	FF	35.15%	32596	0.8359
FL	FF	33.63%	45366	0.9569
FL	FF	34.12%	29075	0.7634
FL	FF	.	17350	0.7925
FL	FF	.	20110	0.9696
FL	FF	.	40691	1.0101
FL	FF	.	43531	1.2400
FL	RF	43.31%	26688	0.4749
FL	RF	41.02%	41665	0.4986
FL	RF	43.37%	40512	0.5338
FL	RF	43.20%	32906	0.5400
FL	RF	42.86%	51970	0.5892
FL	RF	.	25643	1.7741
FL	RF	.	28858	1.1996
FL	RF	.	26764	1.5200
FL	RF	.	27694	1.5920
FL	RF	.	17556	0.8503
FL	RF	.	21259	1.3552
CB	FF	27.86%	40248	0.3895
CB	FF	15.97%	39568	0.5284
CB	FF	23.60%	72072	0.3597
CB	FF	30.70%	38455	0.4936
CB	FF	.	41191	0.4791
CB	FF	.	30875	0.3352
CB	RF	40.71%	43276	1.0641
CB	RF	39.35%	41486	1.0357
CB	RF	36.98%	51989	1.3693
CB	RF	40.77%	39223	0.9995
CB	RF	39.41%	35633	1.0603
CB	RF	40.97%	34141	0.8152
CB	RF	.	37567	0.9482

TABLE A.2. CONTINUED

Brand	Fat content	RE	Fracture stress (Pa)	Fracture strain
Cabot	FF	31.03%	55164	0.5410
Cabot	FF	31.76%	41303	0.6829
Cabot	FF	34.11%	48608	0.4395
Cabot	FF	33.47%	52242	0.6194
Cabot	FF	33.35%	35020	2.2343
Cabot	FF	34.10%	31308	1.4972
Cabot	FF	35.74%	.	.
Cabot	FF	32.24%	.	.
Cabot	FF	37.30%	.	.
Cabot	RF	41.59%	47015	0.9408
Cabot	RF	42.78%	36806	1.1004
Cabot	RF	41.44%	63753	1.0620
Cabot	RF	47.92%	28994	1.1224
Cabot	RF	48.62%	41962	1.9782
Cabot	RF	49.59%	.	.
Cabot	RF	49.18%	.	.
Cabot	RF	48.65%	.	.
Cabot	LF	44.03%	57762	0.7877
Cabot	LF	41.90%	44380	0.6695
Cabot	LF	49.02%	45509	0.7412
Cabot	LF	49.55%	29883	0.4177
Cabot	LF	49.59%	49999	1.0896
Cabot	LF	49.81%	.	.
Cabot	LF	49.23%	.	.

TABLE A.3. EFFECT OF TEMPERATURE AND FAT CONTENT ON COMMERCIAL CHEESE STORAGE MODULI AT 8.3 RAD/S
Brands: FL (Food Lion), CB (Cracker Barrel), and Cabot. Values in table are storage moduli (G') obtained from frequency sweeps.

Temperature (°C)	BRAND--FAT CONTENT						
	CB-FF	CB--RF	FL--FF	FL--RF	Cabot--FF	Cabot--RF	Cabot--LF
10	2.91E+06	9.22E+05	4.04E+06	1.39E+06	3.32E+06	5.38E+05	6.95E+05
15	2.29E+06	8.12E+05	3.42E+06	1.28E+06	2.47E+06	4.90E+05	5.83E+05
20	1.59E+06	6.61E+05	2.40E+06	1.11E+06	1.80E+06	4.45E+05	4.59E+05
25	1.04E+06	5.32E+05	1.91E+06	9.19E+05	1.28E+06	3.79E+05	3.38E+05

TABLE A.4. EFFECT OF TEMPERATURE AND FAT CONTENT ON COMMERCIAL CHEESE STORAGE MODULI AT 62.8 RAD/S
Brands: FL (Food Lion), CB (Cracker Barrel), and Cabot. Values in table are storage moduli (G') obtained from frequency sweeps; G' in units of Pa.

Temperature (°C)	BRAND--FAT CONTENT						
	CB--FF	CB--RF	FL--FF	FL--RF	Cabot--FF	Cabot--RF	Cabot--LF
10	2.88E+06	9.13E+05	2.86E+06	1.22E+06	2.62E+06	7.88E+05	8.58E+05
15	2.22E+06	7.47E+05	2.51E+06	1.03E+06	2.00E+06	6.87E+05	7.18E+05
20	1.30E+06	5.42E+05	1.66E+06	8.09E+05	1.33E+06	5.60E+05	5.61E+05
25	8.47E+05	4.15E+05	1.25E+06	6.53E+05	9.55E+05	4.59E+05	4.16E+05

TABLE A.5. CORRELATION BETWEEN CREEP AND FREQUENCY SWEEPS
Brands: FL (Food Lion), CB (Cracker Barrel), and Cabot. Complex modulus (G*) obtained from frequency sweeps at 25°C and 0.013 rad/s. All values in table are in units of Pa.

Brand--Fat content	G*	$1/J_{max}$ at 100 Pa	$1/J_{max}$ at 500 Pa
FL--FF	8.65E+05	1.11E+05	8.46E+04
FL--RF	4.19E+05	6.56E+04	6.37E+04
CB--FF	4.15E+05	5.91E+04	5.22E+04
CB--RF	2.44E+05	5.59E+04	4.77E+04
Cabot--FF	5.93E+05	1.14E+05	1.01E+05
Cabot--RF	1.60E+05	4.33E+05	4.18E+04
Cabot--LF	1.34E+05	6.67E+04	7.41E+04

Appendix B

Raw Data for Chapter 3 (Retail Cheese Received Directly from Manufacturer)

Figures and Tables

TABLE B.1. RAW DATA FROM STRESS SWEEPS AND UNIAXIAL COMPRESSION

Fat Content	Critical stress (Pa)	Critical Strain	RE
LF	1278	2.26E-03	46.62%
	697	1.99E-03	47.81%
	886	2.44E-03	47.69%
	706	2.01E-03	46.57%
	.	.	47.34%
	.	.	46.18%
RF	1001	1.82E-03	49.53%
	1800	1.14E-03	48.38%
	1003	8.80E-04	48.78%
	.	.	48.14%
	.	.	47.12%
	.	.	48.09%
FF	1222	9.42E-04	40.33%
	1025	6.97E-04	40.14%
	1439	7.37E-04	39.60%
	.	.	40.77%
	.	.	39.68%
	.	.	40.59%

TABLE B.2. RAW DATA FROM CREEP/RECOVERY TESTS AT DIFFERENT APPLIED LOADS

Fat Content	Load (Pa)	J_{max} (μPa^{-1})	J_r ($\mu Pa-1$)	crp
LF	100	18.82	11.50	38.89%
		40.90	24.47	40.17%
		26.92	16.08	40.27%
	500	30.21	19.19	36.48%
		23.51	10.73	54.36%
		17.46	11.19	35.91%
	2200	17.21	12.67	26.38%
		31.93	11.30	64.61%
		29.65	10.66	64.05%
RF	100	36.60	12.09	66.97%
		29.02	15.77	45.66%
		29.03	12.69	56.29%
	500	31.64	11.90	62.39%
		49.11	27.45	44.11%
		43.32	16.43	62.07%
	2200	61.08	39.23	35.77%
		47.44	27.28	42.50%
		54.08	33.37	38.30%
FF	100	11.09	5.86	47.33%
		23.19	10.59	54.33%
		20.30	11.52	43.25%
	500	17.09	11.72	31.42%
		13.52	8.87	34.42%
		17.23	9.54	44.65%
	2200	18.53	10.76	41.88%
		22.50	16.11	28.36%
		19.22	10.50	45.32%

