The Efficacy of Sodium Gluconate as a Calcium Lactate Crystal Inhibitor in Cheddar Cheese

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE UNIVERSITY OF MINNESOTA

BY

Chanokphat Phadungath

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Dr.Lloyd Ernst Metzger, Adviser

August, 2011

Acknowledgements

First and foremost, I offer my sincerest gratitude to my academic adviser, Dr. Lloyd E. Metzger, who has supported me from the beginning of my research project until the very end of my dissertation writing with his patience and knowledge while allowing me the room to work in my own way. In addition, he was always accessible and willing to help all his advisees, including me, with our research. As a result, my research life was very smooth and very rewarding.

I am very thankful to my graduate committee members, Dr. David E. Smith, Dr.Francisco Diez-Gonzalez, Dr.Philippe Buhlmann, and Dr.Nancy Jo Ehlke, for their guidance and suggestions, and Dr. Don A. Grindstaff for his advice.

I am truly grateful for financial support from the Midwest Dairy Foods Research Center.

Special thanks go to Peggy for teaching and guiding me how to first run HPLC and AAS once I started my research; Terry, Ray and Jody from the Dairy Plant at Department of Food Science and Nutrition, University of Minnesota for helping through my very first Cheddar cheese making, Akimoto from the Dairy Plant at South Dakota State University for his help with the Cheddar cheese making.

I would not have had a wholesome joy throughout my PhD research if it was not because of all my labmates. I am very thankful to have known and to have worked with

everyone; thus, special thanks to everyone, which includes Liu, Rohit, Shiva, Praveen, Melissa, and Alexa from University of Minnesota, and Prafulla, Huimin, Jayendra, Anil, Marella, and Ananya from South Dakota State University.

My deepest gratitude goes to my family, my mother – Suwakhon Siriwongworawat, my dad – Chana Phadungath, my sister – Chonnakan Sombatsiri, and my brother – Komchart Phadungath, for their unconditional love and support throughout my life; this dissertation would have been impossible without them.

Dedication

I would like to dedicate this research project to my mother, Suwakhon Siriwongworawat for believing in me and supporting me wholeheartedly from the begging to the end.

Mom, I love you.

Abstract

Calcium and lactate are present in excess of their solubility in Cheddar cheese. Consequently, calcium lactate crystals are a common defect in Cheddar cheese. A novel approach for preventing calcium lactate crystals is the addition of sodium gluconate. Sodium gluconate has the potential to increase the solubility of calcium and lactate by forming soluble complexes with calcium and lactate ions, and preventing them from being available for the formation of calcium lactate crystals. This research project was to determine the efficacy of sodium gluconate as a calcium lactate crystal inhibitor in Cheddar cheese. The first part of this study was to determine if sodium gluconate could increase the solubility of calcium lactate. Seven calcium lactate solutions (5.31% w/w) with seven levels of sodium gluconate (0, 0.5, 1, 1.5, 2, 3, and 4% w/w) were made in triplicate. Solutions were stored at 7°C for 21 days, and were visually inspected for calcium lactate crystal formation. Subsequently, they were filtered to remove calcium lactate crystals and the supernatant was analyzed for lactic acid and gluconic acid by HPLC and for calcium by Atomic Absorption Spectroscopy. The visual inspection demonstrated that calcium lactate crystals were formed in the solution with 0% gluconate after the first day of storage and calcium lactate crystals continued to accumulate over time. A minute amount of calcium lactate crystals was also visible in the solution with 0.5% gluconate after 21 days of storage, while calcium lactate crystals were not visible in the other solutions. The HPLC results indicated that there was a higher concentration of calcium and lactic acid in the filtrate from the solutions containing added gluconate. Thus, sodium gluconate can increase the solubility of calcium lactate.

The second part of this study was to determine the manufacture and composition of Cheddar cheeses with different levels of sodium gluconate addition. Six Cheddar cheeses with two levels of salting (2 and 2.5%) and three sodium gluconate addition levels (0, 0.5 and 1%) were manufactured. All cheeses were made using a stirred-curd procedure and replicated three times. Two levels were obtained by dividing cheese curd (at pH 5.6) into two equal-weight halves; each half was salted with 2 and 2.5% (by weight of cheese curd) sodium chloride. Subsequently, each of the salted halves was separated into three equal-weight batches and mixed with 0 (control), 0.5, and 1.0% sodium gluconate, respectively. After sodium gluconate addition, the curds were hooped, pressed for 16 hour, vacuum-sealed in polyethylene bags, and transferred to a ripening room at 6 to 8°C. After 1 week of storage, compositional analyses (pH, moisture, salt, fat and protein) and gluconic acid concentration were determined. Mean pH, moisture, salt, fat and protein content of the cheeses ranged from 5.06 to 5.32, 36.98 to 38.15%, 1.65 to 2.13%, 30.96 to 32.98%, and 25.6 to 26.1%, respectively. At both salting levels, the pH and moisture contents were significantly ($p<0.05$) increased in the treatments with added sodium gluconate. The concentration of gluconic acid in the low salt treatments was 0.33 and 0.59% for the 0.5 and 1.0% addition level, respectively, whereas the concentration in the high salt levels was 0.33 and 0.58%, respectively.

The third part of this study was to determine if the level and type of residual sugar and organic acids produced during ripening was impacted by sodium gluconate addition to Cheddar cheeses. Six cheeses with two salting rates (2 and 2.5%) and three sodium gluconate addition levels (0, 0.5 and 1%) were manufactured. The cheeses were analyzed for lactose and water-soluble organic acids (acetic, butanoic, citric, formic, gluconic,

lactic, orotic, propanoic, and uric) at 1-week, 3-month and 6-month, 9-month, and 12-month of ripening by using a cation-exchange-column HPLC externally equipped with a refractive index detector . The organic acids were detected using the UV detector set at 210 and 285 nm, and the refractive index was used for quantification of lactose. The results indicated that at 1-week of ripening, Cheddar cheeses with a higher concentration of gluconic acid had lower concentration of lactic acid, but higher concentration of lactose, while there were no differences in acetic, butanoic, citric, formic, orotic, propanoic and uric acids among treatments at all ripening times. The concentrations of butanoic and propanoic acids gradually increased over time in all treatments, whereas the concentrations of orotic acid and lactose gradually decreased over time. Minor changes in the levels of acetic, citric, formic, lactic, and uric were also observed throughout ripening in all treatments.

The fourth part of this study was to determine the effect of sodium gluconate on pH, lactose, lactic acid, and WSC changes during Cheddar cheese ripening. Six Cheddar cheeses with two salting levels (2 and 2.5%) and three sodium gluconate levels (0, 0.5 and 1%) were manufactured in triplicate. Composition and chemical analysis was performed at 1 week of ripening, and at 3, 6, 9, and 12 months of ripening. Cheeses were analyzed for pH, lactose and lactic acid, and WSC. Compositional analyses at 1 week indicated that sodium gluconate addition had a significant effect on cheese pH, moisture, Na, lactose, and lactic acid. Cheddar cheeses from both 2% and 2.5% salt levels with 0.5 and 1.0% sodium gluconate exhibited higher pH than the control cheeses throughout the ripening time. HPLC results from Cheddar cheeses from both 2% and 2.5% salt levels indicated that cheeses with higher concentration of sodium gluconate addition had a

higher concentration of lactose, but lower concentration of lactic acid when compared to the control cheeses throughout the ripening time. WSC results indicated that Cheddar cheeses from both 2% and 2.5% salt levels with higher concentration of sodium gluconate addition had lower WSC concentration when compared to the control cheeses throughout the ripening time. From the results, we concluded that sodium gluconate could have an effect on starter culture activity and could also act as buffering agent, which would cause a higher cheese pH. A higher cheese pH resulted in less soluble of calcium in the cheese serum; thus, resulting in less calcium and lactate ions in the cheese serum.

The final part of this study was to determine the effect of sodium gluconate on the extent of proteolysis, textural properties and sensory evaluation during Cheddar cheese ripening. Six Cheddar cheeses with two salting levels (2 and 2.5%) and three sodium gluconate levels (0, 0.5 and 1%) were manufactured in triplicate. Cheeses were analyzed for the extent of proteolysis by measuring pH 4.6 soluble N and 12% TCA soluble N at 3, 6, 9, and 12-month of ripening. Textural properties were determined by Texture Profile Analysis (TPA) using a TA.XTplus Texture Analyzer at 3, 6, 9, and 12-month of ripening. TPA parameters generated were fracturability, hardness, cohesiveness, springiness, chewiness, and resilience. Descriptive sensory analysis was used to monitor Cheddar cheese flavors in this study at 6 and 12-month of ripening. An increase in soluble N and decreases in textural properties (fracturability, hardness, cohesiveness, springiness, gumminess, chewiness, and resilience) were observed throughout the ripening time for all treatments. At both salting levels, cheeses with added sodium gluconate exhibited a trend for a higher level of proteolysis and lower TPA hardness at 6

and 9 months. The overall flavor intensity scores at 6 months of ripening were lower in cheeses with added sodium gluconate, which could relate to their lower bitterness scores. A similar trend was observed at 12 months of ripening, where cheeses with sodium gluconate addition had lower overall flavor intensity and lower bitterness scores. This present study provides an understanding of how sodium gluconate impacts cheese characteristics during ripening.

Table of Contents

Acknowledgements ..... **i**
Dedication ..... **iii**
Abstract ..... **iv**
Table of Contents ..... **ix**
List of Tables ..... **xiii**
List of Figures ..... **xv**
Chapter 1: Review of Literature **1**
Casein protein in milk **2**
Casein micelle structure **3**
The importance of calcium-phosphate and κ-casein to the casein micelle **8**
Cheddar cheese manufacture **10**
Rennet coagulation of milk **11**
Cheese ripening **13**
Microbiology of cheese ripening **13**
Critical biochemical reactions during cheese ripening **17**
Proteolysis in cheese during ripening **19**
Calcium lactate crystal in Cheddar cheese **21**
A racemic mixture of L(+) and D(-) calcium lactate in Cheddar cheese **32**
Other possible crystals **34**
The formation of calcium lactate crystals **37**
Solubility of calcium lactate **39**
Why is Ca-DL-lactate less soluble than Ca-L-lactate? **43**
Methods for estimating surface crystal development **46**
Factors influencing calcium lactate crystal formation in Cheddar cheese **47**
Recommendation methods for limiting calcium lactate crystal formation in Cheddar cheese **48**
Sodium gluconate as a calcium lactate crystal inhibitor **48**
Cheese with sodium gluconate to inhibit calcium lactate crystal Formation **50**
Sodium gluconate as a Ca-sequestrant **54**
The possible mechanisms for calcium lactate gluconate (CLG) formation ... **57**
Safety and legality of sodium gluconate as a food additive **62**
References **63**

Chapter 2. Effect of Sodium Gluconate On the Solubility of Calcium Lactate **80**
Introduction **81**
Materials and Methods **84**
Solubility model **84**

Analysis of lactic and gluconic acids **85**
Analysis of calcium content **86**
Statistical analyses **87**
Results and Discussion **87**
Visual inspection **87**
Analyses of lactic acid, gluconic acid, and calcium **88**
Sodium gluconate as a Ca-sequestrant **92**
Possible formation of CLG complexes **92**
Conclusions **94**
References **94**

Chapter 3. Cheddar Cheese Manufacture and Composition **98**
Introduction **99**
Materials and Methods **101**
The selection of sodium gluconate level for Cheddar cheese manufacture **101**
Experimental design **102**
Cheese manufacture **102**
Cheese sampling **105**
Chemical and compositional analyses **105**
Statistical analyses **106**
Results and Discussion **107**
Milk composition **107**
Cheese composition **107**
Na and Cl **110**
Ca and P **110**
Fat and protein **112**
Lactose and lactic acid at 1 week **112**
S/M and moisture **113**
pH at 1 week **115**
Gluconic acid **117**
The possible effect of sodium gluconate on Cheddar cheese composition **117**
Conclusions **118**
References **119**

Chapter 4. Changes in Residual Lactose and Water-soluble Organic Acids During Cheese Ripening **125**
Introduction **126**
Materials and Methods **129**
Experimental design **129**
Analysis of organic acids and lactose **130**
Statistical analyses **135**
Results and Discussion **135**
Changes in gluconic acid **135**

Changes in lactose **139**
Changes in lactic acid **141**
Changes in citric acid **144**
Changes in orotic acid **146**
Changes in succinic acid **147**
Changes in formic acid **149**
Changes in uric acid **151**
Changes in acetic acid **152**
Changes in propanoic acid **154**
Changes in butanoic acid **156**
Conclusions **157**
References **158**

Chapter 5. The Effect of Sodium Gluconate on pH, Water-soluble Calcium, Lactose, Lactic Acid, and Calcium Lactate Crystal Formation During Cheddar Cheese Ripening **164**
Introduction **165**
Materials and Methods **168**
Experimental design **168**
Analyses of pH and water-soluble Ca **169**
Analyses of lactic acid and lactose **171**
Surface crystal formation study **172**
Statistical analyses **173**
Results and Discussion **176**
Changes in pH **176**
Changes in lactose and lactic acid **177**
Changes in water-soluble Ca **180**
Surface crystal formation **184**
The possible effect of sodium gluconate as a calcium lactate crystal inhibitor in Cheddar cheese **189**
Conclusions **190**
References **191**

Chapter 6. The Effect of Sodium Gluconate on Proteolysis, Textural Properties and Sensory Evaluation During Cheddar Cheese Ripening **196**
Introduction **197**
Materials and Methods **201**
Experimental design **201**
Proteolysis in cheese during ripening **202**
pH 4.6 soluble N **203**
12% TCA soluble N **203**
Textural properties **204**
Statistical analyses **204**
Sensory evaluation **205**
Panelist training **205**

Testing **205**
Data analysis **207**
Results and Discussion **207**
Proteolysis in cheese during ripening **207**
Changes in pH 4.6 soluble nitrogen **207**
Changes in 12% TCA soluble nitrogen **208**
Factors influencing proteolysis **209**
Textural properties **213**
TPA parameters **213**
Factors influencing textural properties **214**
Sensory evaluation **221**
Sensory attributes **221**
Factors affecting sensory evaluation **227**
Conclusions **230**
References **231**
Biblography **239**

List of Tables

Chapter 1.

1. Effect of temperature on the solubility of Ca-L-lactate and Ca-DL-lactate …… **42**
2. Effect of pH on solubility of CaL_2 in water at 10°C …… **43**
3. Properties of calcium lactate gluconate compared to commonly used soluble organic calcium salts (in water at room temperature) …….. **58**

Chapter 2.

1. Mean squares and probabilities (in parentheses) for % Ca, % lactic acid, and % gluconic acid in CaL_2 solutions at 0 day and after 21 days of storage …… **90**
2. Mean calcium, lactic acid, and gluconic acid in CaL_2 solutions at 0 day of storage …….. **91**
3. Mean calcium, lactic acid, and gluconic acid in CaL_2 solutions after 21 days of storage …….. **91**

Chpter 3.

1. Mean squares and probability values (in parentheses) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition at 1 week of ripening …….. **108**
2. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition at 1 week of ripening …….. **111**

Chapter 4.

1. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition …… **130**
2. Retention time (RT) for different analytes, their detection, and calibration curve ……... **134**
3. Mean squares and probabilities (in parentheses) of changes in organic acids and lactose in Cheddar cheese with added sodium gluconate during storage at 6 – 8°C for 12 months …… **136**

Chapter 5.

1. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition …… **169**
2. Mean squares and probabilities (in parentheses) of changes in pH, %WSC, lactose and lactic acid in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months ……... **175**
3. Number of cheese slices (from the total of 30 slices) that showed calcium lactate crystal (CLC) formation during 12 months of ripening …….. **185**

Chapter 6.

1. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition …… **202**

2. Cheddar cheese lexicon (sensory descriptors and their definitions) used for the sensory evaluation of Cheddar cheeses with and without sodium gluconate addition at 6 and 12-month of ripening ……. **206**
3. Mean squares and probabilities (in parentheses) of changes in pH 4.6 soluble N and 12% TCA soluble N in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months …….. **208**
4. Mean squares and probabilities (in parentheses) of changes in TPA parameters in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months ……... **217**
5. Means (across the row) of sensory attributes of Cheddar cheeses with sodium gluconate addition at 6-month of ripening …… **222**
6. Means (across the row) of sensory attributes of Cheddar cheeses with sodium gluconate addition at 12-month of ripening …….. **223**
7. Mean squares and probabilities (in parentheses) of selected sensory attribute in Cheddar cheese with added sodium gluconate at 6 and 12 months of ripening ……. **224**

List of Figures

Chapter 1.

1. The structure of casein micelle in the sub-micelles model showing the protruding C-terminal parts of κ-casein as proposed by Walstra …….. **4**
2. Hairy casein micelle model proposed by Holt, where a tangled web and open structure of polypeptide chains cross-linked by calcium phosphate nanocluster (colloidal calcium phosphate) in the core provides rise to an external region of lower segment density known as the hairy layer. The gray circles represent the calcium phosphate nanoclusters …… **5**
3. The dual bonding model of casein micelle structure, with α-, β-, κ-casein portrayed as indicated. Bonding appears between the hydrophobic regions, shown as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to colloidal calciumphosphate clusters. Molecules of κ-casein (K) limit further growth of the structure …… **7**
4. Possible pathway for calcium lactate crystal formation on Cheddar cheese ….. **40**
5. Stereochemistry of the Ca ion hydration shell in (a) eight-fold (square-antiprismatic) structure and (b) seven-fold (pentagonal bipyramidal) structure …… **44**
6. The geometry of Ca-carboxylate interactions; (a) the unidentate mode, (b) the α-mode, and (c) the bidentate mode …….. **45**
7. The possible configurations of (a) Ca-L-lactate with α-mode and unidentate chelation and (b) Ca-DL-lactate with α-mode chelation …… **46**
8. A diagram describing an oxidation process of D-glucose to D-gluconic acid and sodium gluconate …….. **49**
9. The structure of Ca-lactobionate complex. Gal represents the galactose residues that are omitted for clarity …… **55**
10. The structure of Ca-gluconate complex. Ca-O2 indicated the bond between Ca ion and oxygen atom from α-hydroxyl group of gluconate. Ca-O12 indicated the bond between Ca ion and oxygen atom from carboxyl group of gluconate. Ca-OW indicated the bonds among Ca ion and water molecules …… **57**
11. The possible mechanisms for the calcium lactate gluconate formation. Hydrated ca ions bind with oxygen from carboxyl groups of both gluconate and lactate, and oxygen from hydroxyl groups of gluconate in two forms; (a) 8-fold hydration and (b) 7-fold hydration …….. **60**
12. Possible configurations of calcium lactate gluconate with (a) α-mode chelation only and (b) α-mode and unidentate mode chelation ……... **61**

Chapter 2.

1. CaL_2 solutions after 21 days of storage. The solutions contained different amount of sodium gluconate; 1) 0% (control), 2) 0.5%, 3) 1%, 4) 1.5%, 5) 2%, 6) 3%, and 7) 4% …….. **88**

2. Proposed mechanism of Ca-lactate-gluconate formation with lactate and gluconate ions competing for weaker Ca-hydration bonds resulting in Ca-lactate-gluconate complexes …….. **93**

Chapter 3.

1. manufacture diagram of Cheddar cheese with sodium gluconate addition ….. **104**

Chapter 4.

1. Typical chromatograms of Cheddar cheese with sodium gluconate addition for a). UV-285 nm detection, b). UV-210 nm detection, and c). RI detection. The organic acids detected are represented using numbers; 1 = lactose, 2 = citric acid, 3 = orotic acid, 4 = gluconic acid, 5 = pyruvic acid, 6 = galactose, 7= succinic acid, 8= lactic acid, 9 = formic acid, 10 = uric acid, 11 = acetic acid, 12 = propanoic acid, and 13 = butanoic acid …… **133**
2. % Gluconic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **138**
3. % Lactose in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate …… **141**
4. % Lactic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **143**
5. % Citric acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **145**
6. % Orotic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **147**

7. % Succinic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **148**
8. % Formic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **150**
9. % Uric acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **152**
10. % Acetic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **153**
11. % Propanoic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **155**
12. % Butanoic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate ……. **157**

Chapter 5.

1. The surface crystal formation study diagram for Cheddar cheese with sodium gluconate addition during 12-month of ripening ……..... **174**
2. Changes in pH in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 =

2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate **177**

3. Changes in (a) % lactose and (b) % lactic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate **179**
4. Changes in (a) % WSC and (b) % WSC (expressed as a % of total Ca) in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate **182**
5. Digital images of calcium lactate crystals on Cheddar cheese surface. The cheese slices represent; (TRT1-6, TRT1-9, TRT1-12). 2% salting level + 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT2-6, TRT2-9, TRT2-12). 2% salting level + 0.5% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT3-6, TRT3-9, TRT3-12). 2% salting level + 1. 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively **187**
6. Digital images of calcium lactate crystals on Cheddar cheese surface. The cheese slices represent; (TRT4-6, TRT4-9, TRT4-12). 2.5% salting level + 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT5-6, TRT5-9, TRT5-12). 2.5% salting level + 0.5% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT6-6, TRT6-9, TRT6-12). 2.5% salting level + 1.0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively **188**
7. Possible effects of sodium gluconate as calcium lactate crystals inhibitor in Cheddar cheese **190**

Chapter 6.

1. Changes in (a). pH 4.6 soluble N (as a percentage of total N) and (b). 12% TCA soluble N (as a percentage of total N) in Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate **210**
2. Changes TPA attributes, (a). fracturability, (b). hardness, (c). adhesiveness,

(d). cohesiveness, (e). springiness, (f). gumminess, (g). chewiness, (h). resilience, in Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate …… **219**

3. Selected sensory attributes at 6-month of ripening of Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate …….. **225**
4. Selected sensory attributes at 12-month of ripening of Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate …….. **226**

Chapter 1. Review of Literature

Cheese production has increased substantially in the past 30 years, and the consumption of all types of cheese has risen. Per capita cheese consumption in the United States has increased from 18.2 pounds in 1981 to 32.5 pounds in 2006, which accounts for an increase of 78.7% in the last 25 years. As a result, a higher percentage of milk is utilized to make cheese, and over all milk demand has become increasingly dependent on cheese production. About two-thirds of total U.S. cheese production in 2006 was from Cheddar and Mozzarella cheeses. Cheddar cheese is ranked the second behind Mozzarella with a difference of only 19,808 lbs (USDA, 2008).

Although cheese making is a relatively simple process, it is still challenging to produce Cheddar cheese with consistent quality (Lawrence et al., 1984). The characteristics of Cheddar cheese change during ripening as a result of a variety of physico-chemical changes, which include: metabolism of residual lactose, lactate and citrate, the release of free fatty acid (lipolysis), the association of catabolic reactions and the degradation of the casein matrix to peptides and free amino acids (proteolysis), and pH change (Creamer and Olsen, 1982; Lawrence et al., 1987; Fox, 1989; McSweeney, 2004a; McSweeney, 2004b; Upreti, 2006). According to USDA (1956) the U.S. grade AA and grade A medium cured and aged Cheddar cheese should have a smooth texture and appearance, preferably bright with smooth surface. However, an appearance of white specks on the surface of Cheddar cheese, which has been reported since the early 1900's, has been one of many important problems faced by cheese manufacturers. Although the non-mold white specks, which have been identified as calcium lactate crystals, neither

show health hazards, nor affect cheese flavor, they lower Cheddar cheese grade and cause a noteworthy expense to cheese manufactures due to trimming losses during the cutting and wrapping processes (Creamer et al., 1972; Pearce, et al., 1973; Dybing et al., 1988; Rajbhandari and Kindstedt, 2008). Therefore, it is important to limit calcium lactate crystals formation in Cheddar cheese.

Casein protein in milk

Originally, milk proteins were believed to be a simple homogeneous protein, but about a century or more ago, milk proteins were divided into two broad classes (Fox and McSweeney, 1998). The first fraction, which is about 80% of the protein in bovine milk, is precipitated at pH 4.6 (isoelectric pH) at 30ºC, and is now called casein. The second minor fraction, makes up about 20% of protein, is soluble under those conditions, and is now referred to as whey protein, serum protein, or non-casein nitrogen (Dalgleish, 1982; Fox and McSweeney, 1998).

The unique characteristic of caseins is their post-translational modifications, which results phosphorylation at seryl and infrequently threonyl residues (Swaisgood, 1992). Hence, caseins are phosphoproteins (Brunner, 1977). Casein is made up of several fractions including α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein (Walstra et al., 1999). There are also trace amounts of γ-casein occurring naturally on account of limited proteolysis of β-casein by plasmin (Swaisgood, 1992). The main casein components have several genetic variants and contain variable numbers of phosphoseryl residues. In addition, all casein proteins have different hydrophobic and hydrophilic regions along the protein chain. α_s-Caseins are the major casein proteins containing 8-10 seryl phosphate

groups, while β- casein contains about 5 phosphoserine residues. β-caseins is more hydrophobic than α_s-caseins and κ-casein. Because α_s-caseins and β-caseins are highly phosphorylated, they are very sensitive to the concentration of calcium salts, that is, they will precipitate with excess Ca^{2+} ions. κ-Casein contains only one phosphoseryl residue and is also glycosylated. Hence, they are stable in the presence of calcium ions, and they play an important role in protecting other caseins from precipitation and make casein micelles stable (Whitney, 1988; Walstra et al., 1999). Another unique feature of caseins is the large amount of proline residues, especially in β-casein, which greatly affects the structure of caseins. This occurs because proline disrupts the formation of α-helical and β-sheet secondary structure (Swaisgood, 1992). Casein is not heat sensitive; only temperatures up to or above 120ºC causes the casein to gradually become insoluble, whereas it is sensitive to pH and will precipitate at its isoelectric pH (Walstra et al., 1999).

Casein micelle structure

About 80-95% of the casein in normal milk is in the form of colloidally dispersed particles, known as micelles. Casein micelles consist of the caseins protein (α_{s1}-, α_{s2}-, β-, and κ- caseins) connecting by inorganic ions referred to as colloidal calcium phosphate. The shape of casein micelles as observed by electron microscopy is spherical with diameters ranging from 50-500 nm (average about 120 nm) and molecular mass from 10^6-10^9 Da. Casein micelles are able to scatter light; therefore, the white color in milk is mainly because of light scattering by casein micelles (Brunner, 1977; Fox and McSweeney, 1998).

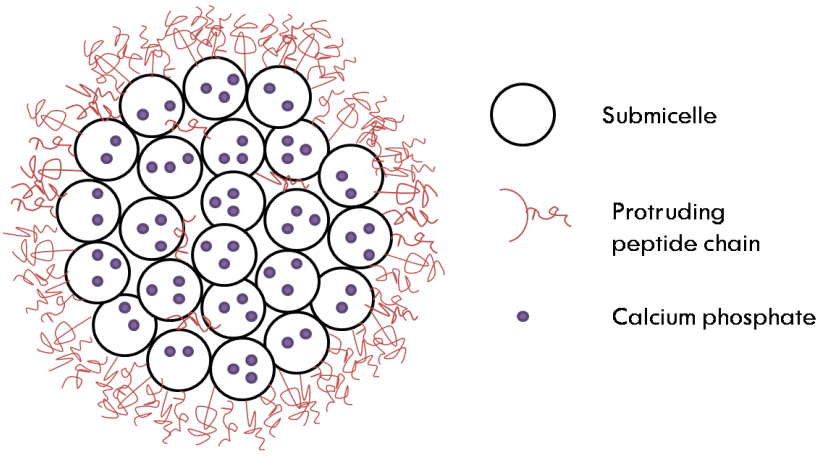

Figure 1. The structure of casein micelle in the sub-micelles model showing the protruding C-terminal parts of κ-casein as proposed by Walstra.

Source: Walstra, 1999

Although the nature and structure of casein micelles have been studied extensively, the exact structure of casein micelles is still hypothetical and not yet determined. The most commonly accepted model for casein micelle structure is the sub-micelle model (Figure 1), which was proposed by Walstra in 1984 (Rollema, 1992). This model suggests that casein micelles are built of roughly spherical subunits or sub-micelles. The composition of sub-micelles is variable and the size is in the range of 12-15 nm in diameter, and each sub-micelle has 20-25 casein molecules. The sub-micelles are kept together by hydrophobic interactions between proteins, and by calcium phosphate linkages. There are two main types of sub-micelles; one mainly consisting of α_s- and β-caseins, hydrophobic regions buried in the center of the sub-micelle, another type consisting of α_s- and κ-caseins, which is more hydrophilic because of the sugar residues on κ-caseins. The κ-caseins are located near the outside of the micelle with the

hydrophilic part of the C-terminal end protruding from the micelle surface to form a 'hairy' layer that will avoid further aggregation of sub-micelles by steric and electrostatic repulsion. Consequently, micelles are stable, and they do not usually flocculate (Walstra, 1999; Walstra et al., 1999).

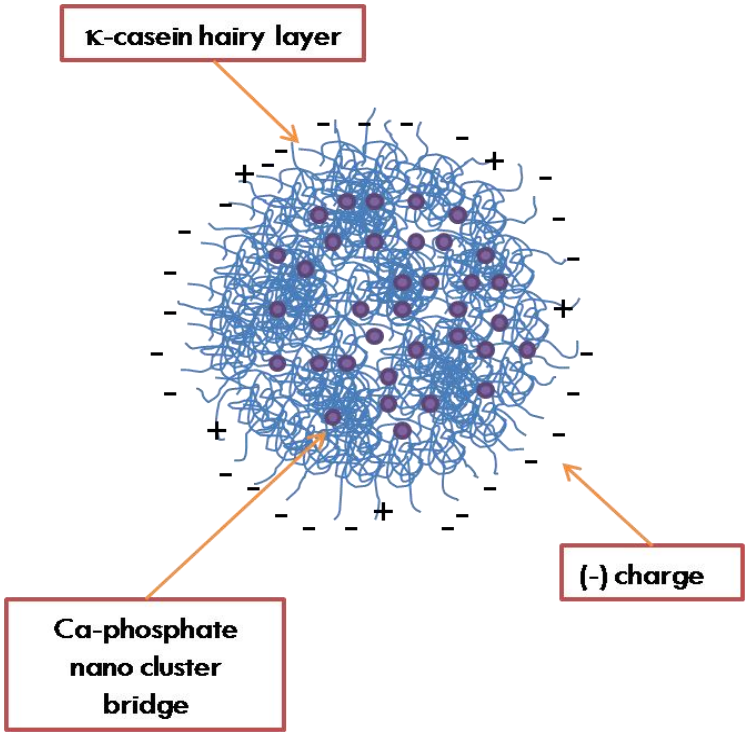

Figure 2. Hairy casein micelle model proposed by Holt, where a tangled web and open structure of polypeptide chains cross-linked by calcium phosphate nanocluster (colloidal calcium phosphate) in the core provides rise to an external region of lower segment density known as the hairy layer. The gray circles represent the calcium phosphate nanoclusters.

Source: Goff, 1995

Although the sub-micelle casein model as extended by Walstra (1999) has been widely accepted, the small casein subunits have never been detected by any other researchers (McMahon and Oommen, 2008). Thus, two alternative models have been proposed by Holt in 1992 and by Horne (1998). Holt delineated the casein micelle as a tangled web of flexible casein networks forming a gel-like structure with micro-granules of colloidal calcium phosphate through the casein phosphate center, and the C-terminal region of κ-casein extends to form a hairy layer (Figure 2). The two main features of this model are the cementing role of colloidal calcium phosphate and the surface location of hairy layer of κ-casein. In addition, casein micelles are stabilized by two main factors, which are a surface (zeta) potential of approximately -20mV at pH 6.7, and steric stabilization owing to the protruding κ-casein layer hairs (Holt, 1994; Holt and Horne, 1996; Fox and McSweeney, 1998).

The dual bonding model was proposed by Horne in 1998. This model can be considered an extension from the aforementioned Holt model (Farrell Jr. et al., 2006), and it is suggested that the proteins in casein micelles are bound together by two types of bonding, and it is a balance between the attractive hydrophobic interactions and electrostatic repulsion. Hydrophobic interaction is the driving force for the formation of casein micelles, while electrostatic repulsions are limiting the growth of polymers or in other words defining the degree of polymerization. The conformation of α_{s1}- and β-caseins when they are adsorbed at hydrophobic interfaces form a train-loop-train and a tail-train structure, respectively, and both caseins polymerize or self-associate, by hydrophobic interactions. Accordingly, the self-association of caseins makes it possible

for polymerization to occur. Calcium phosphate nanoclusters, or CCP, are considered to be one of the linkages between casein micelles and act as neutralizing agents of the negative charge of the phosphoserine residues. Consequently, electrostatic repulsion is reduced and the hydrophobic interaction between caseins is still dominant, resulting in more associations of proteins. Unlike the other caseins, κ-caseins can only interact hydrophobically and acts as a propagation terminator, because they do not have a phosphoserine cluster to bind calcium and also another hydrophobic point to prolong the chain.

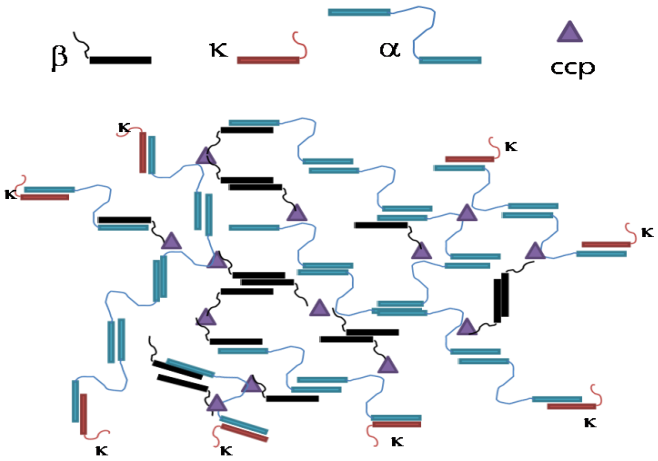

Figure 3. The dual bonding model of casein micelle structure, with α-, β-, κ-casein portrayed as indicated. Bonding appears between the hydrophobic regions, shown as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to colloidal calciumphosphate clusters. Molecules of κ-casein (K) limit further growth of the structure.

Source: Horne, 1998

The dual bonding model for the casein micelle structure is shown in Figure 3. The α_s-, β-, and κ-caseins are shown as indicated. Bonding between caseins first takes place in the hydrophobic regions, shown as rectangular bars, and also the linkage between CCP and phosphoserine residues of casein molecules. κ-Caseins, marked as letter K, limit further growth (Horne, 1998).

The importance of calcium-phosphate and κ-casein to the casein micelle

One of many important functions of the casein micelle is to solubilize calcium phosphates in milk (Farrell Jr. et al., 2006). The dry matter of bovine casein has been found to consist of about 94% protein and 6% mineral, which is colloidal calcium phosphate (CCP) (Horne, 2006). The relationship between CCP and casein micelles has been vigorously investigated for over a century. However, this relationship has not yet been fully understood (Fox and Brodkorb, 2008). As hypothesized by De Kruif and Holt (2004), CCP could be bound and stabilized by phosphopeptide portions of α_s- and β-caseins, resulting in the formation of calcium-phosphate nanoclusters or CCP. This CCP would randomly grow and precipitate without bridging with peptides. In addition, the formation of CCP is believed to generate casein micelle structure by randomly binding with phosphoproteins until a size limited colloid is formed. According to Horne and his dual-binding casein micelle structure model, CCP is considered to begin the process of casein micelle formation by acting like a bridge and neutralizing agent for the phosphoproteins, which hydrophobically interact to each other. The hydrophobic blocks of protein-protein interactions and the CCP linkage further generate the casein micelle formation. This casein micelle has a gel-like structure with embedded CCP and κ-caseins

as chain terminator (Horne, 1998; Farrell Jr. et al., 2006). CCP along with hydrogen bonds, hydrophobic and electrostatic interactions are responsible for casein micelle stability. It was found that the micelles dissolve into small particles in milk solution once the CCP is removed by acidification, dialysis or Ca chelator; thus this phenomenon suggests that CCP play an important role in cementing the micelles together (Fox and Brodkorb, 2008).

At the concentration of protein and calcium found in bovine milk, Ca-sensitive caseins (α_{s1}-, α_{s2}- and β-caseins) are readily precipitated by calcium bound to their phosphoserine residues. However, κ-casein, which is soluble in calcium, can interact and stabilize about 10 times its mass around the core of Ca-sensitive caseins. In addition, because of the negative charge obtained from oligosaccharide chains at the carboxy-terminal ends of κ-caseins, they can provide steric stabilization for the casein micelles in milk. It has long been believed that κ-casein is the only type of casein protein in the surface layer. This has been confirmed by the decrease in hydrodynamic diameter during renneting, since chymosin removes the protruding macropeptide portion of κ-casein. However, other researchers have found that N-terminal residues of all caseins are released by super-polymerized aminopeptidase that cannot diffuse into the micelle. These phenomena suggest that κ-casein is not very exposed and that the surface of the micelle is not exclusively covered with κ-casein. Consequently, some of the other casein fractions are also located on the surface of the micelle (Horne, 2003; Horne, 2006; Farrell Jr. et al., 2006; Fox and Brodkorb, 2008). A recent study by Dalgleish et al. (2004) suggests that the casein micelle surface is more complex than just a simple hard sphere covered by a

'hairy layer'. The electron micrograph from their study also suggested that the micelles consist of seemingly casein tubules with the end protruding from the bulk structure that protects the micelles. They then hypothesized that κ-casein is probably only located at the ends of the tubes and not evenly covering the entire surface, since the amount of κ-casein in milk is not sufficient to cover the entire micelle surface on its own.

Cheddar cheese manufacture

The production of Cheddar cheese can be divided into two stages, which are manufacture and ripening. The manufacture of Cheddar cheese consists of preparing and standardizing milk, adding starter culture with rennet, coagulating milk, cutting the coagulum into small cubes, heating and agitating the cubes, removing the whey, fusing the curd into slabs followed by milling or continuously stirring the Cheddar curd, salting and pressing (Hill, 1995; Lawernce et al., 2004; Fox and McSweeney, 2004).

Traditionally, Cheddar cheese utilizes the cheddaring process, where the curd is allowed to fuse into slabs, which are turned, piled, and re-piled at regular intervals for 1 to 2 hr until reaching the desired pH. This process causes the curd granules to fuse together under gravity, which leads to a close-knit and fibrous cheese structure. The cheddared curds are followed by milling, which involves mechanically cutting curds into small pieces. The milling process facilitates uniform salt distribution into the curds and promotes whey drainage from the curds. However, because of the development of hooping and pressing of salted granular curd under vacuum, the stirred-curd method has become more commonly accepted. In this method, drained curds are continuously stirred until reaching the desired pH. Although the constant agitating does not allow knitting of

curds, the use of ‘block former’ hooping and vacuum pressing system yields Cheddar cheese with a close-texture characteristic. Thus, the stirred-curd method facilitating by hooping and vacuum pressing eliminate the need for cheddaring and milling. The stirred-curd method requires shorter time than the traditional method; thus this is the method of choice in highly mechanized cheese plants (Lawrence et al., 2004; Serrano et al., 2004; Rehman et al., 2008).

Rennet coagulation of milk

Rennet is a general term for proteinase used to coagulate milk. Milk coagulants from several sources including those from vegetable, animal, bacteria and fungi have been used in cheese making. Rennet, which is a natural coagulant extracted from the fourth stomach of the calf was the main choice for the early cheesemakers (Scott, 1986). Traditionally, rennet extracted from young calf stomachs are used to make Cheddar cheese. This rennet contains about 88 – 94% chymosin, which provides about 90% of total milk clotting activity, and about 6 – 12% pepsin, which provides about 10% of milk clotting activity. Bovine chymosin is an aspartyl proteinase containing approximately 320 amino acid residues. Its physiological role is to coagulate milk in the young mammal stomach, which increases the digestion efficiency. An important characteristic of chymosin is due to its proteolytic activity, because its general proteolytic activity is low when compared with its milk clotting activity (Horne and Banks, 2004; McSweeney, 2007).

The cheese production has increased, while the supply of calf rennet has decreased; thus this led to the use of alternative products, which are other types of

aspartyl proteinases. Rennet substitutes should have high milk clotting activity compared to general proteolytic activity and specific activity on κ-casein. Rennet substitutes include bovine and porcine pepsins, microbial aspartyl proteinases, and fermentation-produced chymosin cloned from microorganisms. Rennet extracted from older bovine contains about 6 – 10% chymosin and 90 – 94% pepsin. Bovine pepsin is mainly used, and it is quite effective once blended with chymosin. Porcine pepsin is unstable at pH above 6; thus it is usually used along with calf rennet as 50/50 blend. Microbial aspartyl proteinases are naturally produced from some yeasts and molds including *Rhizomucor meihei*, which is most widely used, *Rhizomucor pusillus*, and *Cryphonectria parasitica*. Fermentation-produced chymosin or recombinant chymosin is produced by fermentation of identical calf protein chymosin obtained from cloning calf chymosin into host microorganisms (*Kluyveromyces lactis, Aspergillus niger, and Escherichia coli*). These rennets have shown excellent results in cheese processing, but their use is still subject to regulation (Scott, 1986; McSweeney, 2007).

The coagulation reaction can be divided into two phases, which are the primary phase where the proteolytic enzymes (chymosin, pepsin or microbial proteinases) cleaved κ-caseins at a specific bond, and the secondary phase where casein micelles start to aggregate. These coagulation phases are in fact overlapping, since casein micelles may begin to aggregate before the κ-casein hydrolysis is completed. As previously mentioned, κ-caseins are glycosylated with hydrophilic short sugar chain in the carboxy-terminal ends called glycomacropeptide (GMP). This GMP consists of residues 106-169, and the other part of κ-casein is the hydrophobic para-κ-casein (residues 1-105). In order for the

bovine casein micelles to aggregate, κ-casein has to be hydrolyzed at the junction between para-κ-casein and GMP at the $Phe_{105} – Met_{106}$ bond. Proteases capable of doing so are aspartic proteinases, and chymosin is considered suitable for cheese manufacture, because it is specifically active in hydrolysis of the $Phe_{105} – Met_{106}$ bond of κ-casein. Thus, during milk coagulation, κ-casein is hydrolyzed at the $Phe_{105} – Met_{106}$ bond, and the hydrophilic GMP is released into the serum phase, while para-κ-casein remains bound to the casein network. The ongoing loss of GMP results in the decrease in the micelles zeta potential from -20 mV to about -10 mV, and destabilization of the micelles. Once κ-caseins have been sufficiently hydrolyzed; casein micelles will begin to aggregate (Dalgleish, 1993; Horne and Banks 2004). In addition, once the pH is lowered, the micellar calcium and phosphate are dissolved into the serum phase (Dejmek and Walstra, 2004).

Cheese Ripening

Microbiology of Cheese Ripening

The microorganisms of Cheddar cheese can be divided into two groups: starter lactic acid bacteria, which are involved in acid production during cheese manufacture and ripening, and nonstarter lactic acid bacteria (NSLAB), which do not cause acid production during manufacture but contribute significantly during ripening. In Cheddar cheese manufacture, defined mesophilic cultures comprising two or more strains of *Lactococcus lactis* are generally used. They are usually added to the milk at the beginning of cheese manufacture, and the primary role of the starter bacteria is to

produce acid during the fermentation process. They further provide a proper environment that allows enzyme activity from chymosin and starter to continue favorably in the cheese (Cogan and Beresford, 2002; Parente and Cogan, 2004).

Starter Bacteria The most commonly used starter LAB for commercial Cheddar cheese making are *Lactococci* (*L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremois*) because of their ability to convert about 95% of fermented sugar to L-lactate (Thomas et al., 1979).The phosphoenolpyruvate-dependent phosphotransferase system is the main system for lactose metabolism by lactococci. In this system, lactose is phosphorylated and transferred inside the cell as lactose-6-phosphate; then phospho-β-galactosidase breaks lactose-6-phosphate down into glucose and galactose-6-phosphate. Glucose is metabolized to pyruvate via Embden-Meyerhoff pathway, while galactose-6-phosphate is metabolized to triose phosphate intermediates via D-galactose-6-phosphate pathway; then these intermediates are converted to pyruvate via Embden-Meyerhoff pathway. Pyruvate is then converted to lactate by lactate dehydrogenase (LDH), and the amount of lactate produced in the system is determined by the activity of LDH (McKay, 1969).

During cheese-making, acid production is the direct result of the starter growth. Acid production during the first few hours depends mainly on the inoculation rate, the renneting time, and the rate of curd cooking. If the inoculum rate is low, this will cause lower lactate concentration and a higher pH value. Cooking temperature is also important in controlling starter culture growth. Mesophilic bacteria, which have optimum growth temperature of 30°C, are used in Cheddar cheese manufacture. However, the cooking temperature of Cheddar cheese is about 38 - 40°C, which will reduce acid production by

mesophilic starter. The acid production is quite fast in Cheddar cheese. Normally, the pH decreases from 6.6, which is the initial milk pH, to pH 6.1, which is the typical whey draining pH approximately 3 hr after the starter culture is added. Faster acid production results in more whey and lactose expulsion from the cheese curd (Cogan and Beresford, 2002).

Although about 90% of the lactose is lost during whey drainage, there is still enough lactose retained in the curd for continuing lactic acid production. Typically, 24 hours after starter addition, most of the lactose is transformed to lactate with L-lactate as the main isomer. Starter LAB is the dominant microflora in the young curd, thus, representing biocatalytic potential for cheese ripening process. However, because Cheddar cheese is dry-salted, this reduces the ability of starter LAB to produce lactate. Salt is one of the major factors that control microbial growth in cheese. Thus, metabolism of lactose depends directly on salt and salt-to-moisture ratio. It has been found that lactose was utilized within 8 days in the cheese with low salt-to-moisture ratio (4.1%), while lactose remained high for several weeks after manufacture in the cheese with high salt-to-moisture ratio (6.3%). *Lc. lactis* ssp. *lactis* is able to survive much better than *Lc. lactis* ssp. *cremoris* in the cheese with about 4 – 5% salt-to-moisture ratio. In addition, because the optimum pH of starter lactococci is 6.3, once the final cheese pH is reached, which generally ranges between 4.8 and 5.3, within 1 day of manufacture, the growth rate of starter cultures is dramatically decreased (Cogan and Beresford, 2002; Beresford and Williams, 2004).

Non-starter Bacteria After about 3 month of cheese ripening, starter LAB are unable to survive in the cheese environment (low moisture, low pH, low temperature, and high salt). On the contrary, the number of NSLAB increases during cheese ripening. Because NSLAB are not intentionally added during cheese manufacture, their source is probably contamination after pasteurization from air, cheese making equipments or ingredients, or survival of pasteurization (Fitzsimons et al., 2001). The majority of NSLAB found in Cheddar cheese is *Lactobacilli* with predominating species including *L. casei*, *L. paracasei*, *L. plantarum*, and *L. brevis*, and occasionally *S. thermophilus* (Peterson and Marshall, 1990; Fitzsimons *et al.*, 2001; Swearingen et al., 2001; Beresford and Williams, 2004). The non-starter lactobacilli in Cheddar cheese are facultative heterofermenters. They are acid-tolerant, and they can grow at 2 - 53^{o}C. Because lactose is fermented to lactic acid by starter LAB within the first few days of ripening process, several substrates for NSLAB growth have been suggested, which include citrate, peptides, amino acids, sugars released from enzymatic hydrolysis of casein, sugars of the glycoproteins of the milk fat globule membrane, and ribose released from RNA after starter autolysis (Peterson and Marshall, 1990; Cogan and Beresford, 2002).

NSLAB play an important role in flavor and texture development in mature cheese. NSLAB proteolytic enzyme activities increase the production of free peptides and amino acids, which subsequently influence flavor development in cheese during ripening. Citrate utilization by NSLAB also enhances flavor development from production of diacetyl, acetoin, and acetate. Apart of their benefits, NSLAB also have negative effects on Cheddar cheese quality. Certain NSLAB are able to racemize L-lactate to D-lactate, which consequently causes calcium lactate crystal formation in aged cheese.

Additionally, end products from lactose hetero-fermentation, which include ethanol, acetic acid, and carbon dioxide, can contribute to off-flavors and texture defects (slits and openness) (Peterson and Marshall, 1990; Swearingen et al., 2001; Cogan and Beresford, 2002; Beresford and Williams, 2004).

Critical biochemical reactions during cheese ripening

Glycolysis of residual lactose during ripening. The metabolism of lactose to lactate is an essential biochemical pathway for the production of all cheese varieties. Cheese curd has a low level of residual lactose, because most of the lactose is lost with whey during drainage in the cheese making process. The residual lactose is readily metabolized to lactate by both starter and non-starter lactic acid bacteria (LAB) during early ripening. The three categorizes of LAB (i.e. lactococci, lactobacilli, and S. *thermophilus*) utilize different systems of lactose transport and catabolism, resulting in differences in their biochemical characteristics and an accumulation of intracellular and extracellular products. The metabolism of lactose to lactate is normally complete at the end of manufacture or during the early ripening stage by starter LAB mainly lactococci. However, in dry-salted cheese particularly Cheddar cheese, the activity of starter cultures is greatly reduced due to the combination of low pH and high NaCl, resulting in further lactose metabolism by non-starter lactic acid bacteria (lactobacilli, and S. *thermophilus*), which consequently influences cheese flavor (McSweeney, 2004a, b).

Contribution of glycolytic end products to cheese characteristics. The different compounds formed during the ripening process play important roles in the flavor of Cheddar cheese. It has been confirmed that the water-soluble fraction in cheese

contributes more to the flavor intensity than the fat fraction (McGugan et al., 1979). The water-soluble fraction consists mainly of short-chain, water soluble organic acids and residual sugars. These organic acids can originate from lactose metabolism by starter LAB, hydrolysis of fatty acids, direct addition as acidulant (Marsli et al., 1981; Adda et al., 1982), and from glycolysis by NSLAB (Crow et al., 2001). Many studies have been conducted to monitor changes in organic acids and residual sugar profiles during cheese ripening (Harvey et al., 1981; Marsili 1985; Akalin et al., 2002; Upreti et al., 2006), and these profiles have been used as a parameter of cheese maturity (Panari, 1986; Bevilacqua and Califano, 1989; Bouzas et al., 1991b; Upreti et al., 2006).

Quantification of water-soluble acids and residual sugars in cheese.

Several methods for sugars and organic acids analyses have been reported, which include enzyme catalysis, colorimetry, wet chemistry, and chromatography (Upreti et al., 2006). High Performance Liquid Chromatography (HPLC) methods gained popularity for identifying organic acids and sugars in cheese with the development of suitable stationary phases. HPLC methods do not require extensive extraction and derivatization, thus minimizing analysis time and potential analyte loss during derivatization. HPLC methods also make it possible to simultaneously analyze sugars and organic acids without causing waste-disposal problems from the use of toxic chemicals (Mullin and Emmons, 1997). HPLC analysis of organic acids in cheese can be executed by using either ion-exchange chromatography (Panari, 1986; Bouzas et al., 1991; Lombardi et al., 1994; Zeppa et al., 2001; Upreti et al., 2006) or reversed-phase chromatography (Pham and Nakai, 1984; Akalin et al., 2002). Although the reversed-phase method has a shorter separation time, yield linear calibration curves for aqueous standards over wide concentration range, and

has a long column life, ion-exchange method for organic acids analysis in cheese is proven to be better in terms of resolution, duration of separation, and ease of analysis. The disadvantage of ion-exchange HPLC method is the co-elution of compounds. However this problem can be solved by combining different modes of detection (UV or RI at different wavelength) during the same analysis (Lues et al., 1998a; Upreti et al., 2006).

Sample extraction for organic acids and sugars is a critical step. However, because of their high water solubility, these substances can be extracted by simply blending the food sample in lukewarm water, acidified water, 80% ethanol or 80% acetronitrile (Gomis 1992). The use of dilute acid solutions ranging from 0.0049 to 0.013 N of sulfuric acid as an extract agent for organic acids and sugars in cheese are found to be suitable for analyzing these compounds with an ion-exchange column (Bouzas et al., 1991; Lombardi et al., 1994; Lues et al., 1998b). Several researchers have reported different ways to efficiently extract sugars and acids, which include: stirring the solution using magnetic stirrer for 1 h (Bouzas et al., 1991), agitating the solution for 1 h on a shaker (Akalin et al., 2002), extracting the solution with stomacher for 10 min (Zeppa et al., 2001), and using high-speed mechanical blenders for homogenizing the cheese and acid mixture ranging from 30 sec to 5 min (Marsili, 1985; Panari, 1986; Mullin and Emmons, 1997; Upreti et al., 2006).

Proteolysis in cheese during ripening

Proteolysis is the most complex and the most important biochemical pathways during cheese ripening. During proteolysis, a peptide bond is cleaved and two new ionic

groups are generated. These ionic groups then compete for water in the system, resulting in an increase in water-binding and a decrease in a_w. This phenomenon influences cheese characteristics including texture, bacterial growth, and enzymatic activity. Proteolysis contributes to the development of cheese texture by hydrolysis of the protein matrix into polypeptides and smaller water-soluble peptides that are not a part of the protein matrix. The production of short peptides and free amino acids contribute to cheese flavor either directly or indirectly by acting as a substrate for a range of catabolic reactions, which later generate volatile flavor compounds. The proteolytic process in Cheddar cheese during ripening is catalyzed by indigenous enzymes from milk mainly plasmin, coagulant (chymosin, pepsin, or other fungal derived coagulants), LAB and NSLAB, or exogenous enzymes added to accelerate ripening (Sousa et al., 2001; Upadhyay et al., 2004; Upreti et al., 2006).

***Methods for monitoring proteolysis in cheese*.** The methods for quantifying proteolysis in cheese can be categorized into two groups, which are specific and non-specific methods. Specific methods have been used to identify specific peptides produced during cheese ripening as well as provide peptide profiles of cheese extracts. These methods include electrophoresis (Urea-PAGE, SDS-PAGE and capillary electrophoresis), chromatography (ion-exchange chromatography, size exclusion chromatography and reversed-phase HPLC), and new techniques such as fluorescence spectroscopy and ultrasound. Non-specific methods provide information regarding the degree of proteolysis and the proteolytic agent activities. These methods include determination of soluble nitrogen or nitrogen that can be extracted by various solvents or buffers (Upadhyay et al., 2004).

Two of the most common solvents for extracting soluble nitrogen are pH 4.6 acetate buffer and 12% trichloroacetic acid solution (TCA) (Bynum and Barbano, 1985). Because proteolysis hydrolyses protein matrix into large and small peptides, and amino acids, it is essential to separate those proteins and peptides into more homogenous fractions in order to characterize their constituents. Extraction of cheese with pH 4.6 acetate buffer will precipitate intact casein, whereas large and small peptides remain soluble in solution. This soluble fraction contains whey proteins, low molecular weight peptides from casein hydrolysis and free amino acids. Extraction with 12% TCA will precipitate large peptides and proteins, whereas small peptides remain soluble. The 12% TCA soluble fraction contains small peptides and amino acids, while the precipitate contains medium and large peptides, and intact casein. These soluble portions are typically analyzed for nitrogen content using Kjeldahl analysis. This analysis provides and index for the extent of proteolysis by comparing the ratio of soluble nitrogen to total nitrogen (Ottman and Metzger, 2002). It has been reported that soluble N extracted from both solvents correlates significantly with the age and flavor intensity of Cheddar cheese (Farkye and Fox, 1990).

Calcium lactate crystal in Cheddar cheese

Calcium lactate is one of most important and most widely used salts in the pharmaceutical industry due to its good solubility and bioavailability. It is normally used in both human and animals with calcium deficiency therapies for the bone mineralization. It is also used as an additional filler-binder in the drug tablets because of its short disintegration time and fast drug release (Chemaly et al., 1999; Sakata et a., 2005). Even though the calcium lactate salt is important in pharmaceutical industry, the presence of

white calcium lactate deposits is considered a defect in hard-type cheeses especially Cheddar cheese.

The appearance of white deposits in Cheddar cheese has been a continuing problem since the early 1900's. In 1903, Babcock and others reported the appearance of white spots on Cheddar cheese. They reported that these white deposits increased when sweet-curd cheeses were manufactured with skimmed or low-fat milk, with low concentration of rennet and salt, and cheeses were stored at 4.4°C as opposed to 15.5°C. In 1910, Van Slyke and Publow suggested that a reaction between calcium and free fatty acid released by bacteria caused white calcium soap deposits on the surface of hard-type cheeses at low temperature (Washam et al., 1985). Tuckey et al. (1938) reported their x-rays analysis from the white deposits isolated from aged Cheddar cheese that the x-rays had the same crystal structure spacings as calcium lactate. The following year, McDowall and McDowell (1939) confirmed the white calcium lactate crystal finding. They examined the white specks picked out from a mature Cheddar cheese by chemical analysis, and reported that calcium, lactate, and moisture were the main constituents obtained from the white deposits. They speculated that if calcium lactate contained five water molecules as crystal form, the composition of the white deposits would compose of 60% calcium lactate pentahydrate, and 30% of moisture, protein, and fat, which could be the cheese residues, adhered to the white deposits. However, Conochie et al. (1960) claimed from their findings that the white deposits consisted not only of calcium lactate but also tyrosine. The possibility of tyrosine crystals will be discussed in the next section.

Farrer and Hollberg (1960) supported the calcium lactate crystal finding by analyzing the white deposits on the surface of Cheddar cheese that was wrapped in flexible material and identifying those white spots as calcium lactate. The white calcium lactate spots composed of 72% Calcium lactate · $5H_2O$, 20% moisture, 6% free lactic acid, and 0.5% P_2O_5. They suggested that the solubility product of calcium lactate could be easily exceeded during cheese maturation, and this could result in calcium lactate crystals. They additionally reported that 1 gm. of water could be lost from 8 oz. of cheese, mainly from the cheese surface, with flexible wrapping during shelf life. This could probably cause cheeses wrapped with flexible packages to be more prone to the formation of the white calcium lactate deposits on the cheese surface.

Scientists in New Zealand (Creamer et al., 1972) suggested that any factors that could influence the concentration and solubility of calcium and lactate ions could initiate the formation of calcium lactate crystals. Those factors included the higher lactose content in milk from different bovine breeds, the higher acid development during ripening, and the lower storage temperature during cheese ripening. They further suggested the methods of reducing calcium lactate crystals, which were by increasing the storage temperature, and changing the processing process. The formation of crystals decreased when cheese was ripened at 12.8°C instead of 7.2°C. However, according to their flavor trials, cheese could be ripened at 12.8°C for up to 6 months. Thus, storing cheese samples at higher temperature would be suitable for cheeses that would be consumed not too long after 6 months. The crystals were found to be eliminated by replacing whey right after cutting with hot water. This dilution method would dilute the

whey and facilitate the loss of calcium and lactose. Thus, less concentration of calcium and lactose/ lactate would be available to form calcium lactate crystals during cheese ripening. However, the dilution method tended to give Cheddar cheese with 0.1 – 0.2 pH units higher than normal.

The following year, the same group of New Zealand scientists (Pearce et al., 1973) studied the calcium lactate deposits on rindless Cheddar cheese in greater details. Their research trials included studying the composition of white deposit, studying the solubility of calcium lactate, experimenting with Cheddar cheeses making process, studying the seasonal effect, and studying the storage temperature of cheese samples. The X-ray diffraction analysis of the white deposit that was handpicked from cheese samples showed that the white crystals were calcium lactate. This was confirmed by both ultra-violet absorption and amino acid analysis that tyrosine and tryptophan were not present. The solubility of calcium lactate was influenced by salt, calcium, lactate ions, and temperature.. Sodium chloride concentration ranged from 0 – 2.0M had minimal effect on the solubility of calcium lactate, while at least 0.3M of lactate ions was required in order for solid calcium lactate to present within the calcium lactate equilibrium solution. The solubility of calcium lactate increased with increasing temperature from 0.2°C to 16.9°C. Several Cheddar cheese making processes were modified for their cheese making experiment. The modifications that affected the presence of calcium lactate crystals included increasing the lactose level in the cheese milk, diluting cheese milk with water, and diluting whey with water after cutting. They reported that an increase in milk lactose increased the calcium lactate crystal deposits, decreased a cheese pH, and decreased calcium content, whereas milk dilution decreased the white crystal deposits. Whey

dilution minimized the white deposits and also raised the cheese pH. Their results on seasonal effects indicated that cheeses made during October through November showed more white deposits than cheeses made in December, while cheeses made in March did not show any deposits. Finally, they reported that cheese samples that were stored at a lower temperature (4.5°C) showed extensive white deposits, while cheeses that were stored at a higher temperature (13°C) showed no or minimal deposits.

