PRODUCTION OF LOW SODIUM CHEDDAR CHEESE: IMPROVING FLAVOR THROUGH THE USE OF FLAVOR ENHANCERS AND SALT REPLACERS

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Dedication

This thesis is dedicated to everyone who has helped me get to where I am now, in particular my parents who have always supported me in everything I do.

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CHAPTER 1: Introduction and Literature Review

1.1 Introduction

Sodium chloride (salt), an essential mineral, was originally used in foods as a preservative. Today it continues to be one of the most effective and versatile antimicrobial agents used in foods (Taormina, 2010). Two driving forces for this preservation are the lowering of water activity (a_w) and increased osmotic pressure. A lower a_w indicates less chemical potential of water for use by microorganisms. An increased osmotic pressure causes cells to contract and become misshapen due to the solution having a higher concentration of salt than the organism and the organism losing water via osmosis. It is known that salt inhibits the growth of many bacterial pathogens and spoilage organisms. Examples include *Listeria monocytogenes* (Nolan et al., 1992; Vogel et al., 2010), *Clostridium Botulinum* (Barbut et al., 1986), *Escherichia coli* (Abdulkarim et al., 2009), *Campylobacter jejuni* (Doyle and Roman, 1982), *Bacillus cereus* (Olmez and Aran, 2005), *Saccharomyces cerevisiae* (Bautista-Gallego et al., 2008), *Aeromonas hydrophila*, *Enterobacter sakazakii*, *Shigella flexneri*, *Yersinia enterocolitica* and three strains of *Staphylococcus aureus* (Bidlas and Lambert, 2008).

Due to the effectiveness of sodium chloride as a preservative, as well as additional benefits including taste, texture modification, fermentation control, and low cost, sodium is prevalent in the food supply, especially in processed foods. Mattes and Donnelly (1991) reported that in the United States, 77% of sodium in food was added during processing while only 12% was inherent to the food, 5% was added during cooking, and 6% was from table use.

Brown et al. (2009) conducted a comprehensive global review of salt intake. They reported that the studies from the United States, United Kingdom and Australia indicated

that between 1995 and 2006, mean sodium consumption ranged from 160.9 to 182.7 mmol/day (3700 to 4200 mg/day) for men and 118 to 142.3 mmol/day (2710 to 3270 mg/day) for women. According to the review, in the United States alone, mean sodium consumption between 1996 and 2000 was 168.6 to 182.7 mmol/day (3880 to 4200 mg/day) for men and 125.9 to 142.3 mmol/day (2890 mg/day to 3270 mg/day) for women. The current daily reference value for sodium in the United States is 2400 mg (21 CFR 101.9).

As a result of the prevalence of salt in food, sodium consumption is higher than recommended, and the health consequences of a high sodium diet have recently received much public attention. The public attention has largely focused on the link between sodium and hypertension, which is highly prevalent in the United States. The incidence of hypertension increased from 23.9% of the population in 1988-1994 to 28.5% in 1999-2000 and did not change between 1999-2000 and 2007-2008 (Egan et al., 2010). Despite recent attention, the recommendation of a low sodium diet as a means to reduce hypertension and cardiovascular disease (CVD) has existed for over 60 years (Kempner, 1944). Much research has since been conducted, and it is now believed that low-salt diets may also reduce the risk of stroke, left ventricular hypertrophy, osteoporosis, renal stones, asthma, cataract, gastric pathology, and possibly senile dementia (McCarty, 2004).

The connection between reducing sodium intake and lowering CVD appears to be one of logic: if reducing sodium intake lowers blood pressure (BP) and lower BP reduces the risk of CVD, then reducing sodium intake reduces CVD. However, as with most general assumptions, this logical connection is not as simple as it appears. Available data

does not provide conclusive evidence that increased sodium intake correlates with CVD or morbidity and mortality, the two common outcomes measured in studies that assess the consequences of sodium in the diet in the U.S. population. Of eight such studies (Kagan et al., 1985; Alderman et al., 1995; Tunstall-Pedoe et al., 1997; Alderman et al., 1998; Ascherio et al., 1998; He et al., 1999; Tuomilehto et al., 2001; Nagata et al., 2004), six examined participants with a mean sodium intake similar to the U.S. (~3 g/day) and two examined participants with a higher mean intake (4.4 and 5.5 g/day). Of the studies of similar mean intake, three found no significant relationship between sodium and CVD, two found an inverse relationship, and one found varying results between overweight (body mass index > 27) and non-overweight participants. No significant relationship was observed in non-overweight participants while significant relationships with stroke and various types of fatal CVD were observed in overweight participants. Of the studies of higher mean intake, varying CVD incidences were associated with higher sodium intake within the studied populations.

The varied conclusions from these studies depict the complexity of defining an overall relationship between increased sodium intake and CVD. Significant physiological and behavioral variation within a population limits the possibility of establishing such a relationship. However, as demonstrated by the aforementioned eight studies, it is possible that some subsets of the population could be affected more drastically by their dietary sodium intake than others, in particular hypertensive, overweight, and high-sodium consuming individuals as well as those in which sodium sharply affects BP.

While increased sodium intake cannot conclusively be associated with increased CVD, it does appear that reducing sodium intake leads to a lower systolic and diastolic

BP. Comprehensive reviews have shown that a reduction of 75-100 mmol sodium/day resulted in a lower systolic and diastolic BP of 1-6.7 and 0.1-3.5 mm Hg respectively (Midgley et al., 1996; Graudal et al., 1998; Hooper et al., 2002). Reduced BP has been associated with reduced CVD events. Cutler et al. (1997) reported that a 3 mm Hg decrease in mean systolic BP was associated with 11% fewer strokes, 7% fewer coronary artery disease events and 5% fewer total deaths. Macmahon et al. (1990) reported that, "prolonged differences in diastolic BP of 5, 7.5, and 10 mm Hg were respectively associated with at least 34%, 46%, and 56% less stroke and at least 21%, 29%, and 37% less coronary heart disease," and that a lower risk of CVD due to lower BP should be observed for both hypertensive and normotensive individuals.

While the necessary sodium reduction needed to result in an observed lower mean BP is significant based on the mean sodium intake in the U.S., it is relatively less significant for those that consume higher levels of sodium. In addition, it is possible that the more sodium-sensitive subsets of the population could benefit from a less dramatic level of sodium reduction. Despite the level of sodium reduction needed, evidence suggests that a reduced sodium diet would reduce the risk of CVD in certain individuals.

The literature does not indicate that a universal sodium reduction is appropriate. In fact, sodium reduction has been shown to have negative effects such as an increase in sympathetic nerve activity (Grassi et al., 2002) and insulin resistance (Petrie et al., 1998) as well as activation of the rennin-angiotensin system (Alderman et al., 1991), which regulates BP and water balance. Sodium, like other essential minerals, is biologically required by the human body at a certain level. Deficiency effects are observed at too low of a level and toxic effects are seen at too high of a level. Therefore, it is logical that

Cohen and Alderman (2007) explained that the relationship between sodium intake and CVD is likely "J"-shaped. They speculated that reduced sodium intake may be beneficial to those whom consume 4-5 g sodium/day or more. The level of sodium consumption at which negative effects are observed, whether it be too low or too high, is unknown and varies between individuals. Nonetheless, there are undoubtedly individuals that consume a harmful level of sodium. These individuals would benefit from a reduced sodium diet.

One challenge of lowering sodium intake is the previously described prevalence of sodium in processed foods. To reduce sodium in a diet, a conscious effort must be made to select foods with lower sodium contents. This requires that consumers understand the association between a reduced sodium diet and a lower risk of CVD. It appears that this understanding is increasing: hypertension and BP control improved from 1988-2008 in the U.S. (Egan et al., 2010). Awareness of the issue was a key contributor to these increases. In addition, 57% of respondents in the 2010 Mintel U.S. report on Attitudes Toward Sodium and High Fructose Corn Syrup Reduction stated that they limit use of packaged foods because they believed them to be high in sodium.

This awareness, if acted upon through reduced sodium intake, likely serves as beneficial to subsets of the population. Therefore, there is a need for reduced sodium foods. The top four contributors of sodium in the U.S. diet are yeast bread (10.2% of total dietary sodium), cheese (5.5%), ham (3.4%) and salad dressings/mayonnaise (3.2%) (Jacobson, 2005). These products are, consequently, the products for which sodium reduction could be most impactful. This research explores the reduction of sodium in cheese, specifically Cheddar. Cheddar cheese has a distinct flavor profile, and flavor is a significant factor of cheese acceptance and quality (Young et al., 2004). Therefore, due to

the impact of sodium on flavor, altering the sodium content of cheese can adversely affect its viability in the market. In addition, it may pose significant challenges to the safety, stability, and texture of the cheese.

The overall objective of this research is to improve the flavor of low sodium Cheddar cheese through the use of salt replacers and flavor enhancers. Under current regulations, a "low sodium" claim may only be made if the food (excluding meals and main dishes) contains 140 mg sodium/50 g product or less when the reference amount customarily consumed is 30 g or less (21 CFR 101.61). The reference amount customarily consumed for cheese is 30 g (21 CFR 101.12). Therefore, "low sodium" refers to a sodium content of 280 mg/100 g or less hereafter in this document. Also in accordance with 21 CFR 101.61, "reduced sodium" refers to a sodium content of at least 25% less per reference amount customarily consumed.

One challenge inherent to this research is determining the concentration of salt replacers to use. Another challenge is selecting flavor enhancers with high potential to improve flavor and determining what concentrations to use. To understand the effects of the salt replacers and flavor enhancers, chemical and sensory measurements can be made through aging, including a consumer acceptability test at the end of aging, to observe differences that may occur and determine which treatments are more preferred.

1.2 Literature Review

1.2.1 Cheese Manufacture

Cheese manufacturing is a process that involves the controlled removal of water from milk to produce a product with a concentrated level of fat and protein. This can be accomplished by coagulating protein in the milk and then removing water, or by concentration of the proteins and fat by removal of water through centrifugation (i.e. fromage frais) or vacuum condensing (i.e. ghetost). The most common methods involve the acid or enzymatic coagulation of the milk and manipulation of the curd to allow for water removal. A general procedure that describes the common methods of cheese manufacture is shown in Figure 1.1 (adapted from Fox et al., 2000d). Milk is standardized to meet certain requirements before it is pasteurized (commonly, but optional under specific regulations). Optional ingredients (color, calcium chloride) can be added, and pre-acidification with food-grade acid or starter cultures (various strains of bacteria) occurs. The cheese milk is then coagulated with acid or rennet (enzymes) to form a gel matrix. The coagulum is cut into small pieces to begin the separation of curd from whey before it is stirred and cooked. Stirring and cooking occurs until proper acid development and/or curd properties are achieved. Once proper conditions are reached, the whey portion is removed to yield cheese curd. Acid development may continue and special operations such as cheddaring, stretching, or spice/flavor addition may be applied. In the case of dry-salted cheeses, salt is applied to the curd to arrest acid development by the cultures (if cultured), promote syneresis of the curd, add a salty flavor, and act as a preservative. The curd is then molded to a shape in a mold under its own weight, or pressure is added for a period of time to assist with water removal. For cheeses that are

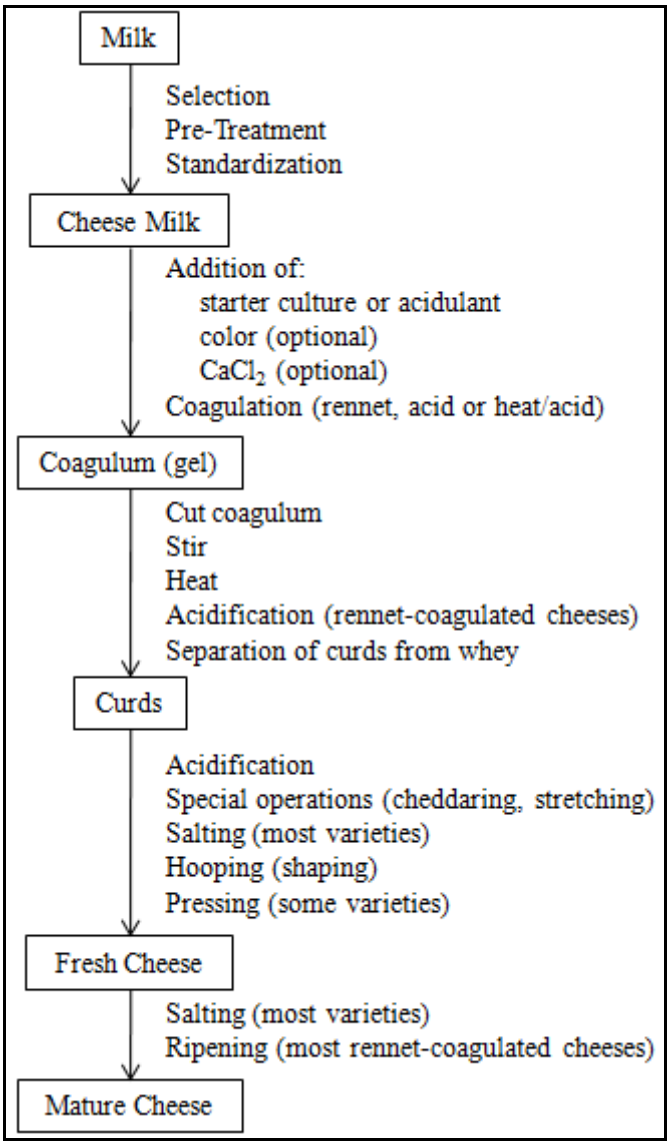

Figure 1.1. A general procedure for cheese making.

not dry salted and are instead brine salted, the formed cheeses are floated in a brine solution and the salt penetrates the cheese via osmosis. The fresh cheese can be consumed or aged under given conditions to produce a matured cheese.

The critical steps in Cheddar cheese manufacture are:

a) Coagulation of cultured cheese milk (typically with the enzyme chymosin)
b) Cutting the coagulum into small cubes to release whey
c) Cooking and stirring curds until proper acid development is achieved
d) Whey removal
e) For traditional matted curd Cheddar:
    a. Cheddaring: fusing curds into slabs to allow for acid and texture development
    b. Milling: cutting cheddared slabs of curd into smaller pieces to allow for better diffusion of salt into curd to arrest acidification by the culture
f) Dry salting: applying salt to curd before pressing
g) Hopping: putting curd into cheesecloth-lined hoops
h) Pressing: applying pressure to the curd to fuse it into blocks
i) Packaging and ripening of the pressed blocks

Cheddar cheese is considered matted curd if step e) is performed. Two other popular variations in the cheese making procedure that do not involve matting are termed "washed curd" and "stirred curd" Cheddar.

1.2.2 Cheddar Cheese Composition and Chemical Properties

According to the USDA Nutrient Database (2011), typical Cheddar cheese contains 36.75% moisture, 33.14% fat, 24.90% protein, 3.93% ash, and 1.55% salt. This calculates

to a salt-to-moisture ratio (S/M), which is the percent sodium chloride divided by the percent moisture, of 4.2%. According to Fox et al. (2000a), typical Cheddar cheese has a pH of 5.5 and a a_w of 0.95. However, due to differences in cheese making procedures and to the fact that different moisture contents can be targeted, there are no concrete targets for Cheddar cheese other than legal requirements and what is desired by the manufacturer in terms of quality and yield. Table 1.1 provides summary of the composition of full fat, full sodium control Cheddar cheese or industry-produced samples from previous studies whether it was stirred-curd, milled-curd, or washed-curd. Only cheese that could legally be labeled Cheddar cheese in United States was included (requirements included in Table 1.1). It should be noted that the measurements were taken at a variety of ages so Table 1.1 should serve as a summary of the composition through aging. It is also worth noting that the pH mean and range is lower than that reported by Fox et al. (2000a).

Table 1.1. Summary of the composition of full fat, full sodium Cheddar cheeses

Measurement	Mean	SD	n	Range	Sources[1]	Legal Note[2]
Moisture, %	36.7	1.3	20	34.9-39.0	1-12	Must be ≤ 39%
Fat, %	33.9	1.7	18	31.0-36.4	1-11	Must be > 50% by weight of solids
Protein, %	24.6	1.3	17	22.7-27.4	1-10	None
Total Ash, %	3.52	0.32	6	3.21-4.10	1-4	None
Salt, %	1.55	0.19	20	1.24-1.93	1-12	None
S/M, %	4.23	0.55	20	3.32-5.42	Calculated	None
pH	5.17	0.06	9	5.07-5.24	3, 8, 10, 12	None
Water Activity	0.958	0.004	2	0.955-0.960	13-14	None

[1]Sources: 1 = Nair et al., 2004; 2 = Azarnia et al., 2010; 3 = Fitzgerald and Buckley, 1985; 4 = Fox et al., 2000a; 5 = Agarwal et al., 2005; 6 = Bansal et al., 2009; 7 = Kilcawley et al., 2007; 8 = Neocleous et al., 2002; 9 = Schroeder et al, 1988; 10 = Whetstine et al., 2007; 11 = Lindsay et al., 1982; 12 = Reddy and Marth, 1993; 13 = Costa et al., 2010; 14 = Marcos et al., 1981
[2]21 CFR 133.113
NA: Not applicable

1.2.3 Role of Salt in Cheddar Cheese

The two main functions of salt in cheese are to act as a preservative and to contribute to flavor and quality (Guinee, 2007). It also provides a dietary source of sodium. Preservation occurs as a result of increased osmotic pressure and decreased a_w. Salt concentration, specifically the S/M, is a key contributor to the a_w of young Cheddar cheese and affects reactions that influence cheese quality (Lawrence et al., 1993; Guinee, 2007). The desired perception of saltiness in cheese is a direct result of salt. Indirect effects of salt on flavor result from influencing microbial and enzymatic activity (Guinee, 2007). These effects of salt and others are detailed in the subsequent sections.

1.2.3.1 During Manufacture

When salt is applied to the curd, the crystals dissolve and create a brine which diffuses into the curd. The curd undergoes syneresis and whey is expelled which results in additional salt crystals being dissolved which continues the cycle (Lawrence et al., 1993). For each kilogram of salt that is absorbed into curd, approximately 2 kg of water is expelled (Fox et al., 2000a). Many factors during cheese manufacture affect salt uptake and ultimately the S/M of Cheddar cheese. The factors are summarized in Table 1.2. A certain amount of salt is lost to the whey that is expelled as a result of syneresis regardless of the control of these factors. Percent of salt lost can range from approximately 10-15% at a very low salting rate (0.5%) (Fox et al., 2000a) to up to 50% at higher salting rates (2.7%) (Nair, 2004). The cheese making procedure and milk quality largely affect the salt uptake (Nair, 2004). In addition, roughly 0.25 kg of fat per 100 kg cheese is lost upon salting and this loss increases significantly as temperature increases (Fox et al., 2000a).

Acid production in the vat is a critical factor to control during Cheddar cheese making due to its close association to the final pH and the basic structure of the cheese (Lawrence et al., 1993). Dry salting results in the retardation of the starter culture activity, and the pH of the curd at salting is, therefore, near the final pH of the cheese (Fox et al., 2000a). The pH of the cheese affects the colloidal calcium phosphate concentration, dissociation of the casein micelles into smaller aggregates, and whey expulsion (Lawrence et al., 1993). At a consistent curd pH at salting, the S/M largely controls the final pH of the cheese by influencing the activity of the starter culture after salting.

Table 1.2. Factors and their effects of salt uptake in Cheddar cheese (Lawrence et al., 1993; Fox et al., 2000a; Guinee 2007)

Factor	Effect on Salt Uptake (SU)
Amount of salt added to curd	Increased SU with increased amount added (non-linearly)
Size of salt crystal	Variable if crystal size is not uniform when using an automated process[1]
Mixing time of salt and curd	Increased SU as mixing time increased from 20 s to 6 min[2]
Post-salting, pre-pressing holding time	Increased SU with increased time up to 30 min[2]
Curd temperature at salting	Decreased SU with increased temperature from 24 to 41°C
Curd size to surface area ratio	Decreased SU with increased ratio
Curd moisture level	Decreased SU with increased moisture
Curd acidity at salting	Increased SU with lower acidity
Depth of the bed of curd	Decreased SU with increased depth[3]

[1]Due to uneven/inconsistent delivery from the salting equipment
[2]The increased time reduces the amount of salt lost to the whey
[3]Moisture level and salt-to-moisture ratio are also decreased

1.2.3.2 During Aging

Cheddar cheese manufacturers typically aim for a S/M between 4 and 6% to produce high quality cheese. The effect of salt on cheese quality is extensive because S/M impacts the rates of major enzymatic and bacterial reactions involved in cheese making, the effects of which are discussed below.

Proteolysis is considered to be the most significant biochemical reaction in Cheddar cheese ripening. A proper S/M facilitates a positive balance of the proteolytic activity of residual coagulant, natural milk proteinases such as plasmin, and enzymes from starter and non-starter bacteria; an imbalance of the proteolytic activity leads to off-flavors and aromas plus texture defects (De Wit et al., 2005; Lane and Fox, 1997). Excessive bitterness is a typical off flavor attributed to proteolysis (Fox, 1989). Intermediate-sized peptides are created by the proteolysis of casein due to the coagulant chymosin and native enzyme plasmin. The coagulant and the proteinases and peptidases from starter and non-starter bacteria continue to hydrolyze the peptides into smaller peptides and free amino acids which positively or negatively contribute to flavor and aroma (Lane and Fox, 1997). The inhibitory effect of salt on bitterness decreases below a S/M of 4.9% (Fox et al., 2000a).

Proteolysis also leads to a softer texture due to weakening of the protein network, increased pH as a result of exposed amine groups, and greater water binding capacity due to exposed amine and carboxyl groups (Fox, 1989).

Lipolysis is also an important biochemical reaction that contributes to the flavor of Cheddar cheese, and the rate of reaction is influenced by the S/M (Guinee, 2007). Flavor is directly influenced by the formation of free fatty acids (FFA) (Adda et al., 1982) and indirectly influenced by precursors to flavor compounds such as methylketones, alcohols, and lactones (Smit et al., 2002). Lipases are present in milk and rennet (Chilliard and Lamberet, 1984), and formed by lactic acid bacteria (LAB) (Fox et al., 2000b). Of these lipases, the LAB lipases appear to be primary lipolytic agents in pasteurized-milk Cheddar cheese. Lipoprotein lipase, a powerful lipase in raw milk, has

more significant effects in raw-milk Cheddar cheese than in cheese made with pasteurized milk (Collins et al., 2003). Like proteolysis, a certain degree of lipolysis is considered important as FFAs serve as constituents to Cheddar cheese flavor (De Wit et al., 2005).

The S/M of Cheddar cheese also impacts the bacterial metabolism of lactose after salting. When residual lactose is present and the S/M is below 5%, starter bacteria ferment the sugar and produce lactate, the conjugate base of lactic acid which lowers the pH (Fox et al., 2000b). At a higher S/M, starter activity is halted, resulting in a high level of residual lactose and higher pH. The effect of residual lactose on the quality of mature Cheddar cheese is inconclusive, but is generally not considered to be positive in regard to product quality (Lawrence et al., 1993; Fox et al., 2000a).

Of the factors that influence microbial stability of Cheddar cheese, a number are impacted by S/M including water activity (a_w). Water activity is one of the most fundamental properties related to the control of microbial growth (Tapia et al., 2007). It is defined as the vapor pressure at the surface of a substance divided by the vapor pressure of pure water at the same temperature and ranges from 0 to 1.0 (1.0 is equal to pure water). Water activity describes the availability of the water in a system with values approaching 1.0 indicating increased availability. Microorganisms will not grow or produce toxins below a limiting a_w, and a value of 0.95 is generally accepted for most spoilage bacteria with the exception of *Staphylococcus aureus*, which can produce toxins down to 0.85 at room temperature (Tapia et al., 2007). Because food systems are complex and variable, the limiting a_w is generally determined in a laboratory under ideal conditions (Tapia et al, 1007). Therefore, the limiting a_w in a given food may effectively

be higher because ideal conditions likely do not exist. In young Cheddar cheese a_w is primarily determined by the S/M (Lawrence et al, 1993). During aging, the continued lowering of a_w due to the production of lactic acid and products of proteolysis is also indirectly influenced by the S/M (Marcos et al., 1981). These combined effects are significant, but certain spoilage bacteria grow below a a_w of 0.95 and osmophilic yeasts and xerophilic molds can grow down to a a_w of ~0.60 (Tapia et al., 2007). Other factors that assist in the control of microorganism growth, and are indirectly affected by the S/M, include pH and oxidation-reduction potential, both of which are related to starter culture fermentation of lactose to lactic acid (Fox et al, 2000c). The above hurdles to microbial growth in Cheddar cheese combine to have a larger summed effect and are further assisted by non-salt related factors such as refrigeration and vacuum or wax packaging.

1.2.3.3 Effect on Safety

Tainted cheese products have been implicated in causing food-borne illness. The majority of outbreaks have been caused by raw milk and soft varieties (McSweeney, 2007). *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and enteropathogenic strains of *Escherichia coli* are the organisms most often responsible for outbreaks in cheese (McSweeney, 2007). Sodium is one of the key hurdles against food-borne pathogens (Taormina, 2010). It affects the above organisms to varying extents.

Salmonella and *E. coli* are relatively salt sensitive. Goepfert et al. (1968) found that the population of *Salmonella typhimurium* in Cheddar cheese made with inoculated cheese milk increased 130 times through the curd production stage due to release of whey (responsible for ~10-fold increase in the curd) and growth. They reported that growth ceased or slowed during salting which they attributed to direct contact with salt. The

Salmonella populations decreased by a factor of 3 logs at 12 to 16 weeks of age depending on storage temperature. Messier et al. (1989) found populations of *Salmonella typhimurium* to be eliminated at 11 days after the production of 2%-salted salami (similar composition to Cheddar cheese). Hosein et al. (2011) observed *E. coli* O157:H7 populations to be reduced by approximately three logs in 20 hours and four logs in 10 hours in pH 3.2 broth solutions with 2 and 4% salt respectively. Glass et al. (1992) found enteropathogenic *E. coli* growth to be inhibited in fermented sausage with a pH of 4.8 with 69 ppm of sodium nitrite. While the conditions in the *E. coli* studies were harsher than in cheese, they nonetheless demonstrate that sodium has an inhibitory effect.

Listeria monocytogenes and *Staphylococcus aureus* are salt tolerant and salt resistant respectively (Taormina, 2010). Of bacterial food-borne pathogens, *L. monocytogenes* is only exceeded by *S. aureus* in osmolality tolerance (Nolan et al., 1992). While salt tolerances of these organisms are significantly higher than the concentrations in Cheddar cheese (Buchanan et al., 1989), data indicates that salt tolerance is lower in food matrices than in laboratory conditions (Nolan et al, 1992; Messier et al., 1989) due to additional variables or hurdles to the microorganisms. Of particular interest to cheese makers are the findings of Rajkowski, et al. (1994) that growth of *L. monocytogenes* and *S. aureus* was inhibited by 4.5% sodium chloride in inoculated UHT sterilized milk. Factoring in the water content of milk, this is a S/M of 5.2% which is a reasonable approximation to Cheddar cheese. The sodium ion has been implicated as the lethal agent for heat-stressed *S. aureus* (Erwin and Haight, 1973). In addition, as discussed above, salt affects the pH and a_w of cheese, both of which influence the growth and survival of these pathogens. At salt concentrations from 0.5 to 8%,

McClure et al. (1991) saw that growth of *L monocytogenes* did not occur in Tryptone Soya Broth at or below pH 5.3 and 4.8 at 5°C and 10°C respectively. While Valero et al. (2009) saw that *S. aureus* can grow at pH 4.5 and at a a_w 0.867 under certain conditions, they concluded that growth was inhibited below 8°C regardless of pH and a_w. At 8°C growth only occurred at optimal pH and a_w.

An important factor to consider when evaluating the safety of Cheddar cheese in regard to the above pathogens and others is the fact that the vast majority of the related research studied inoculated systems. Barring post-pasteurization contamination from gross violations of plant sanitation or intentional contamination, the concentrations of pathogens in cheese does not reach those levels. Therefore, the starter culture also provides inhibition via competitive exclusion. For example, Staphylococci do not compete favorably against fermentative lactic acid bacteria (Genigeorgis, 1976).

1.2.4 Sodium Reduction Strategies

Interest in sodium reduction of cheese has increased, but many challenges exist in doing so due to the previously described effects of sodium in cheese. Nonetheless, various strategies exist to reduce the sodium content of food. Relevant strategies to natural cheese are discussed in the subsequent sections.

1.2.4.1 Reduction of Sodium to the Minimum Level Required to Maintain Quality

Many foods contain higher sodium levels than required to achieve consumer acceptance. Therefore, the sodium levels of these foods could be reduced without significant negative impact. The level of acceptable reduction would be unique to each food product. In full fat Cheddar cheese this topic has been explored by Lindsay et al. (1982) and Schroeder et al. (1988). Schroeder et al. (1988) found that a trained sensory panel could correctly

identify cheese in order from highest to lowest salt content (equivalent to 4.1, 3.1, 2.0, 1.0, and 0.2% S/M). The panel did not observe any other discriminating flavor differences between the cheese with a 4.1 and 3.1% S/M. Below a 3.1% S/M, higher acidity and bitterness, lower Cheddar intensity, and aftertaste were detected. These findings corresponded well with the untrained consumer panel in which the cheeses with a 4.1 and 3.1% S/M were liked equally while cheese with a lower S/M were less liked through seven months of aging. Lindsay et al. (1982) observed a lower consumer liking score for nine-month old cheese with a 3.5% S/M than cheese with a 4.2% S/M. The two studies do not agree on the level of sodium reduction at which consumer liking decreases. Mistry and Kasperson (1998) conducted a descriptive sensory analysis of body/texture, flavor intensity, acid, bitterness, and appearance in reduced fat Cheddar cheese. Only flavor intensity was significantly different between cheese with a 4.5% S/M and cheese with a 3.7% S/M while body/texture, flavor intensity, acid, and bitterness were significantly different between the cheese with a 4.5% S/M and cheese with a 2.7% S/M. While no consumer test was conducted, these results indicate that liking may have been similar between the cheese with 4.5 and 3.7% S/M, but not between the cheese with 4.5 and 2.7% S/M.