TABLE B.3. RAW DATA FROM TORSION GELOMETRY

	Rate (s^{-1})	Fracture stress (Pa)	Fracture strain	Angle of fracture (degrees)
LF	0.041	48028	1.425	40.0
		40827	1.107	43.0
		35819	1.298	22.0
		39412	1.506	38.0
		42533	0.982	32.0
	0.41	49690	2.263	43.0
		41636	2.185	55.0
		71426	2.131	19.0
		70081	3.239	34.0
		73886	3.430	41.0
	4.1	108437	1.529	35.0
		75581	1.645	41.0
		84214	2.097	40.0
		63231	1.641	39.0
		83008	2.076	0.0
RF	0.041	30791	1.437	47.0
		15490	0.918	35.0
		24949	1.330	50.0
		29103	1.283	45.0
		27510	1.145	49.0
	0.41	32623	1.451	32.0
		40072	1.222	44.0
		37701	1.178	32.0
		35950	1.583	47.0
		32640	1.464	35.0
	4.1	61458	1.528	34.0
		65357	1.843	41.0
		92578	1.871	63.0
		69600	2.052	46.0
		28655	2.237	32.0
		73624	2.653	50.0
FF	0.041	20549	0.855	14.0
		21724	0.740	51.0
		23654	0.780	46.5
		28525	0.889	30.0
		33041	1.127	39.0
	0.41	49690	2.263	43.0
		41636	2.185	55.0
		71426	2.131	19.0
		70081	3.239	34.0
		73886	3.430	41.0
	4.1	40765	1.951	6.0
		40273	1.618	18.0
		29290	1.349	5.0
		63448	1.129	28.0
		59740	1.150	0.0
		26405	2.180	31.0

TABLE B.4. RAW DATA FROM SENB TESTING

		K_c (kPa•m^0.5)	G_c (J/m^2)	Max. Extension (mm)	Max. Load (N)	Work to Max. Load (J)	Slope (N/mm), E, up to load 0.1 N
LF	Notched	2.25	12.97	2.799	0.128	2.20E-04	0.1340
		2.56	15.47	3.000	0.184	3.10E-04	0.0949
		2.97	16.85	2.900	0.220	3.40E-04	0.1371
		3.65	23.19	3.400	0.265	4.60E-04	0.1450
		2.49	13.02	3.003	0.199	3.00E-04	0.0783
		1.62	10.07	3.501	0.115	2.10E-04	0.0436
		3.06	23.43	3.299	0.238	5.00E-04	0.1024
	Non-notched	.	.	5.903	0.692	2.58E-03	0.1535
		.	.	8.000	0.486	2.55E-03	0.1135
		.	.	6.302	0.409	1.58E-03	0.0925
		.	.	4.101	0.472	1.18E-03	0.1024
		.	.	4.203	0.403	1.08E-03	0.1716
		.	.	5.100	0.490	1.56E-03	0.0938
RF	Notched	1.95	19.21	3.900	0.126	3.60E-04	0.1457
		2.58	7.63	1.801	0.184	1.60E-04	0.1120
		2.19	19.44	4.001	0.159	4.00E-04	0.1322
		2.72	11.93	2.800	0.136	1.70E-04	0.0606
		2.27	8.43	2.300	0.152	1.60E-04	0.1129
		2.07	5.70	2.101	0.161	1.20E-04	0.0976
		2.08	6.38	2.700	0.142	1.30E-04	0.0805
	Non-notched	.	.	13.402	0.402	3.54E-03	0.0704
		.	.	12.499	0.390	3.06E-03	0.0708
		.	.	13.100	0.545	4.74E-03	0.1275
		.	.	13.299	0.590	5.38E-03	0.1732
		.	.	11.700	0.270	1.45E-03	0.1023
		.	.	11.001	0.504	3.60E-03	0.0519
FF	Notched	2.32	8.46	2.100	0.180	1.80E-04	0.0454
		2.18	27.37	4.999	0.156	5.40E-04	0.2095
		2.65	35.40	4.800	0.185	6.70E-04	0.1743
		3.22	9.10	1.800	0.248	2.00E-04	0.0988
		2.25	11.58	2.803	0.168	2.40E-04	0.2015
		2.55	26.84	5.100	0.141	4.40E-04	0.0323
		3.98	36.55	4.500	0.285	7.30E-04	0.1361
	Non-notched	.	.	13.200	0.663	6.03E-03	0.1708
		.	.	14.300	0.686	6.36E-03	0.1741
		.	.	15.001	0.675	6.87E-03	0.0890
		.	.	9.602	1.116	6.60E-03	0.1861
		.	.	13.600	0.508	4.22E-03	0.1793
		.	.	13.139	0.730	6.01E-03	0.1720

TABLE B.5. DESCRIPTIVE ANALYSIS SCORES (15-POINT SCALE) OF CHEESE BASED ON FAT CONTENT AND SAMPLE SIZE

Fat Content	Thickness (mm)	Panel Rep	Panelist	Hfirm	Hspring	Hhard	Firm	Fracturability	Deg. Break	Cohes	Adhes	Smth. Of Mass	Smth. Of Mouth
FF	2	1	2	5.5	6.0	5.0	4.5	4.0	10.0	10.5	9.5	9.0	9.0
FF	2	1	3	4.0	5.0	4.0	6.0	5.0	11.0	11.0	10.0	11.0	11.0
FF	2	1	4	5.0	7.0	5.0	5.0	5.0	11.0	11.5	9.0	11.5	11.0
FF	2	1	5	5.5	9.0	6.8	5.8	4.0	11.5	12.0	10.3	10.5	10.5
FF	2	1	7	5.0	10.0	6.0	4.5	3.0	10.0	11.0	10.0	10.0	9.0
FF	2	1	11	5.0	8.0	5.5	4.0	5.0	10.5	11.0	9.0	9.0	11.0
FF	2	2	2	6.5	8.0	6.0	5.0	4.0	10.5	10.5	9.5	9.0	9.0
FF	2	2	3	4.0	5.5	4.0	6.0	5.5	11.5	11.0	10.0	11.0	11.0
FF	2	2	4	5.5	8.0	5.5	5.5	5.0	11.0	11.5	9.0	11.5	11.0
FF	2	2	5	5.5	9.0	6.0	6.5	5.0	11.0	12.5	9.8	10.5	10.5
FF	2	2	7	5.0	10.0	6.0	4.0	3.0	11.0	11.0	9.0	10.0	9.0
FF	2	2	11	5.0	8.0	5.0	4.0	5.0	10.0	11.0	9.0	9.0	11.0
FF	2	3	2	5.5	6.0	5.0	4.5	4.0	10.0	10.5	9.5	9.0	9.0
FF	2	3	3	4.0	6.0	4.0	6.0	4.0	11.5	11.0	10.5	11.0	11.5
FF	2	3	4	4.0	7.0	5.0	5.0	5.0	11.0	11.0	9.0	11.0	11.0
FF	2	3	5	5.5	10.0	6.5	6.0	4.0	11.0	12.0	10.0	10.5	11.0
FF	2	3	7	5.0	8.0	6.5	4.0	3.0	11.0	12.0	10.5	9.0	9.0
FF	2	3	11	4.5	7.0	4.5	4.0	5.0	10.0	10.5	9.5	9.0	10.0
FF	8	1	2	6.0	3.5	7.0	5.0	5.0	10.5	11.0	10.0	9.5	10.0
FF	8	1	3	6.0	6.0	9.0	6.5	5.5	11.0	11.0	10.0	10.5	10.0
FF	8	1	4	6.0	6.0	11.0	6.5	6.5	10.5	11.5	9.5	11.0	11.0
FF	8	1	5	6.0	2.0	11.0	6.0	5.0	10.0	10.5	9.0	9.5	10.5
FF	8	1	7	7.5	5.0	10.0	5.0	4.0	9.0	9.0	8.0	10.0	11.0
FF	8	1	11	7.5	3.5	11.0	6.5	5.0	10.0	11.0	9.0	9.0	10.0
FF	8	2	2	7.3	3.0	7.3	5.5	5.0	11.0	11.0	11.0	9.5	9.5
FF	8	2	3	5.5	7.0	10.5	6.0	5.0	10.5	10.0	9.5	11.0	11.0
FF	8	2	4	7.5	7.0	10.5	6.5	5.0	10.5	11.0	9.5	10.5	10.0
FF	8	2	5	6.5	4.0	9.5	5.0	5.5	11.0	11.5	10.0	11.0	10.5
FF	8	2	7	7.0	6.0	7.5	5.0	4.0	9.0	9.0	8.0	10.0	10.0
FF	8	2	11	7.0	3.5	10.5	6.0	5.0	9.5	11.0	9.0	8.5	10.0
FF	8	3	2	6.5	3.5	8.0	5.0	5.3	10.0	11.0	9.5	9.5	9.5
FF	8	3	3	5.5	6.0	7.0	5.0	4.0	11.0	11.5	9.5	11.5	11.5
FF	8	3	4	7.0	6.5	9.0	6.0	6.0	10.0	11.0	9.5	10.5	10.5
FF	8	3	5	6.0	3.0	9.5	5.0	5.0	11.5	11.3	10.3	11.0	11.0
FF	8	3	7	7.0	6.0	6.0	5.0	4.0	9.0	10.0	9.0	9.0	9.5
FF	8	3	11	6.0	3.5	9.0	5.0	5.0	11.0	11.5	9.5	10.5	10.0