From their results, Pearce et al. (1973) explained that calcium and lactate concentration in Cheddar cheese was probably close to saturation. A small alteration of either one or both calcium and lactate concentration could cause an under saturated calcium lactate concentration to become a supersaturated calcium lactate concentration, which would result in a formation of calcium lactate crystals in Cheddar cheese. They implied that the lactate concentration could be adjusted by adjusting the level of lactose concentration in milk. However, an adjustment of calcium concentration would not be preferred, since removal of calcium would affect the rennet coagulation of the milk, the syneresis of the curd, and most importantly the texture of the final cheese. They recommended the methods of reducing calcium lactate crystal deposits by diluting cheese milk with water, washing the curd between milling and salting, and increasing storage temperature of cheese samples.

A group of scientists in Italy (Bianchi et al., 1974) reported their findings for the white deposits on the surface of Grana Padano cheeses that different physical form of white deposits would contribute to different types of crystals. The granule type of white deposits contributed to mainly tyrosine crystals, while the spot type of white deposits

contributed to amino acid crystals of leucine and isoleusine. The physical form of calcium lactate crystals would also be granule type but in more amorphous forms. The observations on the microscopic crystalline in Cheddar cheese by light and electron microscope (Brooker et al., 1975) reported two distinct type of crystalline, which were macroscopic and microscopic crystalline. The macroscopic crystalline exhibited an irregular shape of large needle-like crystals, which were identified as calcium lactate. The microscopic crystalline appeared in cheese immediately after pressing and accumulated until cheeses were one month old. They were more abundant than the macroscopic crystalline and were more concentrated along the line of milled curd fusion. Brooker et al. (1975) suggested that the microscopic crystalline were calcium phosphate. The incidence of calcium phosphate in Cheddar cheese will be discussed in the next section.

Washam et al. (1982; 1985) studied the microstructure of crystalline in Cheddar cheese in greater details. They started by studying the crystalline structures of five known chemical compounds (calcium lactate, sorbic acid, sodium chloride, tyrosine, and calcium phosphate) that were reported as crystals in Cheddar and other cheeses. Those stated chemical compounds were induced and crystallized on the young Cheddar cheese surface, and the crystals were observed by scanning electron microscopy. Calcium lactate crystals formed bundles or sheets of parallel flat and needle-like fibers. Sorbic acid exhibited tangled-web of thread-like crystals. Sodium chloride crystals exhibited both individual and clusters of cubic crystals. Tyrosine crystals were also exhibited fibrous crystals but of larger fiber component and more compact aggregate than calcium lactate crystals. Calcium phosphate exhibited a mixed matrix of pore and aggregate amorphous granules, which appeared like sea coral morphology. They later studied a scanning

electron microscopy of crystalline structures and X-ray diffractions on various commercial brands of consumer-cut Cheddar, sliced Cheddar, marble Cheddar, and smoked processed Swiss cheeses. The crystals picked from the surface of consumer-cut Cheddar and sliced Cheddar cheeses showed long and granular unstructured materials with thread and needle-like structures, which were characteristic of calcium lactate crystals. The crystals were also confirmed by the X-ray diffraction as calcium lactate. Unlike the crystals from consumer-cut and sliced Cheddar cheeses, the crystals on the surface of marble Cheddar cheese did not show long and thread-like structure. The crystals were acicular, and were confirmed by the X-ray diffraction as calcium lactate. The modification of crystal structure on the surface of marble Cheddar cheeses could be due to the manufacturing procedure of separately making yellow and white Cheddar and mixing those two cheeses before hooping. The differences in yellow and white Cheddar compositions could possibly modify crystal structures and rate of crystal growth. Finally, the crystals from the surface of processed Swiss cheese were identified to be the combination of potassium tartrate, epsomite, and some other trace components.

Washam et al. (1985) explained that during cheese processing, as milk pH decreased and milk coagulated, colloidal calcium phosphate became more soluble and along with lactose, they were lost during whey drainage after cutting. However, a prolonged stirred-curd prior to whey drainage could promote lactose diffusion back into the curd. The higher concentration of curd lactose would provide greater lactic acid production and greater decrease of cheese pH, which would cause greater solubilization of calcium phosphate. Because the water phase inside the curd composed primarily of an aggregated casein micelle networks, microbial fermentation during cheese ripening

would cause a subsequent drop in cheese pH, an additional release of the calcium phosphate, and a formation of calcium lactate in the water phase. During cheese ripening, the water phase would equilibrate throughout the cheese. However, the diffusion of water from the liquid-filled inter-curd spaces would leave the area with high concentration of calcium lactate and calcium phosphate. It was possible that calcium lactate was the first component to crystallize as cheeses were kept in cold storage room during ripening.

Processing variables and compositional factors also play vital roles in the formation of calcium lactate crystals in the cheese. Sutherland and Jameson (1981) studied the composition of hard cheese manufactured by ultrafiltration. Whole milk was concentrated approximately 4.8-fold by ultrafiltration. Milk was also adjusted by different levels diafiltration and pH to achieve different lactose and mineral levels in the retentates, respectively. The retentates were used to make Cheddar cheeses by the traditional cheese making procedures. The moisture contents in cheeses made from milk ultrafiltered below pH 6.0 ranged from 40 to 44%, which were excessive for the normal Cheddar cheese. The fat content in the cheese sample made with the most acidified retentate (pH 5.62) was significantly lower (24.5%) than fat content from other cheese samples (30 – 33%). The levels of both calcium and phosphate in cheeses decreased with the reduction in initial milk pH, which also correlated with the levels of these elements in the retentate. Both protein and salt contents did not show wide variation among cheese samples. The levels of lactose in the final cheeses varied greatly with different diafiltration factor. Cheeses that were not diafiltered had significantly higher lactose contents (1 – 2.15%) than cheeses that were diafiltered (0.07 – 0.54%). The levels of lactose in final cheeses also correlated with the levels of lactose in the retentates. The

white deposits of calcium lactate were identified on the surface of cheese samples made from non-diafiltered retentate, whereas cheese samples made from diafiltered retentate did not show any white deposits on the surface. They explained that excessive levels of lactose in non-diafiltered retentates could lead to final cheese samples with supersaturated with calcium lactate, thus, causing white deposits of calcium lactate crystals on the surface of final cheeses.

Dybing et al. (1988) studied the effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. In their study, Cheddar cheeses were manufactured according to the manufacture procedures. Cheeses were ripened for 2, 5, 8, or 12 months before packaging and stored at 2, 4, 6, or 12°C. White crystals were determined by X-ray diffraction and HPLC. They reported that the crystals found on the cheese surfaces were identified by X-ray as calcium lactate pentahydrate. The HPLC analysis showed that the main component of the crystals was 65 – 85% calcium lactate, and the remainder was mostly water. They did not detect any level of calcium phosphate, calcium citrate or tyrosine. The crystals started to appear after about 2 months of aging, and the rate of crystal growth increased dramatically after 3 to 5 months of aging. The effect of cheese age at different packaging and storage temperatures reported that cheese samples that were packaged after 8 and 12 months of ripening developed significantly higher levels of surface crystals than cheese samples packaged after 2 and 5 months of ripening. Crystals were minimally occurred on the surface of cheeses stored at 2 and 12°C, while the crystals were heavily occurred on the cheeses stored at 4 and 6°C with the maximum amount of crystals at 6°C. Finally, the relationship between manufacturing

parameters and the crystal formation reported that fast acid production, high milling acidity level, and low salt content associated with the lower level of crystal formation during ripening.

From their results, Dybing et al. (1988) explained that although calcium and lactate ions were indigenous components in milk, calcium lactate was not. This meant that the concentration of both calcium and lactate ions had to be high enough for the calcium lactate crystals to occur. During cheese ripening, because the majority of serum calcium would be removed during the whey drainage, it was highly likely that the main source of calcium for the crystal formation would be from the casein-bound calcium. The source of lactate for the crystal formation would be from the lactose fermentation. They further explained that any factors that could affect the concentration of calcium and lactate ions and the solubility of calcium lactate would result in the greater crystal development during ripening. Those factors could be higher salting levels, higher milk lactose, and lower storage temperature. Because it was speculated that the main source of calcium for the crystal formation was from the casein-bound calcium, higher salting levels would release more concentration of calcium from casein, thus, resulting in higher available calcium ions and greater crystal development. Higher milk lactose could provide additional substrate for the bacteria and cause higher available lactate ions and greater crystal development. Finally, the low storage temperature could not only provide better atmosphere for bacterial growth, which would result in more lactate production, but also affect the solubility of calcium lactate, which would result in more crystal development.

Rajbhandari and Kindstedt (2005a) recently studied the compositional factors associated with calcium lactate crystallization in smoked Cheddar cheese. They intended to study the causes of within vat variation in crystallization behavior since it was reported that the white crystals sometimes deposited on the surface of some Cheddar cheese samples but not on other samples from the same vat of milk. In their study, six pairs of 5 to 6 months old crystallized and non-crystallized retail cheese samples from the same cheese plant were collected. All the cheese samples were stored for an additional 5 to 13 months at 4°C. The crystalline deposits were removed from the cheese surface and analyzed for lactic acid, calcium, phosphate, sodium chloride, moisture and crude protein. The cheese samples were sectioned into three subsamples according to their depth from the surface (0 to 5 mm, 6 to 10 mm, and more than 10 mm depth), and were subjected to compositional analyses. The crystalline materials composed of 52.1% lactate, 8.1% calcium, 0.17% phosphate, 28.5% water, and 8.9% crude protein. Both crystallized and non-crystallized cheese samples exhibited a significant moisture gradient decreasing from the center to the surface. The crystallized samples had higher moisture, lactate and water soluble calcium, but lower pH and sodium chloride content with compared with the non-crystallized cheese samples. They suggested that the formation of calcium lactate crystals could have been influenced by within-vat variation due to the salting efficiency. During salting, some parts of the cheese curd could have absorbed lower amount of salt than others, which could cause pockets of higher moisture and higher lactose within the final cheese. Once the cheese block was cut into the retailed chunks, the lower salt and higher moisture chunks could result in cheese with higher lactate and lower pH, which would shift the calcium to soluble state. The higher concentration of calcium and late ions in the

cheese samples could increase at the cheese surface because of the moisture loss during cheese ripening and smoking, and would finally trigger the formation of calcium lactate crystals.

After a century of research, it is now believed that the white deposits on the surface of Cheddar cheese is calcium lactate pentahydrate. Several factors have been linked to influence the formation of calcium lactate crystals, and several methods have also been suggested to limit the formation of calcium lactate crystals. Those factors and methods will be discussed in the later section.

A racemic mixture of L(+) and D(-) calcium lactate in Cheddar cheese

Although lactic acid produced from lactose is mainly an L isomer, once the cheese is aged, D-lactic acid can be formed. D-lactic acid can be produced from residual lactose in cheese by lactobacilli or from racemization of L-lactate by non-starter lactobacilli and pediococci (Thomas and Crow, 1983). The formation of D-lactate has also been linked to calcium lactate crystal formation, because the solubility of Ca-DL-lactate is lower than that of only Ca-L-lactate (McSweeney and Fox, 2004). Thus, calcium lactate crystals could likely be found in L- and D-lactate isomers or a mixture of both isomers.

Severn et al. (1985) established the more accurate method for quantifying L- and D-lactic acid in cheese and calcium lactate crystals on the surface of Cheddar cheese. This new protocol was modified through an existing L-lactic acid assay procedure. Nicotinamide adenine dinucleotide was taken out of the reaction mixture in order to

determin D-lactic acid. Their results reported a high percentage of both lactic acids were recovered without racemization of both lactic acids. The percentage recovered was 97.7 for L-lactic acid, and 99.5 for D-lactic acid.

The following researchers have established the relationship between nonstarter lactic acid bacteria (NSLAB) with D-lactic acid and the formation of calcium lactate crystal in Cheddar cheese. Rengpipat and Johnson (1989) isolated organisms from the white-crystal deposits on the surface of aged Cheddar cheese. The organism was characterized as an atypical *Lactobacillus* strain UW 1. This strain had a limited ability to ferment carbohydrates, but could produced significant quantities of D-lactate. It was confirmed that Cheddar cheese prepared from milk contaminated with this UW 1 strain formed heavy crystal deposits during ripening. Johnson et al. (1990a) studied the influence of nonstarter bacteria on calcium lactate crystallization on the surface of Cheddar cheese. In their study, Cheddar cheeses were manufactured from milk that contained racemase-positive *Lactobacillus* to induce calcium lactate crystal formation. During cheese ripening, a predominant L-lactic acid was converted to a racemic mixture of L- and D-lactic acid as the NSLAB counts increased. The crystal formation also started to occur when the L-lactic was racemized to the D-form. The racemic mixture of L- and D-lactic acid was finally found in both white crystal deposits and cheese samples with crystal formation. Chou et al. (2003) studied the effect of nonstarter lactic acid bacteria on calcium lactate crystallization in Cheddar cheese. In their study, Cheddar cheeses were manufactured with milk inoculated with *Lactococcus lactis* starter culture, with or without *Lactobacillus curvatus* or *Lb. helveticus* WSU 19 adjunct cultures. Cheese samples without adjunct cultures or with only *Lb. helveticus* exhibited

predominantly L-lactate (95%), and the crystal formation did not appear until after almost 4 months of aging. On the contrary, a racemic mixture of D- and L-lactate was identified in cheese samples containing *Lb. curvatus*, and the white crystal deposit started to appear concomitantly with the D-lactate production. Recently, Agarwal et al. (2006) observed calcium lactate crystal formation after 2 months of aging in Cheddar cheese containing *Lb. curvatus*. This stated cheese samples also contained significantly higher concentration of D-lactate when compared with control cheeses without *Lb. curvatus.*

Although several researchers have confirmed the relationship between NSLAB with D-lactate and crystal formation, the following researcher stated the opposite. Swearingen et al. (2004) studied factors affecting Cheddar cheese defects. In their study, Cheddar cheeses were manufactured at a commercial cheese plant according to standard Cheddar cheese making procedures. Five different commercial mixed mesophilic culture blends, which were combinations of *Lactococcus lactis* ssp. *cremoris* and *L. lactis* ssp. *lactis*, were used for the cheese making. The results indicated that less than 0.04% D-lactic acid was found in cheese samples. Thus, they concluded that the conversion of L-lactic acid to D-lactic acid did not influence the appearance of calcium lactate crystals during ripening.

Other possible crystals

Although it is now believed that the white deposits on the surface of Cheddar cheese primarily composed of calcium lactate pentahydrate, the white deposits have also been identified as calcium phosphate (Conochie and Sutherland, 1965; Brooker et al.,

1975; Olson, 1983), tyrosine (Don and Dahlberg, 1942; Harper et al., 1953; Conochie at al., 1960), and mixtures of amino acids (Harper et al., 1953; Bianchi et al., 1974).

Conochie and Sutherland (1965) studied the nature and cause of steaminess in Cheddar cheese. Their microscopic study reported heavy layers of white crystal defects along the seams of milled Cheddar curds. The X-ray diffractions identified the crystals as calcium orthophosphate. They speculated that after the salting cheese curds were pressed, some of the curd that covered with undissolved sodium chloride did not fuse; thus, causing an increase in calcium, phosphorus, and water release along the curd seams. However, as cheeses aged, the solubility of calcium phosphate along the seam decreased due to the diffusion of sodium chloride from the surface into the particles, which resulted in a formation of calcium orthophosphate crystal layers along the seams of cheese curds. Brooker et al. (1975) confirmed the calcium phosphate finding. Their microscopic crystalline study in Cheddar cheese reported aggregate crystals concentrated along the lines of the curd particle fusion. These crystals appeared immediately after pressing. They speculated that the crystals occurred along the curd spaces, which was a resulted from pockets of residual whey, and the crystals were likely to be calcium phosphate. Olsen (1983) suggested that calcium phosphate crystals could normally occurred in Cheddar cheese during aging. The crystals were reported as early as a few days after cheese manufacture, and were more numerous than calcium lactate crystals. Unlike the calcium lactate crystal, the calcium phosphate crystal appeared as a small and compact aggregate.

Don and Dahlberg (1942) collected white particles from the surface of several month old canned Cheddar cheese. The white materials gave a completely negative test

for lactic acid, and only trace amount of calcium was present. The microscopic examination showed the particles to be in an arrangement of tyrosine, and the chemical analyses proved the particles to be mainly tyrosine. Harper et al. (1953) studied the chemical compositions of the white deposits from seven different lots of Cheddar cheeses ranging from 18 to 150 months. The white deposits were collected from both the interior and the surface of the cheese samples. The particles were washed with ethyl ether to remove fat, and were analyzed by microbiological assay and X-ray diffraction methods. Results from microbiological assays reported tyrosine in all seven samples, lactic acid and leucine in six samples, and cystine in four samples. Results from X-ray diffraction also supported the microbiological assays. The quantity of lactic acid was not analyzed in this study. Thus, it was not possible to correlate the amount of lactic acid present with different ages of cheese samples or with different locations of white deposits. The quantity of tyrosine was analyzed from all cheese samples, and it was suggested that tyrosine was abundant in very mature cheese samples (103 to 150 months). Tyrosine was also abundant in the samples where the white deposits were collected from the interior of the cheese. Conochie et al. (1960) analyzed the white particles on the surface of 15-month old Cheddar cheese and reported that the crystals composed mainly of tyrosine. They also reported that the white particles from less mature cheese were identified as calcium lactate. Bianchi et al. (1974) reported two types of amino acid deposits in Grana Padano cheese. The bigger granule-type deposits composed mainly of tyrosine, phenylalanine, and glutamic acid. The smaller spot-type deposits composed mainly of leucine and isoleucine. In fact, it is not surprising to see the deposits of tyrosine and other amino acid crystal in cheese especially a very mature cheese. As cheese becomes very

mature, a proportion of protein will be degraded to amino acids, which results in an increase in the concentration of free tyrosine (Silverman and Kosikowski, 1955). Since tyrosine has a considerably low solubility, it is then likely to crystallize during cheese ripening (Pearce et al., 1973).

The formation of calcium lactate crystals

In milk, about two-third of the calcium is bound with phosphate as CCP in the casein micelles. The remaining calcium is present as complexes with either phosphate or citrate or as ionic calcium in the serum phase of milk. A large portion of the serum calcium is removed during whey drainage. Consequently, it has been suggested that casein bound calcium is the major source of calcium contributing to the formation of calcium lactate crystal (CLC) (Dybing et al., 1988). During Cheddar cheese curd formation, lactose is converted to lactic acid. Indigenous concentrations of calcium and lactate ions present in cheese serum are very close to saturation. Small increase of either ion can result in supersaturation and crystallization. Thus, throughout the aging process of hard-type cheese especially Cheddar cheese, small white deposits that can be seen without magnification, appear within and on the cheese surface after about six months of aging.

It has been reported that the solubility of anhydrous Ca-L-lacte is 3.38 g /100 g of water at 4°C (Kubantseva et al., 2004), which provides 0.62g of calcium and 2.76g of lactate per 100g of water. Thus, it is believed that calcium lactate crystals only form when greater than 0.62g of calcium and 2.76 g of lactate per 100g of water are present in cheese. However, the typical concentration of soluble calcium and lactate in the serum

phase of Cheddar cheese is 1.06 g/ 100 g and 3.73 g /100 g, respectively (Morris et al., 1988). Consequently, the concentration of soluble calcium and lactate are in excess of their solubility at 4°C in a typical Cheddar cheese. Since calcium and lactate ions are in a supersaturate state in the cheese serum phase, crystallization at nucleation sites occurs. A continuous movement of calcium and lactate ions to the nucleation sites then causes the sites to become macro crystals (Dybing et al., 1988; Kubantseva and Hartel, 2002; Swearingen et al., 2004). The formation of CLC in cheese can be depicted by the following equation (Dybing et al., 1988; Kubantseva and Hartel, 2002; Kubantseva et al., 2004).

$$Ca^{2+} + 2L^{-} \Leftrightarrow \underset{\text{Soluble}}{CaL_2} \Leftrightarrow \underset{\text{Nucleated crystals}}{CaL_2} \Rightarrow \underset{\text{Macro crystals}}{CaL_2}$$

While CLC are not harmful to consumers, they are perceived as grittiness in the cheese, and more importantly, they are considered a quality defect because consumers often believe that they are mold or some other type of spoilage (Chou et al., 2003; Rajbhandari and Kindstedt, 2005a). In addition, this can lower the reputation of cheese products, which will lead to financial loss for cheese manufacturers (Washam et al., 1982; Swearingen et al., 2004).

A potential pathway for the formation of calcium lactate crystals is shown in Figure 4. This figure suggested that the main source of calcium for the crystal formation is from casein bound calcium. During ripening, casein is hydrolyzed, which results in the release of calcium. The decrease in pH during manufacture and ripening also results in the release of casein bound calcium (Kubantseva and Hartel, 2002). The crystals have

previously been identified as calcium lactate crystals, particularly calcium lactate pentahydrate ($Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$), where five molecules of water are incorporated into the crystal lattice (Tuckey et al., 1938; McDowall and McDowell, 1939; Farrer and Hollberg, 1960; Dybing et al., 1988).

Solubility of calcium lactate

Calcium lactate (CaL_2) is moderately soluble. The equilibrium of saturated solution of CaL_2 consists of two steps.

$$Ca(CH_3CHOHCOO^-)_2\ (s) \Leftrightarrow CaCH_3CHOHCOO^+\ (aq) + CH_3CHOHCOO^-\ (aq) \quad \text{(eq.1)}$$

$$CaCH_3CHOHCOO^+\ (aq) \Leftrightarrow Ca^{2+} + CH_3CHOHCOO^-\ (aq) \quad \text{(eq.2)}$$

The first step completely dissociate, while the second step does not completely dissociate. An incomplete dissociate of CaL^+ in the second step is due to the presence of the negatively charged alcohol groups in anion lactate molecules, which results in back association of Ca^{2+} and lactate ions. In general, in order to determine the solubility of CaL_2 in water, the concentration of calcium ions is measured. The methods used to measure calcium ions include Atomic Absorption Spectroscopy (AAS), complexometric (EDTA) titration, oxalic method, and the conductivity method (Kubantseva and Hartel, 2002).

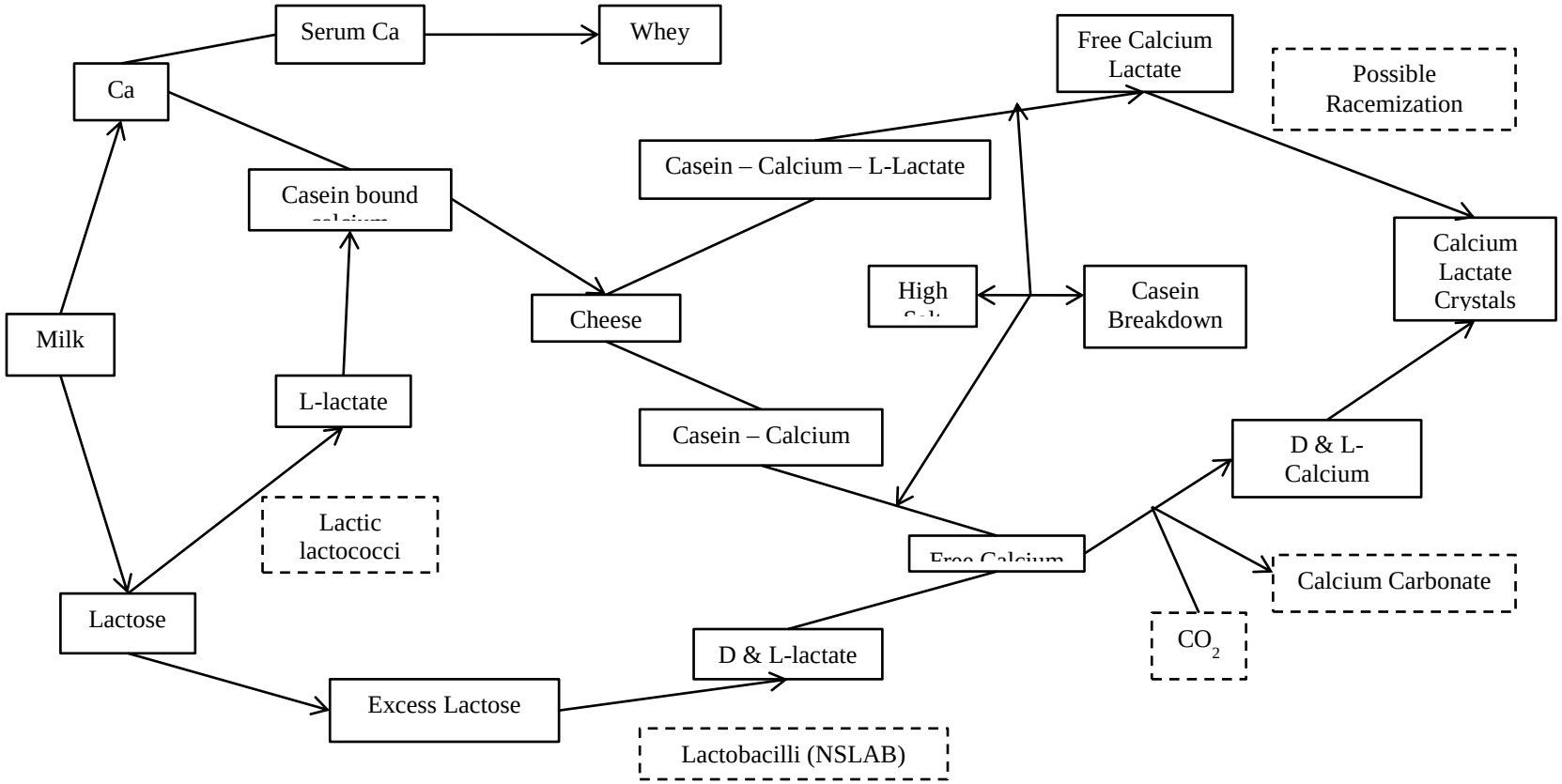

Figure 4. Possible pathway for calcium lactate crystal formation on Cheddar cheese.

Source: Dybing et al., 1988

The solubility of CaL_2 in water varies depending on several factors including temperature, pH, and the presence of other compounds. The solubility of calcium lactate increases with an increase in temperature and pH. The presence of 'uncommon ions' also increases the solubility of CaL_2, because uncommon ions increase the total ionic concentration in the solution, which results in larger stoichiometric concentration compared to the ion activity. Thus, CaL_2 will be more soluble in order to restore the equilibrium (Kubantseva and Hartel, 2002). Cao et al. (2001) studied the solubility of Ca-L-lactate and Ca-DL-lactate in water in the range of 5 to 80°C. The results are shown in Table 1, and these results show that the solubility of both Ca-L-lactate and Ca-DL-lactate increases with increasing temperature. These results also support the statement that the solubility of Ca-DL-lactate is less than that of pure Ca-L-lactate, since the solubility of Ca-DL-lactate is less than that of Ca-L-lactate throughout the temperature range.

Kubantseva and Hartel (2002) studied the effect of pH to the solubility of Ca-L-lactate. The experiments were executed by adjusting the pH of water to different levels with lactic acid. Ca-L-lactate crystals were then added to each solution, and the solubility of Ca-L-lactate was measured after the equilibrium of the solutions was reached. They reported that the solubility of Ca-L-lactate increases with increasing pH (Table 2), and they explained that at lower pH, the higher concentration of lactic acid led to an increase in lactate ions. Thus, the equilibrium shifted towards the formation of Ca-L-lactate crystals, which results in less soluble Ca-L-lactate at the equilibrium state.

Table 1 Effect of temperature on the solubility of Ca-L-lactate and Ca-DL-lactate

Temperature (°C)	**Solubility of Ca-L-lactate (g Anhydrous CaL_2/100g solution)**	**Solubility of Ca-DL-lactate (g Anhydrous of CaL_2/ 100g solution)**
5	4.20	2.90
10	4.50	3.00
15	5.00	3.00
20	6.00	3.40
25	6.25	3.60
30	8.00	4.00
40	10.00	6.20
45	10.70	8.40
50	13.60	9.80
55	16.20	11.00
60	21.80	14.10
65	26.90	17.30
70	32.00	-
75	39.00	26.30
80	46.00	36.40

Adapted from: Cao et al., 2002

Table 2 Effect of pH on solubility of CaL_2 in water at 10°C

pH	**Solubility of Ca-L-lactate (g Anhydrous CaL_2/ 100 g solution)**
5.00	1.88
5.12	2.34
6.00	2.78
7.00 (no lactic acid added)	2.93

Adapted from: Kubantseva and Hartel, 2002

Why is Ca-DL-lactate less soluble than Ca-L-lactate?

As previously mentioned, the formation of D-lactate has also been linked to calcium lactate crystal formation, because the solubility of Ca-DL-lactate is lower than that of only Ca-L-lactate (McSweeney and Fox, 2004). We speculate that Ca-DL-lactate is less soluble than Ca-L-lactate due to the differences between their configurations. In order to fully explain our speculation, a brief background of calcium ion behaviors is necessary.

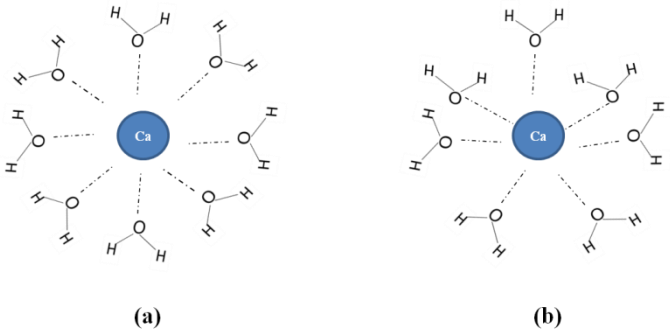

Figure 5. Stereochemistry of the Ca ion hydration shell in (a) eight-fold (square-antiprismatic) structure and (b) seven-fold (pentagonal bipyramidal) structure.

Source: Cook and Bugg, 1977

Ca ions in aqueous solutions are highly hydrated. The strong ion-dipole interaction tends to orient the water molecules around Ca ions (Israelachvili, 1992). Cook and Bugg (1977) suggested that the two most stable hydrated Ca ions structures are the eight-fold (square-antiprismatic arrangement) and the seven-fold (pentagonal bipyramidal arrangement), as shown in Figure 5(a) and 5(b), respectively. In these structures, the water molecules are positioned with their oxygen atoms directly toward the Ca ions. The geometry of seven- and eight-fold hydrated Ca ions allow water molecules to effectively pack around Ca ions that are not closer than normal van der Waals separations, thus resulting in stable Ca-water formation. In addition, three most commonly observed modes of Ca-carboxylate interactions are suggested as shown in Figure 6; the unidentate mode: the Ca ion interacts with only one of the two O atoms of the carboxylate group, the α mode: the Ca ion is chelated by a carboxylate O atom combined with a suitable ligand

atom attached to the α position, and the bidentate: the Ca ion is chelated by the pair of carboxylate O atoms. It is suggested that the α-mode chelation is the favorable configuration for binding Ca ions (Einspahr and Bugg, 1980).

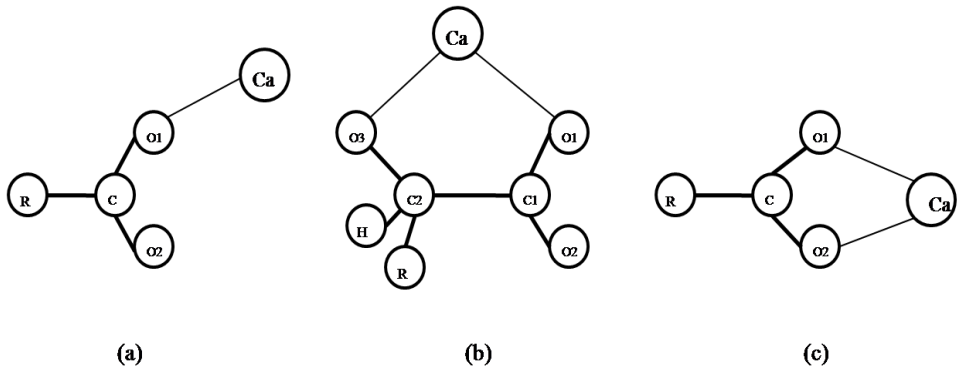

Figure 6. The geometry of Ca-carboxylate interactions; (a) the unidentate mode, (b) the α-mode, and (c) the bidentate mode.

Source: Einspahr and Bugg, 1980.

The difference between L- and D-lactate is due to their configuration. For L – lactate, the OH group is placed to the left of highest numbered chiral carbon atom, while the OH group is placed to the right of highest numbered chiral carbon atom for D-lactate. From Figure 7, we speculate that both the D- and L-lactate from Ca-DL-lactate are able to bind Ca ion with the α-mode chelation. However, only one of the L-lactate from Ca-L-lactate is able to bind Ca ion with the α-mode, while the other L-lactate is able to bind Ca ion with other chelation mode. Thus, it is possible that the Ca-DL-lactate configuration is

thermodynamically favored, which leads to favorable and more compact crystal structures. This then causes Ca-DL-lactate to be less soluble.

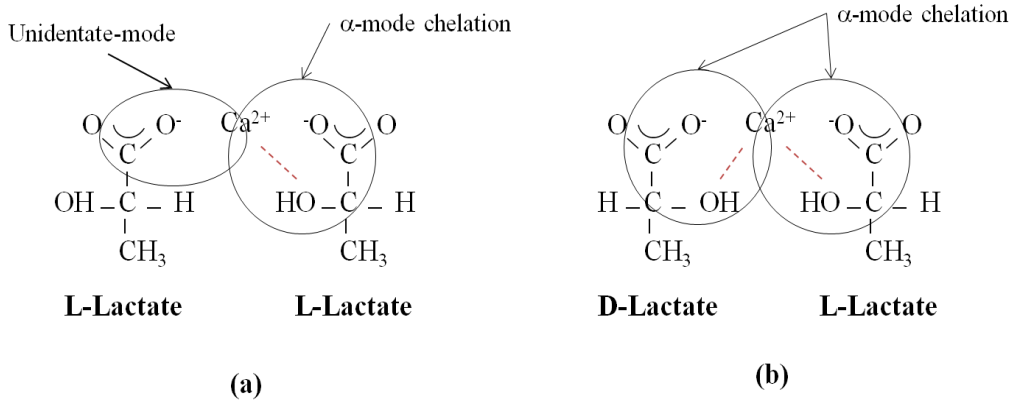

Figure 7. The possible configurations of (a) Ca-L-lactate with α-mode and unidentate chelation and (b) Ca-DL-lactate with α-mode chelation.

Methods for estimating surface crystal development

In order to routinely evaluate the surface crystal development in the cheese plant, a simple method would be useful. Dybing et al. (1986) developed a simple method for estimating the extent of surface crystal on colored Cheddar cheese. The surface of Cheddar cheese wrapped with clear film was photocopied by a copier that was previously calibrated to clearly show the surface crystal. The photocopies of the cheese surface were compared to a series of photocopies that were designated an increase in crystal growth. Crystal growth was rated from 0 to 4 as follows: 0 = no crystals, 1 = light, 2 = medium, 3 = heavy and 4 = very heavy. The advantage of this method was that it did not destroy or disrupt the cheese samples. Thus, it was possible to study the extended crystal growth on

the same piece of cheese. However, this method was not suitable for the cheese samples that did not have even and flat surfaces. A recent study by Rajbhandari and Kindstedt (2005b; 2006) developed a quantitatively and nondestructively method to characterize calcium lactate crystals on Cheddar cheese by using image analysis of the digital photographs of the cheese surface. The image analysis was utilized to measure changes in the number of discrete crystal regions, which might be used as an indicator of crystal formation rate. Changes in the area and circularity of individual discrete crystal regions were also measured and used as indicators the crystal growth rate and morphology, respectively. Their results indicated that the discrete crystal region measurement underestimated the true number of the surface crystals because neighboring crystals merged as they grew and became a single crystal region over time. The individual crystals were almost perfect circle and their growth rate increased in a near-linear manner. They concluded that the image analysis method could be a useful tool to measure crystal formation and growth rates on cheese over time.

Factors influencing calcium lactate crystal formation in Cheddar cheese

Several factors have been shown to influence CLC including dead lactic acid bacteria cells as nucleation sites for the crystal formation in cheese (Kalab, 1980); high lactose levels in milk (Pearce et al., 1973); ultrafiltrated milk without diafiltration (Sutherland and Jameson, 1981); milk citrate levels and the subsequent utilization of citrate by microorganism (Morris et al., 1988); the conversion of L– lactate into a racemic mixture of L– and D– lactate caused by contamination of nonstarter lactic acid bacteria and biofilm formation (Thomas and Crow, 1983; Johnson et al., 1990a; Somers et al.,

2001; Chou et al., 2003; Agarwal et al, 2006); loose packaging and low storage temperature (Johnson et al., 1990b); and certain starter culture stains and low salt-in-moisture levels in cheese (Swearingen et al., 2004).

Recommendation methods for limiting calcium lactate crystal formation in Cheddar cheese

Previous recommendations for limiting CLC formation in cheese include: 1) reducing the concentration of lactic acid in the final curd, 2) reducing or eliminating undesirable non-starter lactic acid bacteria from the cheese-making process, 3) controlling storage temperature, and 4) vacuum packaging cheese to lessen the airspace around the outer cheese surface (Dybing et al., 1988; Olsen and Johnson, 1989; Johnson et al, 1990a; Johnson et al., 1990b). Although all the above recommended methods are incorporated into the Cheddar cheese-making process, CLC can still occur.

Sodium gluconate as a calcium lactate crystal inhibitor

Sodium gluconate is a salt form of gluconic acid, which is a polyhydroxycarboxylic acid obtained through an oxidation procedure of glucose, as shown in Figure 8 (Abbadi et al, 1999). Because of its polyhydroxycarboxylic ligand, sodium gluconate has a high sequestering power (Ramachandran et al., 2006), and it is able to sequester calcium ions via the carboxylic oxygen and the α-hydroxylic groups (α-mode chelation) by means of coordinate and covalent bonds. One potential approach to preventing CLC is to add sodium gluconate to Cheddar cheese. Sodium gluconate can increase the solubility of calcium and/or lactate in the serum phase by forming complexes

with one or both of the calcium and lactate ions, and removing one or both of the calcium and lactate ions from being available for the formation of CLC.

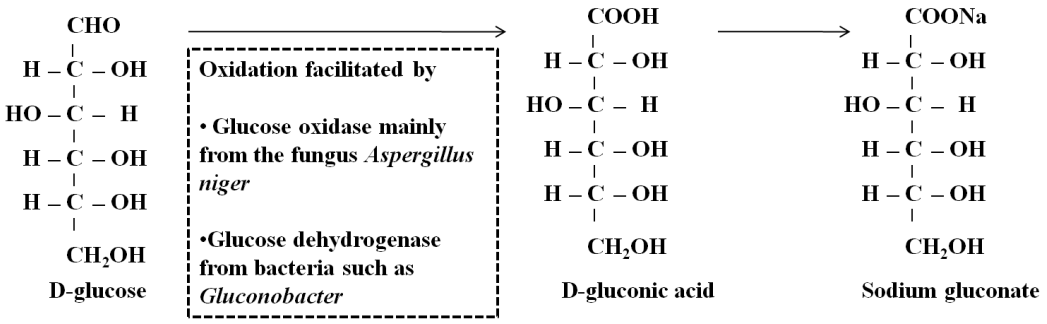

Figure 8. A diagram describing an oxidation process of D-glucose to D-gluconic acid and sodium gluconate.

Adapted from: Abbadi et al., 1999.

Abbadi et al. (1999) studied the Ca-sequestering capability of polyhydroxycarboxylic acids, which were aldonic and aldaric acids. Sodium gluconate and sodium galactonate were used as representatives of aldanic acids, while disodium glucarate and disodium galactarate were used as representatives of aldaric acids. Their results indicated that all aldonates and aldarates were able to sequester calcium ions via their hydroxycarboxylate groups. Gluconate has also been used in other areas because of its chelating ability. For examples, it has been used to prevent the milkstone formation in milk can (Prescott et al., 1953), inhibit corrosion of carbon steel in chloride solutions (Lahodny-Šarc et al., 2000), remove heavy metals from soil components and soils (Fischer and Bipp, 2002), decrease paint stripping time by solubilizing the surface

piqment adhering to metal after stripping, and eliminating rust spots on bottle necks thus increasing efficiency in bottle washing. Gluconic acid and gluconate has also been used in the food industry as food additives and ingredients (Sodium gluconate handbook, 1994).

Cheese with sodium gluconate to inhibit calcium lactate crystal formation

Metzger and Grindstaff (2005) proposed the use of sodium gluconate as a CLC inhibitor in Cheddar cheese. They described the preferable Cheddar cheese manufacture with sodium gluconate addition as follow. The starting milk mixture should have total solids within the range of 14 to 15% by adjusting additional cream and non-fat milk solids to the starting skim milk. Calcium chloride and colorant are added to the milk mixture. The milk ingredient is then acidified with mesophilic starter culture, incubated at 31 - 32°C for 30 min. After incubation, rennet at about 0.02 – 0.1% is added to the mixture, and the mixture is further incubated at at 31 - 32°C for 30 min. Rennet causes the milk mixture to coagulate into a curd. After coagulation, the curd is cut, stirred and heated at 31 - 39°C for 30 min. Then whey is drained, and the curd is either matted into a slab for the traditional Cheddaring method, or immediately stirred for the stirred-curd method. The curd is then salted with sodium chloride at about 2.75%. Normally, about 65 – 90% of salt is retained in the curd, which results in 1.5 – 2.0% of salt in the typical Cheddar cheese. They suggested that sodium gluconate could be added to the starting milk mixture, the concentrated milk, the starter culture, or the rennet. However, the most preferred method is to add sodium gluconate during or immediately after the salting step, because a granule form of sodium gluconate can be used, and this method also minimizes

the amount of sodium gluconate that will be lost during whey drainage. The preferable amount of sodium gluconate is within the range of 0.32 – 4.73%by weight, which results in about 0.26 – 2.8% gluconate in the final cheese.

In order to obtain sufficient amount of sodium gluconate to prevent the CLC formation, Metzger and Grindstaff (2005) suggested that the amount of sodium gluconate added into the cheese should be based on the lactate content of the cheese, and the amount of sodium gluconate retained in the cheese. The normal range of lactate in Cheddar cheese is 1.1 – 1.9%, and the amount of sodium gluconate, if added during or immediately after salting step, is believed to be retained at the similar rate as that of sodium chloride salt, which is about 65 – 90%. The preferred amount of sodium gluconate should be within the range of about 1/4 to 5/3 the lactate content of the cheese. For instance, if Cheddar cheese contains 1.1% lactate, the amount of sodium gluconate added should be within the range of 0.32 – 2% by weight. If the gluconate retention is about 90%, this results in 0.29 – 1.8% sodium gluconate to inhibit the CLC formation. If the Cheddar cheese contains 1.9% lactate, the amount of sodium gluconate added should then be within the range of 0.77 – 4.7% by weight. If the gluconate retention is about 65%, this results in 0.50 – 3.1% sodium gluconate to inhibit the CLC formation.

In this study, six Cheddar cheeses were made at three different batches. In each batch, concentrated milk mixture was prepared by adding 10 pounds of non-fat dry milk and 10 pounds of cream (40% butterfat) to the 500 pounds whole milk (3.8% butterfat). 15.6 mL of color was added to the milk mixture. Then, starter culture was added to the milk mixture at the different levels for each batch; 36 mL of a direct vat set, frozen,

concentrated cultures strain M30 and M42 was added to the first batch; 2% of bulk starter culture strain M46 was added to the second batch; 64 mL of a direct vat set, frozen, concentrated cultures strain M62 was added to the third batch. The milk mixture was incubated at 31°C for 45 min, and 24 mL of rennet diluted with 500 mL of de-ionized water were added to the milk mixture. The mixture was set about 30 min until the visually-clotting time was reached, and the coagulum was cut, allowed to heal for 5 min and gently stirred for another 5 min. The curds and whey were cooked and stirred to 38°C in 30 min and were held at 38°C for another 30 min. After the pH of the curds reached about 6.25 – 6.30, whey was drained. In the first batch (treatment 1 and 2), the traditional milled-curd method was used. After the pH of the cheese curd reached 5.4, the cheese curd was divided into two equal halves. Each half was salted with 2.75% sodium chloride. The first half (treatment 1) contained no sodium gluconate, and 2.4% sodium gluconate was added to the second half (treatment 2) immediately after the salting step. The stirred-curd method was used for the second (treatment 3 and 4) and the third (treatment 5 and 6) batches. The cheese curd from each batch was divided into two equal halves after the pH of the curd reached 5.4. The two cheese-curd halves from the second batch were salted with 2.75% sodium chloride. No additional sodium gluconate was added to the first half (treatment 3), and 2.4% sodium gluconate was added to the second half (treatment 4) immediately after the salting step. The two cheese-curd halves from the third batch were salted with 2.5% sodium chloride. No additional sodium gluconate was added to the first half (treatment 5), and 3.0% sodium gluconate was added to the second half (treatment 6) right after the salting step. The cheeses from all treatments were then

pressed, hooped overnight, vacuum-sealed in clear wrapping, and aged under refrigeration for two months.

After two months, cheese samples were measured for the pH, lactic acid, gluconic acid, moisture content, and were inspected for the CLC formation. The pH, % lactic acid, and % moisture in treatment 1 (milled-curd with 2.75% salt and 0% sodium gluconate) was 5.13, 1.87%, and 38.84%, respectively, while the pH, % lactic acid, % gluconate, and % moisture in treatment 2 (milled-curd with 2.75% salt and 2.4% sodium gluconate) was 5.44, 1.51%, 1.29%, and 40.32%, respectively. The pH, % lactic acid, and % moisture in treatment 3 (stirred-curd with 2.75% and 0% sodium gluconate) was 5.35, 1.08% and 39%, respectively, whereas the pH, % lactic acid, % gluconate and % moisture in treatment 4 (stirred-curd with 2.75% and 2.4% sodium gluconate) was 5.42, 1.01%, 0.79% and 42.51%, respectively. The pH, % lactic acid, and % moisture in treatment 5 (stirred-curd with 2.5% salt and 0% sodium gluconate) was 5.25, 1.24%, and 33.05%, whereas the pH, % lactic acid, % gluconate and % moisture in treatment 6 (stirred-curd with 2.5% salt and 3.0% sodium gluconate) was 5.41, 1.03%, 1.40% and 34.5%, respectively. Cheeses obtained from the treatment 1, 3 and 5 (no sodium gluconate addition) had visible CLC on the cheese surface and cheese interior, while cheeses with sodium gluconate addition (treatment 2, 4, and 6) did not show and visible CLC formation present. Thus, this suggests that sodium gluconate is effective as CLC inhibitor. However, the effect of sodium gluconate on Cheddar cheese during ripening, which include glycolysis, proteolysis, textural properties and sensory properties has not yet been investigated.

Sodium gluconate as a Ca-sequestrant

In aqueous solution, sugars can form complexes with metal cations by displacing water molecules in the solvation sphere of cations by their –OH groups. At least three –OH groups are required for complex formation in this system. The complex-forming ability of sugars is considerably enhanced when an anchoring group such as –COOH is introduced into the sugar molecules and they become sugar acids (Gyurcsik and Nagy, 2000; Nagy and Szorcsik, 2002). Because of an increasing interest in utilization of organic sequestering agents, sugar acids, which are compounds that have promising ability for this purpose, have become the subject of interest for more than 50 years (Mehltretter et al., 1953; Sawyer, 1964). Gluconic acid, which is a sugar acid, and its salts have been used as sequestering agent for metal ions in industrial, medical and agricultural area (Escandar and Sala, 1992). Gluconic acid can be produced by chemical and biological processes. The chemical processes include the chemical oxidation of glucose in alkaline media and electrolytic oxidation of an alkaline solution of glucose. The biological processes include the fermentation of glucose solution by fungi and bacteria. The chemical method is a simple one-step process, but because of the low selectivity, this method is not suitable for producing gluconic acid on an industrial scale. Thus, the fermentation process utilizing fungi *Aspergillus niger* is the dominant technique for producing gluconic acid in the industry (Prescott et al., 1953; Ramachandran et al., 2006). Recent developments in gluconic acid production include finding an alternative and cheaper carbohydrate source such as agro-food by products, which include sugarcane molasses, beet molasses, grape must and banana must, to meet higher demand for gluconic acid (Singh and Kumar, 2007).

Sodium gluconate is a sodium salt of gluconic acid, and it is able to chelate and form stable complexes with metal ions, which include Ca (II), Cu (II), Fe (II), Fe (III), Co (II), Ni (II), Cd (II), Hg (II), Pb (II), Al (III), Ga (III), and In (III) (Pecsok and Juvet Jr., 1955; Pecsok and Sandera, 1955; Pecsok and Juvet Jr., 1956; Escandar and Sala, 1992; Gyurcsik and Nagy, 2000). The ability of gluconate to form a water-soluble complex with metal ions is suggested to be from both the carboxylic oxygen and the α-hydroxylic group that can bind cations in a ring by the α-mode chelation. The functional groups are in positions that allow the formation of stable five-membered chelate rings, and it is assumed that the complex between sugar acids and metal ions are one-to-one complexes, where one molecule of saccharic acid has formed a five-membered ring complex with one molecule of Ca ion (Mehltretter et al., 1953; Sawyer, 1964; Saladini et al., 2001).

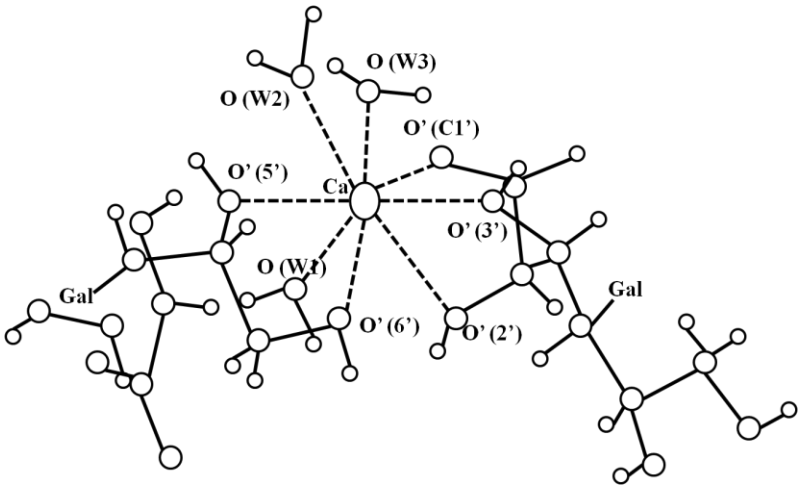

Figure 9. The structure of Ca-lactobionate complex. Gal represents the galactose residues that are omitted for clarity.

Source: Cook and Bugg, 1977

Cook and Bugg (1977) suggested the structure of Ca-lactobionate complex as can be seen in Figure 9. Lactobionate is an ion form of lactobionic acid. Lactobionic acid is a sugar acid obtained from an oxidation process of lactose, where the glucose residue of lactose is converted to gluconate anion and the galactose residue is unaltered. The lactobionate ion contains functional groups of lactose and additional carboxyl group in the gluconate residue. In this complex structure, two lactobionate ions are bound to a Ca ion via their gluconate residues. One lactobionate ion chelates Ca ion by an oxygen atom from carboxyl group and two other oxygen atoms from hydroxyl groups in the α and β positions (O(2′) and O(3′)). The other lactobionate ion chelates the Ca ion via the oxygen pairs of hydroxyl groups (O(5′) and O(6′)) located at the end of the gluconate residue. Three water molecules (O(W1), O(W2), and O(W3)) complete the Ca ion shell, which results in the total of eight oxygen atoms coordinated to Ca that looks like a square-antiprism structure shown in Figure 5(a). As extended from Cook and Bugg, Wiezorek et al., (1996) studied the crystal and molecular structure of Ca-gluconate hydrate, and they suggested that the Ca ion binds to two gluconate ions via an oxygen atom from the carboxyl group (O12) and an oxygen atom from α-hydroxyl group (O2), and three oxygen atoms from three water molecules (O1W, $O1W_a$, and O3W), as shown in Figure 10. This results in the structure similar to the seven-fold pentagonal bipyramidal structure as shown in Figure 5(b).

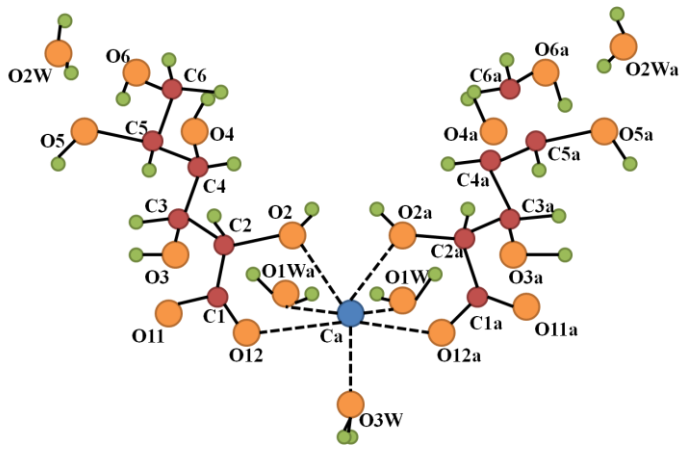

Figure 10. The structure of Ca-gluconate complex. Ca-O2 indicated the bond between Ca ion and oxygen atom from α-hydroxyl group of gluconate. Ca-O12 indicated the bond between Ca ion and oxygen atom from carboxyl group of gluconate. Ca-OW indicated the bonds among Ca ion and water molecules.

Source: Wieczorek et al., 1996

The possible mechanisms for calcium lactate gluconate (CLG) formation

Although CLG has been widely used as a soluble pharmaceutical calcium source, its definite complex structure is still not clear. CLG was first developed by Sandoz, and is now well-known as Calcium-Sandoz® tablets with the chemical formula of $Ca_5(C_3H_5O_3)_6.(C_6H_{11}O_7)_4.2H_2O$. CLG has the highest solubility when compared with calcium lactate and calcium gluconate, as shown in Table 3. The reason for its high solubility is due to the ability of gluconate to form complex with calcium and lactate ions in solution. In addition, CLG provides a neutral taste, whereas calcium lactate tends to provide a bitter taste at high concentration, which could be due to free calcium ion

concentration (Jungbunzlauer, 2002). This indicates that the formation of CLG complexes in Cheddar cheese may not only increase the solubility of the calcium lactate, but may also limit the bitter taste of Cheddar cheese.

Table 3. Properties of calcium lactate gluconate compared to commonly used soluble organic calcium salts (in water at room temperature).

Product	**Ca content**	**Solubility (g/ L water)**	**Solubility (g Ca/ L water)**
Calcium lactate gluconate	13%	400	52
Calcium lactate pentahydrate	13%	66	9
Calcium gluconate	9%	35	3

Adapted from: Jungbunzlauer, 2002

From the chemical formula of CLG that was mentioned above, it is not possible for the structure of CLG to be as simple as one Ca ion binds with one lactate and one gluconate. Two possible mechanisms for the formation of CLG in Cheddar cheese are proposed. In the first mechanism as shown in Figure 11, Ca ions are fully hydrated with either 7-fold or 8-fold, and lactate and gluconate are in ionic forms. In this system, Ca ions are able to bind with oxygen from water molecules, oxygen from carboxyl groups of both gluconate and lactate, and oxygen from hydroxyl groups of gluconate. It was suggested by Cook and Bugg (1977) that the Ca-oxygen bond strength from Ca-water is

weaker than that from Ca-carboxyl and Ca-hydroxyl. Thus, it is possible that once lactic acid and gluconic acid dissociate, their ions are competing to replace weaker Ca-water bonds from hydrated Ca ions, resulting in CLG complexes that are more soluble in the solution.

In the second possible mechanism, gluconate ions are introduced to Cheddar cheese that may already have calcium lactate crystals. As previously mentioned, calcium lactate crystals that appear in Cheddar cheese have been identified as calcium lactate pentahydrate. Thus, it is possible that the structure of the calcium lactate crystal is in the seven-fold pentagonal bipyramidal arrangement, where a Ca ion binds to two lactate ions via carboxyl groups and five oxygen atoms from water molecules. Once gluconate ions are introduced to the system, their carboxyl and hydroxyl groups are able to bind with Ca ions. As already mentioned, the Ca-oxygen bond strength from Ca-water is weaker than that from Ca-carboxyl and Ca-hydroxyl. Thus, it is possible that gluconate ions replace water molecules that are bound to the calcium lactate complex. According to the equilibrium of saturated solution of CaL_2 (eq.1 and eq.2), CaL^+ ions do not completely dissociate. It is then possible that gluconate ions bind with CaL^+ ions and make them unavailable for the further formation of CLC. As can be explained by Le Châtelier's principle, once CaL^+ ions are removed from the equilibrium (eq.1), the equilibrium shifts towards the right direction, which is the dissociation of CaL_2 in order to balance for the CaL^+ decrease. Thus, this results in more soluble CaL_2 in the solution. In addition, the gluconate ions can be considered as 'uncommon ions'. As already mentioned, uncommon ions increase the total ionic concentration in the solution; thus, CaL_2 will be more soluble to restore the equilibrium of the system.

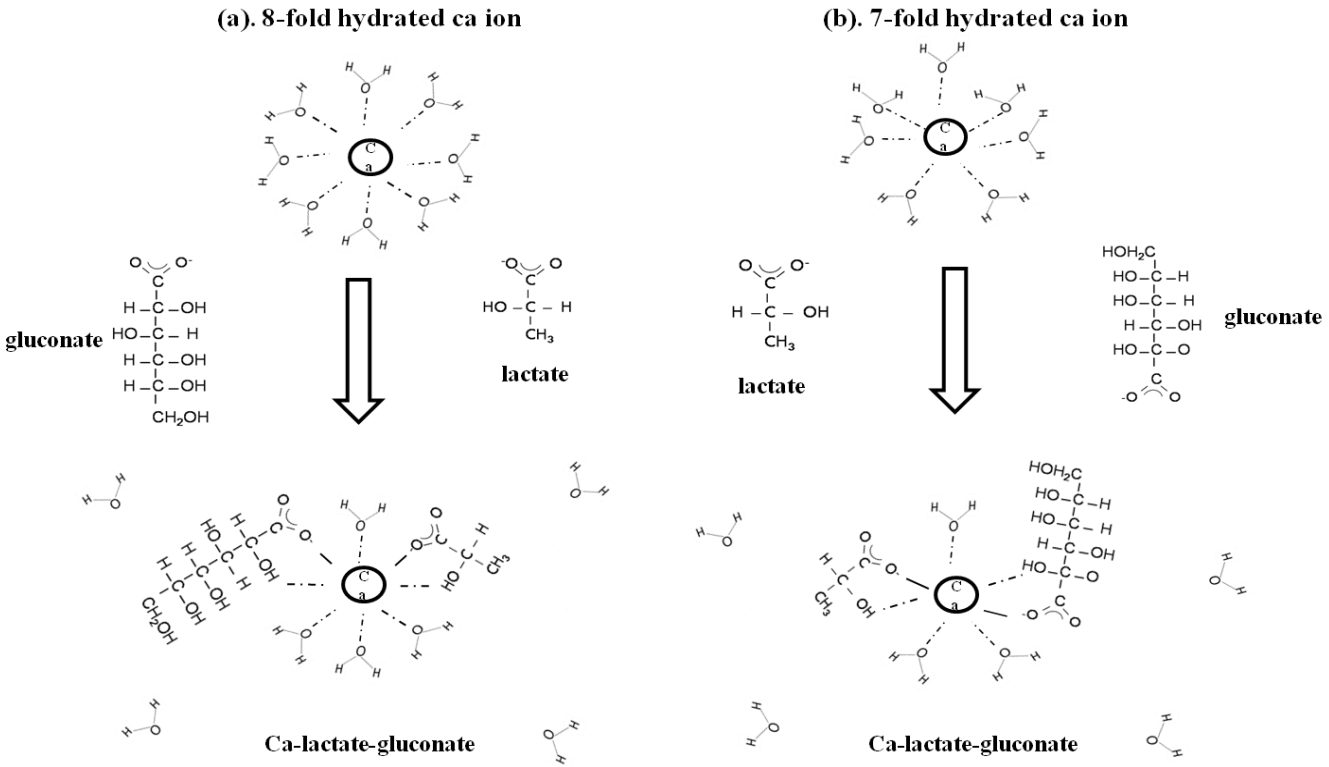

Figure 11. The possible mechanisms for the calcium lactate gluconate formation. Hydrated ca ions bind with oxygen from carboxyl groups of both gluconate and lactate, and oxygen from hydroxyl groups of gluconate in two forms; (a) 8-fold hydration and (b) 7-fold hydration.