The variation in sodium reduction that resulted in lower consumer liking demonstrates that this strategy of sodium reduction would be specific to individual cheese manufacturers. Determining the minimum level of salt required to maintain quality would be required of each manufacturer, and the definition of quality would likely vary. Additionally, tight control and understanding of the cheese making procedure would be required to consistently achieve the target salt content. This would involve factors such as

standardization of cheese milk, addition of culture at a rate dependent on milk composition rather than by weight or volume, the pH of milk or curd throughout manufacture, firmness of the gel at cutting, cook temperature and profile, time of whey drainage after reaching cook temperature, and the Cheddaring process (for milled-curd). Tight control of the factors affecting salt uptake (Table 1.2) would also be required.

Similar to reducing sodium to a level required to maintain quality is reducing sodium to meet the label claim of "reduced sodium." Under current regulations, reduced sodium foods are foods with 25% less sodium per reference amount customarily consumed (21 CFR 101.61). Reduced sodium Cheddar cheese is currently marketed by various cheese manufacturers. While the quality of these cheeses may be acceptable, the lower level raises other concerns, particularly safety, because other anti-microbials are typically not added due to conflict with the standard of identity for Cheddar cheese. NaCl is one of the most effective and versatile anti-microbials in food (Taormina, 2010). Therefore, its removal without reinforcement of microbial stability poses a considerable threat to the safety of reduced sodium food. Taormina (2010) explained that pathogen growth and survival may increase and that accelerated spoilage of certain foods may occur as a result of haphazard sodium reduction. In cheese, these effects would occur due to a lower S/M and its consequences.

1.2.4.2 Gradual Reduction of the Sodium Content

One strategy to reduce sodium, whether to a level to maintain quality or another level, can be done through gradually reducing the sodium content without the consumer's knowledge, i.e. the reduction is not explicitly marketed or promoted. This can be particularly effective due to the concept of a Just Noticeable Difference (JND) which

refers to the difference threshold, or the minimum level of difference that must be present to be detected by an observer (Lawless and Heymann, 1999). Therefore the sodium content of a food could be reduced at a level below the JND with minimal notice from consumers. Bertino et al. (1982) demonstrated that the preference for sodium can be lowered after a reduction in sodium intake. Consequentially, after a period of adaption to the initial decrease in sodium, it is likely that an additional decrease in sodium content could be implemented, again below the JND. However, the JND for the subsequent reductions would possibly change because the JND only applies to the food at the original concentration (Drake et al., 2011). In cottage cheese, Drake et al. (2011) found that an untrained panel could detect an 8% reduction in cottage cheese (original concentration was 450 mg/113 g product) and a 14% reduction in cheese sauce (original concentration was 360 mg/28 g product, approximately twice that of Cheddar). Wyatt (1983) found that untrained panelists did not notice a difference in 25% reduced sodium cottage cheese (original concentration was 325 mg/100 g product), but a 50% reduction was noticed. These studies demonstrate, like the previous strategy, that a gradual reduction in sodium would be specific to individual cheese manufacturers. The series of gradual reductions would eventually encounter insurmountable challenges due to the effect of a lower S/M. Also, like the previous strategy, the safety of the cheese would be of concern.

1.2.4.3 Use of Alternative Mineral Salts

When reducing sodium in foods, a common initial concern is a decrease in the salty taste of that food. To compensate for a lower saltiness, mineral salt replacers can be used to maintain a salty flavor (Murphy et al., 1981). Multiple types of salt taste receptors are

present on the tongue including at least one that responds to a variety of cations (DeSimone and Lyall, 2006). In addition, the ions from the replacers interact with free water and lower the a_w (Taormina, 2010), which assists in microbial stability. This is an advantage of this strategy of sodium reduction. The readily apparent disadvantage of salt replacers, in terms of quality, is the associated bitterness. Due to the suppression of saltiness and direct contribution to flavor of phosphates and citrates, chloride-based salts are more commonly used as salt replacers (Guinee and O'Kennedy, 2007).

In cheese, potassium chloride (KCl), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$) have been investigated (Lindsay et al., 1982; Taylor, 1983; Fitzgerald and Buckley, 1985; Lefier et al., 1987; Aly, 1995; Reddy and Marth, 1995; Katsiari et al., 1997; Katsiari et al., 2001). Research of $CaCl_2$ and $MgCl_2$ is more limited, which is likely due to the higher observed metallic flavor and bitterness. Fitzgerald and Buckley (1985) investigated Cheddar cheese salted individually with $CaCl_2$ and $MgCl_2$ at an ionic strength equivalent to the 2.5% NaCl control which equated to 1.58 and 1.35% respectively. Cheese was also salted with the two replacers in a 1:1 mixture with NaCl, although it was not specified if the ratio was based on an ionic strength, weight, or molar equivalent. The cheeses with only the replacers were determined to be excessively bitter and were not included in the study's sensory evaluation. The cheese with the 1:1 mixtures were found by an untrained panel to be metallic and bitter with a flaky, crumbly, and greasy texture. In a Gruyere-type cheese studied by Lefier et al. (1987), bitterness was higher and the body was softer than control in cheese salted with a $MgCl_2$ brine. However, acceptability was high as determined by the taste panel.

The use of KCl has produced more positive results than $CaCl_2$ and $MgCl_2$ and has been more widely studied (Koenig and Marth, 1982; Lindsay et al., 1982; Taylor, 1983; Fitzgerald and Buckley, 1985; Reddy and Marth, 1993; Reddy and Marth, 1994; Aly, 1995; Reddy and Marth, 1995; Katsiari et al., 1997; Laborda and Rubiolo, 1999; Katsiari et al., 2001, Ayyash and Shah, 2011). The amount of KCl as a percent of total salt has ranged from 100% to 25%, and the total percent salt has also varied, but it is difficult to determine the exact range because final concentrations were not always listed or listed clearly. In general, consumer liking was high in these studies, although Lindsay et al. (1982), Aly (1995), and Katsiari (1997) observed a higher preference or total rating score for the treatments with less KCl within their respective studies. This was attributed mostly to higher bitterness. These results indicate that KCl may not practical for total substitution of NaCl, but it is still a useful tool for reducing sodium in cheese.

In addition to liking and flavor, other aspects of the cheese were examined. Among the aforementioned studies, moisture was similar between control treatments and KCl treatments, with an overall range of -0.8 to +0.5% (most between -0.2 and +0.2%). Among the aforementioned studies that examined Cheddar cheese, the difference in pH between control and KCl treatments was typically small (between -0.06 and +0.05), but Fitzgerald and Buckley (1985) saw a difference of +0.16. Among seven of the aforementioned studies, proteolysis was not found to be different between control and KCl treatments, as measured by various indicating measurements (discussed in section A.1.7 of the appendix) with the exception of Fitzgerald and Buckley (1985) who found proteolysis to be lower by an average of 2.87% over 16 weeks of aging in cheese salted

with only KCl (3.18% wt/ wt).. Among the three studies that measured texture attributes, via texture profile analysis and/or sensory analysis, very few differences were observed.

In addition to the chemical and physical characteristics of cheese salted with KCl, the resultant microbial stability is another important topic as this directly relates to the safety of the food. The use of KCl appears to affect microorganisms similarly to NaCl, although this is not definitively established. In Cheddar cheese, Reddy and Marth (1995) observed no significant difference ($p < 0.05$) between cheese salted with various ratios of KCl and NaCl in the counts of aerobic microorganisms, LAB (starter or non-starter), aerobic spores, coliforms, yeasts, and molds. In rennet whey that was salted with NaCl, KCl, or a 1:1 molar ratio mix of the two, Larson et al. (1993) observed no effect of the salt treatments on the growth of *Listeria monocytogenes* or *Salmonella heidelberg*. In Kimchi, a fermented cabbage product, Choi et al. (1994) observed similar growth rates of LAB during fermentation. These findings are generally supported by studies in laboratory conditions: Nielsen and Zeuthen (1987), Boziaris et al. (2007), and Bidlas and Lambert (2008) found KCl to inhibit various pathogenic and non-pathogenic bacteria to the same extent as NaCl when used on an equivalent molar or a_w basis in nutrient broths. Nielsen and Zeuthen (1987) also observed the same effect in meat emulsions. However, Bautista-Gallego et al. (2008) found KCl to inhibit *Lactobacillus pentosus* and *Saccharomyces cerivisiae* to a lesser extent than NaCl, but conclusions were made on a weight-weight basis. It is difficult to determine if equal inhibition would have been observed on an equivalent molar basis.

The above studies are not capable of describing the effects of KCl on all microorganisms so a definitive conclusion about the effectiveness of KCl cannot be

reached. Additionally, when bacteria experience osmotic stress they, as a preservation technique, uptake potassium or other ions without an interference in cell metabolism in order to maintain cellular turgor pressure with the surrounding media (Welsh, 2000).

Despite the role of potassium in cellular preservation, KCl was shown to have a high level of inhibitory effect in cheese, rennet whey, Kimchi, meat emulsions, and nutrient broths (Reddy and Marth 1995; Larson et al., 1993; Choi et al., 1994; Nielsen and Zeuthen, 1987; Boziaris et al., 2007; Bidlas and Lambert, 2008). Additionally, $MgCl_2$ and $CaCl_2$ have been shown to have an inhibitory effect on microorganisms: Nielsen and Zeuthen (1987) found $MgCl_2$ and $CaCl_2$ to have a higher inhibition effect than NaCl in meat emulsions and nutrient broths. Bautista-Gallego et al. (2008) found $CaCl_2$ to have similar inhibition to NaCl while $MgCl_2$ showed slightly lower inhibition.

Due to the demonstrated inhibitory capability of salt replacers, the technique of using them to reduce sodium in food may be more practical than the previously described techniques when all factors are considered rather than solely taste and texture.

1.2.4.4Addition of Flavor Enhancers

A disadvantage of the previous strategy is the inherent off flavors from salt replacers when used at high concentrations. To overcome this issue, flavor enhancers are currently used in reduced sodium foods such as soups, frozen meals, processed meats, seasoned dry snacks, and canned vegetables to reduce off flavors associated with salt replacers and enhance the salty perception of the foods. Examples of flavor enhancers include monosodium glutamate (MSG), hydrolyzed vegetable protein (HVP), yeast extract (YE), disodium inosinate (IMP), disodium guanylate (GMP), and compounds that reduce

bitterness (Institute of Medicine, 2010). If flavor enhancers are used appropriately, products that are acceptable to consumers may be achieved (Reddy and Marth, 1991).

To the author's knowledge there has been no research conducted on the use of flavor enhancers in cheese to enhance saltiness or reduce off flavors of salt replacers. Yeast extract was used by Shakeel-Ur-Rehman et al. (2003) as a source of nutrients for non-starter LAB in reduced fat Cheddar cheese in an attempt to enhance flavor development in ripened cheeses. They found non-starter LAB counts to be higher in the experimental cheese through 30 days of aging, but after 60 days the counts were similar. The cheese with yeast extract had differences in its flavor profile, as determined by a six-member trained sensory panel, including higher sulfur and lower brothy flavor intensities. This indicates that flavor enhancers may be capable of modifying flavors in cheese.

Monosodium glutamate contains a large amount of glutamic acid, which Drake et al. (2007) found to naturally play the largest role in umami taste in Cheddar and Swiss cheese. Drake et al. (2001) and Young et al. (2004) found umami intensity to increase with cheese age and to correlate positively with a number of aged Cheddar cheese flavor attributes. Therefore, umami appears to be an important factor in Cheddar cheese flavor.

Due to the public concern of MSG causing health issues including possible nervous system damage (Meadows, 2003), cheese manufacturers likely would not want to use MSG as an ingredient to increase umami taste in their cheese. Fortunately, other compounds such as IMP, GMP, HVP, and YE elicit umami taste or similar characteristics (Kojima, 1974; Dall Aaslyng et al., 1998; Institute of Medicine, 2010). The umami or umami-type characteristics of these flavor enhancers are likely largely responsible for increasing consumer acceptability of the low sodium foods in which they are used. The

umami could contribute directly to a more favorable taste or mask undesirable tastes and flavors. Also, these compounds are capable of enhancing the salty taste of food (Fuke and Ueda, 1996; Institute of Medicine, 2010). One mechanism for enhanced salty taste is by stimulating the magnitude of a current through the epithelial-sodium channel, the sodium-specific salt taste receptor (Desimone and Lyall, 2006), so it is possible that these compounds are capable of doing so.

Other compounds are developed with the intent to reduce bitterness. Such compounds are typically proprietary to the companies that produce them so it is difficult to understand their mechanism behind their function. However it is possible that the compounds bind to a bitter substance which prevents it from triggering a bitter taste signal to the brain (Funasaki et al., 2006) or bind to bitter taste receptors which blocks bitter substances from interacting with them (Ming et al., 1999).

Regardless of the mechanism by which flavor enhancers operate, they offer the potential to modify the taste or flavor of reduced sodium foods that suffer from bitterness, and could be a useful tool in reducing sodium in cheese. This is a particularly promising strategy when paired with the use of alternative mineral salts because the potential of greater sodium reduction exists due to the possibilities of reducing off flavors associated with the alternative salts and enhancing the salty perception. Additionally, the safety of the cheese would likely be higher than cheese made using strategies 1.2.4.1 and 1.2.4.2 because the alternative salts have an inhibitory effect on microorganisms.

1.2.4.5 Additional Strategies

Sea salt sales have increased recently and companies have incorporated sea salt into products (Mintel, 2009, Mintel, 2011). Many people believe sea salt is lower in sodium

because it contains other minerals from the sea water it is derived from. While other minerals are often present in sea salt, the sodium reduction is usually small. However, some suppliers do provide sea salt with substantial reductions, up to 45% (A and B Ingredients, Fairfield, NJ) and possibly higher. These sea salts are potentially useful for reducing sodium in foods, but it is possible that the unique minerals could affect the taste, flavor, and texture of a product. For example, Fitzgerald and Buckley (1985) observed significant flavor and texture differences between Cheddar cheese salted with KCl, $MgCl_2$, and CaC_2. Therefore, testing should be done with specific sources of sea salt to understand the effects.

Another strategy to reduce sodium in cheese has been to increase the protein content of cheese milk via supplementation with ultrafiltered whole milk retentate (UFMR). Kosikowski (1983) found that reduced sodium Cheddar cheese without supplementation was more bitter, acidic, and pasty plus lacked normal Cheddar flavor compared to reduced sodium cheese made with various levels of UFMR. The latter cheeses rated as being good to excellent quality Cheddar cheese. No age of the cheese at testing was specified. The difference was attributed to the increased level of calcium and phosphate which act as buffers and prevent a low pH which in turn limits bitterness due to proteolysis. This strategy appears to be beneficial to the acceptance of reduced sodium Cheddar cheese, but as with previous methods, reducing the sodium content of the cheese without reinforcement of microbial stability should be cautioned.

1.3 Research Hypotheses

Based on the effects of sodium in cheese and the strengths and weaknesses of the above sodium reduction strategies, the following hypotheses were established:

1. A model system will determine the appropriate concentrations of salt and mineral salt replacers to achieve equal a_w in experimental cheese production of cheeses with varying sodium contents.
2. Producing low sodium Cheddar-style cheese with mineral salt replacers with equivalent a_w to full sodium Cheddar cheese will result in similar biochemical and microbiological reactions during cheese aging
3. The incorporation of flavor enhancers into low sodium Cheddar-style cheese with mineral salt replacers will reduce the negative sensory attributes associated with the replacers
4. The incorporation of flavor enhancers into low sodium Cheddar-style cheese with mineral salt replacers will not alter the biochemical, microbial, and physical characteristics of the cheese during production and aging.

To test these hypotheses, the research described in the following chapters was performed to scientifically advance the sodium reduction technology of Cheddar cheese. As Sofos (1983) concluded, reduction of the sodium content in food should only occur if appropriate research supports doing so due to the complexity of the subject.

CHAPTER 2: Determination of Salt and Salt Replacer Concentrations to Achieve Equal Water Activity in Cheddar Cheese with Varying Sodium Content

2.1 Introduction

The objective of this research was to quickly and economically determine salt (sodium chloride) and mineral salt replacer concentrations needed to produce reduced sodium cheese with equivalent water activity (a_w) to full sodium control, without making cheese. Previous studies of reduced sodium cheese have used mineral salt replacers when reducing sodium in cheese (Lindsay et al., 1982; Fitzgerald and Buckley, 1985; Aly, 1995; Reddy and Marth, 1995; Katsiari et al., 1997; Katsiari et al., 2001). However, most of them have used levels too low to produce equal a_w to full sodium control. One reason for this is that it is difficult to predict final a_w of cheese. A quick and economical method to determine salt and salt replacer treatments necessary to produce equal a_w in Cheddar cheese would therefore be beneficial to cheese makers.

It was hypothesized that a model cheese system could be developed in which direct a_w measurement could be made to assess whether or not pre-calculated salt and mineral salt replacer concentrations produce equivalent a_w to control. In addition, the concentrations determined with the model system could be applied to cheese making to yield the same result in finished cheese.

To test the hypothesis, all-purpose flour, salt and salt replacers (potassium chloride, modified potassium chloride, magnesium chloride and calcium chloride) were blended with butter and water at concentrations that approximated the solids, fat, and moisture contents of typical Cheddar cheese. Salt and salt replacers were applied to the model systems at concentrations predicted by Raoult's Law, a predictive a_w equation. The a_w of the model samples was measured on a a_w meter, and concentrations were adjusted using Raoult's Law if they differed from the full sodium model. Based on the results

determined using the model system, stirred-curd pilot scale batches of reduced and full sodium Cheddar cheese were manufactured in duplicate. Water activity of the cheese was measured and evaluated statistically by linear mixed model.

2.2 Materials and Methods

2.2.1 Materials

Sodium chloride (**N**) (Top-Flo® Evaporated Salt, Cargill, Inc., Minneapolis, MN) and the salt replacers potassium chloride (**K**) (Premier™ Potassium Chloride 8799, Cargill, Inc., Minneapolis, MN), a modified potassium chloride (**MK**) (Modified Potassium Chloride 14510, Nu-tek Products, Inc., Minnetonka, MN), magnesium chloride (**MG**) (magnesium chloride, 6-Hydrate 5956-06, Mallinckrodt Baker, Inc., Phillipsburg, NJ), calcium chloride (**C**) (calcium chloride, dihydrate, granular 4616-06, Mallinckrodt Baker, Inc., Phillipsburg, NJ), and a 45% reduced-sodium sea salt (**S**) (SS45, A and B Ingredients, Fairfield, NJ), all-purpose flour (Pillsbury BEST Flour, The J.M Smucker Company, Orrville, OH), and salted butter (Land O'Lakes, Inc., St. Paul, MN) were used to prepare model systems. All chemicals were Food Chemicals Codex (FCC) or United States Pharmacopeia (USP) grade.

2.2.2 Salt and Salt Replacer Concentration Determination

Raoult's law (Figure 2.1) was used to calculate concentrations of salt and replacement salts. An activity coefficient of one was used. Typical Cheddar cheese values of 1.6% salt and 36.8% moisture (wt/wt) were used as the values for the full sodium control, and Raoult's law predicted a a_w of 0.974 using those values. For reduced sodium treatments, excluding the S treatment, a 53% salt reduction was used as the target, which equates to

300 mg sodium/100 g sample. The amount of salt replacer (K, MK, MG or C) needed to equal the same a_w as the full sodium target (0.974) and was calculated using Raoult's Law. For the S treatment, information provided by the manufacturer was used to calculate an equal concentration of solutes as the control treatment. It was not possible to calculate MK concentrations because the percent formula of all the solutes in the product was not known. Therefore, the predicted concentrations were based on analogous K concentrations.

$$a_w = \gamma_s X_{water} = \gamma_s \frac{n_{water}}{n_{water} + n_{solutes}}$$

$$\gamma_s = Activity\ Coefficient$$

$$X = Mole\ Fraction$$

$$n = Number\ of\ Moles$$

Figure 2.1. Raoult's Law (Labuza and Altunakar, 2007).

2.2.3 Preparation of Model Cheese Systems

The general scheme for model system preparation is outlined in Figure 2.2. All-purpose flour and salt plus salt replacer were dry blended before butter and water were added to achieve 36.8% moisture and 36% fat (wt/wt). Moisture from the flour, butter, salt and salt replacers was taken into account when formulating. Ingredients were blended in a

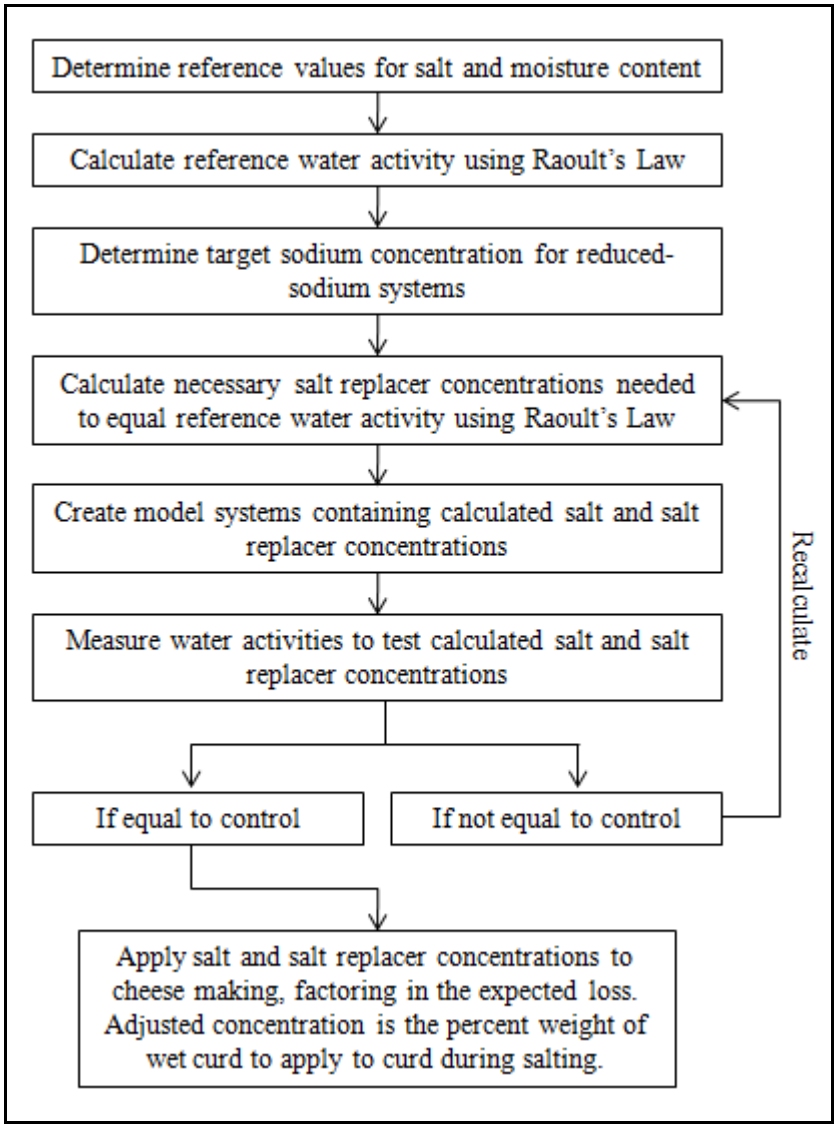

Figure 2.2. General scheme of salt and salt replacer concentration determination.

236.6-ml cup on an Osterizer 12 Speed Blender (Sunbeam Products, Inc., Boca Raton, FL) at high speed for approximately 45 s in 5 s intervals until uniform. The a_w of the model cheeses were measured with an Aqua Lab 3TE a_w meter (Decagon Devices, Pullman, WA) at 23°C. If the difference in a_w between the reduced sodium and the full

sodium treatments was greater than 0.006, the absolute value of the difference was subtracted from 0.974 if the a_w was higher than control and added to 0.974 if it was lower. The new reference a_w was used in Raoult's law in place of 0.974 to determine a new salt replacer concentration needed to achieve equal a_w to control. The new salt replacer concentrations were blended into a new model system, and the formula was adjusted to achieve the same moisture and fat targets. The a_w was measured to determine if equivalency to control was achieved, and the process was repeated until it was achieved.

2.2.4 Cheese Making

A full fat, stirred curd Cheddar cheese procedure was used at the University of Minnesota's Joe Warthesen Food Processing Center under the guidance of the head cheese maker. Once the desired titratable acidity was reached for salting, curd was weighed and divided into treatment groups which were salted using the determined salt and salt replacer concentrations from the model systems (Table 2.2). For treatments in which models were not created (S/K, S/MK, SS/MG), the amounts used in cheese making were calculated by applying the modifications required in S (for the S component), N/MG (for the MG component), and N/MK (for the MK component) during model testing. Salt and salt replacer was added in 3 additions, separated by five min intervals, to either 30 or 60 lbs of drained curd in individual plastic containers with drain holes. Based on the standard Cheddar cheese making procedure in the Joe Warthesen Food Processing Center in which drained curd is salted at 2.5% and results in ~1.6% salt in the final cheese, concentrations of salt and salt replacer targeted for the final cheese were multiplied by 1.56 (2.5/1.6) to match the loss expected during salting and pressing,. Curd

was manually stirred during the salting intervals to prevent matting and then transferred to cheesecloth-lined, 9.1-kg Wilson style cheese hoops and pressed overnight at 276 kPa. After removal from hoops, blocks were vacuum packaged. Water activity of the 9.1-kg blocks was measured after one week of refrigerated storage at 4 to 5°C. Cheese making was replicated on two days with different lots of milk. Note: this cheese was used for the research in the proceeding chapter, and the full cheese making procedure is described there.

2.2.5 Compositional Analysis

The butter and flour used in model systems were analyzed for mineral content on an Optima 3000 inductively coupled plasma atomic emission spectrometer (Perkin Elmer Inc., Waltham, MA) following AOAC method 985.01 (AOAC, 1990).

2.2.6 Statistical Analysis

Cheese making was replicated, and four a_w measurements, at minimum, of each cheese from both cheese makes were made. Linear mixed model with least significant difference as a summary test was conducted as statistical analysis (SPSS Statistics ver. 17.0.2 (IBM SPSS, Chicago, IL)).

2.3 Results and Discussion

Predicted salt and salt replacer levels for use in the model systems are shown in Table 2.1. In the model cheese system testing, deviation of the a_w from the N control treatment was measured in the N/MG, N/C, and S treatments. These differences were most likely due to a variation in the salts from the labeled composition. Factoring in the differences

Table 2.1. Salt and salt replacer concentrations predicted using Raoult's law to create equivalent water activity in model systems

	Treatments[1]								
	Full Sodium		Reduced Sodium[3]						
Item, %	N[2]	S	N/K	N/MK	N/MG	N/C	S/K[4]	S/MK[4]	S/MG[4]
N	1.6	0	0.76	0.76	0.76	0.76	0	0	0
S	0	2.47	0	0	0	0	1.36	1.36	1.36
K	0	0	1.07	0	0	0	0.92	0	0
MK	0	0	0	1.07[5]	0	0	0	0.92[6]	0
MG	0	0	0	0	1.95	0	0	0	1.67
C	0	0	0	0	0	1.41	0	0	0

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified KCL; MG = magnesium chloride hexahydrate; C = calcium chloride dihydrate
[2]640 mg sodium/100 g sample target
[3]300 mg sodium/100 g sample target
[4]Treatment not tested in model cheese system
[5]Value based on K concentration in N/K treatment
[6]Value based on K concentration in S/K treatment

Table 2.2. Concentrations of salt and salt replacers to create reduced sodium model cheese with the same water activity as full sodium model cheese containing only sodium chloride (Treatment N)

	Treatments[1]					
	Full Sodium		Reduced Sodium[3]			
Item, %	N[2]	S	N/K	N/MK	N/MG	N/C
N	1.6	0	0.76	0.76	0.76	0.76
S	0	2.13[4]	0	0	0	0
K	0	0	1.07	0	0	0
MK	0	0	0	1.07	0	0
MG	0	0	0	0	2.48[4]	0
C	0	0	0	0	0	1.63[4]

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified KCL; MG = magnesium chloride hexahydrate; C = calcium chloride dihydrate
[2]640 mg sodium/100 g sample target
[3]300 mg sodium/100 g sample target
[4]Value differed from the calculated value, and was modified as a result of testing in the model system

into Raoult's Law and re-testing, the salt and salt replacer concentrations resulting in equal a_w are shown in Table 2.2. The measured a_w of the model systems were lower than the predicted 0.974 (Table 2.3). To evaluate the effect of solutes from the flour and butter, Raoult's Law, using values from Table 2.1 and the mineral content data from flour and butter, showed that the additional solutes did not account for the difference; a_w was only lowered by 0.001 to 0.002 in all models. Raoult's Law allows for an activity coefficient because it is expected that systems will vary from ideality. Deviation from ideality, and therefore deviation from an activity coefficient of one, can result from water-solute interactions and a difference in solute size compared to the size of the water molecule (Labuza and Altunakar, 2007). The observed difference is likely a result of the activity coefficient of the model system being different from one.

Despite the discrepancy between predicted and measured a_w in the model systems, the described method was able to determine appropriate concentrations of salt and salt replacers to produce reduced sodium cheeses with equal a_w to a full sodium target. The a_w of the manufactured cheeses was higher than that of the model systems (Table 2.3). This discrepancy also did not limit the effectiveness of the model system method because it was not necessary for the values to be equal as differences in the composition could result in varying a_w. The critical aspect was for the a_w to be equal between treatments in a given matrix (model system or cheese).

It is likely that this model system could be used to determine concentrations of other solutes used in cheese making to yield equivalent a_w, such as acids. This would be useful when trying to predict changes to cheese making procedures to create equivalent a_w.