TABLE B.5 CONTINUED

Fat Content	Thickness (mm)	Panel Rep	Panelist	Hfirm	Hspring	Hhard	Firm	Fracturability	Deg. Break	Cohes	Adhes	Smth. Of Mass	Smth. Of Mouth
FF	14	1	2	7.0	2.5	7.0	5.0	4.0	11.5	11.0	10.0	10.0	11.0
FF	14	1	3	7.5	2.0	8.5	6.0	5.0	11.0	10.0	10.0	10.0	9.0
FF	14	1	4	7.0	2.0	8.0	6.0	6.5	10.5	11.0	10.0	11.0	11.0
FF	14	1	5	8.0	1.0	8.5	6.0	6.0	10.5	11.0	10.0	11.0	10.0
FF	14	1	7	7.0	2.0	7.0	6.0	4.0	10.0	10.0	10.0	11.0	10.0
FF	14	1	11	7.5	3.0	8.0	6.5	5.0	10.0	11.0	10.0	10.0	11.0
FF	14	2	2	8.0	3.0	8.5	6.5	5.0	11.0	11.0	10.0	10.0	9.5
FF	14	2	3	7.0	1.0	7.0	6.0	4.0	11.0	11.0	10.0	11.0	10.0
FF	14	2	4	7.5	1.0	8.5	7.5	5.5	10.0	11.0	10.0	11.0	11.0
FF	14	2	5	8.0	3.0	8.0	6.0	5.0	10.5	12.3	11.0	11.0	9.3
FF	14	2	7	6.5	2.0	8.0	6.0	4.0	11.0	10.0	11.0	11.0	10.0
FF	14	2	11	8.0	2.0	8.5	7.0	5.5	10.0	11.0	10.0	10.0	10.0
FF	14	3	2	6.0	2.0	7.0	5.5	4.0	10.5	10.0	10.5	10.0	10.3
FF	14	3	3	6.5	2.0	7.0	6.5	4.0	12.0	11.5	11.0	11.5	11.0
FF	14	3	4	7.5	1.0	7.5	6.0	5.0	11.0	11.5	10.0	11.0	11.0
FF	14	3	5	8.3	3.5	8.5	6.3	5.5	11.0	12.0	11.0	11.5	9.5
FF	14	3	7	6.5	3.5	7.0	5.5	4.0	11.0	10.0	11.0	11.0	10.0
FF	14	3	11	7.0	3.0	8.0	6.0	5.0	11.0	11.0	10.0	10.5	11.0
FF	20	1	2	7.0	12.0	9.0	5.5	5.0	9.5	11.5	10.5	9.5	10.0
FF	20	1	3	6.0	11.0	9.0	6.0	5.0	11.0	10.0	10.0	11.0	10.5
FF	20	1	4	6.0	14.0	7.5	7.0	5.5	9.5	12.0	11.0	11.0	11.0
FF	20	1	5	6.5	11.0	8.5	7.0	5.5	10.0	11.0	11.0	10.8	10.0
FF	20	1	7	7.0	9.5	8.0	5.5	4.5	9.0	10.0	9.0	9.0	9.0
FF	20	1	11	6.5	10.0	8.0	5.5	5.0	9.5	11.0	10.5	10.5	11.0
FF	20	2	2	8.0	12.0	9.0	5.5	5.5	10.5	11.0	10.5	9.5	10.5
FF	20	2	3	5.5	10.0	9.0	7.0	5.5	10.0	10.0	10.0	11.0	11.0
FF	20	2	4	8.0	13.5	8.0	7.0	5.0	9.5	12.0	11.5	11.5	11.5
FF	20	2	5	6.0	11.5	7.0	6.5	5.5	11.0	11.0	11.0	11.5	11.0
FF	20	2	7	7.0	9.5	7.5	5.5	4.5	9.0	10.0	9.0	9.5	9.0
FF	20	2	11	7.0	9.5	9.0	6.0	5.0	9.0	10.0	10.0	10.0	11.0
FF	20	3	2	8.0	12.0	9.0	5.5	5.0	10.0	11.5	10.5	9.5	10.5
FF	20	3	3	6.5	10.0	8.0	7.0	5.5	9.0	10.0	10.0	10.5	10.0
FF	20	3	4	6.5	13.5	7.0	7.0	5.0	10.5	12.0	11.5	11.5	11.5
FF	20	3	5	6.0	11.0	7.0	7.3	5.0	11.0	11.3	11.0	11.0	10.5
FF	20	3	7	7.0	10.0	8.0	5.0	4.5	9.0	10.0	9.0	9.0	9.0
FF	20	3	11	7.0	10.0	8.0	5.5	5.5	10.0	11.0	10.5	11.0	11.0

TABLE B.5 CONTINUED

Fat Content	Thickness (mm)	Panel Rep	Panelist	Hfirm	Hspring	Hhard	Firm	Fracturability	Deg. Break	Cohes	Adhes	Smth. Of Mass	Smth. Of Mouth
RF	2	1	2	7.5	13.0	12.0	8.0	5.0	7.0	6.8	6.0	6.5	7.5
RF	2	1	3	6.0	12.0	12.0	8.0	4.0	5.0	6.0	6.0	7.0	9.0
RF	2	1	4	6.5	14.5	10.5	7.0	5.0	5.5	6.5	5.5	7.5	8.0
RF	2	1	5	6.8	14.5	12.5	8.5	5.0	6.5	5.5	5.0	6.0	9.0
RF	2	1	7	7.0	13.0	10.0	6.5	3.5	6.0	6.0	4.0	6.0	7.0
RF	2	1	11	6.5	14.0	12.0	6.0	5.0	5.0	5.5	3.5	6.5	7.5
RF	2	2	2	8.0	14.0	13.0	8.5	5.0	7.0	6.0	6.0	6.5	7.0
RF	2	2	3	6.0	12.0	13.0	8.0	4.0	5.0	5.5	5.0	6.0	9.0
RF	2	2	4	7.5	14.0	11.0	7.0	5.0	6.0	6.5	5.5	7.5	8.0
RF	2	2	5	7.5	13.5	12.0	8.5	4.0	6.0	7.0	6.0	7.5	9.0
RF	2	2	7	7.0	13.0	11.0	6.5	3.5	6.0	6.0	4.0	6.0	7.5
RF	2	2	11	6.5	13.0	11.5	6.0	5.0	5.0	5.5	4.0	6.0	8.0
RF	2	3	2	7.0	14.0	14.5	8.5	5.5	5.0	6.0	5.0	6.0	7.5
RF	2	3	3	5.5	12.0	12.0	8.0	4.0	5.0	6.0	5.0	7.0	9.0
RF	2	3	4	6.5	14.0	10.5	7.5	5.0	5.0	5.5	5.0	7.0	8.0
RF	2	3	5	5.8	13.8	14.3	7.0	3.5	7.0	7.0	6.5	6.5	9.0
RF	2	3	7	6.5	12.0	11.0	6.0	3.5	5.0	5.5	4.0	6.0	7.0
RF	2	3	11	5.5	14.0	12.0	6.0	5.0	5.5	6.0	5.0	7.0	8.0
RF	8	1	2	8.0	12.5	12.5	9.5	6.0	8.0	5.5	5.5	6.5	8.0
RF	8	1	3	6.0	12.0	13.0	9.5	5.0	9.0	6.0	7.0	7.0	10.0
RF	8	1	4	6.0	12.5	12.0	8.0	6.0	6.0	4.0	3.0	9.5	9.0
RF	8	1	5	8.0	13.5	13.5	9.8	5.0	6.0	4.0	3.0	6.0	9.5
RF	8	1	7	6.5	12.0	10.0	8.0	5.0	6.0	5.0	5.0	6.0	7.0
RF	8	1	11	6.0	12.5	10.5	9.0	5.0	7.5	7.5	7.0	8.0	8.0
RF	8	2	2	8.5	12.5	12.5	9.5	6.0	8.0	5.5	5.5	6.0	7.5
RF	8	2	3	7.0	12.0	13.0	9.0	5.0	8.5	6.0	7.0	9.5	9.0
RF	8	2	4	6.0	12.5	11.5	8.0	6.0	6.0	4.5	3.5	9.5	9.0
RF	8	2	5	8.5	14.0	12.5	10.0	5.0	6.0	4.3	3.0	6.5	10.0
RF	8	2	7	6.5	11.0	10.0	8.0	4.5	6.0	5.0	5.0	6.5	7.0
RF	8	2	11	6.5	12.5	11.5	8.5	5.0	8.0	6.0	7.0	8.0	8.0
RF	8	3	2	8.0	12.5	12.0	9.0	5.5	7.5	4.5	5.0	6.0	6.5
RF	8	3	3	7.0	12.0	13.0	10.0	5.0	8.0	5.5	6.5	9.5	9.0
RF	8	3	4	6.5	12.0	11.5	8.0	5.0	5.5	4.0	3.0	7.0	9.5
RF	8	3	5	8.0	14.0	13.3	9.8	5.0	6.0	5.0	3.5	6.0	9.3
RF	8	3	7	5.0	12.0	10.5	7.5	4.5	7.0	6.0	5.0	6.5	7.0
RF	8	3	11	6.0	12.0	11.0	8.0	5.0	8.5	7.0	7.0	8.0	8.0