Finally, we propose the possible explanation why CLG has higher solubility than Ca-D-lactate and Ca-L-lactate. As seen in Figure 12, ca ions are able to bind lactate and gluconate with the α-mode chelation alone or with the combination of α-mode and unidentate mode. It is possible that the CLG configurations are not thermodynamically favored for the crystal formation. In addition, the bulky chains of gluconate might provide the steric hindrance and prevent the CLG to close-pack, stack and form crystals. This then causes CLG to be more soluble.

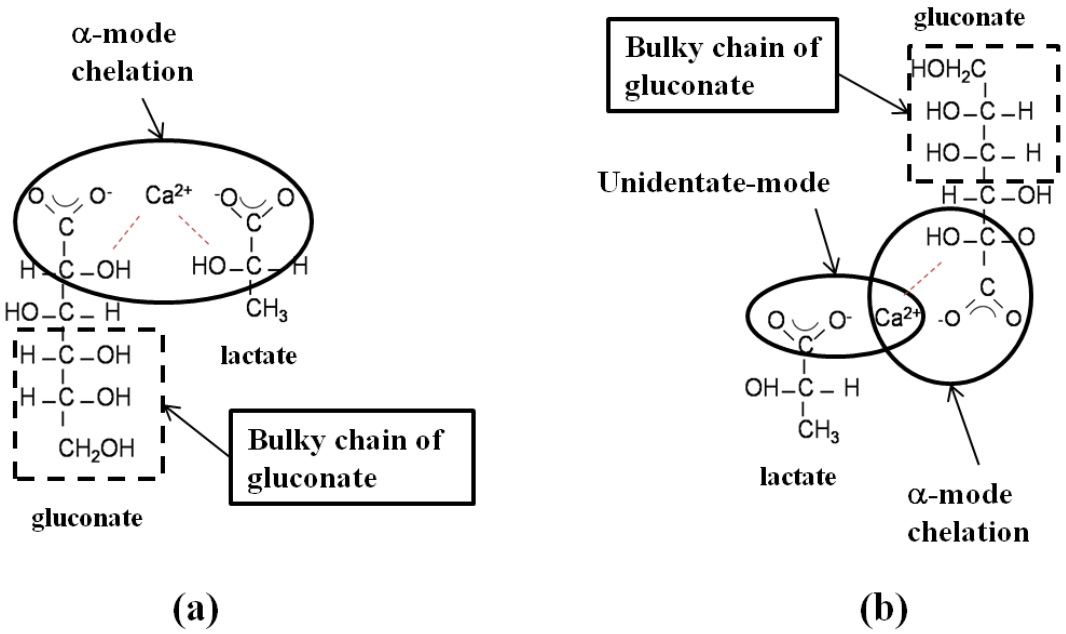

Figure 12. Possible configurations of calcium lactate gluconate with (a) α-mode chelation only and (b) α-mode and unidentate mode chelation.

Safety and legality of sodium gluconate as a food additive

The acute toxicity study of sodium gluconate was conducted. 10 rats of both sexes were given sodium gluconate orally as single doses of 0.625, 1.25, or 2.5% w/w sodium gluconate after an overnight fast. The rats were observed for 14 days for mortality, abnormal clinical signs, body-weight changes and gross pathological changes. There was no evidence of toxicity in all rats. The only clinical effects found after 2-3 hr of treatment was soft feces seen in one male and three female given 2.5% w/w sodium gluconate. In the four-week study, groups of 10 males and 10 female rats were fed basal diet containing sodium gluconate at the concentrations of 0, 1.25, 2.5, or 5% w/w per day. Qualitative and quantitative urinary examinations were performed at the end of treatment, and water intake was measured over 24 h. Haematological and clinical chemical examinations were performed on all animals at necropsy. The effects were an increased water intake, an increased prothrombin time, and increased relative kidney weights, but these effects were not dose-related. Qualitative urine analyses revealed effects related to the high sodium intake arising from the sodium gluconate (World Health Organization, 1999). There were no evidence of gastric or renal irritation in humans treated with gluconic acid and its derivatives; thus, gluconic acid and its derivatives are non-toxic and well tolerated in humans (Prescott et al., 1953).

According to U.S. FDA, sodium gluconate is a food additive that is ‘Generally Recognized As Safe’ (GRAS) under the ‘Sequestrants’ category. Sodium gluconate is permitted to use as a food additive in Canada under pH-adjusting agents and stabilizers categories, and is also an approved food additive that can be used in European countries

(Health Canada, 2006; U.S. FDA, 2006; Food Standards Agency, 2007). In addition, sodium gluconate is readily biodegradable; thus, it will not cause waste-water problem if being used in the food industry (Dweck, 2005).

REFERENCES

Abbadi, A., K. F. Gotlieb, J. B. M. Meiberg, J. A. Peters, and H. van Bekkum, H. 1999. New Ca-sequestering materials based on the oxidation of the hydrolysis products of lactose. Green Chemistry. October 1999: 231–235.

Acharya, M. R. and V. V. Mistry. 2004. Comparison of effect of vacuum-condensed and ultrafiltered milk on Cheddar cheese. J. Dairy. Sci. 87: 4004 – 4012.

Adda, J., J. C. Gripon, and L. Vassal. 1982. The chemistry of flavor and texture generation in cheese. Food Chem. 9: 115-129.

Akalin, A. S., S. Gonc¸ and Y. Akba. 2002. Variation in organic acids content during ripening of pickled white cheese. J. Dairy Sci. 85: 1670-1676.

Agarwal, S., K. Sharma, B. G. Swanson, G. Ű. Yűksel, and S. Clark. 2006. Nonstarter lactic acid bacteria biofilms and calcium lactate crystals in Cheddar cheese. J. Dairy Sci. 89: 1452 – 1466.

Beresford, T. and A. Williams. 2004. The microbiology of cheese ripening, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 287-317.

Bevilacqua, A. E. and A. N. Califano. 1989. Determination of organic acids in dairy products by high performance liquid chromatography. J. Food Sci. 54: 1076-1079.

Bianchi, A., G. Beretta, G. Caserio, and G. Giolitti. 1974. Amino acid composition of granules and spots in Grana Padano cheeses. J. Dairy Sci. 57: 1504 – 1508.

Bouzas, J., F. W. Bodyfelt, and A. Torres. 1991a. A potential analytical assessment of Cheddar cheese flavor defects. Int. Dairy J. 1: 263-271.

Bouzas, J., C. A. Kantt, F. Bodyfelt, and J. A. Torres. 1991b. Simultaneous determination of sugars and organic acids in Cheddar cheese by high-performance liquid chromatography. J. Food Sci. 56: 276-278.

Brooker, B. E., D. G. Hobbs, and A. Turvey. 1975. Observations on the microscopic crystalline inclusions in Cheddar cheese. J. Dairy Res. 42: 341 – 348.

Brunner, J. R. 1977. Milk proteins, In Food Proteins (ed. J.R. Whitaker and S.R. Tannenbaum), AVI Publishing Company, Inc., Connecticut, pp. 175-208.

Bynum, O. G. and D. M. Barbano, D.M. 1985. Whole milk reverse osmosis retentates for Cheddar cheese manufacture: chemical changes during aging. J. Dairy Sci. 68: 1-10.

Cao, X., H. J. Lee, H. S. Yun, and Y. M. Koo. 2002. Solubilities of calcium and zinc lactate in water and water-ethanol mixture. Korean J. Chem. Eng. 18: 133-135.

Chemaly, Z., H. Muhr., and M. Fick. 1999. Crystallization kinetics of calcium lactate in a mixed-suspension-mixed product removal crystallizer. Ind. Eng. Chem. Res. 38: 2803 – 2808.

Chou, Y. -E., C. G. Edwards, L. O. Luedecke, M. P. Bates, and S. Clark. 2003. Nonstarter lactic acid bacteria and aging temperature affect calcium lactate crystallization in Cheddar cheese. J. Dairy Sci. 86:2516–2524.

Cogan, T. M. and T. P. Beresford. 2002. Microbiology of hard cheese, In Dairy Microbiology Handbook: the microbiology of milk and milk products, 3rd edn, (ed. Robinson, R.K.), Wiley-Interscience, Inc., New York, pp. 515-560.

Cook, W. J. and C. E. Bugg. 1977. Structure of calcium-carbohydrate complexes, In Metal-ligand interactions in organic chemistry and biochemistry, Part 2, (eds. Pullman, B. and Goldblum, N.), D. Reidel Publishing Company, Dordrecht, Holland, pp. 231-256.

Conochi, J., J. Czulak, A. J. Lawrence, and W. F. Cole. 1960. Tyrosine and calcium lactate crystals on rindless cheese. Aust. J. Dairy Technol. 15: 120.

Conochi, J. and B. J. Sutherland. 1965. The nature and cause of steaminess in Cheddar cheese. J. Dairy Res. 32: 35 – 44.

Creamer, L. K., K. N. Pearce. J. Gilles, and R. C. Lawrence. 1972. White deposits on the surface of rindless Cheddar cheese. N. Z. J. Dairy Sci. Tech. 7: 21.

Creamer, L. K. and N. F. Olsen. 1982. Rheological evaluation of maturing Cheddar cheese. J. Food Sci. 47: 631-636, 646.

Creamer, L. K., R. C. Lawrence, and J. Gilles. 1985. Effect of acidification of cheese milk on the resultant Cheddar cheese. N. Z. J. Dairy Sci. Tech. 20: 185 – 203.

Crow, V., B. Curry, and M. Hayes. 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. Int. Dairy J. 11: 275-283.

Dalgleish, D. G. 1982. Milk proteins - chemistry and physics, In Food Proteins (ed. P.F. Fox and J.J. Condon), Applied Science Publishers, Ltd., Essex, pp. 155-178.

Dalgleish, D. G. 1993. The enzymatic coagulation of milk, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 2rd edn, (ed. Fox, P.F.), Chapman & Hall, London, UK, pp. 69-100.

Dalgleish, D. G., P. A. Spaguolo, and H. D. Goff. 2004. A possible structure of the casein micelle based on high-resolution field-emission scanning electron microscopy. Int. Dairy J. 14: 1025-1031.

Dejmek, P. and P. Walstra. 2004. The syneresis of rennet-coagulated curd, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 71-103.

De Kruif, C. G. and C. Holt. 2004. Casein micelle structure, functions, and interactions, In Advanced Dairy Chemistry Vol 1: Proteins, 3rd edn. (eds. P. F. Fox and P. L. H. McSweeney), Kluwer Academic/ Plenum Publishers, New York, New York, pp. 233 – 276.

Don, F. L., and A. C. Dahlberg. 1942. Identification of the white particles found on the ripened Cheddar cheese. J. Dairy Sci. 25: 31 – 36.

Dweck, A. C. 2005. An update on natural preservatives. Personal Care. September: 11-15.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701–1710.

Dybing, S. T., S. A. Brudvig, J. A. Wiegand, and E. A. Huang. 1986. A simple method for estimating the extent of surface crystal development on colored Cheddar cheese. J. Food Protect. 49: 421 – 422.

Einspahr, H. and C. E. Bugg. 1980. The geometry of ca-carboxylate interactions in crystalline complexes. Acta Cryst. B37: 1044 – 1052.

Escandar, G. M. and L. F. Sala. 1992. Complexes of Cu(II) with D-aldonic and D-alduronic acids in aqueous solution. Can. J. Chem. 70: 2053-2057.

Farkye, N. Y and P. F. Fox. 1990. Objective indices of cheese ripening. Trends in Food Sci and Tech. 1: 37-40.

Farrell Jr., H. M., E. L. Malin, E. M. Brown, and P.X. Qi. 2006. Casein micelle structure: what can be learned from milk synthesis and structural biology? Curr. Opin. Colloid Interface Sci. 11: 135-147.

Farrer, K. T. H., and W. C. J. Hollberg. 1960. Calcium lactate on rindless cheese. Aust. J. Dairy Technol. 15: 151 – 152.

Fischer, K. and H. –P. Bipp. 2002. Removal of heavy metals from soil components and soils by natural chelating agents. Part II. Soil extraction by sugar acids. Water, Air, and Soil Pollution. 138: 271 – 288.

Fitzsimons, N. A., T. M. Cogan, S. Condon, and T. Beresford. 2001. Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. J. Appl. Micro. 90: 600-608.

Food Standards Agency. 2007. Current EU approved additives and their E numbers. From Internet website: http://www.food.gov.uk/

Fox, P. F. 1989. Proteolysis during cheese manufacturing and ripening. J Dairy Sci. 72: 1379-1400.

Fox, P.F. and A. Brodkorb. 2008. The casein micelle: historical aspects, current concepts and significance. Int. Dairy J. 18:677-684

Fox, P. F. and P. L. H. McSweeney. 1998. Dairy Chemistry and Biochemistry. Blackie Academic & Professional, London.

Fox, P.F. and P. L. H. McSweeney. 2004. Cheese: an overview, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 1-18.

Goff, D. (1995) Dairy Chemistry and Physics. Dairy Science and Technology Education Series, University of Guelph. From Internet website: http://www.foodsci.uoguelph.ca/dairyedu/

Gomis, D. B. 1992. HPLC analysis of organic acids, In Food Analysis by HPLC, (ed. L.M.L. Nollet). Marcel Dekker, New York, NY, pp. 371-385.

Gyurcsik, B. and L. Nagy. 2000. Carbohydrates as ligands: coordination equilibria and structure of the metal complexes. Coord. Chem. Rev. 203: 81-149.

Harper, W. J., A. M. Swanson, and H. H. Sommer. 1953. Observations on the chemical composition of white particles in several lots of Cheddar cheese. J. Dairy Sci. 36: 368 – 372.

Harvey, C. D., R. Jenness and H. A. Morris. 1981. Gas chromatographic quantitation of sugars and nonvolatile water-soluble organic acids in commercial Cheddar cheese. J. Dairy Sci. 64: 1648-1654.

Health Canada. 2006. Food additives permitted for use in Canada. From Internet website: http://www.hc-sc.gc.ca/index-eng.php

Hill, A.R. (1995) Cheese. Dairy Science and Technology Education Series, University of Guelph. From Internet website: http://www.foodsci.uoguelph.ca/dairyedu/

Holt, C. 1994. The biological function of casein: Research reviews, In Hannah Research Yearbook, Hannah Research Institute, Scotland, pp. 60-68.

Holt, C. and D. S. Horne. 1996. The hairy casein micelle: Evolution of the concept and its implications for dairy technology. Neth. Milk Dairy J., 50: 85-111.

Horne, D. S. 1998. Casein interactions: casting light on the black boxes, the structure in dairy products. Int. Dairy J., 8: 171-177.

Horne, D. S. 2003. Casein micelles as hard spheres: limitations of the model in the acidified gel formation. Colloids and Surfaces A: Physiochem. Eng. Aspects. 213: 255-263.

Horne, D. S. 2006. Casein micelle structure: models and muddles. Curr. Opin. Colloid Interface Sci. 11: 148-153.

Horne, D. S. and J. M. Banks. 2004. Rennet-induced coagulation of milk, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 46-70.

Israelachvili, J. 1992. Intermolecular & surface forces. Academic Press Limited, London.

Jacques, J., A. Collet, and S.H. Wilen 1981. Enantiomers, Racemates, and Resolutions. Wiley, New York

Johnson, M. E., B. A. Riesterer, C. Chen, B. Tricomi, and N. F. Olson. 1990b. Effect of packaging and storage conditions on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73:3033 – 3041.

Johnson, M. E., B. A. Riesterer, and N. F. Olsen. 1990a. Influence of nonstarter bacteria on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73: 1145 – 1149.

Jungbunzlauer. 2002. Calcium lactate gluconate – the innovative solution for extra calcium. Innovations in Food Technology. 16: 1-3.

Kalab, M. 1980. Decayed lactic bacteria – a possible source of crystallization nuclei in cheese. J. Dairy Sci. 63(2): 301-304.

Kubantseva, N. and R. W. Hartel, R.W. 2002. Solubility of calcium lactate in aqueous solution. Food Rev. Intl. 18: 135–149.

Kubantseva, N., R. W. Hartel, P. A. Swearingen. 2004. Factors affecting solubility of calcium lactate in aqueous solutions. J. Dairy Sci. 87: 863–867.

Lahodny-Šarc, O., F. Kapor, and R. Halle. 2000. Corrosion inhibition of carbon steel in chloride solutions by blends of calcium gluconate and sodium benzoate. Materials and Corrosion. 51: 147–151.

Lawrence, R.C., H. A. Heap and J. Gilles. (1984) A controlled approach to cheese technology. J. Dairy Sci. 67: 1632-1645.

Lawrence, R. C., L. K. Creamer and J. Gilles. 1987. Texture development during cheese ripening. J. Dairy Sci. 70: 1748-1760.

Lawrence, R. C., J. Gilles, L. K. Creamer, V. L. Crow, H. A. Heap, C. G. Honoré, K. A. Johnston, and P. K. Samal. 2004. Cheddar cheese and related dry-salted cheese

varieties, In In Cheese: Chemistry, Physics and Microbiology, Vol 2: Major Cheese Groups, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 71-102.

Lombardi, A. M., A. E. Bevilacqua, and A. N. Califano. 1994. Variation in organic acids content during ripening of Reggianito cheese in air-tight sealed bags. Food Chem. 51: 221-226.

Lues, J. F. R., W. C. Botha, and E. J. Smit. 1998a. Ion-exchange HPLC analysis and assessment of extraction methods. Food Res. Int. 31: 441-447.

Lues, J. F. R., W. C. Botha, and E. J. Smit. 1998b. Ion-exchange HPLC of cheese-related organic acids in comparison with reverse-phase HPLC. Int. Dairy J. 8: 959-965.

Marsili, R.T. 1985. Monitoring chemical changes in Cheddar cheese during aging by high-performance liquid chromatography and gas chromatography technique. J. Dairy Sci. 68: 3155-3161.

Marsili, R. T., H. Ostapenko, R. E. Simmons, and D. E. Green. 1981. High performance liquid chromatographic determination of organic acids in dairy products. J. Food Sci. 46: 52-57.

McDowall, F. H., and A. K. R. McDowell. 1939. The white particles in mature Cheddar cheese. J. Dairy Res. 10: 118 – 119.

McGugan, W. A., D. B. Emmons. and E. Larmond. 1979. Influence of volatile and nonvolatile fractions on intensity of Cheddar cheese flavor. J. Dairy Sci. 62: 398-403.

McKay, L. L., R. A. Walter, W. E. Sandine, and P. R. Elliker. 1969. Involvement of phosphoenolpyruvate in lactose utilization by group N streptococci. J. Bacteriol. 99: 603-610.

McMahon, D. J. and B. S. Oommen. 2008. Supramolecular structure of the casein micelle. *J. Dairy Sci.* 91(5): 1709-1721.

McSweeney, P. L. H. 2004a. Biochemistry of cheese ripening. Int. J. Dairy Tech. 57: 127-144.

McSweeney, P. L. H. 2004b. Biochemistry of cheese ripening: introduction and overview, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 347-360.

McSweeney, P. L. H. and P. F. Fox. 2004. Metabolism of residual lactose and of lactate and citrate, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp.361-371.

Mehltretter, C .L., B. H. Alexander, and C. E. Rist. 1953. Sequestration by sugar acids. Ind. Eng. Chem. 45: 2782-2784.

Metzger, L. E. and D. A. Grindstaff. 2005. Cheese with sodium gluconate to inhibit calcium lactate crystal formation. U.S. Patent Application Publication No. US 2005/ 0281915 A1

McSweeney, P. L. H. 2007. Conversion of milk to curd, In Cheese problem solved. (ed. McSweeney, P.L.H.), CRC Press LLC., Boca Raton, FL, pp. 50-71.

Morris, H. A., C. Holt, B. E. Brooker, J. M. Banks, and W. Manson. 1988. Inorganic constituents of cheese: analysis of juice from a one-month-old Cheddar cheese and the use of light and electron microscopy to characterize the crystalline phases. J. Dairy Res. 55: 255-268.

Mullin, W. J. and D. B. Emmons. 1997. Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromatography. Food Res. Int. 30: 147-151.

Nagy, L. and A. Szorcsik. 2002. Equilibrium and structural studies on metal complexes of carbohydrates and their derivatives. J. Inorg. Biochem. 89: 1-12.

Olsen, N. F. 1983. Minimizing salt crystal formation. Dairy Field. 54 – 55.

Olsen, N. F. and M. E. Johnson. 1989. Controlling calcium lactate crystallization. WMMB Res. Rev. 1: 1 – 4.

Ottman, K. and L. E. Metzger. 2002. Soluble protein procedure for cheese. Testing Cheese Quality Workshop, May 23 – 24, 2002, North Central Cheese Industries Association, St Paul, MN.

Panari, G. 1986. HPLC of organic acids: an approach to cheese analysis. Milchwissenchaft. 41: 214-216.

Parente, E. and T. M. Cogan. 2004. Starter cultures: general aspects, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 123-147.

Pearce, K. N., L. K. Creamer, and J. Gilles. 1973. Calcium lactate deposits on rindless Cheddar cheese. N.Z.J. Dairy Sci. Tech. 8: 3-7.

Pecsok. R. L. and J. Sandera. 1955. The gluconate complexes. II. the ferric-gluconate system. J. Am. Chem. Soc. 77: 1489 – 1494.

Pecsok, R. L. and R. S. Juvet Jr. 1955. The gluconate complexes. I. copper gluconate complexes in strongly basic media. J. Am. Chem. Soc. 77: 202 – 206.

Pecsok. R. L. and R. S. Juvet Jr. 1956. The gluconate complexes. III. the lead gluconate system. J. Am. Chem. Soc. 78: 3967 – 3972.

Peterson, S. D. and R. T. Marshall. 1990. Nonstarter lactobacilli in Cheddar cheese: a review. J. Dairy Sci. 73: 1395-1410.

Pham, A. and S. Nakai. 1984. Application of stepwise discriminant analysis to high pressure liquid chromatography profiles of water extract for judging ripening of Cheddar cheese. J. Dairy Sci. 67: 1390-1396.

Prescott, F. J., J. K. Shaw, J. P. Bilello, and G.O. Cragwall. 1953. Gluconic acid and its derivatives. Ind. Eng. Chem. 45: 338-342.

Rajbhandari, P. and P. S. Kindstedt. 2005a. Compositional factors associated with calcium lactate crystallization in smoked Cheddar cheese. J. Dairy Sci. 88: 3737–3744.

Rajbhandari, P. and P. S. Kindstedt. 2005b. Development and application of image analysis to quantify calcium lactate crystallization in smoked Cheddar cheese. J. Dairy Sci. 88: 4157 – 4164.

Rajbhandari, P. and P. S. Kindstedt. 2008. Characterization of calcium lactate crystals on Cheddar cheese by image analysis. J. Dairy Sci. 91: 2190 – 2195.

Ramachandran, S., P. Fontanille, A. Pandey, and C. Larroche. 2006. Gluconic acid: properties, applications and microbial production. Food Technol. Biotechnol. 44: 185-195.

Rehman, S. U., M. A. Drake, and N. Y. Farkye. 2008. Differences between Cheddar cheese manufactured by milled-curd and stirred-curd methods using different commercial starters. J. Dairy Sci. 91: 76-84

Rengpipat, S. and E. A. Johnson. 1989. Characterization of *Lactobacillus* strain producting white crystals on Cheddar cheese. Appl. Environ. Microbiol. 55: 2579 – 2582.

Rollema, H. S. 1992. Casein association and micelle formation, In Advanced Dairy Chemistry, Vol. 1: Proteins (ed. P.F. Fox), Elsevier Science Publisher, Ltd., Essex, pp. 111-140.

Sakata, Y., S. Shiraishi, M. Otsuka. 2005. Characterization of dehydration and hydration behavior of calcium lactate pentahydrate and its anhydrate. Colloids Surf., B. 46: 135 – 141.

Saladini, M., L. Menabue, and E. Ferrari. 2000. Sugar complexes with metal^{2+} ions: thermodynamic parameters of associations of Ca^{2+}, Mg^{2+} and Zn^{2+} with galactaric acid. Carbohydr. Res. 336: 55-61.

Sawyer, D. T. 1964. Metal-gluconate complexes. Chem. Rev. 64: 633 – 643.

Scott, R. 1986. Cheesemaking practice. Elsevier Applied Science Publishers, LTD., New York, NY.

Serrano, J., G. Velazquez, K. Lopetcharat, J. A. Ramı´rez, and J. A. Torres. 2004. Effect of moderate pressure treatments on microstructure, texture, and sensory properties of stirred-curd Cheddar shreds. J. Dairy Sci. 87: 3172-3182.

Severn, D. J., M. E. Johnson, and N. F. Olson. 1986. Determination of lactic acid in Cheddar cheese and calcium lactate crystals. J. Dairy Sci. 69: 2027 – 2030.

Silverman, G. J. and F. V. Kosikowski. 1955. Tyrosine in Cheddar cheese. J. Dairy Sci. 38: 950 – 958.

Singh, O. V. and R. Kumar. 2007. Biotechnological production of gluconic acid: future implications. Appl. Microbiol. Biotechnol. 75: 713-722.

Sodium gluconate handbook. 1994. PMP Fermentation Products, Inc., Chicago, IL.

Somers, E. B., M. E. Johnson, and A. C. L. Wong. 2001. Biofilm formation and contamination of cheese by nonstarter lactic acid bacteria in the dairy environment. J. Dairy Sci. 84:1926–1936.

Sousa, M. J., Y. Ardo, and P. L. H. McSweeney. 2001. Advances in the study of proteolysis during cheese ripening. Int. Dairy J. 11: 327-345.

Sutherland, B. J. and G. W. Jameson. 1981. Composition of hard cheese manufactured by ultrafiltration. Aust. J. Dairy Technol. 36:136–142.

Swaisgood, H. E. 1992. Chemistry of caseins, In Advanced Dairy Chemistry, Vol. 1: Proteins (ed. P.F. Fox), Elsevier Science Publisher, Ltd., Essex, pp. 63-110.

Swearingen, P. A., D. E. Adams, and T. L. Lensmire. 2004. Factors affecting calcium lactate and liquid expulsion defects in Cheddar cheese. J. Dairy Sci. 87:574–582.

Swearingen, P. A., D. J. O'Sullivan, and J. J. Warthesen. 2001. Isolation, characterization, and influence of native nonstarter lactic acid bacteria on Cheddar cheese quality. J. Dairy Sci. 84: 50-59.

Thomas, T. D. and V. L. Crow. 1983. Mechanism of D(−)-lactic acid formation in Cheddar cheese. N.Z. J. Dairy. Sci. Technol. 18:131–141.

Thomas, T. D., D. C. Ellwood, and V. M. C. Longyear. 1979. Change from homo- to heterolactic fermentation by Streptococcus lactis resulting from glucose limitation in anaerobic chemostat cultures. J. Bacteriol. 138: 109-117.

Tuckey, S. L., H. A. Ruehe, and G. L. Clark. 1938. X-ray diffraction analysis of white specks in Cheddar cheese. J. Dairy Sci. 21: A161 (Abstr)

Upadhay, V. K., P. L. H. McSweeney, A. A. A. Magboul, and P. F. Fox. 2004. Proteolysis in cheese during ripening, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 391-433.

Upreti, P. and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: manufacture and composition. J. Dairy Sci. 89: 420 – 428.

Upreti, P., L. L. McKay, and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. J. Dairy Sci. 89:429–443.

USDA. 1956. United States Standards for Grades of Cheddar cheese. Dairy Division. Agricultural Marketing Service. United States Department of Agriculture

USDA. 2008. *Dairy Products 2007 Summary*. National Agricultural Statistics Service. United States Department of Agriculture

U.S. FDA. 2006. FDA list of food additives that are 'Generally Recognized As Safe' (GRAS). From Internet website: http://www.cfsan.fda.gov

Walstra, P. 1999. Casein sub-micelles: do they exist? *Int. Dairy J*., 9: 189-192.

Walstra, P., T. J. Geurts, A. Noomen, A. Jellema, and M. A. J. S. van Boekel. 1999. Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc., New York.

Washam, C. J., T. J. Kerr, and V. J. Hurst. 1982. Microstructure of various chemical compounds crystallized on Cheddar cheese. J. Food Prot. 45:594–596.

Washam, C. J., T. J. Kerr, V. J. Hurst, and W. E. Rigsby. 1985. A scanning electron microscopy study of crystalline structures on commercial cheese. Dev. Ind. Microbiol. 26: 749 – 761.

Whitney, R. M. 1988. Proteins of milk, In Fundamental of Dairy Chemistry, 3rd edn. (ed. N.P. Wong), Van Nostrand Reinhold, New York, pp. 81-169.

Wieczorek, M. W., J. Blaszczyk, and B. W. Karol. 1996. Effects of cation interactions on sugar anion conformation in complexes of lacobionate and gluconate with calcium, sodium, or potassium. Acta. Cryst. C52: 1193-1198.

World Health Organization. 1999. WHO Food Additives Series: 42, Safety Evaluation of certain cood cdditives: glucono delta lactone and the calcium, magnesium, potassium, and sodium salts of gluconic acid. Geneva.

Zeppa, G., L. Conterno, and V. Gerbi. 2001. Determination of organic acids, sugars, diacetyl and acetoin in cheese by high-performance liquid chromatography. J. Agric. Food Chem. 49: 2722-2726.

Chapter 2. Effect of sodium gluconate on the solubility of calcium lactate

Calcium and lactate are present in excess of their solubility in Cheddar cheese. Consequently, calcium lactate crystals are a common defect in Cheddar cheese. A novel approach for preventing calcium lactate crystals is the addition of sodium gluconate. Sodium gluconate has the potential to increase the solubility of calcium and lactate by forming soluble complexes with calcium and lactate ions, and preventing them from being available for the formation of calcium lactate crystals. The objective of this study was to determine if sodium gluconate could increase the solubility of calcium lactate. Seven calcium lactate solutions (5.31% w/w) with seven levels of sodium gluconate (0, 0.5, 1, 1.5, 2, 3, and 4% w/w) were made in triplicate. Solutions were stored at 7°C for 21 days, and were visually inspected for calcium lactate crystal formation. Subsequently, they were filtered to remove calcium lactate crystals and the supernatant was analyzed for lactic acid and gluconic acid by HPLC and for calcium by Atomic Absorption Spectroscopy. The visual inspection demonstrated that calcium lactate crystals were formed in the solution with 0% gluconate after the first day of storage and calcium lactate crystals continued to accumulate over time. A minute amount of calcium lactate crystals was also visible in the solution with 0.5% gluconate after 21 days of storage, while calcium lactate crystals were not visible in the other solutions. The HPLC results indicated that there was a higher concentration of calcium and lactic acid in the filtrate from the solutions containing added gluconate. Thus, sodium gluconate can increase the solubility of calcium lactate.

INTRODUCTION

Calcium lactate crystals (CLC) can appear within and on the surface of hard-type cheese especially Cheddar cheese after approximately six months of aging. CLC have been reported in Cheddar cheese as early as 1938 and are primarily composed of calcium lactate pentahydrate ($Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$), where five molecules of water are incorporated into the crystal lattice (Tuckey et al., 1938; McDowall and McDowell, 1939; Dorn and Dahlberg, 1942; Shock et al., 1948; Farrer and Hollberg, 1960; Severn et al., 1986). A potential pathway for the formation of calcium lactate crystals was proposed by Dybing et al. (1998). The solubility of anhydrous Ca-L-lactate is 3.38 g /100 g of water at 4°C (Kubantseva et al., 2004), which provides 0.62 g of calcium and 2.76g of lactate per 100 g of water. Thus, it is believed that calcium lactate crystals only form when greater than 0.62 g of calcium and 2.76 g of lactate per 100 g of water are present in cheese. However, the typical concentration of soluble calcium and lactate in the serum phase of aged Cheddar cheese is 1.06 g/ 100 g and 3.73 g /100 g, respectively (Morris et al., 1988). Consequently, the concentration of soluble calcium and lactate are in excess of their solubility at 4°C in a typical Cheddar cheese. Since calcium and lactate ions are in a supersaturate state in the cheese serum phase, crystallization at nucleation sites occurs. A continuous movement of calcium and lactate ions to the nucleation sites then causes the sites to become macro crystals (Dybing et al., 1988; Kubantseva and Hartel, 2002; Swearingen et al., 2004).

In an aqueous solution, sugars can form complexes with metal cations by displacing water molecules in the solvation sphere of cations with their –OH groups. At

least three –OH groups are required for complex formation in this system. The complex-forming ability of sugars is considerably enhanced when an anchoring group such as –COOH is introduced into the sugar molecules and they become sugar acids (Gyurcsik and Nagy, 2000; Nagy and Szorcsik, 2002). Because of an increasing interest in utilization of organic sequestering agents, sugar acids, which are compounds that have promising ability for this purpose, have become the subject of interest for more than 50 years (Mehltretter et al., 1953). Gluconic acid, which is a sugar acid, and its salts have been used as sequestering agent for metal ions in industrial, medical and agricultural applications (Escandar and Sala, 1992). Sodium gluconate is a sodium salt of gluconic acid, and it is able to chelate and form stable complexes with metal ions, which include Ca (II), Cu (II), Fe (II), Fe (III), Co (II), Ni (II), Cd (II), Hg (II), Pb (II), Al (III), Ga (III), and In (III) (Escandar and Sala, 1992; Gyurcsik and Nagy, 2000). Gluconic acid can be produced by chemical and biological processes. The chemical processes include the chemical oxidation of glucose in alkaline media and electrolytic oxidation of an alkaline solution of glucose. The biological processes include the fermentation of glucose solution by fungi and bacteria. The chemical method is a simple one-step process, but because of the low selectivity, this method is not suitable for producing gluconic acid on an industrial scale. Thus, the fermentation process utilizing fungi Aspergillus niger is the dominant technique for producing gluconic acid in the industry (Prescott et al., 1953; Ramachandran et al., 2006). Recent developments in gluconic acid production include finding an alternative and cheaper carbohydrate source such as agro-food by products, which include sugarcane molasses, beet molasses, grape must and banana must, to meet the higher demand for gluconic acid (Singh and Kumar, 2007).

Abbadi et al. (1999) studied the Ca-sequestering capability of polyhydroxycarboxylic acids (aldonic and aldaric acids). Sodium gluconate and sodium galactonate were used as representatives of aldonic acids, while disodium glucarate and disodium galactarate were used as representatives of aldaric acids. Their results indicated that all aldonates and aldarates were able to sequester calcium ions via their hydroxycarboxylate groups. Gluconate has also been used in other areas because of its chelating ability. For examples, it has been used to prevent the milkstone formation in the milk tank (Prescott et al., 1953), inhibit corrosion of carbon steel in chloride solutions (Lahodny-Šarc et al., 2000), remove heavy metals from soil components and soils (Fischer and Bipp, 2002), decrease paint stripping time by solubilizing the surface pigment adhering to metal after stripping, and eliminating rust spots on bottle necks thus increasing efficiency in bottle washing. Gluconic acid and gluconate have also been used in the food industry as food additives and ingredients and have generally recognized as safe status (FDA, 2010). It was recently reported that the solubility of calcium lactate with a combination of gluconate was 33.4% higher than the solubility of calcium lactate alone (Jungbunzlauer, 2002). This previous research indicates that a potential approach for preventing CLC formation in Cheddar cheese is to add sodium gluconate during the manufacture process. Sodium gluconate addition could increase the solubility of calcium and/or lactate in the serum phase by forming complexes with one or both of the calcium and lactate ions, and removing one or both of the calcium and lactate ions from being available for the formation of CLC. The objective of this study was to determine if sodium gluconate could increase the solubility of calcium and/ or lactate in model solutions.

MATERIALS AND METHODS

Solubility Model

Three replications of seven calcium lactate (CaL_2) solutions were prepared by dissolving 7.5% (w/w) calcium L-lactate pentahydrate powder ($Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$, USP grade, Fisher Chemicals, Fair Lawn, NJ), 0.3% (w/w) potassium sorbate (99%, Alfa Aesar®, Ward Hill, MA), and one of seven levels of sodium gluconate powder (NaC6H11O7, PMP Fermentation Products, Inc., Chicago, IL) – 0 (control), 0.5, 1, 1.5, 2, 3, and 4% (w/w) in distilled water to get a total weight of 100 g. The 7.5% calcium lactate pentahydrate powder provided 5.31% (w/w) calcium lactate in each solution and the potassium sorbate was utilized for mold inhibition. A piece of 1.5 × 3 cm sand paper (Wetordry™ Sandpaper 9085NA, Super Fine 400 grit, 3M™, St. Paul, MN) was also placed in each solution to provide a nucleation site for crystal development.

The solutions were stored for 21 days at 7°C, which is a typical aging temperature for Cheddar cheese. The storage period of 21 days was selected because previous research has demonstrated that the true equilibrium of CaL_2 solution was reached in 21 days (Kubantseva et al., 2004). Each solution was visually inspected for CLC formation throughout the storage time. After 21 days, the solutions were filtered (Whatman #4; Whatman International Ltd., Maidstone, England) at 7°C to remove CLC. The supernatant was analyzed for lactic acid, gluconic acid, and calcium content. The pH of each solution was measured before and after storage, and the pH values for all solutions

were similar and ranged from 6.87 to 6.91. Previous research has reported that this range in pH does not impact the solubility of CaL_2 (Kubantseva and Hartel, 2002).

Analysis of Lactic and Gluconic Acids

Lactic and gluconic acids were determined in the filtered supernatant using an HPLC procedure adapted from Upreti et al. (2006). For the sample preparation, 2 mL of the CaL_2 supernatant was mixed with 10 mL of 0.013 N sulfuric acid. The mixture was filtered through 0.22 μm Filter Unit (Millex® -GV Syringe Driven Filter Unit (non-sterile), Millipore Corporation, Bedford, MA). Approximately 0.5 to 1 ml of the collected filtrate was directly injected into the HPLC system using a 1 cc syringe (Tuberculin Syringes without needle, Monoject®, Sherwood Medical, St. Louis, MO).

The HPLC system (AKTA^{TM} design, Phamacia Biotech AB, Uppsala, Sweden) was composed of a solvent delivery unit (Pump P-900) and sample injection loop, a variable wavelength UV/Visible detector (UV-900), and data processing software (Unicorn version 4.0, Amersham Pharmacia Biotech AB, Uppsala, Sweden). The system was externally equipped with a refractive index detector (Beckman 156 Refractive Index detector, Beckman, Berkeley, CA), and a column heater (Hot Pocket^{TM}, Thermo Hypersil-Keystone, Bellefonte, PA). The column used for separation of the analytes was a cation exchange column with the dimension 300 × 7.8 mm i.d. (Aminex HPX – 87H) held at 65°C, with a cation H^+ microguard cartridge (Bio-Rad Laboratories, Hercules, CA). Analysis was performed at 0.8 mL/ min flow rate using 0.013 N sulfuric acid (Fisher Scientific, Fair Lawn, NJ) as the mobile phase. Lactic acid was detected using the

UV detector set at 210 nm, and the refractive index detector was used for quantification of gluconic acid.

Quantification of analytes was based on the external standard method. L-(+)-Lactic acid solution (30% in water by weight, Sigma-Aldrich, Inc., St. Louis, MO), and sodium gluconate powder (PMP Fermentation Products, Inc., Chicago, IL) were used to make stock solutions of lactic acid, and gluconic acid, respectively. The stock solutions were sufficiently diluted with 0.013 N sulfuric acid to obtain the desired concentration range of analytes to establish standard curves. The analytes were then quantified by the best-fit standard curves prepared by linear regression analysis of peak area vs. different concentrations of the analytes.

Analysis of Calcium Content

Calcium was determined by an atomic absorption spectroscopy procedure adapted from Metzger et al. (2000). For sample preparation, 1 mL of supernatant was diluted with 19 mL distilled water. Then 1 mL of the dilution was mixed with 29 g of 12% (w/v) trichloroacetic acid (Fisher Scientific, Fair Lawn, NJ). After 30 min, the mixture was filtered through filter paper (Whatman 541; Whatman International Ltd., Maidstone, England). Subsequently, 10 g of filtrate was added to 9.6 g of distilled water and 0.4 g of 5% lanthanum oxide (Sigma Chemical Co., St. Louis, MO). The final concentration of trichloroacetic acid and lanthanum oxide in each sample was 6% (w/v) and 0.02% (w/v), respectively. Calibration standards were prepared using a calcium reference solution (SC191-500, Fisher Scientific, Fair Lawn, NJ). The calcium reference solution was mixed with trichloroacetic acid, lanthanum oxide, and distilled water to obtain calibration

standards containing 0, 4, 6, 8, 10, 12, and 16 mg/kg of calcium. All standards also contained 6% (w/v) trichloroacetic acid and 0.02% (w/v) lanthanum oxide. Each calibration standard and sample was aspirated into an AAS (Model 2380; Perkin Elmer Corp., Norwalk, CT), which was equipped with a calcium lamp (0303-6017 Perkin Elmer Corp., Norwalk, CT) for calcium determination. A standard curve was prepared from the calibration standards and the calcium content of the samples was quantified from linear regression analysis of absorbance vs concentration of the standards.

Statistical Analyses

A 7 × 2 factorial model with 3 replications was used for statistical analysis. The PROC GLM procedure of SAS, which involved 2 factors (treatment – 7 levels of sodium gluconate, and storage – before and after storage) as class variables, was utilized to compare lactic acid, gluconic acid, and calcium content. When significant differences occurred ($P \leq 0.05$), the treatment means were compared by Tukey HSD Test (SAS Institute, 1990).

RESULTS AND DISCUSSION

Visual Inspection

A visual inspection of the solutions demonstrated that CLC were formed in the solution with 0% gluconate after the first of storage day and CLC continued to accumulate over time. A minute amount of CLC was also visible in the solution with 0.5% gluconate after 21 days of storage, while CLC were not visible in the other solutions at any time. The seven solutions after 21 days of storage are shown in Figure 1.

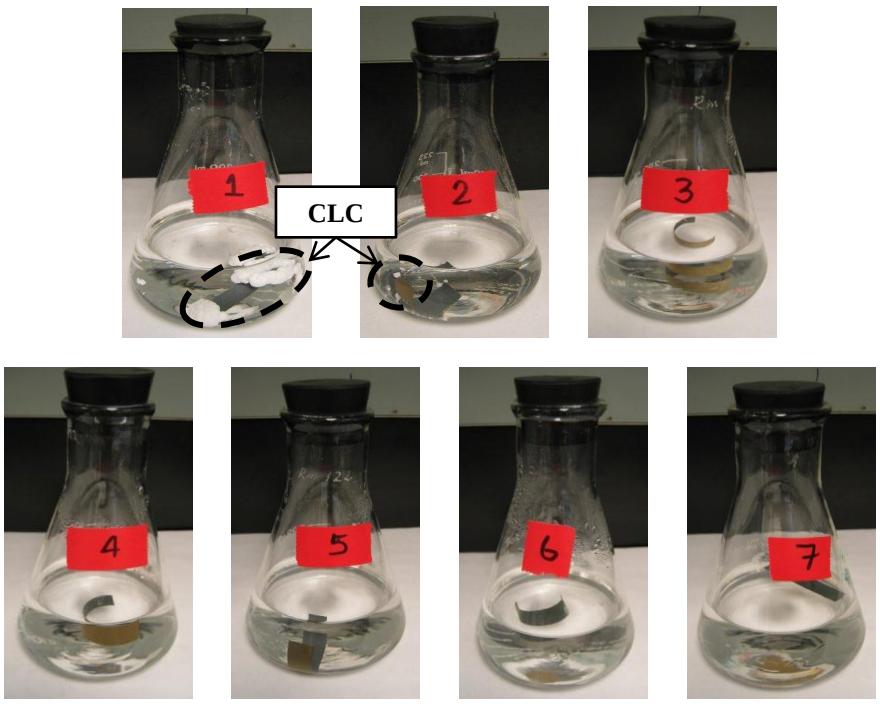

Figure 1. CaL_2 solutions after 21 days of storage. The solutions contained different amount of sodium gluconate; 1) 0% (control), 2) 0.5%, 3) 1%, 4) 1.5%, 5) 2%, 6) 3%, and 7) 4%.

Analyses of Lactic Acid, Gluconic Acid, and Calcium

Table 1 shows mean squares and probabilities of % Ca, % lactic acid, and % gluconic acid. From Table 1, treatment, storage, and the interaction of term of treatment and storage had significant effects ($p<0.05$) on % Ca, % lactic acid, and % gluconic acid, while replications had significant effect ($p<0.05$) on % Ca. Table 2 shows the mean Ca, lactic acid, and gluconic acid contents of each treatment at 0 day of storage. As previously described that CaL_2 solutions were prepared by dissolving 7.5% (w/w) calcium $_L$-lactate pentahydrate powder ($Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$). This concentration

of calcium lactate pentahydrate powder provided 5.31% (w/w) calcium lactate in each solution. Calcium lactate ($Ca(CH_3CHOHCOO)_2$) contains approximately 18.33% Ca and 81.67% lactate, which corresponds to 0.97% Ca and 4.34% lactate. At 0 day of storage, the values ranged from 1.026 to 1.033% for Ca and from 4.39 to 4.45% for lactate. There were no differences ($P > 0.05$) in the Ca or lactate concentration among the treatments. Sodium gluconate (NaC6H11O7) contains approximately 89% gluconic acid. As expected, the concentrations of gluconic acid measured by HPLC were relatively close to 89% of the amount of sodium gluconate added to each solution (i.e. $0.5 \times 89\% = 0.45$). Since the actual concentration of Ca, lactic acid, and gluconic acid at day 0 were close to the targeted levels for each treatment, this indicated that all solutions were properly prepared.

Table 3 shows the mean Ca, lactic acid, and gluconic acid values of each treatment at 21 days of storage. The Ca and lactic acid contents of the control solution (treatment 1) were lower ($P < 0.05$) then the other treatments which contained sodium gluconate. When comparing the solutions before and after storage (Table 2 vs. Table 3), both Ca and lactic acid in the control solutions after 21 days of storage were significantly lower ($P < 0.05$) than at day 0 (1.028 vs. 0.836 and 4.42 vs. 3.59 for Ca and lactic acid, respectively). There were no differences ($P > 0.05$) difference in Ca and lactate contents in treatments 2, 3, 4, 5, 6, and 7 at 0 and 21 days of storage. The decrease of Ca and lactic acid in the control solution after 21 days of storage is in accordance with results with visual inspection, where CLC were visible in the control solutions after 21 days of storage. The minute amount of CLC in treatment 2 did not ($P < 0.05$) influence the concentration of Ca and lactate in the filtrate. These results indicate that sodium

gluconate addition to calcium lactate solutions increase the solubility of calcium lactate. It is possible that the increase in the solubility of calcium lactate is a result of complex formation with gluconate.

Table 1. Mean squares and probabilities (in parentheses) for % Ca, % lactic acid, and % gluconic acid in CaL_2 solutions at 0 day and after 21 days of storage.

Factors	df	% Calcium	% Lactic acid	% Gluconic acid
Treatment†	6	0.791×10^{-2}* (<0.01)	0.1365* (<0.01)	10.44* (<0.01)
Replications	2	0.545×10^{-3}* (0.006)	0.0037 (0.1845)	0.710×10^{-3} (0.6618)
Storage††	1	0.847×10^{-2}* (<0.01)	0.2417* (<0.01)	0.0387* (<0.01)
Treatment × Storage	6	0.768×10^{-2}* (<0.01)	0.1339* (<0.01)	0.439×10^{-2}* (0.0417)
Error	26	0.870×10^{-4}	0.20×10^{-2}	0.169×10^{-2}

*Statistically significant at p-value < 0.05.

†Solutions contained different amount of sodium gluconate; 1) 0% (control), 2) 0.5%, 3) 1%, 4) 1.5%, 5) 2%, 6) 3%, and 7) 4%.

††Solutions were analyzed for lactic acid, gluconic acid, and calcium before (0 day) and after storage (21 days).

Table 2. Mean calcium, lactic acid, and gluconic acid in CaL_2 solutions at 0 day of storage.

Component	Treatments (% Sodium gluconate)						
	1 (0%)	**2 (0.5%)**	**3 (1%)**	**4 (1.5%)**	**5 (2%)**	**6 (3%)**	**7 (4%)**
Calcium (%)	1.028	1.033	1.027	1.026	1.027	1.029	1.031
Lactic acid (%)	4.42	4.42	4.43	4.44	4.39	4.45	4.39
Gluconic acid (%)	0.00^{g}	0.43^{f}	0.91^{e}	1.40^{d}	1.81^{c}	2.89^{b}	3.76^{a}

[a–g] Means within the solutions across the row without common superscripts differ (Tukey HSD at $P < 0.05$).

Table 3. Mean calcium, lactic acid, and gluconic acid in CaL_2 solutions after 21 days of storage.

Components	Treatments (% Sodium gluconate)						
	1 (0%)	**2 (0.5%)**	**3 (1%)**	**4 (1.5%)**	**5 (2%)**	**6 (3%)**	**7 (4%)**
Calcium (%)	0.836^{b}	1.028^{a}	1.031^{a}	1.026^{a}	1.026^{a}	1.024^{a}	1.026^{a}
Lactic acid (%)	3.59^{b}	4.42^{a}	4.41^{a}	4.40^{a}	4.36^{a}	4.35^{a}	4.34^{a}
Gluconic acid (%)	0.00^{g}	0.42^{f}	0.86^{e}	1.31^{d}	1.79^{c}	2.79^{b}	3.62^{a}

[a–g] Means within the solutions across the row without common superscripts differ (Tukey HSD at $P < 0.05$).

Sodium Gluconate as a Ca-sequestrant

The three most commonly observed modes of Ca-carboxylate interactions are 1). the unidentate mode where the Ca ion interacts with only one of the two oxygen atoms of the carboxylate group; 2). the α mode where the Ca ion is chelated by a carboxylic oxygen atom combined with a suitable ligand atom attached to the α position; and 3). the bidentate mode where the Ca ion is chelated by the pair of carboxylic oxygen atoms. If the suitable ligand is attached to the carboxylate groups, the α-mode chelation is found to be the most favorable Ca-chelating mode (Einspahr and Bugg, 1981). Sodium gluconate is a polyhydroxycarboxylic acid, and it has the ability to form water-soluble complexes with calcium ions, which could result in calcium-lactate-gluconate (CLG) complexes. Its chelating ability is suggested to be from the α-mode chelation with one of the carboxylic oxygen and the α-hydroxylic ligand. This α-mode chelation allows both functional groups to be in a position to bind a Ca ion in the formation of a stable five-membered chelate ring (Wieczorek et al., 1996; Saladini et al., 2001; Ramachandran et al., 2006).

Possible Formation of CLG Complexes

Our proposed mechanism for the formation of CLG complexes in the model solution is shown in Figure 2. In this mechanism, Ca ions are able to interact with oxygen from water molecules, oxygen from carboxyl groups of both gluconate and lactate, and oxygen from hydroxyl groups of gluconate. It was suggested by Cook and Bugg (1977) that the Ca-oxygen bond strength from Ca-water (ion-dipole bond) is weaker than that from Ca-carboxyl and Ca-hydroxyl (electro static bond). Thus, it is possible that once lactic acid and gluconic acid dissociate, their ions are competing to replace weaker Ca-

water bonds from hydrated Ca ions, resulting in CLG complexes. In order for crystals to form, a substance has to be in a position that will allow it to stack and layer in the most compact way possible (Jacques et al., 1981). It is possible that the bulky side chain of gluconic acid may provide steric hindrance that prevents calcium lactate crystal formation.

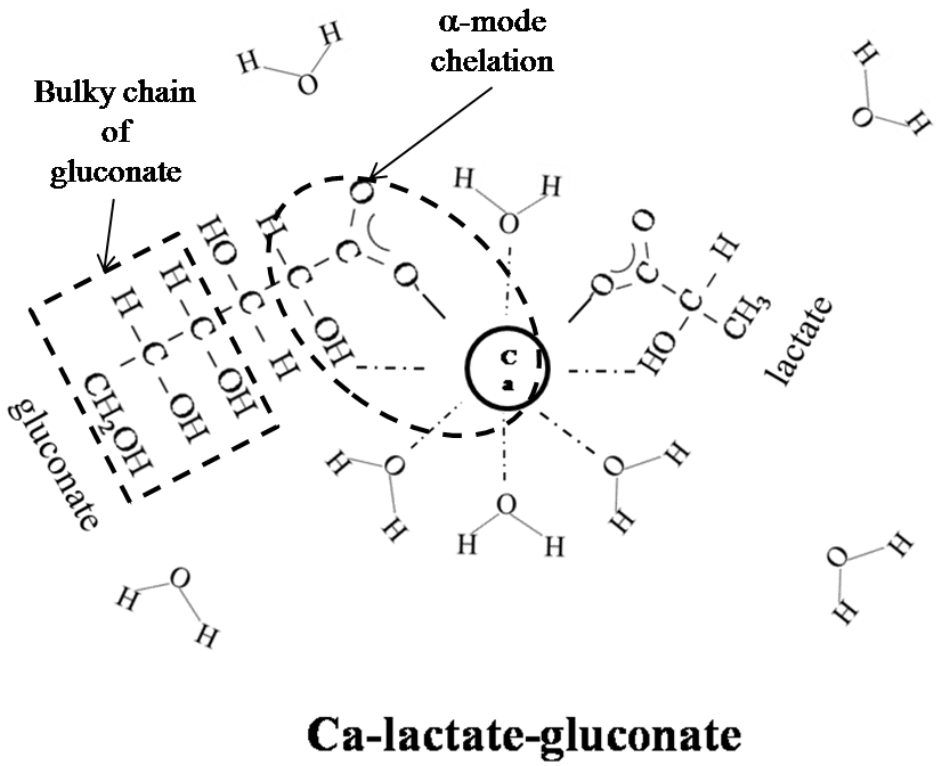

Figure 2. Proposed mechanism of Ca-lactate-gluconate formation with lactate and gluconate ions competing for weaker Ca-hydration bonds resulting in Ca-lactate-gluconate complexes.

CONCLUSIONS

It is apparent that sodium gluconate can significantly increase the solubility of calcium lactate. However, the solution in this study was simple a model containing only calcium and lactate ions, while the real Cheddar cheese serum is a complex system containing several other constituents such as sodium, chloride, phosphorus, potassium, magnesium, citrate, acetate, and sulfate ions.

REFERENCES

Abbadi, A., K. F. Gotlieb, J. B. M. Meiberg, J. A. Peters, and H. van Bekkum. 1999. New Ca-sequestering materials based on the oxidation of the hydrolysis products of lactose. Green Chemistry. October 1999: 231–235.

Cook, W. J., and C. E. Bugg. 1977. Structure of calcium-carbohydrate complexes, In Metal-ligand Interactions in Organic Chemistry and Biochemistry, Part 2, (eds. Pullman, B. and Goldblum, N.), D. Reidel Pub. Co., Dordrecht, Holland, pp. 231-256.

Dorn, F. L., and A. C. Dahlberg. 1942. Identification of the white particles found on ripened Cheddar cheeses. J. Dairy Sci. 25: 31-36.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701–1710.

Einspahr, H., and C. E. Bugg. 1981. The geometry of calcium-carbohydrate interactions in crystalline complexes. Acta Cryst. B37: 1044-1052.

Escandar, G. M., and L. F. Sala. 1992. Complexes of Cu(II) with D-aldonic and D-alduronic acids in aqueous solution. Can. J. Chem. 70: 2053-2057.

Farrer, K. T. H., and W. C. J. Hollberg. 1960. Calcium lactate on rindless cheese. Aust. J. Dairy Technol. 15:151–152.

FDA. 2010. 21 CFR, Part 182.6757. United States Food and Drug Adminsitration. Department of Health and Human Services, Washington, D.C.

Fischer, K., and H. -P. Bipp. 2002. Removal of heavy metals from soil components and soils by natural chelating agents. Part II. Soil extraction by sugar acids. Water, Air, and Soil Pollution. 138: 271 – 288.

Gyurcsik, B., and L. Nagy. 2000. Carbohydrates as ligands: coordination equilibria and structure of the metal complexes. Coord. Chem. Rev. 203: 81-149.

Jungbunzlauer (2002) Calcium lactate gluconate – the innovative solution for extra calcium. Innovations in Food Technology. 16: 1-3.

Kubantseva, N., and R. W. Hartel. 2002. Solubility of calcium lactate in aqueous solution. Food Rev. Intl. 18: 135–149.

Kubantseva, N., R. W. Hartel, and P. A. Swearingen. 2004. Factors affecting solubility of calcium lactate in aqueous solutions. J. Dairy Sci. 87: 863–867.

Lahodny-Šarc, O., F. Kapor, and R. Halle. 2000. Corrosion inhibition of carbon steel in chloride solutions by blends of calcium gluconate and sodium benzoate. Materials and Corrosion. 51: 147–151.

McDowall, F. H., and A. K. R. McDowell. 1939. The white particles in mature Cheddar cheese. J. Dairy Res. 10:118–119.

Mehltretter, C. L., B. H. Alexander, and C. E. Rist. 1953. Sequestration by sugar acids. Ind. Eng. Chem. 45: 2782-2784.

Metzger, L. E., D. M. Barbano, M. A. Rudan, and P. S. Kinstedt, 2000. Effect of milk preacidification on low fat Mozzarella cheese. I. Composition and yield. J. Dairy Sci. 84: 1357 – 1366.

Morris, H. A., C. Holt, B. E. Brooker, J. M. Banks, and W. Manson. 1988. Inorganic constituents of cheese: analysis of juice from a one-month-old Cheddar cheese and the use of light and electron microscopy to characterize the crystalline phases. J. Dairy Res. 55: 255-268.

Nagy, L., and A. Szorcsik. 2002. Equilibrium and structural studies on metal complexes of carbohydrates and their derivatives. J. Inorg. Biochem. 89: 1-12.

Prescott, F. J., J. K. Shaw, J. P. Bilello, and G.O. Cragwall. 1953. Gluconic acid and its derivatives. Ind. Eng. Chem. 45: 338-342.

Ramachandran, S., P. Fontanille, A. Pandey, and C. Larroche. 2006. Gluconic acid: properties, applications and microbial production. Food Technol. Biotechnol. 44: 185-195.

Saladini, M., L. Menabue, and E. Ferrari. 2001. Sugar complexes with metal^{2+} ions: thermodynamic parameters of associations of Ca^{2+}, Mg^{2+} and Zn^{2+} with galactaric acid. Carbohydr. Res. 336: 55-61.

SAS Institute. 1990. SAS User's Guide: Statistics, Version 6.0 ed. SAS lnst. Inc., Cary, NC.

Severn, D.J., M.E. Johnson, and N.F. Olson. 1986. Determination of lactic acid in Cheddar cheese and calcium lactate crystals. J Dairy Sci. 69:2027-2030.

Shock, A. A., W. J. Harper, A. M. Swanson, and H. H. Sommer. 1948. What's in those "white specks" on Cheddar? Wisc Agr. Exp. Sta. Bull. 474. Univ. Wisconsin, Madison.

Singh, O. V., and R. Kumar. 2007. Biotechnological production of gluconic acid: future implications. Appl. Microbiol. Biotechnol. 75: 713-722.

Sodium gluconate handbook. 1994. PMP Fermentation Products, Inc., Chicago, IL

Swearingen, P. A., D. E. Adams, and T. L. Lensmire. 2004. Factors affecting calcium lactate and liquid expulsion defects in Cheddar cheese. J. Dairy Sci. 87:574–582.

Tuckey, S. L., H. A. Ruehe, and G. L. Clark. 1938. X-ray diffraction analysis of white specks in Cheddar cheese. J. Dairy Sci. 21:A161. (Abstr)

Upreti, P., L. L. McKay, and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. J. Dairy Sci. 89:429–443

Wieczorek, M. W., J. Blaszczyk, and B. W. Karol. 1996. Effects of cation interactions on sugar anion conformation in complexes of lacobionate and gluconate with calcium, sodium, or potassium. Acta. Cryst. C52: 1193-1198.

Chapter 3. Cheddar cheese manufacture and composition

During the aging process of hard-type cheese especially Cheddar cheese, small white spots that are visible without magnification, can appear within and on the surface of cheese between two and six months of aging. The crystals have previously been identified as calcium lactate crystals (CLC). One potential approach for preventing CLC is to add sodium gluconate to the cheese during salting. Sodium gluconate increases the solubility of calcium and lactate in the cheese serum phase. The objective of this study was to determine the manufacture and composition of Cheddar cheeses with different levels of sodium gluconate addition. Six Cheddar cheeses with two levels of salting (2 and 2.5%) and three sodium gluconate addition levels (0, 0.5 and 1%) were manufactured. All cheeses were made using a stirred-curd procedure and replicated three times. Two levels were obtained by dividing cheese curd (at pH 5.6) into two equal-weight halves; each half was salted with 2 and 2.5% (by weight of cheese curd) sodium chloride. Subsequently, each of the salted halves was separated into three equal-weight batches and mixed with 0 (control), 0.5, and 1.0% sodium gluconate, respectively. After sodium gluconate addition, the curds were hooped, pressed for 16 hour, vacuum-sealed in polyethylene bags, and transferred to a ripening room at 6 to 8°C. After 1 week of storage, compositional analyses (pH, moisture, salt, fat and protein) and gluconic acid concentration were determined. Mean pH, moisture, salt, fat and protein content of the cheeses ranged from 5.06 to 5.32, 36.98 to 38.15%, 1.65 to 2.13%, 30.96 to 32.98%, and 25.6 to 26.1%, respectively. At both salting levels, the pH and moisture contents were

significantly ($p<0.05$) increased in the treatments with added sodium gluconate. The concentration of gluconic acid in the low salt treatments was 0.33 and 0.59% for the 0.5 and 1.0% addition level, respectively, whereas the concentration in the high salt levels was 0.33 and 0.58%, respectively.