Table 2.3. Comparison of measured water activity (a_w) between model systems and pilot plant produced Cheddar cheese

	Treatments[1]										
	Full Sodium		Reduced Sodium[3]								
a_w	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Model system[6]	0.930	0.930	0.925	0.925	0.935	0.930	-	-	-	NA	NA
Cheddar cheese	0.955	0.955	0.955	0.955	0.955	0.960	0.955[7]	0.955[7]	0.955[7]	0.002	0.074

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified KCL; MG = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown

[5]Sensitivity of the a_w meter is ±0.003

[6]Model systems not created for S/K, S/MK, S/MG treatments

[7]Salt and salt replacer levels used were based on those from the S treatment for the S component, and the salt replacer concentration from the corresponding N treatment

NA: Statistics not calculated

2.4 Conclusions

Reduced sodium Cheddar-style cheeses with various salt and salt replacer concentrations were manufactured that yielded equivalent a_w to the existing full sodium Cheddar cheese produced at the University of Minnesota's Joe Warthesen Food Processing Center. The described method of using Raoult's law and verification with a model was a valuable tool for determining the appropriate salt and salt replacer concentrations prior to cheese making. Additionally, the model system was used to determine the concentrations of salt replacers when prediction by Raoult's law was not possible (when percent quantities of each solute in the ingredient /were unknown).

A reference a_w of 0.974 was calculated based on a cheese salt content of 1.6% and a moisture of 36.8%. These values can be altered to represent a cheese maker's existing "gold standard" cheese recipe.

Without the model system, it would be necessary to manufacture experimental batches of cheese to determine the salt and salt replacer concentrations that produce equivalent a_w to full sodium control. The described method of determining salt and salt replacer concentrations could be applied to many varieties of dry-salted cheese to reduce experimental time and cost.

CHAPTER 3: Manufacture and Analysis of Reduced Sodium Cheddar-Style Cheese with Mineral Salt Replacers

3.1 Introduction

The use of mineral salt replacers to reduce the sodium content in cheese has been investigated as a way of maintaining both the salty flavor and the preservative effects of salt. Most previous studies of sodium reduction in cheese have used mineral salt replacers at levels too low to produce equal water activity (a_w) to control in the finished cheese (Lindsay et al., 1982; Aly, 1995; Reddy and Marth, 1995; Katsiari et al., 1997; Katsiari et al., 2001). This can cloud the evaluation of the salt replacers as unequivocal a_w itself could cause cheese to be of low quality due to differences in biochemical reactions during aging. By equaling a_w of the control to simulate the salt-to-moisture ratio, it is anticipated that an objective evaluation of the salt replacers can be made.

The objective of this research was to investigate the use of mineral salt (NaCl) replacers to produce reduced sodium cheese with similar flavor and texture to full sodium control.

It was hypothesized that reduced sodium Cheddar-style cheese could be made with mineral salt replacers and that by maintaining the a_w of full-sodium control cheese, the effect of the salt-to-moisture ratio would be duplicated and result in similar biochemical reactions during cheese aging. This would eliminate the difference in the reaction rates of microorganisms and enzymes, and the effect of the salt replacers would be observed.

To test the hypothesis, stirred-curd full and reduced sodium Cheddar-style cheeses were produced using the salt replacers potassium chloride (KCl), modified KCL, magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), and a naturally-reduced-sodium

sea salt. Salt and salt replacers were applied at concentrations to achieve an equivalent a_w to full sodium cheese.

3.2 Materials and Methods

3.2.1 Cheese Making Materials

A blend of *Lactococcus lactis ssp. cremoris* and *L. lactis ssp. lactis* (CHOOZIT™ Superstart® direct-to-vat-set strain M30, Danisco USA, Inc., Madison, WI), annato (AFC W/S 1X 70463, Chr. Hansen, Inc., Milwaukee, WI), 45% (wt/vol) calcium chloride (CAL-SOL 71257, Chr. Hansen, Inc., Milwaukee, WI), and liquid chymosin (CHY-MAX® 73863, Chr. Hansen, Inc., Milwaukee, WI) were used to manufacture the cheese.

Sodium chloride (**N**) (Top-Flo® Evaporated Salt, Cargill, Inc., Minneapolis, MN) and the salt replacers potassium chloride (**K**) (Premier™ Potassium Chloride 8799, Cargill, Inc., Minneapolis, MN), a modified potassium chloride (**MK**) (Modified Potassium Chloride 14510, Nu-tek Products, Inc., Minnetonka, MN), magnesium chloride (**MG**) (magnesium chloride 6-Hydrate 5956-06, Mallinckrodt Baker, Inc., Phillipsburg, NJ), calcium chloride (**C**) (calcium chloride dihydrate, granular 4616-06, Mallinckrodt Baker, Inc., Phillipsburg, NJ), and a 45% reduced sodium sea salt (**S**) (SS45, A and B Ingredients, Fairfield, NJ) were used to salt the cheese curd. All were Food Chemicals Codex (FCC) or United States Pharmacopeia (USP) grade.

3.2.2 Cheddar-Style Cheese Manufacture

Cheddar-style cheese was manufactured at the University of Minnesota's Joe Warthesen Food Processing Center. The procedure is outlined in Figure 3.1. Raw whole milk was

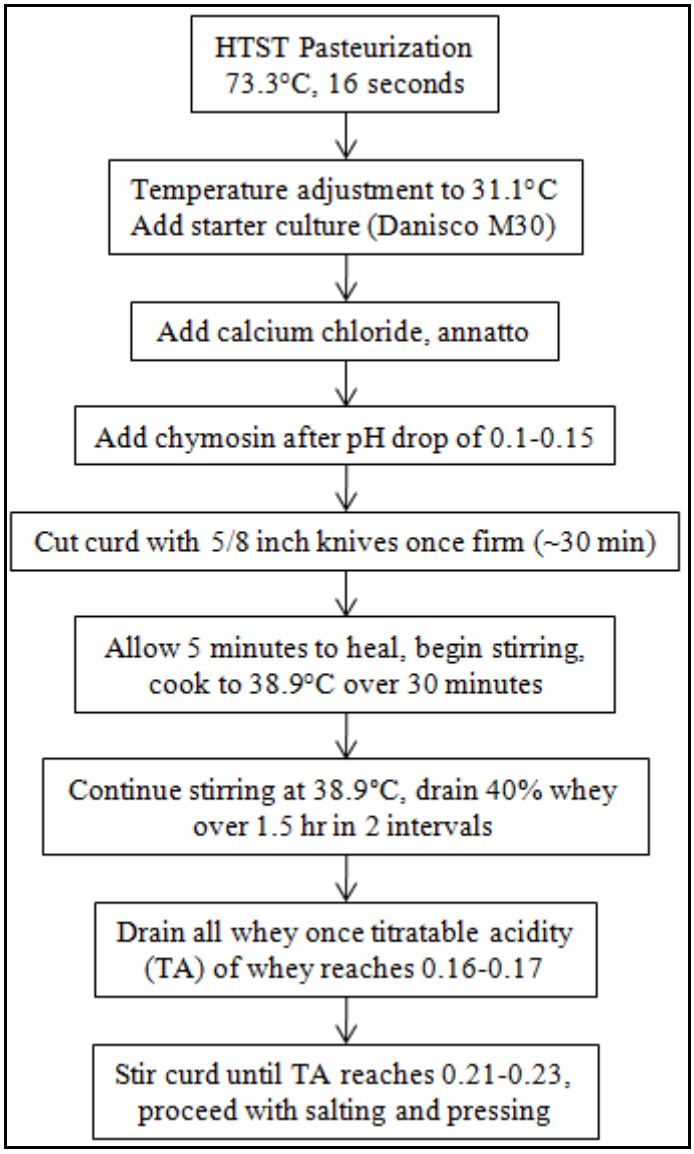

Figure 3.1. Flow diagram of the cheese making procedure.

pasteurized at 73.3°C for 16 s on a plate and frame-style milk pasteurizer and pumped into a Damrow 2,260 Kg rectangular vat (Tetra Damrow, Vernon Hills, IL) at 31.1°C. One hour before renneting, the lactic acid starter culture was added at 231 ml/1000 kg milk with

gentle agitation. Twenty minutes before renneting, Calcium chloride was added at 198 ml/1000 kg milk and annatto was added at 66 ml/1000 kg milk. After a pH drop in the milk of 0.1-0.15, chymosin was added at 99 ml/1000 kg milk by diluting with water 1:50 and adding with gentle agitation. Once mixed, agitation ceased and the vat was allowed to coagulate until the gel was properly firm (~30 min). The coagulum was cut into small pieces with 5/8 inch wire knives and allowed to heal for 5 minutes prior to stirring commenced. Ten min after cutting, curd and whey were gradually heated to 38.9°C over a 30 min period while stirring. Temperature was maintained and stirring continued for 1.5 hr during which approximately 40% of the whey was drained in two intervals. The majority of the remaining whey was drained once the titratable acidity (TA) reached 0.16-0.17 (lactic acid basis); enough whey was maintained in the vat to prevent the curd from drying. Curd was stirred until the TA reached 0.21-0.23, at which point it was divided into treatment groups that were salted with salt and salt replacer treatments (Table 3.1) based on concentrations found to produce equivalent a_w between reduced sodium and full sodium model cheese systems (Chapter 2). Table 3.2 contains a description of each treatment. Drained curd in either 13.6 or 27.2 kg portions was manually salted in individual plastic bins with drain holes in three additions spaced five min apart and continuously stirred by hand to prevent clumping and aid in salt absorption into curd. Curd was transferred to cheesecloth-lined, 9.1 kg Wilson-style cheese hoops and pressed overnight at 276 kPa. Blocks were vacuum packaged in a Multivac vacuum packager (Koch, Kansas City, MO) and stored at 4-5°C. Cheese making was replicated on two days with different lots of milk.

Table 3.1. Percent of salt and salt replacers applied to drained curd

	Treatment[1]								
	Full Sodium		Reduced Sodium[3]						
Item, %	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG
N	2.50	0	1.21	1.21	1.21	1.21	0	0	0
S	0	3.33	0	0	0	0	2.16	2.16	2.16
K	0	0	1.69	0	0	0	1.31	0	0
MK	0	0	0	1.69	0	0	0	1.31	0
MG	0	0	0	0	3.92	0	0	0	3.05
C	0	0	0	0	0	2.57	0	0	0

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate
[2]640 mg sodium/100 g sample target
[3]300 mg sodium/100 g sample target

Table 3.2. Descriptions of the treatments in Chapter 3

Treatment	Description
N	Full sodium control
S	Full sodium cheese made with sea salt
N/K	Reduced sodium cheese with sodium chloride as the source of sodium and added potassium chloride
N/MK	Reduced sodium cheese with sodium chloride as the source of sodium and added modified potassium chloride
N/MG	Reduced sodium cheese with sodium chloride as the source of sodium and added magnesium chloride
N/C	Reduced sodium cheese with sodium chloride as the source of sodium and added calcium chloride
S/K	Reduced sodium cheese with sea salt as the source of sodium and added potassium chloride
S/MK	Reduced sodium cheese with sea salt as the source of sodium and added modified potassium chloride
S/MG	Reduced sodium cheese with sea salt as the source of sodium and added magnesium chloride

3.2.3 Compositional and Chemical Analysis

To measure titratable acidity during cheese making, three drops of phenolphthalein indicator was added to 9.00 g milk or whey. 0.1 N NaOH from an automatic burette standardized for lactic acid basis was dispensed into the sample until the phenolphthalein end point was reached (~ pH 8.3, faint pink color). The volume of dispensed NaOH corresponded to the TA value as indicated by the burette.

Fat and ash content were determined following standard methods 18.8A2c and 18.4A respectively (Richardson, 1985). Ash was measured after step 18.4A3d. Moisture content was determined using a vacuum oven following standard method 15.111 (Wehr and Frank, 2004). Water activity was measured at 23°C on an Aqua Lab 3TE a_w meter (Decagon Devices, Pullman, WA). The pH was measured on an Acorn® pH 6 Meter (Oakton Instruments, Vernon Hills, IL) with an Orion 8172BNWP Ross Sure-Flow pH electrode (Thermo Fisher Scientific, Inc., Waltham, MA). Approximately 0.2 g lab-grade water was added to 4 g grated cheese then the pH probe was inserted for measurement. Total protein (nitrogen x 6.38) was determined using a TruSpec® N (Leco Corporation, St. Joseph, MI) based on the Dumas method of combustion. Sodium, potassium, magnesium, and calcium content were measured by atomic absorption spectrometry using a Perkin Elmer AAnalyst 100 using IDF method 119:2007(E) (IDF, 2007) with the exception of using the hot plate digestion time and temperature of Kira et al. (2004). All measurements were performed in duplicate at minimum. An expanded methods section is in section A.1 of the Appendix.

3.2.4 Texture Profile Analysis

Texture profile analysis (TPA) was performed on a TA.XT*Plus* texture analyzer (Texture Technologies Corporation, Scarsdale, NY). Hardness, springiness, cohesiveness, adhesiveness, resilience, chewiness were calculated according to Bourne (1978). Additional information about calculating the texture attributes is in section A.1.9 in the Appendix. Cheese was equilibrated to 7^{o}C for 16 hours then cylinders (11.75mm diameter, 50-60mm height) were cut with a steel cork borer and placed in an air-tight bag. Multiple samples of 12mm height were cut from the cylinders, excluding 5mm on each end, using a guided blade and returned to the bag. Samples were individually removed from the refrigerator, and a thin layer of light white mineral oil (151694, MP Biomedicals, LLC, Solon, OH) was applied to both ends. Within 20 s of removal, samples were compressed twice, with 2 s between compressions, to 20% of original height at a crosshead speed of 1 mm/s between a 25 mm diameter polycarbonate probe (TA11, Texture Technologies Corporation, Scarsdale, NY) and a polycarbonate stage; room temperature was 23^{o}C. A minimum of five replicates per cheese sample were analyzed.

3.2.5 Statistical Analysis of Compositional, Chemical, and Texture Profile Analysis

Cheese making was conducted in duplicate. Fat, ash, moisture, a_w, pH, total protein, mineral content, and TPA measurements of the cheese were duplicated, at minimum. Linear Mixed Model analysis with Fisher's Least Significant Difference as a summary test (alpha = 0.05) was performed using SPSS Statistics ver. 17.0.2 (IBM SPSS, Chicago, IL). In the case of varying standard error between treatments, the largest is reported. All

correlations were determined using Pearson's correlation (alpha = 0.05) in XLSTAT version 2011.1.04 (Addinsoft USA, New York, NY).

3.2.6 Descriptive Sensory Analysis

Sensory evaluation and data analysis was conducted by the University of Minnesota's Sensory Center in the Department of Food Science and Nutrition. Testing was conducted at one month and six months of age with 11 and nine panelists respectively.

3.2.6.1 Subjects

Members of the trained panel from the Sensory Center at the University of Minnesota participated in these tests. All were PROP tasters or supertasters and were compensated for participating. All recruiting and experimental procedures were approved by the University of Minnesota's Institutional Review Board.

3.2.6.2 Products

A total of 14 cheeses were included consisting of the seven treatments from both cheese make replicates. Panelists received three cubes (1.5 cm^3) of each cheese at room temperature (22°C) in a random three-digit coded four ounce plastic cup with a lid.

3.2.6.3 Training

Panelists participated in five training sessions in which a lexicon of sensory attributes (Table 3.3) was collectively developed and refined. Taste, flavor, aroma, texture, and sensation references used for the descriptive sensory analysis are listed in the lexicon. All chemicals were Food Chemicals Codex or United States Pharmacopeia grade. Panelists practiced evaluating the cheeses on SIMS® Sensory Evaluation Software (Sensory

Table 3.3. Cheddar-style cheese lexicon

Descriptive Term[1]	Definition	Reference
Aroma and Flavor		
Overall Flavor or Aroma Intensity	The overall intensity of aroma and flavor	
Diacetyl	Aromatic or flavor associated with buttery popcorn	Unsalted butter
Cooked	The note associated with heated or cooked milk	Evaporated milk, Nestle Carnation with added Vitamin. D
Metallic	Aromatic or flavor associated with metals (tin or iron)	0.005% Ferrous Sulfate
Milky	Aromatic or flavor associated with skim milk or milk derived products.	Whole milk
Whey	Off-flavors or off-aromas in cheese associated with retained cheese whey	Thawed whey from cheese making
Malty	Sweet slightly fermented or sour grain note associated with freshly kilned malt	Barley malt extract
Earthy	Aromatic or flavor characteristic of damp soil, wet foliage	Wet potting soil
Moldy	The flavor and aromatics associated with molds- usually earthy, dirty, stale, musty and slightly sour	English stilton cheese
Sulfur	Aromatic or flavor associated with hydrogen sulfide, rotten egg	Cracker Barrel Vermont Sharp White Cheddar cheese
Unclean	Off-flavor/aroma that has also been described as a post-vomit flavor	No reference
Yeasty	Flavor/aroma associated with fresh yeast and yeast fermentation	1 tablespoon Red Star active dry yeast soaked in 400ml warm water.
Brothy	Flavor/aroma associated with boiled meat or vegetable soup stock	Solution of 75% Swanson's Vegetable broth, 25% H2O
Pineapple	Flavor/aroma associated with canned pineapple	Canned Pineapple Chunks
Hydrolytic Rancidity	Flavor/aroma associated with short-chain fatty acids, similar to those in blue cheese	Blue Cheese
Fermented	Flavor/aroma associated with fermenting fruits	No reference
Nutty	Flavor/aroma associated with nuts or nut meat	Planters mixed nuts
Sour dairy	Flavor/aroma associated with fermented milk character.	Sour cream, buttermilk
Soapy/detergent	Flavor/aroma associated with unscented soap or detergent	Ivory unscented bar soap
Tastes		
Sweet	The taste stimulated by sucrose and other sugars	500 ml water, 25 g sugar
Salty	The taste stimulated by sodium chloride, and in part by other salts, such as potassium chloride	500 ml water, 2.2 g NaCl
Sour	The taste stimulated by acids, such as citric or malic	500 ml water, 0.375g citric acid

[1]Scale for all terms ranges from 0 (none) to 20 (intense)

Table 3.3 Continued

Descriptive Term[1]	Definition	Reference
Bitter	The taste stimulated by substances such as quinine, caffeine, and hop bitters	500 ml water, 0.285g caffeine
Umami	Oral sensation stimulated by monosodium glutamate (MSG)	500 ml water, 5 g MSG
Sensations		
Astringent	Chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as rough or dry and associated with tannins and alum	1.25 g alum in 500 ml water
Pungent	A sharp, irritating and penetrating sensation in the nasal cavity	Vinegar
Numbing	Numbing or loss of sensation in the mouth. May appear as an aftertaste	5% clove in room temperature spring water
In-Hand Texture		
Firmness	The force needed to compress a cheese cube when squeezed between thumb and index finger	Reference cheese (Cracker Barrel Natural Sharp Cheddar Cheese) is a 15
Springiness	The rate at which a slightly compressed (5-20%) cheese cube returns to its original shape	Reference cheese is a 5
Stickiness	The degree to which a sample held between thumb and two fingers sticks to the index finger as it is removed from the sample	Reference cheese is a 3
Cohesiveness	The degree to which a sample holds together after pressing and rolling it between thumb and two fingers	Reference cheese is a 13
Slipperiness	The extent of enhanced lubricating or friction-reducing quality when a sample is rubbed between two fingers	Reference cheese is a 10
First-Bite Texture		
First Bite Firmness	The force required to completely bite through a sample using incisors	Reference cheese is a 5
First Bite Stickiness	The degree to which the sample sticks to incisors during the first bite.	Reference cheese is a 15
First Bite Brittleness	The level of crumbling/breakdown after completely biting through a sample with incisors	Reference cheese is a 2
Five-Chew Texture		
Five Chew Firmness	The extent of resistance offered by the cheese, assessed after five chews using incisors.	Reference cheese is a 7
Five Chew Stickiness	The adhesion or stickiness of the cheese against the palate and around the teeth after five chews.	Reference cheese is a 15
Five Chew Brittleness	The extent of crumbling or breakdown after five chews	Reference cheese is a 2
Five Chew Curdiness	The extent to which a lumpy or curdy texture is perceived in the mouth after five chews	Reference cheese is a 1

Computer Systems, Morristown, NJ) which was used during testing. Results were discussed as a group. Practice sessions were repeated to finalize the lexicon.

3.2.6.4 Testing

Panelists participated in four test sessions. Panelists evaluated a complete set of the samples from the first replicate in the first two sessions (the second session served as a sensory replicate) and a complete set of the samples from the second replicate in the third and fourth sessions (the fourth session served as the sensory replicate). Within a session, serving orders were balanced for order and carryover effects. During the testing sessions each panelist evaluated each sample by rating the intensity of the attributes on a 20 point line scale labeled '*none*' at the left end and '*intense*' at the right end. Intensity ratings of flavor and taste were made on the standard citric acid scale developed by the University of Minnesota Sensory Center using concentrations of 0.03, 0.05, 0.08, and 0.14%. Ratings of odors were made on the standard butanol scale using concentrations of 0.02, 0.16, 2.56, and 20.48 g/L (ASTM, 2010). Texture ratings were made on a 20 point scale anchored with references. Panelists were instructed to wear nose clips when evaluating the taste attributes.

3.2.6.5 Data Analysis

General linear model analysis was performed using SAS® PROC GLM ver. 9.1 (SAS Institute, Inc., Carry, NC). Student-Newman-Keuls was used as a summary test (alpha = 0.05). Principle components analysis (PCA) with varimax rotation was performed in XLSTAT® (Addinsoft USA, New York, NY) using the mean attribute scores for each cheese. Only attributes that differed significantly among the treatments were included in the PCA.

3.2.7 Comparison of Compositional, Chemical, and Texture Profile Analysis to Descriptive Sensory Analysis

Correlations between the means of compositional and chemical measurements with significant differences between treatments and descriptive sensory attributes in Table 3.9 were determined using Pearson's correlation (alpha = 0.05) in XLSTAT version 2011.1.04 (Addinsoft USA, New York, NY). The same method was used to determine correlations between texture profile analysis and descriptive sensory texture attributes.

3.3 Results and Discussion

3.3.1 Compositional, Chemical, and Texture Profile Analysis

Compositional data is shown in Table 3.4. Moisture tended to be lower in treatments containing sea salt compared to analogous treatments containing NaCl, with the exception of the treatment S/MG. Correspondingly fat and protein were higher in the treatments lower in moisture. Treatments S/MG and N/MG contained more than 39% moisture, which is above the legal maximum to be labeled as Cheddar cheese in the United States. The differences in moisture indicate that differences in syneresis occurred during salting. Fitzgerald and Buckley (1985) also found that Cheddar cheese salted with $MgCl_2$ only (1.35% to equal ionic strength of control) and a NaCl/$MgCl_2$ mixture (1:1 mixture, but it was not specified on what basis) had higher moisture contents than full sodium control. The full sodium treatment S was higher in potassium and lower in sodium than the full sodium treatment N, as expected based on the composition of the sea salt.

Table 3.4. Compositional analysis of Cheddar-style cheese

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Measurement	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Moisture, %	36.87^{bcd}	34.56^{d}	35.81^{cd}	38.28^{bc}	39.33^{ab}	34.59^{d}	35.26^{d}	36.65^{cd}	40.89^{a}	1.05	<0.01
Fat, %	35.3^{abc}	36.6^{a}	35.8^{ab}	34.4^{bcd}	33.4^{cd}	36.6^{a}	36.3^{ab}	35.1^{abc}	32.7^{d}	0.7	0.02
Protein, %	23.90^{ab}	24.68^{a}	24.33^{ab}	22.38^{c}	22.62^{c}	24.55^{ab}	24.19^{ab}	23.61^{b}	22.12^{c}	0.32	<0.001
Total Ash, %	3.838^{bc}	3.989^{abc}	4.133^{a}	4.051^{ab}	3.748^{c}	4.242^{a}	4.122^{a}	4.094^{ab}	4.190^{a}	0.082	0.03
Sodium, mg/100g	664.7^{a}	508.4^{b}	358.3^{c}	386.6^{c}	355.0^{c}	298.4^{d}	348.8^{cd}	356.9^{c}	387.7^{c}	19.1	<0.001
Potassium, mg/100g	89.1^{e}	202.2^{d}	707.0^{a}	665.1^{ab}	90.2^{e}	86.5^{e}	597.2^{bc}	554.9^{c}	162.9^{d}	24.3	<0.001
Calcium, mg/100g	225.2^{b}	225.7^{b}	210.0^{b}	205.0^{b}	196.3^{b}	991.1^{a}	222.0^{b}	218.6^{b}	204.4^{b}	58.2	<0.001
Magnesium, mg/100g	10.6^{c}	16.6^{c}	8.9^{c}	11.4^{c}	183.7^{a}	18.8^{c}	14.5^{c}	21.5^{c}	146.1^{b}	11.7	<0.001

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each parameter

[a-e]Means without a common superscript letter within the same row are significantly different ($p < 0.05$)

The a_w of full sodium control was 0.956 at one week of age, and no treatments were significantly different from the control (alpha = 0.05) as shown in Table 3.5. At a constant salt concentration, one would expect a higher a_w in cheese with a higher moisture content. However, there was a positive trend between moisture and sodium concentration among the reduced sodium cheeses, leading to nearly equal sodium-to-moisture ratios. This can be explained by less moisture being expelled from the curd and less salt being lost with that moisture to the whey stream. This may explain why differences in the moisture levels did not lead to varying a_w.

The pH of the cheeses ranged from 4.96 to 5.19 at one month of age (Table 3.5). The reduced sodium cheeses were lower in pH than the full sodium control, with the exception of N/K and S/MG, indicating that the salt replacers and sea salt did not inhibit the starter cultures at salting as much as NaCl. The lower pH in treatments N/MG and N/C is supported by Fitzgerald and Buckley (1985) who observed a 0.07 to 0.14 lower pH value in Cheddar cheeses salted with $MgCl_2$ and $CaCl_2$, both with and without NaCl. The lower pH in cheese with KCl is supported by Reddy and Marth (1995) who found the pH to be lower by 0.04 to 0.07 in 3 d Cheddar with various NaCl/KCl mixtures. These differences are not as large as the differences observed in this study (0.12 to 0.35). Lindsay et al. (1982) and Fitzgerald and Buckley (1985) do not support the lower inhibitory effect of KCl on the starter culture. They found that Cheddar cheese with various NaCl/KCl mixtures resulted in a similar or higher pH than control (range of +0.01 to +0.16).

Table 3.5. Water activity (a_w) and pH of Cheddar-style cheese

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Measurement	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
a_w	0.956	0.957	0.955	0.957	0.953	0.959	0.954	0.957	0.956	0.002	0.07
pH	5.17[a]	4.97[b]	5.07[ab]	4.96[b]	4.99[b]	4.97[b]	5.00[b]	4.97[b]	5.19[a]	0.04	0.02

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each parameter

[a-b]Means without a common superscript letter within the same row are significantly different ($p < 0.05$)

The hardness values of the treatments with sea salt were higher than in cheeses containing NaCl, with the exception of N/C, which was the hardest, as shown in Table 3.6. The increased hardness of treatment N/C is supported by Pastorino et al. (2003) who found the hardness of cheese similar to low-moisture part-skim mozzarella increased with an increased concentration of calcium resulting from an injection with a 40% (wt/wt) $CaCl_2$ solution after pressing. The increased hardness is not supported by Fitzgerald and Buckley (1985). They observed lower hardness and firmness in Cheddar cheese salted with $CaCl_2$ only (1.58% to equal ionic strength of control) and a NaCl/$CaCl_2$ mixture (1:1 ratio, but it was not specified on what basis). However, they did not report a_w values so it is possible that the opposing result could be due to inequivalent a_w between treatments. Treatment N/C was also more springy, less adhesive, and more chewy than control ($p < 0.01$). Treatments with $MgCl_2$ were generally less hard than all other treatments, with the exception of N/MK. The reduction in firmness corroborates what was reported by Lefier et al. (1987) and Chamba and Derby (1994) in reduced sodium gruyere and emmental when $MgCl_2$ was used to replace sodium. Treatments with KCl generally had similar texture to control, with the exceptions of lower cohesiveness ($p < 0.001$) and higher adhesiveness in treatments N/K and N/MK ($p < 0.001$). This is in agreement with Fitzgerald and Buckley (1985), Katsiari et al. (1997), and Katsiari (1998) who found no significant difference in textural properties due to KCl in Cheddar, feta, and kefalograviera cheeses respectively.

Table 3.6. Mean values from texture profile analysis (TPA) of Cheddar-style cheese

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
TPA Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Hardness, g	6045^{cde}	7715^{ab}	6230^{cd}	4535^{f}	5580^{def}	8495^{a}	7415^{abc}	6930^{bcd}	4665^{ef}	480	<0.001
Springiness	0.375^{bcd}	0.433^{b}	0.304^{cd}	0.257^{d}	0.477^{ab}	0.567^{a}	0.382^{bc}	0.384^{bc}	0.489^{ab}	0.041	<0.001
Cohesiveness	0.207^{ab}	0.136^{e}	0.175^{cd}	0.159^{cde}	0.233^{a}	0.189^{bc}	0.150^{de}	0.141^{e}	0.175^{cd}	0.014	<0.001
Adhesiveness, g x sec	198.8^{b}	90.7^{bc}	436.5^{a}	393.7^{a}	107.2^{bc}	15.6^{c}	170.3^{b}	175.9^{b}	47.0^{c}	41.3	<0.001
Resilience	0.0437^{bc}	0.0382^{bc}	0.0344^{c}	0.0292^{c}	0.0674^{a}	0.0625^{a}	0.0363^{bc}	0.0341^{c}	0.0527^{ab}	0.0058	<0.001
Chewiness, g	473^{bc}	454^{bc}	325^{c}	181^{c}	731^{ab}	925^{a}	432^{bc}	365^{c}	413^{c}	104	<0.01

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each parameter

[a- f]Means without a common superscript letter within the same row are significantly different ($p < 0.05$)

Pearson's correlations between chemical and texture profile analysis measurements are shown in Table 3.7. Among all cheeses, hardness correlated negatively with moisture and positively with fat and protein. Potassium content correlated positively with adhesiveness and negatively with springiness, resilience, and chewiness. Calcium content correlated positively with chewiness. Magnesium content correlated positively with resilience.