TABLE B.5 CONTINUED

Fat Content	Thickness (mm)	Panel Rep	Panelist	Hfirm	Hspring	Hhard	Firm	Fracturability	Deg. Break	Cohes	Adhes	Smth. Of Mass	Smth. Of Mouth
RF	14	1	2	10.5	14.5	14.3	10.0	6.5	4.5	5.5	6.0	6.5	7.5
RF	14	1	3	8.0	14.0	14.5	11.5	6.5	4.0	6.0	6.0	6.0	7.5
RF	14	1	4	10.0	14.5	13.0	9.0	6.5	6.0	6.0	6.5	7.5	8.0
RF	14	1	5	8.0	13.8	12.0	9.0	5.0	4.0	6.5	6.0	6.5	9.0
RF	14	1	7	8.0	13.0	13.0	10.5	4.0	6.0	5.0	4.0	5.0	7.0
RF	14	1	11	9.5	12.0	12.5	9.0	5.5	5.5	5.5	5.0	5.5	9.0
RF	14	2	2	9.5	14.5	14.5	9.0	6.5	6.0	6.0	5.5	6.5	7.5
RF	14	2	3	8.0	14.5	14.5	9.5	6.0	4.0	6.0	5.5	7.0	8.0
RF	14	2	4	10.5	13.5	12.0	9.0	6.0	6.0	6.0	6.0	6.5	8.0
RF	14	2	5	8.0	14.5	12.0	9.0	6.0	4.0	5.0	4.5	6.5	7.5
RF	14	2	7	8.0	12.0	12.5	10.5	4.0	5.5	5.0	4.0	5.0	7.0
RF	14	2	11	10.0	14.0	13.5	9.0	6.0	4.5	5.0	4.5	5.0	8.5
RF	14	3	2	9.5	14.5	14.5	9.0	6.5	6.0	6.5	6.0	6.5	7.5
RF	14	3	3	8.0	14.5	14.5	9.5	6.0	4.0	6.0	5.5	7.0	8.0
RF	14	3	4	10.5	13.5	12.0	9.0	6.5	6.0	6.0	6.0	6.5	8.0
RF	14	3	5	7.5	14.5	12.0	9.0	6.0	4.0	5.0	4.5	6.5	7.5
RF	14	3	7	8.0	12.0	12.5	10.5	4.0	5.5	5.0	4.0	5.0	7.0
RF	14	3	11	10.0	14.0	13.5	9.0	6.0	4.5	5.0	4.5	5.0	8.5
RF	20	1	2	10.8	14.0	14.5	9.8	6.0	6.0	6.0	6.0	6.5	7.0
RF	20	1	3	10.0	13.5	14.5	10.0	5.5	4.0	5.0	5.0	6.0	7.0
RF	20	1	4	9.0	14.0	13.0	10.0	5.5	4.5	4.0	5.0	4.5	5.5
RF	20	1	5	8.0	14.3	14.3	9.3	6.0	4.8	6.0	6.0	6.0	7.0
RF	20	1	7	9.0	13.0	13.5	9.0	4.0	4.0	4.0	4.5	5.0	6.0
RF	20	1	11	8.0	14.0	13.0	8.0	6.0	6.0	6.0	6.0	6.0	7.0
RF	20	2	2	9.8	14.0	14.0	9.8	6.5	6.0	6.5	6.5	6.0	7.0
RF	20	2	3	10.0	14.0	14.5	10.0	6.0	4.0	5.0	5.0	6.0	7.0
RF	20	2	4	8.5	14.5	13.0	10.0	5.5	5.0	4.0	5.0	4.5	5.5
RF	20	2	5	8.0	14.0	11.5	9.0	5.5	4.0	4.5	5.0	6.0	6.0
RF	20	2	7	9.0	13.0	13.0	8.0	4.0	4.0	4.0	4.5	5.0	6.0
RF	20	2	11	8.5	14.0	13.5	8.0	6.0	6.0	6.0	6.0	6.5	7.0
RF	20	3	2	9.0	14.5	13.0	9.0	5.5	5.0	6.5	6.0	5.5	6.5
RF	20	3	3	9.0	14.0	14.5	9.0	6.0	4.0	5.5	5.0	6.0	7.0
RF	20	3	4	8.0	14.0	12.5	10.0	6.0	5.0	5.0	5.0	5.5	5.5
RF	20	3	5	9.0	14.5	11.0	8.5	5.5	4.0	6.5	5.0	5.5	7.0
RF	20	3	7	8.0	14.0	14.0	8.0	4.0	4.5	5.5	4.5	5.5	6.5
RF	20	3	11	9.0	14.5	13.0	8.0	5.5	5.0	6.0	5.0	5.5	7.0

TABLE B.5 CONTINUED

Fat Content	Thickness (mm)	Panel Rep	Panelist	Hfirm	Hspring	Hhard	Firm	Fracturability	Deg. Break	Cohes	Adhes	Smth. Of Mass	Smth. Of Mouth
LF	2	1	2	8.0	14.5	14.5	9.5	5.5	3.0	4.5	3.5	5.0	5.5
LF	2	1	3	6.5	14.0	14.5	9.0	4.0	3.0	5.0	3.0	6.0	6.0
LF	2	1	4	8.0	14.0	14.5	8.0	4.5	3.0	4.5	4.5	5.5	6.5
LF	2	1	5	7.3	13.8	14.0	9.0	5.3	3.5	5.0	4.0	6.0	7.0
LF	2	1	7	6.5	13.0	13.0	7.0	3.5	4.5	4.5	3.5	5.0	6.0
LF	2	1	11	8.0	14.5	14.5	8.5	5.0	3.0	3.0	3.5	5.0	7.0
LF	2	2	2	8.0	14.5	14.5	9.5	5.0	3.0	4.5	3.5	5.0	5.5
LF	2	2	3	7.0	13.5	13.5	9.0	3.0	2.5	4.0	3.0	6.0	7.0
LF	2	2	4	8.0	13.5	14.0	8.0	5.0	2.5	3.5	3.0	4.5	5.5
LF	2	2	5	6.5	14.5	14.5	9.0	5.0	3.5	3.5	5.0	4.5	7.0
LF	2	2	7	6.5	13.0	13.0	8.0	3.5	4.5	4.5	3.5	4.5	5.5
LF	2	2	11	8.0	14.0	14.5	8.5	5.0	3.5	4.0	3.0	5.5	6.5
LF	2	3	2	8.0	14.5	14.5	9.5	5.0	3.0	4.5	3.5	5.0	5.5
LF	2	3	3	7.5	14.0	14.0	9.0	3.0	2.5	4.0	3.0	5.0	7.0
LF	2	3	4	8.0	13.5	14.5	8.0	4.5	2.5	3.5	3.5	4.5	6.0
LF	2	3	5	6.5	14.0	14.5	9.5	5.0	4.0	3.0	4.5	5.0	7.0
LF	2	3	7	6.5	13.0	13.0	8.5	3.5	4.5	5.0	5.0	5.0	6.0
LF	2	3	11	7.0	14.0	14.0	8.0	5.0	3.0	3.0	3.0	5.0	7.0
LF	8	1	2	10.0	14.0	12.0	10.5	6.0	4.0	6.0	5.5	4.0	5.5
LF	8	1	3	9.0	14.0	14.0	10.0	6.0	4.0	4.0	5.0	4.0	5.0
LF	8	1	4	8.5	13.5	13.8	10.5	6.0	6.0	7.5	6.5	6.0	6.0
LF	8	1	5	9.0	14.0	13.0	10.0	4.5	4.0	4.5	5.5	5.0	6.5
LF	8	1	7	9.0	12.0	13.0	9.0	5.0	6.0	5.0	6.0	6.0	6.5
LF	8	1	11	9.5	11.5	13.5	9.5	6.0	6.5	8.0	6.0	6.5	6.5
LF	8	2	2	9.5	14.5	13.0	10.0	5.8	4.0	5.5	5.0	4.0	5.5
LF	8	2	3	8.5	14.0	14.0	9.0	6.0	4.0	4.0	6.0	4.0	6.0
LF	8	2	4	8.5	14.0	13.0	9.8	6.0	6.5	8.0	8.0	6.5	7.0
LF	8	2	5	9.0	13.5	14.5	9.5	5.5	4.0	6.0	5.0	6.0	6.5
LF	8	2	7	9.0	10.0	13.0	10.0	5.0	6.0	5.0	5.0	6.0	6.5
LF	8	2	11	10.5	13.0	13.5	9.5	5.5	6.0	7.5	6.5	5.0	7.0
LF	8	3	2	10.5	14.5	14.0	11.5	6.5	4.0	5.8	5.0	4.5	5.5
LF	8	3	3	9.0	14.0	14.0	9.0	5.0	4.0	4.5	4.0	4.5	6.5
LF	8	3	4	9.0	13.0	13.5	10.0	6.0	6.5	7.5	7.0	6.0	6.5
LF	8	3	5	8.3	13.5	14.8	9.5	5.0	4.0	6.0	4.0	5.0	6.0
LF	8	3	7	9.0	10.0	13.0	10.0	5.0	5.0	5.0	5.0	6.0	6.0
LF	8	3	11	9.0	13.0	13.0	9.5	5.5	6.5	7.5	6.0	6.0	7.0