INTRODUCTION

Calcium lactate crystals (CLC) are not harmful to consumers. They are, however, perceived as grittiness in the cheese, and more importantly, they are considered a quality defect because consumers often believe that they are mold or some other type of spoilage (Dybing et al., 1988; Chou et al., 2003). In addition, this can lower the reputation of cheese products, which will lead to financial loss for cheese manufacturers (Washam et al., 1982; Swearingen et al., 2004). Several factors have been shown to influence CLC including dead lactic acid bacteria cells acting as nucleation sites for the crystal formation in cheese (Kalab, 1980); high lactose levels in milk (Pearce et al., 1973); ultrafiltrated milk without diafiltration (Sutherland and Jameson, 1981); milk citrate levels and the subsequent utilization of citrate by microorganism (Morris et al., 1988); the conversion of L– lactate into a racemic mixture of L– and D– lactate caused by contamination of nonstarter lactic acid bacteria and biofilm formation (Thomas and Crow, 1983; Johnson et al., 1990a; Somers et al., 2001); loose packaging and low storage temperature (Johnson et al., 1990b); and certain starter culture stains and low salt-in-moisture levels in cheese (Swearingen et al., 2004). Recommendations for limiting CLC formation in cheese include: 1) reducing the concentration of lactic acid in the final curd, 2) reducing or eliminating undesirable non-starter lactic acid bacteria from the cheese-making process, 3) controlling storage temperature, and 4) vacuum packaging cheese to lessen the

airspace around the outer cheese surface (Dybing et al., 1988; Olsen and Johnson, 1989; Johnson et al, 1990a; Johnson et al., 1990b). Although all the above recommended methods are incorporated into the Cheddar cheese-making process, CLC can still occur.

One potential approach for preventing CLC is to add sodium gluconate to Cheddar cheese. Sodium gluconate can increase the solubility of calcium and lactate in the serum phase by forming complexes with one or both of the calcium and lactate ions, and removing the calcium and lactate ions from being available for the formation of CLC (Phadungaht and Metzger, 201x). Metzger and Grindstaff (US 2005/ 0281915 A1) studied the effect of sodium gluconate as a CLC inhibitor in Cheddar cheese. From their patent, Cheddar cheeses were manufactured with sodium gluconate addition in the range of 0 to 10%. The sodium gluconate addition was immediately after the salting step, and after the cheeses were pressed and hooped overnight, they were ripened under refrigeration. Cheeses were inspected for the CLC formation after two months of aging. It was reported that cheeses obtained from the control treatment (no sodium gluconate addition) had visible CLC on the cheese surface and cheese interior, while cheeses with sodium gluconate addition did not show any visible CLC formation present. Thus, this suggests that sodium gluconate is effective as CLC inhibitor. However, the effect of sodium gluconate on Cheddar cheese composition, glycolysis, proteolysis, textural properties and sensory properties of Cheddar cheese with added sodium gluconate during ripening has not yet been investigated.

According to U.S. FDA, sodium gluconate is a food additive that is ‘Generally Recognized As Safe’ (GRAS) under the ‘Sequestrants’ category. Sodium gluconate is

permitted for use as a food additive in Canada under pH-adjusting agents and stabilizers categories, and is also an approved food additive that can be used in European countries (Health Canada, 2006; U.S. FDA, 2006; Food Standards Agency, 2007). The study of sodium gluconate and calcium gluconate infusion in rates indicated that up to 85% of gluconic acid was readily removed by kidneys and excreted in urine (Stetten and Topper, 1953; Crawford, et al., 1959). The acute toxicity of sodium gluconate in rats showed no evidence of toxicity. The observed negative effects were an increased water intake, an increased prothrombin time, and increased relative kidney weights, but these effects were not dose-related. Qualitative urine analyses revealed effects related to the high sodium intake arising from sodium gluconate (World Health Organization, 1999). There were no evidence of gastric or renal irritation in humans treated with gluconic acid and its derivatives; thus, gluconic acid and its derivatives are non-toxic and well tolerated in humans (Prescott et al., 1953). In addition, sodium gluconate is readily biodegradable; thus, it will not cause waste-water problem when used in the food industry (Dweck, 2005). The objective of this study was to determine the manufacture and composition of Cheddar cheeses with different levels of sodium gluconate addition.

MATERIALS AND METHODS

The Selection of Sodium Gluconate Level for Cheddar Cheese Manufacture

From our previous study (Phadungath and Metzger, 201x), the effective concentration of sodium gluconate to inhibit calcium lactate crystals in calcium lactate model solutions was in the range of 0.5 – 1.0%. Therefore, the three levels of sodium gluconate, which were 0, 0.5, and 1%, were selected for the cheese making study.

Experimental Design

A replicated 2×3 factorial designs was utilized for a Cheddar cheese making study. This design utilized three levels of sodium gluconate (0, 0.5 and 1%) and two levels of salt (2 and 2.5%) for a total of six treatments. The treatments were; TRT1 - 0% sodium gluconate + 2% salt, TRT2 – 0.5% sodium gluconate + 2% salt, TRT3 – 1.0% sodium gluconate + 2% salt, TRT4 - 0% sodium gluconate + 2.5% salt, TRT5 – 0.5% sodium gluconate + 2.5% salt, TRT6 - 1% sodium gluconate + 2.5% salt. Each treatment was manufactured in triplicate from three different lots of milk.

Cheese Manufacture

Six Cheddar cheeses with two levels of salting (2 and 2.5%) and three sodium gluconate addition levels (0, 0.5 and 1%) were manufactured, as shown in Figure 1. For each replicate, the cheese milk was standardized to 14% total solids, 3.9% protein, and 3.79% fat by mixing 18.35 kg non-fat dry milk (Extra-grade low-heat, Associated Milk Products Inc., New Ulm, MN), 16.34 kg raw cream (South Dakota State University Dairy Plant, Brookings, SD), and 42.82 kg water with 603.29 kg raw whole milk (South Dakota State University Dairy Plant, Brookings, SD) to obtain 680.4 kg milk mixture. The milk mixture was pasteurized at 73°C for 16 s and then cooled to 30°C in a plate-heat exchanger (Tetra Pak, Vernon Hills, IL), and the pasteurized milk was transferred to 1270-kg cheese vats (Kusel Equipment Co., Watertown, WI). Color (AFC-WS-1X, Chr. Hansen, Inc., Milwaukee, WI) was added at a rate of 6.61 mL/100 Kg of cheese milk. The Cheddar cheese was prepared with starter culture in the form of a direct vat set, deep-frozen, concentrated lactic starter culture (DVS® 970, Chr. Hansen, Inc., Milwaukee, WI). Rennet (Chy-max®, Chr. Hansen, Inc., Milwaukee, WI) was added at a

rate of 9.9 mL/100 Kg of cheese milk. The coagulant was gently stirred into the milk, agitation was subsequently stopped, and the vats were allowed to set quiescently. The cut time was determined by subjective assessment of the firmness of the coagulum. The coagulum was cut with 0.63-cm knif and allowed to heal for 5 min before initiating the cooking procedure, which was heating the coagulum to 37°C for 30 min. On completion of cooking, a stirred-curd procedure for Cheddar cheese making was used. Two salting rates were obtained by dividing cheese curd (at pH 5.6) into two equal-weight halves; each half was salted with 2 and 2.5% (by weight of cheese curd) sodium chloride (Morton Salt, Chicago, IL). In order to obtain six treatments, each of the salted halves was separated into three equal-weight batches and mixed with 0 (control), 0.5, and 1.0% sodium gluconate ($NaC_6H_{11}O_7$, PMP Fermentation Products, Inc., Chicago, IL), respectively. After sodium gluconate addition, the curds were hooped in 9 kg cheese hoops; then pressed at 138 Kpa (AFVS-Spec., Kusel Equipment Co., Watertown, WI). The cheeses were removed from the press after 16 h, vacuum-sealed (Model 620A, Sipromac, St. Germain, Canada) in polyethylene bags (3 mil Nylon/PE pouches, Prime Source vacuum pouches, KOCH Supplies LLC, North Kansas City, MO), and transferred to a ripening room at 6 to 8°C.

.

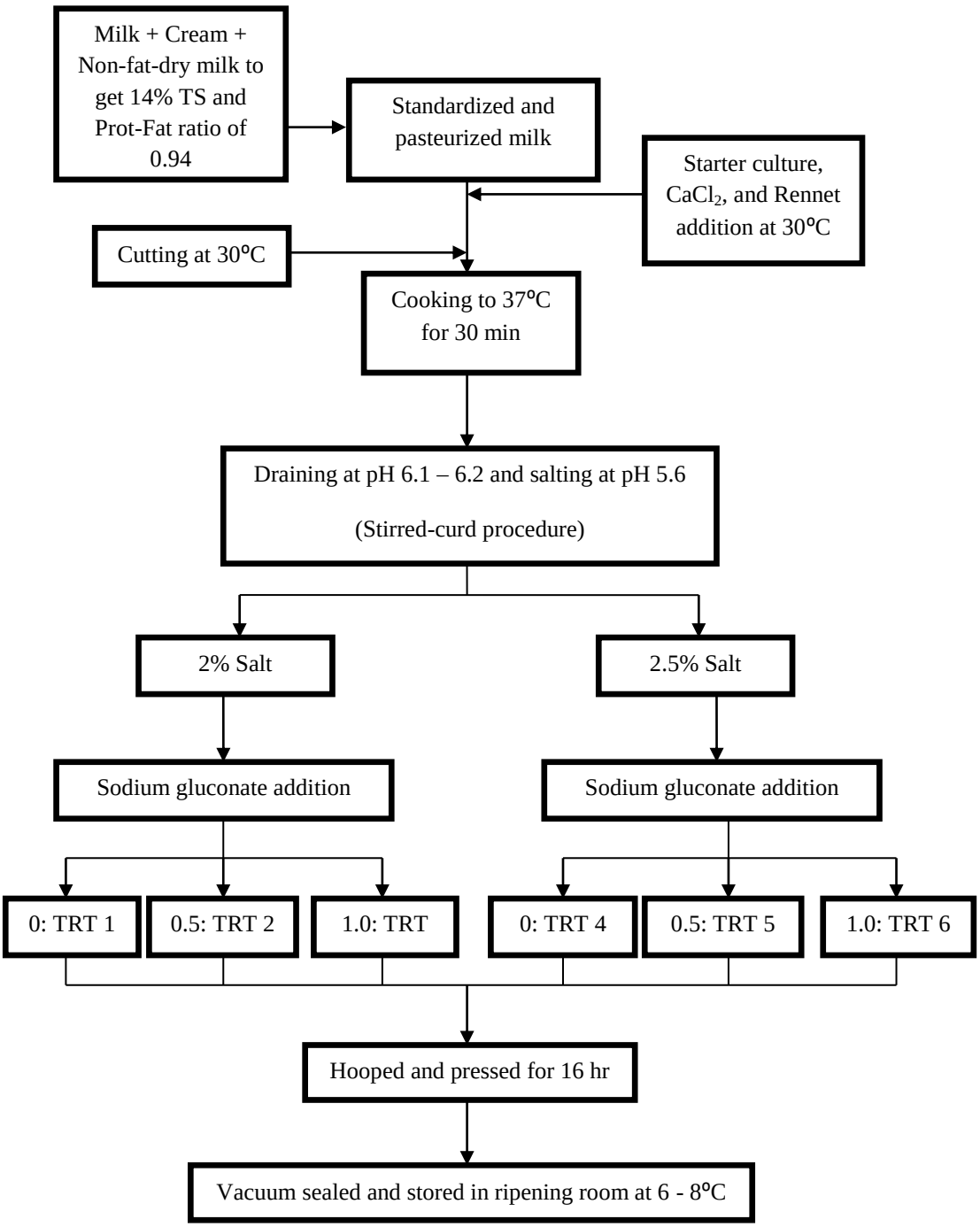

Figure 1. The manufacture diagram of Cheddar cheese with sodium gluconate addition.

Cheese Sampling

For each treatment, one 9.1 kg block of cheese was obtained. This cheese block was cut into three equal portions that were randomly designated for different analyses: one portion was assigned for compositional and chemical/microbiological analysis during ripening. Another portion was kept frozen for further study, and the third portion was used for monitoring textural changes, surface crystal formation, and sensory analysis during ripening. The first portion was subsequently cut into several portions and different pieces were randomly allocated to different time points. To prepare cheeses for chemical/microbiological analysis, samples were ground in a blender (Osterizer Galaxie, Sunbeam Products, Inc., Boca Raton, FL) to a particle size of 2 to 3 mm. The ground cheese particles were packed into 50-mL plastic snap-lid vials (leaving no head space) and stored at 4°C until analyzed.

Chemical and Compositional Analyses

The milk samples were analyzed for fat, protein and total solids. Milk fat was determined using the Mojonnier ether extraction method (Atherton and Newlander, 1977). Total protein in milk was determined by measuring total nitrogen in the milk using the Kjeldahl method (Kjeltec™ 2200 Auto Distillation Unit, FOSS, Eden Prarie, MN). The total solids in milk was analyzed gravimetrically, by drying 3 g of milk at 100°C in a forced draft oven (Lindberg/Blue M, Asheville, NC) for 4 h. Chemical and compositional analyses (pH, moisture, fat, protein, chloride, sodium, calcium, phosphorus, lactic acid and gluconic acid) of cheese samples were determined after 1 week of ripening. The pH was measured using a combination glass electrode (accumet® accuCap™ Combination

pH Electrodes, Fisher Scientific, Fair Lawn, NJ) and pH meter (Corning pH/ion meter 450, Corning, Inc., Corning, NY). The moisture content was analyzed gravimetrically, by drying 1.5 g of cheese at 100°C in a forced draft oven (Lindberg/Blue M, Asheville, NC) for 24 h. Chloride content was determined using a Corning Chloride Analyzer (Ciba Corning Diagnostics, Medfield, MA), based on the Volhard test (Marshall, 1992). Fat content was determined by using the Mojonnier ether extraction method. Total protein in cheeses was determined by measuring total nitrogen in the cheeses using the Kjeldahl method (Kjeltec™ 2200 Auto Distillation Unit, FOSS, Eden Prarie, MN). Total Ca in cheeses was measured using an Atomic Absorption Spectroscopy (Model 200; Perkin Elmer Corp., Norwalk, CT) procedure adapted from Brooks et al. (1970). Phosphorus and sodium content were determined using inductively coupled plasma - optical emission spectrophotometer method (AOAC, 1995). Lactose, gluconic acid and lactic acid were determined by using a HPLC-based method adapted from Upreti et al. (2006).

Statistical Analyses

A 2 × 3 factorial model with 3 replications was used for statistical analysis. The PROC GLM procedure of SAS, which involved 3 factors (salt, gluconic acid, and replication) as class variables, and mean separation ($P < 0.05$) by Tukey HSD Test, were used for the data analyses (SAS Institute, 1990).

RESULTS AND DISCUSSION

Milk Composition

The mean total solids, fat, protein, and pH of milk used to manufacture the Cheddar cheese was 13.97%, 3.92%, 3.7%, and 6.6, respectively. Although, the concentrated milk utilization for cheese making provides milk with higher proportion of protein content and more consistent quality for the final cheese (Acharya and Mistry, 2004), the concentration of lactose in milk also increase, which will provide more potential for crystal formation in Cheddar cheese during ripening.

Cheese Composition

The mean squares and *P*-values and means of chemical and compositional attributes of the Cheddar cheese samples from the six treatments are shown in Table 1 and Table 2, respectively. Salt had a significant effect ($P<0.05$) on pH, % Na, % Cl, % lactose and % lactic acid. Sodium gluconate level had a significant effect ($P<0.05$) on pH, % moisture, % fat, % Ca, % fat on a dry basis, % Ca on a dry basis, % lactose and % lactic acid. The replication effect was significant ($P<0.05$) for % moisture, % fat, % protein, % S/MC, % Ca, % fat on a dry basis, % protein on a dry basis, and %P on a dry basis. The interaction of salt and sodium gluconate addition had a significant effect ($P<0.05$) on % lactic acid, and all other interaction terms were not significant ($P<0.05$).

Table 1. Mean squares and probability values (in parentheses) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition at 1 week of ripening.

Sources of variation	df	Chemical and compositional attributes							
		pH (1 wk)	% Moisture	% Fat	% Protein	% Na	% Cl	% S/M	% Ca
Salt (S)	1	0.04* (0.02)	0.16 (0.21)	0.32 (0.23)	0.0002 (0.97)	0.02 (0.022)	0.17* (< 0.01)	3.50* (< 0.01)	8.89×10^{-7} (0.93)
Sodium gluconate level (G)	2	0.04* (0.01)	1.48* (< 0.01)	3.27* (< 0.01)	0.10 (0.65)	0.03* (0.047)	0.02 (0.07)	0.19 (0.20)	1.35×10^{-3}* (0.01)
Replication (R)	2	0.01 (0.10)	17.90* (< 0.01)	13.70* (< 0.01)	6.51* (< 0.01)	0.004 (0.47)	0.01 (0.14)	0.64* (0.04)	1.74×10^{-3}* (< 0.01)
S × G	2	0.0005 (0.83)	0.06 (0.50)	0.48 (0.16)	0.26 (0.38)	0.001 (0.71)	0.0005 (0.88)	0.01 (0.91)	2.37×10^{-5} (0.78)
S × R	2	0.003 (0.39)	0.28 (0.12)	0.08 (0.63)	0.55 (0.19)	0.001 (0.79)	0.008 (0.21)	0.25 (0.16)	4.61×10^{-5} (0.64)
G × R	4	0.001 (0.81)	0.11 (0.34)	0.66 (0.10)	0.07 (0.86)	0.001 (0.92)	0.002 (0.65)	0.05 (0.67)	2.32×10^{-4} (0.19)
Error	4	0.002	0.07	0.16	0.21	0.02	0.003	0.08	9.06×10^{-5}

*Statistically significant at *p*-value < 0.05.,

**DB or % dry basis = [attribute ÷ (100 − moisture)] × 100

Table 1 (continue). Mean squares and probability values (in parentheses) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition at 1 week of ripening.

Sources of variation	df	Chemical and compositional attributes						
		% P	% Fat (DB) **	% Protein (DB)	% Ca (DB)	% P (DB)	% Lactose (1 wk)	% Lactic acid (1 wk)
Salt (S)	1	8.89×10^{-5} (0.28)	1.56 (0.10)	0.05 (0.81)	0.03×10^{-3} (0.68)	1.07×10^{-4} (0.35)	0.12* (0.03)	0.39* (< 0.01)
Gluconic acid (G)	2	3.30×10^{-4} (0.07)	3.93* (0.02)	0.24 (0.74)	1.39×10^{-3}* (0.04)	1.83×10^{-4} (0.26)	0.14* (0.02)	0.29* (< 0.01)
Replication (R)	2	2.29×10^{-4} (0.11)	9.34* (< 0.01)	14.21* (< 0.01)	0.77×10^{-3} (0.09)	9.86×10^{-4}* (0.03)	0.07 (0.06)	0.01 (0.23)
S × G	2	2.61×10^{-5} (0.67)	0.94 (0.18)	0.81 (0.42)	0.03×10^{-3} (0.85)	2.69×10^{-5} (0.77)	3.2×10^{-4} (0.97)	0.06* (0.03)
S × R	2	2.02×10^{-5} (0.72)	0.42 (0.38)	2.24 (0.16)	0.12×10^{-3} (0.55)	8.05×10^{-5} (0.49)	7.3×10^{-4} (0.94)	0.02 (0.15)
G × R	4	3.09×10^{-5} (0.72)	1.76 (0.07)	0.27 (0.83)	0.61×10^{-3} (0.12)	1.37×10^{-4} (0.36)	1.4×10^{-2} (0.44)	0.002 (0.82)
Error	4	5.76×10^{-5}	0.34	0.76	0.16×10^{-3}	9.45×10^{-5}	1.1×10^{-2}	0.006

*Statistically significant at p-value < 0.05.,

**DB or % dry basis = [attribute ÷ (100 − moisture)] × 100

Na and Cl. The Na content in the cheeses was significantly affected ($P<0.05$) by the level of sodium gluconate addition (Table 1). From table 2, the Na content in cheeses at 2% salt level ranged from 0.61% in cheese without sodium gluconate addition to 0.77%in cheese with 1% sodium gluconate addition. The Na content in cheeses at 2.5% salt level ranged from 0.72% in cheese without sodium gluconate addition to 0.82% in cheese with 1% sodium gluconate addition. An increase in Na content is probably due to the contribution of Na from sodium gluconate addition. The Na content range (0.61% to 0.82%) of Cheddar cheeses in this study falls within the Na content range for commercial Cheddar cheese, which is about 0.48% to 0.85% (Kindstedt and Kosikowski, 1988).

The Cl content in cheeses was significantly affected ($P<0.05$) by the level of salt addition (Table 1). This is clearly due to the different salt content (2% and 2.5%) that was applied to the cheese curds. From table 2, the Cl content in cheeses at 2% salt level ranged from 0.99% to 1.12%, and the Cl content in cheeses at the 2.5% salt level ranged from 1.20% to 1.29%. The small difference in Cl content with each salting level could be due to an uneven salt application and uptake by the cheese curds during manufacture, and also due to the differences in moisture content among cheese treatments.

Ca and P. The Ca content in cheeses was significantly affected ($P<0.05$) by the level of sodium gluconate addition (Table 1). From table 2, the Ca content in cheeses with 2% salt level ranged from 0.73% in cheese without sodium gluconate addition to 0.69% in cheese with 1% sodium gluconate addition. The Ca content in cheeses with 2.5% salt level ranged from 0.73% in cheese without sodium gluconate addition to 0.70% in cheese with 1% sodium gluconate addition. However, Ca on a dry basis was not significant different ($P<0.05$) among treatments. Thus, a reduction of Ca content in

cheeses with sodium gluconate addition could be merely due to a dilution effect of higher moisture content and higher sodium gluconate content in these treatments. The Ca content range (0.69% to 0.73%) of Cheddar cheeses in this study falls within the Ca content range for commercial Cheddar cheese, which is about 0.61% to 0.83% (Kindstedt and Kosikowski, 1988).

Table 2. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition at 1 week of ripening.**

Mean value (%)*	Treatments					
	2% Salt			2.5% Salt		
	Sodium gluconate (%)			Sodium gluconate (%)		
	0	0.5	1.0	0	0.5	1.0
pH (1 wk)	5.06^{a}	5.16^{ab}	5.24^{ab}	5.16^{ab}	5.28^{b}	5.32^{b}
Moisture	36.98^{a}	37.79^{ab}	38.14^{b}	37.01^{a}	37.54^{ab}	37.79^{ab}
Fat	32.98^{a}	32.18^{ab}	30.96^{b}	32.26^{ab}	31.73^{ab}	31.33^{b}
Protein	25.72	26.1	25.6	26.08	25.65	25.72
Na	0.61^{a}	0.74^{ab}	0.77^{b}	0.72^{ab}	0.78^{b}	0.82^{b}
Cl	0.99^{a}	1.08^{ab}	1.12^{ab}	1.20^{ab}	1.29^{b}	1.29^{b}
S/M	4.47^{a}	4.73^{a}	4.85^{ab}	5.36^{b}	5.68^{b}	5.66^{b}
Total Ca	0.73^{a}	0.72^{ab}	0.69^{b}	0.73^{ab}	0.72^{ab}	0.70^{ab}
P	0.5	0.49	0.48	0.5	0.49	0.49
Fat (DB)**	52.33^{a}	51.74^{ab}	50.06^{b}	51.22^{ab}	50.80^{ab}	50.38^{ab}
Protein (DB)	40.82	41.96	41.4	41.41	41.06	41.35
Total Ca (DB)	1.16	1.15	1.13	1.16	1.15	1.13
P (DB)	0.79	0.78	0.78	0.79	0.79	0.79
Lactose (1 wk)	0.53^{a}	0.69^{ab}	0.85^{ab}	0.71^{ab}	0.85^{ab}	1.00^{b}
Lactic acid (1 wk)	1.56^{a}	1.35^{ab}	0.95^{c}	1.17^{b}	0.93^{c}	0.89^{c}
Gluconic acid	0	0.33	0.59	0	0.33	0.58

* ^{a,b,c} Means within the column not sharing common superscripts are different (Tukey HSD at $P < 0.05$).

** n = 3 replicates.

The P content and the P on a dry basis content were not affected by both salt and sodium gluconate addition (Table 1). From table 2, the P content in cheeses at both 2% and 2.5% salt level ranged from 0.48% to 0.50%, and this range falls within the P content range for commercial Cheddar cheese, which is about 0.44% to o.56% (Kindstedt and Kosikowski, 1987).

Fat and Protein. The fat content of the cheeses at 2% salt level ranged from 32.98% in cheese without sodium gluconate addition to 30.96% in cheese with 1% sodium gluconate addition. The fat content in cheeses at 2.5% salt level ranged from 32.26% in cheese without sodium gluconate addition to 31.33% in cheese with 1% sodium gluconate addition. A reduction of fat content in cheeses with sodium gluconate addition could be owing to a dilution effect of higher moisture content and higher sodium gluconate content in these treatments. The fat content on a dry basis of the cheeses in this study ranged from 50.06% to 52.33%, and this range falls within the recommended range for Cheddar cheese, which is about 50% to 57% (Lawrence et al., 2004).

The protein content and the protein on a dry basis content were not affected by both salt and sodium gluconate addition (Table 1). From table 2, the protein content of cheeses at 2% salt level ranged from 25.72% to 26.1%, and the protein content of cheeses at 2.5% salt level ranged from 25.65% to 26.08%.

Lactose and Lactic Acid at 1 Week. The lactose content in cheeses at the 2% salt level ranged from 0.53% in cheese without sodium gluconate addition to 0.85% in cheese with 1% sodium gluconate addition. The lactose content in cheeses at 2.5% salt level ranged from 0.71% in cheese without sodium gluconate addition to 1.00% in cheese with 1% sodium gluconate addition. The lactic acid content in cheeses at 2% salt level ranged

from 1.56% in cheese without sodium gluconate addition to 0.95% in cheese with 1% sodium gluconate addition. The lactic acid content in cheeses at 2.5% salt level ranged from 1.17% in cheese without sodium gluconate addition to 0.89% in cheese with 1% sodium gluconate addition.

An inverse relationship exists between the lactose and lactic acid contents. At both 2.0% and 2.5% salt level, the lactose content in cheeses with sodium gluconate addition were higher than the lactose content in cheeses without sodium gluconate addition, whereas, the lactic acid content in cheeses with sodium gluconate addition were lower than the lactic acid content in cheeses without sodium gluconate addition. As stated earlier, sodium gluconate contributed to a higher concentration of Na in cheeses, and this could possibly inhibit starter culture growth, which would cause less utilization of lactose in the cheeses. Thus, sodium gluconate addition could result in a reduction in the lactic acid production and a higher amount of residual lactose content in cheeses. Additionally, cheeses with 2.5% salt addition had higher lactose content than cheeses with 2.0% salt addition, and vice versa for lactic acid content. A higher level of lactose content in cheeses with 2.5% salt is probably due to inhibition of starter culture activity by the high salt level. Thus, less lactose is fermented and corespondingly lactic acid production is also reduced. This result is also in agreement with Upreti et al. (2006), where they reported a higher lactose content and a lower lactic acid content in Cheddar cheese with a high salt treatment as compared to cheese with a low salt treatment.

S/M and Moisture. The S/M in cheeses was significantly affected ($P<0.05$) by the level of salt addition (Table 1). This is due to the different salt content (2% and 2.5%) that was applied to the cheese curds, since the S/M in cheeses with 2%

salt level was significantly lower ($P<0.05$) than the S/M in cheeses with 2.5% salt level. From table 2, The S/M in cheeses at 2% salt level ranged from 4.47% to 4.85%, and the S/M in cheeses at 2.5% salt level ranged from 5.36% to 5.68%. The S/M range (4.47% to 5.68%) of Cheddar cheeses in this study falls within the recommended S/M range for Cheddar cheese, which is about 4.0% to 6.0% (Lawrence et al., 2004). Higher S/M, which is caused by higher salt content, inhibits starter culture activity, and results in lower lactic acid production, and a higher pH value.

The moisture content in cheeses at 2% salt level ranged from 36.98% in cheese without sodium gluconate addition to 38.14% in cheese with 1% sodium gluconate addition. The moisture content in cheeses at 2.5% salt level ranged from 37.01% in cheese without sodium gluconate addition to 37.79% in cheese with 1% sodium gluconate addition. The moisture content of all treatment was in compliance with the standard of identity for Cheddar cheese, which is not more than 39% (USDA, 1956).

The moisture content in cheeses with sodium gluconate addition were higher than the moisture content in cheeses without sodium gluconate addition at both 2% and 2.5% salt level. Gluconate ions have buffering capacity, which could cause the cheese pH to shift to a higher pH. As the cheese pH is shifted away from the isoelectric pH (pH 4.6) to pH 5.3, the electrostatic repulsion is increased, which caused a reduction in protein-protein interactions (De Kruif and Holt, 2003). It was reported that at pH 5.3, there is an increase in solubilization of CCP and the voluminosity of casein micelles is at the maximum (van Hooydonk et al., 1986). Thus, the protein hydration is increased, resulting in an increase in moisture content in cheeses. Similar results were found by Pastorino et

al., (2003a). In their study, they high-pressure injected a 20% glucono-delta-lactone one to five times to Cheddar cheese blocks after 14 days of storage. They reported a decrease in pH from 5.3 in control cheese to 4.7 in cheese with 5 injections, and also a decrease in moisture from 34% in control cheese to 31% in cheese after 5 injections. In addition, sodium gluconate contributed to a higher concentration of Na in cheeses. The binding of Na+ by the micelles and a sodium-calcium ion exchange effect with the para-casein could cause more casein hydration (Guinee and Fox, 2004), and hence an increase in moisture content of cheeses.

pH at 1 Week. The pH values in cheeses at 2% salt level ranged from 5.06 in cheese without sodium gluconate addition to 5.24 in cheese with 1% sodium gluconate addition. The pH values in cheeses at 2.5% salt level ranged from 5.16 in cheese without sodium gluconate addition to 5.32 in cheese with 1% sodium gluconate addition. The pH range (5.06 to 5.32) of Cheddar cheeses in this study falls within the recommended pH range for Cheddar cheese, which is about pH 5.0 to 5.4 (Lawrence et al., 2004).

The pH values in cheeses with sodium gluconate addition were higher than the pH values in cheeses without sodium gluconate addition at both 2% and 2.5% salt level. The pH of the cheese curd is determined by both lactic acid production and the buffering capacity of the curd. It is believed that young Cheddar cheese curd has high buffering capacity at the pH range of 4.5 to 5.5. Thus, the acid production after curd salting might only have a minor effect on the final cheese pH (Lucey and Fox, 1993; Salaün et al., 2005). Gluconate ions have buffering capacity, and thus, they can modify the acid-base equilibrium of the proteins and affect the buffering capacity of the cheese. A similar

condition was reported with an addition of citrate salts to milk. Since citrate salts are calcium chelators, their addition to milk causes solubilization of CCP, and the buffering capacity is shifted to the higher pH (Salaün et al., 2005). In addition, sodium gluconate contributed to a higher concentration of Na in cheeses, and this could possibly inhibit starter culture growth and cause a reduction in the lactic acid production. This explanation is in agreement with Roy (1991), where his research was to determine the effect of adding various salts (NaCl, $CaCl_2$, and KCl) on growth and acid production of *L. helveticus*. He reported a growth inhibition with salts addition of up to 6% with NaCl being most inhibitory and KCl being the least, and lactic acid formation still continued.

Cheeses with 2.5% salt addition had higher pH than cheeses with 2% salt addition. It has been established that two of the most important purposes of salt in cheese is to control microbial growth and regulate the final pH in cheeses since the high salt level can inhibit starter activity and retard the lactic acid production (Guinee and Fox, 2004; Guinee, 2005). The higher pH values in cheeses with 2.5% salt content are in agreement with the lower concentration of lactic acid produced in cheeses. Thus, the higher salt content could contribute to the higher pH in cheeses. A similar influence of salt content on cheese pH was observed by Rajbhandari and Kindstedt (2005) where they reported commercial smoked Cheddar cheese samples that had higher salt content and lower lactic acid content also had higher pH values. Other researchers also reported higher lactic acid bacteria populations in Cheddar cheese with sodium chloride reduction (Schroeder et al., 1988). However, Pastorino et al., (2003b) exhibited different results. In their study, sodium chloride was injected in Muenster cheese after 14 days of storage, and

they reported that cheese pH was not affected by the sodium chloride injection. Their result is probably due to the fact that the sodium chloride injection was not until after 14 days of storage. Normally, lactose content in cheese is depleted before two weeks after manufacture; thus, the sodium chloride injection after 14 days would not affect a change in pH because of lactose metabolism by starter culture (McSweeney and Fox, 2004.)

Gluconic Acid. According to the previous study (Phadungath and Metzger, 201x), it was speculated that the amount of sodium gluconate required for cheese manufacture is within the range of 0.375% to 0.75%. Sodium gluconate is about 89% gluconate by weight. Therefore, 0.375% to 0.75% sodium gluconate results in approximately (0.375 × 0.89) = 0.33% to (0.75 × 0.89) = 0.67% gluconate in the final cheese. From table 2, the % gluconic acid in the final cheese is in the range of 0.33% to 0.59%, which is close to the range that was speculated.

The Possible Effect of Sodium Gluconate on Cheddar Cheese Composition

From the results, sodium gluconate has a significant impact ($P<0.05$) on the cheese pH, moisture, lactose and lactic acid. Cheeses with sodium gluconate addition have higher pH, higher moisture content, higher lactose content and lower lactic acid when compared to cheeses without sodium gluconate addition. After sodium gluconate was applied to the cheese curds, two circumstances could probably occur. Firstly, sodium gluconate contributed to more Na content cheese, which might inhibit starter culture activity. This would lead to less fermentation of lactose, a reduction in lactic acid production, and consequently a higher cheese pH. A second possible effect is that sodium gluconate could act as buffering agent, and cause pH to shift to a higher pH. Once the

cheese pH is shifted further away from the isoelectric point, the electrostatic repulsion is decreased, which caused a reduction in protein-protein interactions. Thus, the protein hydration is increased, and a subsequent increase in moisture content in cheeses.

CONCLUSIONS

Results from this study suggest that sodium gluconate addition caused an increase in the cheese pH, moisture, Na, lactose, and a decrease in fat and lactic acid. Sodium gluconate contributed to a higher Na content in cheese, which inhibit starter culture activity and cause less fermentation of lactose. This would result in a reduction in lactic acid production and a higher pH. Sodium gluconate could also act as buffering agent, and thus result in a higher pH. A higher cheese pH contributed to an increase in protein hydration and a higher in cheese moisture. A decrease in fat content could be due to a dilution effect from higher moisture content. Different salting levels had an impact on pH, Cl, S/M, lactose and lactic acid. When comparing to cheeses with 2.0% salt, cheeses with 2.5% salt had higher pH, higher S/M, higher lactose, and lower lactic acid. A higher salt level in cheese inhibited starter culture activity, which caused less lactose fermentation. This would result in a decrease in lactic acid production and a higher pH. Although sodium gluconate and salt addition had an impact on the aforementioned cheese composition, the overall cheese composition is still in the recommended range for commercial Cheddar cheese.

REFERENCES

Acharya, M. R. and V. V. Mistry. 2004. Comparison of effect of vacuum-condensed and ultrafiltered milk on Cheddar cheese. J. Dairy. Sci. 87: 4004 – 4012.

Association of Official Analytical Chemists, International. 1995. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

Atherton, H. V., and J. A. Newlander. 1977. Tests for fat: Babcock, Gerber, and Mojonnier, In Chemistry and Testing of Dairy Products. 4th ed. AVI Publ. Co., Inc., Westport, CT, pp. 71 – 116.

Brooks, I. B., G. A. Luster, and D. G. Easterly. 1970. A procedure for the rapid determination of the major cations in milk by atomic absorption spectrophotometry. Atomic Absorption Newsletter 9:93–94.

Chou, Y. -E., C. G. Edwards, L. O. Luedecke, M. P. Bates, and S. Clark. 2003. Nonstarter lactic acid bacteria and aging temperature affect calcium lactate crystallization in Cheddar cheese. J. Dairy Sci. 86:2516–2524.

Crawford, M. A., L. Loughridge, M. D. Milne, and B. H. Scribner. 1959. Organic acid excretion after calcium gluconate infusions. J. Clin. Path. 12: 524 – 529.

De Kruif, C. G. and C. Holt. 2004. Casein micelle structure, functions, and interactions, In Advanced Dairy Chemistry Vol 1: Proteins, 3rd edn. (eds. P. F. Fox and P. L. H. McSweeney), Kluwer Academic/ Plenum Publishers, New York, New York, pp. 233 – 276.

Dweck, A. C. 2005. An update on natural preservatives. Personal Care. September: 11-15.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701–1710.

Food Standards Agency. 2007. Current EU approved additives and their E numbers. From Internet website: http://www.food.gov.uk/

Guinee, T. P. 2005. Salting and the role of salt in cheese. Int. J. Dairy Tech. 57: 99 – 109.

Guinee, T. P. and P.F. Fox. 2004. Salt in Cheese: Physical, Chemical and Biological Aspects, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp. 207-259.

Health Canada. 2006. Food additives permitted for use in Canada. From Internet website: http://www.hc-sc.gc.ca/index-eng.php

Johnson, M. E., B. A. Riesterer, C. Chen, B. Tricomi, and N. F. Olson. 1990b. Effect of packaging and storage conditions on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73:3033 – 3041.

Johnson, M. E., B. A. Riesterer, and N. F. Olsen. 1990a. Influence of nonstarter bacteria on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73: 1145 – 1149.

Kalab, M. 1980. Decayed lactic bacteria – a possible source of crystallization nuclei in cheese. J. Dairy Sci. 63(2): 301-304.

Kindstedt, P. S. and F. V. Kosikowski. 1988. Calcium, phosphorus, and sodium concentration in Cheddar cheese. J. Dairy Sci. 71: 285 – 289.

Lawrence, R. C., J. Gilles, L. K. Creamer, V. L. Crow, H. A. Heap, C. G. Honoré, K. A. Johnston, and P. K. Samal. 2004. Cheddar cheese and related dry-salted cheese varieties, In In Cheese: Chemistry, Physics and Microbiology, Vol 2: Major Cheese Groups, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp. 71-102

Lucey, J. A., and P. F. Fox. 1993. Importance of calcium and phosphate in cheese manufacture: A review. J. Dairy Sci. 76: 1714 – 1724.

Marshall, R. T. 1992. Chemical and physical methods, In Standard Methods for the Examination of Dairy Products. 16th ed. American Public Health Association, Washington, DC, pp. 433 – 529.

McSweeney, P. L. H. and Fox, P. F. 2004. Metabolism of residual lactose and of lactate and citrate, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp.361-371

Metzger, L. E. and D. A. Grindstaff. 2005. Cheese with sodium gluconate to inhibit calcium lactate crystal formation. U.S. Patent Application Publication No. US 2005/ 0281915 A1

Morris, H. A., C. Holt, B. E. Brooker, J. M. Banks, and W. Manson. 1988. Inorganic constituents of cheese: analysis of juice from a one-month-old Cheddar cheese and the use of light and electron microscopy to characterize the crystalline phases. J. Dairy Res. 55: 255-268.

Olsen, N. F. and M. E. Johnson. 1989. Controlling calcium lactate crystallization. WMMB Res. Rev. 1: 1 – 4.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003a. Effect of pH on the chemical composition and structure-fuction relationships of Cheddar cheese. J. Dairy Sci. 86: 2751 – 2760.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003b. Effect of salt on structure-fuction relationships on cheese. J. Dairy Sci. 86: 60 – 69.

Pearce, K. N., L. K. Creamer, and J. Gilles. 1973. Calcium lactate deposits on rindless Cheddar cheese. N.Z.J. Dairy Sci. Tech. 8: 3-7.

Phadungath, C. and L. E. Metzger. 201x. Effect of sodium gluconate on the solubility of calcium lactate. J. Dairy Sci. (submitted)

Prescott, F. J., J. K. Shaw, J. P. Bilello, and G.O. Cragwall. 1953. Gluconic acid and its derivatives. Ind. Eng. Chem. 45: 338-342.

Rajbhandari, P. and P. S. Kindstedt. 2005. Compositional factors associated with calcium lactate crystallization in smoked Cheddar cheese. J. Dairy Sci. 88:3737–3744.

Roy, D. 1991. Salt stress in growth and acid production of *Lactobacillus helveticus* strain Milano. Letters Appl. Microbiol. 12: 207 – 211.

Salaün, F., B. Mietton, and F. Gaucheron. 2005. Buffering capacity of dairy products. Int. Dairy. J. 15: 95 – 109.

SAS Institute. 1990. SAS User's Guide: Statistics, Version 6.0 ed. SAS lnst. Inc., Cary, NC.

Schroeder, C. L., F.W. Bodyfelt, C. J. Wyatt., and M. R. McDaniel. 1988. Reduction of sodium chloride in Cheddar cheese: effect on sensory, microbiological and chemical properties. J. Dairy Sci. 71: 2010 – 2020.

Somers, E. B., M. E. Johnson, and A. C. L. Wong. 2001. Biofilm formation and contamination of cheese by nonstarter lactic acid bacteria in the dairy environment. J. Dairy Sci. 84:1926–1936.

Stettenm M. R. and Y. J. Topper. 1953. Pathways from gluconic acid to glucose in vivo. J. Biol. Chem. 203: 653 – 664.

Sutherland, B. J. and G. W. Jameson. 1981. Composition of hard cheese manufactured by ultrafiltration. Aust. J. Dairy Technol. 36:136–142.

Swearingen, P. A., D. E. Adams, and T. L. Lensmire. 2004. Factors affecting calcium lactate and liquid expulsion defects in Cheddar cheese. J. Dairy Sci. 87:574–582.

Thomas, T. D. and V. L. Crow. 1983. Mechanism of D(−)-lactic acid formation in Cheddar cheese. N.Z. J. Dairy. Sci. Technol. 18:131–141.

Upreti, P. and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: manufacture and composition. J. Dairy Sci. 89: 420 – 428.

Upreti, P., L. L. McKay, and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. J. Dairy Sci. 89:429–443.

USDA. 1956. United States Standards for Grades of Cheddar cheese. Dairy Division. Agricultural Marketing Service. United States Department of Agriculture

U.S. FDA. 2006. FDA list of food additives that are 'Generally Recognized As Safe' (GRAS). From Internet website: http://www.cfsan.fda.gov

van Hooydonk, A. C. M., H. G. Hagedoorn, and I. J. Boerrigter. 1986. pH-Induced physic-chemical changes of casein micelles in milk and their effect on renneting. 1.

Effect of acidification on physic-chemical properties. Neth. Milk. Dairy J. 40: 281 – 296.

Washam, C. J., T. J. Kerr, and V. J. Hurst. 1982. Microstructure of various chemical compounds crystallized on Cheddar cheese. J. Food Prot. 45:594–596.

World Health Organization. 1999. WHO Food Additives Series: 42, Safety Evaluation of certain cood cdditives: glucono delta lactone and the calcium, magnesium, potassium, and sodium salts of gluconic acid. Geneva.

Chapter 4. Changes in residual lactose and water-soluble organic acids during Cheddar cheese ripening

During Cheddar cheese ripening, lactose is converted to glucose and galactose or galactose-6-phosphate by starter and non-starter lactic acid bacteria. These sugars are primarily converted into lactic acid during the manufacturing process. However, under stressed condition (low pH and high salt) present during cheese ripening, bacteria utilize alternative pathways, which can result in formation of various organic acids. The objective of this study was to determine if the level and type of residual sugar and organic acids produced during ripening was impacted by sodium gluconate addition to Cheddar cheeses. Six cheeses with two salting rates (2 and 2.5%) and three sodium gluconate addition levels (0, 0.5 and 1%) were manufactured. The cheeses were analyzed for lactose and water-soluble organic acids (acetic, butanoic, citric, formic, gluconic, lactic, orotic, propanoic, and uric) at 1-week, 3-month and 6-month, 9-month, and 12-month of ripening by using a cation-exchange-column HPLC externally equipped with a refractive index detector . The organic acids were detected using the UV detector set at 210 and 285 nm, and the refractive index was used for quantification of lactose. The results indicated that at 1-week of ripening, Cheddar cheeses with a higher concentration of gluconic acid had lower concentration of lactic acid, but higher concentration of lactose, while there were no differences in acetic, butanoic, citric, formic, orotic, propanoic and uric acids among treatments at all ripening times. The concentrations of butanoic and propanoic acids gradually increased over time in all treatments, whereas the concentrations of orotic

acid and lactose gradually decreased over time. Minor changes in the levels of acetic, citric, formic, lactic, and uric were also observed throughout ripening in all treatments.

INTRODUCTION

Cheese ripening is a complicated process, which involves a range of biochemical reactions. To obtain homogeneous and high quality cheese, uniform lactose fermentation is required (Beresford et al., 2001). Thus, the metabolism of lactose to lactate is an essential biochemical pathway for the production of all cheese varieties. Cheese curd has a low level of residual lactose, because most of the lactose is lost with whey during drainage in the cheese making process. The residual lactose is readily metabolized to lactate by both starter and non-starter lactic acid bacteria (LAB) during early ripening. The three categorizes of LAB (i.e. lactococci, lactobacilli, and *S. thermophilus*) utilize different systems of lactose transport and catabolism, resulting in differences in their biochemical characteristics and an accumulation of intracellular and extracellular products. The metabolism of lactose to lactate is normally complete at the end of manufacture or during the early ripening stage by starter LAB mainly lactococci. However, in dry-salted cheese particularly Cheddar cheese, the activity of starter cultures is greatly reduced due to the combination of low pH and high NaCl, resulting in further lactose metabolism by non-starter lactic acid bacteria (lactobacilli, and S. *thermophilus*), which consequently influences cheese flavor (McSweeney, 2004a, b).

LAB are heterotrophic chemo-organotrops; thus, the growth and metabolic pathways of LAB are influenced by the available substrate. *L. lactis* are originally considered to be homolactic fermenters of glucose and lactose. However, once they are

grown in limited carbohydrate condition, they will become heterolactic, which will lead to a production of not only lactate but also formate, acetate, and ethanol (Thomas et. al., 1979). During cheese ripening, the lactose concentration is limited; thus this condition will cause some LAB to become heterolactic and produce a varietoy of compounds. Consequently, different organic acids formed during the ripening process play important roles in the flavor of Cheddar cheese. It has been confirmed that the water-soluble fraction in cheese contributes more to the flavor intensity than the fat fraction (McGugan et al., 1979). The water-soluble fraction consists mainly of short-chain, water soluble organic acids and residual sugars. These organic acids can originate from lactose metabolism by starter lactic acid bacteria (LAB), hydrolysis of fatty acids, direct addition as acidulant (Marsli et al., 1981; Adda et al., 1982), and from glycolysis by non-starter lactic acid bacteria (NSLAB) (Crow et al., 2001). Many studies have been conducted to monitor changes in organic acids and residual sugar profiles during cheese ripening (Harvey et al., 1981; Marsili 1985; Akalin et al., 2002; Upreti et al., 2006), and these profiles have been used as a parameter of cheese maturity (Panari, 1986; Bevilacqua and Califano, 1989; Bouzas et al., 1991b; Upreti et al., 2006).

Changes in organic acids in cheese during ripening could be affected by ripening time, milk treatment, and compositional factors. Lues and Botha (1998) investigated the relationships between South African young (6 to 10-week) and mature (8 to 12-month) Cheddar cheese and organic acid content by ion-exclusion HPLC. They reported that both young and mature Cheddar cheese had a similar pattern of chromatograms. The differences observed were that mature Cheddar cheese exhibited a higher number and a higher concentration of organic acids when compared to the young cheese. The effect of

different milk treatments on changes in organic acids during cheese ripening were conducted by Buffa et al. (2004). In their study, nine organic acids (citric, pyruvic, malic, lactic, formic, acetic, uric, propionic, and butyric acids) in cheese made from raw, pasteurized, and pressure-treated goat milk were measured at 1, 30 and 60-day of ripening. They reported that milk treatments did not affect organic acid contents of 1-day old cheeses. At the end of ripening, raw milk and pressure-treated milk cheeses exhibited higher concentration of organic acids compared to pasteurized milk cheese. A recent study by Upreti et al. (2006) reported that during Cheddar cheese ripening, lactose content was influenced by Ca and P, residual lactose, and salt-to-moisture ratio, and lactic acid concentration was affected by salt-to-moisture ratio.

Calcium lactate crystals (CLC) can appear within and on the surface of hard-type cheese especially Cheddar cheese after approximately four to six months of aging. In a typical Cheddar cheese, the concentration of soluble calcium and lactate are in excess of their solubility at 4°C. Since calcium and lactate ions are in a supersaturate state in the cheese serum phase, crystallization at nucleation sites occurs. CLC are not harmful to consumers. They are, however, perceived as grittiness in the cheese, and more importantly, they are considered a quality defect because consumers often believe that they are mold or some other type of spoilage (Dybing et al., 1988). One potential approach for preventing CLC is to add sodium gluconate to Cheddar cheese. In a previous study (Phadungath and Metzger, 201x), we determined that sodium gluconate can increase the solubility of calcium and lactate by forming complexes with calcium and lactate ions, and removing the calcium and lactate ions from being available for the formation of CLC. In the previous study, seven calcium lactate solutions (5.31% w/w)

with seven level of sodium gluconate (0, 0.5, 1, 1.5, 2, 3, and 4% w/w) were made. Solutions were stored at 7°C for 21 days. The solutions were visually inspected for CLC formation, analyzed for lactic acid and gluconic acid by HPLC and for calcium by Atomic Absorption Spectroscopy. We determined that CLC were formed in the solution with 0% gluconate after the first day of storage, and the HPLC results indicated that there was a higher concentration of calcium and lactic acid in the filtrate from the solutions containing added gluconate. These results demonstrated that sodium gluconate can increase the solubility of calcium lactate. However the effect of sodium gluconate on Cheddar cheese characteristics during ripening has not been studied. The objective of this study was to determine if the level and type of residual sugar and organic acids produced during ripening was impacted by sodium gluconate addition to Cheddar cheeses.

MATERIALS AND METHODS

Experimental Design

A replicated 2×3 factorial designs was utilized for a Cheddar cheese making study. This design utilized three levels of sodium gluconate (0, 0.5 and 1%) and two levels of salt (2 and 2.5%) for a total of six treatments. The treatments were; TRT1 - 0% sodium gluconate + 2% salt, TRT2 – 0.5% sodium gluconate + 2% salt, TRT3 – 1.0% sodium gluconate + 2% salt, TRT4 - 0% sodium gluconate + 2.5% salt, TRT5 – 0.5% sodium gluconate + 2.5% salt, TRT6 - 1% sodium gluconate + 2.5% salt. Each treatment was manufactured in triplicate from three different lots of milk. The manufacturing protocols and cheese composition for Cheddar cheese in this study have been previously reported and shown in Table 1(Phadungath and Metzger, 2008). The cheeses were

ripened for 12 months, and changes in lactose and water-soluble organic acids were monitored during ripening.

Table 1. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition**

Mean value (%)*	Treatments					
	2% Salt			2.5% Salt		
	Sodium gluconate (%)			Sodium gluconate (%)		
	0	**0.5**	**1.0**	**0**	**0.5**	**1.0**
pH (1 wk)	5.06^{a}	5.16^{ab}	5.24^{ab}	5.16^{ab}	5.28^{b}	5.32^{b}
Moisture	36.98^{a}	37.79^{ab}	38.14^{b}	37.01^{a}	37.54^{ab}	37.79^{ab}
Fat	32.98^{a}	32.18^{ab}	30.96^{b}	32.26^{ab}	31.73^{ab}	31.33^{b}
Protein	25.72	26.1	25.6	26.08	25.65	25.72
Na	0.61^{a}	0.74^{ab}	0.77^{b}	0.72^{ab}	0.78^{b}	0.82^{b}
Cl	0.99^{a}	1.08^{ab}	1.12^{ab}	1.20^{ab}	1.29^{b}	1.29^{b}
S/M	4.47^{a}	4.73^{a}	4.85^{ab}	5.36^{b}	5.68^{b}	5.66^{b}
Total Ca	0.73^{a}	0.72^{ab}	0.69^{b}	0.73^{ab}	0.72^{ab}	0.70^{ab}
P	0.5	0.49	0.48	0.5	0.49	0.49
Lactose (1 wk)	0.53^{a}	0.69^{ab}	0.85^{ab}	0.71^{ab}	0.85^{ab}	1.00^{b}
Lactic acid (1 wk)	1.56^{a}	1.35^{ab}	0.95^{c}	1.17^{b}	0.93^{c}	0.89^{c}
Gluconic acid	0	0.33	0.59	0	0.33	0.58

* ^{a,b,c} Means within the column not sharing common superscripts are different (Tukey HSD at $P < 0.05$).

** n = 3 replicates.

Analyses of Organic Acids and Lactose

The cheese samples from six treatments were analyzed for lactose and water-soluble organic acids (citric acid, orotic acid, gluconic acid, succinic acid, lactic acid, formic acid, uric acid, acetic acid, propanoic acid, and butanoic acid) at 1-week, 3-month,

6-month, 9-month, and 12-month of ripening using an HPLC procedure adapted from Zeppa et al. (2001) and Upreti et al. (2006). For the sample preparation, about 5-g cheese were manually homogenized for 90 s with 10-mL of 0.013N sulfuric acid (at 65°C) using a high shear Omni mixer-homogenizer (model 17105, Omni International, Waterbury, CT). The extract was centrifuged (Jouan CR4-12 Centrifuges, Jouan, Inc., Winchester, VA) at 7,000 ×g for 10 min. Then the samples were held at 4°C for 20 min to solidify the fat layer, and the top fat layer was removed using a spatula. The supernatant was filtered through filter paper (Whatman 4; Whatman International Ltd., Maidstone, England). A 0.5 mL aliquot of filtered supernatant was poured into a 0.5-mL Microcon® (Millipore Corporation, Bedford, MA) centrifugal filter device with a molecular weight cut-off of 3000D then micro-centrifuged (Jouan A14 Microcentrifuges, Jouan, Inc., Winchester, VA) at 14,000 ×g for 20 min to remove the soluble peptides present in the filtrate. The collected filtrate from the micro-centrifuge was directly injected into the HPLC system.

The HPLC system (Beckman Coulter Inc., Fullerton, CA) was equipped with a photodiode array detector set at 210 and 285nm, and data processing software (System Gold® HPLC, 32 Karat^{TM} Software, Beckman Coulter Inc., Fullerton, CA). The system was externally equipped with an intelligent refractive index detector (JASCO Model RI-2031 Refractive index detector, Jasco, Inc., Easton, MD), and a column heater (Alltech® Model 6301 Column Heater, Alltech Associates Inc., Deerfield, IL). The column used for separation of the analytes was an Rezex ROA-organic acid H^+ column (300x7mm, 8µm, Phenomenex) held at 65°C, with a cation H^+ microguard cartridge (Bio-Rad Laboratories, Hercules, CA). The analysis was performed isocratically at 0.6 mL/ min

flow rate using 0.013 N sulfuric acid (Fisher Scientific, Fair Lawn, NJ) as the mobile phase. The refractive index detector was used for quantification of lactose (Figure 1). The separated short-chain, water-soluble organic acids (citric acid, orotic acid, gluconic acid, succinic acid, lactic acid, formic acid, uric acid, acetic acid, propanoic acid, and butanoic acid) were detected using the UV detector set at 210 and 285 nm (Figure 1). Although galactose and pyruvic acid could be detected by HPLC, galactose was co-eluted with gluconic acid at the RI detection, and pyruvic acid was co-eluted with gluconic acid at UV-210 nm detection; thus, both galactose and pyruvic acid are not reported in this study.

Quantification of analytes was based on the external standard method. Lactose (Fisher Scientific), citric acid (Sigma-Aldrich, Inc., St. Louis, MO), sodium isocitrate (M.P. Biomedicals, LLC, Aurora, OH), orotic acid (Sigma), sodium gluconate (PMP Fermentation Products, Inc., Chicago, IL), succinic acid (Sigma), lactic acid (Sigma), formic acid (Sigma), uric acid (Acros Organics, Fairlawn, NJ), acetic acid (Sigma), propanoic acid (Sigma), and butanoic acid (Sigma) were used for making stock solutions. The stock solutions were sufficiently diluted with 0.013 N sulfuric acid to obtain desired concentration range of analytes to establish standard curves. The analytes were then quantified by the best-fit standard curves prepared by linear regression of peak area vs. different concentrations of the analytes, as shown in Table 2. However, quantification of coeluting analytes (e.g., citric acid, orotic acid, and lactose) was done using multiple regression equations as suggested by Bouzas et al. (1991) and Upreti et al. (2006).

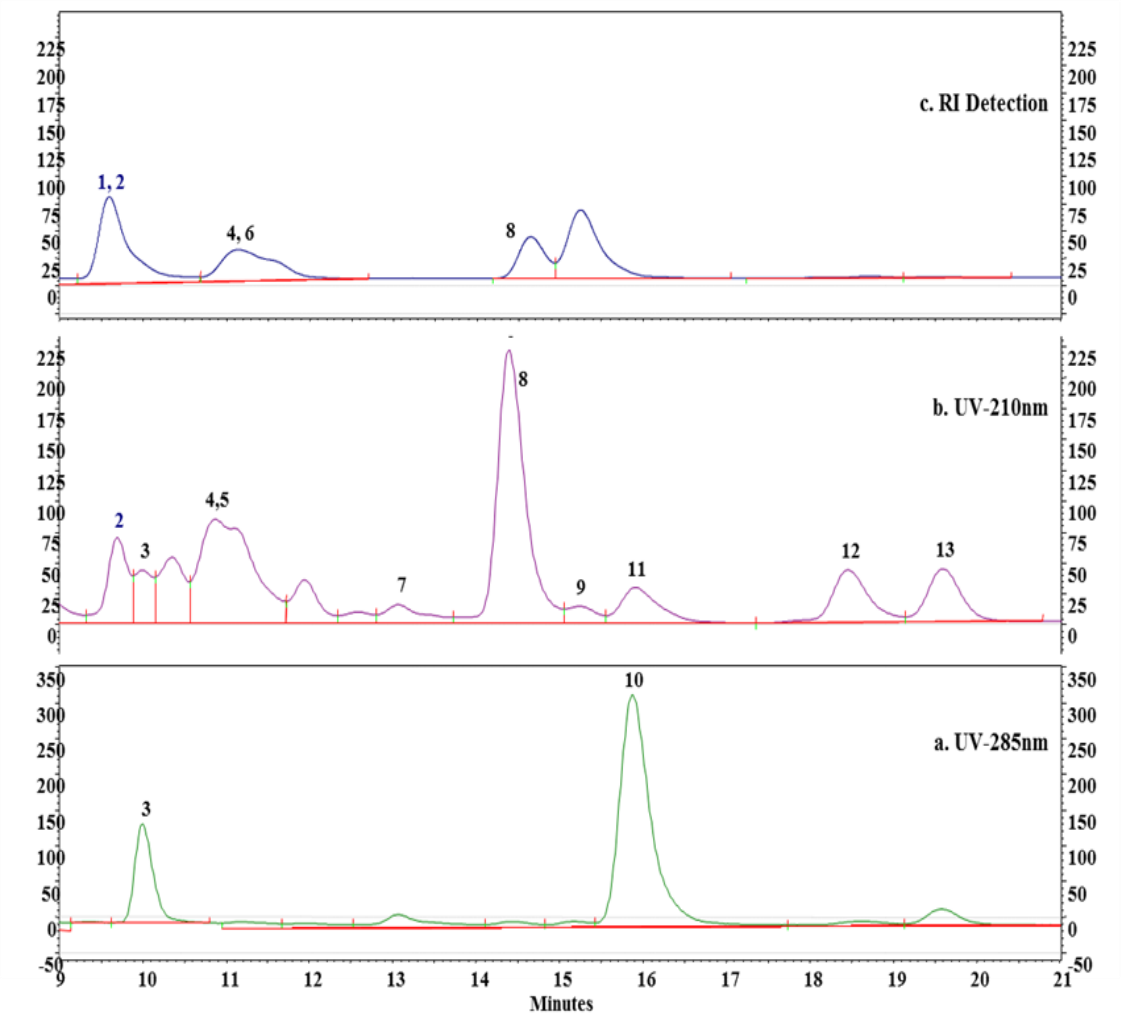

Figure 1. Typical chromatograms of Cheddar cheese with sodium gluconate addition for a). UV-285 nm detection, b). UV-210 nm detection, and c). RI detection. The organic acids detected are represented using numbers; 1 = lactose, 2 = citric acid, 3 = orotic acid, 4 = gluconic acid, 5 = pyruvic acid, 6 = galactose, 7= succinic acid, 8= lactic acid, 9 = formic acid, 10 = uric acid, 11 = acetic acid, 12 = propanoic acid, and 13 = butanoic acid.