3.3.2 Descriptive Sensory Analysis

There were many differences identified during the descriptive analysis due to the large number of attributes evaluated. Only the most important and surprising are discussed in the text due to the large amount of data.

Sensory attributes with significant differences ($p < 0.05$) between treatments and means greater than 1 (for at least one treatment) on the 20 point scale for one-month and six-month analysis are shown in Tables 3.8 and 3.9 respectively. Expanded data tables for all means at one and six months are shown in Tables A.2 and A.3 of the Appendix, respectively.

The use of $CaCl_2$ and $MgCl_2$ resulted in considerable off flavors in the cheese as treatments N/MG, N/C, and S/MG were more bitter, metallic, unclean, and soapy than the other treatments ($p < 0.001$). Treatment N/C was the most bitter, unclean, and astringent of all samples. The findings of Fitzgerald and Buckley (1985) agree with the off flavors due to $MgCl_2$ and $CaCl_2$. Cheddar cheese salted only with $MgCl_2$ or $CaCl_2$ was not included in sensory evaluation in their study due to extreme bitterness, and cheese salted with a 1:1 molar ratio mixture of NaCl and $MgCl_2$ or $CaCl_2$ was found to be bitter and metallic. In this study, bitter, metallic, unclean, and soapy correlated negatively with sweet, diacetyl flavor, and milky flavor. Therefore, a cheese maker could select starter or

Table 3.7. Pearson's correlations between chemical measurements and texture profile analysis measurements. Values shown are Pearson's correlation coefficient (r)

Variables	Ash	Sodium	Potassium	Calcium	Magnesium	Moisture	Fat	Protein	pH
Hardness	0.274	-0.122	-0.137	0.603	-0.472	**-0.882***	**0.858***	**0.886***	-0.447
Springiness	0.119	-0.205	**-0.798***	0.621	0.472	0.012	-0.038	0.098	0.046
Cohesiveness	-0.547	0.114	-0.570	0.157	0.574	0.405	-0.416	-0.287	0.330
Adhesiveness	-0.032	0.030	**0.792***	-0.431	-0.428	-0.034	0.052	-0.051	-0.045
Resilience	-0.209	-0.193	**-0.803***	0.486	**0.705**	0.284	-0.313	-0.171	0.115
Chewiness	-0.064	-0.213	**-0.722***	**0.750***	0.312	-0.195	0.163	0.269	-0.148

***Values in bold are different from zero ($p < 0.05$)

Table 3.8. Mean values of descriptive sensory analysis of Cheddar-style cheese at one month of age. Only attributes with significant differences between treatments (alpha = 0.05) and means greater than 1 (for at least one treatment) at one month or six months of age are shown

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Aroma											
Overall	9.0^{a}	8.9^{a}	8.8^{a}	8.8^{a}	8.5^{a}	8.1^{a}	8.8^{a}	9.0^{a}	8.3^{a}	0.4	0.02
Sour Dairy	2.7^{a}	2.7^{a}	2.4^{ab}	2.7^{a}	2.0^{b}	2.3^{ab}	2.6^{ab}	3.0^{a}	2.5^{ab}	0.4	<0.001
Taste											
Sweet	1.1^{a}	0.6^{abc}	0.8^{ab}	0.8^{ab}	0.7^{abc}	0.2^{c}	0.7^{abc}	0.5^{abc}	0.4^{bc}	0.2	<0.001
Salty	3.6^{b}	4.2^{b}	3.8^{b}	4.5^{ab}	3.6^{b}	4.5^{ab}	4.1^{b}	4.5^{ab}	5.4^{a}	0.5	<0.001
Sour	2.0^{c}	3.3^{a}	2.8^{ab}	3.7^{a}	1.8^{c}	1.9^{c}	3.3^{a}	3.2^{a}	2.3^{bc}	0.4	<0.001
Bitter	0.1^{d}	0.8^{d}	0.7^{d}	0.6^{d}	5.2^{b}	6.3^{a}	0.8^{d}	0.9^{d}	4.4^{c}	0.5	<0.001
Umami	1.4^{ab}	1.1^{ab}	1.4^{a}	1.2^{ab}	0.6^{b}	0.7^{b}	1.3^{ab}	1.4^{a}	1.3^{ab}	0.3	<0.01
Flavor											
Overall	7.8^{d}	8.2^{cd}	8.0^{cd}	8.9^{ab}	8.8^{abc}	9.4^{a}	8.6^{bc}	8.5^{bc}	9.0^{ab}	0.4	<0.001
Diacetyl	4.0^{ab}	3.2^{b}c	4.0^{ab}	4.1^{a}	3.0^{cd}	2.4^{d}	3.9^{ab}	3.8^{abc}	3.1^{cd}	0.3	<0.001
Cooked	1.1^{a}	1.0^{ab}	1.2^{a}	1.1^{a}	0.6^{b}	0.6^{b}	1.0^{ab}	1.1^{a}	0.8^{ab}	0.2	<0.001
Metallic	0.1^{b}	0.2^{b}	0.2^{b}	0.2^{b}	1.6^{a}	2.0^{a}	0.2^{b}	0.1^{b}	1.6^{a}	0.3	<0.001
Milky	1.8^{a}	1.4^{ab}	1.9^{a}	1.9^{a}	1.0^{bc}	0.8^{c}	1.8^{a}	1.6^{a}	1.3^{ab}	0.3	<0.001
Whey	2.8^{ab}	2.7^{ab}	2.8^{a}	3.0^{a}	2.2^{b}	1.5^{c}	3.0^{a}	2.9^{a}	2.1^{b}	0.3	<0.001

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each attribute

[a-e]Means with without a common superscript letter within the same row are significantly different ($p < 0.05$)

Table 3.8 Continued

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Unclean	0.1^{c}	0.2^{c}	0.1^{c}	0.1^{c}	2.5^{b}	3.6^{a}	0.4^{c}	0.2^{c}	2.8^{b}	0.4	<0.001
Sour Dairy	2.6^{b}	3.3^{ab}	3.1^{ab}	3.6^{a}	1.7^{c}	1.8^{c}	3.1^{ab}	3.6^{a}	2.5^{b}	0.4	<0.001
Soapy	0.3^{c}	0.4^{c}	0.3^{c}	0.3^{c}	1.6^{b}	2.4^{a}	0.4^{c}	0.4^{c}	1.9^{ab}	0.3	<0.001
Sensation											
Astringency	0.3^{b}	0.7^{ab}	0.5^{b}	0.7^{ab}	0.5^{b}	1.1^{a}	0.7^{ab}	0.7^{ab}	0.8^{ab}	0.3	<0.001
Pungent	0.2^{b}	0.5^{ab}	0.3^{ab}	0.6^{ab}	0.4^{ab}	0.7^{a}	0.5^{ab}	0.6^{ab}	0.6^{ab}	0.3	0.03
Numbing	0.2^{c}	0.3^{c}	0.2^{c}	0.3^{c}	1.3^{b}	2.2^{a}	0.4^{c}	0.2^{c}	1.4^{b}	0.4	<0.001
In-Hand Texture											
Firmness	9.8^{cd}	16.1^{a}	10.7^{c}	8.3^{e}	7.5^{e}	14.4^{b}	14.0^{b}	14.3^{b}	8.8^{de}	0.6	<0.001
Springiness	8.9^{b}	4.5^{d}	8.3^{b}	9.4^{b}	10.6^{a}	5.9^{c}	5.9^{c}	5.6^{c}	9.3^{b}	0.4	<0.001
Stickiness	3.8^{bcd}	3.0^{d}	4.0^{bc}	5.0^{a}	4.5^{ab}	3.3^{cd}	3.5^{cd}	3.4^{cd}	4.0^{bc}	0.4	<0.001
Cohesiveness	11.4^{a}	11.5^{a}	11.6^{a}	10.1^{a}	10.6^{a}	10.8^{a}	11.5^{a}	11.4^{a}	8.4^{b}	0.6	<0.001
Slipperiness	10.3^{cd}	12.0^{b}	10.5^{cd}	10.0^{cd}	11.3^{bc}	11.8^{b}	9.8^{d}	10.1^{cd}	13.5^{a}	0.6	<0.001
First-Bite Texture											
Firmness	5.8^{c}	10.0^{a}	6.1^{c}	4.5^{d}	5.7^{c}	9.7^{a}	7.8^{b}	7.9^{b}	5.7^{c}	0.5	<0.001
Stickiness	8.1^{c}	4.9^{d}	9.5^{b}	11.7^{a}	6.9^{c}	4.4^{d}	7.3^{c}	7.3^{c}	5.1^{d}	0.6	<0.001
Brittleness	3.8^{cd}	8.0^{a}	3.6^{cd}	2.8^{d}	4.6^{bc}	7.2^{a}	5.3^{b}	5.3^{b}	7.8^{a}	0.5	<0.001
Five-Chew Texture											
Firmness	7.6^{c}	10.3^{a}	7.3^{c}	5.8^{d}	7.4^{c}	10.4^{a}	8.7^{b}	8.9^{b}	7.1^{c}	0.4	<0.001
Stickiness	9.0^{bc}	5.4^{e}	9.7^{b}	11.9^{a}	6.9^{d}	4.9^{e}	8.6^{bc}	7.9^{cd}	5.7^{e}	0.6	<0.001
Brittleness	4.6^{c}	9.1^{a}	3.9^{c}	2.8^{d}	5.7^{b}	9.0^{a}	6.0^{b}	6.5^{b}	8.1^{a}	0.5	<0.001
Curdiness	3.9^{c}	7.9^{a}	3.1^{cd}	2.3^{d}	5.8^{b}	8.6^{a}	5.1^{b}	5.1^{b}	8.9^{a}	0.6	<0.001

Table 3.9. Mean values of descriptive sensory analysis of Cheddar-style cheese at six months of age. Only attributes with significant differences between treatments (alpha = 0.05) and means greater than 1 (for at least one treatment) at one month or six months of age are shown

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Aroma											
Overall	7.7	8.1	7.3	7.9	7.4	7.5	7.3	8.1	7.4	0.5	NS
Sour Dairy	1.7	1.8	1.7	1.7	1.6	1.9	1.6	1.7	1.5	0.3	NS
Taste											
Sweet	1.3^{a}	0.7^{abc}	1.1^{ab}	0.8^{abc}	0.6^{abc}	0.3^{c}	1.0^{abc}	0.7^{abc}	0.4^{bc}	0.3	<0.001
Salty	4.5^{a}	3.9^{a}	4.5^{a}	4.5^{a}	3.3^{a}	3.1^{a}	3.9^{a}	3.9^{a}	4.6^{a}	0.6	0.04
Sour	2.3	2.7	2.7	3.3	4.1	3.4	2.5	3.3	3.5	0.6	NS
Bitter	1.1^{c}	1.7^{c}	1.6^{c}	2.3^{c}	6.3^{b}	8.0^{a}	1.7^{c}	2.2^{c}	5.6^{b}	0.7	<0.001
Umami	1.3	1.2	1.2	1.3	0.8	1.0	1.2	1.3	1.3	0.3	NS
Flavor											
Overall	8.4^{c}	7.9^{c}	8.9^{abc}	10.0^{a}	9.1^{abc}	9.7^{ab}	8.2^{c}	8.4^{bc}	10.0^{a}	0.6	<0.001
Diacetyl	5.4^{a}	4.7^{ab}	5.3^{a}	5.3^{a}	3.5^{bc}	2.9^{c}	4.2^{abc}	4.3^{abc}	3.9^{abc}	0.5	<0.001
Cooked	2.3^{abc}	2.1^{abc}	2.4^{ab}	1.8^{abc}	1.7^{bc}	1.1^{c}	1.8^{abc}	2.9^{a}	1.9^{abc}	0.8	<0.01
Metallic	0.7^{c}	1.1^{c}	0.9^{c}	1.7^{c}	3.8^{ab}	4.3^{a}	0.8^{c}	1.3^{c}	3.0^{b}	0.7	<0.001
Milky	1.2^{a}	0.8^{ab}	1.0^{ab}	1.2^{a}	0.7^{ab}	0.4^{b}	0.9^{ab}	0.7^{ab}	0.6^{ab}	0.2	<0.01
Whey	1.8	2.1	2.1	2.4	1.5	1.3	2.1	2.2	2.4	0.6	NS

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each attribute

NS: No significant differences between treatments (alpha = 0.05)

[a-e]Means with without a common superscript letter within the same row are significantly different ($p < 0.05$)

Table 3.9 Continued

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Unclean	0.3^{c}	0.6^{c}	0.4^{c}	0.7^{c}	2.0^{b}	4.6^{a}	0.5^{c}	0.7^{c}	2.5^{b}	0.5	<0.001
Sour Dairy	2.2	2.6	2.4	2.9	2.7	3.5	2.1	2.4	2.2	0.7	NS
Soapy	0.5^{c}	1.1^{c}	0.7^{c}	0.8^{c}	2.0^{b}	3.2^{a}	1.0^{c}	1.1^{c}	2.7^{a}	0.4	<0.001
Sensation											
Astringency	0.2^{b}	0.9^{b}	0.6^{b}	0.5^{b}	1.1^{b}	1.9^{a}	0.4^{b}	0.9^{b}	0.7^{b}	0.6	<0.001
Numbing	0.1^{c}	0.2^{bc}	0.3^{bc}	0.2^{bc}	1.0^{a}	0.7^{ab}	0.2^{bc}	0.3^{bc}	0.7^{ab}	0.2	<0.001
Pungent	0.4^{a}	0.5^{a}	0.5^{a}	1.0^{a}	1.3^{a}	1.5^{a}	0.4^{a}	1.0^{a}	1.1^{a}	0.5	0.03
In-Hand Texture											
Firmness	12.1^{c}	18.2^{a}	15.2^{b}	14.0^{b}	9.0^{d}	15.2^{b}	17.8^{a}	18.1^{a}	14.0^{b}	0.7	<0.001
Springiness	6.4	6.4	6.0	5.9	8.8	6.4	5.9	6.5	6.2	1.2	NS
Stickiness	3.7^{ab}	2.1^{cd}	3.2^{bc}	3.5^{ab}	4.3^{a}	2.9^{bcd}	1.8^{d}	2.2^{cd}	3.1^{bc}	0.5	<0.001
Cohesiveness	13.2	12.6	12.3	11.6	12.7	12.3	13.2	13.0	10.9	1.0	NS
Slipperiness	10.4^{bcd}	8.0^{e}	9.5^{cde}	10.6^{bcd}	11.6^{b}	11.4^{bc}	9.2^{de}	9.5^{cde}	13.6^{a}	0.8	<0.001
First-Bite Texture											
Firmness	4.8^{e}	12.8^{a}	6.1^{e}	5.5^{e}	3.4^{f}	9.7^{c}	11.2^{b}	12.1^{ab}	7.5^{d}	0.7	<0.001
Stickiness	12.8^{a}	5.4^{c}	12.9^{a}	13.3^{a}	12.1^{a}	7.6^{bc}	6.6^{bc}	6.4^{bc}	7.9^{b}	0.9	<0.001
Brittleness	2.2^{c}	10.1^{a}	3.3^{c}	3.6^{c}	2.0^{c}	7.4^{b}	7.9^{b}	9.0^{ab}	7.1^{b}	1.0	<0.001
Five-Chew Texture											
Firmness	5.9^{fg}	12.9^{a}	7.5^{e}	6.5^{ef}	4.9^{g}	10.4^{c}	11.4^{bc}	11.9^{ab}	8.8^{d}	0.6	<0.001
Stickiness	13.7^{a}	6.5^{b}	12.6^{a}	13.6^{a}	12.0^{a}	6.9^{b}	7.1^{b}	7.3^{b}	8.5^{b}	0.9	<0.001
Brittleness	2.1^{c}	10.7^{a}	3.0^{c}	3.1^{c}	2.8^{c}	8.1^{b}	8.5^{b}	9.5^{ab}	7.7^{b}	0.9	<0.001
Curdiness	1.6^{b}	7.9^{a}	2.3^{b}	2.2^{b}	2.5^{b}	7.4^{a}	5.9^{a}	7.3^{a}	6.9^{a}	0.9	<0.001

adjunct cultures that produce these attributes in an attempt to reduce these flavors. All of the reduced sodium treatments, with the exception of N/K, had significantly more overall flavor than full-sodium control. This is contrast with Lindsay et al. (1982) who found that reduced sodium Cheddar cheese with KCl (1:1 molar ratio with NaCl) lacked full flavor compared to the full sodium reference at 3, 6, and 9 months of age. Shakeel-ur-Rehman et al. (2008) found salty taste to be higher in stirred-curd Cheddar than in milled-curd Cheddar so this could explain the difference between the present study and the study of Lindsay, which used milled-curd Cheddar

Treatments containing sea salt, with the exception of S/MG, were firmer and more brittle than control in first-bite and five-chew texture ($p < 0.001$), and N/C was as firm as the sea salt treatments. The least firm treatment at six months was N/MG; it was the least firm in in-hand and first-bite firmness and in five-chew firmness ($p < 0.001$). At one month of age, the stickiest treatment was N/MK, but by 6 months of age there was no difference between treatments containing N. In general, treatments containing N were significantly more sticky than treatments containing S at six months. The differences in texture between analogous treatments containing N and S may have been due to sulfate anions (SO_4^{2-}) as this was the only unique component of the sea salt.

Differences between one-month and six-month analysis are shown in Table 3.10. An expanded table is shown in Table A.4 in the appendix. Attributes which had significant differences between treatments in month one, but not month six were overall aroma, sour dairy aroma, sour, umami, whey flavor, sour dairy flavor, numbing, in-hand springiness, and in-hand cohesiveness. Significant differences between treatments existed for pungent at month six, but not month one. No significant aroma differences between

Table 3.10. Differences between one-month and six-month Cheddar-style cheese descriptive sensory analysis. A negative value indicates a lower rating in month six and a positive value indicates a higher rating in month six.

	Treatment[1]								
	Full Sodium		Reduced Sodium[3]						
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG
Aroma									
Overall	-1.3	-0.8	**-1.6***	**-0.9***	-1.2	-0.6	**-1.5***	-1.0	-0.9
Sour dairy	-1.0	-0.9	-0.7	-1.1	-0.4	-0.4	**-1.0***	**-1.3***	-0.9
Taste									
Sweet	0.2*	0.2	0.3	0.0	-0.1	0.1	0.3*	0.2	-0.1
Salty	0.8	-0.3	0.7	0.0	-0.3	**-1.4***	-0.1	**-0.6***	-0.7
Sour	**0.2***	-0.6	**-0.1***	-0.4	**2.4***	**1.5***	-0.7	**0.1***	**1.3***
Bitter	**0.9***	0.8	0.9	**1.8***	1.1	**1.7***	0.8	**1.3***	1.2
Umami	-0.1	0.1	-0.2	0.1	0.2	0.3	-0.1	-0.1	0.0
Flavor									
Overall	0.6	-0.3	0.9	1.1	0.3	0.2	-0.5	-0.1	1.0
Diacetyl	**1.4***	**1.5***	**1.3***	1.2	0.4	**0.5***	0.2	**0.5***	**0.8***
Cooked	**1.2***	1.2	1.2	0.6	1.0	0.5	0.8	1.9	**1.1***
Metallic	**0.6***	**0.8***	**0.7***	1.5	**2.2***	**2.4***	**0.6***	**1.2***	**1.4***
Milky	-0.7	-0.6	**-0.9***	-0.7	-0.4	-0.4	**-0.8***	**-0.9***	**-0.8***
Whey	**-0.9***	**-0.6***	**-0.7***	-0.6	**-0.6***	-0.2	**-0.9***	**-0.7***	0.3
Unclean	**0.2***	**0.3***	0.3	**0.6***	-0.5	**1.1***	**0.1***	**0.5***	-0.3
Sour dairy	-0.5	-0.7	-0.7	-0.6	**0.9***	**1.7***	-1.0	-1.2	-0.3
Soapy	**0.2***	**0.7***	0.4	0.5	**0.4***	0.8	0.5	**0.7***	0.7
Sensation									
Astringency	-0.1	0.2	0.1	-0.2	0.6	0.8	-0.3	0.2	-0.1
Numbing	-0.1	-0.1	**0.1***	-0.1	-0.3	**-1.4***	-0.2	0.1	**-0.7***
Pungent	0.3	0.0	0.2	0.4	0.9	0.8	-0.1*	0.4	0.6

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

*****Values in bold are significant ($p < 0.05$)

Table 3.10 Continued

	Treatment[1]								
	Full Sodium		Reduced Sodium[3]						
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG
In-Hand Texture									
Firmness	**2.3***	**2.1***	**4.5***	**5.6***	1.5	0.8	**3.8***	**3.8***	**5.2***
Springiness	**-2.5***	**1.9***	**-2.3***	**-3.5***	**-1.8***	0.5	0.0	**0.9***	**-3.0***
Stickiness	-0.1	-0.9	-0.9	**-1.6***	-0.2	-0.4	**-1.7***	**-1.2***	-0.9
Cohesiveness	**1.7***	**1.1***	0.7	1.4	2.2	1.5	**1.7***	1.6	2.5
Slipperiness	0.1	-4.0	-0.9	0.6	0.4	-0.5	-0.6	-0.5	0.1
First-Bite Texture									
Firmness	-1.0	2.9	0.0	**1.0***	**-2.4***	-0.1	**3.4***	**4.2***	1.8
Stickiness	**4.8***	0.5	**3.4***	1.6	**5.3***	**3.2***	-0.7	-0.9	2.8
Brittleness	**-1.6***	2.0	-0.3	0.8	**-2.5***	**0.2***	2.6	**3.7***	-0.6
Five-Chew Texture									
Firmness	-1.7	2.6	0.2	**0.7***	**-2.5***	0.0	**2.6***	**3.0***	1.6
Stickiness	**4.7***	**1.1***	**2.9***	1.7	**5.0***	**2.0***	-1.5	**-0.6***	**2.8***
Brittleness	**-2.5***	1.6	**-0.9***	0.4	**-2.9***	**0.8***	2.5	3.0	-0.4
Curdiness	**-2.4***	0.0	-0.8	0.0	**-3.4***	-1.2	0.8	**2.1***	-1.9

treatments existed at month six. Bitterness increased from month one to month six in treatments N, N/MK, N/C, and S/MK ($p < 0.05$). Metallic flavor increased in all treatments, except N/MK ($p < 0.05$), and the increase was numerically the greatest in N/MG, N/C, and S/MG. Unclean flavor increased in treatments all treatments except N/K, N/MG and S/MG ($p < 0.05$). Saltiness decreased in treatments N/C and S/MK ($p < 0.05$). Sulfur flavor increased in all treatments ($p < 0.05$) which is consistent with what is expected in an aged Cheddar-style cheese. In-hand firmness increased in all treatments

except N/MG, N/C; first-bite and five-chew firmness increased in N/MK, N/MG, S/K, and S/MK ($p < 0.05$). The differences in sensory attribute changes over time indicate that biochemical reactions during aging are different between treatments.

Principal component analysis (PCA) of the treatments at six months (Figure 3.2) showed treatments N/K and N/MK were most similar to control. The three treatments (N, N/K, and N/MK) were negatively related to principal component one, and thus had more diacetyl, milky and sweet flavors and less metallic, unclean, fermented and soapy flavors, and numbing intensity, than did products that were positively related to principal component one (cheeses containing $MgCl_2$ or $CaCl_2$). The three treatments were also negatively related to principal component two, and thus were less firm, brittle and curdy and were more sticky than were products that were positively related to principal component two (cheeses containing sea salt). PCA results at one month showed similar results.

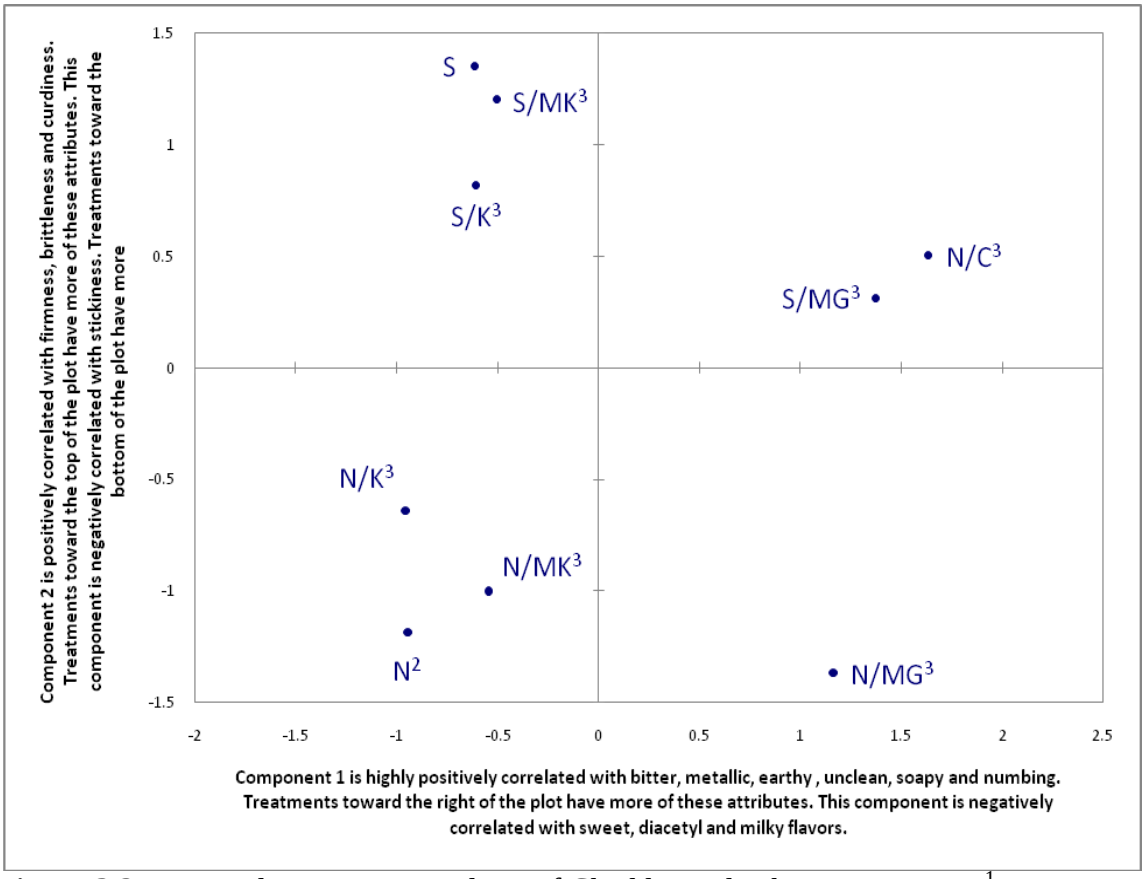

Figure 3.2. Principal component analysis of Cheddar-style cheese treatments[1] at six months of age. Principal component one and two explained 45% and 34% of the variability in the data respectively.
[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate
[2]640 mg sodium/100 g sample target
[3]300 mg sodium/100 g sample target

3.3.3 Comparison of Texture Profile Analysis to Descriptive Sensory Texture Analysis

Correlations between texture profile analysis and descriptive sensory texture attributes are shown in Table 3.11. TPA hardness correlated positively with in-hand, first-bite, and five-chew firmness ($p \leq 0.001$). TPA adhesiveness correlated positively with first-bite

and five-chew stickiness ($p < 0.001$). TPA chewiness correlated negatively to first-bite and five-chew stickiness ($p \leq 0.04$). TPA springiness and cohesiveness did not correlate to in-hand springiness and cohesiveness respectively. TPA resilience did not correlate to in-hand springiness, or any firmness measurement.

Table 3.11. Pearson's correlations between texture profile analysis (TPA) measurements and descriptive sensory texture attributes. Values shown are Pearson's correlation coefficient (r)

	TPA Measurement					
Sensory Attribute	Hardness	Springiness	Cohesiveness	Adhesiveness	Resilience	Chewiness
In-Hand Texture						
Firmness	**0.893***	0.235	-0.656	-0.323	-0.237	0.177
Springiness	**-0.850***	-0.182	**0.722***	0.290	0.305	-0.100
Stickiness	**-0.854***	-0.458	0.420	0.531	0.000	-0.330
Cohesiveness	0.641	-0.278	-0.175	0.271	-0.342	0.039
Slipperiness	-0.082	**0.700***	0.142	-0.642	0.571	0.359
First-Bite Texture						
Firmness	**0.931***	0.528	-0.414	-0.554	0.104	0.479
Stickiness	-0.548	**-0.918***	-0.026	**0.927***	-0.609	**-0.682***
Brittleness	0.448	**0.775***	-0.261	**-0.827***	0.380	0.430
Five-Chew Texture						
Firmness	**0.941***	0.601	-0.294	-0.620	0.204	0.571
Stickiness	-0.498	**-0.933***	-0.086	**0.905***	**-0.667***	**-0.711***
Brittleness	0.581	**0.846***	-0.185	**-0.879***	0.461	0.575
Curdiness	0.379	**0.905***	-0.013	**-0.905***	0.608	0.605

***Values in bold are different from zero ($p < 0.05$)

3.4 Conclusions

Despite achieving a_w that was not significantly different between treatments, the various salt and salt replacer treatments had varying effects on composition, chemical properties, texture, taste, and flavor. The use of $CaCl_2$ and $MgCl_2$ to reduce sodium in Cheddar-style cheese causes numerous differences from full sodium control while the use of KCl and modified KCl causes fewer and less objectionable differences. Naturally-reduced sodium sea salt has different effects on Cheddar-style cheese than sodium chloride. In general, cheeses with KCl and modified KCl showed many compositional, chemical, sensory and textural similarities to control, and Treatments N/K and N/MK were rated as most similar to control in sensory analysis. Similarly, other investigators have found favorable results of using KCl when reducing sodium in Cheddar (Lindsay et al., 1982; Reddy and Marth, 1994), Colby (Taylor, 1983), Kefalograviera (Katsiari et al., 1998), process American (Karahadian and Lindsay, 1984), Swiss (Jameson, 1987), and Feta (Katsiari et al., 1997) cheeses. Therefore, N/K and N/MK were selected to be investigated further, and the results of this work are shown in Chapter 5.