TABLE B.5 CONTINUED

Fat Content	Thickness (mm)	Panel Rep	Panelist	Hfirm	Hspring	Hhard	Firm	Fracturability	Deg. Break	Cohes	Adhes	Smth. Of Mass	Smth. Of Mouth
LF	14	1	2	10.0	14.5	14.5	10.5	6.5	5.5	6.0	5.0	5.5	6.5
LF	14	1	3	9.0	14.0	14.0	11.0	6.0	3.0	5.0	4.5	6.0	7.0
LF	14	1	4	12.0	14.5	13.5	11.5	6.5	5.0	4.5	4.5	6.5	7.5
LF	14	1	5	9.0	14.5	14.0	10.0	6.5	3.0	4.0	4.5	6.5	7.5
LF	14	1	7	9.0	13.5	13.0	11.0	5.5	5.5	5.0	3.0	4.0	6.0
LF	14	1	11	12.0	13.5	13.5	10.5	6.0	3.0	4.0	3.5	4.3	7.0
LF	14	2	2	11.0	14.5	14.5	10.5	6.5	5.5	6.5	5.0	6.0	7.5
LF	14	2	3	9.0	14.5	14.0	10.5	6.5	3.0	4.5	4.5	5.0	6.5
LF	14	2	4	11.5	14.5	13.8	10.5	7.0	5.5	5.5	5.5	6.0	7.5
LF	14	2	5	10.0	13.8	13.5	10.3	5.5	3.0	4.0	4.0	6.5	7.5
LF	14	2	7	10.0	12.0	13.0	11.0	5.5	5.5	5.0	3.0	4.0	6.0
LF	14	2	11	11.5	14.0	13.0	10.5	6.0	3.5	4.0	3.5	4.0	7.5
LF	14	3	2	11.0	14.5	14.8	10.5	6.5	5.5	6.5	6.0	6.5	7.5
LF	14	3	3	9.0	14.0	14.5	11.5	6.5	3.5	4.5	4.0	5.0	7.0
LF	14	3	4	12.5	14.5	13.0	10.5	6.0	4.5	4.0	4.0	6.5	7.5
LF	14	3	5	9.0	14.5	12.3	10.3	6.5	3.5	5.3	4.3	6.5	7.0
LF	14	3	7	9.0	14.0	13.5	12.0	5.5	5.5	6.0	3.0	5.0	7.0
LF	14	3	11	12.0	13.5	13.5	10.0	6.5	3.0	4.0	3.5	4.0	7.0
LF	20	1	2	9.0	14.5	14.0	9.0	5.5	4.5	5.0	5.0	5.5	6.5
LF	20	1	3	9.0	14.0	14.0	10.0	6.0	4.0	6.0	5.0	6.0	8.0
LF	20	1	4	8.5	14.0	13.5	12.0	6.5	4.0	4.0	4.0	6.0	6.0
LF	20	1	5	9.0	13.5	13.0	9.0	5.0	3.0	4.5	5.3	6.5	8.0
LF	20	1	7	8.5	13.5	14.5	9.0	4.5	5.0	5.0	4.0	5.0	6.5
LF	20	1	11	8.5	14.5	13.5	9.5	6.0	3.0	4.0	3.5	5.0	8.0
LF	20	2	2	9.0	14.5	13.5	9.0	5.5	4.5	5.0	5.0	5.0	5.5
LF	20	2	3	9.0	14.0	14.0	10.0	6.0	4.5	6.0	4.5	6.0	8.0
LF	20	2	4	9.0	14.5	13.0	12.5	6.0	4.5	4.0	4.0	5.5	6.0
LF	20	2	5	8.8	14.5	13.5	9.3	5.5	3.3	5.5	5.0	6.5	7.0
LF	20	2	7	9.0	13.0	14.5	9.0	4.5	4.5	5.0	3.5	5.5	6.5
LF	20	2	11	9.0	14.5	13.5	10.0	6.0	3.0	4.0	4.0	5.0	7.5
LF	20	3	2	9.5	14.5	14.5	10.0	6.0	3.0	4.0	4.0	5.0	5.5
LF	20	3	3	8.5	14.0	14.0	9.0	6.5	3.5	5.0	4.0	5.0	7.5
LF	20	3	4	8.5	14.0	13.5	12.0	6.3	4.0	4.0	4.0	5.5	6.0
LF	20	3	5	8.0	14.0	13.0	9.5	5.0	4.0	6.0	5.0	6.5	7.5
LF	20	3	7	9.0	14.0	14.5	9.0	4.5	4.5	5.0	3.5	5.0	6.5
LF	20	3	11	8.5	14.5	13.0	9.0	5.5	4.0	4.0	4.5	5.5	8.0

TABLE B.6. TOP LINE COMPUSENSE RESULTS FOR CONSUMER SENSORY PANEL

Liking attributes are scored on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely. Different letters in rows following means signify significant differences ($p < 0.05$)

Results for LF cheddar cheese (n=107)

	2mm	8mm	14mm
Overall Liking	5.2a	5.3a	5.5a
Flavor Liking	5.4a	5.3a	5.6a
Texture Liking	4.8a	5.0a	5.2a
Preferred sample	28.0% c	32.7% b	39.3% a

Results for RF Cheddar Cheese (n=107)

	2mm	8mm	14mm
Overall Liking	5.8a	5.9a	6.2a
Flavor Liking	5.8b	5.9ab	6.3a
Texture Liking	5.9a	6.0a	6.2a
Preferred sample	29.9% b	27.1% c	43.0% a

Results for FF Cheddar Cheese (n=107)

	2mm	8mm	14mm
Overall Liking	6.5a	6.8a	6.7a
Flavor Liking	6.5a	6.9a	6.8a
Texture Liking	6.5a	6.8a	6.8a
Preferred sample	24.3% c	42.1% a	33.7% b

Appendix C

Raw Data for Chapter 4 (Filled Gel Cheese Model with Uncharged Sephadex Beads)

Figures and Tables

TABLE C.1. PROPERTY AVERAGES FROM BATCH 1 AND 2

Two batches of cheese were made for each treatment and subsampled. Values in this table are averages of that subsampling; these values were used for all statistical analysis.