Table 2. Retention time (RT) for different analytes, their detection, and calibration curves

Anayte	RT (min)	Detection*			Calibration equation
		210	285	RI	
Lactose	9.58			×	(0.000001963 × RI area) - (0.8510 × citric) + 0.007476
Citric acid	9.86	×		×	(0.00000004433 × UV210 area) - (51.25 × orotic) + 0.000831
Orotic acid	10.03	×	×		(0.000000001109 × UV 285 area) - 0.0000004286
Gluconic acid	11.0	×			(0.000003194 × UV 210 height) + 0.0009321
	10.88			×	(0.00008384 × RI height) + 0.002638
Succinic acid	13.16	×			(0.00000005234 × UV210 area) + 0.0001148
Lactic acid	14.43	×		×	(0.00000007807 × UV 210 area) + 0.006809
Formic acid	14.99	×	×		(0.00000005085 × UV210 area) + 0.0003196
Uric acid	15.83		×		0.000000001089 × UV285 area - 0.000003328
Acetic acid	15.96	×			(8.717E-08 × UV210 area) - (38.24 × uric) + 0.0005581
Propanoic acid	18.27	×			(0.0000001540 × UV210 area) + 0.0002705
Butanoic acid	21.62	×			(0.0000001025 × UV210 area) + 0.00009970

*UV detection at 210 and 285 nm, and refractive index (RI) detection.

Statistical Analyses

A 2 × 3 factorial model with 3 replications was used for statistical analysis and changes in the concentration of lactose and organic acids during ripening were analyzed using a repeated measures design (Table 3). The PROC GLM procedure of SAS, which involved 2 factors (salt, and sodium gluconate level) as class variables and changes in the concentration of lactose and organic acids during ripening were analyzed using a repeated measures design (SAS Institute, 1990).

RESULTS AND DISCUSSION

Changes in Gluconic Acid

Gluconic acid content was significantly ($P < 0.05$) affected only by sodium gluconate addition, and was not affected by salting levels, time, and interaction terms (Table 3). According to the previous study (Phadungath and Metzger, 201xa), it was speculated that the amount of sodium gluconate required for cheese manufacture is within the range of 0.375% to 0.75%. Sodium gluconate is about 89% gluconate by weight. Therefore, 0.375% to 0.75% sodium gluconate results in approximately (0.375 × 0.89) = 0.33% to (0.75 × 0.89) = 0.67% gluconate in the final cheese. From figure 6, the % gluconic acid in the final cheese is in the range of 0.33% to 0.59%, which is close to the range that was speculated.

Table 3. Mean squares and probabilities (in parentheses) of changes in organic acids and lactose in Cheddar cheese with added sodium gluconate during storage at 6 – 8 °C for 12 months.

Factor	df	Lactose	Lactic acid	Citric acid	Orotic acid	Gluconic acid	Succinic acid
Whole plot							
Salt (S)	1	0.37* (< 0.01)	0.89* (< 0.01)	1.25×10^{-3} (0.231)	8.84×10^{-8} (0.815)	0.10×10^{-4} (0.9732)	1.17×10^{-3} (0.338)
Gluconic acid (G)	2	0.37* (< 0.01)	0.49* (< 0.01)	0.12×10^{-3} (0.865)	1.61×10^{-8} (0.989)	2.63* (< 0.01)	1.51×10^{-3} (0.312)
S × G	2	2.21×10^{-3} (0.922)	1.42×10^{-2} (0.762)	0.05×10^{-3} (0.944)	4.11×10^{-8} (0.974)	0.10×10^{-8} (0.998)	1.47×10^{-3} (0.322)
Error	12	2.67×10^{-2}	5.12×10^{-2}	0.79×10^{-3}	1.54×10^{-6}	5.88×10^{-3}	1.18×10^{-3}
Subplot							
Time (T)	4	0.30* (< 0.01)	0.14* (< 0.01)	7.84×10^{-3}* (< 0.01)	4.69×10^{-6}* (< 0.01)	1.65×10^{-3} (0.522)	2.52×10^{-2}* (< 0.01)
T × S	4	1.92×10^{-3} (0.945)	1.76×10^{-2} (0.057)	0.16×10^{-3} (0.968)	5.14×10^{-8}* (0.023)	0.58×10^{-8} (0.887)	0.14×10^{-3} (0.943)
T × G	8	8.24×10^{-3} (0.611)	2.14×10^{-2}* (< 0.01)	0.47×10^{-3} (0.919)	5.54×10^{-8}* (< 0.01)	0.79×10^{-8} (0.922)	0.46×10^{-3} (0.752)
T × S × G	8	0.91×10^{-3} (0.999)	1.36×10^{-2} (0.080)	0.12×10^{-3} (0.999)	5.42×10^{-9} (0.951)	0.96×10^{-8} (0.870)	0.41×10^{-3} (0.805)
Error	48	1.04×10^{-2}	7.12×10^{-3}	1.19×10^{-3}	1.65×10^{-8}	2.03×10^{-3}	0.73×10^{-3}

*Statistically significant at *P*-value < 0.05.

Table 3. (continue) Mean squares and probabilities (in parentheses) of changes in organic acids and lactose in Cheddar cheese with added sodium gluconate during storage at 6 – 8 °C for 12 months.

Factor	df	Formic acid	Uric acid	Acetic acid	Propanoic acid	Butanoic acid
Whole plot						
Salt (S)	1	1.42×10^{-8} (0.989)	5.68×10^{-6}* (0.010)	2.01×10^{-3}* (0.047)	2.36×10^{-2} (0.682)	0.22×10^{-3} (0.839)
Gluconic acid (G)	2	1.00×10^{-5} (0.886)	1.91×10^{-7} (0.737)	0.11×10^{-3} (0.774)	1.77×10^{-3} (0.987)	4.06×10^{-3} (0.470)
S × G	2	1.35×10^{-5} (0.849)	1.19×10^{-7} (0.825)	0.41×10^{-3} (0.395)	9.41×10^{-3} (0.932)	0.05×10^{-3} (0.990)
Error	12	8.18×10^{-5}	6.09×10^{-7}	0.41×10^{-3}	0.13	5.05×10^{-3}
Subplot						
Time (T)	4	3.14×10^{-4}* (< 0.01)	4.62×10^{-6}* (< 0.01)	1.74×10^{-2}* (< 0.01)	4.08* (< 0.01)	0.145* (< 0.01)
T × S	4	4.74×10^{-5} (0.099)	9.12×10^{-7} (0.090)	0.16×10^{-3} (0.618)	3.22×10^{-3} (0.970)	1.18×10^{-3} (0.729)
T × G	8	1.09×10^{-5} (0.864)	1.13×10^{-7} (0.974)	0.22×10^{-3} (0.507)	2.44×10^{-3} (0.999)	1.09×10^{-3} (0.869)
T × S × G	8	1.14×10^{-5} (0.864)	2.02×10^{-8} (0.999)	0.80×10^{-4} (0.945)	2.00×10^{-3} (0.999)	0.96×10^{-3} (0.906)
Error	48	2.29×10^{-5}	4.26×10^{-7}	0.24×10^{-3}	2.46×10^{-2}	2.31×10^{-3}

*Statistically significant at *P*-value < 0.05.

According to Hegazi and Abo-Elnaga (1990), *L. lactis* is able to produce CO_2 from gluconate. However, there was no indication of CO_2 in any of our vacuum cheese packages throughout the ripening time, which would suggest that gluconate in our cheese samples was not metabolized by L. *lactis.* In addition, gluconate could also be metabolized by heterofermentative lactobacilli with a presence of enzyme gluconokinase

(Hegazi and Abo-Elnaga, 1990); however, from figure 2, the concentration of gluconic acid remained constant throughout the ripening time, which suggested that gluconate was not metabolized to any other compounds.

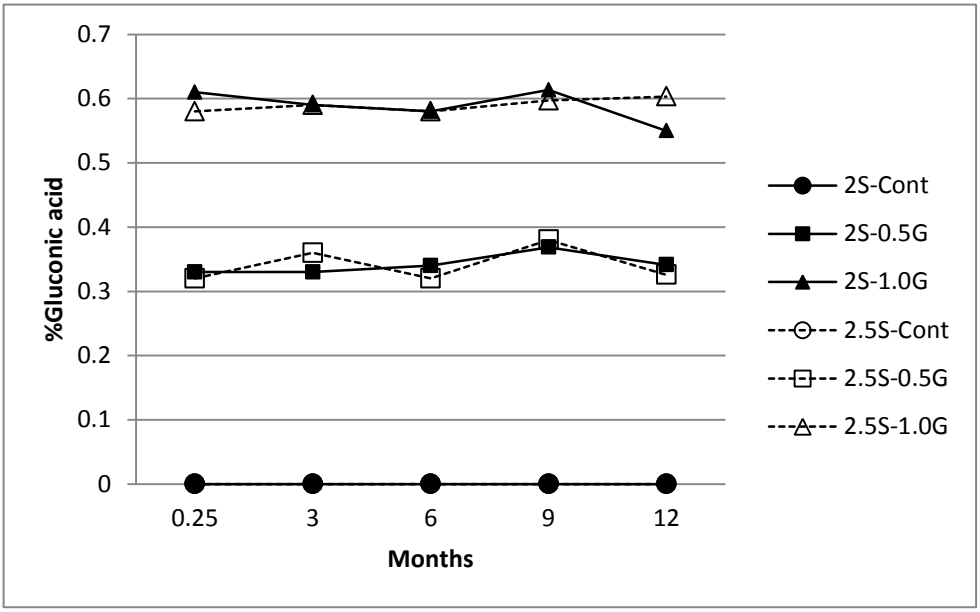

Figure 2. % Gluconic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Changes in Lactose

Lactose content was significantly ($P < 0.05$) affected by salting levels, sodium gluconate addition, and time, but was not affected by interaction terms (Table 3). Figure 3 shows that the lactose content in all treatments decreased from 1-week to 12-month of ripening. According to McSweeney and Fox (2004), Cheddar cheese curd contains only about 0.8 – 1.0% of lactose before salting, and lactose is normally depleted between 2 - 7 weeks of ripening (Bouzas et al., 1991; McSweeney and Fox, 2004). However, 1-week of ripening, the lactose concentration in our cheeses were relatively high (0.5 to 1.0%), and lactose was not completely metabolized by the 12-month of ripening. This could be mainly due to the high solids in the initial cheese milk mixture that contributed to higher lactose content. A similar result was observed by Harvey et al (1981), where they reported a residual lactose in commercial cheddar cheese after about 6-month of ripening, and Upreti et al. (2006) also reported a relative high concentration of residual lactose (up to 1.50%) in Cheddar cheese after 12-month of ripening. Turner and Thomas (1980) suggested that lactose is normally fermented by starter LAB within the first 8 days, and afterwards NSLAB is responsible for lactose fermentation. Thus, the relative high lactose concentration in our cheeses throughout the ripening time could indicate minimal LAB and NSLAB activity to metabolize residual lactose.

Cheeses with 2.5% salting level had higher residual lactose than cheeses with 2% salting level throughout the ripening time. Cheeses with higher % of salt addition also had higher S/M, as shown in Table 1. A similar influence of S/M on residual lactose was reported by Upreti et al. (2006). S/M alters a_w of the cheese, and thus, affects bacterial

activities. Guinee and Fox (2004) suggested that the growth of lacotocci in Cheddar curd is inhibited by ≥ a 4% S/M, as confirmed by Upreti et al. (2006), where they reported that a S/M of 4.5% can inhibit *L. lactis* ssp. *lactis* to ferment lactose in Cheddar cheese that is ripened at 6 to 8°C. The residual lactose content in cheeses with sodium gluconate addition were higher than the residual lactose content in cheeses without sodium gluconate addition at both 2% and 2.5% salting level throughout the ripening time. Sodium gluconate contributed to a higher concentration of Na in cheeses (Table 1), and this could possibly inhibit starter culture growth and cause higher residual lactose in cheeses. This explanation is in agreement with Roy (1991), where his research was to determine the effect of adding various salts (NaCl, $CaCl_2$, and KCl) on growth and acid production of *L. helveticus.* He reported a growth inhibition with salts addition of up to 6% with NaCl being most inhibitory and KCl being the least, and lactic acid formation still continued.

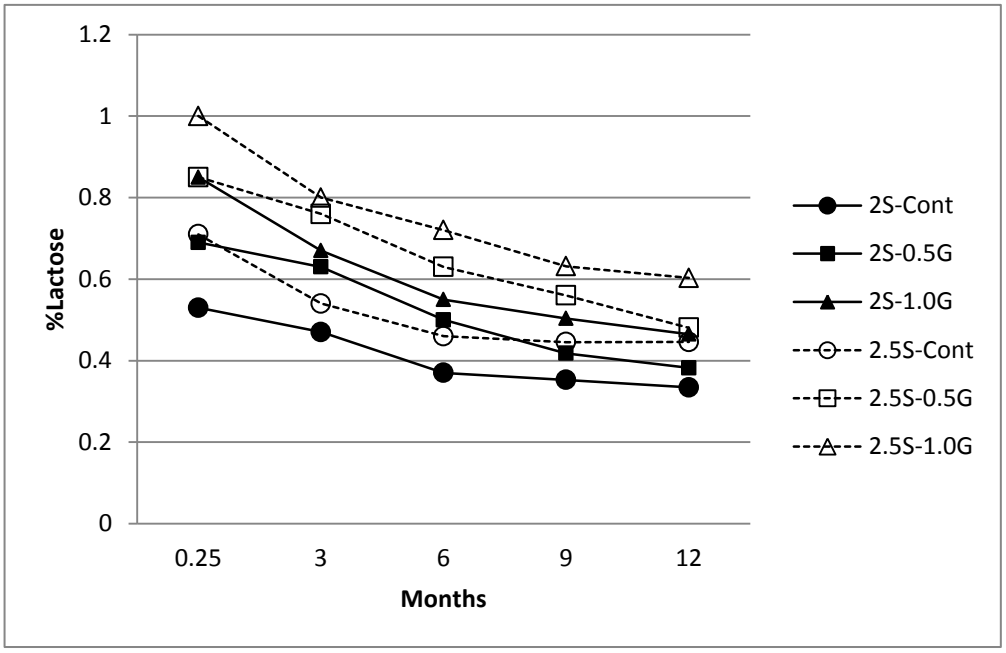

Figure 3. % Lactose in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Changes in Lactic Acid

Lactic acid was detected in a greater concentration than other organic acids since the primary role of dairy starter cultures is to convert lactose to lactic acid. Formation of lactic acid is important for proper cheese manufacture and flavor development of cheese (Califano and Bevilacqua, 2000). Lactic acid content was significantly ($P < 0.05$) affected by salting levels, sodium gluconate addition, time, and an interaction of time × sodium gluconate addition (Table 3). From figure 4, lactic acid in Cheddar cheeses with

2% salting level and 0% and 0.5% sodium gluconate addition was constant from 1-week to 3-month of ripening, while lactic acid in other treatments increased from 1-week to 3-month of ripening. Lactic acid content from all treatments gradually increased from 3-month to 9-month of ripening, followed by a slight decrease from 9-month to 12-month of ripening. A similar pattern was observed by Akalin et al. (2002), where they reported an initial decrease of lactic acid in Pickled White cheese from 0 to 1-month of ripening, followed by an increase from 1-month to 9-month of ripening, and a slight decrease from 9-month to 12-month of ripening. An increase in lactic acid content during ripening was also reported by others in Cheddar cheese (Marsili, 1985; Lues and Bekker, 2002; Ong and Shah, 2008), Port Salut Argentino cheese (Bevilacqua and Califano, 1992), Afuega'l Pitu cheese (de Llano et al., 1996), and goat's milk cheese (Buffa et al., 2004). In addition, lactic acid content in Cheddar cheese with 0% sodium gluconate addition dropped dramatically from 9-month to 12-month of ripening. This higher reduction in lactic acid from Cheddar cheese without sodium gluconate addition could be due to the formation of CLC during cheese ripening.

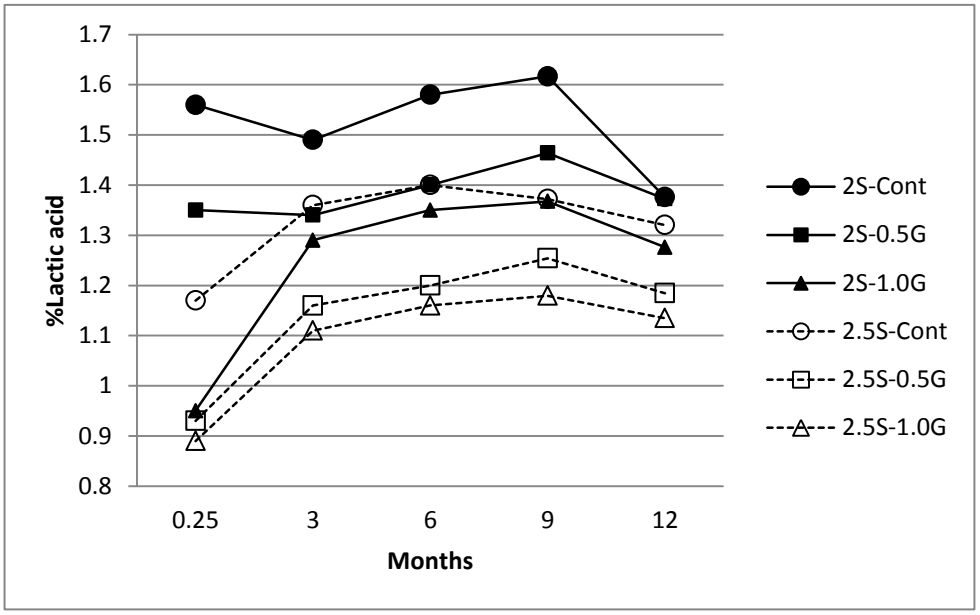

Figure 4. % Lactic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Cheeses with 2.5% salting level had lower lactic acid content than cheeses with 2.0% salting level throughout the ripening time. This is in agreement with the lactose content in cheeses, where cheeses with 2.5% salting level had higher lactose content, and vice versa for cheeses with 2.0% salting level, which was probably due to inhibition of starter culture activity by the high salt level. Thus, lactose content is less fermented and lactic acid production is also reduced. Lactic acid content in cheeses with sodium gluconate addition were lower than the lactic acid content in cheeses without sodium gluconate addition at both 2% and 2.5% salting level throughout the ripening time. As

stated earlier, sodium gluconate contributed to a higher concentration of Na in cheeses (Table 1), and this could possibly inhibit starter culture growth, which would cause less utilization of lactose in the cheeses. Thus, sodium gluconate addition could result in a reduction in the lactic acid production and a higher amount of residual lactose content in cheeses.

Changes in Citric Acid

Citric acid content was significantly ($P < 0.05$) affected by time but was not affected by salting levels, sodium gluconate addition, and interaction terms (Table 3). Figure 5 shows that citric acid from all treatments slightly decreased from 1-week to 3-month of ripening, followed by an increase from 3-month to 6-month of ripening and a decrease from 6-month to 12-month of ripening. A similar pattern was observed by Akalin et al. (2002), where they reported an initial decrease of citric acid in Pickled White cheese from 0 to 1-month of ripening, followed by an increase from 1-month to 3-month and a decrease from 3-month to 6-month of ripening.

An increase in citric acid was observed during ripening for Gouda cheese (Califano and Bevilacqua, 2000) and Cheddar cheese (McGregor and White, 1990; Lues and Bekker, 2002; Upreti et al., 2006). Lues and Bekker indicated that an increase in citric acid during ripening could be a result of a co-elution with 2-ketoglutaric acid, and Upreti et al. (2006) suggested that this phenomenon could be due to an absence of citrate-fermenting strains of LAB in cheese. On the contrary, Bevilacqua and Califano (1992), de Llano et al. (1996), and Ong and Shah (2008) reported a decrease in citric acid during ripening for Port Salut Argentino cheese, Afuega'l Pitu cheese, and Cheddar cheese,

respectively. The decrease in citric acid during ripening is probably due to its role as a substrate in the Krebs or citric acid cycle for producing pyruvic acid and carbon dioxide, and acetic acid by citrate-fermenting strains of LAB (Ong and Shah, 2008). NSLAB was also suggested to metabolize citrate as reported by Díaz-Muñiz et al. (2006) that *Lact. casei* is able to convert citric acid to acetic acid, l/d-lactic acid, acetoin, diacetyl, ethanol, and formic acid under conditions similar in cheese ripening.

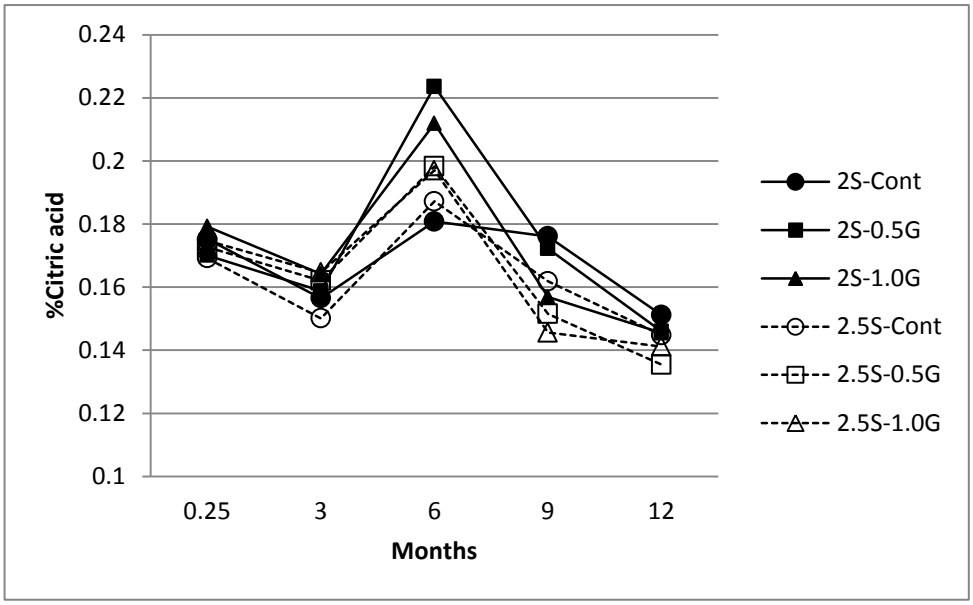

Figure 5. % Citric acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Changes in Orotic Acid

Orotic acid content was significantly ($P < 0.05$) affected by time, an interaction of time × salting levels, and time × sodium gluconate addition, and was not affected by salting levels and sodium gluconate addition (Table 3). Figure 6 shows that orotic acid content from all treatments dramatically decreased from 1-week to 6-month of ripening, and a slight decrease occurred from 6-month to 12-month of ripening. The result is in agreement with McGregor and White (1990), and Upreti et al. (2006) as they reported a decrease in orotic acid content in Cheddar cheese over time. A decrease in orotic acid content over time was also observed in Port Salut Argentino cheese (Bevilacqua and Califano, 1992) and Afuega'l Pitu cheese (de Llano et al., 1996).

Orotic acid is an intermediate component for biosynthesis; thus it is a commonly found in all living cells especially in milk fluid (Akalin and Gönç, 1996). Orotic acid is more prevalent in bovine milk (Larson and Hegarty, 1979), and it was reported to be heat-stable under both HTST and UHT conditions (Saidi and Warthesen). Therefore, it is possible that the initial concentration of orotic acid in cheese is a result of orotic acid retention in cheese from the milk that was used for cheese making. Orotic acid can be readily utilized by various bacteria, thus causing the reduction in its content during cheese ripening (Navder et al., 1990; Zeppa and Rolle, 2008).

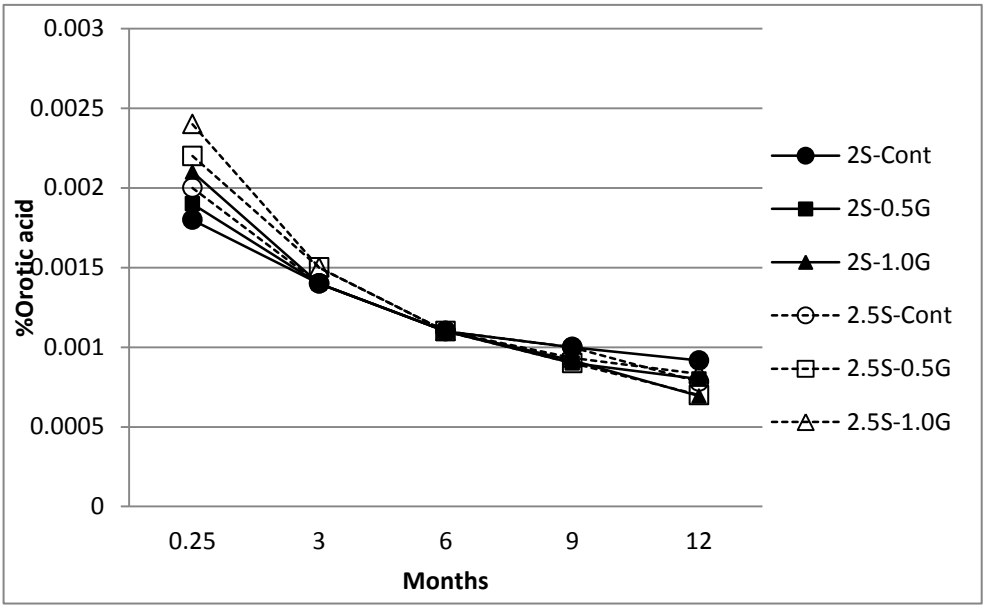

Figure 6. % Orotic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Changes in Succinic Acid

Succinic acid content was significantly ($P < 0.05$) affected by time, and was not affected by salting levels, sodium gluconate addition, and interaction terms (Table 3). Figure 7 shows that succinic acid content from cheeses with 2.5% salting level and cheese with 2% salt level and 1% sodium gluconate addition remained constant from 1-week to 6-month of ripening. A slight increase in succinic acid from 1-week to 6-month of ripening was found in cheeses with 2% salting level without sodium gluconate

addition and 0.5% sodium gluconate addition. Then the succinic acid content from all treatments dramatically decreased from 6-month to 12-month of ripening.

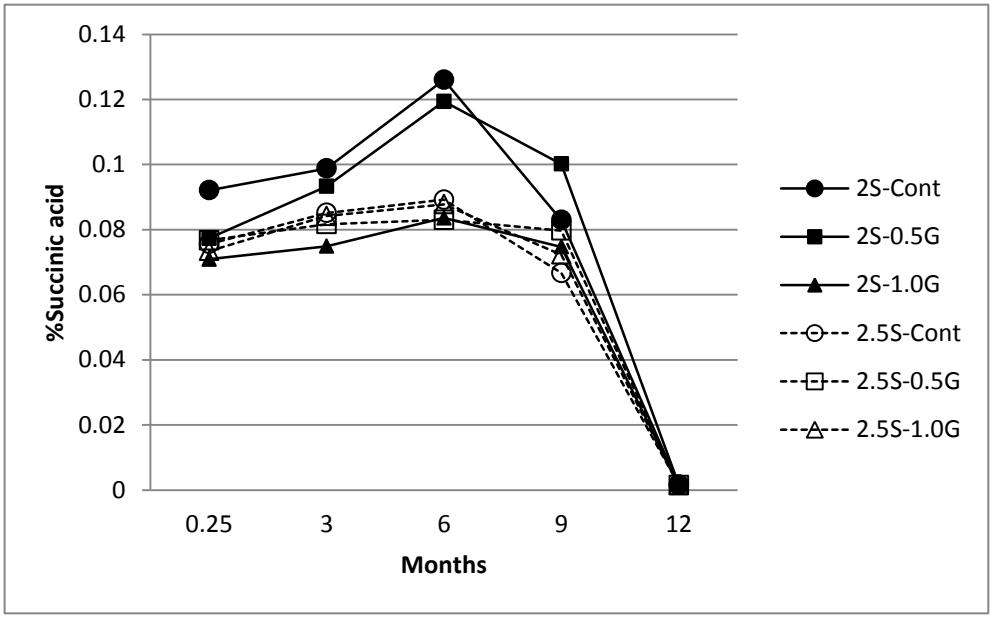

Figure 7. % Succinic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

It is suggested that succinic acid gives a savory characteristic to Cheddar cheese and enhances overall flavor of Cheddar cheese (Dudley and Steele, 2004). A concentration of up to 0.20% of succinic acid, which is substantially higher than the concentration detected in this study, has been reported in some European cheeses

(Harvey et al., 1981). A trace amount of succinic acid was detected in young and aged commercial Cheddar cheeses reported by Harvey et al. (1981), and they also indicated that the concentration of succinic acid in Cheddar cheese was not closely related to the age of cheese. Succinic acid can be synthesized from citrate by NSLAB *Lact. plantarum* via the reductive tricarboxylic acid (TCA) cycle, and an occurrence of succinic acid was also suggested to be from gas-forming cultures (Mullin and Emmons, 1997; Dudley and Steele, 2004).

Changes in Formic Acid

Formic acid content was significantly ($P < 0.05$) affected by time, and was not affected by salting levels, sodium gluconate addition, and interaction terms (Table 3). Formic acid content in cheeses with 2% salting level and 0% and 0.5% sodium gluconate addition remained constant from 1-week to 3-month of ripening, followed by an increase from 3-month to 9-month of ripening and a decrease from 9-month to 12-month of ripening. The acid in cheese with 2% salting level and 1% sodium gluconate addition increased from 1-week to 6-month of ripening, followed by a decrease from 6-month to 12-month of ripening. The acid in cheeses with 2.5% salting level and 0% and 0.5% sodium gluconate addition increased from 1-week to 9-month of ripening followed by a slight decrease from 9-month to 12-month of ripening. Finally, the acid in cheese with 2.5% salting level and 1.0% sodium gluconate addition dramatically increased from 1-week to 6-month of ripening and followed by a sharp decrease from 6-month to 12-month of ripening.

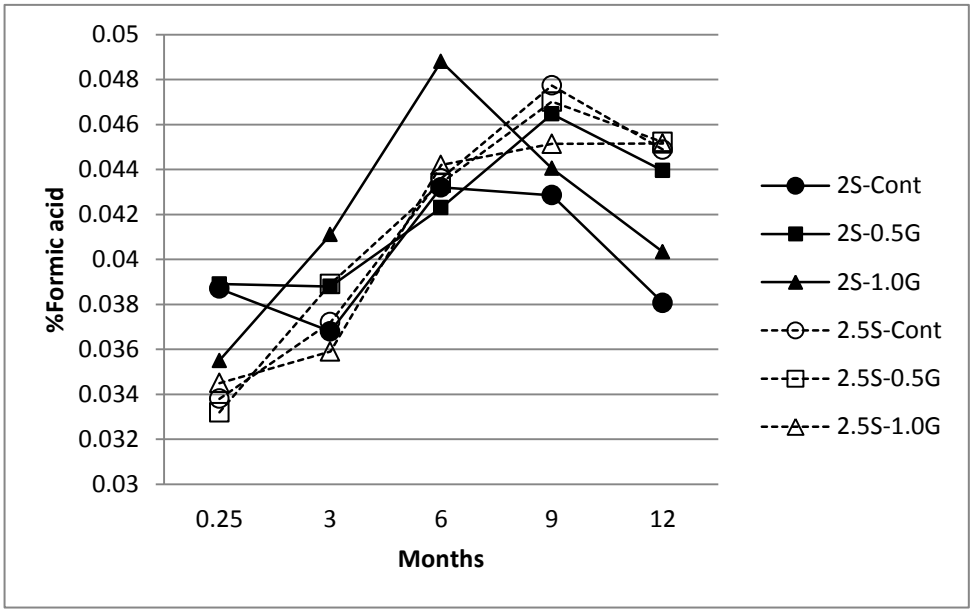

Figure 8. % Formic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Califano and Bevilacqua (2000) observed an initial increase in formic acid followed by a slight decrease in Gouda cheese during ripening. A similar pattern was observed by Upreti et al. (2006), where they reported a constant formic acid content in Cheddar cheese from 4-week to 12-week of ripening, followed by a slight increase from 12-week to 32-week and a decrease from 32-week to 48-week of ripening. Bevilacqua and Califano (1992), de Llano et al. (1996), and Akalin et al. (2002) reported a slight increase in concentration of formic acid during ripening of Port Salut Argentino cheese,

Afuega'l Pitu cheese, and Pickled White cheese, respectively. The increase in formic acid during ripening has been suggested to be form the presence of *S. thermophilus* and *L. bulgaricus*, which produce formic acid from lactose (Bevilacqua and Califano, 1992; Akalin et al., 2002).

Changes in Uric Acid

Uric acid content was significantly ($P < 0.05$) affected by salting level, and time, and was not affected by sodium gluconate addition, and interaction terms (Table 3). Figure 9 shows that uric acid content in cheeses from all treatments increased from 1-week to 9-month of ripening followed by a decrease from 9-month to 12-month of ripening. Cheeses with 2% salting level had higher uric acid content than cheeses with 2.5% salting level from 6-month to 12-month of ripening. De Llano et al. (1996), Akalin et al. (2002), and Upreti et al. (2006) reported a constant uric acid concentration during early ripening followed by an increase during ripening in Afuega'l Pitu cheese, Pickled White cheese, and Cheddar cheese, respectively. On the contrary, Bevilacqua and Califano reported a decrease in uric acid content during ripening of Port Salut Argentino cheese, and Buffa et al. (2004) reported a very low concentration of uric acid in 1-day-old goat's milk cheese, but uric acid could not be detected as the cheese aged.

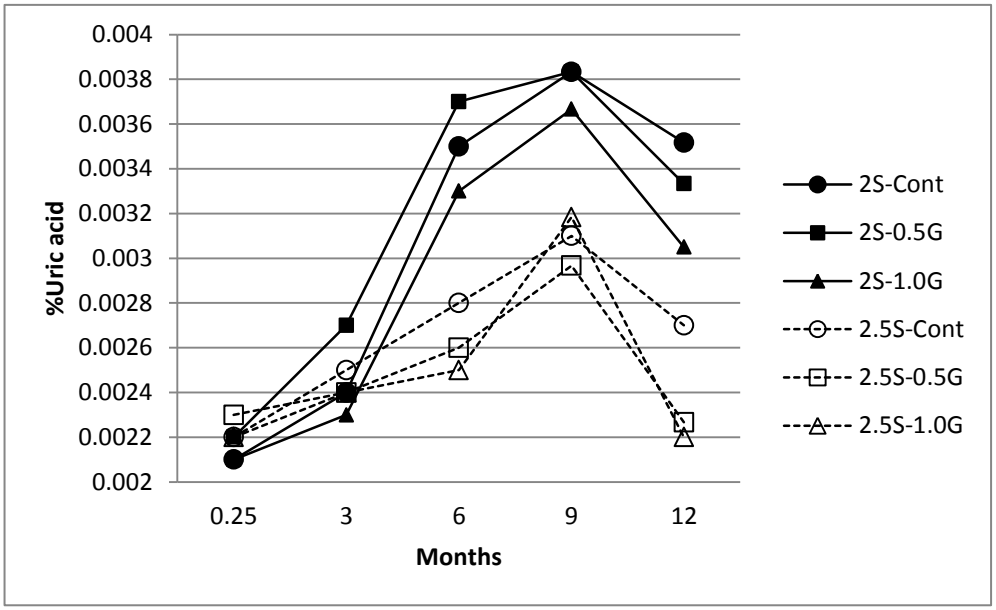

Figure 9. % Uric acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Changes in Acetic Acid

Acetic acid content was significantly ($P < 0.05$) affected by salting level, and time, and was not affected by sodium gluconate addition, and interaction terms (Table 3). Figure 10 shows that acetic acid content in cheese with 2% salting level and 0% sodium gluconate addition increased from 1-week to 6-month of ripening, then decreased from 6-month to 12-month of ripening. Acetic acid content in other treatments increased from 1-week to 9-month of ripening, then decreased from 9-month to 12-month of ripening. A

similar pattern was observed by Upreti et al. (2006), where they reported an increase of acetic acid in Cheddar cheese from 4-week to 32-week of ripening followed by a decrease from 32-week to 48-week of ripening.

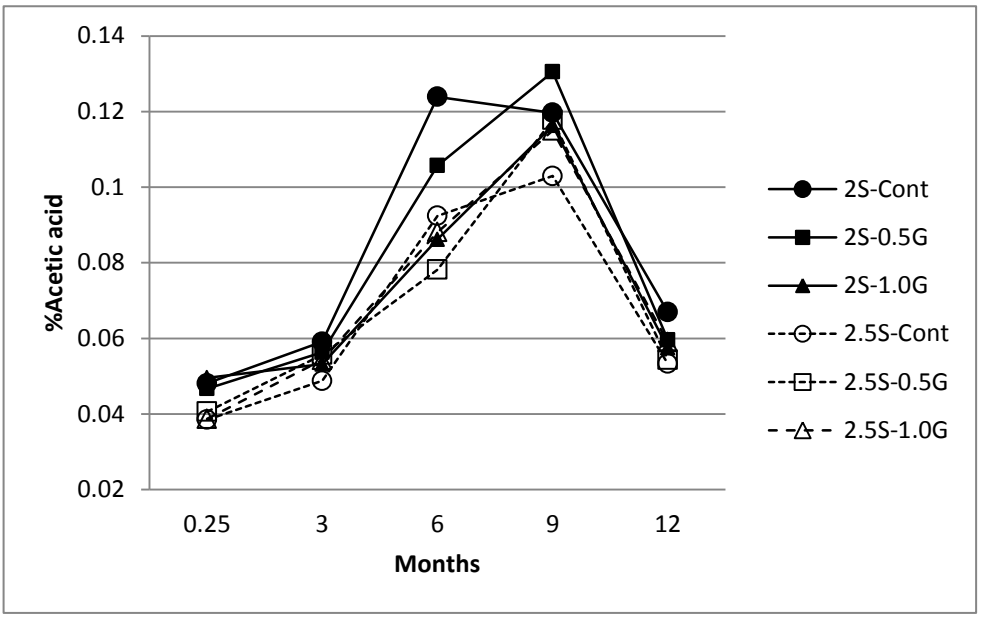

Figure 10. % Acetic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

St-Gelais et al. (1991) and Lues and Bekker (2002) reported an initial decrease of Acetic acid content in Cheddar cheese-like products and Cheddar cheese, respectively, from about 3-week to 4-month followed by a slow increase up to 6-month of ripening.

Marsili (1985), McGregor and White (1990), and Bouzas et al. (1993) observed a steady increase in Acetic acid during Cheddar cheese ripening, which was confirmed by Ong and Shah (2008). An increase in acetic acid over time suggests that acetic acid can be produced from citrate and lactose by *Leuconostoc* ssp., or amino acids by lactobacilli, which provide an indicator of the degree of hetero-fermentative metabolism (Lues and Bekker, 2002; Buffa et al., 2004). In contrast to other studies, the increase followed by the decrease of acetic acid content in our cheeses could indicate the role of acetic acid as an intermediate in biochemical pathways (Upreti et al., 2006).

Changes in Propanoic Acid

Propanoic acid content was significantly ($P < 0.05$) affected only by time, and was not affected by salting levels, sodium gluconate addition, and interaction terms (Table 3). Figure 11 shows that propanoic acid content in all cheese treatments steeply increased approximately 11 to 12-fold from 1-week to 12-month of ripening. An increase in propanoic acid content in Cheddar cheese during ripening was also reported by others (Marsili, 1985; McGregor and White, 1991; Lues and Botha, 1998; Upreti et al., 2006). The increase in propanoic acid content over time was also observed in Cheddar cheese-like products (St.-Galais et al., 1991), Port Salut Argentino cheese (Bevilacqua and Califano, 1992), Afuega'l Pitu cheese (de Llano et al., 1996), Gouda type cheese (Califano and Bevilacqua, 2000), Pickled White cheese (Akalin et al., 2002), and goat's milk cheese (Buffa et al., 2004).

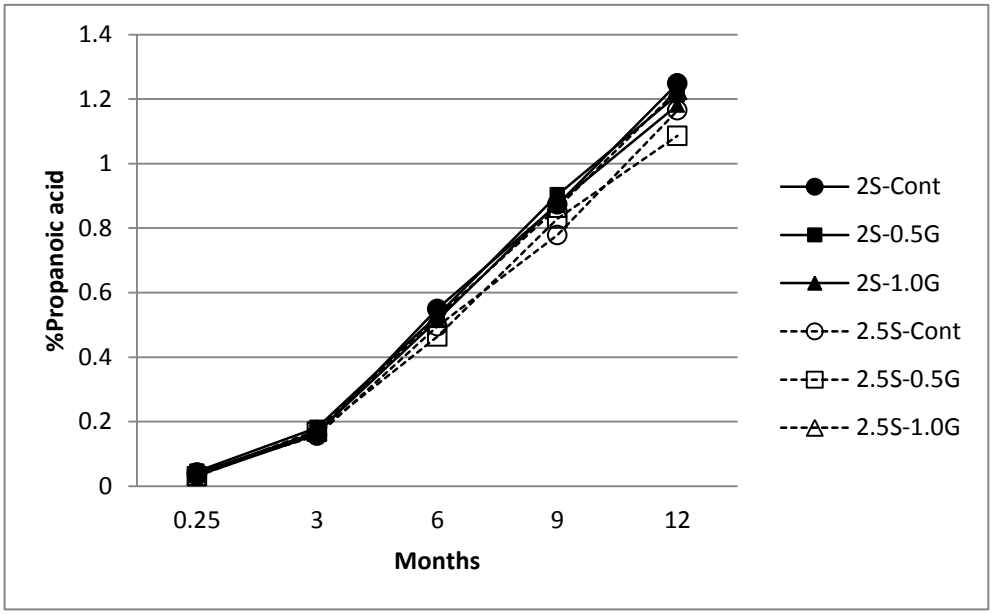

Figure 11. % Propanoic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

It was suggested that propanoic is the best predictor for glycolytic activity during Cheddar cheese ripening, because the concentration of propanoic acid gradually increases over time (Marsili, 1985). It has been reported that the presence of propanoic acid is closely related to the intracellular lipases excreted during the death of starter lactococci, the intensity secondary microflora and theirs esterase and lipase activities that took place during ripening, non-specific esterase activities, and proteolysis of side-chain from casein

amino acids (St.-Galais et al., 1991; Califano and Bevilacqua, 2000; Akalin et al., 2002; Upreti et al., 2006).

Changes in Butanoic Acid

Butanoic acid content was significantly ($P < 0.05$) affected only by time, and was not affected by salting levels, sodium gluconate addition, and interaction terms (Table 3). Figure 12 shows that butanoic acid content in all cheese treatments sharply increased from 1-week to 9-month of ripening. Butanoic acid in some treatments including all treatments from 2% salting level, and cheese with 2.5% salting level and 0.5% sodium gluconate addition slightly decreased from 9-month to 12-month of ripening. An increase in butanoic acid content in Cheddar cheese during ripening was also observed by others (Marsili, 1985; Lues and Botha, 1998; Upreti et al., 2006). The increase in butanoic acid content over time was also observed in Cheddar cheese-like products (St.-Galais et al., 1991), Port Salut Argentino cheese (Bevilacqua and Califano, 1992), Afuega'l Pitu cheese (de Llano et al., 1996), Gouda type cheese (Califano and Bevilacqua, 2000), and Pickled White cheese (Akalin et al., 2002). The activities of lipases and esterases from starter lactococci and secondary microflora have been suggested to be the source of an increase in butanoic acid content during ripening (Califano and Bevilacqua, 2000; Akalin et al., 2002). Lactose as a source of butanoic acid in Cheddar cheese has also been suggested (Upreti et al., 2006).

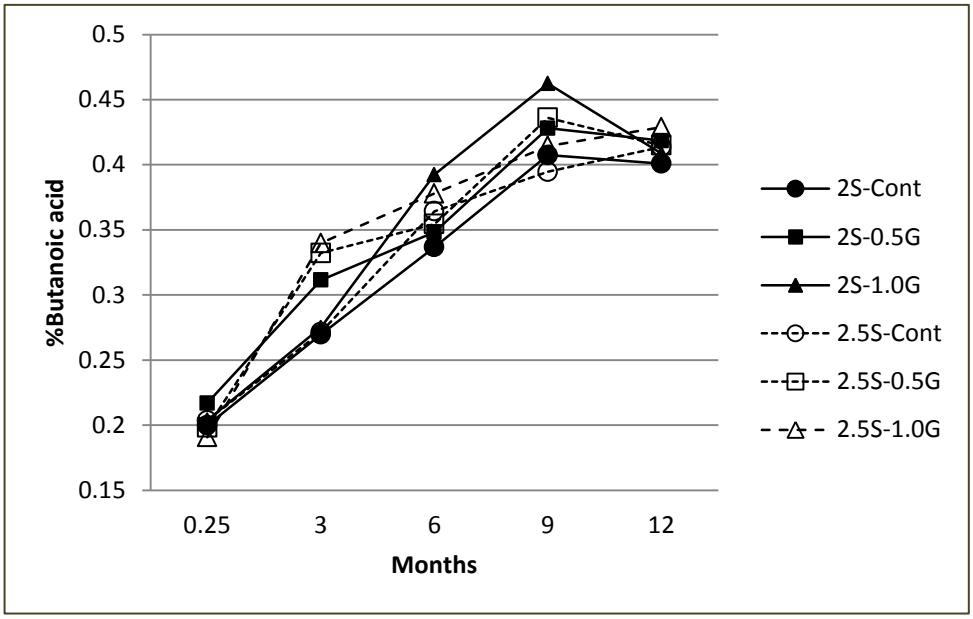

Figure 12. % Butanoic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

CONCLUSIONS

Results from this study suggest that sodium gluconate addition caused an increase in the lactose content and a decrease lactic acid content, while sodium gluconate did not affect other organic aids (citric, orotic, formic, uric, acetic, propanoic, and butyric acids) during Cheddar cheese ripening. Although sodium gluconate had an effect on lactic acid, which is main organic acid found in Cheddar cheese during ripening, a combination and a balance of different water-soluble organic acids influence the flavor development of

Cheddar cheese. Thus, it is possible that sodium gluconate addition did not have any negative impact on flavor development of Cheddar cheese during ripening resulting from organic acids.

REFERENCES

Adda, J., J. C. Gripon, and L. Vassal. 1982. The chemistry of flavor and texture generation in cheese. Food Chem. 9: 115-129.

Akalin, A. S., S. Gönç, and Y. Akbaş. 2002. Variation in organic acids content during ripening of Pickled White cheese. J. Dairy Sci. 85: 1670 – 1676.

Akalin, A. S. and S. Gönç. 1996. The determination of orotic acid in ruminant milks using HPLC. Milchwissenschaft. 51: 554 – 556.

Beresford, T. P., N. A. Fitzsimons, N. L. Brennan, and T. M. Cogan. 2001. Recent advances in cheese microbiology. Int. Dairy. J. 11: 259 – 274.

Bevilacqua, A. E. and A. N. Califano. 1989. Determination of organic acids in dairy products by high performance liquid chromatography. J. Food Sci. 54: 1076-1079

Bevilacqua, A. E. and A. N. Califano. 1992. Changes in organic aids during ripening of Port Salut Argentino cheese. Food Chem. 43: 345 – 349.

Bouzas, J., C. A. Kantt, F. Bodyfelt, and J. A. Torres. 1991. Simultaneous determination of sugars and organic acids in Cheddar cheese by high-performance liquid chromatography. J. Food Sci. 56: 276-278.

Buffa, M., B. Guamis, J. Saldo, A. J. Trujillo. 2004. Changes in organic acids during ripening of cheeses made from raw, pasteurized or high-pressure-treated goat's milk. Lebensm.-Wiss. u.-Technol. 37: 247 – 253.

Califano, A. N. and A. E. Bevilacqua. 2000. Multivariate analysis of the organic acids content of Gouda type cheese during ripening. J. Food Compos. Anal. 13: 949 – 960.

Crow, V., B. Curry, and M. Hayes. 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. Int. Dairy J. 11: 275-283.

de Llano, D. G., A. Rodriguez, and P. Cuesta. 1996. Effect of lactic starter cultures on the organic acid composition of milk and cheese during ripening – analysis by HPLC. J. Applied Bact. 80: 570 – 576.

Díaz-Muñiz, I., D. S. Banavara, M. F. Budinich, S. A. Rankin, E. G. Dudley, and J. L. Steele. 2006. *Lactobacillus casei* metabolic potential to utilize citrate as an energy source in ripening cheese: a bioinformatics approach. J. Applied Micro. 101: 872 – 882.

Dudley, E. G. and J. L. Steele. 2004. Succinate production and citrate catabolism by Cheddar cheese nonstarter lactobacilli. J. Applied Micro. 98: 14 – 23.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701–1710.

Guinee, T. P. and P.F. Fox. 2004. Salt in Cheese: Physical, Chemical and Biological Aspects, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp. 207-259.

Harvey, C. D., R. Jenness, and H. A. Morris. 1981. Gas chromatographic quantitation of sugars and nonvolatile water-soluble organic acids in commercial Cheddar cheese. J. Dairy Sci. 64: 1648-1654.

Hegazi, F. Z. and I. G. Abo-Elnaga. 1990. Dissimilation of organic acids by dairy lactic acid bacteria. Die Nahrung. 34: 791 – 801.

Larson, B. L. and H. M. Hegarty. 1979. Orotic acid in milks of various species and commercial dairy products. J. Dairy Sci. 62: 1641 – 1644.

Lues, J. F. R. and A. C. M. Bekker. 2002. Mathematical expressions for organic acids in early ripening of a Cheddar cheese. J. Food Compos. Anal. 15: 11 – 17.

Lues, J. F. R. and W. C. Botha. 1998. Relationship amongst South African processed, young and matured Cheddar cheese pertaining to organic acid content and non-starter population. Food Res. Int. 31: 449 – 457.

Marsili, R.T. 1985. Monitoring chemical changes in Cheddar cheese during aging by high-performance liquid chromatography and gas chromatography technique. J. Dairy Sci. 68: 3155-3161.

Marsili, R.T., H. Ostapenko, R. E. Simmons, and D. E. Green. 1981. High performance liquid chromatographic determination of organic acids in dairy products. J. Food Sci. 46: 52-57.

McGregor, J. U. and C. H. White. 1990. Effect of enzyme treatment and ultrafiltration on the quality of low fat Cheddar cheese. J. Dairy Sci. 73: 571 – 578.

McGugan, W.A., D. B. Emmons, and E. Larmond. 1979. Influence of volatile and nonvolatile fractions on intensity of Cheddar cheese flavor. J. Dairy Sci. 62: 398-403.

McSweeney, P.L.H. 2004a. Biochemistry of cheese ripening. Int. J. Dairy Tech. 57: 127-144.

McSweeney, P.L.H. 2004b. Biochemistry of cheese ripening: introduction and overview, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 347-360.

McSweeney, P. L. H. and P. F. Fox. 2004. Metabolism of residual lactose and of lactate and citrate, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 361 – 371.

Mullin, W. J. and D. B. Emmons. 1997. Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromatography. Food Res. Int. 30: 147 – 151.

Navder, K. P., R. S. Huang, E. B. Fryer, and H. C. Fryer. 1990. Effects of fermentation and storage on the concentration of orotic acid and uric acid in skim milk. J. Food Sci. 55: 585 – 586.

Ong, L. and N. P. Shah. 2008. Influence of probiotic *Lactobacillus acidophilus* and *L. helveticus* on proteolysis, organic acid profiles, and ACE-inhibitory activity of Cheddar cheeses ripened at 4, 8, and 12°C. J. Food Sci. 73: M111 – M120.

Panari, G. 1986. HPLC of organic acids: an approach to cheese analysis. Milchwissenchaft. 41: 214-216.

Phadungath, C. and L. E. Metzger. 201X. Effect of sodium gluconate on the solubility of calcium lactate. J. Dairy Sci. (submitted)

Phadungath, C. and L. E. Metzger. 2008. Manufacture of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN.

Roy, D. 1991. Salt stress in growth and acid production of *Lactobacillus helveticus* strain Milano. Letters Appl. Microbiol. 12: 207 – 211.

Saidi, B. and J. J. Warthesen. 1989. Analysus and stability of orotic acid in milk. J. Dairy Sci. 72: 2900 – 2905.

SAS Institute. 1990. SAS User's Guide: Statistics, Version 6.0 ed. SAS lnst. Inc., Cary, NC.

St-Gelais, D., G. Doyon, J. R. Rolland, and J. Goulet. 1991. Sugar and organic acid concentrations during ripening of Cheddar cheese-like products. Milchwissenchaft. 46: 288 – 291.

Thomas. T. D., D. C. Ellwood, and V. M. C. Longyear. 1979. Change from homo- to heterolactic fermentation by Streptococcus *lactis* resulting from glucose Limitation in anaerobic chemostat cultures. J. Bacteriol. 138: 109 – 117.

Turner, K. W. and T. D. Thomas. 1980. Lactose fermentation in Cheddar cheese and the effect of salt. N. Z. J. Dairy Sci. Technol. 15: 265 – 276.

Upreti, P., L. L. McKay, and L. E. Metzger, L.E. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. J. Dairy Sci. 89: 429-443.

Zeppa, G., L. Conterno, and V. Gerbi. 2001. Determination of organic acids, sugars, diacetyl, and acetoin in cheese by high-performance liquid chromatography. J. Agric. Food Chem. 49: 2722 – 2726.

Zeppa, G. and L. Rolle. 2008. A study on organic acid, sugar and ketone contents in typical Piedmont cheeses. Ital. J. Food Sci. 1: 127 – 139.

Chapter 5. The effect of sodium gluconate on pH, water-soluble calcium, lactose, lactic acid and calcium lactate crystal formation during Cheddar cheese ripening

Sodium gluconate increases the solubility of calcium lactate in model solutions by forming soluble sodium-lactate-gluconate complexes. However, its effect on the main components responsible for calcium lactate crystals in Cheddar cheese, which are lactic acid and water-soluble calcium (WSC), has not been reported. The objective of this study was to determine the effect of sodium gluconate on pH, lactose, lactic acid, and WSC changes during Cheddar cheese ripening. Six Cheddar cheeses with two salting levels (2 and 2.5%) and three sodium gluconate levels (0, 0.5 and 1%) were manufactured in triplicate. Composition and chemical analysis was performed at 1 week of ripening, and at 3, 6, 9, and 12 months of ripening. Cheeses were analyzed for pH, lactose and lactic acid, and WSC. Compositional analyses at 1 week indicated that sodium gluconate addition had a significant effect on cheese pH, moisture, Na, lactose, and lactic acid. Cheddar cheeses from both 2% and 2.5% salt levels with 0.5 and 1.0% sodium gluconate exhibited higher pH than the control cheeses throughout the ripening time. HPLC results from Cheddar cheeses from both 2% and 2.5% salt levels indicated that cheeses with higher concentration of sodium gluconate addition had a higher concentration of lactose, but lower concentration of lactic acid when compared to the control cheeses throughout the ripening time. WSC results indicated that Cheddar cheeses from both 2% and 2.5% salt levels with higher concentration of sodium gluconate addition had lower WSC concentration when compared to the control cheeses throughout the ripening time. From

the results, we concluded that sodium gluconate could have an effect on starter culture activity and could also act as buffering agent, which would cause a higher cheese pH. A higher cheese pH resulted in less soluble of calcium in the cheese serum; thus, resulting in less calcium and lactate ions in the cheese serum.

INTRODUCTION

Calcium lactate crystals (CLC) can appear within and on the surface of hard-type cheese especially Cheddar cheese after approximately four to six months of aging. In a typical Cheddar cheese, the concentration of soluble calcium and lactate are in excess of their solubility at 4°C. Since calcium and lactate ions are in a supersaturate state in the cheese serum phase, crystallization at nucleation sites occurs. A continuous movement of calcium and lactate ions to the nucleation sites causes the sites to become macro crystals (Dybing et al., 1988; Kubantseva and Hartel, 2002; Swearingen et al., 2004). One potential approach for preventing CLC is to add sodium gluconate to Cheddar cheese. In a previous study (Phadungath and Metzger, 201x), we determined that sodium gluconate can increase the solubility of calcium and lactate by forming complexes with calcium and lactate ions, and removing the calcium and lactate ions from being available for the formation of CLC. In the previous study, seven calcium lactate solutions (5.31% w/w) with seven level of sodium gluconate (0, 0.5, 1, 1.5, 2, 3, and 4% w/w) were made. Solutions were stored at 7°C for 21 days. The solutions were visually inspected for CLC formation, analyzed for lactic acid and gluconic acid by HPLC and for calcium by Atomic Absorption Spectroscopy. We determined that CLC were formed in the solution with 0% gluconate after the first day of storage, and the HPLC results indicated that there

was a higher concentration of calcium and lactic acid in the filtrate from the solutions containing added gluconate. These results demonstrated that sodium gluconate can increase the solubility of calcium lactate.

One of many important functions of the casein micelle is to solubilize calcium phosphates in milk (Farrell Jr. et al., 2006). The dry matter of bovine casein has been found to consist of about 94% protein and 6% mineral, which is colloidal calcium phosphate (CCP) (Horne, 2006). The relationship between CCP and casein micelles has been vigorously investigated for over a century. However, this relationship has not yet been fully understood (Fox and Brodkorb, 2008). As hypothesized by De Kruif and Holt, CCP could be bound and stabilized by phosphopeptide portions of α_s- and β-caseins, resulting in the formation of calcium-phosphate nanoclusters or CCP. In addition, the formation of CCP is believed to generate casein micelle structure by randomly binding with phosphoproteins until a size limited colloid is formed. According to Horne and his dual-binding casein micelle structure model, CCP is considered to begin the process of casein micelle formation by acting like a bridge and neutralizing agent for the phosphoproteins, which hydrophobically interact to each other. The hydrophobic blocks of protein-protein interactions and the CCP linkage further generate the casein micelle formation. This casein micelle has a gel-like structure with embedded CCP and κ-caseins as chain terminator (Horne, 1998; Farrell Jr. et al., 2006). CCP along with hydrogen bonds, hydrophobic and electrostatic interactions are responsible for casein micelle stability. It was found that the micelles dissolve into small particles in milk solution once the CCP is removed by acidification, dialysis or Ca chelator; thus this phenomenon

suggests that CCP play an important role in cementing the micelles together (Fox and Brodkorb, 2008).

In milk, about two-third of the calcium is bound with phosphate as colloidal calcium phosphate (CCP) in the casein micelles. The remaining calcium is present as complexes with either phosphate or citrate or as ionic calcium in the serum phase of milk. During cheese manufacture, a large portion of the serum calcium is removed during whey drainage, and approximately 80 – 90% of the CCP is retained in the curd (Lucey and Fox, 1993). It has been suggested that casein bound calcium is the major source of calcium contributing to the CLC formation (Dybing et al., 1988). As the cheese pH decreases, CCP is solubilized into the cheese serum, thus causing an increase in water-soluble calcium (WSC) (Lucey and Fox, 1993), resulting in more Ca available for CLC formation. The transition from CCP to WSC has become the subject of interest, primarily on cheese texture and functionality, especially Cheddar cheese (Lee et al., 2005; Lucey et al., 2005; O'Mahoney et al., 2006; Upreti and Metzger, 2007) and Mozzarella cheese (Guinee et al., 2002; Joshi et al., 2003; Sheehan and Guinee, 2004). It is, however, difficult to study the shift in WSC and CCP in the cheese matrix, since WSC and CCP are influenced by the rate of acid production during cheese manufacture and proteolysis during cheese ripening. Acid production and changes in pH during ripening are influenced primarily by utilization of lactose and the balance of different organic acid production mainly lactic acid, causing the pH drop (Fox 1990), and buffering capacity of the cheese that resist pH change (Salaün et al, 2005). We recently reported the effect of sodium gluconate on the Cheddar cheese manufacture and composition (Phadungath and Metzger, 2008a), and determined that sodium gluconate influenced cheese pH and

moisture content. The differences in cheese pH and moisture content among treatments could influence further changes in lactic acid and WSC, which are the two main components responsible for CLC formation in Cheddar cheese during ripening. Thus, the objective of this study was to determine the effect of sodium gluconate on changes in pH, WSC, lactose and lactic acid during Cheddar cheese ripening.