While previous studies have implicated mineral salt replacers as causing bitterness and metallic off flavors in cheese (Fitzgerald and Buckley, 1985; Lindsay et al., 1982), the data suggests that the salt replacers may not be the sole cause; cheeses with KCl and modified KCl were not significantly more bitter than control. It is possible that the direct off flavors from $CaCl_2$ and $MgCl_2$ in combination with a difference in cheese aging reactions compared to control led to the bitterness and metallic flavor seen in this study for the treatments containing those salt replacers. $CaCl_2$ may have more of a direct off flavor than $MgCl_2$ as evidenced by the positive correlation between calcium content

and unclean flavor, soapy flavor, and astringency that did not exist with magnesium content. While a_w was not significantly different between treatments, it is possible that the divalent nature of calcium and potassium could cause different effects on cheese aging compared to the monovalent sodium and potassium.

The microbial stability of reduced sodium cheese with added salt replacers should be investigated due to the probable reduced starter culture inhibition of mineral salt replacers. The same effect might also result in reduced inhibition of food-borne pathogens

Minor modifications in a cheese making procedure may be needed when using mineral salt replacers as the pH of all but one reduced sodium cheese was lower than full sodium control. To achieve a final pH equivalent to control, the target pH at salting could be increased in order to reach the same final target at packaging.

CHAPTER 4: Evaluation of Flavor Enhancer Types and Concentrations for Use in Low Sodium Cheddar-Style Cheese

4.1 Introduction

Flavor enhancers are used in foods to achieve a certain flavor profile or enhance the salty perception of reduced sodium foods. While mineral salt replacers have been studied as a means to reduce sodium in cheese, no published research has investigated the use of FE in these cheeses to improve the observed negative sensory characteristics. Therefore, it is unknown which flavor enhancers would be suitable for use in reduced sodium cheese, and a screening process was needed before incorporating them into cheese.

The objective of this research was to determine which flavor enhancers and what concentrations would be selected for use in manufacturing low sodium Cheddar-style cheese with potassium chloride (KCl) in the proceeding chapter.

It was hypothesized that a bench top-scale method could be developed to quickly evaluate and screen flavor enhancers to accomplish the objective and eliminate the need for making cheese to do so.

To test the hypothesis, flavor enhancers were incorporated into finely grated reduced sodium Cheddar-style cheese containing KCl from Chapter 3 to screen for useful ingredients. The most promising ingredients were incorporated in the same cheese at various usage levels to determine a level to use in cheese making trials.

4.2 Materials and Methods

4.2.1 First Evaluation of Flavor Enhancers

Reduced sodium Cheddar-style cheese containing KCl from Chapter 3 (treatment N/K), was finely grated with a Waring Commercial WFP14 food processor. Thirty g of cheese was added to a Waring Commercial WSG30 spice grinder before adding flavor enhancers at concentrations shown in Table 4.1. Use levels of flavor enhancers were 25 to 50% below product recommendation, if available, or the recommended level of a similar product, if not available. An additional 15 g of cheese was added, and contents were hand-stirred for 10 s then blended for approximately 30 s in 5 s intervals until slightly pasty. An additional 10 s of hand stirring was performed to facilitate even mixing. The mixture was rolled into a tight ball, vacuum packaged with a Multivac vacuum packager (Koch, Kansas City, MO), and stored at 7°C for 2 d before evaluation.

Three experienced cheese tasters and five regular consumers of Cheddar cheese evaluated all cheeses for general flavor profile and off flavors. Group discussion occurred to determine flavor characteristics of each sample. Based on the results, the flavor enhancers were categorized, and half were selected to evaluate further.

Table 4.1. Product information and amounts of flavor enhancers used in the first evaluation period

Sample Number	Supplier	Label Name	Product Description	mg /45 g cheese
1	INNOVA[1]	Sav Nat Fl Enhancer 0188404	HVP[8]/YE[9]	59.2
2	INNOVA	Sav Salt Reducer/Ehancer 0188807	YE	59.2
3	INNOVA	Sav Salt Reducer/Ehancer 0189154	YE and natural flavor	59.2
4	INNOVA	Sav MSG Replacer 0187641	HVP/YE/IMP[10]/GMP[11]	33.8
5	David Michael[2]	50/50 Disodium Inosinate/Disodium Guanylate	IMP/GMP blend	101.3
6	David Michael	DM Choice® Natural Flavor "Potassium Blocker Type"	Natural flavor	65.7
7	Gold Coast[3]	Natural salt replacer #2 (#319678)	Salts, sugar, natural flavors, and whey	67.5
8	Gold Coast	Natural Salt replacer #3 (#319679)	YE, natural flavor	67.5
9	Edlong[4]	Natural Cheddar-Type Flavor #1411344- Powder	Natural flavor	112.5
10	Edlong	Natural Masking Flavor #1411308- Powder	Natural flavor	90.0

[1]INNOVA, Lombard, IL
[2]David Michael & Co., Philadelphia, PA
[3]Gold Coast Ingredients, Inc., Commerce, CA
[4]Edlong Corp., Elk Grove Village, IL
[5] Ajinomoto Food Ingredients LLC, Chicago, IL
[6] Balchem Corp., New Hampton, NY
[7]Premium Ingredients International (US), LLC, Carol Stream, IL
[8]HVP: hydrolyzed vegetable protein
[9]YE: yeast extract
[10]IMP: disodium 5' inosinate
[11]GMP: disodium 5' guanylate

Table 4.1 Continued

Sample Number	Supplier	Label Name	Product Description	mg /45 g cheese
11	Edlong	Natural and Artificial Masking Flavor #1411662-Powder	Natural and artificial flavor	90.0
12	Edlong	Natural Cheddar Flavor WONF #1411086- Spray Dry	Natural flavor	225.0
13	Edlong	Natural Cheese Flavor WONF #2406- Powder	Natural flavor	112.5
14	Ajinomoto [5]	AJITIDE IMP Disodium 5' Inosinate	IMP	105
15	Balchem[6]	Flavorshure C-Salt CC98	Choline chloride	45.0
16	Premium Ingredients[7]	CJTIDE Disodium 5' Guanylate	GMP	105

4.2.2 Second Evaluation of Flavor Enhancers

Flavor enhancers were incorporated into cheese in the same manner as the first evaluation period. Three concentrations (75%, 50%, and 25% of the concentration from the first evaluation period) of eight flavor enhancers were applied to the cheese (Table 4.2). Negative control without flavor enhancers was also created. Seven of the eight panelists from the first evaluation period sampled eight cheeses plus the negative control in each of three sessions. Samples were balanced across sessions, and sample order was randomized within sessions. Room temperature (~22°C) cheese samples were presented in 2-ounce soufflé cups with three-digit codes. Panelists marked their rating of overall Cheddar cheese flavor quality, ignoring other factors, on a 16.8 cm line scale anchored with "poor quality" on the left end and "excellent quality" on the right end. The full sodium cheese

Table 4.2. Usage levels of flavor enhancers (mg added to 45 g cheese) in the second evaluation period and the corresponding sensory scores

Sample Number	Sample	Sample Label	Usage Level[1]	mg/45 g Cheese	Sensory Score[2]
None	Reduced sodium Cheddar-style cheese containing KCl (No flavor enhancers)	Negative Control	NA[3]	NA	11.4
1	Sav Nat Fl Enhancer 0188404[4]	1-H	High	44.4	12.8
		1-M	Medium	29.6	15.8
		1-L	Low	14.8	7.2
2	Sav Salt Reducer/ Enhancer 188807[4]	2-H	High	44.4	10.1
		2-M	Medium	29.6	13.3
		2-L	Low	14.8	11.5
4	Sav Salt Reducer/ Enhancer 0187641[4]	4-H	High	25.4	11.0
		4-M	Medium	16.9	8.3
		4-L	Low	8.5	7.9
6	DM Choice® Natural Flavor "Potassium Blocker Type" #33150[5]	6-H	High	49.3	11.7
		6-M	Medium	32.9	11.1
		6-L	Low	16.4	11.4

[1]High = 75% of the level used in the first evaluation period; Medium = 50% of the level used in the first evaluation period; Low = 25% of the level used in the first evaluation period.
[2]Highest score possible = 16.8, lowest score possible = 0
[3]NA: Not applicable
[4]Supplier: INNOVA, Lombard, IL
[5]Supplier: David Michael & Co., Philadelphia, PA
[6]Supplier: Edlong Corp., Elk Grove Village, IL
[7]Supplier: Ajinomoto Food Ingredients LLC, Chicago, IL
[8] Supplier: Balchem Corp., New Hampton, NY
[9]Supplier: Premium Ingredients International (US), LLC, Carol Stream, IL

Table 4.2 Continued

Sample Number	Sample	Sample Label	Usage Level[1]	mg/45 g Cheese	Sensory Score[2]
11	Natural and Artificial Masking Flavor #1411662[6]	11-H	High	67.5	3.9
		11-M	Medium	45.0	4.6
		11-L	Low	22.5	5.9
14	AJITIDE IMP disodium 5' inosinate[7]	14-H	High	78.8	12.1
		14-M	Medium	52.5	10.7
		14-L	Low	26.3	9.8
15	Flavorshure C-Salt CC98[8]	15-H	High	33.8	8.8
		15-M	Medium	22.5	11.3
		15-L	Low	11.25	11.0
16	GMP disodium 5' guanylate[9]	16-H	High	78.8	11.3
		16-M	Medium	52.5	12.0
		16-L	Low	26.3	11.9

made with sodium chloride from Chapter 3 (treatment N) was given as a high quality reference and marked on the scale at 12.5 cm from the left end. No training occurred to define high and low quality. Panelists were asked to rinse their mouth with filtered water between samples. Sensory scores for each sample were determined by measuring the distance (cm) of the marking from the left end of the scale. Based on the scores and other considerations, flavor enhancers and their concentrations were selected to use in the future research.

4.3 Results and Discussion

4.3.1 First Evaluation of Flavor Enhancers

Based on the group discussion, the samples were categorized with the following terms to describe the flavor: buttery, beefy, brothy/umami, unclean, fruity, and process cheese-like (Table 4.3). Samples 1, 2, and 4 were selected in order to include samples containing HVP and YE, plus the samples may have reduced bitterness and had clean flavors. Sample 3 was not selected as a source of YE due to an unclean flavor. Sample 6 was selected because it was described as pleasantly non-descript while maintaining Cheddar cheese flavor. It was agreed that sample 14 was potentially useful, but a lower use level was needed; it was selected based on flavor potential. Samples 15 and 16 were selected due to their smooth clean flavor, plus sample 15 had a unique chemical identity. The final sample, sample 11, was selected for further evaluation due to its unique fruity flavor. While fruity was not considered to be Cheddar-like, it was agreed that it could potentially cause interesting or useful effects at a lower use level.

Table 4.3. Observed flavor categories of the flavor enhancers in reduced sodium Cheddar-style cheese during the first evaluation period

Sample Label	Supplier	Label Name	Product Description	Flavor Categories					
				Buttery	Beefy	Brothy/ Umami	Unclean	Fruity	Process Cheese
None	Not Applicable	Reduced sodium Cheddar-style cheese containing KCl (No flavor enhancers)	Negative control	NR	NR	NR	NR	NR	NR
1	INNOVA[1]	Sav Nat Fl Enhancer 0188404	HVP[8]/YE[9]	NR	NR	*	NR	NR	NR
2	INNOVA	Sav Salt Reducer/Ehancer 0188807	YE	NR	NR	*	NR	NR	NR
3	INNOVA	Sav Salt Reducer/Ehancer 0189154	YE and natural flavor	NR	NR	NR	*	NR	NR
4	INNOVA	Sav MSG Replacer 0187641	HVP/YE/IMP[10]/GMP[11]	NR	NR	NR	NR	NR	*
5	David Michael[2]	50/50 Disodium Inosinate/Disodium Guanylate	IMP/GMP blend	NR	*	*	*	NR	NR

∗Sample was rated as having the above characteristic
NR: Sample was not rated with the flavor category
[1]INNOVA, Lombard, IL
[2]David Michael & Co., Philadelphia, PA
[3]Gold Coast Ingredients, Inc., Commerce, CA
[4]Edlong Corp., Elk Grove Village, IL
[5] Ajinomoto Food Ingredients LLC, Chicago, IL
[6] Balchem Corp., New Hampton, NY
[7]Premium Ingredients International (US), LLC, Carol Stream, IL
[8]HVP: hydrolyzed vegetable protein
[9]YE: yeast extract
[10]IMP: disodium 5' inosinate
[11]GMP: disodium 5' guanylate

Table 4.3 Continued

Sample Label	Supplier	Label Name	Product Description	Flavor Categories					
				Buttery	Beefy	Brothy/ Umami	Unclean	Fruity	Process Cheese
6	David Michael	DM Choice® Natural Flavor "Potassium Blocker Type"	Natural flavor	NR	NR	NR	NR	NR	NR
7	Gold Coast[3]	Natural salt replacer #2 (#319678)	Salts, sugar, natural flavors, and whey	*	NR	NR	NR	NR	NR
8	Gold Coast	Natural Salt replacer #3 (#319679)	YE, natural flavor	NR	NR	NR	NR	NR	NR
9	Edlong[4]	Natural Cheddar-Type Flavor #1411344- Powder	Natural flavor	NR	NR	NR	*	NR	NR
10	Edlong	Natural Masking Flavor #1411308- Powder	Natural flavor	NR	NR	NR	NR	NR	*
11	Edlong	Natural and Artificial Masking Flavor #1411662- Powder	Natural and artificial flavor	NR	NR	NR	NR	*	NR
12	Edlong	Natural Cheddar Flavor WONF #1411086- Spray Dry	Natural flavor	NR	NR	NR	*	NR	NR
13	Edlong	Natural Cheese Flavor WONF #2406- Powder	Natural flavor	NR	NR	*	NR	NR	NR
14	Ajinomoto[5]	AJITIDE IMP Disodium 5' Inosinate	IMP	NR	*	*	NR	NR	NR
15	Balchem[6]	Flavorshure C-Salt CC98	Choline chloride	*	NR	NR	NR	NR	NR
16	Premium Ingredients[7]	CJTIDE Disodium 5' Guanylate	GMP	*	NR	NR	NR	NR	NR

4.3.2 Second Evaluation of Flavor Enhancers

Sensory scores for the second evaluation period are shown in Table 4.2. Of the samples containing HVP, YE, or both (1, 2, and 4), sample 1 and the usage level of 1-M were chosen because 1-M scored the highest of all samples. While sample 2 did score highly, it was not further evaluated so that a variety of ingredient types could be used. Samples 14 and 16 at usage levels 14-H and 16-M respectively were selected based on their scores being the top two of remaining samples under consideration and in the top five of all samples tested. Due to the anticipated design of the future research, one additional flavor enhancer, sample 6 and the usage level of 6-H, was selected based on having the highest score of the remaining samples under consideration. The chosen samples and their use level are shown in Table 4.4.

Table 4.4. Flavor enhancers and their use level (mg added to 45 g cheese) selected for use in future research

Supplier	Label Name	Product Description	mg/45 g cheese
INNOVA[1]	Sav Nat Fl Enhancer 0188404	HVP[5]/YE[6]	29.6
David Michael[2]	DM Choice® Natural Flavor "Potassium Blocker Type"	Natural flavor	49.3
Ajinomoto[3]	AJITIDE IMP Disodium 5' Inosinate	IMP[7]	78.8
Premium Ingredients[4]	CJTIDE Disodium 5' Guanylate	GMP[8]	52.5

[1]INNOVA, Lombard, IL
[2]David Michael & Co., Philadelphia, PA
[3]Ajinomoto Food Ingredients LLC, Chicago, IL
[4]Premium Ingredients International (US), LLC, Carol Stream, IL
[5]HVP: hydrolyzed vegetable protein
[6]YE: yeast extract
[7]IMP: disodium 5’ inosinate
[8]GMP: disodium 5’ guanylate

4.4 Conclusions

The described methods were used to determine levels of flavor enhancers to use in the manufacture of the low sodium Cheddar-style cheese with potassium chloride (KCl) in Chapter 5. The four flavor enhancers selected, at their respective usage levels, all scored higher in overall Cheddar cheese flavor quality than the negative control without flavor enhancers when evaluated by experience cheese tasters, and experienced cheese consumers. This study indicated that the addition of flavor enhancers to reduced sodium cheese might reduce negative sensory characteristics due to salt replacers. Additionally, flavor enhancers may impart their own desirable flavor characteristics into the cheese that could counteract a flat flavor that can be noted in reduced sodium products.

CHAPTER 5: Manufacture and Analysis of Low Sodium Cheddar-Style Cheese with Mineral Salt Replacers and Flavor Enhancers

5.1 Introduction

The use of KCl was shown in the previous study, when maintaining the same water activity (a_w) as full sodium Cheddar cheese, to have promising sensory results. Flavor enhancers are currently used in reduced sodium foods such as soups and frozen meals to enhance the salty perception and reduce off flavors associated with mineral salt replacers, in particular, KCl. Previous research of sodium reduction in cheese has studied various mineral salt replacers, but replacers in combination with flavor enhancers have not been investigated as a way to improve undesirable flavors resulting from the use of replacers. Therefore, this topic would be beneficial to explore. Some flavor enhancers are amino acids or small peptides and could influence culture growth in addition to imparting flavor into the cheese.

Another observation from the previous study was that there were differences between the two KCl ingredients used in sensory, compositional, chemical, and texture properties of the cheese. These differences would be interesting to understand further by monitoring the cheeses during aging.

For this study the following hypotheses were proposed:

1. The incorporation of flavor enhancers will reduce the negative sensory attributes associated with the use of KCl in low sodium Cheddar-style cheese.
2. Flavor enhancers will not alter the biochemical, microbial, and physical reactions in the cheese during production and aging
3. Differences in flavor and texture between KCl and modified KCl are due to differences in cheese chemistry during aging

To test these hypotheses, full fat, stirred-curd Cheddar-style cheese was produced with less than 280 mg sodium/100 g cheese using KCl or modified KCl as salt replacers. Four different flavor enhancers were added to four treatments that contained KCl. Salt, salt replacers, and flavor enhancers were applied at concentrations to achieve equivalent a_w to full sodium cheese, as in Chapter 3, to produce reduced sodium cheeses of similar quality to full sodium cheese. Compositional, chemical, microbial, and physical analysis was performed through four months of aging to evaluate if there were differences in cheese chemistry. Data was analyzed by linear mixed model to evaluate if there were differences. Descriptive sensory analysis was conducted throughout the aging period to evaluate if the flavor enhancers changed the flavor profile and if there were differences between the two KCl sources. A consumer acceptability test was also conducted to see if consumers had a preference for any of the low sodium treatments. Data was analyzed by general linear model to evaluate if there were differences. The University of Minnesota's Sensory Center in the Department of Food Science and Nutrition conducted the sensory testing and resultant data analysis.

5.2 Materials and Methods

5.2.1 Cheese Making Materials

5.2.1.1 Culture

A direct-to-vat-set culture of *Lactococcus lactis ssp. cremoris* and *L. lactis ssp. Lactis* (CHOOZIT™ Superstart® M30, Danisco USA, Inc., Madison, WI) was added to sterilized whole milk at 1% (wt/wt). The culture was maintained at 22.2°C for 16 hr to create a

bulk culture. The bulk culture was frozen and held at -80°C until needed. Sixteen hr before cheese making, the bulk culture was added to sterilized whole milk at 1% (wt/wt) and maintained at 22.2°C to create the bulk culture used in cheese making.

5.2.1.2 Ingredients

Annatto (AFC W/S 1X 70463, Chr. Hansen, Inc., Milwaukee, WI), 45% (wt/vol) calcium chloride (CAL-SOL 71257, Chr. Hansen, Inc., Milwaukee, WI), and liquid chymosin (CHY-MAX® 73863, Chr. Hansen, Inc., Milwaukee, WI) were used in manufacturing the cheese curd. The salt and salt replacers used were sodium chloride (**N**) (Top-Flo® Evaporated Salt, Cargill, Inc., Minneapolis, MN), potassium chloride (**K**) (Premier™ Potassium Chloride 8799, Cargill, Inc., Minneapolis, MN), and a modified potassium chloride (**MK**) (Modified Potassium Chloride 14510, Nu-tek Products, Inc., Minnetonka, MN). The flavor enhancers used were a blend of hydrolyzed vegetable protein and yeast extract (**HY**) (SAVOR Notes Sav. Nat. Fl. Enhancer 0188404, INNOVA, Lombard, IL), a natural flavor (**BB**) (DM Choice® Natural Flavor "Potassium Blocker Type" 33150 Powder, David Michael and Co., Philadelphia, PA), disodium 5' inosinate (**I**), (AJITIDE IMP, Ajinomoto Food Ingredients LLC, Chicago, IL), and disodium 5' guanylate (**G**), (CJTIDE, PT. CJ Indonesia). All were Food Chemicals Codex (FCC) or United States Pharmacopeia (USP) grade.

5.2.2 Cheddar-Style Cheese Making Procedure

Cheddar-style cheese was manufactured at the University of Minnesota's Joe Warthesen Food Processing Center. The procedure is outlined in Figure 5.1. Raw whole milk was pasteurized at 73.3°C for 16 s on a plate and frame-style milk pasteurizer and pumped into a Damrow 2,260 Kg rectangular vat (Tetra Damrow, Vernon Hills, IL) at 31.1°C.

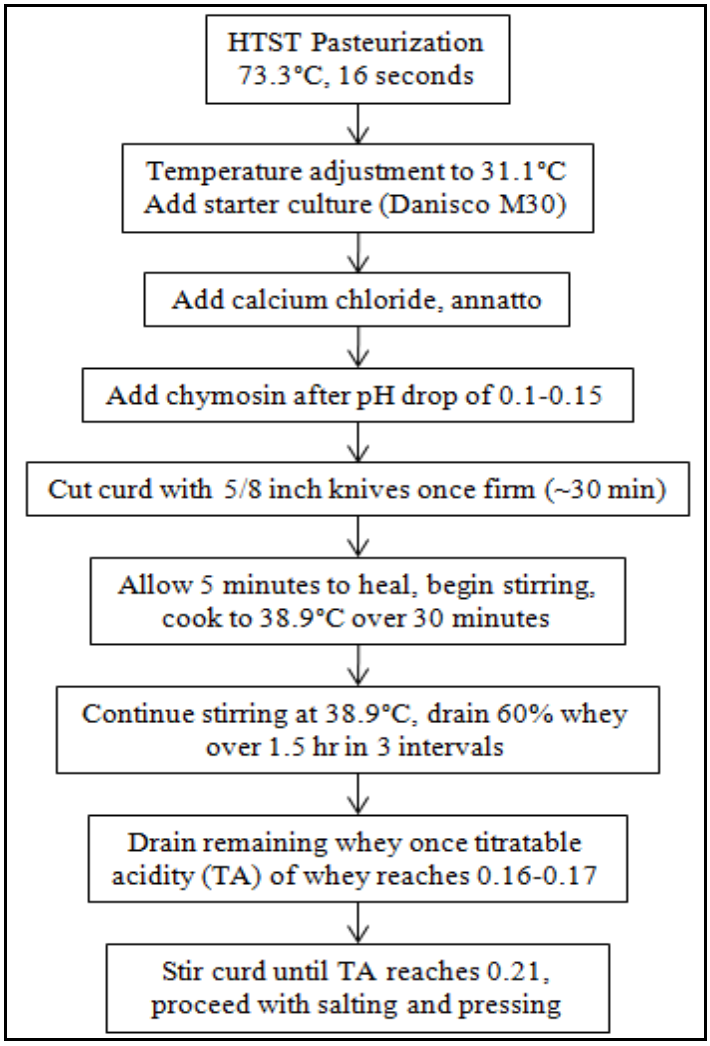

Figure 5.1. Flow diagram of the cheese making procedure.

Approximately one hour before renneting, lactic acid bulk starter culture was added at 1% (wt/wt) with gentle agitation. Twenty minutes before renneting, Calcium chloride was added at 198 ml/1000 kg milk and annatto was added at 66 ml/1000 kg milk. After a pH drop in the milk of 0.1-0.15, chymosin was added at a rate of 99 ml/1000 kg milk by diluting with water 1:50 and adding with gentle agitation. Once mixed, agitation ceased and the vat

was allowed to coagulate until the gel was properly firm (~30 min). The coagulum was cut into small pieces with 5/8 inch wire knives and allowed to heal for 5 minutes prior to stirring commenced. Ten min after cutting, curd and whey were gradually heated to 38.9°C over a 30 min period while stirring. Temperature was maintained and stirring continued for 1.5 hr during which approximately 60% of the whey was drained in three intervals. The majority of the remaining whey was drained once the titratable acidity (TA) reached 0.16-0.17 (lactic acid basis); enough whey was maintained in the vat to prevent the curd from drying. Curd was stirred until the TA reached 0.21 at which point it was divided into treatment groups that were salted with salt and salt replacer/flavor enhancer treatments (Table 5.1). Concentrations of KCl and modified KCl were determined based on concentrations found to produce equivalent a_w between low sodium and full sodium model cheese systems, as described in Chapter 2. Concentrations of flavor enhancers were determined through the testing as described in Chapter 4. Table 5.2 contains a description of each treatment. Drained curd in 27.2 kg portions was manually salted in individual plastic bins with drain holes in three additions spaced five min apart and continuously stirred by hand to prevent clumping and aid in salt absorption into curd. Curd was transferred to cheesecloth-lined, 9.1 kg Wilson-style cheese hoops and pressed overnight at 276 kPa. Blocks were vacuum packaged in a Multivac vacuum packager (Koch, Kansas City, MO) and stored at 4-5°C. Cheese making was replicated on two days with different lots of milk.

Table 5.1. Salt, salt replacer, and flavor enhancer concentrations for salting during cheese making

	Treatment[1]						
	Full Sodium[2]	Low Sodium[3]					
Item[1], %	N	K	HY	BB	I	G	MK
N	2.04	0.70	0.70	0.70	0.70	0.70	0.70
K	0	1.70	1.70	1.70	1.70	1.70	0
MK	0	0	0	0	0	0	1.70
HY	0	0	0.064	0	0	0	0
BB	0	0	0	0.107	0	0	0
I	0	0	0	0	0.171	0	0
G	0	0	0	0		0.114	0

[1]Items: N = sodium chloride; K = potassium chloride; HY = hydrolyzed vegetable protein/yeast extract blend; BB = potassium blocker type flavoring; I = disodium 5' inosinate; G = disodium 5' guanylate; MK = modified K
[2]640 mg sodium/100 g sample target
[3]220 mg sodium/100 g sample target

Table 5.2. Descriptions of the treatments in Chapter 5

Treatment	Description
N	Full sodium control
K	Low sodium cheese with sodium chloride as the source of sodium and with added potassium chloride
HY	Low sodium cheese with sodium chloride as the source of sodium and with added potassium chloride and a hydrolyzed vegetable protein/yeast extract blend
BB	Low sodium cheese with sodium chloride as the source of sodium and with added potassium chloride and potassium blocker type flavoring
I	Low sodium cheese with sodium chloride as the source of sodium and with added potassium chloride and disodium 5' inosinate
G	Low sodium cheese with sodium chloride as the source of sodium and with added potassium chloride and disodium 5' guanylate
MK	Low sodium cheese with sodium chloride as the source of sodium and with added modified potassium chloride

5.2.3 Compositional, Chemical, and Microbial Analysis

To measure titratable acidity during cheese making, three drops of phenolphthalein indicator was added to 9.00 g milk or whey. 0.1 N NaOH from an automatic burette standardized for lactic acid basis was dispensed into the sample until the phenolphthalein end point was reached (~pH 8.3, faint pink color). The volume of dispensed NaOH corresponded to the TA value as indicated by the burette.

Moisture content, pH, a_w, water soluble nitrogen (WSN), and lactic acid bacteria (LAB) enumeration were measured within two weeks of cheese making and monthly for four months (weeks 4, 8, 12, and 16) after cheese making. Fat, ash, protein, and mineral (sodium, potassium, magnesium, calcium) contents were measured once at approximately 16 weeks of age.

Moisture content was determined using a vacuum oven following method 15.111 in Standard Methods for the Analysis of Dairy Products (Wehr and Frank, 2004). The pH was measured on an Acorn® pH 6 Meter (Oakton Instruments, Vernon Hills, IL) with an Orion 8172BNWP Ross Sure-Flow pH electrode (Thermo Fisher Scientific, Inc., Waltham, MA). Approximately 0.2 g lab-grade water was added to 4 g grated cheese then the pH probe was inserted for measurement. Water activity was measured at 23°C on an Aqua Lab 3TE a_w meter (Decagon Devices, Pullman, WA). The WSN method was based on the single extraction of Kuchroo and Fox (1982) followed by nitrogen analysis using a TruSpec® N (Leco Corporation, St. Joseph, MI) based on the Dumas method of combustion. Extracts were centrifuged in a GS-6R centrifuge (Beckman Coulter, Inc., Brea, CA) at 3500 RPM for 30 minutes and filtered through Whatman #4 filter paper (Whatman plc., Maidstone, Kent, UK). WSN was expressed as the percent of the total

nitrogen in the cheese that was soluble in the first extraction. LAB counts were performed based on method 8.071, the total LAB method in Standard Methods for the Analysis of Dairy Products (Wehr and Frank, 2004). 11.00 g grated cheese was stomached in 99.0 ml 0.1% peptone water for 2 min. Serial dilutions were prepared in 0.1% peptone water, and 0.1 ml was streak plated on Lactobacilli MRS agar (Difco Laboratories, Detroit, MI) with a sterilized hockey stick. Plates were aerobically incubated for 48 ± 3 hr at 32°C. Fat and ash content were determined following methods 18.8A2c and 18.4A respectively in Standard Methods for the Analysis of Dairy Products (Richardson, 1985). Ash was measured after step 18.4A3d. Total protein (nitrogen x 6.38) was determined using the TruSpec® N. Sodium, potassium, magnesium, and calcium content were measured by atomic absorption spectrometry using a Perkin Elmer AAnalyst 100 using IDF method 119:2007(E) (IDF, 2007) with the exception of using the hot plate digestion time and temperature of Kira et al. (2004). All measurements were performed in duplicate, at minimum. An expanded methods section is in section A.1 of the Appendix.