Filler	Volume	Age	RE	Critical stress (Pa)	Critical strain	$J_{max,\ 100\ Pa}$ (μPa^{-1})	$J_{r,\ 100\ Pa}$ (μPa^{-1})	$crp_{100\ Pa}$	$J_{max,\ 500\ Pa}$ (μPa^{-1})	$J_{r,\ 500\ Pa}$ (μPa^{-1})	$crp_{500\ Pa}$
Control	LF	8	44.90%	540	2.58E-03	41.87	19.16	54.12%	81.82	56.46	30.70%
		8	42.40%	328	2.44E-03	56.36	19.01	66.36%	40.55	7.84	81.01%
		12	42.71%	283	1.77E-03	60.76	35.40	40.17%	39.50	24.47	37.71%
		12	40.18%	366	2.13E-03	53.92	25.23	54.35%	67.72	35.96	47.00%
		18	42.07%	162	1.32E-03	47.41	17.68	61.74%	49.95	31.69	38.95%
		18	40.42%	576	2.24E-03	105.17	36.61	65.64%	132.92	57.82	59.33%
	RF	8	45.85%	309	1.48E-03	78.46	46.35	39.34%	65.51	44.35	32.30%
		8	41.01%	445	1.57E-03	47.69	12.10	74.90%	49.35	10.98	77.49%
		12	42.11%	218	1.03E-03	65.53	39.29	40.39%	71.07	43.65	39.37%
		12	39.16%	279	2.84E-03	138.38	45.47	67.78%	109.28	34.16	69.76%
		18	39.31%	271	1.68E-03	70.85	33.17	54.08%	67.82	42.73	37.12%
		18	39.81%	258	2.44E-03	174.80	89.42	47.80%	151.15	68.14	57.64%
	FF	8	42.66%	102	5.66E-04	47.73	36.29	23.06%	49.75	33.13	33.29%
		8	35.90%	607	1.21E-03	35.52	7.85	78.38%	41.21	19.33	53.40%
		12	38.52%	195	4.70E-04	26.33	12.15	54.93%	27.38	16.57	40.44%
		12	37.09%	1168	2.11E-03	34.80	11.56	64.78%	46.16	13.65	70.13%
		18	38.52%	167	6.18E-04	14.44	6.19	50.40%	21.93	8.83	59.71%
		18	34.45%	197	1.01E-03	29.59	16.67	43.36%	40.88	16.51	59.85%
Superfine SF	LF	8	46.08%	492	4.38E-03	56.62	41.83	26.30%	82.85	60.67	26.93%
		8	43.02%	431	2.09E-03	119.00	63.64	47.62%	134.75	92.44	31.53%
		12	44.14%	330	2.54E-03	57.73	39.68	31.54%	55.28	37.89	31.29%
		12	42.90%	392	2.39E-03	107.56	58.72	48.15%	72.34	22.36	69.20%
		18	42.27%	182	1.81E-03	50.98	21.18	59.12%	68.92	50.09	27.36%
		18	40.09%	837	3.04E-03	117.59	22.95	80.09%	187.10	97.23	47.95%
	RF	8	46.74%	301	3.83E-03	73.69	49.78	30.55%	110.20	72.64	34.11%
		8	43.29%	789	1.83E-03	37.16	14.32	62.15%	37.62	11.19	69.75%
		12	44.40%	296	1.56E-03	56.80	30.67	46.35%	54.38	36.27	33.33%
		12	40.72%	538	2.90E-03	94.47	33.18	65.58%	99.71	48.93	51.02%
		18	41.25%	207	1.95E-03	65.34	50.47	22.76%	92.71	63.08	34.52%
		18	41.84%	283	3.20E-03	125.35	61.49	52.04%	71.77	28.09	60.94%
	FF	8	48.60%	571	1.40E-03	14.06	9.88	29.71%	21.46	16.35	23.64%
		8	47.15%	390	2.87E-03	35.12	13.59	61.68%	36.81	16.35	55.58%
		12	42.98%	515	7.54E-04	8.20	6.15	23.69%	9.64	7.97	17.15%
		12	45.90%	592	1.28E-03	35.12	13.59	61.68%	36.81	20.41	45.12%
		18	47.98%	922	3.70E-04	4.49	3.87	14.58%	8.65	6.40	25.95%
		18	44.72%	1092	3.01E-03	24.20	15.02	37.36%	30.38	18.67	39.81%

TABLE C.1 CONTINUED

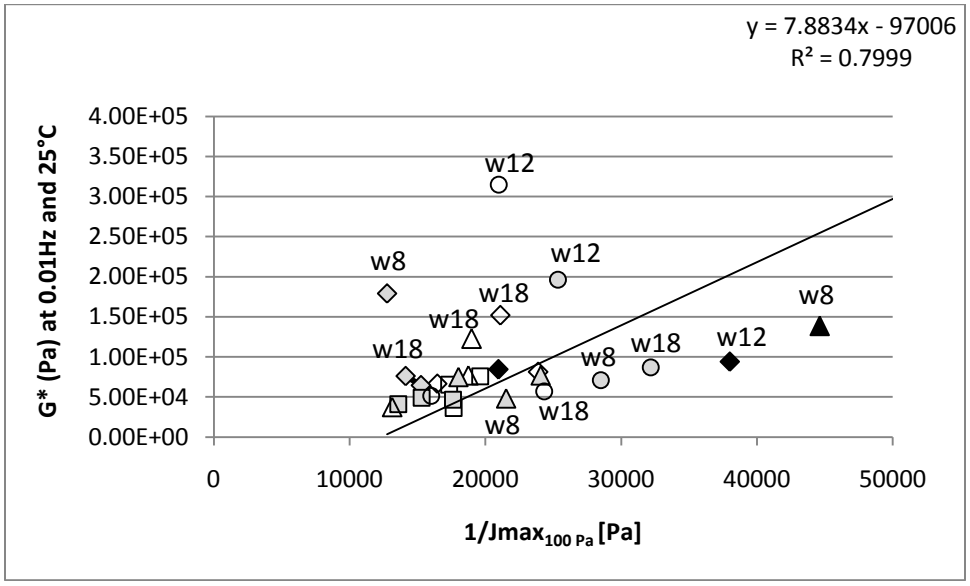
Filler	Volume	Age	RE	Critical stress (Pa)	Critical strain	$J_{max,\ 100\ Pa}$ (μPa^{-1})	$J_{r,\ 100\ Pa}$ (μPa^{-1})	$crp_{100\ Pa}$	$J_{max,\ 500\ Pa}$ (μPa^{-1})	$J_{r,\ 500\ Pa}$ (μPa^{-1})	$crp_{500\ Pa}$
Fine	LF	8	46.48%	451	4.81E-03	76.12	48.40	36.34%	70.75	35.66	50.99%
		8	43.89%	412	2.50E-03	64.35	39.69	42.49%	65.04	23.65	63.58%
		12	43.21%	298	3.06E-03	53.34	42.55	19.97%	56.18	41.84	25.61%
		12	42.98%	411	1.87E-03	74.74	28.56	61.41%	76.30	36.97	51.84%
		18	41.99%	226	1.98E-03	52.67	23.45	55.44%	56.04	34.95	37.71%
		18	41.26%	901	2.73E-03	79.67	28.45	64.25%	81.06	39.06	52.34%
	RF	8	47.74%	240	2.68E-03	46.48	37.42	19.08%	57.80	37.14	36.20%
		8	45.87%	628	2.17E-03	21.58	6.60	68.75%	36.80	18.16	49.16%
		12	44.94%	358	1.56E-03	41.56	27.92	34.58%	61.97	43.58	29.65%
		12	41.84%	487	3.12E-03	69.79	43.92	37.26%	64.26	32.73	49.56%
		18	40.59%	221	1.31E-03	55.52	32.17	42.02%	42.13	23.95	44.16%
		18	43.80%	870	3.41E-03	61.09	23.38	64.62%	64.44	28.25	56.26%
	FF	8	48.61%	530	1.12E-03	22.41	11.19	48.68%	19.61	12.67	35.77%
		8	47.39%	994	2.62E-03	21.51	8.25	61.67%	28.95	14.68	48.85%
		12	44.94%	802	7.35E-04	12.13	7.79	35.63%	9.67	7.08	27.36%
		12	46.07%	1296	2.60E-03	21.51	8.25	61.67%	28.95	14.68	48.85%
		18	48.88%	1000	4.40E-04	10.05	5.57	44.60%	7.28	4.46	38.66%
		18	45.48%	894	1.75E-03	21.98	9.68	55.42%	23.69	12.12	49.00%
Medium	LF	8	47.31%	583	3.51E-03	62.44	44.85	26.58%	59.76	40.14	32.59%
		8	46.88%	446	1.36E-03	26.04	12.73	51.90%	32.15	11.48	64.41%
		12	44.41%	372	1.17E-03	47.68	26.33	44.94%	44.50	29.19	34.51%
		12	44.39%	747	2.70E-03	51.91	34.13	33.83%	50.25	34.08	32.94%
		18	41.79%	255	1.63E-03	41.11	30.53	25.30%	43.95	25.04	43.17%
		18	42.42%	326	3.18E-03	64.66	23.18	65.44%	69.17	30.58	56.65%
	RF	8	50.26%	270	1.84E-03	35.10	26.34	25.08%	48.64	35.13	27.57%
		8	48.24%	984	1.34E-03	25.50	11.85	53.63%	24.28	13.03	46.53%
		12	46.49%	572	2.09E-03	39.46	26.22	33.55%	34.94	25.77	26.30%
		12	44.98%	1204	3.01E-03	46.67	24.20	47.01%	44.38	29.44	33.79%
		18	43.43%	361	1.44E-03	31.08	14.46	55.46%	27.35	15.28	46.74%
		18	44.95%	1355	2.55E-03	38.34	21.59	42.91%	40.15	17.94	55.80%
	FF	8	51.65%	840	7.78E-04	11.90	5.05	57.46%	9.71	6.14	36.79%
		8	50.30%	1034	2.07E-03	21.00	8.59	58.84%	18.55	6.13	65.61%
		12	45.10%	647	1.16E-03	10.74	7.09	32.08%	10.29	6.95	32.31%
		12	48.28%	906	1.88E-03	18.55	6.13	65.61%	21.00	8.59	58.84%
		18	50.18%	786	3.76E-04	7.49	4.04	45.83%	7.27	3.69	45.97%
		18	42.99%	561	1.51E-03	19.94	7.38	63.62%	22.02	13.93	39.92%