MATERIALS AND METHODS

Experimental Design

A replicated 2×3 factorial designs was utilized for a Cheddar cheese making study. This design utilized three levels of sodium gluconate (0, 0.5 and 1%) and two levels of salt (2 and 2.5%) for a total of six treatments. The treatments were; TRT1 - 0% sodium gluconate + 2% salt, TRT2 – 0.5% sodium gluconate + 2% salt, TRT3 – 1.0% sodium gluconate + 2% salt, TRT4 - 0% sodium gluconate + 2.5% salt, TRT5 – 0.5% sodium gluconate + 2.5% salt, TRT6 - 1% sodium gluconate + 2.5% salt. Each treatment was manufactured in triplicate from three different lots of milk. The manufacturing protocols and cheese composition for Cheddar cheese in this study have been previously reported and shown in Table 1(Phadungath and Metzger, 2008a). The cheeses were ripened for 12 months, and changes in pH, water-soluble Ca, lactose lactic acid were monitored during ripening.

Table 1. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition**

Mean value (%)*	Treatments					
	2% Salt			2.5% Salt		
	Sodium gluconate (%)			Sodium gluconate (%)		
	0	0.5	1.0	0	0.5	1.0
pH (1 wk)	5.06^{a}	5.16^{ab}	5.24^{ab}	5.16^{ab}	5.28^{b}	5.32^{b}
Moisture	36.98^{a}	37.79^{ab}	38.14^{b}	37.01^{a}	37.54^{ab}	37.79^{ab}
Fat	32.98^{a}	32.18^{ab}	30.96^{b}	32.26^{ab}	31.73^{ab}	31.33^{b}
Protein	25.72	26.1	25.6	26.08	25.65	25.72
Na	0.61^{a}	0.74^{ab}	0.77^{b}	0.72^{ab}	0.78^{b}	0.82^{b}
Cl	0.99^{a}	1.08^{ab}	1.12^{ab}	1.20^{ab}	1.29^{b}	1.29^{b}
S/M	4.47^{a}	4.73^{a}	4.85^{ab}	5.36^{b}	5.68^{b}	5.66^{b}
Total Ca	0.73^{a}	0.72^{ab}	0.69^{b}	0.73^{ab}	0.72^{ab}	0.70^{ab}
P	0.5	0.49	0.48	0.5	0.49	0.49
Lactose (1 wk)	0.53^{a}	0.69^{ab}	0.85^{ab}	0.71^{ab}	0.85^{ab}	1.00^{b}
Lactic acid (1 wk)	1.56^{a}	1.35^{ab}	0.95^{c}	1.17^{b}	0.93^{c}	0.89^{c}
Gluconic acid	0	0.33	0.59	0	0.33	0.58

* ^{a,b,c} Means within the column not sharing common superscripts are different (Tukey HSD at $P < 0.05$).

Analyses of pH and water-soluble Ca

The cheese samples from the six treatments were analyzed for pH and water-soluble Ca at 1-week, and 3, 6, 9, and 12 months of ripening. The pH was measured using a combination glass electrode (accumet® accuCap™ Combination pH Electrodes, Fisher Scientific, Fair Lawn, NJ) and pH meter (Corning pH/ion meter 450, Corning, Inc., Corning, NY). Calcium was determined by an Atomic Absorption Spectroscopy (AAS) (Model 200; Perkin Elmer Corp., Norwalk, CT) at 1 week, 3, 6, 9 and 12 months of ripening with procedure adapted from Metzger et al. (2000). About 1.5-g cheese was

manually homogenized for 30 s with 15-mL of 60°C double distilled water using a high shear Omni mixer-homogenizer (model 17105, Omni International, Waterbury, CT). The extract was centrifuged (Jouan CR4-12 Centrifuges, Jouan, Inc., Winchester, VA) at 7,000 ×g for 10 min. Then the samples were held at 4°C for 10 min to solidify the fat layer, and the top fat layer was removed using a spatula. The supernatant was filtered through filter paper (Whatman #541, Whatman International Ltd., Maidstone, England). A 5-mL aliquot of filtered supernatant was transferred into a clean, pre-weighed centrifuge tube (Fisherbrand Higher-Speed Easy Reader Plastic Centrifuge Tubes, Fisher Scientific, Fair Lawn, NJ); 25-mL of 12% Trichloroacetic acid (TCA) (Fisher Scientific, Fair Lawn, NJ) was added to the tube, and the sample was centrifuged and filtered the same as the previous step. A 4-mL sample of collected filtrate from the previous step was transferred into a clean, pre-weighed snap-lid vial (Capitol Vial Inc., Auburn, Al); then 6-mL of 12% TCA, 0.4-mL of 5% lanthanum oxide (Sigma-Aldrich, Inc., St. Louis, MO), and 9.6-mL of double distilled water was added into the vial. The final solution for the sample was then analyzed for Ca using AAS. For sample quantification, each sample was aspirated into the AAS, which was equipped with a calcium lamp (0303-6017 Perkin Elmer Corp., Norwalk, CT). The reference standards prepared from calcium reference solution (1000ppm +/-1%/Certified, Fisher Scientific, Fair Lawn, NJ) were used to calibrate the AAS. The calcium reference solution was mixed with TCA, 5% lanthanum oxide, and distilled water to obtain reference standard containing 0, 4, 6, 8, 10, 12, and 16 mg/kg of calcium. All reference standards also contained 6% (w/v) trichloroacetic acid and 0.02% (w/v) lanthanum oxide.

Analyses of Lactic Acid and Lactose

The cheese samples from each treatment were analyzed for lactose and lactic acid at 1-week, 3, 6, 9, and 12 months of ripening using an HPLC procedure as described in a previous study (Phadungath and Metzger, 2008b). For the sample preparation, about 5-g cheese were manually homogenized for 90 s with 10-mL of 0.013N sulfuric acid (at 65°C) using a high shear Omni mixer-homogenizer (model 17105, Omni International, Waterbury, CT). The extract was centrifuged at (Jouan CR4-12 Centrifuges, Jouan, Inc., Winchester, VA) 7,000 ×g for 10 min. Subsequently, the samples were held at 4°C for 20 min to solidify the fat layer, whichwas removed with a spatula, and the supernatant was filtered through filter paper (Whatman #4; Whatman International Ltd., Maidstone, England). A 0.5 mL aliquot of filtered supernatant was poured into a 0.5-mL Microcon® (Millipore Corporation, Bedford, MA) centrifugal filter device with a molecular weight cut-off of 3000D and micro-centrifuged (Jouan A14 Microcentrifuges, Jouan, Inc., Winchester, VA) at 14,000 ×g for 20 min to remove the soluble peptides present in the filtrate. The collected filtrate from the micro-centrifuge was directly injected into the HPLC system.

The HPLC system (Beckman Coulter Inc., Fullerton, CA) was equipped with a photodiode array detector set at 210 nm, and data processing software (System Gold® HPLC, 32 Karat™ Software, Beckman Coulter Inc., Fullerton, CA). The system was externally equipped with an intelligent refractive index detector (JASCO Model RI-2031 Refractive index detector, Jasco, Inc., Easton, MD), and a column heater (Alltech® Model 6301 Column Heater, Alltech Associates Inc., Deerfield, IL). The column used

for separation of the analytes was a Rezex ROA-organic acid H^+ column (300x7mm, 8µm, Phenomenex) held at 65°C, with a cation H^+ microguard cartridge (Bio-Rad Laboratories, Hercules, CA). The analysis was performed isocratically at 0.6 mL/ min flow rate using 0.013 N sulfuric acid (Fisher Scientific, Fair Lawn, NJ) as the mobile phase. The refractive index detector was used for quantification of lactose. The separation of lactic acid was detected using the UV detector set at 210.

Surface Crystal Formation Study

Cheese samples were analyzed for surface crystal formation during ripening as shown in Figure 1. The cheese block from each treatment was sliced using a commercial meat-cheese slicer into 30 slices with 90 × 140 × 3.2 mm (3.5 × 5.5 × 0.125 inch) dimension, and all the slices were individually vacuum-sealed (Multivac® vacuum packaging machine; Koch, Inc., Kansas City, MO), in polyethylene bags (3 mil Nylon/PE pouches, Prime Source vacuum pouches, KOCH Supplies LLC, North Kansas City, MO) and stored at 7°C. Cheese slices were transferred into a room temperature environment (23°C) for 3-5 hours until the temperature of the entire cheese slice reached room temperature. This application was done on a weekly-basis for 4 weeks. Then the slices were transferred and stored in a refrigerator (4°C) and were transferred into the room temperature environment on a monthly-basis for 12 month. The slices were monthly evaluated visually for calcium lactate crystal (CLC) formation. At every month of ripening, both surfaces of all cheese slices were digitally scanned with optical 1200 x 2400 dpi resolution (Canon imageClass MF 5770 Laser Multifunction Printer - Copier - Fax – Scanner, Canon U.S.A., Inc., Lake Success, NY). The image processing was

adapted from Caccamo et al. (2004) and Rajbhandari and Kindstedt (2005). The JPEG files containing cheese images were processed using the Adobe Photoshop Elements 6 (Adobe Systems Incorporated, San Jose, CA). The images were first manually cropped to eliminate the excess packaging area, which result in the entire cheese surface as the active region to be further processed. The active region was subjected to the 'Spot Healing Brush' followed by the 'Unsharp Mask' to eliminate the glare from packaging crease. Then, the active region was undergone 'Auto Smart Fix' to highlight the surface crystal on the cheese slice.

Statistical Analyses

A 2 × 3 factorial model with 3 replications was used for statistical analysis and changes in the concentration of lactose and acids during ripening were analyzed using a repeated measures design (Table23). The PROC GLM procedure of SAS, which involved 2 factors (salt, and sodium gluconate level) as class variables and changes in the concentration of lactose and acids during ripening were analyzed using a repeated measures design (SAS Institute, 1990).

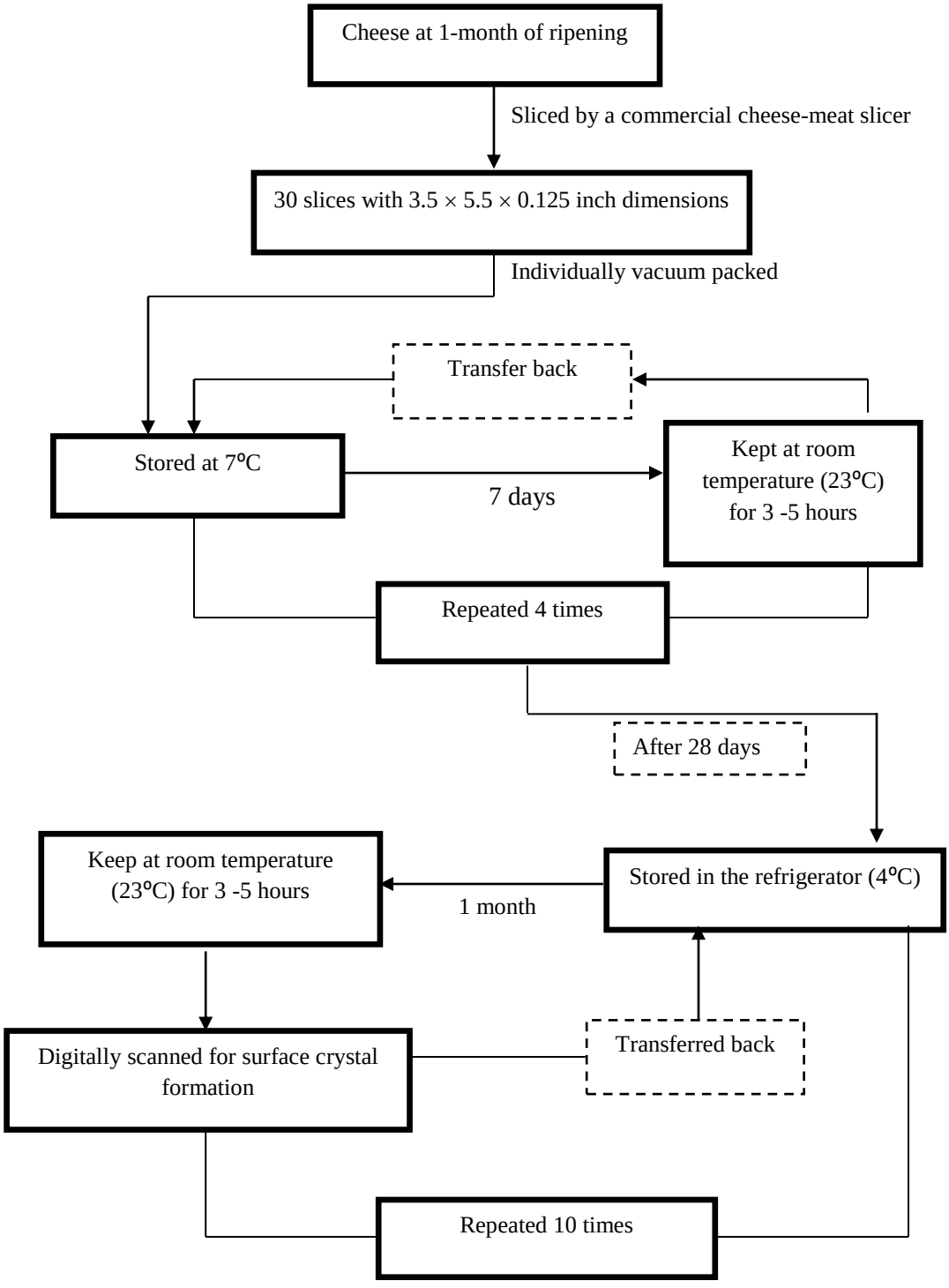

Figure 1. The surface crystal formation study diagram for Cheddar cheese with sodium gluconate addition during 12-month of ripening.

Table 2. Mean squares and probabilities (in parentheses) of changes in pH, %WSC, lactose and lactic acid in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months.

Factor	df	pH	%WSC	Lactose	Lactic acid
Whole plot					
Salt (S)	1	0.16 (0.103)	5.52×10^{-2}* (<0.01)	0.37* (< 0.01)	0.89* (< 0.01)
Gluconic acid (G)	2	0.19* (0.049)	1.97×10^{-2}* (0.014)	0.37* (< 0.01)	0.49* (< 0.01)
S × G	2	0.30×10^{-3} (0.994)	0.70×10^{-3} (0.802)	2.21×10^{-3} (0.922)	1.42×10^{-2} (0.762)
Error	12	5.16×10^{-2}	3.15×10^{-3}	2.67×10^{-2}	5.12×10^{-2}
Subplot					
Time (T)	4	0.061* (<0.01)	1.28×10^{-2}* (<0.01)	0.30* (< 0.01)	0.14* (< 0.01)
T × S	4	0.19×10^{-2} (0.792)	0.32×10^{-3} (0.805)	1.92×10^{-3} (0.945)	1.76×10^{-2} (0.057)
T × G	8	0.24×10^{-2} (0.836)	0.42×10^{-3} (0.828)	8.24×10^{-3} (0.611)	2.14×10^{-2}* (< 0.01)
T × S × G	8	1.3110^{-3} (0.969)	0.47×10^{-3} (0.783)	0.91×10^{-3} (0.999)	1.36×10^{-2} (0.080)
Error	48	0.47×10^{-2}	0.79×10^{-3}	1.04×10^{-2}	7.12×10^{-3}

*Statistically significant at *P*-value < 0.05.

RESULTS AND DISCUSSION

Changes in pH

The cheese pH during ripening was significantly ($P < 0.05$) affected by sodium gluconate addition and time, but was not affected by salting levels and interaction terms (Table 2). Figure 2 shows that the pH values in cheeses with sodium gluconate addition were higher than the pH values in cheeses without sodium gluconate addition at both 2% and 2.5% salt level throughout the ripening time. This phenomenon could be due to the buffering capacity of gluconate ions, which could modify the buffering capacity of the cheese matrix. As the cheese pH is shifted away from the isoelectric pH (pH 4.6), the electrostatic repulsion is increased, which caused a reduction in protein-protein interactions and an increase in protein-water interactions (De Kruif and Holt, 2003). Thus, the protein hydration is increased, which results in a subsequent increase in moisture content in cheeses.

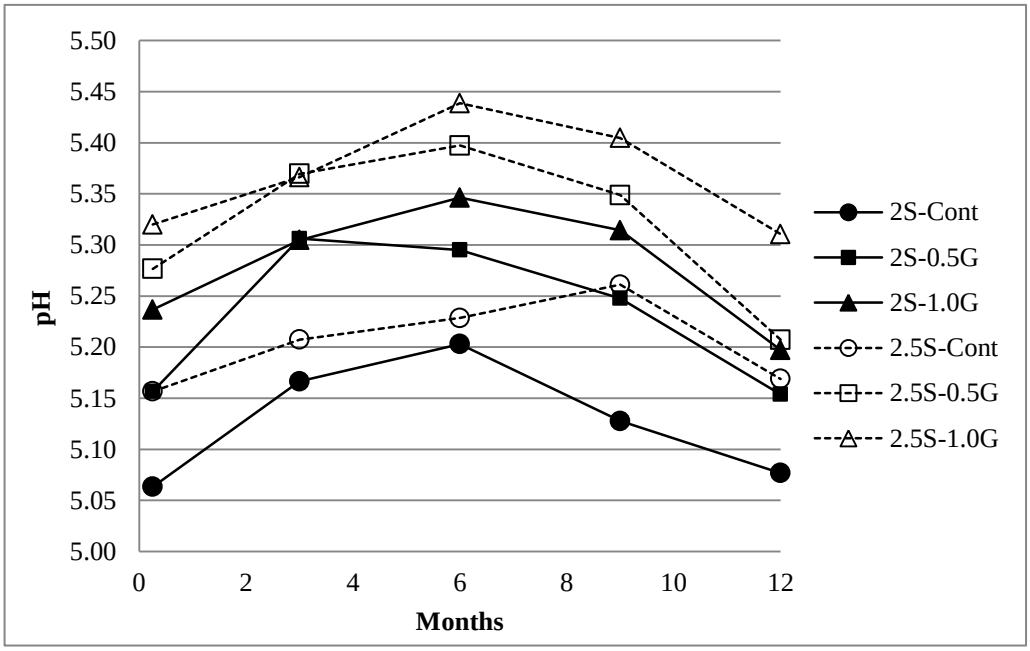

Figure 2. Changes in pH in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Changes in Lactose and Lactic acid Contents

Lactose content was significantly ($P < 0.05$) affected by salting levels, sodium gluconate addition, and time, but was not affected by interaction terms (Table 2). Lactic acid content was significantly ($P < 0.05$) affected by salting levels, sodium gluconate addition, time, and an interaction of time × sodium gluconate addition (Table 2). Changes in lactose and lactic acid content during ripening are shown in Figure 3(a) and 3(b), respectively. A reverse relationship exists between the lactose and lactic acid

concentration during ripening. Lactose content in all treatments decreased between 1-week and 12-month of ripening, while lactic acid content increased. Cheeses with 2.5% salting level had higher residual lactose than cheeses with 2% salting level throughout the ripening time, and vice versa for lactic acid content. It is shown in Table 1 that cheeses with 2.5% salting level also had a higher level of S/M (5.36 to 5.66% S/M) as compared to cheeses with 2% salting level (4.47 to 4.85% S/M). It has been previously reported that the growth of lactocci in Cheddar cheese is inhibited by a S/M higher than 4%, thus, resulting in a retardation in lactose fermentation during cheese ripening (Guinee and Fox 2004; Upreti et al. 2006). Thus, a higher residual lactose content and a lower lactic acid content in Cheddar cheese with 2.5% salting level as compared to those from cheese with 2% salting level was likely due to an impact from a higher S/M.

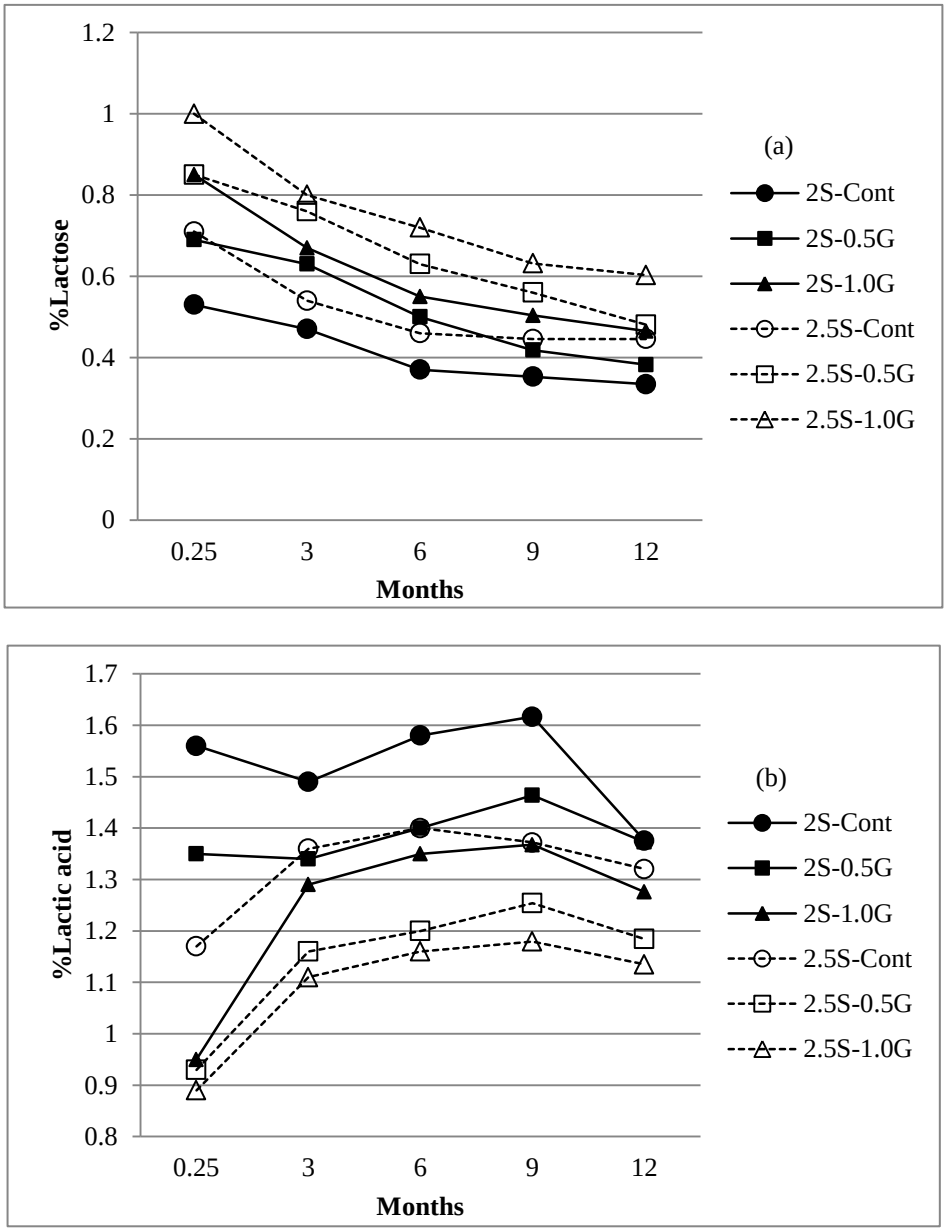

Figure 3. Changes in (a) % lactose and (b) % lactic acid in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Residual lactose content in cheeses with sodium gluconate addition were higher than the residual lactose content in cheeses without sodium gluconate addition at both salting levels throughout the ripening time, whereas, the lactic acid content in cheeses with sodium gluconate addition were lower than the lactic acid content in cheeses without sodium gluconate addition. As aforementioned, sodium gluconate contributed to a higher moisture content in cheese because its buffering capacity caused an increase in the cheese pH. The higher in moisture content could result in a higher salt retention in the cheese (higher Na and Cl as shown in Table 1). An increase in salt retention could decrease lactose fermentation by starter cultures, resulting in a higher residual lactose, and a reduction in lactic acid production. The reduction in lactic acid production in cheeses with sodium gluconate addition eventually causes an increase in cheese pH during ripening. In addition, sodium gluconate contributed to a higher Na content in cheese (Table 1). According to Roy (1991), NaCl had the most inhibitory effect on lactic acid bacteria growth when compared to $CaCl_2$, and KCl. Therefore, an increase in Na content could inhibit starter culture activity during cheese ripening, resulting in an increase in residual lactose and a reduction in lactic acid production. As suggested by Turner and Thomas (1980), lactose is normally fermented by starter LAB within the first week, and afterwards NSLAB is responsible. Thus, the residual lactose concentration left in our cheeses throughout the ripening time could indicate minimal LAB and NSLAB activity to metabolize residual lactose.

Changes in Water-soluble Ca

WSC was significantly ($P < 0.05$) affected by salting level, sodium gluconate addition, and time, but was not affected by interaction terms (Table 2). As shown in

Figure 4(a), the WSC increased between 1-week and 6-month, and remained stable after 6-month of ripening in all treatments, except 2% salt with no sodium gluconate addition, which decreased between 1-week and 3-month of ripening, then increased between 3 and 6-month of ripening. An increase in the WSC during cheese ripening is in agreement with other studies (Hassan et al., 2004; Lee et al., 2005; Upreti and Metzger, 2007). This phenomenon is believed to occur because insoluble and soluble Ca in cheese are not in the equilibrium state immediately after cheese manufacture, resulting in a driving force of the solubilization of CCP during initial cheese ripening (Lee et al, 2005). An increase in cheese pH during ripening from all treatments is in agreement with Upreti and Metzger (2007). They explained that this phenomenon is due to the restricted mobility and its slow equilibrium of Ca and phosphate in cheese. They further explained that during cheese ripening, proteolysis could cause a slow release of Ca and phosphate from CCP. If the Ca and phosphate ions are in the excess amount of lactic acid produced during ripening, phosphate ions could buffer the cheese pH to the original pH after salting; thus causing an increase in the cheese pH during ripening. This is also in accordance with our pH and lactic acid results, where cheeses with sodium gluconate addition had a lower lactic acid production and a higher pH. A shift in the ratio of WSC to total Ca is shown in Figure 4(b), and the result demonstrated that in most treatments, a shift of more than 10% occurred between 1-week and 6-month of ripening, which corresponded to the shift in pH during this ripening time. This is also in agreement with Upreti and Metzger (2007), where they reported a linear relationship between WSC (expressed as a % of total Ca) and the cheese pH.

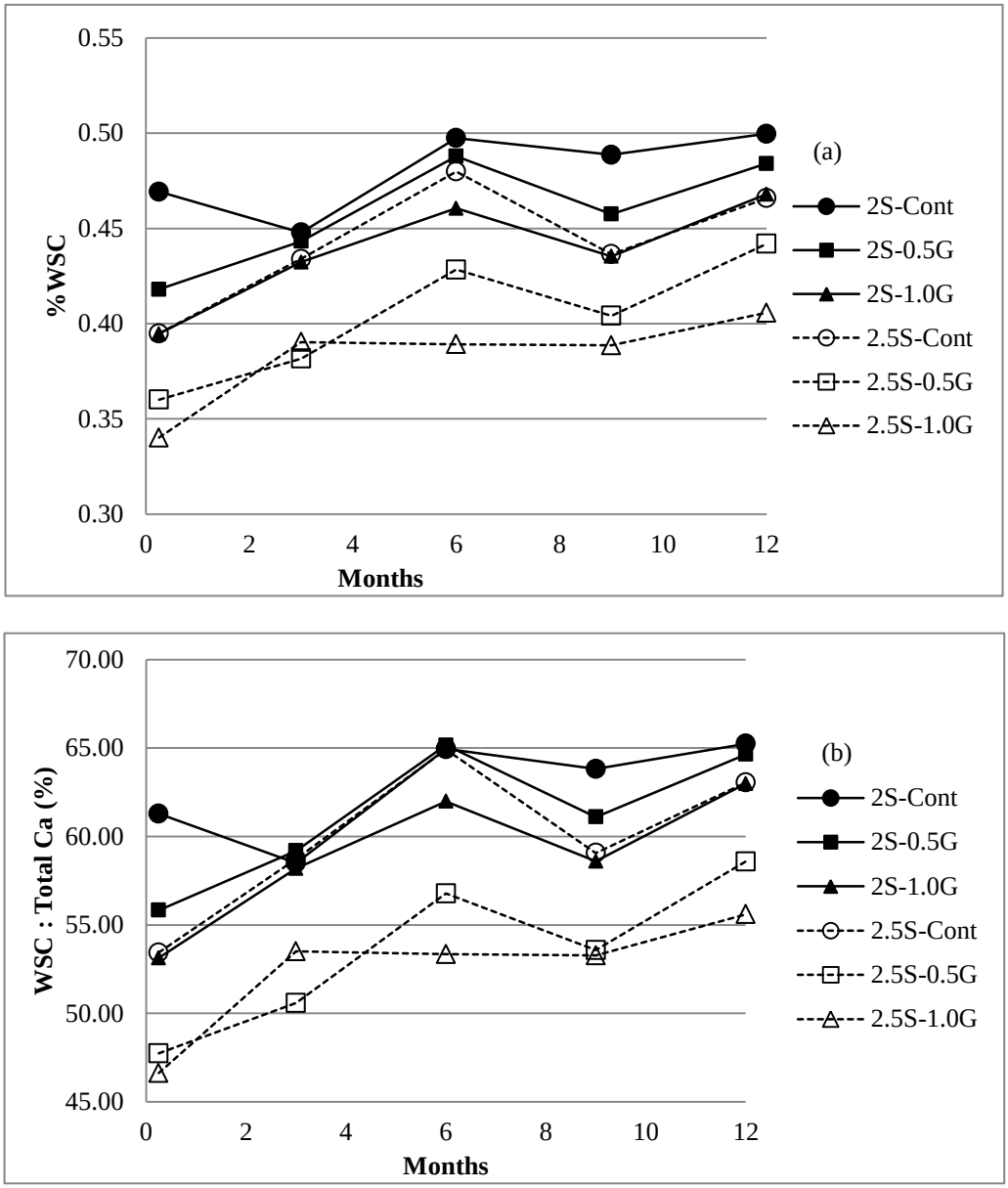

Figure 4. Changes in (a) % WSC and (b) % WSC (expressed as a % of total Ca) in Cheddar cheeses with added sodium gluconate during 1-week, 3, 6, 9, and 12-month of ripening. Six treatments are; (•)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

WSC in cheeses with 2.5% salt were lower than that in cheeses with 2% salt throughout the ripening time. As already mentioned, higher salt content results in higher S/M, which would affect starter culture activity and cause a higher pH. Because the decrease in pH from the range of 6.0 to 5.0 encourages more solubilization of Ca from CCP (insoluble state) to the water-soluble state (Pyne and Mcgann, 1960), cheeses with higher salt content and higher pH would have lower WSC. In addition, WSC in cheeses from both salting levels with sodium gluconate addition were lower than WSC in cheese without sodium gluconate addition throughout the ripening time. According to our Ca lactate model solution study, we hypothesized that sodium gluconate addition could chelate Ca and cause CCP to be solubilized into the cheese serum phase, which should result in an increase in WSC concentration. However, the present results indicate the opposite phenomenon. Because the ability of chelators to bind Ca ions depends on several factors, including salt concentration and electrostatic interaction (Svensson et al., 1992), and the competition between other ions and Ca to interact with the chelators (Patton et al., 2004), the ability of sodium gluconate to chelate Ca in the complex cheese matrix is not as simple as in the model system. The observed differences in WSC appear to be related to the differences in the cheese pH caused by the addition of sodium gluconate. From the results, cheeses with sodium gluconate addition had higher pH values and lower WSC content when compared to cheeses without sodium gluconate at both salting levels. This effect of pH was supported by Pastorino et al. (2003), where they reported an increase in soluble Ca content in Cheddar cheese as the cheese pH decreased from 5.3 to 4.7. A recent study by Karlsson et al. (2007) on the influence of pH on rennet-induced casein gels also indicated a decrease in CCP as the pH decreased from

6.21 to 5.90, which implied a transition from insoluble Ca to water-soluble state. Therefore, our results indicate that sodium gluconate addition causes an increase in the cheese pH, which causes a decrease in WSC.

Surface Crystal Formation

Table 3 represents the number of cheese slices from the total of 30 slices that had surface crystal formation during 12 months of ripening. Figure 5 and 6 are examples of the digital images of calcium lactate crystals on Cheddar cheese surface at 6-month, 9-month, and 12-month of ripening from 2% and 2.5% salting level, respectively. The capital D indicated definite crystals, which meant that the crystals were on the cheese surface and were visible without any magnification (Figure 5 – TRT1-6, TRT1-9, TRT1-12, and Figure 6 – TRT4-9, TRT4-12). The capital S indicated slight crystals, which meant that the crystals appeared around the edge of packaging or on the surface that required close inspection or the use of magnification to be seen (Figure 5 – TRT2-9, TRT2-12, and Figure 6 – TRT5-9, TRT5-12). For the 2% salting level, 17 cheese slices from the control cheese had definite crystals at 6 months of ripening, and by 10 months of ripening, all the cheese slices had definite crystals (Figure 5 – TRT1-6, -9, -12). Only 10 slices of cheese from 2% salting level with 0.5% sodium gluconate addition had slight crystals at 7 months of ripening, and by 12 months of ripening, 6 slices had definite crystals, 17 slices had slight crystals and 7 slices did not have and crystals (Figure 5 – TRT2-6, -9, -12). None of 2% cheese slices with 1% sodium gluconate addition had any crystals throughout the ripening time (Figure 5 – TRT3-6, -9, -12). For cheeses with the 2.5% salting level, 6 slices of cheese without sodium gluconate addition had slight

crystals at 6-month of ripening, and by 12-month of ripening, all slices had crystals with 11 slices for definite crystals and 19 slices for slight crystals (Figure 6 – TRT4-6, -9, -12). Cheese with 2.5% salting level with 0.5% sodium gluconate addition had 6 slices with slight crystals at 7-month of ripening, and by 12-month of ripening 12 slices had slight crystals (Figure 6 – TRT5-6, -9, -12). Cheeses with both salting levels 1% sodium gluconate addition did not have any crystals at all time (Figure 6 – TRT6-6, -9, -12).

Table 3. Number of cheese slices (from the total of 30 slices) that showed calcium lactate crystal (CLC) formation during 12 months of ripening.

	Treatments		**Number of slices during 12-month ripening**											
	Salt (%)	**Sodium gluconate (%)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	**2**	**0**	-	-	-	-	-	17D*	24D	25D	29D	30D	30D	30D
2	**2**	**0.5**	-	-	-	-	-	-	10S	14S	18S	1D, 18S	4D, 19S	6D, 17S
3	**2**	**1**	-	-	-	-	-	-	-	-	-	-	-	-
4	**2.5**	**0**	-	-	-	-	-	10S**	2D, 16S	2D, 18S	2D, 20S	8D, 14S	10D, 15S	11D, 19S
5	**2.5**	**0.5**	-	-	-	-	-	-	6S	6S	7S	10S	10S	12S
6	**2.5**	**1**	-	-	-	-	-	-	-	-	-	-	-	-

***D (definite crystals)** = Cheese slices shown to have crystals on the cheese surface and edges of the vacuum packaging that were visible without any magnification.

****S (slight crystals)** = Cheese slices shown to have very small amount of crystals on the edges of the packaging that required close inspection or magnification to be observed.

The cheese slices were temperature abused monthly and stored at the refrigerated temperature (~ 4°C) prior to digitally scanned; the chemical and compositional attributes could be altered and not exactly the same as those from the cheese blocks that were stored in the storage room (6-8°C) prior to analyses. Thus, it might not be entirely accurate to directly relate the present WSC and lactic acid results to the CLC formation on the cheese slices. In a similar study conducted by Johnson et el. (1990), Cheddar cheese samples were stored at either 4.4 or 7.2°C, were visually inspected for CLC formation and analyzed for lactic acid content. They reported that CLC developed faster and to a greater surface coverage in cheeses stored at 4.4°C than in cheeses stored at 7.2°C. In addition, they also reported that cheeses with CLC formation had higher total lactic acid and D-lactic acid content compared to cheeses without CLC formation. It has been previously reported that D-lactic acid is less soluble than L-lactic acid, thus causing more possibility for CLC formation in cheese (McSweeney and Fox, 2004). Although D-lactic acid was not analyzed in our study, the lactic acid results were in agreement with Johnson et al. (1990), where cheeses with CLC formation (TRT 1, 2, 4, and 5) had higher lactic acid content than cheeses without CLC formation (TRT 3 and 6). In addition, the surface crystal formation results indicate that visible crystals on the cheese surface started to occur at approximately 6-month of ripening, and surface crystal accumulated to a greater extent from 6-month to 12-month of ripening. This is in agreement with %WSC results, where % WSC (expressed as a % of total Ca) in cheeses with CLC formation (TRT 1, 2, 4, and 5) dramatically increased from 1-week to 6-month of ripening, then remained stable (TRT 1 and 2) or gradually increased (TRT 4 and 5) until 12-month of ripening.

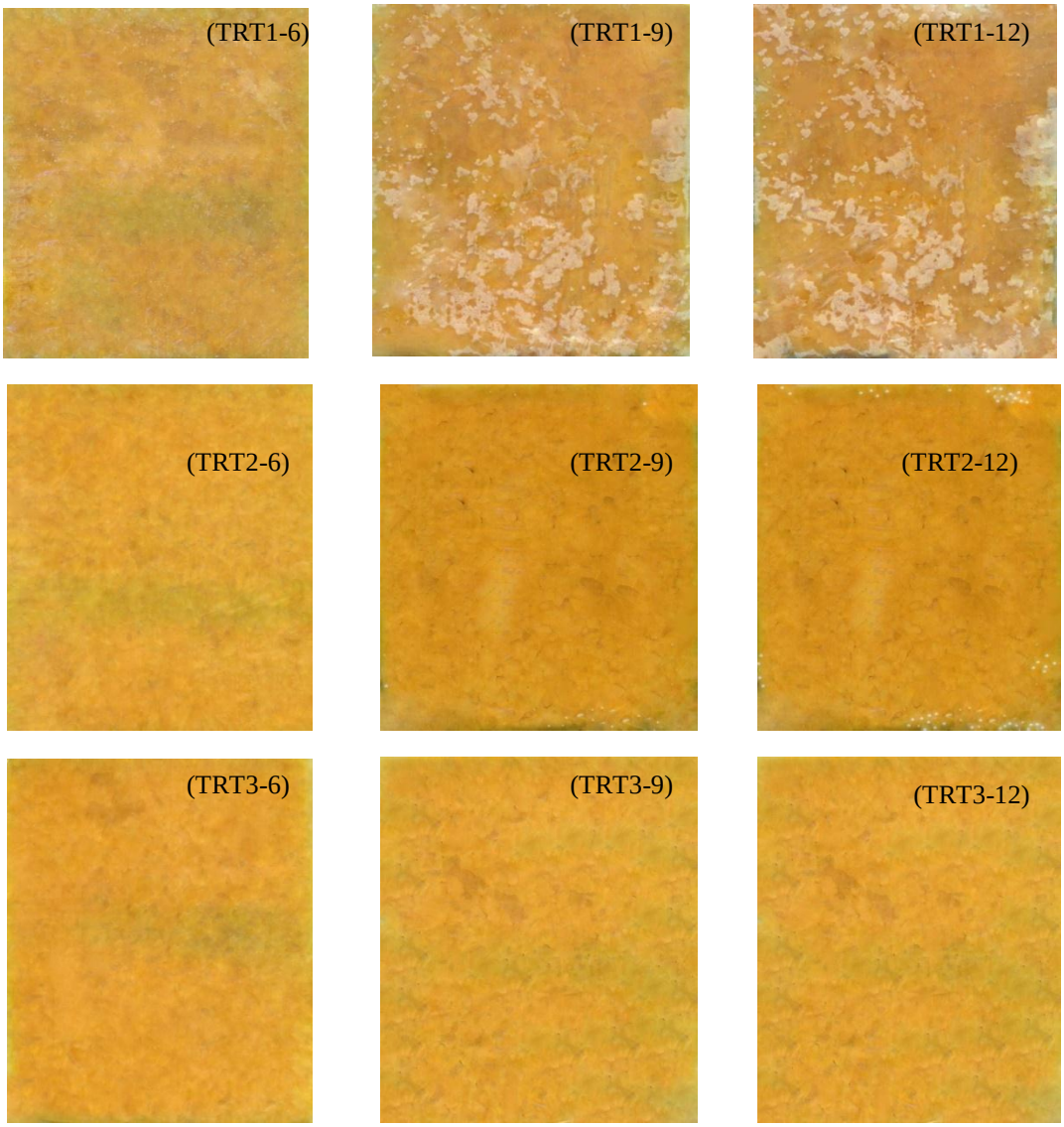

Figure 5. Digital images of calcium lactate crystals on Cheddar cheese surface. The cheese slices represent; (TRT1-6, TRT1-9, TRT1-12). 2% salting level + 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT2-6, TRT2-9, TRT2-12). 2% salting level + 0.5% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT3-6, TRT3-9, TRT3-12). 2% salting level +1. 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively.

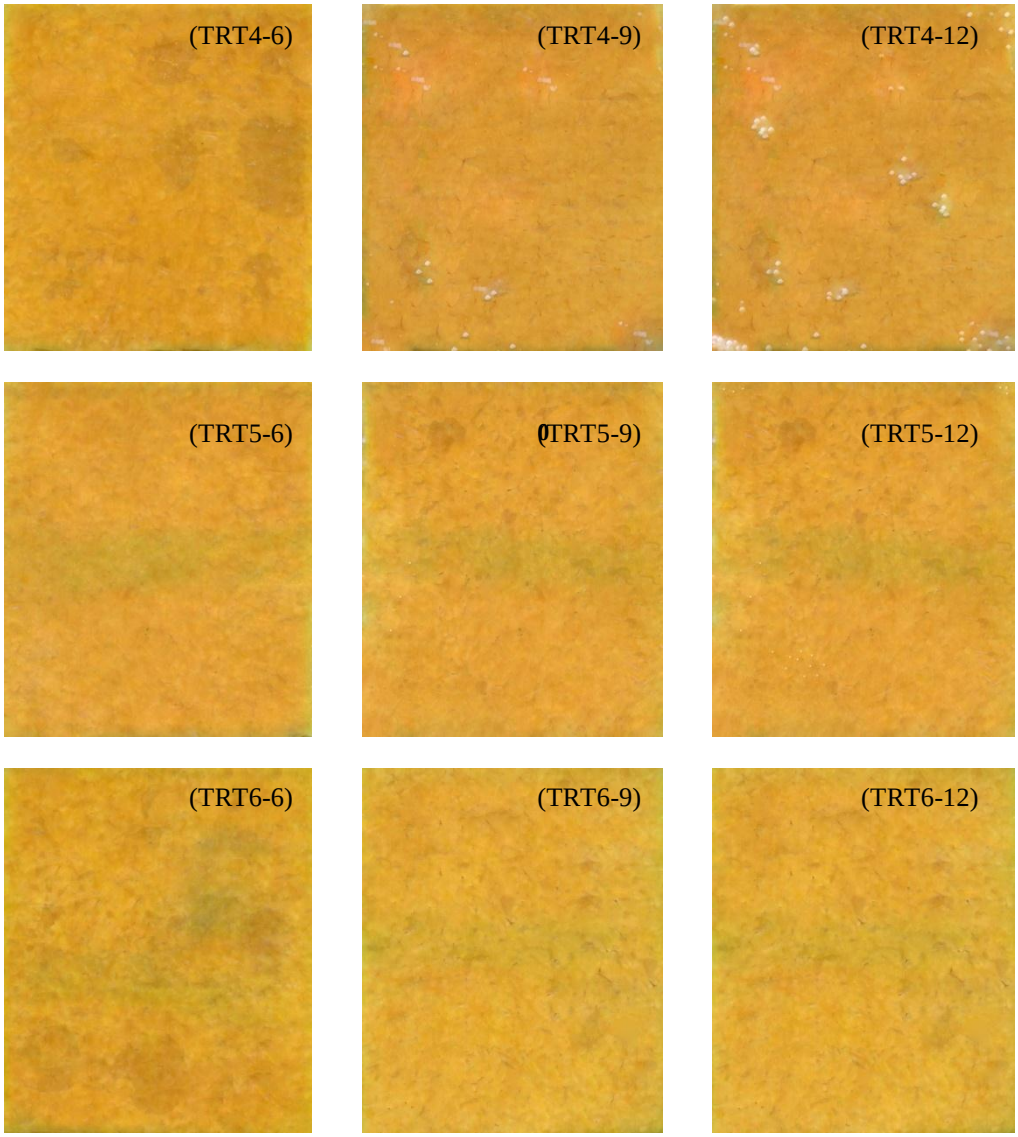

Figure 6. Digital images of calcium lactate crystals on Cheddar cheese surface. The cheese slices represent; (TRT4-6, TRT4-9, TRT4-12). 2.5% salting level + 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT5-6, TRT5-9, TRT5-12). 2.5% salting level + 0.5% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively; (TRT6-6, TRT6-9, TRT6-12). 2.5% salting level +1. 0% sodium gluconate addition at 6-month, 9-month, and 12-month of ripening, respectively.

The Possible Effect of Sodium Gluconate as a Calcium Lactate Crystal Inhibitor in Cheddar Cheese

From the results, sodium gluconate significantly ($P<0.05$) affect pH, WSC, lactose and lactic acid in Cheddar cheese during ripening. Cheeses with sodium gluconate addition had higher pH, lower WSC, higher lactose, and lower lactic acid content when compared to cheeses without sodium gluconate addition. Figure 7 summarizes the possible effects of sodium gluconate on CLC inhibition in Cheddar cheese. After sodium gluconate was applied to the cheese curds after salting, two circumstances probably occur. Firstly, sodium gluconate contributed to more Na content in cheese, which retarded the growth of starter lactococci. The retardation of starter lactococci growth caused a reduction in the fermentation of lactose, which then led to a reduction in lactic acid production and a higher cheese pH. A second factor is that sodium gluconate acts as buffering agent and resists a pH reduction. The higher pH in cheeses with sodium gluconate addition results in a less WSC. Both of these factors result in lower lactic acid and WSC content in the serum phase of the cheese. Thus, the use of sodium gluconate reduced calcium lactate crystal formation by limiting the concentration of substrates involved in crystal formation as well as increasing the solubility of calcium lactate.

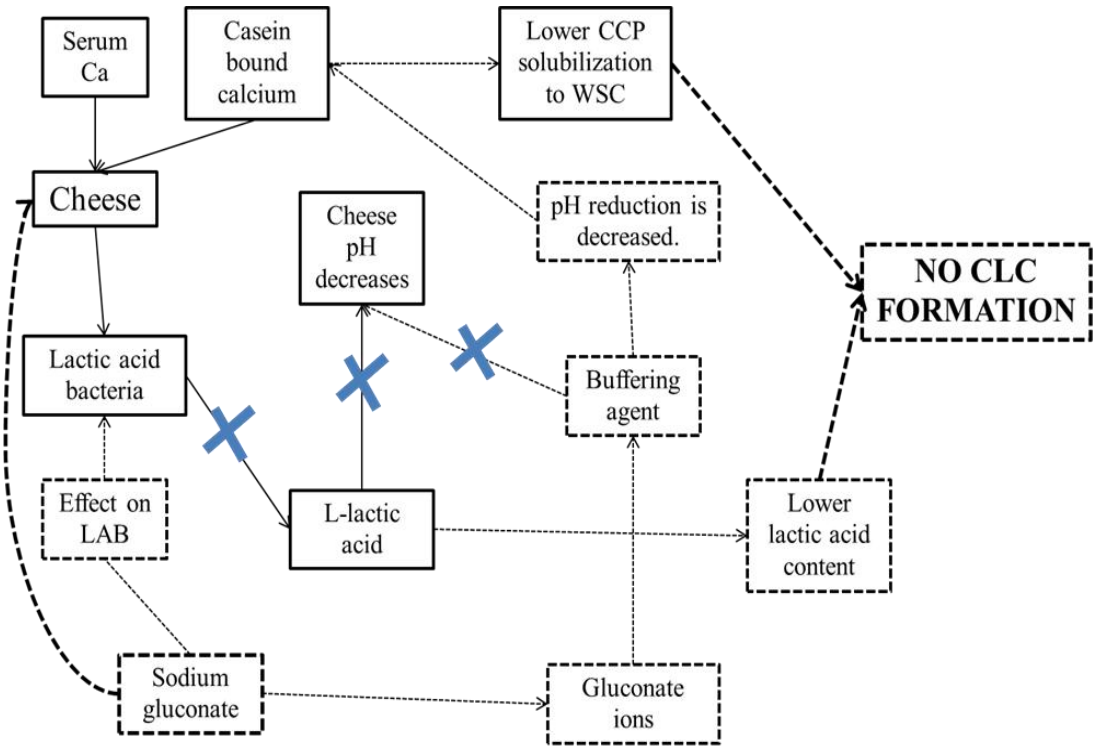

Figure 7. Possible effects of sodium gluconate as calcium lactate crystals inhibitor in Cheddar cheese.

CONCLUSIONS

Sodium gluconate addition caused an increase in the pH values and lactose content and a decrease in WSC and lactic acid content. In addition, results from surface crystal formation study indicate that cheeses without sodium gluconate addition from both salting levels had visible surface crystals starting from 6-month of ripening, while cheeses with 0.5% sodium gluconate addition from both salting levels had minute surface crystals that required magnification starting from 7-month of ripening, and cheeses with 1.0% sodium gluconate addition did not have any surface crystals throughout the ripening time. Thus, the results imply that sodium gluconate is effective as calcium lactate crystals

inhibitor in Cheddar cheese. The effects of sodium gluconate are a result of its ability to reduce WSC and lactic acid in cheese as well as its ability to increase the solubility of calcium lactate.

REFERENCES

Caccamo, M., C. Melilli, D. M. Barbano, and G. Portelli. 2004. Measurement of gas holes and mechanical openness in cheese by image analysis. J. Dairy Sci. 87: 739 – 748.

De Kruif, C. G. and C. Holt. 2004. Casein micelle structure, functions, and interactions, In Advanced Dairy Chemistry Vol 1: Proteins, 3rd edn. (eds. P. F. Fox and P. L. H. McSweeney), Kluwer Academic/ Plenum Publishers, New York, New York, pp. 233 – 276.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701–1710.

Farrell Jr., H. M., E. L. Malin, E. A. Brown, and P.X. Qi 2006. Casein micelle structure: what can be learned from milk synthesis and structural biology? Curr. Opin. Colloid Interface Sci. 11: 135-147.

Fox, P. F. and A. Brodkorb. 2008. The casein micelle: historical aspects, current concepts and significance. Int. Dairy J. 18:677-684.

Fox, P. F., J. A. Lucey, and T. M. Cogan. 1990. Glycolysis and related reactions during cheese manufacture and ripening. Crit. Rev. Food Sci. Nutr. 29: 237 – 253.

Guinee, T. P., E. P. Feeney, M. A. E. Auty, and P. F. Fox. 2002. Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. J. Dairy Sci. 85: 1655 – 1669.

Guinee, T. P. and P.F. Fox. 2004. Salt in Cheese: Physical, Chemical and Biological Aspects, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp. 207-259.

Hassan, A., M. E. Johnson, and J. A. Lucey. 2004. Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. J. Dairy Sci. 87: 854 – 862.

Horne, D. S. 1998. Casein interactions: casting light on the black boxes, the structure in dairy products. Int. Dairy J., 8: 171-177.

Horne, D. S. 2006. Casein micelle structure: models and muddles. Curr. Opin. Colloid Interface Sci. 11: 148-153.

Johnson, M. E., B. A. Riesterer, C. Chen, B. Tricomi, and N. F. Olsen. 1990. Effect of packaging and storage conditions on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73: 3033 – 3041.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2003. Understanding the role of calcium in functionality of part-skim Mozzarella cheese. J. Dairy Sci. 86: 1918 – 1926.

Karlsson, A. O., R. Ipsen, and Y. Ardö. 2007. Influence of pH and NaCl on rheological properties of rennet-induced casein gels made from UF concentrated skim milk. Int. Dairy J. 17: 1053 – 1062.

Kubantseva, N., and R. W. Hartel. 2002. Solubility of calcium lactate in aqueous solution. Food Rev. Intl. 18: 135–149.

Lee, M. –R. , M. E. Johnson, and J. A. Lucey. 2005. Impact of modifications in acid development on the insoluble calcium content and rheological properties of Cheddar cheese. J. Dairy Sci. 88: 3798 – 3809.

Lucey, J. A. and P. F. Fox. 1993. Importance of calcium and phosphate in cheese manufacture: A review. J. Dairy Sci. 76: 1714 – 1724.

Lucey, J. A., R. Mishra, A. Hassan, and M. E. Johnson. 2005. Rheological and calcium equilibrium changes during ripening of Cheddar cheese. Int. Dairy J. 15: 645 – 653.

McSweeney, P.L.H. and P. F. Fox. 2004. Metabolism of residual lactose and of lactate and citrate, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp.361-371.

Metzger, L. E., D. M. Barbano, M. A. Rudan, and P. S. Kinstedt. 2000. Effect of milk preacidification on low fat Mozzarella cheese. I. Composition and yield. J. Dairy Sci. 84: 1357 – 1366.

O'Mahoney, J. A., P. L. H. McSweeney, and J. A. Lucey. 2006. A model system for studying the effect of colloidal calcium phosphate concentration on the rheological properties of Cheddar cheese. J. Dairy Sci. 89: 892 – 904.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003a. Effect of pH on the chemical composition and structure-fuction relationships of Cheddar cheese. J. Dairy Sci. 86: 2751 – 2760.

Patton, C., S. Thompson, and D. Epel. 2004. Some precautions in using chelators to buffer metals in biological solutions. Cell Calcium. 35: 427 – 431.

Phadungath, C. and L. E. Metzger. 201X. Effect of sodium gluconate on the solubility of calcium lactate. J. Dairy Sci. (submitted)

Phadungath, C. and L. E. Metzger. 2008a. Changes in residual sugar and water-soluble organic acids during ripening of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN

Phadungath, C. and L.E. Metzger. 2008b. Manufacture of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN

Pyne, G. T. and T. C. A. McGann. 1960. The colloidal phosphate of milk II. Influence of citrate. J. Dairy Res. 27: 9 – 17.

Rajbhandari, P. and P. S. Kindstedt. 2005. Development and Application of Image Analysis to Quantify Calcium Lactate Crystals on the Surface of Smoked Cheddar Cheese. J. Dairy Sci. 88: 4157 – 4164.

Roy, D. 1991. Salt stress in growth and acid production of *Lactobacillus helveticus* strain Milano. Letters Appl. Microbiol. 12: 207 – 211.

Salaün, F., B. Mietton, and F. Gaucheron. 2005. Buffering capacity of dairy products. Int. Dairy. J. 15: 95 – 109.

Sheehan, J. J., and T. P. Guinee. 2004. Effect of pH and calcium level on the biochemical, textural and functional properties of reduced-fat Mozzarella cheese. Int. Dairy J. 14: 161 – 172.

Svensson, B., B. Jönsson, M. Fushiki, and S. Linse. 1992. Ionic strength effects on the binding constant of calcium chelators: experiment and theory. J. Phys. Chem. 96: 3135 – 3138.

Swearingen, P. A., D. E. Adams, and T. L. Lensmire. 2004. Factors affecting calcium lactate and liquid expulsion defects in Cheddar cheese. J. Dairy Sci. 87:574–582.

Turner, K. W. and T. D. Thomas. 1980. Lactose fermentation in Cheddar cheese and the effect of salt. N. Z. J. Dairy Sci. Technol. 15: 265 – 276.

Upreti, P. and L. E. Metzger. 2007. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: pH changes during ripening. J. Dairy Sci. 90: 1 – 12.

Upreti, P., L. L. McKay, and L. E. Metzger, L.E. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. J. Dairy Sci. 89: 429-443.

Chapter 6. The effect of sodium gluconate on proteolysis, textural properties and sensory evaluation during Cheddar cheese ripening

Proteolysis is the most important biochemical pathway during cheese ripening. This phenomenon influences cheese characteristics including textural and sensory properties during cheese ripening. The objective of this study was to determine the effect of sodium gluconate on the extent of proteolysis, textural properties and sensory evaluation during Cheddar cheese ripening. Six Cheddar cheeses with two salting levels (2 and 2.5%) and three sodium gluconate levels (0, 0.5 and 1%) were manufactured in triplicate. Cheeses were analyzed for the extent of proteolysis by measuring pH 4.6 soluble N and 12% TCA soluble N at 3, 6, 9, and 12-month of ripening. Textural properties were determined by Texture Profile Analysis (TPA) using a TA.XTplus Texture Analyzer at 3, 6, 9, and 12-month of ripening. TPA parameters generated were fracturability, hardness, cohesiveness, springiness, chewiness, and resilience. Descriptive sensory analysis was used to monitor Cheddar cheese flavors in this study at 6 and 12-month of ripening. An increase in soluble N and decreases in textural properties (fracturability, hardness, cohesiveness, springiness, gumminess, chewiness, and resilience) were observed throughout the ripening time for all treatments. At both salting levels, cheeses with added sodium gluconate exhibited a trend for a higher level of proteolysis and lower TPA hardness at 6 and 9 months. The overall flavor intensity scores at 6 months of ripening were lower in cheeses with added sodium gluconate, which could relate to their lower bitterness scores. A similar trend was observed at 12 months of ripening, where cheeses

with sodium gluconate addition had lower overall flavor intensity and lower bitterness scores. This present study provides an understanding of how sodium gluconate impacts cheese characteristics during ripening.

INTRODUCTION

Calcium lactate crystals (CLC) can appear within and on the surface of hard-type cheese especially Cheddar cheese after approximately four to six months of aging. In a typical Cheddar cheese, the concentration of soluble calcium and lactate are in excess of their solubility at 4°C. Since calcium and lactate ions are in a supersaturate state in the cheese serum phase, crystallization at nucleation sites occurs. A continuous movement of calcium and lactate ions to the nucleation sites causes the sites to become macro crystals (Dybing et al., 1988). One potential approach for preventing CLC is to add sodium gluconate to Cheddar cheese. In a previous study (Phadungath and Metzger, 201x), we determined that sodium gluconate can increase the solubility of calcium and lactate by forming complexes with calcium and lactate ions, and removing the calcium and lactate ions from being available for the formation of CLC. In the previous study, seven calcium lactate solutions (5.31% w/w) with seven level of sodium gluconate (0, 0.5, 1, 1.5, 2, 3, and 4% w/w) were made. Solutions were stored at 7°C for 21 days. The solutions were visually inspected for CLC formation, analyzed for lactic acid and gluconic acid by HPLC and for calcium by Atomic Absorption Spectroscopy. We determined that CLC were formed in the solution with 0% gluconate after the first day of storage, and the HPLC results indicated that there was a higher concentration of calcium and lactic acid in

the filtrate from the solutions containing added gluconate. These results demonstrated that sodium gluconate can increase the solubility of calcium lactate.

Proteolysis contributes to the development of cheese texture by hydrolysis of the protein matrix into polypeptides and smaller water-soluble peptides that are not a part of the protein matrix. The production of short peptides and free amino acids contribute to cheese flavor either directly or indirectly by acting as a substrate for a range of catabolic reactions, which later generate volatile flavor compounds (Sousa et al., 2001; Upadhyay et al., 2004). Two of the most common solvents for extracting soluble nitrogen are pH 4.6 acetate buffer and 12% trichloroacetic acid solution (TCA) (Bynum and Barbano, 1985). Because proteolysis hydrolyses the protein matrix into large and small peptides, and amino acids, it is essential to separate those proteins and peptides into more homogenous fractions in order to characterize their constituents. Extraction of cheese with pH 4.6 acetate buffer will precipitate intact casein, whereas large and small peptides remain soluble in solution. This soluble fraction contains whey proteins, low molecular weight peptides from casein hydrolysis and free amino acids. Extraction with 12% TCA will precipitate large peptides and proteins, whereas small peptides remain soluble. The 12% TCA soluble fraction contains small peptides and amino acids, while the precipitate contains medium and large peptides, and intact casein. These soluble portions are typically analyzed for nitrogen content using Kjeldahl analysis. This analysis provides and index for the extent of proteolysis by comparing the ratio of soluble nitrogen to total nitrogen (Upadhyay et al., 2004). It has been reported that soluble N extracted from both solvents correlates significantly with the age and flavor intensity of Cheddar cheese (Farkye and Fox, 1990).