5.2.4 Texture Profile Analysis

Texture profile analysis (TPA) was performed at 4, 8, 12, and 16 weeks of age on a TA.XT*Plus* texture analyzer (Texture Technologies Corporation, Scarsdale, NY). Hardness, springiness, cohesiveness, adhesiveness, resilience, and chewiness were calculated according to Bourne (1978). Additional information about calculating the texture attributes is in section A.1.9 of the Appendix. Testing was in accordance with IDF/RM method 205:2006(E) (IDF, 2006) with the exception of cutting slabs of appropriate height from the larger block of cheese prior to taking cylindrical samples.

Cheese was equilibrated to 6^{o}C for 16 hours then a slab of cheese (15 mm height) was cut from the middle of the block of cheese. Cylinders (11.5mm diameter) were cut with a #8 standard cork borer and placed in an air-tight bag. Samples were individually removed from the refrigerator and a thin layer of light white mineral oil (151694, MP Biomedicals, LLC, Solon, OH) was applied to both ends. Within 20 s of removal, samples were compressed twice, with 1 s between compressions, to 20% of original height at crosshead speed of 0.83 mm/s between a 25 mm diameter polycarbonate probe (TA11, Texture Technologies Corporation, Scarsdale, NY) and a polycarbonate stage; room temperature was 23^{o}C. A minimum of five replicates per cheese sample were analyzed.

5.2.5 Statistical Analysis of Compositional, Chemical, Microbial, and Texture Profile Analysis

Cheese making was conducted in duplicate. All measurements were duplicated, at minimum. Linear Mixed Model analysis with Least Significant Difference as a summary test (alpha = 0.05) was performed using SPSS Statistics ver. 17.0.2 (IBM SPSS, Chicago, IL). In the case of varying standard error between treatments, the largest is reported. All correlations between means were determined using Pearson's correlation (alpha = 0.05) in XLSTAT version 2011.1.04 (Addinsoft USA, New York, NY), and only measurements with significant differences between treatments ($p < 0.05$) were included. In the case of measurements over time, the means of all measurements within each treatment were used for the correlations.

5.2.6 Descriptive Sensory Analysis

Sensory evaluation and data analysis was conducted by the University of Minnesota's Sensory Center in the Department of Food Science and Nutrition. Evaluation was

conducted monthly for four months (weeks 4, 8, 12, and 16) by 10 trained panelists (6 to 10 participated in each separate test).

5.2.6.1 Subjects

Members of the trained descriptive analysis panel from the Sensory Center at the University of Minnesota participated in the tests. All were PROP tasters or supertasters and were compensated for participating. All recruiting and experimental procedures were approved by the University of Minnesota's Institutional Review Board.

5.2.6.2 Products

A total of 14 cheeses were included consisting of the seven treatments from both cheese make replicates Each panelist received 3 cubes ($1.5cm^3$) of each cheese at room temperature (22°C) in a random three-digit coded four ounce plastic cup with lid. Products were refrigerated until one hour prior to serving.

5.2.6.3 Training

Panelists participated in three training sessions during the first month of the study to familiarize themselves with the test cheeses. In each training session the panelists received a lexicon (Table 5.3) developed in Chapter 3. Taste, flavor, aroma, texture, and sensation references used for the descriptive sensory analysis are listed in the lexicon. All chemicals were FCC or USP grade. Panelists individually described several pairs of the test cheeses using the lexicon. They then participated in a group discussion about the rating intensities of the sensory attributes for each cheese and clarified definitions of the established references.

5.2.6.4 Testing

Panelists participated in a total of 16 testing sessions, completing 4 sessions per week for 1 week during each month of the four-month aging period. Panelists evaluated a complete set of the 14 samples (seven from both cheese making replicates) during the first two sessions of the week. This was repeated during the second two sessions of the week to serve as a sensory replicate. Month 3 (week 12) tests did not include the sensory replicate. Within a group of two sessions, serving orders were balanced for order and carryover effects. During the testing sessions each panelist evaluated samples by rating the intensity of the attributes on 20 point line scales labeled 'none' at the left end and 'intense' at the right end. Intensity ratings of flavor and taste were made on the standard citric acid scale developed by the University of Minnesota Sensory Center using concentrations of 0.03, 0.05, 0.08, and 0.14%. Ratings of odors were made on the standard butanol scale using concentrations of 0.02, 0.16, 2.56, and 20.48 g/L (ASTM, 2010). Texture ratings were made on a 20 point scale anchored with references. Panelists were instructed to wear nose clips when evaluating the taste attributes.

Table 5.3. Cheddar-style cheese lexicon

Descriptive Term[1]	Definition	Reference
Aroma and Flavor		
Overall Flavor or Aroma Intensity	The overall intensity of aroma and flavor	
Diacetyl	Aromatic or flavor associated with buttery popcorn	Unsalted butter
Cooked	The note associated with heated or cooked milk	Evaporated milk, Nestle Carnation with added Vitamin. D
Metallic	Aromatic or flavor associated with metals (tin or iron)	0.005% Ferrous Sulfate
Milky	Aromatic or flavor associated with skim milk or milk derived products.	Whole milk
Whey	Off-flavors or off-aromas in cheese associated with retained cheese whey	Thawed whey from cheese making
Malty	Sweet slightly fermented or sour grain note associated with freshly kilned malt	Barley malt extract
Earthy	Aromatic or flavor characteristic of damp soil, wet foliage	Wet potting soil
Moldy	The flavor and aromatics associated with molds- usually earthy, dirty, stale, musty and slightly sour	English stilton cheese
Sulfur	Aromatic or flavor associated with hydrogen sulfide, rotten egg	Cracker Barrel Vermont Sharp White Cheddar cheese
Unclean	Off-flavor/aroma that has also been described as a post-vomit flavor	No reference
Yeasty	Flavor/aroma associated with fresh yeast and yeast fermentation	1 tablespoon Red Star active dry yeast soaked in 400ml warm water.
Brothy	Flavor/aroma associated with boiled meat or vegetable soup stock	Solution of 75% Swanson's Vegetable broth, 25% H2O
Pineapple	Flavor/aroma associated with canned pineapple	Canned Pineapple Chunks
Hydrolytic Rancidity	Flavor/aroma associated with short-chain fatty acids, similar to those in blue cheese	Blue Cheese
Fermented	Flavor/aroma associated with fermenting fruits	No reference
Nutty	Flavor/aroma associated with nuts or nut meat	Planters mixed nuts
Sour dairy	Flavor/aroma associated with fermented milk character.	Sour cream, buttermilk
Soapy/detergent	Flavor/aroma associated with unscented soap or detergent	Ivory unscented bar soap
Tastes		
Sweet	The taste stimulated by sucrose and other sugars	500 ml water, 25 g sugar
Salty	The taste stimulated by sodium chloride, and in part by other salts, such as potassium chloride	500 ml water, 2.2 g NaCl
Sour	The taste stimulated by acids, such as citric or malic	500 ml water, 0.375g citric acid

[1]Scale for all terms ranges from 0 (none) to 20 (intense)

Table 5.3 Continued

Descriptive Term[1]	Definition	Reference
Bitter	The taste stimulated by substances such as quinine, caffeine, and hop bitters	500 ml water, 0.285g caffeine
Umami	Oral sensation stimulated by monosodium glutamate (MSG)	500 ml water, 5 g MSG
Sensations		
Astringent	Chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as rough or dry and associated with tannins and alum	1.25 g alum in 500 ml water
Pungent	A sharp, irritating and penetrating sensation in the nasal cavity	Vinegar
Numbing	Numbing or loss of sensation in the mouth. May appear as an aftertaste	5% clove in room temperature spring water
In-Hand Texture		
Firmness	The force needed to compress a cheese cube when squeezed between thumb and index finger	Reference cheese (Cracker Barrel Natural Sharp Cheddar Cheese) is a 15
Springiness	The rate at which a slightly compressed (5-20%) cheese cube returns to its original shape	Reference cheese is a 5
Stickiness	The degree to which a sample held between thumb and two fingers sticks to the index finger as it is removed from the sample	Reference cheese is a 3
Cohesiveness	The degree to which a sample holds together after pressing and rolling it between thumb and two fingers	Reference cheese is a 13
Slipperiness	The extent of enhanced lubricating or friction-reducing quality when a sample is rubbed between two fingers	Reference cheese is a 10
First-Bite Texture		
First Bite Firmness	The force required to completely bite through a sample using incisors	Reference cheese is a 5
First Bite Stickiness	The degree to which the sample sticks to incisors during the first bite.	Reference cheese is a 15
First Bite Brittleness	The level of crumbling/breakdown after completely biting through a sample with incisors	Reference cheese is a 2
Five-Chew Texture		
Five Chew Firmness	The extent of resistance offered by the cheese, assessed after five chews using incisors.	Reference cheese is a 7
Five Chew Stickiness	The adhesion or stickiness of the cheese against the palate and around the teeth after five chews.	Reference cheese is a 15
Five Chew Brittleness	The extent of crumbling or breakdown after five chews	Reference cheese is a 2
Five Chew Curdiness	The extent to which a lumpy or curdy texture is perceived in the mouth after five chews	Reference cheese is a 1

5.2.6.5 Data Analysis

General linear model analysis was performed using SAS® PROC GLM ver. 9.1 (SAS Institute, Inc., Carry, NC) to determine whether the samples differed in any of the attributes. Student-Newman-Keuls was used as a summary test (alpha = 0.05).

5.2.7 Consumer Acceptability Test

5.2.7.1 Subjects

The University of Minnesota's Sensory Center recruited 117 judges comprised of students and staff at the University of Minnesota who expressed interest in participating in sensory tests. Panelists were 18 years of age or older, had no food allergies or sensitivities, and consumed Cheddar cheese within the month before testing. They were paid for participating. The University of Minnesota's Institutional Review Board approved all recruiting and experimental procedures.

5.2.7.2 Products

A total of 14 cheeses were included. Each of the seven treatments from one of the cheese make replicates was included. The same seven treatments from one of the cheese make replicates of a similar make procedure were included as well. These cheeses have not been discussed to this point because very few differences existed between the two cheese makes. The cheeses were included in all testing before this was discovered. Therefore, they are included in the consumer acceptability test discussion because the main make procedure scores are in context of panelists rating all 14 cheeses. Panelists received 2 cubes ($1.5cm^3$) of each cheese at room temperature (22°C) in a random three-digit coded

two ounce plastic cup with a lid. Products were refrigerated until one hour prior to serving.

5.2.7.3 Experimental Procedure

Samples were balanced for order and carryover effects. Subjects were asked to take one bite of the sample and rate it for overall liking, liking of flavor, and liking of texture. Liking ratings were made on 120 point labeled affective magnitude scales, with the left end labeled '*greatest imaginable disliking'* and the right end labeled '*greatest imaginable liking'* (Table 5.4). Panelists were then instructed to take a second bite of the sample and rate the intensity of off flavor. This rating was made on a 20 point line scale with the left most end labeled '*none'* and the right most end labeled '*extremely intense*.'

Table 5.4. Reference end labels and point values of the labeled affective magnitude scale

Reference Caption	**Point Value**
Greatest imaginable disliking	0
Dislike extremely	13
Dislike very much	25
Dislike moderately	39.5
Dislike slightly	53
Neutral	60
Like slightly	67
Like moderately	81
Like very much	93
Like extremely	104
Greatest imaginable liking	120

5.2.7.4 Data Analysis

Mixed model analysis was performed using SAS® PROC MIXED ver. 9.1 (SAS Institute, Inc., Carry, NC). A Bonferonni correction was used as a summary test (alpha = 0.05)

5.3 Results and Discussion

5.3.1 Compositional, Chemical, Microbial, and Texture Profile Analysis

Means for all compositional, chemical, microbial, and texture profile analysis (TPA) measurements over time for all treatments are shown in Table A.5 in the Appendix. The water activity of all treatments were not different at all time measurements (alpha = 0.05). With the exception of a small (0.46% average) increase in moisture content ($p < 0.01$), flavor enhancers had no significant effect on the measurements (alpha = 0.05). Therefore, only results for treatments N, K, and MK are discussed. Main and interaction effects of treatment and time on compositional, chemical, microbial, and TPA measurements are shown in Table 5.5. Composition data is shown in Table 5.6. Note: moisture values are time averaged across all weeks.

Moisture content was significantly influenced by treatment ($p < 0.001$). Differences in syneresis between the treatments were observed as evidenced by the varying moisture contents between treatments. The time-averaged moisture contents of treatments N, K, and MK were 38.37, 37.74, and 40.89% respectively. Treatment MK contained more than 39% moisture, which is above the legal maximum to be labeled as Cheddar cheese in the United States; it also contained more moisture than treatments N and K ($p < 0.001$). Correspondingly, the percent fat and protein in treatment MK was

Table 5.5. Main and interaction effects of treatment (Treat.) and time on compositional, chemical, microbial and texture profile analysis (TPA) measurements of low sodium Cheddar-style cheese

Measurement	Treat.	Time	Treat.*Time
Moisture	*	NS	NS
pH	*	*	*
Water activity	NS	*	NS
WSN[1]	*	*	NS
LAB[2]	NS	*	NS
Fat	*	#	#
Protein	*	#	#
Total ash	NS	#	#
Sodium	*	#	#
Potassium	*	#	#
Calcium	NS	#	#
Magnesium	NS	#	#
TPA[3]			
Hardness	NS	NS	NS
Springiness	*	NS	NS
Cohesiveness	NS	NS	NS
Adhesiveness	*	*	NS
Resilience	*	NS	NS
Chewiness	*	NS	NS

*Significant effect ($p < 0.05$)
NS: not significant
#Not applicable
[1]WSN: Water soluble nitrogen
[2]LAB: Lactic acid bacteria
[3]Only full sodium control and low sodium cheese with added potassium chloride (KCl) were compared. Low sodium cheese with added modified KCl was too soft to sample.

Table 5.6. Composition data of Cheddar-style cheese. Moisture values are time-averaged across all measurements within each treatment

	Treatment[1]				
	Full Sodium[2]	Low Sodium[3]			
Measurement	N	K	MK	SE[4]	p
Moisture, %	38.37[b]	37.74[c]	40.89[a]	0.31	<0.001
Fat, %	33.0[ab]	33.1[ab]	31.5[c]	0.3	<0.001
Protein, %	24.73[a]	24.69[a]	23.36[b]	0.16	<0.01
Total Ash, %	3.93[ab]	3.83[bc]	3.78[c]	0.09	0.02
Na, mg/100g	612.5[a]	212.2[b]	238.9[b]	11.2	<0.001
K, mg/100g	83.7[b]	674.2[a]	656.0[a]	29.1	<0.001
Ca, mg/100g	232.5	240.7	233.3	13.8	0.56
Mg, mg/100g	8.6	8.8	9.3	0.4	0.58

[1]Treatments: N = full sodium control (sodium chloride); K = sodium chloride + potassium chloride; MK = sodium chloride + modified potassium chloride
[2] 640 mg sodium/100 g cheese target
[3]220 mg sodium/ 100 g cheese target
[4]Largest standard error of all treatments is shown for each measurement
[a-d]Means without a common superscript letter within the same row are significantly different ($p < 0.05$).

lower ($p < 0.01$). Treatment K produced cheese with lower moisture than treatments N and MK ($p < 0.001$). However, the difference in moisture between treatments N and K (0.63%) was not large. Previous studies have also shown little effect of KCl on the moisture content of cheese. In previous studies on reduced sodium Cheddar cheese, the moisture content of cheese with varying ratios of NaCl and KCl has ranged from 0.8% lower to 0.6% higher than full sodium control with the majority between ± 0.2% moisture from control (Lindsay et al., 1982; Fitzgerald and Buckley, 1985; Reddy and Marth, 1995). Similar results have been observed in other cheeses as well such as kefalograviera (a hard sheep's milk cheese) (Katsiari et al., 1998) and feta (Aly, 1995; Katsiari et al., 1997).

The pH of the control was higher than the low sodium cheeses, which were not different from one another ($p < 0.01$) (Figure 5.2). Treatment N decreased in pH between weeks 2 and 4, increased between weeks 4 and 8, and again decreased between weeks 8 and 12. The initial decrease is likely due to residual lactose in the curd as a result of the starter culture fermenting less lactose initially than the low sodium treatments. The subsequent increase and decrease in pH are probably due to exposed amine groups due to proteolysis (Fox, 1989) and the buffering capacity of cheese (Upreti and Metzger 2007) respectively. The buffering effect could explain the convergence in pH at the end of the aging period. The pH of treatments K and MK did not significantly increase or decrease over time (alpha = 0.05). Treatment MK consistently had the lowest pH values, but they were not significantly lower than treatment K. The data indicate that KCl inhibits the starter culture at salting to a lesser extent than NaCl and that microbial activity is affected differently by KCl and NaCl after cheese making

The lower pH in cheese with KCl is supported by Reddy and Marth (1995) who found the pH to be lower by 0.04 to 0.07 units in 3 d Cheddar with various NaCl/KCl mixtures. Koenig and Marth (1982) observed a similar difference in Cheddar through 6 weeks of age. In addition, they saw that after 6 weeks, the pH values converged which is similar to that of this study after 8 weeks. The differences observed by Koenig and Marth (1982) were not as large as the differences observed in this study (0.12 to 0.35). Lindsay et al. (1982) and Fitzgerald and Buckley (1985) do not support the lower inhibitory effect of KCl on the starter culture. They found that Cheddar cheese with various NaCl/KCl mixtures resulted in a similar or higher pH than control (range of +0.01 to +0.16).

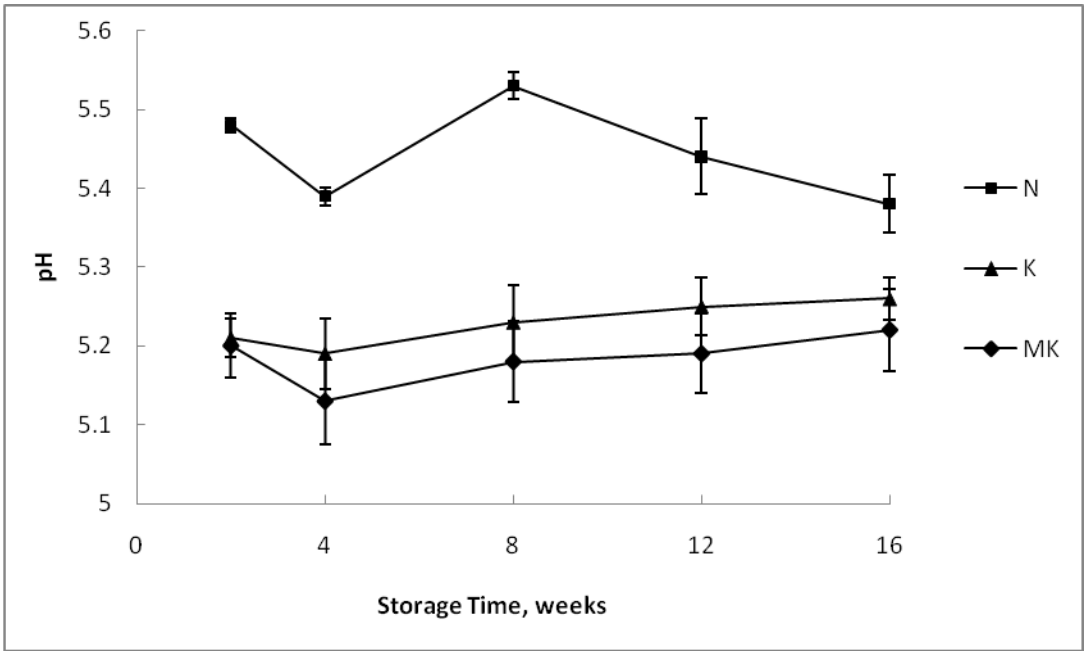

Figure 5.2. Changes in pH through 16 weeks of aging. Treatments: N = full sodium control (640 mg sodium/100 g cheese target); K = sodium chloride + potassium chloride (220 mg sodium/ 100 g cheese target); MK = sodium chloride + modified potassium chloride (220 mg sodium/ 100 g target). Error bars are ± standard errors.

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Proteolysis, as indicated by WSN, during aging was significantly influenced by treatment and time ($p < 0.05$) (Figure 5.3). The amount of WSN increased through the aging period for all treatments ($p < 0.001$). WSN was higher in treatment MK than N and K ($p < 0.01$). The higher moisture content in treatment MK could have contributed to the increased WSN despite the a_w being the same. The WSN data indicate that the proteolytic activity in low sodium Cheddar-style cheese with KCl was not different from full sodium control while the activity in low sodium Cheddar-style cheese with modified KCl was different than control. The increase in WSN over time and no difference in proteolysis as a result of KCl are supported by others (Aly, 1995; Reddy and Marth, 1995; Laborda and Rubiolo, 1999; Ayyash and Shah, 2011; Katsiari, 2011). However, Fitzgerald and

Buckley (1985) reported varied effects of KCl. They observed an average decrease in WSN of 2.9% and an average increase of 1.3% over 16 weeks when KCl only (equivalent ionic strength to control) and NaCl/KCl (1:1 weight ratio), respectively, were used.

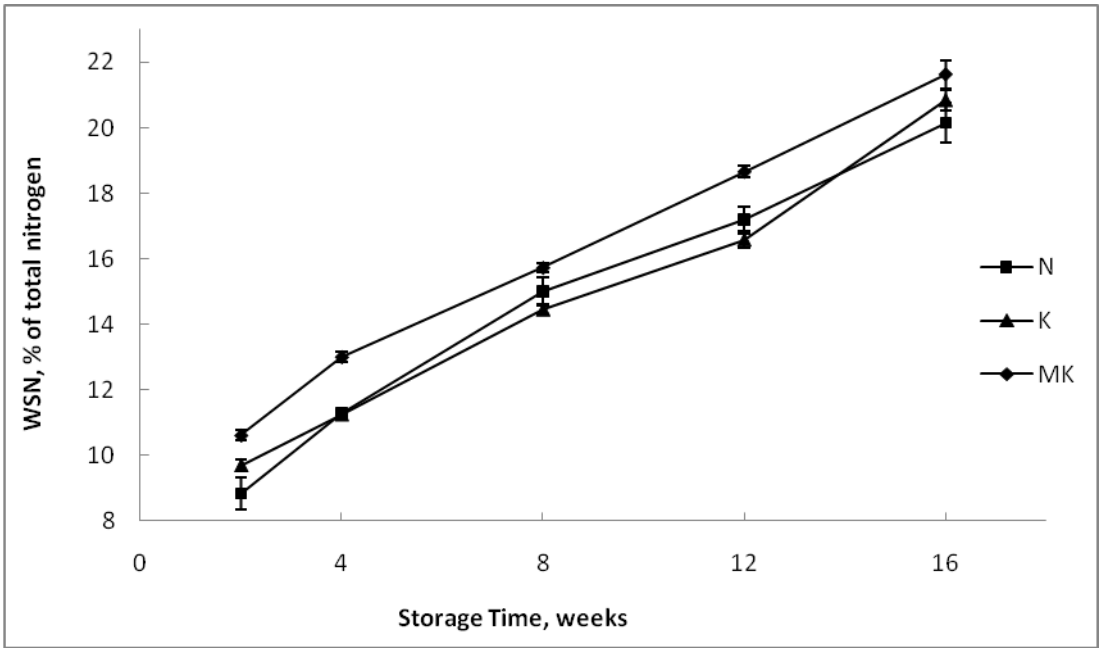


Figure 5.3. Changes in water soluble nitrogen (WSN) through 16 weeks of aging. Treatments: N = full sodium control (640 mg sodium/100 g cheese target); K = sodium chloride + potassium chloride (220 mg sodium/ 100 g cheese target); MK = sodium chloride + modified potassium chloride (220 mg sodium/ 100 g target). Error bars are ± standard errors.

The lactic acid bacteria counts in this study do not support attributing the difference in proteolysis in treatment MK to the activity of starter and non-starter LAB as the counts were not influenced by treatment (alpha = 0.05). However, aerobic incubation

may not facilitate enumeration all the types of LAB that could contribute to proteolysis so a definite conclusion cannot be reached. Reddy and Marth (1995) also observed no difference between LAB counts of Cheddar cheese treatments salted with various NaCl/KCl mixtures through 36 weeks of aging, despite a slightly lower pH in cheeses with KCl (0.04-0.06) at 3 days of age.

Texture was affect by the use of NaCl, KCl, and modified KCl. The texture of cheese with modified KCl was such that samples for measurement by TPA were not obtained because the cheese was too soft to remove samples effectively from the cork borer. Only measurements of treatments N and K through 12 weeks of age were analyzed because the edges of samples of treatment K at 16 weeks fractured upon removal from the cork borer. With the exception of adhesiveness, no TPA parameters were influenced by time (alpha = 0.05). Treatment N was springier, more resilient, chewier, and less adhesive than treatment K ($p \leq 0.02$). Adhesiveness increased through 12 weeks of age for both treatments ($p = 0.02$). Hardness and cohesiveness were not different between treatments (alpha = 0.05), and this is in accordance with Fitzgerald and Buckley (1985), Katsiari et al. (1997), and Katsiari (1998) who found no significant difference in textural properties due to KCl in Cheddar, feta, and kefalograviera cheeses respectively. The observed texture differences in this study are not supported by these studies.

5.3.2 Descriptive Sensory Analysis

Means for monthly descriptive analysis measurements are shown in Table A.6 in the Appendix; only attributes with a significant effect of treatment or time are shown. Main effects of treatment and time on said measurements are shown in Table 5.7. No interactions between treatment and time were significant (alpha = 0.05).

Table 5.7. Main effects of treatment and time on monthly descriptive analysis measurements of Cheddar-style cheese. No interactions between treatment and time were significant (alpha = 0.05)

Attribute	Treatment	Time
Aroma		
Overall	NS	*
Sulfur	NS	*
Sour dairy	NS	*
Taste		
Salty	NS	*
Sour	*	*
Bitter	*	*
Umami	*	NS
Flavor		
Overall	*	*
Diacetyl	NS	*
Cooked	NS	*
Milky	NS	*
Sulfur	*	*
Brothy	*	*
Sour dairy	*	*
In-Hand Texture		
Firmness	*	NS
Springiness	NS	*
Cohesiveness	*	NS
First-Bite Texture		
Firmness	*	*
Stickiness	*	*
Brittleness	*	*
Five-Chew Texture		
Firmness	*	*
Stickiness	*	*
Brittleness	*	*
Curdiness	*	*

*Significant effect ($p < 0.05$)
NS: not significant

The use of KCl and modified KCl did not affect the salty perception of the cheeses as saltiness was not different between treatments (alpha = 0.05). This is contrast with Lindsay et al. (1982) who found that saltiness was lower in cheese with a combined 1.5% salt consisting of NaCl and KCl on a 1:1 molar ratio at 3, 6, and 9 months of age compared to cheese with 1.5% NaCl. Bitterness was low in all cheeses; all mean scores were equal to or less than 1.5 on the 20 point scale. However, the bitterness of control cheese was lower than all low sodium cheeses, which were not different from one another ($p = 0.02$). Other studies have also shown cheese with KCl to be more bitter than full sodium control (Fitzgerald and Buckley, 1985; Lindsay et al., 1982; Katsiari et al., 1997; Katsiari et al., 1998). Sour taste was lower in control cheese than all low sodium cheeses ($p < 0.001$). This is likely a result of a higher pH in control cheese because pH and sour were negatively correlated ($r = -0.994$, $p < 0.001$). The use of flavor enhancers did not affect the five basic tastes of the cheeses with the exception of umami, which was higher in treatment I than the other treatments ($p < 0.001$).

Like umami, brothy flavor was also higher in treatment I than the other treatments ($p < 0.001$). The finding of similar brothy flavor intensity between treatments N and HY does not agree with the findings of Shakeel-Ur-Rehman et al. (2003) who found the intensity of reduced fat Cheddar with yeast extract to have a lower brothy flavor. KCl, modified, KCl, and flavor enhancers enhanced the overall flavor intensity as the rating for control cheese was lower than the other treatments ($p < 0.01$). This is in contrast with Lindsay et al. (1982) who reported panelists commented on a less full flavor in cheese containing KCl. Shakeel-ur-Rehman et al. (2008) found salty taste to be higher in stirred-curd Cheddar than in milled-curd Cheddar so this could explain the difference between

the present study and the study of Lindsay, which used milled-curd Cheddar. Sulfur flavor was also lower in control than the other treatments ($p < 0.001$) and increased in all treatments through the 16 weeks of aging ($p < 0.001$), although control decreased between weeks 4 and 8 before increasing (Figure 5.4). This is additional evidence that NaCl and KCl affect microbial activity differently considering sulfur containing flavor compounds can be produced by starter and non-starter bacteria during aging (Berger et al., 1999). Sour dairy flavor was lower in control cheese than the other treatments with the exception of treatment I which was not different from any treatment ($p < 0.01$). The lower sour dairy flavor in control cheese is likely a result of a higher pH. The lower sour dairy flavor in treatment I could be due to a masking effect from the higher brothy flavor and umami intensity.