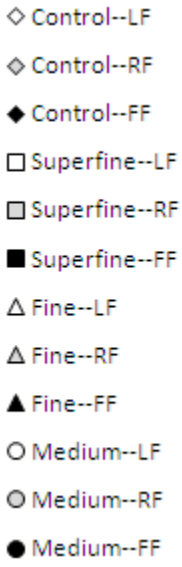
TABLE C.2. EFFECT OF TEMPERATURE AND PHASE VOLUME ON STORAGE MODULUS

Values in table are storage moduli, G' (Pa), which were averaged from frequency sweeps on treatments at different ages (8, 12, and 18 weeks) and two different batches (complete replications) of cheese. Treatments were SF (Superfine Sephadex), Fine Sephadex, Med (Medium Sephadex) and Cont (Control).

	Filler type--Filler Volume											
Temperature (°C)	SF--LF	SF--RF	SF--FF	Fine--LF	Fine--RF	Fine--FF	Med--LF	Med--RF	Med--FF	Cont--LF	Cont--RF	Cont--FF
10	650133	372467	922933	582367	579967	1055733	825560	851133	1034667	553033	653867	1670667
15	649067	355733	895767	590800	571183	992300	787960	832333	945300	521700	637900	1437833
20	639100	324800	832533	562467	541450	892267	725240	749633	812000	452983	574883	1033100
25	591967	289467	763933	519633	497217	806100	676000	668333	681167	390667	512500	813867

FIGURE C.1. CORRELATION BETWEEN FREQUENCY SWEEP AND MAXIMUM CREEP COMPLIANCE
Data not presented in Chapter 4





Appendix D

Raw Data for Chapter 5 (Filled Gel Cheese Model with Charged Sephadex Beads) Figures and Tables

TABLE D.1. RAW DATA FROM STRESS SWEEPS AND UNIAXIAL COMPRESSION

Treatment	Age (weeks)	Critical stress (Pa)	Critical strain	RE
LFC	8	1484	0.001995	55.66%
		1210	0.001809	56.16%
		1353	0.002282	54.59%
		.	.	55.82%
		.	.	54.89%
		.	.	55.51%
	12	1102	0.002724	48.67%
		1484	2.79E-03	48.73%
		985	3.51E-03	48.72%
		.	.	48.47%
		.	.	49.33%
		.	.	49.31%
FFC	8	1279	0.001657	44.87%
		985	0.001364	43.56%
		607	0.001683	44.17%
		.	.	45.14%
		.	.	45.13%
		.	.	46.48%
	12	1082	0.001616	46.14%
		1353	0.002359	43.20%
		788	0.002289	43.53%
		.	.	42.17%
		.	.	42.13%
		.	.	44.66%
FF0	8	705	0.002562	52.35%
		788	0.001717	51.28%
		731	0.002852	48.66%
		.	.	48.33%
		.	.	48.81%
		.	.	48.03%
	12	630	0.004962	42.06%
		973	0.003996	43.69%
		902	0.002373	43.92%
		.	.	42.46%
		.	.	43.59%
		.	.	42.61%

TABLE D.1. CONTINUED

Treatment	Age (weeks)	Critical stress (Pa)	Critical strain	RE
FF-	8	523	0.002678	50.15%
		388	0.002544	48.81%
		1146	0.004032	47.00%
		.	.	47.10%
		.	.	48.85%
		.	.	48.93%
	12	572	0.003704	42.24%
		666	0.003088	38.93%
		836	0.004347	39.38%
		.	.	47.42%
		.	.	41.97%
		.	.	41.24%
FF+	8	184	0.004142	51.39%
		230	0.005599	49.11%
		206	0.005092	51.00%
		.	.	50.93%
		.	.	49.58%
		.	.	54.54%
	12	805	0.004212	40.57%
		363	0.001956	29.31%
		1422	0.003546	19.70%
		.	.	34.55%
		.	.	21.45%
		.	.	26.44%

TABLE D.2. RAW DATA FROM CREEP/RECOVERY TESTS

Treatment	Age (weeks)	Load (Pa)	J_{max} (μPa^{-1})	J_r (μPa^{-1})	crp
LFC	8	100	27.30	4.06	85.11%
			30.00	10.34	65.53%
		500	26.08	12.22	53.14%
			22.07	10.65	51.74%
		2200	22.68	8.31	63.35%
			22.45	8.12	63.84%
	12	100	25.07	10.08	59.79%
			23.34	10.53	54.88%
		500	40.49	28.02	30.80%
			38.97	11.57	70.31%
		2200	40.04	21.76	45.65%
			54.95	40.19	26.86%
FFC	8	100	26.84	8.50	68.35%
			24.53	8.09	67.03%
		500	24.41	12.29	49.65%
			21.35	9.04	57.68%
		2200	25.72	17.30	32.74%
			31.49	20.50	34.90%
	12	100	24.53	12.43	49.33%
			37.15	13.17	64.55%
		500	23.88	9.91	58.52%
			36.83	21.99	40.29%
		2200	48.12	31.36	34.83%
			35.44	24.38	31.21%

TABLE D.2. CONTINUED

Treatment	Age (weeks)	Load (Pa)	J_{max} (μPa^{-1})	J_r (μPa^{-1})	crp
FF0	8	100	33.61	18.48	45.02%
			37.92	22.55	40.53%
		500	33.78	15.71	53.49%
			29.89	11.31	62.16%
		2200	49.85	26.60	46.64%
			40.60	16.95	58.25%
	12	100	33.61	18.48	45.02%
			37.92	22.55	40.53%
		500	33.78	15.71	53.49%
			29.89	11.31	62.16%
		2200	49.85	26.60	46.64%
			40.60	16.95	58.25%
FF-	8	100	48.59	23.38	51.88%
			57.82	24.81	57.09%
		500	61.86	51.66	16.49%
			87.51	21.02	75.98%
		2200	58.45	25.84	55.79%
			86.77	53.40	38.46%
	12	100	48.59	23.38	51.88%
			57.82	24.81	57.09%
		500	61.86	51.66	16.49%
			87.51	21.02	75.98%
		2200	58.45	25.84	55.79%
			86.77	53.40	38.46%
FF+	8	100	76.73	45.67	40.48%
			77.14	53.12	31.14%
		500	43.90	12.80	70.84%
			47.41	20.66	56.42%
		2200	105.00	61.57	41.36%
			125.60	78.04	37.87%
	12	100	76.73	45.67	40.48%
			77.14	53.12	31.14%
		500	43.90	12.80	70.84%
			47.41	20.66	56.42%
		2200	105.00	61.57	41.36%
			125.60	78.04	37.87%