Cheddar cheese texture plays an important part in determining the commercial value and overall acceptance by consumers (Jack et al., 1994). Factors that affect cheese texture during manufacture and storage include cheese pH and proteolysis. Cheese pH during manufacturing significantly affects cheese texture, and it can be controlled by adding salt, decreasing amount of residual lactose in the curd, or changing the buffering capacity of the cheese (Lawrence et al. 1987; Foegeding and Drake, 2006). Proteolysis during cheese ripening and hydrolysis of α_{s1}- and β-caseins result in continuous changes in Cheddar cheese textural properties (Everett and Olson 2003; Sallami et al. 2004). Texture analysis in cheese is performed using various empirical, imitative and fundamental methods. Several mechanical instruments have been used to measure cheese texture. One of the most widely used imitative cheese texture measurements is Texture Profile Analysis (TPA) using a universal texting machine/ texture analyzer. The force-time curve obtained from the TPA test is used to determine hardness, springiness, cohesiveness, adhesiveness, chewiness and gumminess. These TPA parameters are found to correlate well with the sensory parameters (Szczesniak 1963a, b; Bourne 1978; Van Vliet 1991a,b; Tunick 2000). It has been reported that the use of TPA can generate TPA parameters that can differentiate Cheddar cheeses with different characteristics such as different pH, calcium content, phosphorus content, residual lactose content, moisture content, salt-to-moisture ratio and ripening time (Chen et al., 1979; Pollard et al., 2003; Chevanan et al., 2006). TPA parameters also highly correlate with textural characteristics from sensory evaluation (Casiraghi et al., 1989; Antoniou et al., 2000) with the optimal combination of deformation and deformation rate at 70 – 90% and 1.0 mm/s for

maximizing the correlation between sensory and instrumental hardness for cheese products (Xiong et al., 2002).

The taste of Cheddar cheese changes more readily than other sensory attributes during ripening. Therefore, understanding the development and variations in taste changes that occur during the ripening process is an important aspect in defining consumer expectations and preferences (Caspia et al., 2006). Because consumer concepts and preferences for cheese flavor profiles are diverse, grading and judging, which is a fundamental sensory tool used in the dairy industry for quality evaluation of dairy products, is not suitable to be used in research to apply to consumer preference (Singh et al., 2003). Descriptive sensory analysis is a very powerful tool that has been used by academic researchers in cheese flavor research. A standardized descriptive language for Cheddar cheese flavor has been developed in order to facilitate communication among research groups and enhance more precise cheese flavor profile (Murray and Delahunty, 2000; Drake et al., 2001). Descriptive sensory analysis has been used in many areas of Cheddar cheese flavor research. For instance, descriptive sensory analysis was used to assess Cheddar cheese flavor defects (Bouzas et al., 1991), study effects of Cheddar cheese manufacturing modifications on the development of the cheese flavor (Grazier et al., 1991; Hickey et al., 2006), study flavor development during ripening of Cheddar cheese (Roberts and Vickers, 1994; Hort and Le Grys, 2001), characterize flavor of commercial Cheddar cheese and understand consumer acceptability and preferences for Cheddar cheese (Lawlor and Delahunty, 2000; Kilcawley et al., 2007), and study regional and international cross validation of Cheddar cheese flavor languages (Drake et al., 2005; Drake et al., 2008).

We recently reported the effect of sodium gluconate on the Cheddar cheese manufacture and composition (Phadungath and Metzger, 2008b), organic acids (Phadungath and Metzger, 2008a), and water-soluble calcium during cheese ripening (Phadungath and Metzger, 2010). We determined that sodium gluconate influenced cheese pH, moisture, lactose, lactic acid, and water-soluble calcium content. The differences in cheese pH, moisture, lactose, lactic acid, and water-soluble calcium content among treatments could influence further changes in textural and sensory properties during cheese ripening, which are the two main cheese characteristics responsible for consumer preference in cheese consumption. Thus, the objective of this study was to determine the effect of sodium gluconate addition on the extent of proteolysis, textural and sensory properties during Cheddar cheese ripening.

MATERIALS AND METHODS

Experimental Design

A replicated 2×3 factorial designs was utilized for a Cheddar cheese making study. This design utilized three levels of sodium gluconate (0, 0.5 and 1%) and two levels of salt (2 and 2.5%) for a total of six treatments. The treatments were; TRT1 - 0% sodium gluconate + 2% salt, TRT2 – 0.5% sodium gluconate + 2% salt, TRT3 – 1.0% sodium gluconate + 2% salt, TRT4 - 0% sodium gluconate + 2.5% salt, TRT5 – 0.5% sodium gluconate + 2.5% salt, TRT6 - 1% sodium gluconate + 2.5% salt. Each treatment was manufactured in triplicate from three different lots of milk. The manufacturing protocols and cheese composition for Cheddar cheese in this study have been previously reported and shown in Table 1(Phadungath and Metzger, 2008). The cheeses were

ripened for 12 months, and changes in the extent of proteolysis, textural properties and sensory evaluation were monitored during ripening.

Table 1. Means (across the row) of chemical and compositional attributes of Cheddar cheeses with sodium gluconate addition**

Mean value (%)*	Treatments					
	2% Salt			2.5% Salt		
	Sodium gluconate (%)			Sodium gluconate (%)		
	0	0.5	1.0	0	0.5	1.0
pH (1 wk)	5.06^{a}	5.16^{ab}	5.24^{ab}	5.16^{ab}	5.28^{b}	5.32^{b}
Moisture	36.98^{a}	37.79^{ab}	38.14^{b}	37.01^{a}	37.54^{ab}	37.79^{ab}
Fat	32.98^{a}	32.18^{ab}	30.96^{b}	32.26^{ab}	31.73^{ab}	31.33^{b}
Protein	25.72	26.1	25.6	26.08	25.65	25.72
Na	0.61^{a}	0.74^{ab}	0.77^{b}	0.72^{ab}	0.78^{b}	0.82^{b}
Cl	0.99^{a}	1.08^{ab}	1.12^{ab}	1.20^{ab}	1.29^{b}	1.29^{b}
S/M	4.47^{a}	4.73^{a}	4.85^{ab}	5.36^{b}	5.68^{b}	5.66^{b}
Total Ca	0.73^{a}	0.72^{ab}	0.69^{b}	0.73^{ab}	0.72^{ab}	0.70^{ab}
P	0.5	0.49	0.48	0.5	0.49	0.49
Lactose (1 wk)	0.53^{a}	0.69^{ab}	0.85^{ab}	0.71^{ab}	0.85^{ab}	1.00^{b}
Lactic acid (1 wk)	1.56^{a}	1.35^{ab}	0.95^{c}	1.17^{b}	0.93^{c}	0.89^{c}
Gluconic acid	0	0.33	0.59	0	0.33	0.58

* ^{a,b,c} Means within the column not sharing common superscripts are different (Tukey HSD at $P < 0.05$).

** n = 3 replicates.

Proteolysis in Cheeses during Ripening

In our study, the extent of proteolysis was characterized by measuring pH 4.6 soluble N and 12% TCA soluble N. The results have been reported as soluble N (as a

percentage of total N) (Bynum and Barbano, 1985). The extent of proteolysis was determined at 3, 6, 9 and 12 month of ripening.

pH 4.6 soluble N. About 0.75-g cheese was manually homogenized for 30 s with 25-mL pH 4.6 acetate buffer or Sharp's extracting solution (Bynum and Barbano, 1985) using a high shear Omni mixer-homogenizer (model 17105, Omni International, Waterbury, CT). The extract was centrifuged (Jouan CR4-12 Centrifuges, Jouan, Inc., Winchester, VA) at 7,000 ×g for 5 min. Then the samples were held at 4°C for 10 min to solidify the fat layer, and the top fat layer was removed using a spatula. The supernatant was filtered through filter paper (Whatman #2, Whatman International Ltd., Maidstone, England). The precipitate was homogenized with additional acetate buffer, centrifuged and filtered as in the previous step. All the collected filtrate was analyzed for N using the Kjeldahl method (Kjeltec™ 2200 Auto Distillation Unit, FOSS, Eden Prarie, MN).

12% TCA soluble N. About 1.5-g cheese was manually homogenized for 30 s with 25-mL of 12% TCA using a high shear Omni mixer-homogenizer (model 17105, Omni International, Waterbury, CT). The extract was centrifuged (Jouan CR4-12 Centrifuges, Jouan, Inc., Winchester, VA) at 7,000 ×g for 5 min. Then the samples were held at 4°C for 10 min to solidify the fat layer, and the top fat layer was removed using a spatula. The supernatant was filtered through filter paper (Whatman #2, Whatman International Ltd., Maidstone, England). The precipitate was homogenized with additional 12% TCA, centrifuged and filtered as in the previous step. All the collected filtrate was analyzed for N using the Kjeldahl method (Kjeltec™ 2200 Auto Distillation Unit, FOSS, Eden Prarie, MN).

Textural properties

TPA was used to evaluate cheese texture at 3, 6, 9 and 12 months of ripening. The method was modified from Antoniou et al. (2000) and Xiong et al. (2002). Cheese samples were prepared using a 21 mm diameter Cork borer and cheese cutter to obtain a sample with a 21 mm diameter and 20 mm height. The samples were covered in saran wrap, kept in an air-tight container to prevent moisture loss, and stored at 4°C for 18 h before analysis. To quantify the textural properties, cheese samples were tempered at room temperature for 30 min before analysis. The TPA analysis was performed using a TA.XTplus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). The test conditions were uniaxial two-bite compression, 50-mm diameter cylindrical flat probe (TA-25), 80% compression, and 0.8 mm/s crosshead speed. TPA parameters generated are fracturability, hardness, cohesiveness, springiness, chewiness, and resilience (Bourne, 1978; Texture Technologies Corp., 2008).

Statistical Analyses

A 2 × 3 factorial model with 3 replications was used for statistical analysis and changes in the pH 4.6 soluble N and 12% TCA soluble N during ripening were analyzed using a repeated measures design (Table 3). The PROC GLM procedure of SAS, which involved 2 factors (salt, and sodium gluconate level) as class variables and changes in the extent of proteolysis, and TPA attributes during ripening were analyzed using a repeated measures design (Table 3 and 4) (SAS Institute, 1990).

Sensory evaluation

Descriptive sensory analysis was used to monitor Cheddar cheese flavors in this study at 6 and 12 months of ripening with the Cheddar cheese lexicon adapted from Drake et al. (2001). The Cheddar cheese lexicons that were used for the sensory evaluation are shown in Table 2.

Panelist training. 10 judges from students and staff on the University of Minnesota – St. Paul Campus who expressed an interest in participating in sensory tests were recruited. There were training sessions prior to the actual sensory evaluation. During the training sessions, the judges tasted references for a variety of Cheddar cheese flavor attributes chosen from the published lexicon, sampled several pairs of the 6 Cheddar cheese treatments and suggested terms that describe the differences among the cheeses, and practiced rating the 6 Cheddar cheese treatments using computerized data collection software.

Testing. Six treatments with 3 replicates of Cheddar cheese at 6 and 12 months or ripening were tested. The panelists were provided with three cubes (1.5 cm^3) of each cheese at room temperature in a plastic 2 oz. cup. Panelists evaluated one cheese replicate from each treatment per week in duplicate with refresher sessions to review the lexicon prior to evaluating the second and the third replicates. During a testing session, panelists evaluated each product by rating the intensity of each Cheddar cheese lexicon attributes on line scales labeled ‘non’ at the left end and ‘extreme amount’ at the right end.

Table 2. Cheddar cheese lexicon (sensory descriptors and their definitions) used for the sensory evaluation of Cheddar cheeses with and without sodium gluconate addition at 6 and 12-month of ripening.*

Descriptive Term	Definition
Taste	
Acidic/ Sour	The fundamental taste sensation elicited by acids.
Bitter	The fundamental taste sensation elicited by caffeine and quinine.
Salty	The fundamental taste sensation elicited by salts.
Sweet	The fundamental taste sensation elicited by sweets.
Umami	Oral sensation stimulated by monosodium glutamate.
Aroma	
Brothy	Aromas associated with boiled meat or vegetable soup stock.
Diacetyl	Aroma associated with diacetyl.
Earthy	Aroma characteristic of damp soil, wet foliage.
Malty	Sweet slightly fermented or sour grain note associated with freshly kilned malt.
Metallic	Aroma associated with metals, tinny or iron.
Milky	Aroma associated with skim milk or milk derived products.
Moldy	The taste and aromas associated with molds. They are usually earthy, dirty, stale, musty and slightly sour.
Musty	Aroma associated with closed air spaces such as attics and closets (dry) and basements (wet).
Rancid	Aroma associated with sour milk and oxidized fats.
Sulfur	Aroma associated with hydrogen sulfide, rotten egg.
Sweaty	The taste and aroma reminiscent of perspiration generated foot odor which are sour, stale and slightly cheesy.
Unclean/ Barny	Off-flavor in dairy products associated with barny aroma found in unventilated cow barns.
Yeasty	Aromas associated with fermenting yeast.
Flavor	
Overall Flavor Intensity	The overall intensity of aroma and flavor.
Strength	The degree of mildness and maturity for Cheddar cheese. Colby cheese is mild, aged Cheddar is mature.
Whey Taint/ Sour Whey	Off-flavors in cheese associated with retained cheese whey.
Pungent	A sharp, irritating and penetrating sensation in the nasal cavity.
Other	
Astringent	A mouth-drying and harsh sensation. The complex of drying, puckering and shrinking sensations in the lower cavity causing contraction of the body tissues.
Fatty	Fatty, Buttery mouth-feel

*Adapted from Drake et al., 2001.

Data analysis. Analysis of Variance was used to determine whether treatments or replicates affect the sensory attributes. The attribute intensity was the dependent variable. Predictors were judge, treatment, replication, treatment×replication, and sensory replicate. The multiple comparison tests were used to determine which specific treatments differed significantly from others for each sensory attribute.

RESULTS AND DISCUSSION

Proteolysis in Cheese during Ripening

Changes in pH 4.6 Soluble Nitrogen. The pH 4.6 acetate buffer soluble N reported as a percentage of total N was significantly affected ($P<0.05$) by salting level, time, and the interaction of time × salt, but not affected by sodium gluconate addition and other interaction terms (Table 3). The level of pH 4.6 soluble N increased in all treatments during ripening, resulting in an increase of up to 1.5 fold by the end of ripening (Figure 1 (a)). An increase was more prominent during early ripening (3 to 6-month of ripening), after which (6 to 12-month of ripening) the increase was less. The level of soluble N from cheeses with 2% salting level was significantly higher ($P<0.05$) than those from cheese with 2.5% salting level from 6 to 12-month of ripening. Although sodium gluconate addition did not have significant effect on the changes in pH 4.6 soluble N, Figure 1 (a) shows a trend where cheeses with sodium gluconate addition from both 2% and 2.5% salting levels had a higher rate of proteolysis when compared to the rate of proteolysis from cheeses without sodium gluconate addition from 6 to 12-month of ripening.

Table 3. Mean squares and probabilities (in parentheses) of changes in pH 4.6 soluble N and 12% TCA soluble N in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months.

Factor	df	Soluble N (as % of total N)	
		pH 4.6	12% TCA
Whole plot			
Salt (S)	1	24.40* (0.048)	7.30 (0.253)
Gluconic acid (G)	2	2.59 (0.612)	1.07 (0.811)
S × G	2	2.70×10^{-2} (0.995)	0.01 (0.998)
Error	12	5.07	5.058
Subplot			
Time (T)	3	1.39×10^{2}* (<0.01)	1.23×10^{2}* (<0.01)
T × S	3	2.05* (<0.01)	0.21 (0.392)
T × G	6	3.50×10^{-2} (0.983)	0.034 (0.985)
T × S × G	6	0.70×10^{-2} (0.999)	0.50×10^{-2} (0.999)
Error	36	0.20	0.21

*Statistically significant at *P*-value < 0.05.

Changes in 12% TCA Soluble Nitrogen. The 12% TCA soluble N reported as a percentage of total N was significantly affected ($P<0.05$) only by time, but not affected by salting level, sodium gluconate addition, and interaction terms (Table 3). The level of 12% TCA soluble N increased in all treatments during ripening, leading to an increase of

almost 2 fold by the end of ripening (Figure 1 (b)). An increase was more noticeable from 3 to 9-month of ripening, and from 9 to 12-month of ripening, the increase was minor.

Factors Influencing Proteolysis. Proteolysis is influenced by both external factors (i.e. manufacturing and ripening conditions) and internal factors (i.e. cheese composition, mineral equilibrium between casein matrix and cheese serum, and concentration of proteolytic enzymes) (Neocleous et al., 2002). Proteolysis is often roughly divided into two phases, which are primary and secondary proteolysis (Grappin et al., 1985; Rank et al., 1985). Primary proteolysis involves mainly hydrolysis of caseins by residual milk-clotting enzyme (i.e. chymosin), and to a lesser degree by milk proteinase (i.e. plasmin), resulting in the formation of large and medium molecular weight peptides. The primary phase of proteolysis is more notable during early ripening. Primary proteolysis mainly contributes to the production of pH 4.6 soluble N (van den Berg and Exterkate, 1993; Bastian and Brown, 1996; Sousa et al., 2001). As also suggested by Reville and Fox (1978), pH 4.6 soluble N is most suitable for cheese that is less than 6-month old. This explains the larger change in pH 4.6 soluble N during earlier ripening (3 to 6-month of ripening) (Figure 1 (a)) when compared with the later ripening period (6 to 12-month of ripening), which was in agreement with Neocleous et al. (2002) and Upreti et al. (2006).

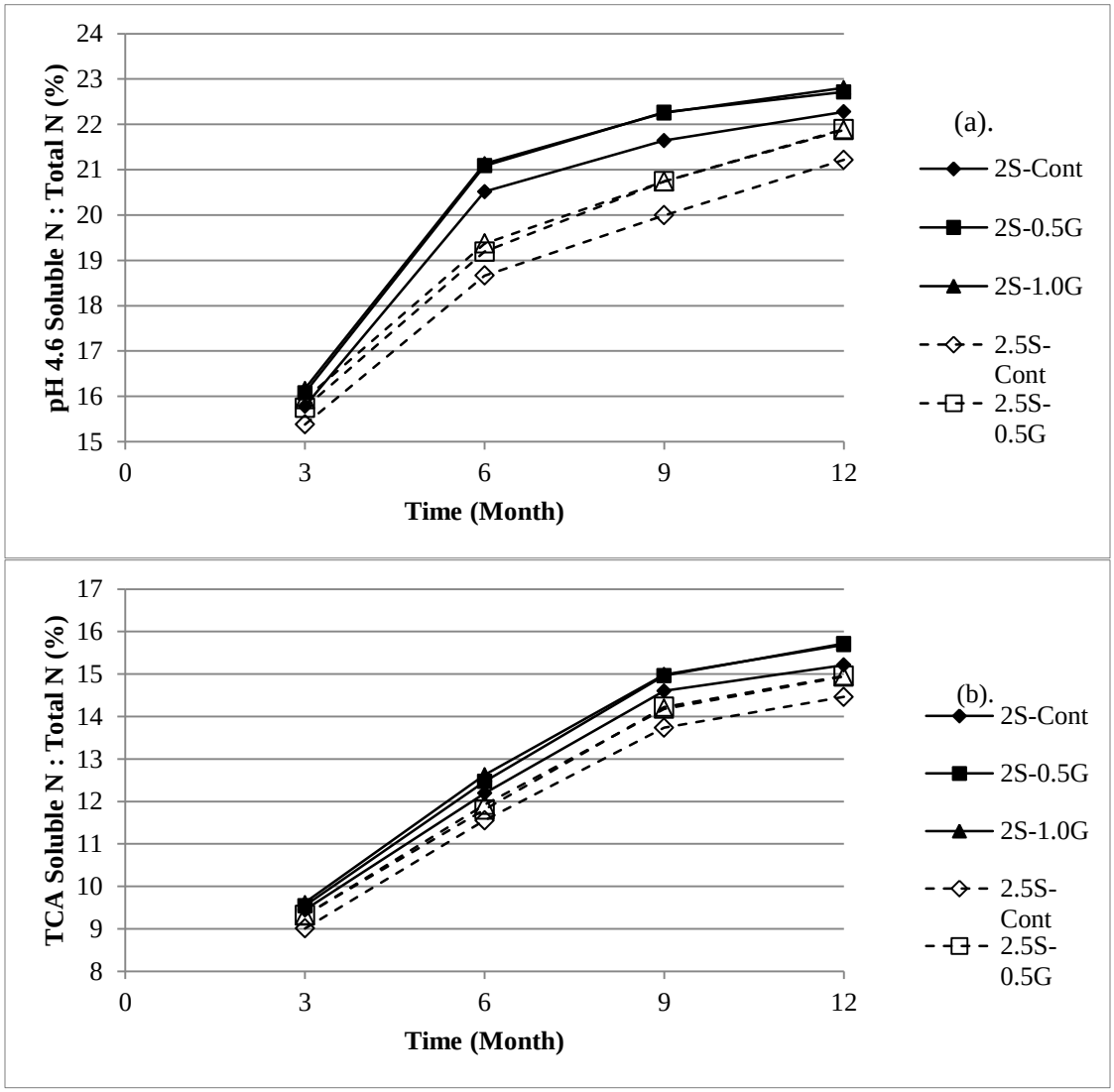

Figure 1. Changes in (a). pH 4.6 soluble N (as a percentage of total N) and (b). 12% TCA soluble N (as a percentage of total N) in Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate

As cheese ages, autolysis of starter cultures will result in a release of proteolytic enzymes into the cheese serum (Crow et al., 1995). Even though starter bacteria are considered to be only weakly proteolytic (Sousa et al., 2001), their complex proteolytic system is important for texture and flavor development during cheese ripening. The proteolytic system of starter bacteria is composed of a proteinase and peptidases. Peptidase enzymes influence the rate of proteolysis during the later stage of ripening, since they are able to hydrolyze large and medium molecular weight peptides derived from the primary phase of proteolysis to smaller peptides and amino acids (Desmazeaud and Gripon, 1977; Law and Haandrikman, 1997). This stage of ripening is often referred to as a secondary proteolysis, and is mainly responsible for the production of 12% TCA soluble N (Rank et al., 1985; Fox, 1989). Thus, this explains a notable increase from 3 to 9-month of ripening in 12% TCA soluble N from our results (Figure 1 (b)), when compared to a large increase upto 6-month of ripening in the pH 4.6 soluble N.

As previously mentioned, cheeses with 2% salting level had higher level of pH 4.6 soluble N when compared to cheeses with 2.5% salting level. Different salting levels contribute to different S/M in cheese samples. Table 1 shows that cheese samples with lower salting level (2% salting level) has lower S/M when compared with cheeses samples with higher salting level (2.5% salting level). A similar result was observed by Fitzgerald and Buckley (1985); Kelly et al. (1996); Mistry and Kasperson (1998), and Upreti et al. (2006). They reported that cheeses with low S/M exhibited higher proteolysis or higher levels of soluble N when compared to cheeses with high S/M. Fitzgerald and Buckley (1985) studied the effect of total and partial substitution of sodium chloride on the quality of Cheddar cheese. They reported that cheeses that were

manufactured with $CaCl_2$ or $MgCl_2$ in place of NaCl exhibited the highest rate of proteolysis throughout the ripening time. They explained that the extensive proteolysis in those cheeses could be due to the lower S/M. Kelly et al. (1996) studied the effect of salt-in-moisture on proteolysis in Cheddar-type cheese. In their study, Cheddar cheese curd was manufactured, divided, and salted from 0 to 3% w/w. They reported that after 24 weeks of ripening, water-soluble N, which represented the rate of proteolysis, decreased with increasing salt concentration. They explained that higher salt concentration contributed to an elevated ionic strength, resulting in an aggregation of β-casein. The cleavage sites of the aggregated β-casein became inaccessible, which causes a limited action of chymosin on β-casein, and results in a slower rate of proteolysis. On the contrary, Mistry and Kasperson (1998) reported in their study on the influence of salt on the quality of reduced fat Cheddar cheese that degradation of α_s-casein decreased with an increase of salt addition from 2.3 to 5%, but there was no difference in the degradation of β-casein with different salting rates. In addition, the effect of salt on the rate of proteolysis could be due to its effect on microbial growth (Guinee, 2004). Schroeder et al. (1988) studied the reduction of sodium chloride in Cheddar cheese and its effect on microbiological properties. They reported that cheese with the least NaCl exhibited an increase in the rate of proteolysis and higher lactic acid bacteria population. Upreti et al. (2006) also reported that cheeses with low S/M had higher activity of lactic acid bacteria when compared to cheeses with high S/M.

Although Table 3 indicates that sodium gluconate addition did not have significant effect on soluble N, Figure 1 exhibited a trend where cheeses with sodium

gluconate addition from both 2% and 2.5% salting levels had higher soluble N when compared with cheeses without sodium gluconate addition. From Table 1, cheeses with sodium gluconate addition had higher moisture content when compared with cheeses without sodium gluconate addition. It is suggested that the moisture in cheese contains an amount of chymosin similar to that in the whey; thus, cheese with higher moisture content should experience a higher rate of proteolysis (van den Berg and Exterkate, 1993). This statement was confirmed by Mistry and Kasperson (1998) and Neocleous et al. (2002), where they reported an increase in the rate of proteolysis with an increase in moisture content in cheese. Table 1 shows that cheeses with sodium gluconate addition have higher residual lactose concentration when compared with cheeses without sodium gluconate addition. It has been suggested that the residual lactose concentration is higher in cheese that has the higher rate of starter autolysis (Crow et al., 1995); thus, it is possible that sodium gluconate might cause more autolysis of starter bacteria. The autolysis of starter bacteria results in a release of proteolytic enzymes of starter bacteria, which could cause an increase in the cheese proteolysis during ripening (Lortal and Chapot-Chartier, 2005). In addition, an increase in the population of non-starter lactobacilli could also accelerate the cheese proteolysis during ripening (Peterson and Marshall, 1990).

Textural Properties

TPA Parameters. The TPA parameters include fracturability, hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness and resilience. Salting level had a significant effect ($P<0.05$) on hardness, and adhesiveness. Time significantly

affected (P<0.05) all parameters, while sodium gluconate addition and interaction parameters did not have any significant effect on all TPA parameters (Table 4). TPA values except for adhesiveness from all cheese treatments decreased with ripening time (Figure 2). Hardness values from cheeses with 2.5% salting level were significantly higher (P<0.05) than hardness values from cheeses with 2% salting level throughout ripening time. Although sodium gluconate addition did not have significant effect on hardness among cheese treatments, Figure 2 shows a trend where cheeses with sodium gluconate addition from both 2% and 2.5% salting levels had lower hardness values when compared to the hardness values from cheeses without sodium gluconate addition throughout the ripening time. Unlike other TPA parameters, adhesiveness values from all cheese treatments increased during ripening. Adhesiveness values from cheeses with 2.5% salting level and 0.5 and 1.0% sodium gluconate addition were significantly lower (P<0.05) than adhesiveness values from other treatments throughout the ripening time.

Factors Influencing Textural Properties. Hardness is the most commonly TPA parameter used to evaluate cheese texture (Bryant et al., 1995). Hardness is obtained from the maximum force during first compression cycle of the 2-bite TPA test (Tunick, 2000). Hardness values from all cheese treatments decreased as cheeses aged (Figure 2). This is in accordance with the proteolysis results where both pH 4.6 and 12% TCA soluble N increased with ripening time, which indicated further breakdown of the casein network in cheese samples. Similar results were also observed by Chevanan and Muthukumarappan (2007), where they reported the reduction in hardness after 6-month of ripening of Cheddar cheeses made with different levels of calcium and phosphorus, residual lactose and salt-to-moisture ratio. As previously mentioned, cheeses with 2.5% salting level had

firmer texture (higher hardness values) when compared to cheeses with 2% salting level (Figure 2 (b)). The effect of salting level on cheese hardness was also observed by Schroeder et al. (1988), Mistry and Kasperson (1998), Pastorino et al. (2003), and Chevanan and Muthukumarappan (2007). Mistry and Kasperson (1998) and Pastorino et al. (2003) explained that an increase in cheese hardness with increasing salting level was due to the decrease in moisture content. Pastorino et al. (2003) further explained that salt can increase ionic strength in the cheese system, which causes a 'salting-in' and a swelling of protein matrix. As protein matrix is swollen, the thickness of the internal structure of the protein strand also increases, and the protein matrix is able to resist the deformation from compression. Consequently, the cheese hardness increases. In addition, the cheese hardness results are also in agreement with our proteolysis results where cheeses with 2% salting level had a higher level of proteolysis when compared to cheeses with 2.5% salting level.

Although Table 3 indicates that sodium gluconate addition did not have significant effect on cheese hardness, Figure 2 (b) exhibited a trend where cheeses with sodium gluconate addition from both 2% and 2.5% salting levels had lower hardness values when compared with cheeses without sodium gluconate addition. From Table 1, cheeses with sodium gluconate addition had higher moisture content when compared with cheeses without sodium gluconate addition. As previously mentioned, Mistry and Kasperson (1998) and Pastorino et al. (2003) reported the decrease in cheese hardness as the moisture content increased. In addition, we speculated that sodium gluconate addition might cause more autolysis of starter bacteria, which could result in an increase in the rate of proteolysis, further breakdown of the protein network, and the decrease in cheese

hardness. The similar phenomenon was observed by Sallaml et al. (2004), where they reported an increase in the rate of proteolysis and the decrease in cheese hardness in Cheddar cheeses made with added autolytic adjunct culture.

Adhesiveness is the relative force that is required to remove the food sample from the palate after a food sample has been chewed (Chen et al., 1979). Adhesiveness is determined by the force area of the negative peak that follows the first compression (Tunick, 2000). Our results indicated that cheeses made with 2.5% salting level with 0.5 and 1% sodium gluconate addition had lower adhesiveness than other treatments. A similar result was observed by Schroeder et al. (1988), where they reported an increase in cheese adhesiveness with the salt reduction in Cheddar cheese manufacture. Bryant et al. (1995) reported from their study on Cheddar cheese texture as influenced by fat reduction that the most adhesive cheeses were obtained from cheeses that had an open and loose protein matrix. Cheeses lost adhesiveness as their protein matrix became more compact. Pastorino et al. (2003) explained that the change in the ability of proteins to interact with water or other proteins can have an effect on cheese adhesiveness. Therefore, it is possible that an increase in salting level from 2% to 2.5% in our Cheddar cheeses cause the decrease in cheese moisture, which would result in cheeses with more compact protein matrix. The more compact protein matrix in cheese will result in the decrease in cheese adhesiveness.

Table 4. Mean squares and probabilities (in parentheses) of changes in TPA parameters in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months.

Factor	df	Fracturability	Hardness	Adhesiveness	Cohesiveness
Whole plot					
Salt (S)	1	2.38×10^{6} (0.302)	3.55×10^{6}* (<0.01)	7.59×10^{7}* (0.024)	0.70×10^{-4} (0.734)
Gluconic acid (G)	2	1.34×10^{5} (0.937)	2.41×10^{6} (0.547)	1.80×10^{7} (0.248)	1.80×10^{-4} (0.732)
S × G	2	1.59×10^{5} (0.925)	2.11×10^{5} (0.946)	1.55×10^{7} (0.295)	0.40×10^{-4} (0.926)
Error	12	2.04×10^{6}	3.79×10^{6}	1.15×10^{7}	5.70×10^{-4}
Subplot					
Time (T)	3	7.67×10^{6}* (<0.01)	8.31×10^{6}* (<0.01)	5.19×10^{7}* (< 0.01)	9.85×10^{-3}* (< 0.01)
T × S	3	1.65×10^{5} (0.395)	1.26×10^{5} (0.854)	2.68×10^{6} (0.809)	0.30×10^{-4} (0.922)
T × G	6	$2.35 \times 10^{6}5$ (0.219)	5.27×10^{5} (0.393)	5.25×10^{6} (0.703)	2.20×10^{-4} (0.378)
T × S × G	6	4.93×10^{4} (0.929)	4.16×10^{4} (0.997)	2.69×10^{6} (0.919)	0.50×10^{-4} (0.946)
Error	36	1.61×10^{5}	4.88×10^{5}	8.29×10^{6}	0.20×10^{-3}

*Statistically significant at *P*-value < 0.05.

Table 4 (continue). Mean squares and probabilities (in parentheses) of changes in TPA parameters in Cheddar cheese with added sodium gluconate during storage at 6 – 8 ^{0}C for 12 months.

Factor	df	Springiness	Gumminess	Chewiness	Resilience
Whole plot					
Salt (S)	1	2.33×10^{-2} (0.270)	1.75×10^{4} (0.621)	1.72×10^{4} (0.120)	4.84×10^{-5} (0.120)
Gluconic acid (G)	2	2.20×10^{-2} (0.318)	1.09×10^{4} (0.853)	2.19×10^{3} (0.708)	1.57×10^{-5} (0.430)
S × G	2	6.11×10^{-3} (0.712)	409 (0.994)	2.87×10^{3} (0.638)	2.27×10^{-6} (0.878)
Error	12	1.74×10^{-2}	6.80×10^{4}	6.16×10^{3}	1.73×10^{-5}
Subplot					
Time (T)	3	2.29×10^{-2}* (< 0.01)	6.67×10^{5}* (<0.01)	6.93×10^{4}* (<0.01)	1.94×10^{-4}* (<0.01)
T × S	3	2.27×10^{-2} (0.204)	2.83×10^{3} (0.897)	5.38×10^{3} (0.078)	1.00×10^{-5} (0.144)
T × G	6	1.52×10^{-2} (0.394)	1.14×10^{4} (0.575)	2.73×10^{3} (0.302)	2.14×10^{-6} (0.867)
T × S × G	6	1.76×10^{-2} (0.305)	1.34×10^{3} (0.996)	3.10×10^{3} (0.232)	4.14×10^{-6} (0.583)
Error	36	1.41×10^{-2}	1.42×10^{4}	2.18×10^{-3}	5.23×10^{-6}

*Statistically significant at *P*-value < 0.05.

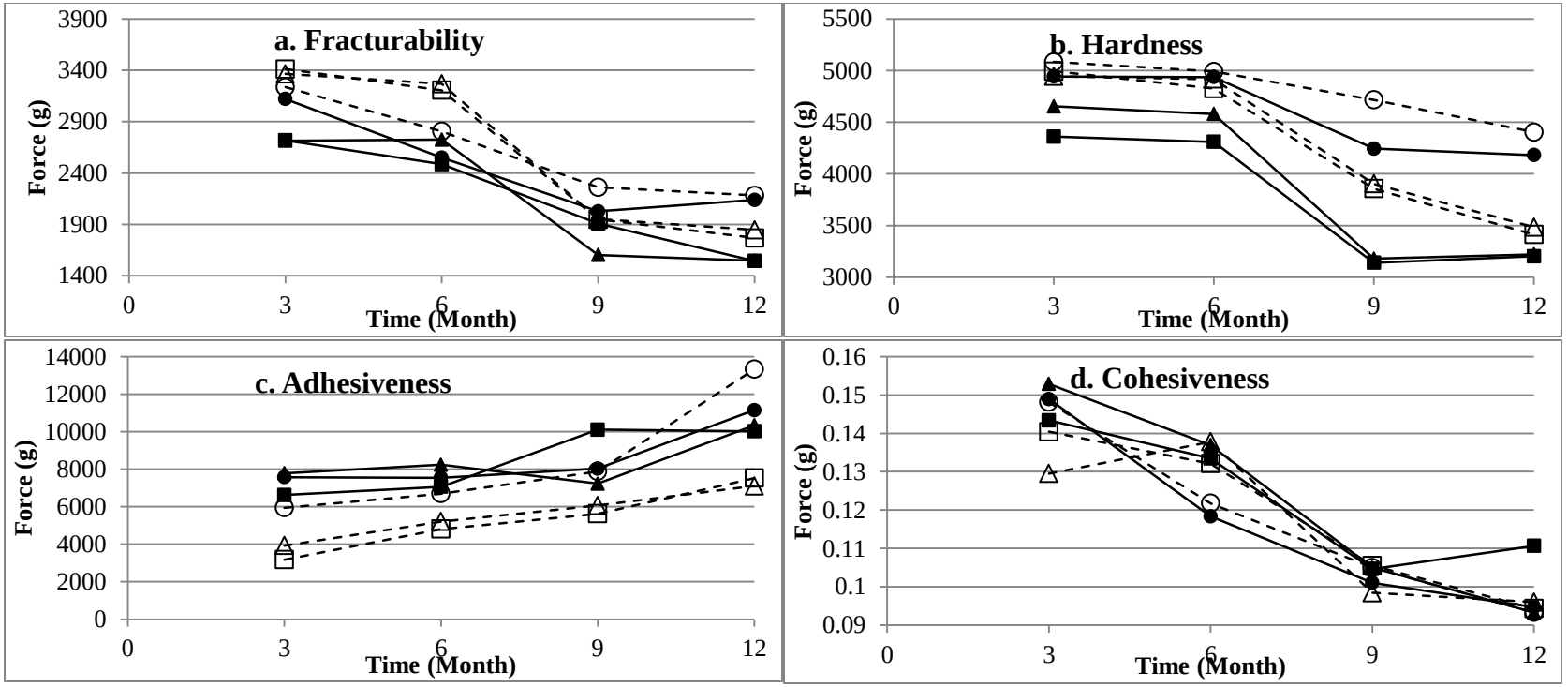

Figure 2. Changes TPA attributes, (a). fracturability, (b). hardness, (c). adhesiveness, (d). cohesiveness, (e). springiness, (f). gumminess, (g). chewiness, (h). resilience, in Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

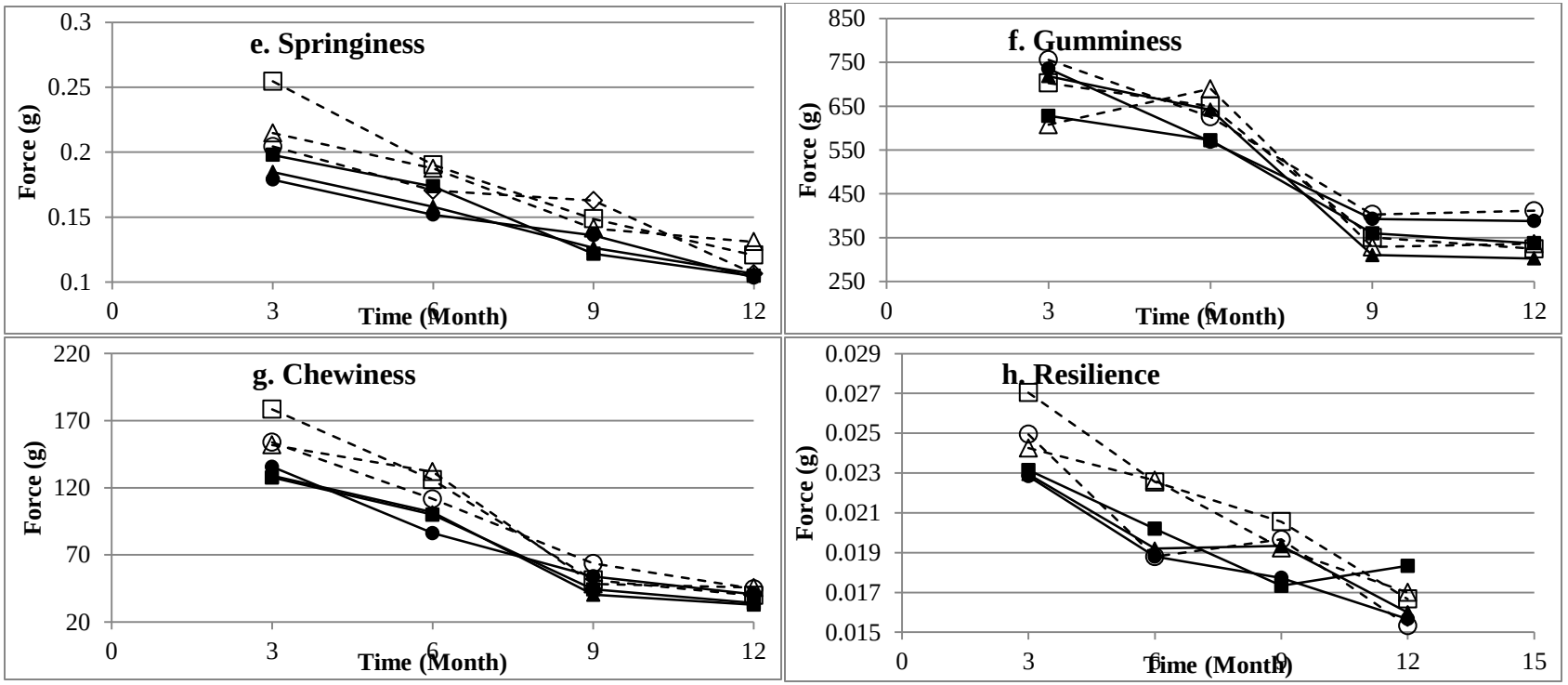

Figure 2 (continue). Changes TPA attributes, (a). fracturability, (b). hardness, (c). adhesiveness, (d). cohesiveness, (e). springiness, (f). gumminess, (g). chewiness, (h). resilience, in Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Sensory Evaluation

Sensory attributes. The Cheddar cheese sensory attributes shown in Table 2 are divided into four categories, which are taste (sour, bitter, salty, sweet, and umami), aroma (brothy, diacetyl, earthy, malty, metallic, milky, moldy, musty, rancid, sulfur, sweaty, unclean/ barny, and yeasty), flavor (overall flavor intensity, strength, whey taint/ sour whey, and pungent), and other (astringent and fatty). Mean scores of all Cheddar cheese sensory attributes rated on a ten-point scale by a trained panel at 6-month and 12-month of ripening are shown in Table 5 and Table 6, respectively. Only selected sensory attributes had significantly different mean scores ($P<0.05$) among treatments (Table 7), and those selected sensory attributes at 6-month and 12-month of ripening are shown in Figure 3 and Figure 4, respectively. The selected sensory attributes at 6-month of ripening are astringent, bitterness, milky, pungent, strength, sulfur, and overall flavor intensity, and the selected sensory attributes at 12-month of ripening are astringent, bitterness, diacetyl, milky, strength, and overall flavor intensity.

From Figure 3 and Figure 4, sensory attributes that had the most significant difference at both 6 and 12-month of ripening were bitterness, strength, and overall flavor intensity. Sodium gluconate addition and sensory evaluation time (6 month VS 12 month) significantly affected ($P<0.05$) bitterness, strength, and overall flavor intensity, while salt only affected strength (Table 7). At both 6 and 12-month of ripening, Cheddar cheese made with 2% salting level and without sodium gluconate addition had the highest bitterness, strength and overall flavor intensity scores. At 6-month of ripening, Cheddar cheese made with 2.5% salting level and 1.0% sodium gluconate addition had the lowest bitterness, strength, and overall flavor intensity scores. Cheddar cheese made with 2%

salting level and 1.0% sodium gluconate addition had the lowest bitterness, strength, and overall flavor intensity scores at 12-month of ripening.

Table 5. Means (across the row) of sensory attributes of Cheddar cheeses with sodium gluconate addition at 6-month of ripening**

Attributes*	Treatments					
	2% Salt			2.5% Salt		
	Sodium gluconate (%)			Sodium gluconate (%)		
	0	0.5	1.0	0	0.5	1.0
Taste						
Acidic/ Sour	3.0^{ab}	3.1^{a}	2.8^{ab}	2.9^{ab}	2.6^{ab}	2.3^{b}
Bitter	3.3^{a}	2.0^{b}	1.7^{b}	2.4^{ab}	2.2^{ab}	1.7^{b}
Salty	3.6	3.5	3.4	3.8	3.6	3.4
Sweet	1.3	1.5	1.7	1.3	1.5	1.6
Umami	1.8^{a}	1.6^{ab}	1.8^{ab}	1.9^{ab}	2.1^{b}	1.9^{b}
Aroma						
Brothy						
Diacetyl	2.1	1.9	2.2	2.5	2.0	2.1
Earthy	0.3	0.3	0.1	0.2	0.3	0.1
Malty	0.5	0.4	0.6	0.5	0.6	0.7
Metallic	1.3	1.3	0.8	1.0	1.1	0.8
Milky	1.2^{b}	1.4^{ab}	1.9^{a}	1.7^{ab}	1.5^{ab}	1.7^{ab}
Moldy	0.7	0.6	0.7	0.4	0.6	0.4
Musty	0.4	0.4	0.5	0.5	0.5	0.4
Rancid	1.1	0.9	1.0	1.0	1.2	0.8
Sulfur	2.4^{a}	1.7^{ab}	1.8^{ab}	1.7^{ab}	2.1^{ab}	1.5^{b}
Sweaty	2.1	1.8	1.9	1.8	1.9	2.0
Unclean/ Barny	0.4	0.4	0.2	0.4	0.2	0.3
Yeasty	0.3	0.5	0.3	0.3	0.4	0.4
Flavor						
Overall Flavor Intensity	6.8^{a}	5.9^{bc}	5.8^{bc}	6.2^{ab}	5.2^{cd}	4.9^{d}
Strength	6.5^{a}	6.0^{a}	5.6^{ab}	5.8^{ab}	5.1^{bc}	4.6^{c}
Whey Taint/ Sour Whey	1.0	1.3	1.4	1.3	1.3	1.1
Pungent	2.4^{a}	2.2^{ab}	2.2^{ab}	2.0^{ab}	1.6^{b}	1.4^{b}
Other						
Astringent	2.1^{a}	1.7^{ab}	1.6^{ab}	1.7^{ab}	1.5^{ab}	1.4^{b}
Fatty	2.5	2.4	2.5	2.6	2.5	2.4

* ^{a,b,c} Means within the column not sharing common superscripts are different (Tukey HSD at $P < 0.05$).

** n = 9 replicates.

Table 6. Means (across the row) of sensory attributes of Cheddar cheeses with sodium gluconate addition at 12-month of ripening**

Attributes*	Treatments					
	2% Salt			2.5% Salt		
	Sodium gluconate (%)			Sodium gluconate (%)		
	0	0.5	1.0	0	0.5	1.0
Taste						
Acidic/ Sour	4.7^{a}	4.3^{ab}	4.0^{b}	4.6^{ab}	4.4^{ab}	4.2^{ab}
Bitter	4.1^{a}	3.3^{abc}	2.6^{c}	3.6^{ab}	2.9^{bc}	3.0^{bc}
Salty	4.4	4.3	4.5	4.5	4.7	4.9
Sweet	0.9^{ab}	1.1^{ab}	1.2^{a}	0.9^{ab}	0.6^{b}	1.0^{ab}
Umami	2.9	3.2	3.1	3.4	3.2	3.4
Aroma						
Brothy	2.0	1.7	1.7	1.6	1.6	1.8
Diacetyl	2.5^{b}	3.1^{ab}	2.7^{ab}	2.5^{b}	2.9^{ab}	3.4^{a}
Earthy	0.1	0.1	0.1	0.2	0.1	0.1
Malty	0.1	0.1	0.1	0.08	0.09	0.1
Metallic	0.4	0.5	0.4	0.3	0.3	0.2
Milky	1.8	2.4	2.5	2.0	2.4	2.5
Moldy	0.3	0.3	0.3	0.3	0.3	0.3
Musty	0.9	0.7	0.8	0.7	0.6	0.7
Rancid	0.9	0.7	0.7	0.9	0.7	0.8
Sulfur	2.3^{a}	1.8^{ab}	1.7^{b}	2.2^{ab}	1.9^{ab}	1.9^{ab}
Sweaty	1.8^{a}	1.5^{ab}	1.1^{b}	1.6^{ab}	1.6^{ab}	1.2^{ab}
Unclean/ Barny	0.2	0.1	0.1	0.1	0.2	0.2
Yeasty	0.08	0.04	0.04	0.01	0.04	0.04
Flavor						
Overall Flavor	7.9^{a}	7.4^{ab}	6.7^{c}	7.8^{ab}	7.1^{bc}	7.2^{bc}
Intensity						
Strength	8.4^{a}	7.5^{abc}	6.8^{c}	8.1^{ab}	7.4^{bc}	7.3^{bc}
Whey Taint/ Sour	2.9	2.7	2.2	2.9	2.7	2.6
Whey						
Pungent	1.2	1.0	0.7	1.0	0.9	0.8
Other						
Astringent	2.2^{a}	2.0^{ab}	1.4^{c}	2.0^{ab}	1.9^{abc}	1.6^{bc}
Fatty	5.5	5.9	6.5	5.3	5.8	6.1

* ^{a,b,c} Means within the column not sharing common superscripts are different (LSD at $P < 0.05$).

Table 7. Mean squares and probabilities (in parentheses) of selected sensory attributes in Cheddar cheese with added sodium gluconate at 6 and 12 months of ripening.

Factor	df	Astringent	Bitterness	Diacetyl	Milky
Salt	1	3.39 (0.274)	7.69 (0.189)	4.56 (0.296)	2.12 (0.373)
Gluconic acid	2	13.45* (<0.01)	64.06* (<0.01)	2.29 (0.579)	10.78* (0.018)
Time	1	5.44 (0.166)	159.79* (<0.01)	72.26* (<0.01)	77.83 (0.926)
Replicate	2	30.64* (<0.01)	28.14* (<0.01)	17.86 (0.153)	4.80 (0.166)
Error	603	2.83	4.44	4.18	2.66
Factor	**df**	**Overall Flavor Intensity**	**Pungent**	**Strength**	**Sulfur**
Salt	1	19.47 (0.054)	15.27* (0.022)	26.73* (0.034)	0.29 (0.753)
Gluconic acid	2	56.06* (<0.01)	6.34 (0.114)	65.24* (<0.01)	9.32* (0.044)
Time	1	377.39* (<0.01)	159.45* (<0.01)	612.18* (<0.01)	2.04 (0.408)
Replicate	2	70.86* (<0.01)	15.36* (<0.01)	101.29* (<0.01)	5.50 (0.158)
Error	603	5.24	2.91	5.95	2.98

*Statistically significant at *P*-value < 0.05.

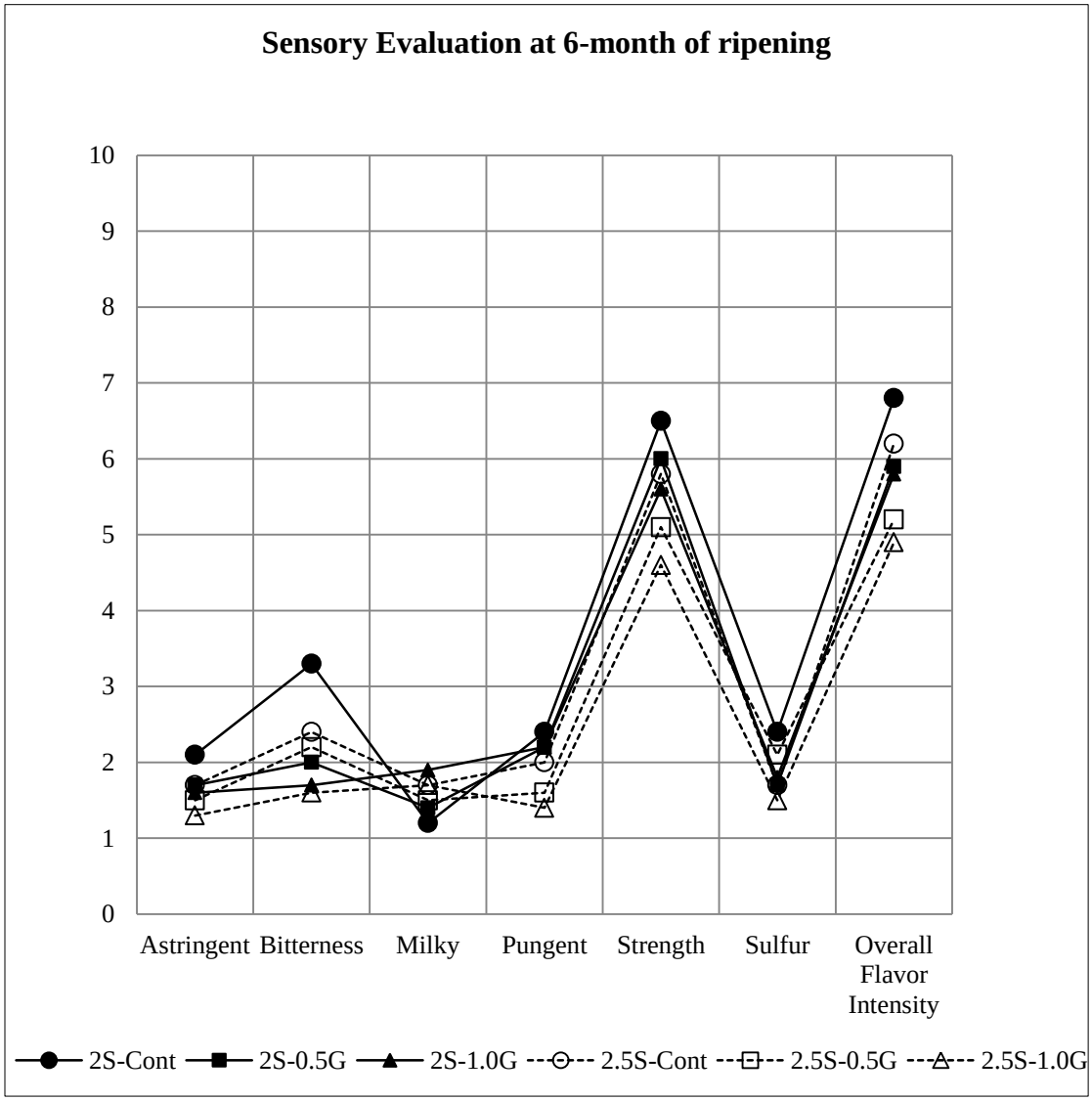

Figure 3. Selected sensory attributes at 6-month of ripening of Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

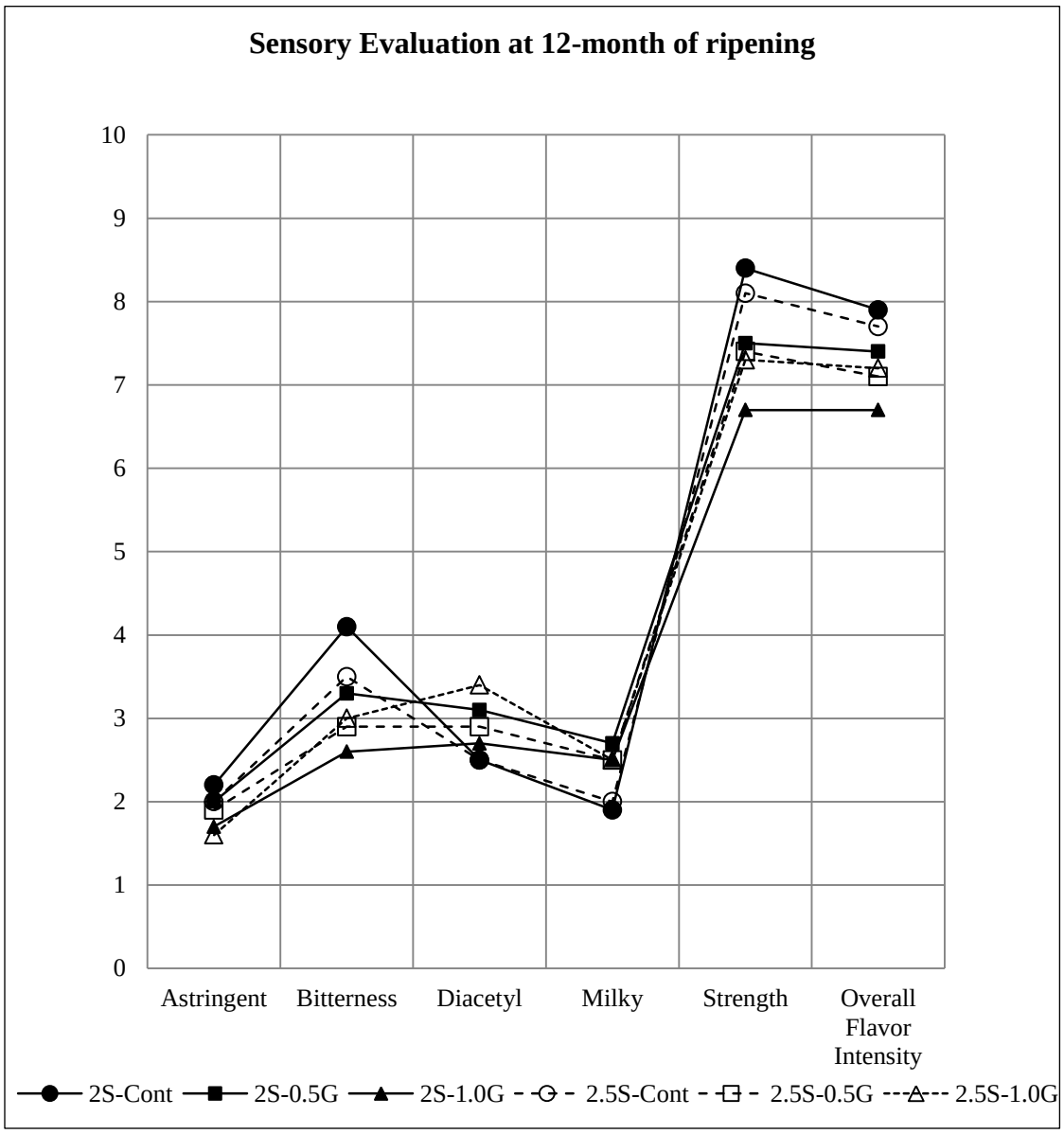

Figure 4. Selected sensory attributes at 12-month of ripening of Cheddar cheeses with added sodium gluconate during 3, 6, 9, and 12-month of ripening. Six treatments are; (●)TRT 1 = 2% salt + 0% sodium gluconate, (■)TRT 2 = 2%Salt + 0.5% sodium gluconate, (▲) TRT 3 = 2%Salt + 1.0% sodium gluconate, (o) TRT 4 = 2.5% salt + 0% sodium gluconate, (□) TRT 5 = 2.5% salt + 0.5% sodium gluconate and (△) TRT 6 = 2.5% salt + 1.0% sodium gluconate.

Factors Affecting Sensory Evaluation. The flavor balance of any particular food largely determines the consumer preferences on that food product (Smit et al., 2005). During ripening of cheese, the flavor development is influenced by the three critical pathways, which are glycolysis, lipolysis and proteolysis. Glycolysis mainly influences the conversion of lactose to lactic acid and the balance of water-soluble organic acid in cheese during ripening. The effect of sodium gluconate addition on the balance of organic acid in Cheddar cheese during ripening was recently reported (Phadungath and Metzger, 2008a). With different microflora and ripening conditions, lactate could be metabolized to varieties of compounds contributing to cheese flavor as well as cheese off-flavors. Lipolysis results in the formation of free fatty acid, which directly contributes to cheese flavor (McSweeney and Sousa, 2000). As already reported in previous section, proteolysis largely affects the texture development of cheese during ripening. Secondary proteolysis is mainly responsible for the formation of small-sized peptides and free amino acids, which probably contribute to the background cheese flavors, and also act as precursor for further production of the volatile compounds (Adda et al., 1982; McSweeney and Sousa, 2000). We can safely assume that the flavor development in cheese during ripening is dynamic since the concentration and the type of flavor compounds are constantly changing due to many chemical, biochemical and microbial activities. Thus, it is not surprising that sensory attributes that had significant differences as shown in Figure 3 and 4 changed between 6 and 12-month of ripening.

Figure 3 and 4 implied that the three most significant sensory attributes from our study are bitterness, strength, and overall flavor intensity. All three sensory attribute scores increased as cheese aged from 6 to 12-month of ripening. This is in agreement

with Mistry and Kasperson (1998), where they reported an increase in bitterness and flavor intensity as cheeses aged in their Cheddar cheeses made with three salting rates (2.3, 3.8, and 5%). Out of the three sensory attributes, salt only had significant effect ($P<0.05$) on strength. Cheddar cheeses from the 2% salting level had higher strength scores when compared with cheeses made with 2.5% salting level at 6-month of ripening (Figure 3). The strength scores for all cheese treatments at 12-month of ripening did not seem to be different (Figure 4). Although salting levels did not affect bitterness and overall flavor intensity scores, at 6-month of ripening, the bitterness score from cheese made with 2% salting level and 0% sodium gluconate was the highest when compared with other treatments, and the overall flavor intensity scores from cheeses with 2% salting level were higher than those from cheeses with 2.5% salting level. At 12-month of ripening, cheese made with 2% salting level and 0% sodium gluconate addition still had the highest bitterness scores when compared with other treatments, while overall flavor intensities did not seem to be different between cheeses with different salting levels. Mistry and Kasperson (1998) also reported lower flavor intensity and bitterness scores in cheeses made with a higher salting level (5%) when compared with cheeses made with a lower salting level (2.3%). However, unlike our results, Schroeder et al. (1988) reported lower flavor intensity scores in Cheddar cheeses made with a salt reduction from 1.44 to 1.12%. We speculated that bitterness contributed to strength and overall flavor intensity. Thus, cheeses with lower bitterness scores would also have lower strength and lower overall flavor intensity scores.