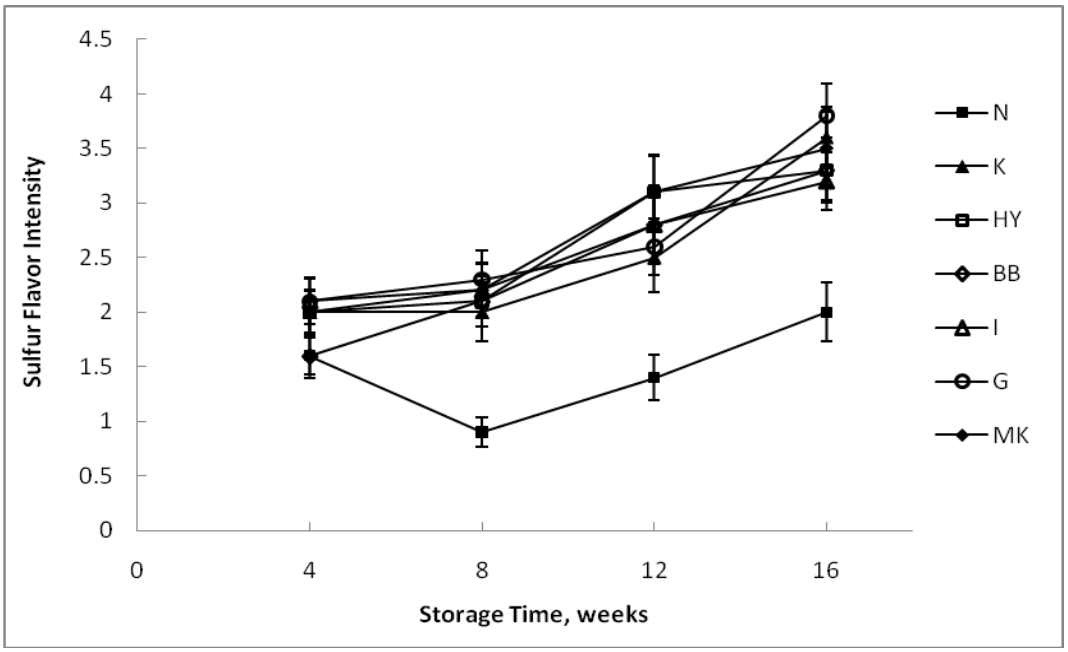


Figure 5.4. Changes in sulfur flavor intensity through 16 weeks of aging. The scale ranged from 'none' (0) to 'intense' (20). Treatments: N = full sodium control (640 mg sodium/100 g cheese target); K = sodium chloride + potassium chloride (220 mg sodium/ 100 g cheese target); MK = sodium chloride + modified potassium chloride (220 mg sodium/ 100 g target). Error bars are ± standard errors.

Descriptive sensory texture evaluation supports the observation that the texture of cheese with modified KCl was different than with NaCl and KCl. In-hand, first-bite, and five-chew firmness were lower in treatment MK than the other treatments ($p < 0.001$). The same was true of in-hand cohesiveness and first-bite stickiness ($p < 0.001$). The softer texture in treatment MK agrees with the higher degree of proteolysis which is considered to result in the softening of Cheddar cheese early on in aging (Creamer and Olson, 1982). However, the moisture content of treatment MK was higher than the legal maximum for Cheddar, and subsequently out of range of typical Cheddar cheese. This would also lead to a softer texture cheese, one more similar to Monterey Jack which has an average moisture content of 41% and a legal maximum of 44% moisture (USDA nutrient database, CFR 133.153)

Texture differences also occurred as a result of all three of the salt or salt replacers used. The addition of flavor enhancers did not result in texture differences ($p < 0.05$). First-bite brittleness was lower in treatment MK than all other treatments ($p < 0.001$) and was numerically highest in N. Additionally, the ratings for five-chew brittleness and curdiness were higher for treatment N than all other cheeses ($p < 0.01$). The reverse was true for five-chew stickiness in which N had the lowest rating ($p < 0.001$). Overall, the texture of cheeses with NaCl and KCl were similar. The same result was found by others (Fitzgerald and Buckley, 1985; Lindsay et al., 1982; Katsiari et al., 1997; Katsiari et al., 1998). The described texture differences provide additional evidence of the difference between the microbial and enzymatic activity in the cheeses as a result of the salt or salt replacer used because microbial and enzymatic activity impact

biochemical reactions that contribute to texture changes during aging (Lawrence et al., 1993).

No aroma differences occurred between treatments, indicating that salt, KCL, and flavor enhancers do not contribute to the aroma of Cheddar cheese.

5.3.3 Consumer Acceptability Test

Mean scores from the consumer acceptability test are shown in Table 5.8. Make procedure B was an additional cheese making procedure that has thus far not been discussed. It was included in Table 8 because the consumers evaluated all 14 cheeses (7 from make procedure A and 7 from make procedure B) at the same time. Therefore, the two make procedures could not be separated for statistical analysis due to a context effect. However, only results from the main make procedure (A) are highlighted hereafter.

Consumer acceptability of the low sodium cheeses was very high. The control cheese, N, scored the highest in overall liking, but was only rated significantly higher than treatment I ($p < 0.001$), likely due to the higher umami and brothy flavor intensity in I. Treatments HY, G, and MK scored slightly lower than treatment K while treatment BB scored slightly higher. It is not surprising to note that the flavor liking differences between the treatments were similar to overall liking because cheese acceptance and quality is highly driven by flavor (Young et al., 2004). Treatment I was least liked in terms of texture and had the highest off flavor ($p < 0.001$). The unfavorable ratings (lower overall, flavor, and texture likings and higher off flavor in treatment I) appear to be driven by the higher umami and brothy flavor intensity; the two intensities were negatively correlated with overall and flavor liking ($r \leq -0.848$, $p = 0.03$) and positively

correlated to off flavor rating ($r \geq 0.887$, $p \leq 0.02$). On the contrary, it appears that the favorable likings of the cheeses were not driven by any measured parameters (sensory or other) as no other correlations were significant (alpha = 0.05).

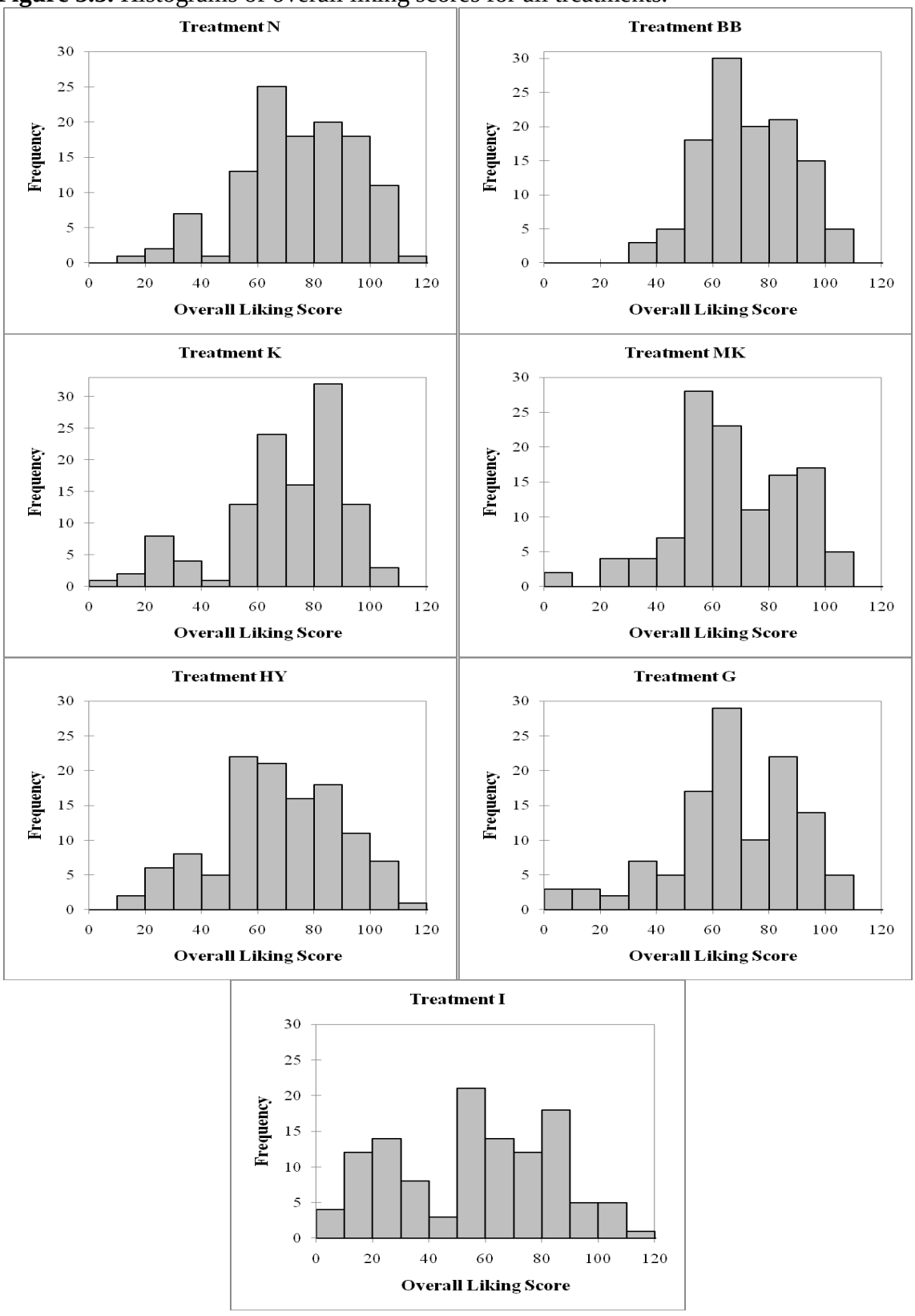
It appears that treatment BB may have masked an unpleasant characteristic in the cheese. Figure 5.5 shows the distribution of the overall liking scores for each treatment, and treatment BB does not have a grouping of scores lower than the main distribution as in treatments N and K.

Table 5.8. Mean scores from the consumer acceptability test

		Attribute			
Make Procedure	Treatment[1]	Overall Liking	Flavor Liking	Texture Liking	Off Flavor
A	N	74.2[a]	73.7[a]	71.7[a]	4.3[d]
	BB	71.7[ab]	70.2[ab]	74.1[a]	4.3[d]
	K	69.2[abc]	68.3[ab]	72.2[ab]	5.5[cd]
	MK	67.7[abc]	66.4[abc]	68.5[ab]	4.7[cd]
	HY	67.2[abc]	64.9[bc]	72.3[ab]	5.3[cd]
	G	67.0[abc]	65.6[abc]	71.6[ab]	6.1[bc]
	I	56.0[d]	52.3[d]	67.3[b]	9.1[a]
B	BB	72.8[ab]	71.3[ab]	73.6[a]	4.6[cd]
	HY	72.3[ab]	71.3[ab]	74.3[a]	5.0[cd]
	G	69.0[abc]	66.6[abc]	73.7[a]	5.3[cd]
	MK	68.7[abc]	66.8[abc]	74.0[a]	5.1[cd]
	K	68.5[abc]	66.8[abc]	74.3[a]	5.9[bcd]
	N	66.3[bc]	64.7[bc]	72.6[ab]	5.6[cd]
	I	61.9[c]	60.1[c]	70.8[ab]	7.1[b]
	SE[2]	2.6	2.7	2.1	0.6
	p	<.001	<.001	<.001	<.001

[1]Treatments: N = full sodium control (640 mg sodium/100 g cheese target); K = sodium chloride + potassium chloride (220 mg sodium/ 100 g cheese target); HY = K + hydrolyzed vegetable protein/yeast extract blend; BB = K + natural potassium blocker type flavor; I = K + disodium 5' inosinate; G = K + disodium guanylate; MK = sodium chloride + modified potassium chloride (220 mg sodium/ 100 g target)
[2]Largest standard error for each attribute is shown
[a-]Means without a common superscript letter within the same column are significantly different ($p < 0.05$)

Figure 5.5. Histograms of overall liking scores for all treatments.



5.4 Conclusions

Low sodium Cheddar-style cheese with KCl or modified KCl can be made that results in high consumer acceptance, low bitterness, and an equivalent saltiness to control when the a_w of full sodium control is maintained. The addition of a natural flavor (BB) to low sodium Cheddar-style cheese with KCl resulted in a higher overall, flavor, and texture liking score plus a numerically lower off flavor rating in the consumer acceptance test, which indicates that flavor enhancers may be a beneficial tool in increasing consumer acceptance of low sodium cheese. However, screening of flavor enhancers and their levels is advised as the addition of HY, G, and particularly I resulted in a lower overall, flavor, and texture liking score. Treatment I also resulted in a higher off flavor rating. Consumer acceptance of the low sodium cheese did not appear to be driven by the vast majority of differences described by the descriptive sensory panel or physical and chemical data.

The mechanism in which flavor enhancers influence consumer acceptability should be explored because the descriptive sensory analysis data in this study do not support the hypothesis of flavor enhancers reducing negative sensory attributes associated with the use of KCl. In fact, the opposite was true for disodium 5' inosinate. The sensory attributes of low sodium treatments with KCl and flavor enhancers (HY, BB, I, G) were not different (alpha = 0.05) than the low sodium treatment with only KCl (K) with the following exception: the use of disodium 5' inosinate resulted in high umami and brothy flavor intensities that appear to have reduced consumer acceptability. Despite the lack of identified differences due to flavor enhancers, the flavor enhancers slightly affected the consumer acceptance scores. While the vast majority of these effects were not

statistically significant, excluding those from treatment I, they do suggest the possibility of altering the consumer acceptance of low sodium cheese. The distribution of scores supports this.

Flavor enhancers had minimal effect on the biochemical, microbial, and physical reactions in low sodium Cheddar-style cheese, as hypothesized. Only moisture content was altered due to the flavor enhancers when compared to treatment K (no flavor enhancers). However, the difference resulted in moisture being no different from control and would therefore not require a change in the cheese making procedure to achieve a desired moisture target. A slight adjustment may be needed if only KCl were used because treatment K had a lower moisture content than control.

Flavor, taste, and texture differences resulted from the use of NaCl, KCl, and modified KCl. This refutes the hypothesis that maintaining a_w between treatments will result in similar biochemical and microbiological reactions during aging. The lower pH in treatments K and MK compared to treatment N indicate that the starter culture is less inhibited by both types of KCl tested than NaCl. Also, sulfur flavor, a result of starter and non-starter bacteria activity, and native and non-native enzymes, was lower in treatment N which indicates the rate of reactions leading to the production of sulfur containing flavor compounds increased as a result of both types of KCl tested. Additionally, the observed texture differences between cheeses with NaCl and KCl compared to modified KCl, as described by descriptive sensory analysis, may be due to the differences in proteolysis and moisture content.

CHAPTER 6: Concluding Remarks

This body of research provides a significant tool for the advancement of creating reduced sodium cheese. The objective of this research was to investigate improvement of the flavor of low sodium Cheddar cheese over what has been previously reported through the use of mineral salt replacers and flavor enhancers. Through a series of screening and testing potentially useful salt replacers and flavor enhancers, formulas were developed that were extensively analyzed throughout aging by descriptive sensory and chemical means, and the work culminated in a consumer sensory panel. Maintaining the a_w between treatments was the basis for how experimental cheese was produced. It was hypothesized that maintaining a_w would simulate the S/M of full sodium control, thereby eliminating differences in biochemical reactions during aging and allowing for a better evaluation of salt replacers and flavor enhancers in cheese.

Initially, the concentrations of salt replacers that would result in equivalent a_w were determined. The concentrations were then used in the production of reduced sodium Cheddar-style cheese to test and screen the replacers in regard to flavor, texture, and chemical properties. KCl and modified KCl were shown to be most similar to full sodium control and were used in subsequent testing. Flavor enhancers were screened to determine which were potentially useful in Cheddar cheese. Low sodium Cheddar-style cheese with KCl or with modified KCl was made, including treatments with KCl that included the most promising flavor enhancers. Chemical and sensory testing was performed to monitor the cheeses through four months of aging, to determine the effect of the flavor enhancers, and to determine the consumer acceptability of the cheese.

Maintaining the same a_w as full sodium control in experimental treatments proved to be an effective technique in reducing the sodium content of Cheddar cheese. While the

hypothesis of equivalent biochemical reactions was refuted, low sodium Cheddar-style cheese with KCl or modified KCl was produced with high consumer acceptance, low bitterness, and equal saltiness to full sodium control. As in this research, others (Lindsay et al., 1982; Fitzgerald and Buckley, 1985; Reddy and Marth, 1994) have observed positive results when using KCl to reduce the salt content in Cheddar cheese. However, the favorable ratings in the other studies were only observed when the sodium content was higher and the potassium content was lower than in the present research (Chapter 5). Considering the relatively low NaCl and relatively high KCl contents in the current research, the indication is that maintaining equivalent a_w between full and low sodium treatments may have contributed to the high consumer acceptance and low bitterness observed in this research. Another factor that could have contributed to the high acceptance and low bitterness in this research is the type of KCl (modified or not) used as the type of KCl was shown to have effects on chemical and sensory attributes (Chapter 5). Because equivalent chemical and textural indications of biochemical reactions were not observed between all treatments, it would be useful for future researchers conduct similar research, but adjust the salt replacer concentrations or cheese making procedure in order to achieve similar cheese chemistry. This would allow for a deeper understanding of the effects of salt replacers on cheese quality.

Some flavor enhancers may improve the acceptability of low sodium Cheddar-style cheese while others may worsen it (Chapter 5). Therefore, future research should explore a wider range of flavor enhancers to understand which ones could further improve the acceptability. However, the flavor enhancers should be screened prior to use in sensory and chemical studies because highly objectionable samples may alter the

perception of the other samples. This research provides such a method to screen flavor enhancers.

Future research should also examine the safety of low sodium cheese made in similar fashion to this study. Both Chapter 3 and Chapter 5 demonstrated that the pH of reduced and low sodium treatments was generally lower than full sodium control. This indicates that the starter culture was less inhibited at salting by salt replacers compared to NaCl. While the growth of most spoilage and pathogenic microorganisms would be more inhibited at a lower pH, a cheese maker would likely adjust the cheese making procedure such that the pH of low sodium cheese matches that of the current full sodium cheese in production. Because the starter culture was less inhibited by salt replacers, it is possible that spoilage and pathogenic microorganisms would also be less inhibited and pose a significant threat to the safety of the cheese.

The present research was conducted in Cheddar-style cheese at one moisture level and, correspondingly, one S/M. Therefore, another area of future research should be to study the effect of applying the methods of the current research to cheese at various moisture levels due to the extensive effects of the S/M on cheese.

The methods of this research could also be applied to other dry-salted cheeses to further advance the reduction of sodium in cheese. In addition, low sodium cheese as a result of such research and the current research could potentially be useful for reducing sodium in other cheese products such as process cheese and cheese spreads.

In conclusion, this body of research significantly advances the subject of sodium reduction in cheese and provides insight into future research on the topic.

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Appendix

A.1 Extended Methods

This section is intended to provide more detail of the methods used in the research. Standard Methods for the Examination of Dairy Products (SMEDP) was an outgrowth from a committee appointed by the American Public Health Association in 1905 in order to standardize the bacteriological examination of milk (Richardson, 1985). Since 1905, numerous editions have been published as new methods were established and old methods were updated. Unless otherwise noted, discussion of SMEDP in this text refers to the 15th edition, published in 1985, as the majority of the compositional or chemical measurements were performed according to this edition. SMEDP does not provide a complete listing of all methods in the dairy field, but is intended to meet the needs of typical dairy laboratories (Richardson, 1985). SMEDP classifies the methods to demonstrate the relative weight or importance. Table A.1 describes the classes.

A.1.1 Fat Content

The Babcock method has been refined through many decades of use to increase its accuracy and applicability (Richardson, 1985). Consequently it has a class O rating in SMEDP. The Gerber, Roese-Gottlieb, and Mojonnier methods are also class O methods. A relatively recent class A1 method is measurement by automated turbidity in which light scattering due to fat globules is measured by a piece of equipment. The Babcock method was selected due to its ease of use and established validity. Also, the Gerber method is not applicable to cheese (Richardson, 1985), and the other methods involve

Table A.1. Descriptions of the classes designated in Standard Methods for the Examination of Dairy Products (SMEDP)

Class	Description[1]
O*	A method or procedure that has been subjected to a thorough evaluation, has been widely used, has thereby demonstrated its value by extensive application, but may not have been formally, collaboratively tested. This classification will include methods that are referred to as standard methods in current APHA (the American Public Health Association) publications; essentially it is a "grandfather clause."
A1*	A method or procedure that has been subjected to a thorough evaluation, has demonstrated its application for a specific purpose on the basis of extensive use, and has been successfully, collaboratively tested
A2*	A method or procedure that has been subjected to a thorough evaluation and has been successfully studied collaboratively
B	A method that has been used successfully in research or other situations, has been devised or modified explicitly for routine examination of samples, has had limited evaluation, and has not been tested collaboratively.
C	An unproved or suggested method not previously used but one that has been proposed by recognized laboratory workers as useful and gives promise of being suitable
D	A method that previously has been placed in Classes O, A, or B but which, through technological advances or significant change in numerical level of acceptable exposure or other circumstances, is being superseded by a method of higher classification. A Class D method may be removed from subsequent editions of SMEDP

[1]Directly quoted from Richardson (1985)
*Class indicates a standard method

potentially harmful solvents or equipment unavailable for use. The Babcock procedure, performed in duplicate (at minimum), was as follows (Richardson, 1985):

1. Accurately weight 9 g of finely grated cheese into a cream or Paley test bottle.

2. Add ~10 ml of 60°C lab grade (LG) water to the bottle and mix thoroughly to suspend the cheese. Cool to 22 ± 1°C.
3. Hold the bottle at a slight angle and add 15 ml of Babcock acid (sulfuric acid, specific gravity 20/20°C 1.825) portion wise in ~8, 4, and 3 ml additions. Addition of acid should take less than 20 s. Swirl vigorously until all chunks disappear, but take caution against chunks entering the neck of the bottle. Shake on mechanical mixer for at least 5 min.
4. Centrifuge (with counterbalancing) for 5 min in Babcock centrifuge then add 60°C LG water until the volume is within 0.6 cm of the neck. Centrifuge for 2 min then add 60°C LG water until the volume approaches the top graduation mark. Centrifuge for 1 min.
5. Place bottles in 55-60°C water bath (preferably 57°C) such that the water level is above the fat column. Temper for at least 5 min then remove bottle and quickly measure the fat content using calipers from the bottom of the lower meniscus to the top of the upper meniscus. For full fat Cheddar, readings are to the nearest 0.5%. Reject fat columns with a cloudy/milky appearance or if there is evidence of charring.

A.1.2 Total Ash

The ashing procedure in SMEDP is a class O method. SMEDP does not include any other procedure beside the dry ashing method, which was as follows and performed in duplicate:

Crucible preparation:

1. Heat the bottom of each ashing crucible over a Bunsen burner and label with an identifying mark (numbers) using a ceramic pen. Allow ink to dry then heat the crucible until first sign of the bottom glowing red. Cool to 22°C.
2. Cautiously submerge the crucibles into aqua regia (3 parts concentrated HCl, 1 part concentrated HNO_3, 4 parts LG water). Minimize splashing: aqua regia is very corrosive and dangerous! After 3 hr (minimum), remove crucibles, rinse with tap water then LG water, and dry in a forced draft oven at 100°C for 1 hr. Place crucibles in a desicator until ready for use.

Note: step 2 must be performed between measurements. Step 1 is only needed if the marking is removed from the crucible or becomes illegible.

Ashing procedure:

1. Accurately record the mass of the empty crucible, but do not tare the scale. Accurately weigh ~2 g of finely grated cheese into the preweighed, labeled crucible. Include two blank crucibles.
2. Heat crucibles at 100°C for 1 hr in an atmospheric oven. Place crucibles in a desicator, and record the location as the marking may disappear in subsequent steps.
3. Flame each crucible over a Bunsen burner to carbonize the sample. Remove the crucible from the flame if fat spatters (preferably before) to prevent losses. The fat will eventually ignite – do not keep the flame under the crucible once this occurs. Return the flame to the crucible once fat is extinguished, and repeat until the sample is completely carbonized (black color). Return crucibles to their original

location. Carbonization should take approximately min per sample. Do not allow sample to carbonize long enough to turn white.

4. Place crucibles in the muffle furnace, again recording the location, and incinerate the samples at 540-550°C for ~12 hr. Turn the furnace off and allow it to cool for at least 8 hr.
5. Cautiously open the furnace and remove samples. Place samples back in the desicator in their original location (use a desicator with a porcelain stand as samples will still be very hot.)
6. Cool crucibles for 1 hr or until 22°C then accurately weigh them.
7. Calculation:

$$\%\text{Total Ash} = \frac{Weight\ of\ residue\ x\ 100}{weight\ of\ sample}$$

$$= \frac{(Weight\ of\ crucible\ after\ ashing - weight\ of\ empty\ crucible - blank)\ x\ 100}{weight\ of\ crucible\ plus\ cheese - weight\ of\ empty\ crucible}$$

Note: If the blank crucible after ashing weighs less than the initial weight, the numerator will increase

A.1.3 Moisture Content

A vacuum oven, atmospheric oven, microwave oven, moisture balance, refractometer, and lactometric method are included in SMEDP, and are classes O, O, A1 (for Cheddar), B, B, and O, respectively. The standard vacuum oven method was selected due to the equipment available in the laboratory and its relative short drying time compared to atmospheric oven drying. The method from the 17th edition of SMEDP (Wehr and Frank, 2004) was used as it provided a more appropriate description of the incoming air flow. The method was as follows and performed in duplicate:

1. Dry labeled aluminum moisture pans in a forced draft oven at 100°C for at least 3 hr and place them in a desicator until ready for use. Include two blank pans. Do not touch pans with hands, even if wearing gloves – always use clean tongs.
2. Accurately record the mass of the empty pan, but do not tare the scale. Accurately weigh ~2.5 g of finely grated cheese into the labeled pan. Once weighed, shake the pan to evenly distribute the cheese across the bottom. Place the pan back on the scale to ensure no loss occurred. Place a sheet of aluminum foil over prepared pans while weighing other samples to limit moisture loss.
3. Place pans on a metal shelf in the vacuum oven at 100 ± 1°C. The temperature should be measured by a thermometer in contact with the shelf or wall of the oven, not in the open space. Do not place samples on the bottom of the oven. Balance the order of samples throughout the oven to limit the effect of varying heating conditions in the oven cavity.
4. Tightly close the oven door then turn on the vacuum. The vacuum should be 25.5 inches of Hg, at minimum, and incoming airflow (dried air) should be ~117 ml/min.
5. Dry samples for 4.75 ± 0.25 hr then turn off the vacuum and slowly allow dry air to enter the oven. Remove the pans with clean tongs and place them in desicators.
6. Cool samples for 30 min and accurately weigh them. Do not wait excessively long to weigh them as they will equilibrate to one another.
7. Calculation:

$$\% \text{ Moisture} = \frac{loss\ in\ weight\ x\ 100}{weight\ of\ sample}$$

$$= \frac{(weight\ of\ pan\ plus\ cheese - weight\ after\ drying - blank)\ x\ 100}{weight\ of\ pan\ plus\ cheese - weight\ of\ empty\ pan}$$

Note: If the blank pan after drying weighs less than the initial weight, the numerator will increase.

A.1.4 Water Activity

SMEDP does not have a method for measuring a_w. However, a number of methods exist including chilled mirror dew point, electric, and hair/polymer hygrometers as well as thermocouple psychrometers, vapor pressure manometers, an isopiestic method, and a freezing point depression method (Fontana, 2007). Each method has advantages and disadvantages, but chilled mirror dew point hygrometers have been used for decades (Fontana, 2007). This could be because they are fast, accurate, precise, and simple to use (Fontana, 2007). However, their use is hindered if samples contain certain volatile compounds which can interfere with the measurement (Fontana, 2007), but many products can be measured, including Cheddar cheese.

Water activity was measured using a chilled mirror dew point hygrometer (Aqua Lab 3TE a_w meter, Decagon Devices, Pullman, WA). Finley grated cheese was lightly packed into sample cups and the cheese was allowed to equilibrate to 23°C. The sample cup was then placed in the a_w meter for measurement. Two sample cups per cheese were measured in duplicate, at minimum.

A.1.5 pH

The standard method for measuring pH is a class O method. Measurement of pH was described in the methods sections of Chapters 3 and 5. SMEDP does not specify to add

the ~0.2 g LG water, but this was done to ensure solid contact between the electrode and cheese. Measurements were made in duplicate.

A.1.6 Total Protein Content

SMEDP includes the Kjeldahl method of measuring total nitrogen as a class A1 method. Faster methods have been developed, including the automation of the Dumas method which is an official method of AOAC International. One piece of equipment that is based on the Dumas method of combustion is the TruSpec® N (Leco Corporation, St. Joseph, MI), which was used for total protein analysis as mentioned in Chapters 3 and 5. The method was as follows and was performed in triplicate with gelatin capsules:

1. Turn on the gases 30 min prior to use.
2. Perform the "system check" as well as the "Whole O2" and "Whole He" leak checks to ensure the equipment is operating properly.
3. Purge the system with high purity glycine (~0.15 g) twice.
4. Analyze blank capsules until readings stabilize and log in the stable readings to calibrate the equipment.
5. Perform drift correct samples with high purity glycine (~0.1 g) until readings stabilize. Log in the stable readings to properly set the standard curve. Use the appropriate method, and enter the conversion factor of 6.38 for dairy proteins.
6. Accurately weigh ~0.15 g of finely grated cheese into capsules. Load capsules into the equipment for measurement.
7. Turn off gases once measurements are completed.

A.1.7 Water Soluble Nitrogen

Water soluble nitrogen (WSN) is a nonspecific indicating method to measure the extent of proteolysis. A number of nonspecific methods exist including those that involve determining the amount of nitrogen that is soluble in a specific solvent (like WSN) or that is permeable through a specific membrane (Fox et al., 2000b). These measurements can be quantified by various methods. However, the methods to analyze proteolysis are not in SMEDP.

WSN of nitrogen soluble in buffers at pH 4.6 are widely used to measure the extent of proteolysis, and WSN is typically expressed as the percentage of total nitrogen that is soluble in water (Fox et al., 2000e). The method of Kuchroo and Fox (1982) to extract the WSN from cheese has become common (Fitzgerald and Buckley, 1985; Aly 1995; Laborda and Rubiolo, 1999), and was used in this research. One extraction per cheese was performed, and two samples from the extraction were prepared for analysis. Two measurements of each sample were performed on the TruSpec® N. The extraction method was as follows:

1. Mix 50.0 g of cheese and 100.0 g of LG water in an Osterizer 12 Speed Blender (Sunbeam Products, Inc., Boca Raton, FL) at high speed for 2 min.
2. Fill Two 50 ml centrifuge tubes per cheese with the mixture then temper them in a 40°C water bath for 1 hr.
3. Centrifuge tubes in a GS-6R centrifuge (Beckman Coulter, Inc., Brea, CA) at 3500 RPM and 40°C for 30 min.
4. Filter samples through Whatman #4 filter paper (Whatman plc., Maidstone, Kent, UK).