TABLE D.3. RAW DATA FROM SENB TESTS

		Age (weeks)	K_c (kPa•m^0.5)	G_c (J/m^2)	Max. Extension (mm)	Max. Load (N)	Work to Max. Load (J)	Slope (N/mm), E, up to load 0.1 N
LFC	Notched	8	3.27	17.13	7.603	0.250	3.60E-04	0.0838
			3.00	8.65	6.902	0.195	1.60E-04	0.0638
			4.37	22.80	10.101	0.271	4.00E-04	0.0720
			3.95	8.41	7.100	0.295	1.70E-04	0.1197
			3.95	8.41	7.699	0.177	1.60E-04	0.0709
			3.31	10.14	7.798	0.239	2.49E-04	0.1049
		12	2.42	15.88	8.100	0.202	3.60E-04	0.0409
			2.48	8.26	7.799	0.173	1.60E-04	0.0634
			2.51	19.84	2.700	0.179	4.00E-04	0.1030
			2.90	8.92	6.900	0.205	1.70E-04	0.1113
			2.32	8.51	5.900	0.155	1.60E-04	0.0944
			2.67	6.17	2.299	0.190	1.20E-04	0.0620
	Non-notched	8	.	.	14.800	0.519	5.47E-03	0.1061
			.	.	15.023	0.462	4.26E-03	0.0837
			.	.	13.900	0.449	4.29E-03	0.1059
			.	.	15.400	0.624	6.92E-03	0.0995
			.	.	.	.	.	.
			.	.	.	.	.	.
		12	.	.	16.000	0.443	3.54E-03	0.0886
			.	.	16.700	0.419	3.06E-03	0.1328
			.	.	14.300	0.405	4.74E-03	0.1445
			.	.	14.601	0.483	5.38E-03	0.0871
			.	.	15.399	0.467	1.45E-03	0.1035
			.	.	15.000	0.348	3.60E-03	0.1051
FFC	Notched	8	4.39	40.46	4.399	0.256	6.70E-04	0.0876
			3.47	34.11	4.700	0.274	7.60E-04	0.0638
			3.73	46.10	5.502	0.292	9.90E-04	0.1264
			3.53	28.30	4.000	0.232	5.20E-04	0.0567
			3.31	42.84	6.101	0.241	8.80E-04	0.0296
			3.84	36.13	4.400	0.289	7.70E-04	0.0560
		12	2.25	27.95	4.999	0.149	5.20E-04	0.1047
			2.39	25.79	4.470	0.175	5.20E-04	0.0484
			1.79	20.92	5.480	0.130	4.30E-04	0.1195
			1.30	16.10	8.701	0.075	2.70E-04	0.0803
			1.64	20.72	6.601	0.102	3.60E-04	0.0867
			2.52	34.15	5.500	0.186	6.70E-04	0.1907
	Non-notched	8	.	.	9.401	0.952	6.48E-03	0.1141
			.	.	5.701	0.429	1.51E-03	0.0734
			.	.	5.700	0.627	2.24E-03	0.0878
			.	.	8.201	0.576	3.30E-03	0.1083
			.	.	6.700	0.511	2.33E-03	0.0619
			.	.	6.199	0.328	1.20E-03	0.1111
		12	.	.	10.201	0.302	2.04E-03	0.1836
			.	.	9.100	0.309	1.83E-03	0.1412
			.	.	8.500	0.226	1.01E-03	0.1289
			.	.	7.200	0.348	1.67E-03	0.1652
			.	.	13.001	0.329	2.99E-03	0.1559
			.	.	7.107	0.394	1.88E-03	0.0835

TABLE D.3 CONTINUED

		Age (weeks)	K_c (kPa•m^0.5)	G_c (J/m^2)	Max. Extension (mm)	Max. Load (N)	Work to Max. Load (J)	Slope (N/mm), E, up to load 0.1 N
FF0	Notched	8	4.83	71.45	7.100	0.308	1.32E-03	0.0675
			3.22	36.32	5.600	0.173	5.80E-04	0.0699
			3.27	42.61	6.605	0.152	6.20E-04	0.0666
			3.45	51.51	6.901	0.224	9.30E-04	0.0693
			3.51	56.85	6.900	0.224	1.02E-03	0.0553
			4.15	48.32	5.901	0.300	1.03E-03	0.0456
		12	1.55	20.02	5.000	0.105	3.80E-04	0.0676
			2.10	34.49	6.000	0.149	6.60E-04	0.0473
			1.58	6.48	1.800	0.097	1.10E-04	0.0314
			1.93	19.77	2.570	0.167	4.60E-04	0.0468
			2.11	21.36	4.299	0.149	4.20E-04	0.0655
			1.29	8.21	3.390	0.077	1.40E-04	0.0871
	Non-notched	8	.	.	15.802	0.619	6.15E-03	0.0627
			.	.	15.302	0.455	4.96E-03	0.0413
			.	.	15.501	0.692	7.17E-03	0.0702
			.	.	15.126	0.622	5.99E-03	0.0626
			.	.	17.099	0.592	7.07E-03	0.0798
			.	.	13.601	0.775	6.46E-03	0.0434
		12	.	.	13.101	0.246	2.29E-03	0.0643
			.	.	10.601	0.165	1.29E-03	0.0696
			.	.	11.413	0.261	2.15E-03	0.0736
			.	.	12.499	0.227	2.14E-03	0.1186
			.	.	13.001	0.306	2.87E-03	0.1059
			.	.	11.501	0.182	1.48E-03	0.0931
FF-	Notched	8	2.46	36.35	6.700	0.147	6.40E-04	0.0338
			3.10	42.62	6.200	0.175	7.10E-04	0.0342
			3.00	37.08	5.999	0.195	7.10E-04	0.0313
			2.97	42.43	6.201	0.187	7.60E-04	0.0422
			3.29	53.43	7.201	0.172	8.00E-04	0.0339
			4.14	70.98	8.199	0.238	1.15E-03	0.0460
		12	1.41	10.26	2.401	0.084	1.80E-04	0.0410
			1.36	26.46	2.998	0.103	5.40E-04	0.0524
			1.58	37.00	3.500	0.105	6.70E-04	0.0471
			1.22	9.45	2.202	0.095	2.00E-04	0.0558
			1.39	13.64	4.700	0.090	2.40E-04	0.0436
			1.61	21.07	3.500	0.123	4.40E-04	0.0467
	Non-notched	8	.	.	18.101	0.304	3.53E-03	0.0202
			.	.	16.700	0.299	3.35E-03	0.0315
			.	.	16.910	0.338	3.81E-03	0.0351
			.	.	15.903	0.410	4.41E-03	0.0272
			.	.	18.407	0.517	6.59E-03	0.0442
			.	.	.	.	.	.
		12	.	.	9.801	0.092	5.80E-04	0.0254
			.	.	12.500	0.188	1.53E-03	0.0461
			.	.	11.300	0.161	1.27E-03	0.0528
			.	.	10.209	0.152	9.90E-04	0.0720
			.	.	14.009	0.119	1.14E-03	0.0828
			.	.	13.800	0.117	1.16E-03	0.0610

TABLE D.3 CONTINUED

		Age (weeks)	K_c (kPa•m^0.5)	G_c (J/m^2)	Max. Extension (mm)	Max. Load (N)	Work to Max. Load (J)	Slope (N/mm), E, up to load 0.1 N
FF+	Notched	8	0.73	5.52	4.402	0.056	1.20E-04	0.0179
			0.75	7.69	4.600	0.064	1.90E-04	0.0229
			0.82	7.68	4.405	0.076	2.00E-04	0.0184
			0.88	9.44	4.699	0.075	2.20E-04	0.0081
			0.57	4.29	4.103	0.046	1.00E-04	0.0250
			0.99	7.73	4.300	0.073	1.60E-04	0.0238
		12	0.52	2.16	3.314	0.044	5.00E-05	0.0264
			0.58	2.60	3.101	0.041	5.00E-05	0.0361
			0.42	1.91	3.001	0.031	4.00E-05	0.0292
			0.56	5.43	4.410	0.045	1.20E-04	0.0253
			.	.	.	.	.	.
			.	.	.	.	.	.
	Non-notched	8	.	.	5.399	0.194	6.30E-04	0.0466
			.	.	4.702	0.202	5.50E-04	0.0367
			.	.	5.401	0.155	5.20E-04	0.0484
			.	.	4.701	0.091	2.30E-04	0.0218
			.	.	5.902	0.197	7.10E-04	0.0531
			.	.	.	.	.	.
		12	.	.	3.401	0.131	2.60E-04	0.0433
			.	.	1.899	0.098	1.00E-04	0.0240
			.	.	2.300	0.092	1.30E-04	0.0514
			.	.	4.601	0.072	1.90E-04	0.0435
			.	.	.	.	.	.
			.	.	.	.	.	.