Bitterness is a quality defect in most of the semi-hard and hard-type cheese that limits consumer acceptance and marketability of the cheese. Bitterness in cheese is

caused mainly by proteolysis in cheese during ripening. Rennet first produces long peptides from casein that will be further hydrolyzed to smaller peptides by starter proteinases or peptidase. The starter proteinases or peptidase hydrolyze the long peptides to smaller peptides with bulky hydrophobic groups that can cause bitterness in cheese if their concentration exceeds the bitter flavor threshold. The accumulation of bitter peptides in cheese is probably due to the inability of starter cultures to hydrolyze bitter peptides to non-bitter peptides (Lemieux and Simard, 1991; McSweeney and Sousa, 2000). From our results, cheddar cheeses from both 2% and 2.5% salting level with sodium gluconate addition had lower bitterness, strength, and overall flavor intensity scores when compared with cheeses made without sodium gluconate addition at both 6 and 12-month of ripening (Figure 3 and Figure 4). We speculated that sodium gluconate addition might cause an increase in starter autolysis. It was suggested by Crow et al. (1995) and Lortal and Chapot-Chartier (2005) that bitterness in cheese is reduced with an increase in the cheese proteolysis when starter autolysis occurs. Sodium gluconate addition might have other effect on starter cultures, such that the starter cultures have become non-bitter starters or proteinase-negative starters, which could result in a lower bitterness perception in cheese. Lemiux and Simard (1991, 1992) suggested that non-bitter starter cultures might have intracellular peptidases that have greater activity than those of bitter starter, which are able to degrade the bitter peptides to non-bitter peptides or amino acids. Lane and Fox (1997) suggested that proteinase-negative starter cultures could result in cheese with less bitterness. In addition, an increase in non-starter lactobacilli during ripening could also accelerate cheese proteolysis without development of the bitterness in cheese (Peterson and Marshall, 1990).

CONCLUSIONS

The level of pH 4.6 soluble N increased in all treatments during ripening, resulting in an increase of up to 1.5 fold by the end of ripening. An increase was more prominent during early ripening (3 to 6-month of ripening), after which (6 to 12-month of ripening) the increase was less. There was a trend where cheeses with sodium gluconate addition from both 2% and 2.5% salting levels had a higher rate of proteolysis when compared to the rate of proteolysis from cheeses without sodium gluconate addition from 6 to 12-month of ripening. The level of 12% TCA soluble N increased in all treatments during ripening, leading to an increase of almost 2 fold by the end of ripening. An increase was more noticeable from 3 to 9-month of ripening, and from 9 to 12-month of ripening, an increase was minor. TPA values except for adhesiveness from all cheese treatments decreased with ripening time. There was a trend where cheeses with sodium gluconate addition from both 2% and 2.5% salting levels had lower hardness values when compared to the hardness values from cheeses without sodium gluconate addition throughout the ripening time. Unlike other TPA parameters, adhesiveness values from all cheese treatments increased during ripening.

Sensory attributes that had the most significant difference at both 6 and 12-month of ripening were bitterness, strength, and overall flavor intensity. Cheddar cheeses from both 2% and 2.5% salting level with sodium gluconate addition had lower bitterness, strength, and overall flavor intensity scores when compared with cheeses made without sodium gluconate addition at both 6 and 12-month of ripening. At both 6 and 12-month of ripening, Cheddar cheese made with 2% salting level and without sodium gluconate

addition had the highest bitterness, strength and overall flavor intensity scores. At 6-month of ripening, Cheddar cheese made with 2.5% salting level and 1.0% sodium gluconate addition had the lowest bitterness, strength, and overall flavor intensity scores. Cheddar cheese made with 2% salting level and 1.0% sodium gluconate addition had the lowest bitterness, strength, and overall flavor intensity scores at 12-month of ripening.

REFERENCES

Adda, J., J. C. Gripon, and L. Vassal. 1982. The chemistry of flavour and texture generation in cheese. Food Chem. 9: 115 – 129.

Antoniou, K. D., D. Petrids, S. Raphaelides, Z. Ben Omar, and R. Kestloot. 2000. Texture assessment of French cheeses. J. Food Sci. 65, 168–172.

Bastian, E. D., and R. J. Brown. 1996. Plasmin in milk and dairy products: an update. Int. Dairy J. 6: 435 – 457.

Bourne, M. C. 1978. Texture profile analysis. Food Technol. (July): 62-66, 72.

Bouzas, J., F. W. Bodyfelt, and A. Torres. 1991. A potential analytical assessment of Cheddar cheese flavor defects. Int. Dairy J. 1: 263-271.

Bryant, A., Z. Ustunol, and J. Steffe. 1995. Texture of Cheddar cheese as influenced by fat reduction. J. Food Sci. 60: 1216 – 1236.

Bynum, O.G. and D. M. Barbano. 1985. Whole milk reverse osmosis retentates for Cheddar cheese manufacture: chemical changes during aging. J. Dairy Sci. 68: 1-10.

Casiraghi, E., M. Lucisano, and C. Pompei. 1989. Correlation among instrumental texture, sensory texture and chemical composition of five Italian cheeses. Ital. J. Food Sci. 1, 53–63.

Caspia, E. L., P. C. Coggins, M. W. Schilling, Y. Yoon, and C. H. White. 2006. The relationship between consumer acceptability and descriptive sensory attributes in Cheddar cheese. J. Sensory Stud. 21: 112-127.

Chen, A. H., J. W. Larkin, C. J. Clark, and W. E. Irwin. 1979. Textural analysis of cheese. J. Dairy Sci. 62: 901-907.

Chevanan, N., and K. Muthukumarappan. 2007. Effect of calcium and phosphorus, residual lactose, and salt-to-moisture ratio on the melting characteristics and hardness of Cheddar cheese during ripening. J. Food Sci. 72: E168 – E176.

Chevanan, N., K. Muthukumarappan, P. Upreti, and L. E. Metzger. 2006. Effect of calcium and phosphorus, residual lactose, and salt-to-moisture ratio on textural properties of Cheddar cheese during ripening. J. Texture Stud. 37:711–730.

Crow, V. L., T. Coolbear, P. K. Gopal, F. G. Martley, L. L. McKay, and H. Riepe. 1995. The role of autolysis of lactic acid bacteria in the ripening of cheese. Int. Dairy J. 5: 855 – 875.

Desmazeaud, M. J. and J. –C. Gripon. 1977. General mechanism of protein breakdown during cheese ripening. Milchwissenschaft. 731 – 734.

Drake, M. A., S. C. McIngvale, P. D. Gerard, K. R. Cadwallader, and G. V. Civille. 2001. Development of a descriptive language for Cheddar cheese. J. Food Sci. 66: 1422-1427.

Drake, M. A., M. D. Yates, and P. D. Gerard. 2008. Determination of regional flavor differences in U.S. Cheddar cheeses aged for 6 mo or longer. J. Food Sci. 73: 199-208.

Drake, M. A., M. D.Yates, P. D. Gerard, C. M. Delahunty, E. M. Sheehan, R. P. Turnbull, and T. M. Dodds. 2005. Comparison of differences between lexicons for descriptive analysis of Cheddar cheese flavor in Ireland, New Zealand, and the United States of America. Int. Dairy J. 15: 473-483.

Everett, D. W. and N. F. Olson. 2003. Free oil and rheology of cheddar cheese containing fat globules stabilized with different proteins. J. Dairy Sci. 86, 755–763.

Farkye, N. Y and P. F. Fox. 1990. Objective indices of cheese ripening. Trends in Food Sci and Tech. 1: 37-40.

Fitzgerald, E. and J. Buckley. 1985. Effect of total and partial substitution of sodium chloride on the quality of Cheddar cheese. J. Dairy Sci. 68: 3127 – 3134.

Foegeding, E. A. and M. A. Drake. 2006. Invited review: Sensory and mechanical properties of cheese texture. J. Dairy Sci. 90:1611–1624.

Fox, P. F. 1989. Proteolysis during cheese manufacture and ripening. J. Dairy Sci. 72: 1379 – 1400.

Grappin, R., T. C. Rank, and N. F. Olsen. 1985. Primary proteolysis of cheese proteins during ripening. A review. J. Dairy Sci. 68: 531 – 540.

Grazier, C. L., F. W. Bodyfelt, M. R. McDaniel, and J. A. Torres. 1991. Temperature effects on the development of Cheddar cheese flavor and aroma. J. Dairy Sci. 74: 3656-3668.

Guinee, T. P. 2004. Salting and the role of salt in cheese. Int. J. Dairy Tech. 57: 99 – 109.

Hickey, D. K., K. N. Kilcawley, T. P. Beresford, E. M. Sheehan, and M. G. Wilkinson. 2006. The influence of a seasonal milk supply on the biochemical and sensory properties of Cheddar cheese. Int. Dairy J. 16: 679-690.

Hort, J. and G. Le Grys. 2001. Developments in the textural and rheological properties of UK Cheddar cheese during ripening. Int. Dairy J. 11: 475-481.

Jack, F. R., J. R. Piggott, and A. Paterson. 1994. Analysis of textural changes in hard cheese during mastication by progressive profiling. J. Food Sci. 59: 539-543.

Kelly, M., P. F. Fox, and P. L. H. McSweeney. 1996. Effect of salt-in-moisture on proteolysis in Cheddar-type cheese. Milchwissenschaft. 51: 498 – 501.

Kilcawley, K.N., P. B. O'Connell, D. K. Hickey, E. M. Sheehan, T. P. Beresford, and P. L. H. McSweeney. 2007. Influence of composition on the biochemical and sensory characteristics of commercial Cheddar cheese of variable quality and fat content. Int. J. Dairy Tech. 60: 81-88.

Lane, C. N. and P. F. Fox. 1997. Role of starter enzyme during ripening of Cheddar cheese made from pasteurized milk under controlled microbiological conditions. Int. Dairy J. 7: 55 – 63.

Lawlor, J. B. and C. M. Delahunty. 2000. The sensory profile and consumer preference for ten speciality cheeses. Int. J. Dairy Technol. 53: 28-36.

Lawrence, R. C., L. K. Creamer, and J. Gilles. 1987. Texture development during cheese ripening. J. Dairy Sci. 70: 1748-1760.

Lemieux, L. and R. E. Simard. 1991. Bitter flavour in dairy products. I. a review of the factors likely to influence its development, mainly in cheese manufacture. Lait. 71: 599 – 636.

Lemieux, L. and R. E. Simard. 1992. Bitter flavour in dairy products. II. a review of bitter peptides from casein: their formation, isolation and identification, structure masking and inhibition. Lait. 72: 335 – 382.

Lortal, S. and M. –P. Chapot-Chartier. 2005. Role, mechanisms and control of lactic acid bacteria lysis in cheese. Int. Dairy J. 15: 857 – 871.

Mistry, V. V., and K. M. Kasperson. 1998. Influence of salt on the quality of reduced fat Cheddar cheese. J. Dairy Sci. 81: 1214 – 1221.

Murray, J. M. and C. M. Delahunty. 2000. Selection of standards to reference terms in a Cheddar-type cheese flavor language. J. Sensory Stud. 15:179-199.

Neocleous, M., D. M. Barbano, and M. Rudan. 2002. Impact of low concentration factor microfiltration on the composition and aging of Cheddar cheese. J. Dairy Sci. 85: 2425 – 2437.

Ottman, K. and L. E. Metzger. 2002. Soluble protein procedure for cheese. Testing Cheese Quality Workshop, May 23 – 24, 2002, North Central Cheese Industries Association, St Paul, MN.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003. Effect of salt on structure-function relationships of cheese. J. Dairy Sci. 86: 60 – 69.

Peterson, S. D. and R. T. Marshall. 1990. Nonstarter lactobacilli in Cheddar cheese: a review. J. Dairy Sci. 73: 1395 – 1410.

Phadungath, C. and L.E. Metzger. 2008a. Changes in residual sugar and water-soluble organic acids during ripening of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN.

Phadungath, C. and L. E. Metzger 201X. Effect of sodium gluconate on the solubility of calcium lactate. J. Dairy Sci. (submitted)

Phadungath, C. and L.E. Metzger. 2008b. Manufacture of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN.

Phadungath, C. and L. E. Metzger. 2010. The effect of sodium gluconate on pH, lactose, lactic and, and water soluble Ca changes during Cheddar cheese ripening, July 11 – 15, 2010, Denver, CO.

Pollard, A., F. Sherkat, M. G. Seuret, and A. L. Halmos. 2003. Textural changes of natural Cheddar cheese during maturation process. J. Food Sci. 68: 2011-2016.

Rank, T. C., R. Grappin, and N. F. Olson. 1985. Secondary proteolysis of cheese during ripening. A review. J. Dairy Sci. 68: 801 – 805.

Reville, W. J. and P. F. Fox. 1978. Soluble protein in Cheddar cheese: a comparison of analytical methods. Ir. J. Fd Sci. Technol. 2: 67 – 76.

Roberts, A. K. and Z. M. Vickers. 1994. Cheddar cheese aging: changes in sensory attributes and consumer acceptance. J. Food Sci. 59: 328-334.

Sallaml, L., E. E. Kheadr, I. Fliss, and J. C. Vuillemard. 2004. Impact of autolytic, proteolytic and nisin-producing adjunct cultures on biochemical and textural properties of Cheddar cheese. J. Dairy Sci. 87, 1585–1594.

Schroeder, C. L., F. W. Bodyfelt, C. J. Wyatt, and M. R. McDaniel. 1988. Reduction of sodium chloride in Cheddar cheese: effect on sensory, microbiological, and chemical properties. J. Dairy Sci. 71: 2010 – 2020.

Singh, T. K., M. A. Drake, and K. R. Cadwallader. 2003. Flavor of Cheddar cheese: a chemical and sensory perspective. Compr Rev in Food Sci and Food Safety. 2: 139-162.

Smit, G., B. A. Smit, and W. J. M. Engels. 2005. Flavor formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiol. Rev. 29: 591 – 610.

Sousa, M. J., Y. Ardo, and P. L. H. McSweeney 2001. Advances in the study of proteolysis during cheese ripening. Int. Dairy J. 11: 327-345.

Szczesniak, A.S. 1963a. Classification of textural characteristics. J. Food Sci. 28: 385-389.

Szczesniak, A.S. 1963b. Objective measurements of food texture. J. Food Sci. 28: 410-420.

Tunick, M.H. 2000. Rheology of dairy foods that gel, stretch and fracture. J. Dairy Sci. 83: 1892–1898.

Upadhay, V.K., P. L. H. McSweeney, A. A. A. Magboul. and P. F. Fox. 2004. Proteolysis in cheese during ripening, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 391-433.

Upreti, P., L. E. Metzger, and K. D. Hayes. 2006. Influence of calcium and phosphorus, lactose and salt-to-moisture ratio on Cheddar cheese quality: Proteolysis during ripening. J. Dairy Sci. 89: 444 – 453.

Van Den Berg, G. and F. A. Exterkate. 1993. Technological parameters involved in cheese ripening. Int. Dairy J. 3: 485 – 507.

Van Vliet, T. (1991a) Terminology to be used in cheese rheology. In Rheological and Fracture Properties of Cheese, Bulletin No. 268, pp. 5–15, International Dairy Federation, Brussels, Belgium.

Van Vliet, T. (1991b) Inventory of test methods. In Rheological and Fracture Properties of Cheese, Bulletin No. 268, pp. 16–25, International Dairy Federation, Brussels, Belgium.

Xiong, R., J –F. Meullenet, J. A. Hankins, and W. K. Chung. 2002. Relationship between sensory and instrumental hardness of commercial cheeses. J. Food Sci. 67: 877 – 883.

Bibliography

Abbadi, A., K. F. Gotlieb, J. B. M. Meiberg, J. A. Peters, and H. van Bekkum, H. 1999. New Ca-sequestering materials based on the oxidation of the hydrolysis products of lactose. Green Chemistry. October 1999: 231–235.

Acharya, M. R. and V. V. Mistry. 2004. Comparison of effect of vacuum-condensed and ultrafiltered milk on Cheddar cheese. J. Dairy. Sci. 87: 4004 – 4012.

Adda, J., J. C. Gripon, and L. Vassal. 1982. The chemistry of flavor and texture generation in cheese. Food Chem. 9: 115-129.

Akalin, A. S. and S. Gönç. 1996. The determination of orotic acid in ruminant milks using HPLC. Milchwissenschaft. 51: 554 – 556.

Akalin, A. S., S. Gönç, and Y. Akbaş. 2002. Variation in organic acids content during ripening of Pickled White cheese. J. Dairy Sci. 85: 1670 – 1676.

Agarwal, S., K. Sharma, B. G. Swanson, G. Ű. Yűksel, and S. Clark. 2006. Nonstarter lactic acid bacteria biofilms and calcium lactate crystals in Cheddar cheese. J. Dairy Sci. 89: 1452 – 1466.

Antoniou, K. D., D. Petrids, S. Raphaelides, Z. Ben Omar, and R. Kestloot. 2000. Texture assessment of French cheeses. J. Food Sci. 65, 168–172.

Association of Official Analytical Chemists, International. 1995. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

Atherton, H. V., and J. A. Newlander. 1977. Tests for fat: Babcock, Gerber, and Mojonnier, In Chemistry and Testing of Dairy Products. 4th ed. AVI Publ. Co., Inc., Westport, CT, pp. 71 – 116.

Bastian, E. D., and R. J. Brown. 1996. Plasmin in milk and dairy products: an update. Int. Dairy J. 6: 435 – 457.

Beresford, T. P. and A. Williams. 2004. The microbiology of cheese ripening, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 287-317. Beresford, T. P., N. A. Fitzsimons, N. L. Brennan, and T. M. Cogan. 2001. Recent advances in cheese microbiology. Int. Dairy. J. 11: 259 – 274.

Beresford, T. P., N. A. Fitzsimons, N. L. Brennan, and T. M. Cogan. 2001. Recent advances in cheese microbiology. Int. Dairy. J. 11: 259 – 274.

Bevilacqua, A. E. and A. N. Califano. 1989. Determination of organic acids in dairy products by high performance liquid chromatography. J. Food Sci. 54: 1076-1079.

Bevilacqua, A. E. and A. N. Califano. 1992. Changes in organic aids during ripening of Port Salut Argentino cheese. Food Chem. 43: 345 – 349.

Bianchi, A., G. Beretta, G. Caserio, and G. Giolitti. 1974. Amino acid composition of granules and spots in Grana Padano cheeses. J. Dairy Sci. 57: 1504 – 1508.

Bourne, M. C. 1978. Texture profile analysis. Food Technol. (July): 62-66, 72.

Bouzas, J., F. W. Bodyfelt, and A. Torres. 1991a. A potential analytical assessment of Cheddar cheese flavor defects. Int. Dairy J. 1: 263-271.

Bouzas, J., C. A. Kantt, F. Bodyfelt, and J. A. Torres. 1991b. Simultaneous determination of sugars and organic acids in Cheddar cheese by high-performance liquid chromatography. J. Food Sci. 56: 276-278.

Brooker, B. E., D. G. Hobbs, and A. Turvey. 1975. Observations on the microscopic crystalline inclusions in Cheddar cheese. J. Dairy Res. 42: 341 – 348.

Brooks, I. B., G. A. Luster, and D. G. Easterly. 1970. A procedure for the rapid determination of the major cations in milk by atomic absorption spectrophotometry. Atomic Absorption Newsletter 9:93–94.

Brunner, J. R. 1977. Milk proteins, In Food Proteins (ed. J.R. Whitaker and S.R. Tannenbaum), AVI Publishing Company, Inc., Connecticut, pp. 175-208.

Bryant, A., Z. Ustunol, and J. Steffe. 1995. Texture of Cheddar cheese as influenced by fat reduction. J. Food Sci. 60: 1216 – 1236.

Buffa, M., B. Guamis, J. Saldo, A. J. Trujillo. 2004. Changes in organic acids during ripening of cheeses made from raw, pasteurized or high-pressure-treated goat's milk. Lebensm.-Wiss. u.-Technol. 37: 247 – 253.

Bynum, O. G. and D. M. Barbano, D.M. 1985. Whole milk reverse osmosis retentates for Cheddar cheese manufacture: chemical changes during aging. J. Dairy Sci. 68: 1-10.

Cao, X., H. J. Lee, H. S. Yun, and Y. M. Koo. 2002. Solubilities of calcium and zinc lactate in water and water-ethanol mixture. Korean J. Chem. Eng. 18: 133-135.

Caccamo, M., C. Melilli, D. M. Barbano, and G. Portelli. 2004. Measurement of gas holes and mechanical openness in cheese by image analysis. J. Dairy Sci. 87: 739 – 748.

Califano, A. N. and A. E. Bevilacqua. 2000. Multivariate analysis of the organic acids content of Gouda type cheese during ripening. J. Food Compos. Anal. 13: 949 – 960.

Casiraghi, E., M. Lucisano, and C. Pompei. 1989. Correlation among instrumental texture, sensory texture and chemical composition of five Italian cheeses. Ital. J. Food Sci. 1, 53–63.

Caspia, E. L., P. C. Coggins, M. W. Schilling, Y. Yoon, and C. H. White. 2006. The relationship between consumer acceptability and descriptive sensory attributes in Cheddar cheese. J. Sensory Stud. 21: 112-127.

Chemaly, Z., H. Muhr., and M. Fick. 1999. Crystallization kinetics of calcium lactate in a mixed-suspension-mixed product removal crystallizer. Ind. Eng. Chem. Res. 38: 2803 – 2808.

Chen, A. H., J. W. Larkin, C. J. Clark, and W. E. Irwin. 1979. Textural analysis of cheese. J. Dairy Sci. 62: 901-907.

Chevanan, N., and K. Muthukumarappan. 2007. Effect of calcium and phosphorus, residual lactose, and salt-to-moisture ratio on the melting characteristics and hardness of Cheddar cheese during ripening. J. Food Sci. 72: E168 – E176.

Chevanan, N., K. Muthukumarappan, P. Upreti, and L. E. Metzger. 2006. Effect of calcium and phosphorus, residual lactose, and salt-to-moisture ratio on textural properties of Cheddar cheese during ripening. J. Texture Stud. 37:711–730.

Chou, Y. -E., C. G. Edwards, L. O. Luedecke, M. P. Bates, and S. Clark. 2003. Nonstarter lactic acid bacteria and aging temperature affect calcium lactate crystallization in Cheddar cheese. J. Dairy Sci. 86:2516–2524.

Cogan, T. M. and T. P. Beresford. 2002. Microbiology of hard cheese, In Dairy Microbiology Handbook: the microbiology of milk and milk products, 3rd edn, (ed. Robinson, R.K.), Wiley-Interscience, Inc., New York, pp. 515-560.

Cook, W. J. and C. E. Bugg. 1977. Structure of calcium-carbohydrate complexes, In Metal-ligand interactions in organic chemistry and biochemistry, Part 2, (eds. Pullman, B. and Goldblum, N.), D. Reidel Publishing Company, Dordrecht, Holland, pp. 231-256.

Conochi, J., J. Czulak, A. J. Lawrence, and W. F. Cole. 1960. Tyrosine and calcium lactate crystals on rindless cheese. Aust. J. Dairy Technol. 15: 120.

Conochi, J. and B. J. Sutherland. 1965. The nature and cause of steaminess in Cheddar cheese. J. Dairy Res. 32: 35 – 44.

Crawford, M. A., L. Loughridge, M. D. Milne, and B. H. Scribner. 1959. Organic acid excretion after calcium gluconate infusions. J. Clin. Path. 12: 524 – 529.

Creamer, L. K., K. N. Pearce. J. Gilles, and R. C. Lawrence. 1972. White deposits on the surface of rindless Cheddar cheese. N. Z. J. Dairy Sci. Tech. 7: 21.

Creamer, L. K. and N. F. Olsen. 1982. Rheological evaluation of maturing Cheddar cheese. J. Food Sci. 47: 631-636, 646.

Creamer, L. K., R. C. Lawrence, and J. Gilles. 1985. Effect of acidification of cheese milk on the resultant Cheddar cheese. N. Z. J. Dairy Sci. Tech. 20: 185 – 203.

Crow, V., B. Curry, and M. Hayes. 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. Int. Dairy J. 11: 275-283.

Crow, V. L., T. Coolbear, P. K. Gopal, F. G. Martley, L. L. McKay, and H. Riepe. 1995. The role of autolysis of lactic acid bacteria in the ripening of cheese. Int. Dairy J. 5: 855 – 875

Dalgleish, D. G. 1982. Milk proteins - chemistry and physics, In Food Proteins (ed. P.F. Fox and J.J. Condon), Applied Science Publishers, Ltd., Essex, pp. 155-178.

Dalgleish, D. G. 1993. The enzymatic coagulation of milk, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 2rd edn, (ed. Fox, P.F.), Chapman & Hall, London, UK, pp. 69-100.

Dalgleish, D. G., P. A. Spaguolo, and H. D. Goff. 2004. A possible structure of the casein micelle based on high-resolution field-emission scanning electron microscopy. Int. Dairy J. 14: 1025-1031.

Dejmek, P. and P. Walstra. 2004. The syneresis of rennet-coagulated curd, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 71-103.

De Kruif, C. G. and C. Holt. 2004. Casein micelle structure, functions, and interactions, In Advanced Dairy Chemistry Vol 1: Proteins, 3rd edn. (eds. P. F. Fox and P. L. H. McSweeney), Kluwer Academic/ Plenum Publishers, New York, New York, pp. 233 – 276.

De Llano, D. G., A. Rodriguez, and P. Cuesta. 1996. Effect of lactic starter cultures on the organic acid composition of milk and cheese during ripening – analysis by HPLC. J. Applied Bact. 80: 570 – 576.

Desmazeaud, M. J. and J. –C. Gripon. 1977. General mechanism of protein breakdown during cheese ripening. Milchwissenschaft. 731 – 734.

Díaz-Muñiz, I., D. S. Banavara, M. F. Budinich, S. A. Rankin, E. G. Dudley, and J. L. Steele. 2006. *Lactobacillus casei* metabolic potential to utilize citrate as an energy

source in ripening cheese: a bioinformatics approach. J. Applied Micro. 101: 872 – 882.

Don, F. L., and A. C. Dahlberg. 1942. Identification of the white particles found on the ripened Cheddar cheese. J. Dairy Sci. 25: 31 – 36.

Drake, M. A., S. C. McIngvale, P. D. Gerard, K. R. Cadwallader, and G. V. Civille. 2001. Development of a descriptive language for Cheddar cheese. J. Food Sci. 66: 1422-1427.

Drake, M. A., M. D. Yates, and P. D. Gerard. 2008. Determination of regional flavor differences in U.S. Cheddar cheeses aged for 6 mo or longer. J. Food Sci. 73: 199-208.

Drake, M. A., M. D.Yates, P. D. Gerard, C. M. Delahunty, E. M. Sheehan, R. P. Turnbull, and T. M. Dodds. 2005. Comparison of differences between lexicons for descriptive analysis of Cheddar cheese flavor in Ireland, New Zealand, and the United States of America. Int. Dairy J. 15: 473-483.

Dudley, E. G. and J. L. Steele. 2004. Succinate production and citrate catabolism by Cheddar cheese nonstarter lactobacilli. J. Applied Micro. 98: 14 – 23.

Dweck, A. C. 2005. An update on natural preservatives. Personal Care. September: 11-15.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701–1710.

Dybing, S. T., S. A. Brudvig, J. A. Wiegand, and E. A. Huang. 1986. A simple method for estimating the extent of surface crystal development on colored Cheddar cheese. J. Food Protect. 49: 421 – 422.

Einspahr, H. and C. E. Bugg. 1980. The geometry of ca-carboxylate interactions in crystalline complexes. Acta Cryst. B37: 1044 – 1052.

Escandar, G. M. and L. F. Sala. 1992. Complexes of Cu(II) with D-aldonic and D-alduronic acids in aqueous solution. Can. J. Chem. 70: 2053-2057.

Everett, D. W. and N. F. Olson. 2003. Free oil and rheology of cheddar cheese containing fat globules stabilized with different proteins. J. Dairy Sci. 86, 755–763.

Farkye, N. Y and P. F. Fox. 1990. Objective indices of cheese ripening. Trends in Food Sci and Tech. 1: 37-40.

Farrell Jr., H. M., E. L. Malin, E. M. Brown, and P.X. Qi. 2006. Casein micelle structure: what can be learned from milk synthesis and structural biology? Curr. Opin. Colloid Interface Sci. 11: 135-147.

Farrer, K. T. H., and W. C. J. Hollberg. 1960. Calcium lactate on rindless cheese. Aust. J. Dairy Technol. 15: 151 – 152.

FDA. 2010. 21 CFR, Part 182.6757. United States Food and Drug Adminsitration. Department of Health and Human Services, Washington, D.C.

Fischer, K. and H. –P. Bipp. 2002. Removal of heavy metals from soil components and soils by natural chelating agents. Part II. Soil extraction by sugar acids. Water, Air, and Soil Pollution. 138: 271 – 288.

Fitzgerald, E. and J. Buckley. 1985. Effect of total and partial substitution of sodium chloride on the quality of Cheddar cheese. J. Dairy Sci. 68: 3127 – 3134.

Fitzsimons, N. A., T. M. Cogan, S. Condon, and T. Beresford. 2001. Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. J. Appl. Micro. 90: 600-608.

Foegeding, E. A. and M. A. Drake. 2006. Invited review: Sensory and mechanical properties of cheese texture. J. Dairy Sci. 90:1611–1624.

Food Standards Agency. 2007. Current EU approved additives and their E numbers. From Internet website: http://www.food.gov.uk/

Fox, P. F. 1989. Proteolysis during cheese manufacturing and ripening. J Dairy Sci. 72: 1379-1400.

Fox, P.F. and A. Brodkorb. 2008. The casein micelle: historical aspects, current concepts and significance. Int. Dairy J. 18:677-684.

Fox, P. F., J. A. Lucey, and T. M. Cogan. 1990. Glycolysis and related reactions during cheese manufacture and ripening. Crit. Rev. Food Sci. Nutr. 29: 237 – 253.

Fox, P. F. and P. L. H. McSweeney. 1998. Dairy Chemistry and Biochemistry. Blackie Academic & Professional, London.

Fox, P.F. and P. L. H. McSweeney. 2004. Cheese: an overview, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 1-18.

Goff, D. (1995) Dairy Chemistry and Physics. Dairy Science and Technology Education Series, University of Guelph. From Internet website: http://www.foodsci.uoguelph.ca/dairyedu/

Gomis, D. B. 1992. HPLC analysis of organic acids, In Food Analysis by HPLC, (ed. L.M.L. Nollet). Marcel Dekker, New York, NY, pp. 371-385.

Grappin, R., T. C. Rank, and N. F. Olsen. 1985. Primary proteolysis of cheese proteins during ripening. A review. J. Dairy Sci. 68: 531 – 540.

Grazier, C. L., F. W. Bodyfelt, M. R. McDaniel, and J. A. Torres. 1991. Temperature effects on the development of Cheddar cheese flavor and aroma. J. Dairy Sci. 74: 3656-3668.

Guinee, T. P. 2005. Salting and the role of salt in cheese. Int. J. Dairy Tech. 57: 99 – 109.

Guinee, T. P., E. P. Feeney, M. A. E. Auty, and P. F. Fox. 2002. Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. J. Dairy Sci. 85: 1655 – 1669.

Guinee, T. P. and P.F. Fox. 2004. Salt in Cheese: Physical, Chemical and Biological Aspects, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp. 207-259.

Gyurcsik, B. and L. Nagy. 2000. Carbohydrates as ligands: coordination equilibria and structure of the metal complexes. Coord. Chem. Rev. 203: 81-149.

Harper, W. J., A. M. Swanson, and H. H. Sommer. 1953. Observations on the chemical composition of white particles in several lots of Cheddar cheese. J. Dairy Sci. 36: 368 – 372.

Harvey, C. D., R. Jenness and H. A. Morris. 1981. Gas chromatographic quantitation of sugars and nonvolatile water-soluble organic acids in commercial Cheddar cheese. J. Dairy Sci. 64: 1648-1654.

Hassan, A., M. E. Johnson, and J. A. Lucey. 2004. Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. J. Dairy Sci. 87: 854 – 862.

Health Canada. 2006. Food additives permitted for use in Canada. From Internet website: http://www.hc-sc.gc.ca/index-eng.php

Hegazi, F. Z. and I. G. Abo-Elnaga. 1990. Dissimilation of organic acids by dairy lactic acid bacteria. Die Nahrung. 34: 791 – 801.

Hickey, D. K., K. N. Kilcawley, T. P. Beresford, E. M. Sheehan, and M. G. Wilkinson. 2006. The influence of a seasonal milk supply on the biochemical and sensory properties of Cheddar cheese. Int. Dairy J. 16: 679-690.

Hill, A.R. (1995) Cheese. Dairy Science and Technology Education Series, University of Guelph. From Internet website: http://www.foodsci.uoguelph.ca/dairyedu/

Holt, C. 1994. The biological function of casein: Research reviews, In Hannah Research Yearbook, Hannah Research Institute, Scotland, pp. 60-68.

Holt, C. and D. S. Horne. 1996. The hairy casein micelle: Evolution of the concept and its implications for dairy technology. Neth. Milk Dairy J., 50: 85-111.

Horne, D. S. 1998. Casein interactions: casting light on the black boxes, the structure in dairy products. Int. Dairy J., 8: 171-177.

Horne, D. S. 2003. Casein micelles as hard spheres: limitations of the model in the acidified gel formation. Colloids and Surfaces A: Physiochem. Eng. Aspects. 213: 255-263.

Horne, D. S. 2006. Casein micelle structure: models and muddles. Curr. Opin. Colloid Interface Sci. 11: 148-153.

Horne, D. S. and J. M. Banks. 2004. Rennet-induced coagulation of milk, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 46-70.

Hort, J. and G. Le Grys. 2001. Developments in the textural and rheological properties of UK Cheddar cheese during ripening. Int. Dairy J. 11: 475-481.

Israelachvili, J. 1992. Intermolecular & surface forces. Academic Press Limited, London.

Jack, F. R., J. R. Piggott, and A. Paterson. 1994. Analysis of textural changes in hard cheese during mastication by progressive profiling. J. Food Sci. 59: 539-543.

Jacques, J., A. Collet, and S.H. Wilen 1981. Enantiomers, Racemates, and Resolutions. Wiley, New York

Johnson, M. E., B. A. Riesterer, C. Chen, B. Tricomi, and N. F. Olson. 1990b. Effect of packaging and storage conditions on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73:3033 – 3041.

Johnson, M. E., B. A. Riesterer, and N. F. Olsen. 1990a. Influence of nonstarter bacteria on calcium lactate crystallization on the surface of Cheddar cheese. J. Dairy Sci. 73: 1145 – 1149.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2003. Understanding the role of calcium in functionality of part-skim Mozzarella cheese. J. Dairy Sci. 86: 1918 – 1926.

Jungbunzlauer. 2002. Calcium lactate gluconate – the innovative solution for extra calcium. Innovations in Food Technology. 16: 1-3.

Kalab, M. 1980. Decayed lactic bacteria – a possible source of crystallization nuclei in cheese. J. Dairy Sci. 63(2): 301-304.

Karlsson, A. O., R. Ipsen, and Y. Ardö. 2007. Influence of pH and NaCl on rheological properties of rennet-induced casein gels made from UF concentrated skim milk. Int. Dairy J. 17: 1053 – 1062.

Kelly, M., P. F. Fox, and P. L. H. McSweeney. 1996. Effect of salt-in-moisture on proteolysis in Cheddar-type cheese. Milchwissenschaft. 51: 498 – 501.

Kilcawley, K.N., P. B. O'Connell, D. K. Hickey, E. M. Sheehan, T. P. Beresford, and P. L. H. McSweeney. 2007. Influence of composition on the biochemical and sensory characteristics of commercial Cheddar cheese of variable quality and fat content. Int. J. Dairy Tech. 60: 81-88.

Kindstedt, P. S. and F. V. Kosikowski. 1988. Calcium, phosphorus, and sodium concentration in Cheddar cheese. J. Dairy Sci. 71: 285 – 289.

Kubantseva, N. and R. W. Hartel, R.W. 2002. Solubility of calcium lactate in aqueous solution. Food Rev. Intl. 18: 135–149.

Kubantseva, N., R. W. Hartel, P. A. Swearingen. 2004. Factors affecting solubility of calcium lactate in aqueous solutions. J. Dairy Sci. 87: 863–867.

Lahodny-Šarc, O., F. Kapor, and R. Halle. 2000. Corrosion inhibition of carbon steel in chloride solutions by blends of calcium gluconate and sodium benzoate. Materials and Corrosion. 51: 147–151.

Lane, C. N. and P. F. Fox. 1997. Role of starter enzyme during ripening of Cheddar cheese made from pasteurized milk under controlled microbiological conditions. Int. Dairy J. 7: 55 – 63.

Larson, B. L. and H. M. Hegarty. 1979. Orotic acid in milks of various species and commercial dairy products. J. Dairy Sci. 62: 1641 – 1644.

Lawlor, J. B. and C. M. Delahunty. 2000. The sensory profile and consumer preference for ten speciality cheeses. Int. J. Dairy Technol. 53: 28-36.

Lawrence, R.C., H. A. Heap and J. Gilles. 1984. A controlled approach to cheese technology. J. Dairy Sci. 67: 1632-1645.

Lawrence, R. C., L. K. Creamer and J. Gilles. 1987. Texture development during cheese ripening. J. Dairy Sci. 70: 1748-1760.

Lawrence, R. C., J. Gilles, L. K. Creamer, V. L. Crow, H. A. Heap, C. G. Honoré, K. A. Johnston, and P. K. Samal. 2004. Cheddar cheese and related dry-salted cheese varieties, In In Cheese: Chemistry, Physics and Microbiology, Vol 2: Major Cheese Groups, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 71-102.

Lee, M. –R. , M. E. Johnson, and J. A. Lucey. 2005. Impact of modifications in acid development on the insoluble calcium content and rheological properties of Cheddar cheese. J. Dairy Sci. 88: 3798 – 3809.

Lemieux, L. and R. E. Simard. 1991. Bitter flavour in dairy products. I. a review of the factors likely to influence its development, mainly in cheese manufacture. Lait. 71: 599 – 636.

Lemieux, L. and R. E. Simard. 1992. Bitter flavour in dairy products. II. a review of bitter peptides from casein: their formation, isolation and identification, structure masking and inhibition. Lait. 72: 335 – 382.

Lombardi, A. M., A. E. Bevilacqua, and A. N. Califano. 1994. Variation in organic acids content during ripening of Reggianito cheese in air-tight sealed bags. Food Chem. 51: 221-226.

Lortal, S. and M. –P. Chapot-Chartier. 2005. Role, mechanisms and control of lactic acid bacteria lysis in cheese. Int. Dairy J. 15: 857 – 871.

Lucey, J. A., and P. F. Fox. 1993. Importance of calcium and phosphate in cheese manufacture: A review. J. Dairy Sci. 76: 1714 – 1724.

Lucey, J. A., R. Mishra, A. Hassan, and M. E. Johnson. 2005. Rheological and calcium equilibrium changes during ripening of Cheddar cheese. Int. Dairy J. 15: 645 – 653.

Lues, J. F. R. and A. C. M. Bekker. 2002. Mathematical expressions for organic acids in early ripening of a Cheddar cheese. J. Food Compos. Anal. 15: 11 – 17.

Lues, J. F. R., W. C. Botha, and E. J. Smit. 1998a. Ion-exchange HPLC analysis and assessment of extraction methods. Food Res. Int. 31: 441-447.

Lues, J. F. R., W. C. Botha, and E. J. Smit. 1998b. Ion-exchange HPLC of cheese-related organic acids in comparison with reverse-phase HPLC. Int. Dairy J. 8: 959-965.

Marshall, R. T. 1992. Chemical and physical methods, In Standard Methods for the Examination of Dairy Products. 16th ed. American Public Health Association, Washington, DC, pp. 433 – 529.

Marsili, R.T. 1985. Monitoring chemical changes in Cheddar cheese during aging by high-performance liquid chromatography and gas chromatography technique. J. Dairy Sci. 68: 3155-3161.

Marsili, R. T., H. Ostapenko, R. E. Simmons, and D. E. Green. 1981. High performance liquid chromatographic determination of organic acids in dairy products. J. Food Sci. 46: 52-57.

McDowall, F. H., and A. K. R. McDowell. 1939. The white particles in mature Cheddar cheese. J. Dairy Res. 10: 118 – 119.

McGregor, J. U. and C. H. White. 1990. Effect of enzyme treatment and ultrafiltration on the quality of low fat Cheddar cheese. J. Dairy Sci. 73: 571 – 578.

McGugan, W. A., D. B. Emmons. and E. Larmond. 1979. Influence of volatile and nonvolatile fractions on intensity of Cheddar cheese flavor. J. Dairy Sci. 62: 398-403.

McKay, L. L., R. A. Walter, W. E. Sandine, and P. R. Elliker. 1969. Involvement of phosphoenolpyruvate in lactose utilization by group N streptococci. J. Bacteriol. 99: 603-610.

McMahon, D. J. and B. S. Oommen. 2008. Supramolecular structure of the casein micelle. *J. Dairy Sci.* 91(5): 1709-1721.

McSweeney, P. L. H. 2004a. Biochemistry of cheese ripening. Int. J. Dairy Tech. 57: 127-144.

McSweeney, P. L. H. 2004b. Biochemistry of cheese ripening: introduction and overview, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 347-360.

McSweeney, P. L. H. and P. F. Fox. 2004. Metabolism of residual lactose and of lactate and citrate, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General

Aspects, 3rd edn, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp.361-371.

Mehltretter, C .L., B. H. Alexander, and C. E. Rist. 1953. Sequestration by sugar acids. Ind. Eng. Chem. 45: 2782-2784.

Metzger, L. E. and D. A. Grindstaff. 2005. Cheese with sodium gluconate to inhibit calcium lactate crystal formation. U.S. Patent Application Publication No. US 2005/ 0281915 A1

Metzger, L. E., D. M. Barbano, M. A. Rudan, and P. S. Kinstedt, 2000. Effect of milk preacidification on low fat Mozzarella cheese. I. Composition and yield. J. Dairy Sci. 84: 1357 – 1366.

McSweeney, P. L. H. 2007. Conversion of milk to curd, In Cheese problem solved. (ed. McSweeney, P.L.H.), CRC Press LLC., Boca Raton, FL, pp. 50-71.

Mistry, V. V., and K. M. Kasperson. 1998. Influence of salt on the quality of reduced fat Cheddar cheese. J. Dairy Sci. 81: 1214 – 1221.

Morris, H. A., C. Holt, B. E. Brooker, J. M. Banks, and W. Manson. 1988. Inorganic constituents of cheese: analysis of juice from a one-month-old Cheddar cheese and the use of light and electron microscopy to characterize the crystalline phases. J. Dairy Res. 55: 255-268.

Mullin, W. J. and D. B. Emmons. 1997. Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromatography. Food Res. Int. 30: 147-151.

Murray, J. M. and C. M. Delahunty. 2000. Selection of standards to reference terms in a Cheddar-type cheese flavor language. J. Sensory Stud. 15:179-199.

Nagy, L. and A. Szorcsik. 2002. Equilibrium and structural studies on metal complexes of carbohydrates and their derivatives. J. Inorg. Biochem. 89: 1-12.

Navder, K. P., R. S. Huang, E. B. Fryer, and H. C. Fryer. 1990. Effects of fermentation and storage on the concentration of orotic acid and uric acid in skim milk. J. Food Sci. 55: 585 – 586.

Neocleous, M., D. M. Barbano, and M. Rudan. 2002. Impact of low concentration factor microfiltration on the composition and aging of Cheddar cheese. J. Dairy Sci. 85: 2425 – 2437.

Olsen, N. F. 1983. Minimizing salt crystal formation. Dairy Field. 54 – 55.

Olsen, N. F. and M. E. Johnson. 1989. Controlling calcium lactate crystallization. WMMB Res. Rev. 1: 1 – 4.

O'Mahoney, J. A., P. L. H. McSweeney, and J. A. Lucey. 2006. A model system for studying the effect of colloidal calcium phosphate concentration on the rheological properties of Cheddar cheese. J. Dairy Sci. 89: 892 – 904.

Ong, L. and N. P. Shah. 2008. Influence of probiotic *Lactobacillus acidophilus* and *L. helveticus* on proteolysis, organic acid profiles, and ACE-inhibitory activity of Cheddar cheeses ripened at 4, 8, and 12°C. J. Food Sci. 73: M111 – M120.

Ottman, K. and L. E. Metzger. 2002. Soluble protein procedure for cheese. Testing Cheese Quality Workshop, May 23 – 24, 2002, North Central Cheese Industries Association, St Paul, MN.

Panari, G. 1986. HPLC of organic acids: an approach to cheese analysis. Milchwissenchaft. 41: 214-216.

Parente, E. and T. M. Cogan. 2004. Starter cultures: general aspects, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 123-147.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003a. Effect of pH on the chemical composition and structure-fuction relationships of Cheddar cheese. J. Dairy Sci. 86: 2751 – 2760.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003b. Effect of salt on structure-fuction relationships on cheese. J. Dairy Sci. 86: 60 – 69.

Patton, C., S. Thompson, and D. Epel. 2004. Some precautions in using chelators to buffer metals in biological solutions. Cell Calcium. 35: 427 – 431.

Pearce, K. N., L. K. Creamer, and J. Gilles. 1973. Calcium lactate deposits on rindless Cheddar cheese. N.Z.J. Dairy Sci. Tech. 8: 3-7.

Pecsok. R. L. and J. Sandera. 1955. The gluconate complexes. II. the ferric-gluconate system. J. Am. Chem. Soc. 77: 1489 – 1494.

Pecsok, R. L. and R. S. Juvet Jr. 1955. The gluconate complexes. I. copper gluconate complexes in strongly basic media. J. Am. Chem. Soc. 77: 202 – 206.

Pecsok. R. L. and R. S. Juvet Jr. 1956. The gluconate complexes. III. the lead gluconate system. J. Am. Chem. Soc. 78: 3967 – 3972.

Peterson, S. D. and R. T. Marshall. 1990. Nonstarter lactobacilli in Cheddar cheese: a review. J. Dairy Sci. 73: 1395-1410.

Phadungath, C. and L. E. Metzger. 201x. Effect of sodium gluconate on the solubility of calcium lactate. J. Dairy Sci. (submitted)

Phadungath, C. and L. E. Metzger. 2008a. Changes in residual sugar and water-soluble organic acids during ripening of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN

Phadungath, C. and L. E. Metzger. 2008. Manufacture of Cheddar cheese with added sodium gluconate. ADSA-ASAS Joint Annual Meeting, July 7-11, 2008, Indianapolis, IN.

Phadungath, C. and L. E. Metzger. 2010. The effect of sodium gluconate on pH, lactose, lactic and, and water soluble Ca changes during Cheddar cheese ripening, July 11 – 15, 2010, Denver, CO.

Pham, A. and S. Nakai. 1984. Application of stepwise discriminant analysis to high pressure liquid chromatography profiles of water extract for judging ripening of Cheddar cheese. J. Dairy Sci. 67: 1390-1396.

Pollard, A., F. Sherkat, M. G. Seuret, and A. L. Halmos. 2003. Textural changes of natural Cheddar cheese during maturation process. J. Food Sci. 68: 2011-2016.

Prescott, F. J., J. K. Shaw, J. P. Bilello, and G.O. Cragwall. 1953. Gluconic acid and its derivatives. Ind. Eng. Chem. 45: 338-342.

Pyne, G. T. and T. C. A. McGann. 1960. The colloidal phosphate of milk II. Influence of citrate. J. Dairy Res. 27: 9 – 17.

Rajbhandari, P. and P. S. Kindstedt. 2005a. Compositional factors associated with calcium lactate crystallization in smoked Cheddar cheese. J. Dairy Sci. 88: 3737–3744.

Rajbhandari, P. and P. S. Kindstedt. 2005b. Development and application of image analysis to quantify calcium lactate crystallization in smoked Cheddar cheese. J. Dairy Sci. 88: 4157 – 4164.

Rajbhandari, P. and P. S. Kindstedt. 2008. Characterization of calcium lactate crystals on Cheddar cheese by image analysis. J. Dairy Sci. 91: 2190 – 2195.

Ramachandran, S., P. Fontanille, A. Pandey, and C. Larroche. 2006. Gluconic acid: properties, applications and microbial production. Food Technol. Biotechnol. 44: 185-195.

Rank, T. C., R. Grappin, and N. F. Olson. 1985. Secondary proteolysis of cheese during ripening. A review. J. Dairy Sci. 68: 801 – 805.

Rehman, S. U., M. A. Drake, and N. Y. Farkye. 2008. Differences between Cheddar cheese manufactured by milled-curd and stirred-curd methods using different commercial starters. J. Dairy Sci. 91: 76-84

Rengpipat, S. and E. A. Johnson. 1989. Characterization of *Lactobacillus* strain producting white crystals on Cheddar cheese. Appl. Environ. Microbiol. 55: 2579 – 2582.

Reville, W. J. and P. F. Fox. 1978. Soluble protein in Cheddar cheese: a comparison of analytical methods. Ir. J. Fd Sci. Technol. 2: 67 – 76.

Roberts, A. K. and Z. M. Vickers. 1994. Cheddar cheese aging: changes in sensory attributes and consumer acceptance. J. Food Sci. 59: 328-334.

Rollema, H. S. 1992. Casein association and micelle formation, In Advanced Dairy Chemistry, Vol. 1: Proteins (ed. P.F. Fox), Elsevier Science Publisher, Ltd., Essex, pp. 111-140.

Roy, D. 1991. Salt stress in growth and acid production of *Lactobacillus helveticus* strain Milano. Letters Appl. Microbiol. 12: 207 – 211.

Saidi, B. and J. J. Warthesen. 1989. Analysus and stability of orotic acid in milk. J. Dairy Sci. 72: 2900 – 2905.

Sakata, Y., S. Shiraishi, M. Otsuka. 2005. Characterization of dehydration and hydration behavior of calcium lactate pentahydrate and its anhydrate. Colloids Surf., B. 46: 135 – 141.

Saladini, M., L. Menabue, and E. Ferrari. 2000. Sugar complexes with metal^{2+} ions: thermodynamic parameters of associations of Ca^{2+}, Mg^{2+} and Zn^{2+} with galactaric acid. Carbohydr. Res. 336: 55-61.

Salaün, F., B. Mietton, and F. Gaucheron. 2005. Buffering capacity of dairy products. Int. Dairy. J. 15: 95 – 109.

Sallaml, L., E. E. Kheadr, I. Fliss, and J. C. Vuillemard. 2004. Impact of autolytic, proteolytic and nisin-producing adjunct cultures on biochemical and textural properties of Cheddar cheese. J. Dairy Sci. 87, 1585–1594.

SAS Institute. 1990. SAS User's Guide: Statistics, Version 6.0 ed. SAS lnst. Inc., Cary, NC.

Sawyer, D. T. 1964. Metal-gluconate complexes. Chem. Rev. 64: 633 – 643.

Schroeder, C. L., F.W. Bodyfelt, C. J. Wyatt., and M. R. McDaniel. 1988. Reduction of sodium chloride in Cheddar cheese: effect on sensory, microbiological and chemical properties. J. Dairy Sci. 71: 2010 – 2020.

Scott, R. 1986. Cheesemaking practice. Elsevier Applied Science Publishers, LTD., New York, NY.

Serrano, J., G. Velazquez, K. Lopetcharat, J. A. Ramı´rez, and J. A. Torres. 2004. Effect of moderate pressure treatments on microstructure, texture, and sensory properties of stirred-curd Cheddar shreds. J. Dairy Sci. 87: 3172-3182.

Severn, D. J., M. E. Johnson, and N. F. Olson. 1986. Determination of lactic acid in Cheddar cheese and calcium lactate crystals. J. Dairy Sci. 69: 2027 – 2030.

Sheehan, J. J., and T. P. Guinee. 2004. Effect of pH and calcium level on the biochemical, textural and functional properties of reduced-fat Mozzarella cheese. Int. Dairy J. 14: 161 – 172.

Shock, A. A., W. J. Harper, A. M. Swanson, and H. H. Sommer. 1948. What's in those "white specks" on Cheddar? Wisc Agr. Exp. Sta. Bull. 474. Univ. Wisconsin, Madison.

Silverman, G. J. and F. V. Kosikowski. 1955. Tyrosine in Cheddar cheese. J. Dairy Sci. 38: 950 – 958.

Singh, O. V. and R. Kumar. 2007. Biotechnological production of gluconic acid: future implications. Appl. Microbiol. Biotechnol. 75: 713-722.

Singh, T. K., M. A. Drake, and K. R. Cadwallader. 2003. Flavor of Cheddar cheese: a chemical and sensory perspective. Compr Rev in Food Sci and Food Safety. 2: 139-162.

Smit, G., B. A. Smit, and W. J. M. Engels. 2005. Flavor formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiol. Rev. 29: 591 – 610.

Sodium gluconate handbook. 1994. PMP Fermentation Products, Inc., Chicago, IL.

Somers, E. B., M. E. Johnson, and A. C. L. Wong. 2001. Biofilm formation and contamination of cheese by nonstarter lactic acid bacteria in the dairy environment. J. Dairy Sci. 84:1926–1936.

Sousa, M. J., Y. Ardo, and P. L. H. McSweeney. 2001. Advances in the study of proteolysis during cheese ripening. Int. Dairy J. 11: 327-345.

Stettenm M. R. and Y. J. Topper. 1953. Pathways from gluconic acid to glucose in vivo. J. Biol. Chem. 203: 653 – 664.

St-Gelais, D., G. Doyon, J. R. Rolland, and J. Goulet. 1991. Sugar and organic acid concentrations during ripening of Cheddar cheese-like products. Milchwissenchaft. 46: 288 – 291.

Sutherland, B. J. and G. W. Jameson. 1981. Composition of hard cheese manufactured by ultrafiltration. Aust. J. Dairy Technol. 36:136–142.

Svensson, B., B. Jönsson, M. Fushiki, and S. Linse. 1992. Ionic strength effects on the binding constant of calcium chelators: experiment and theory. J. Phys. Chem. 96: 3135 – 3138.

Swaisgood, H. E. 1992. Chemistry of caseins, In Advanced Dairy Chemistry, Vol. 1: Proteins (ed. P.F. Fox), Elsevier Science Publisher, Ltd., Essex, pp. 63-110.

Swearingen, P. A., D. E. Adams, and T. L. Lensmire. 2004. Factors affecting calcium lactate and liquid expulsion defects in Cheddar cheese. J. Dairy Sci. 87:574–582.

Swearingen, P. A., D. J. O'Sullivan, and J. J. Warthesen. 2001. Isolation, characterization, and influence of native nonstarter lactic acid bacteria on Cheddar cheese quality. J. Dairy Sci. 84: 50-59.

Szczesniak, A.S. 1963a. Classification of textural characteristics. J. Food Sci. 28: 385-389.

Szczesniak, A.S. 1963b. Objective measurements of food texture. J. Food Sci. 28: 410-420.

Thomas, T. D. and V. L. Crow. 1983. Mechanism of D(−)-lactic acid formation in Cheddar cheese. N.Z. J. Dairy. Sci. Technol. 18:131–141.

Thomas, T. D., D. C. Ellwood, and V. M. C. Longyear. 1979. Change from homo- to heterolactic fermentation by Streptococcus lactis resulting from glucose limitation in anaerobic chemostat cultures. J. Bacteriol. 138: 109-117.

Tuckey, S. L., H. A. Ruehe, and G. L. Clark. 1938. X-ray diffraction analysis of white specks in Cheddar cheese. J. Dairy Sci. 21: A161 (Abstr)

Tunick, M.H. 2000. Rheology of dairy foods that gel, stretch and fracture. J. Dairy Sci. 83: 1892–1898.

Turner, K. W. and T. D. Thomas. 1980. Lactose fermentation in Cheddar cheese and the effect of salt. N. Z. J. Dairy Sci. Technol. 15: 265 – 276.

Upadhay, V. K., P. L. H. McSweeney, A. A. A. Magboul, and P. F. Fox. 2004. Proteolysis in cheese during ripening, In Cheese: Chemistry, Physics and Microbiology, Vol 1: General Aspects, 3rd edn, (eds. Fox, P.F., McSweeney, P.L.H., Cogam, T.M. and Guinee, T.P.), Elsevier Academic Press, Boston, MA, pp. 391-433.

Upreti, P. and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: manufacture and composition. J. Dairy Sci. 89: 420 – 428.

Upreti, P. and L. E. Metzger. 2007. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: pH changes during ripening. J. Dairy Sci. 90: 1 – 12.

Upreti, P., L. E. Metzger, and K. D. Hayes. 2006. Influence of calcium and phosphorus, lactose and salt-to-moisture ratio on Cheddar cheese quality: Proteolysis during ripening. J. Dairy Sci. 89: 444 – 453.

Upreti, P., L. L. McKay, and L. E. Metzger. 2006. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. J. Dairy Sci. 89:429–443.

USDA. 1956. United States Standards for Grades of Cheddar cheese. Dairy Division. Agricultural Marketing Service. United States Department of Agriculture

USDA. 2008. *Dairy Products 2007 Summary*. National Agricultural Statistics Service. United States Department of Agriculture

U.S. FDA. 2006. FDA list of food additives that are 'Generally Recognized As Safe' (GRAS). From Internet website: http://www.cfsan.fda.gov

Van Den Berg, G. and F. A. Exterkate. 1993. Technological parameters involved in cheese ripening. Int. Dairy J. 3: 485 – 507.

van Hooydonk, A. C. M., H. G. Hagedoorn, and I. J. Boerrigter. 1986. pH-Induced physic-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physic-chemical properties. Neth. Milk. Dairy J. 40: 281 – 296.

Van Vliet, T. (1991a) Terminology to be used in cheese rheology. In Rheological and Fracture Properties of Cheese, Bulletin No. 268, pp. 5–15, International Dairy Federation, Brussels, Belgium.

Van Vliet, T. (1991b) Inventory of test methods. In Rheological and Fracture Properties of Cheese, Bulletin No. 268, pp. 16–25, International Dairy Federation, Brussels, Belgium.

Walstra, P. 1999. Casein sub-micelles: do they exist? *Int. Dairy J*., 9: 189-192.

Walstra, P., T. J. Geurts, A. Noomen, A. Jellema, and M. A. J. S. van Boekel. 1999. Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc., New York.

Washam, C. J., T. J. Kerr, and V. J. Hurst. 1982. Microstructure of various chemical compounds crystallized on Cheddar cheese. J. Food Prot. 45:594–596.

Washam, C. J., T. J. Kerr, V. J. Hurst, and W. E. Rigsby. 1985. A scanning electron microscopy study of crystalline structures on commercial cheese. Dev. Ind. Microbiol. 26: 749 – 761.

Whitney, R. M. 1988. Proteins of milk, In Fundamental of Dairy Chemistry, 3rd edn. (ed. N.P. Wong), Van Nostrand Reinhold, New York, pp. 81-169.

Wieczorek, M. W., J. Blaszczyk, and B. W. Karol. 1996. Effects of cation interactions on sugar anion conformation in complexes of lacobionate and gluconate with calcium, sodium, or potassium. Acta. Cryst. C52: 1193-1198.

World Health Organization. 1999. WHO Food Additives Series: 42, Safety Evaluation of certain cood cdditives: glucono delta lactone and the calcium, magnesium, potassium, and sodium salts of gluconic acid. Geneva.

Xiong, R., J –F. Meullenet, J. A. Hankins, and W. K. Chung. 2002. Relationship between sensory and instrumental hardness of commercial cheeses. J. Food Sci. 67: 877 – 883.

Zeppa, G., L. Conterno, and V. Gerbi. 2001. Determination of organic acids, sugars, diacetyl and acetoin in cheese by high-performance liquid chromatography. J. Agric. Food Chem. 49: 2722-2726.

Zeppa, G. and L. Rolle. 2008. A study on organic acid, sugar and ketone contents in typical Piedmont cheeses. Ital. J. Food Sci. 1: 127 – 139.