5. Mix the filtrate until homogenous and collect a portion in a labeled 15 ml centrifuge tube. Freeze at -80°C if possible, otherwise -28.9°C until needed.
6. Thaw samples and analyze them on the TruSpec® N in the same manner as in protein analysis, but with the following exceptions
    a. Use tin capsules, and
    b. Perform the drift correct samples with a precisely prepared 0.2% glycine solution.

A.1.8 Mineral Content (Sodium, Potassium, Calcium, and Magnesium)

SMEDP does not contain a method for analyzing mineral content. However, IDF method 119:2007(E) provides methodology for the analysis of sodium, potassium, calcium, and magnesium. Resources were not available to perform the digestions according to the method so the hot plate method (time and temperature) of Kira et al. (2004) was incorporated. The method was as follows and was performed in duplicate:

1. Soak all glassware in aqua regia for at least 6 hr then rinse with distilled, deionized (DD) water to remove residual minerals.
2. Accurately weight ~0.35 g of finely grated cheese into a dry Erlenmeyer flask (up to 125 ml). Include 2 blank flasks
3. Add 10 ml of 10.2*N* trace element free (TEF) HNO3 to each flask and cover with a loose fitting glass stopper. Prepare all TEF acid solutions with trace metal grade HNO_3 and DD water.
4. Digest samples at 100-105°C for 2 hr. Stirring is not necessary, but add acid back to original volume if the level decreases significantly to prevent charring.

5. Quantitatively transfer the contents of the flasks to 50 ml volumetric flasks. Heat the sample to melt the fat if it solidifies. Use 0.1 *1N* TEF HNO_3 to rinse the flasks while transferring
6. Cool samples to 20°C and fill flasks to volume with 20°C 0.*1N* TEF HNO_3.
7. Thoroughly mix samples then heat them in an oven until the fat melts and remove it with a plastic transfer pipet.
8. Accurately dilute samples further in order to produce a sample of appropriate concentration for analysis.
9. Prepare standard solutions of known concentrations by diluting standard mineral solutions with 0.*1N* TEF HNO_3.
10. Analyze standards and samples via atomic absorption spectrometry on a Perkin Elmer AAnalyst 100 (PerkinElmer, Inc., Waltham, MA). Concentrations are determined by using a standard curve to calculate the concentration of a given mineral in the diluted sample and multiplying by the dilution factor used to reach that dilution. Report results as mg/100 g of cheese.

A.1.9 Texture Profile Analysis

TPA methodology was described in Chapters 3 and 5. Slight differences exist between the two chapters because the IDF/RM method 205:2006(E) was unknown to the author at the time of analysis in Chapter 3. Figure A.1 shows a typical curve from TPA measurements.

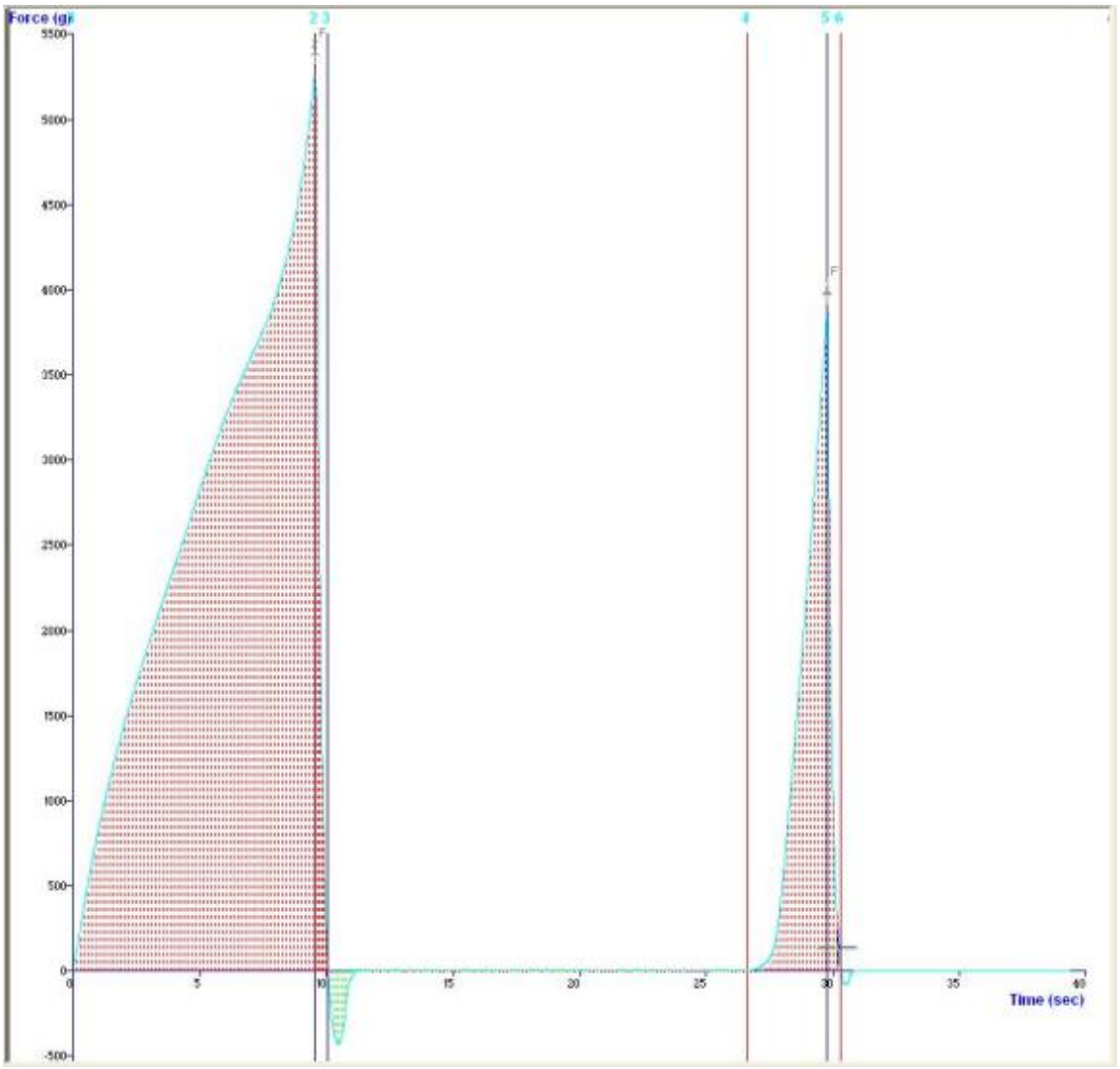

Figure A.1. A typical plot for texture profile analysis measurements. Force (g) is on the y-axis and time (sec) is on the x-axis. Time point (TP) 1 is at 0 s, TP 2 is at the first vertical line right of the y-axis, and TP 3, 4, 5, and 6 are at the subsequent vertical lines.

From Figure A.1:

Area 1 = area under the curve between TP 1 and TP 2

Area 2 = area under the curve between TP 2 and TP 3

Area 3 = area under the curve between TP 3 and TP 4

Area 4 = area under the curve between TP 4 and TP 6

The TPA measurements are calculated in the following ways:

Hardness = the highest recorded force of the left peak

$$\text{Springiness} = \frac{Time\ \ between\ TP\ 4\ and\ TP\ 5}{Time\ between\ TP\ 1\ and\ TP\ 2}$$

$$\text{Cohesiveness} = \frac{Area\ 4}{Area\ 1 + Area\ 2}$$

Adhesiveness = Area 3

$$\text{Resilience} = \frac{Area\ 2}{Area\ 1}$$

Chewiness = Hardness x Springiness x Cohesiveness

A.1.10 Lactic Acid Bacteria Enumeration

The lactic bacteria (LAB) enumeration was based on method 8.071 in the 17th edition of SMEDP (Wehr and Frank, 2004). The method from the 17th edition was used because it was more detailed and current. Similar to WSN, one stomached sample was prepared per cheese from which two samples were taken and prepared for enumeration. All peptone water and media was sterilized. The method was as follows:

1. Add 11.00 ± 0.01 g of cheese to a sterilized stomacher bag then add 99.00 ml of 0.1% peptone water. Stomach contents for 2 min.
2. Pipet 1.00 ml of sample using an automatic pipet into 2 test tubes with 9.00 ml 0.1% peptone water.
3. Prepare serial dilutions down to a dilution factor of 10^{-6} with an automatic pipet using 0.1% peptone water.
4. Pipet 0.1 ml of the serial dilutions, starting with the most dilute, onto Lactobacilli MRS agar (Difco Laboratories, Detroit, MI).
5. Streak the sample evenly across the surface of the plate with a flame-sterilized hockey stick that was allowed to cool.

6. Repeat steps 4 and 5 for the progressively less dilute samples up to the 10^{-4} dilution.
7. Aerobically incubate plates at 32°C for 48 ± 3 hr.
8. Count the plates with 25-250 colonies only after the incubation. Report results as colony forming units/g of cheese.

A.2 Expanded Data Tables

The following data tables are expanded versions of those in Chapters 3 and 5.

Table A.2. Mean values for all attributes in the descriptive sensory analysis at one month of age (Chapter 3)

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Aroma											
Overall	9.0[a]	8.9[a]	8.8[a]	8.8[a]	8.5[a]	8.1[a]	8.8[a]	9.0[a]	8.3[a]	0.4	0.02
Diacetyl	4.7	4.6	5.1	5.0	4.4	4.3	4.7	4.8	4.7	0.5	NS
Cooked	1.1	1.1	1.2	1.2	1.1	1.1	0.8	1.0	1.3	0.2	NS
Metallic	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.0	0.1	NS
Milky	1.7	1.8	1.8	1.9	1.8	1.5	1.9	1.7	1.7	0.3	NS
Whey	3.2	3.5	3.4	3.3	3.2	3.2	3.4	3.6	3.4	0.5	NS
Malty	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS
Earthy	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	NS
Moldy	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	NS
Sulfur	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	NS
Unclean	0.0[a]	0.3[a]	0.0[a]	0.0[a]	0.3[a]	0.2[a]	0.2[a]	0.2[a]	0.4[a]	0.2	0.03
Yeasty	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	NS
Brothy	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.6	0.6	0.2	NS
Pineapple	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	NS
Hydrolytic rancidity	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.2	NS
Fermented	0.0	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.1	NS

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each attribute

[a-e]Means with without a common superscript letter within the same row are significantly different ($p < 0.05$)

NS: No significant differences between treatments (alpha = 0.05)

Table A.2 Continued

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Nutty	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	NS
Sour dairy	2.7^{a}	2.7^{a}	2.4^{ab}	2.7^{a}	2.0^{b}	2.3^{ab}	2.6^{ab}	3.0^{a}	2.5^{ab}	0.4	0.001
Soapy	0.2	0.1	0.3	0.0	0.2	0.2	0.2	0.2	0.3	0.1	NS
Taste											
Sweet	1.1^{a}	0.6^{abc}	0.8^{ab}	0.8^{ab}	0.7^{abc}	0.2^{c}	0.7^{abc}	0.5^{abc}	0.4^{bc}	0.2	<0.001
Salty	3.6^{b}	4.2^{b}	3.8^{b}	4.5^{ab}	3.6^{b}	4.5^{ab}	4.1^{b}	4.5^{ab}	5.4^{a}	0.5	<0.001
Sour	2.0^{c}	3.3^{a}	2.8^{ab}	3.7^{a}	1.8^{c}	1.9^{c}	3.3^{a}	3.2^{a}	2.3^{bc}	0.4	<0.001
Bitter	0.1^{d}	0.8^{d}	0.7^{d}	0.6^{d}	5.2^{b}	6.3^{a}	0.8^{d}	0.9^{d}	4.4^{c}	0.5	<0.001
Umami	1.4^{ab}	1.1^{ab}	1.4^{a}	1.2^{ab}	0.6^{b}	0.7^{b}	1.3^{ab}	1.4^{a}	1.3^{ab}	0.3	<0.01
Flavor											
Overall	7.8^{d}	8.2^{cd}	8.0^{cd}	8.9^{ab}	8.8^{abc}	9.4^{a}	8.6^{bc}	8.5^{bc}	9.0^{ab}	0.4	<0.001
Diacetyl	4.0^{ab}	3.2^{bc}	4.0^{ab}	4.1^{a}	3.0^{cd}	2.4^{d}	3.9^{ab}	3.8^{abc}	3.1^{cd}	0.3	<0.001
Cooked	1.1^{a}	1.0^{ab}	1.2^{a}	1.1^{a}	0.6^{b}	0.6^{b}	1.0^{ab}	1.1^{a}	0.8^{ab}	0.2	<0.001
Metallic	0.1^{b}	0.2^{b}	0.2^{b}	0.2^{b}	1.6^{a}	2.0^{a}	0.2^{b}	0.1^{b}	1.6^{a}	0.3	<0.001
Milky	1.8^{a}	1.4^{ab}	1.9^{a}	1.9^{a}	1.0^{bc}	0.8^{c}	1.8^{a}	1.6^{a}	1.3^{ab}	0.3	<0.001
Whey	2.8^{ab}	2.7^{ab}	2.8^{a}	3.0^{a}	2.2^{b}	1.5^{c}	3.0^{a}	2.9^{a}	2.1^{b}	0.3	<0.001
Malty	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	NS
Earthy	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.4^{a}	0.1^{ab}	0.2^{ab}	0.0^{b}	0.1^{b}	0.2	0.02
Moldy	0.0^{b}	0.0^{b}	0.1^{b}	0.0^{b}	0.2^{ab}	0.4^{a}	0.1^{b}	0.0^{b}	0.4^{a}	0.2	<0.001
Sulfur	0.2	0.3	0.2	0.4	0.5	0.4	0.5	0.2	0.4	0.2	NS
Unclean	0.1^{c}	0.2^{c}	0.1^{c}	0.1^{c}	2.5^{b}	3.6^{a}	0.4^{c}	0.2^{c}	2.8^{b}	0.4	<0.001
Yeasty	0.2	0.1	0.1	0.1	0.1	0.3	0.0	0.1	0.1	0.1	NS
Brothy	1.0	0.8	0.6	0.7	0.6	0.8	0.7	0.9	0.8	0.3	NS
Pineapple	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	NS

Table A.2 Continued

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Hydrolytic rancidity	0.4^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.8^{a}	1.0^{a}	0.4^{a}	0.6^{a}	0.9^{a}	0.2	<0.01
Fermented	0.1	0.1	0.2	0.1	0.0	0.2	0.1	0.0	0.0	0.2	NS
Nutty	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	NS
Sour dairy	2.6^{b}	3.3^{ab}	3.1^{ab}	3.6^{a}	1.7^{c}	1.8^{c}	3.1^{ab}	3.6^{a}	2.5^{b}	0.4	<0.001
Soapy	0.3^{c}	0.4^{c}	0.3^{c}	0.3^{c}	1.6^{b}	2.4^{a}	0.4^{c}	0.4^{c}	1.9^{ab}	0.3	<0.001
Sensation											
Astringency	0.3^{b}	0.7^{ab}	0.5^{b}	0.7^{ab}	0.5^{b}	1.1^{a}	0.7^{ab}	0.7^{ab}	0.8^{ab}	0.3	0.001
Pungent	0.2^{b}	0.5^{ab}	0.3^{ab}	0.6^{ab}	0.4^{ab}	0.7^{a}	0.5^{ab}	0.6^{ab}	0.6^{ab}	0.3	0.03
Numbing	0.2^{c}	0.3^{c}	0.2^{c}	0.3^{c}	1.3^{b}	2.2^{a}	0.4^{c}	0.2^{c}	1.4^{b}	0.4	<0.001
In-Hand Texture											
Firmness	9.8^{cd}	16.1^{a}	10.7^{c}	8.3^{e}	7.5^{e}	14.4^{b}	14.0^{b}	14.3^{b}	8.8^{e}	0.6	<0.001
Springiness	8.9^{b}	4.5^{d}	8.3^{b}	9.4^{b}	10.6^{a}	5.9^{c}	5.9^{c}	5.6^{c}	9.3^{b}	0.4	<0.001
Stickiness	3.8^{bcd}	3.0^{d}	4.0^{bc}	5.0^{a}	4.5^{ab}	3.3^{cd}	3.5^{cd}	3.4^{cd}	4.0^{bc}	0.4	<0.001
Cohesiveness	11.4^{a}	11.5^{a}	11.6^{a}	10.1^{a}	10.6^{a}	10.8^{a}	11.5^{a}	11.4^{a}	8.4^{b}	0.7	<0.001
Slipperiness	10.3^{cd}	12.0^{b}	10.5^{cd}	10.0^{cd}	11.3^{bc}	11.8^{b}	9.8^{d}	10.1^{cd}	13.5^{a}	0.6	<0.001
First-Bite Texture											
Firmness	5.8^{c}	10.0^{a}	6.1^{c}	4.5^{d}	5.7^{c}	9.7^{a}	7.8^{b}	7.9^{b}	5.7^{c}	0.5	<0.001
Stickiness	8.1^{c}	4.9^{d}	9.5^{b}	11.7^{a}	6.9^{c}	4.4^{d}	7.3^{c}	7.3^{c}	5.1^{d}	0.6	<0.001
Brittleness	3.8^{cd}	8.0^{a}	3.6^{cd}	2.8^{d}	4.6^{bc}	7.2^{a}	5.3^{b}	5.3^{b}	7.8^{a}	0.5	<0.001
Five-Chew Texture											
Firmness	7.6^{c}	10.3^{a}	7.3^{c}	5.8^{d}	7.4^{c}	10.4^{a}	8.7^{b}	8.9^{b}	7.1^{c}	0.4	<0.001
Stickiness	9.0^{bc}	5.4^{e}	9.7^{b}	11.9^{a}	6.9^{d}	4.9^{e}	8.6^{bc}	7.9^{cd}	5.7^{e}	0.6	<0.001
Brittleness	4.6^{c}	9.1^{a}	3.9^{c}	2.8^{d}	5.7^{b}	9.0^{a}	6.0^{b}	6.5^{b}	8.1^{a}	0.5	<0.001
Curdiness	3.9^{c}	7.9^{a}	3.1^{cd}	2.3^{d}	5.8^{b}	8.6^{a}	5.1^{b}	5.1^{b}	8.9^{a}	0.6	<0.001

Table A.3. Mean values for all attributes in the descriptive sensory analysis at six months of age (Chapter 3)

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Aroma											
Overall	7.7	8.1	7.3	7.9	7.4	7.5	7.3	8.1	7.4	0.5	NS
Diacetyl	5.2	5.6	5.1	5.9	5.1	4.7	5.2	5.4	5.0	0.7	NS
Cooked	2.3	2.0	1.9	2.1	1.7	2.2	1.9	2.5	2.2	0.8	NS
Metallic	0.4	0.3	0.1	0.3	0.5	0.3	0.4	0.4	0.3	0.2	NS
Milky	0.9	1.2	0.9	1.0	0.7	1.5	1.0	1.0	1.0	0.4	NS
Whey	1.9	2.1	1.8	2.3	2.3	2.1	1.9	2.6	2.2	0.5	NS
Malty	0.2[a]	0.3[a]	0.2[a]	0.0[a]	0.1[a]	0.0[a]	0.2[a]	0.1[a]	0.2[a]	0.1	0.03
Earthy	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	NS
Moldy	0.2	0.3	0.1	0.2	0.2	0.4	0.2	0.3	0.4	0.1	NS
Sulfur	1.6	1.2	1.5	1.2	1.6	1.4	1.4	1.4	1.1	0.3	NS
Unclean	0.2	0.0	0.1	0.3	0.3	0.2	0.1	0.2	0.3	0.2	NS
Yeasty	0.7	0.9	0.8	0.7	0.5	0.7	0.9	1.0	0.7	0.3	NS
Brothy	1.2	1.4	1.4	1.4	1.2	0.9	1.1	1.3	1.2	0.4	NS
Pineapple	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	NS
Hydrolytic rancidity	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	NS
Fermented	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.1	0.1	NS

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

[4]Largest standard error of all treatments is shown for each attribute

[a-g]Means with without a common superscript letter within the same row are significantly different ($p < 0.05$)

NS: No significant differences between treatments (alpha = 0.05)

Table A.3 Continued

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Nutty	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	NS
Sour dairy	1.7	1.8	1.7	1.7	1.6	1.9	1.6	1.7	1.5	0.3	NS
Soapy	0.4	0.4	0.2	0.2	0.5	0.5	0.5	0.2	0.5	0.2	NS
Taste											
Sweet	1.3^{a}	0.7^{abc}	1.1^{ab}	0.8^{abc}	0.6^{abc}	0.3^{c}	1.0^{abc}	0.7^{abc}	0.4^{bc}	0.3	<0.001
Salty	4.5^{a}	3.9^{a}	4.5^{a}	4.5^{a}	3.3^{a}	3.1^{a}	3.9^{a}	3.9^{a}	4.6^{a}	0.6	0.04
Sour	2.3	2.7	2.7	3.3	4.1	3.4	2.5	3.3	3.5	0.6	NS
Bitter	1.1^{c}	1.7^{c}	1.6^{c}	2.3^{c}	6.3^{b}	8.0^{a}	1.7^{c}	2.2^{c}	5.6^{b}	0.7	<0.001
Umami	1.3	1.2	1.2	1.3	0.8	1.0	1.2	1.3	1.3	0.3	NS
Flavor											
Overall	8.4^{c}	7.9^{c}	8.9^{abc}	10.0^{a}	9.1^{abc}	9.7^{ab}	8.2^{c}	8.4^{bc}	10.0^{a}	0.6	<0.001
Diacetyl	5.4^{a}	4.7^{ab}	5.3^{a}	5.3^{a}	3.5^{bc}	2.9^{c}	4.2^{abc}	4.3^{abc}	3.9^{abc}	0.5	<0.001
Cooked	2.3^{abc}	2.1^{abc}	2.4^{ab}	1.8^{abc}	1.7^{bc}	1.1^{c}	1.8^{abc}	2.9^{a}	1.9^{abc}	0.8	<0.01
Metallic	0.7^{c}	1.1^{c}	0.9^{c}	1.7^{c}	3.8^{ab}	4.3^{a}	0.8^{c}	1.3^{c}	3.0^{b}	0.7	<0.001
Milky	1.2^{a}	0.8^{ab}	1.0^{ab}	1.2^{a}	0.7^{ab}	0.4^{b}	0.9^{ab}	0.7^{ab}	0.6^{ab}	0.2	<0.01
Whey	1.8	2.1	2.1	2.4	1.5	1.3	2.1	2.2	2.4	0.6	NS
Malty	0.1	0.1	0.2	0.2	0.1	0.0	0.1	0.1	0.2	0.1	NS
Earthy	0.1^{ab}	0.0^{b}	0.1^{b}	0.1^{ab}	0.3^{ab}	0.4^{a}	0.1^{ab}	0.3^{ab}	0.4^{ab}	0.1	<0.001
Moldy	0.2^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.6^{a}	0.3^{a}	0.8^{a}	0.8^{a}	0.2	0.03
Sulfur	2.0	2.1	2.5	2.2	2.3	2.3	2.0	2.3	2.3	0.5	NS
Unclean	0.3^{c}	0.6^{c}	0.4^{c}	0.7^{c}	2.0^{b}	4.6^{a}	0.5^{c}	0.7^{c}	2.5^{b}	0.5	<0.001
Yeasty	0.7	0.8	0.8	0.7	0.9	1.4	0.8	1.0	1.1	0.6	NS
Brothy	1.7	1.8	1.9	1.5	1.0	1.1	2.0	1.8	1.8	0.4	NS
Pineapple	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	NS

Table A.3 Continued

	Treatment[1]										
	Full Sodium		Reduced Sodium[3]								
Sensory Attribute	N[2]	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG	SE[4]	p
Hydrolytic rancidity	0.2^{b}	0.2^{b}	0.0^{b}	0.2^{b}	0.6^{a}	0.4^{ab}	0.1^{a}	0.2^{b}	0.4^{ab}	0.2	<0.001
Fermented	0.1^{b}	0.3^{b}	0.1^{b}	0.1^{b}	0.8^{ab}	1.3^{a}	0.3^{b}	0.2^{b}	0.2^{b}	0.5	<0.001
Nutty	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	NS
Sour dairy	2.2	2.6	2.4	2.9	2.7	3.5	2.1	2.4	2.2	0.7	NS
Soapy	0.5^{c}	1.1^{c}	0.7^{c}	0.8^{c}	2.0^{b}	3.2^{a}	1.0^{c}	1.1^{c}	2.7^{a}	0.4	<0.001
Sensation											
Astringency	0.2^{b}	0.9^{b}	0.6^{b}	0.5^{b}	1.1^{b}	1.9^{a}	0.4^{b}	0.9^{b}	0.7^{b}	0.6	<0.001
Pungent	0.4^{a}	0.5^{a}	0.5^{a}	1.0^{a}	1.3^{a}	1.5^{a}	0.4^{a}	1.0^{a}	1.1^{a}	0.5	0.03
Numbing	0.1^{c}	0.2^{bc}	0.3^{bc}	0.2^{bc}	1.0^{a}	0.7^{ab}	0.2^{bc}	0.3^{bc}	0.7^{ab}	0.2	<0.001
In-Hand Texture											
Firmness	12.1^{c}	18.2^{a}	15.2^{b}	14.0^{b}	9.0^{d}	15.2^{b}	17.8^{a}	18.1^{a}	14.0^{b}	0.7	<0.001
Springiness	6.4	6.4	6.0	5.9	8.8	6.4	5.9	6.5	6.2	1.2	NS
Stickiness	3.7^{ab}	2.1^{cd}	3.2^{bc}	3.5^{ab}	4.3^{a}	2.9^{bcd}	1.8^{d}	2.2^{cd}	3.1^{bc}	0.5	<0.001
Cohesiveness	13.2	12.6	12.3	11.6	12.7	12.3	13.2	13.0	10.9	1.0	NS
Slipperiness	10.4^{bcd}	8.0^{e}	9.5^{cde}	10.6^{bcd}	11.6^{b}	11.4b^{c}	9.2d^{e}	9.5^{cde}	13.6^{a}	0.8	<0.001
First-Bite Texture											
Firmness	4.8^{e}	12.8^{a}	6.1^{e}	5.5^{e}	3.4^{f}	9.7^{c}	11.2^{b}	12.1^{ab}	7.5^{d}	0.7	<0.001
Stickiness	12.8^{a}	5.4^{c}	12.9^{a}	13.3^{a}	12.1^{a}	7.6^{bc}	6.6^{bc}	6.4^{bc}	7.9^{b}	0.9	<0.001
Brittleness	2.2^{c}	10.1^{a}	3.3^{c}	3.6^{c}	2.0^{c}	7.4^{b}	7.9^{b}	9.0^{ab}	7.1^{b}	1.0	<0.001
Five-Chew Texture											
Firmness	5.9^{fg}	12.9^{a}	7.5^{e}	6.5^{ef}	4.9^{g}	10.4^{c}	11.4^{bc}	11.9^{ab}	8.8^{d}	0.6	<0.001
Stickiness	13.7^{a}	6.5^{b}	12.6^{a}	13.6^{a}	12.0^{a}	6.9^{b}	7.1^{b}	7.3^{b}	8.5^{b}	0.9	<0.001
Brittleness	2.1^{c}	10.7^{a}	3.0^{c}	3.1^{c}	2.8^{c}	8.1^{b}	8.5^{b}	9.5^{ab}	7.7^{b}	0.9	<0.001
Curdiness	1.6^{b}	7.9^{a}	2.3^{b}	2.2^{b}	2.5^{b}	7.4^{a}	5.9^{a}	7.3^{a}	6.9^{a}	0.9	<0.001

Table A.4. All differences between descriptive sensory analysis means from one month and six months of age (Chapter 3). A negative value indicates a lower rating in month 6 and a positive value indicates a higher rating in month 6

	Treatment[1]								
	Full Sodium[2]		Reduced Sodium[3]						
Sensory Attribute	N	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG
Aroma									
Overall	-1.3	-0.8	-1.6*	-0.9*	-1.2	-0.6	-1.5*	-1.0	-0.9
Diacetyl	0.5	1.0	0.1	1.0	0.8	0.4	0.4	0.6	0.2
Cooked	1.2	0.8	0.7	0.9	0.6	1.1	1.1	1.5	0.9
Metallic	0.4*	0.3	0.1	0.3*	0.3	0.3	0.3	0.3	0.2
Milky	-0.8*	-0.7*	-0.9*	-0.9*	-1.1*	0.0	-1.0*	-0.7*	-0.8*
Whey	-1.3*	-1.4*	-1.6*	-1.1*	-0.9*	-1.1*	-1.5*	-1.0	-1.2*
Malty	0.2	0.2	0.2	0.0	0.1	0.0	0.2	0.0	0.2
Earthy	0.0	0.0	0.0	0.0	-0.1	0.0	0.0	0.0	0.1
Moldy	0.2*	0.3*	0.1*	0.2*	0.2*	0.3*	0.2*	0.2	0.3*
Sulfur	1.6*	1.1*	1.4*	1.2*	1.5*	1.2*	1.3*	1.3*	0.9*
Unclean	0.2	-0.2	0.1*	0.3*	0.0	0.0	-0.1	-0.1	-0.1
Yeasty	0.7*	0.8*	0.8*	0.7*	0.5*	0.7*	0.9*	1.0*	0.5*
Brothy	0.8*	1.0*	0.9*	0.9*	0.7*	0.4*	0.5*	0.7*	0.7*
Pineapple	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1
Hydrolytic rancidity	-0.1	-0.2	-0.3	-0.3	-0.1	-0.2	-0.3	-0.2	-0.2
Fermented	0.2	0.1	0.0	0.1	0.1	0.2	0.2	0.1	0.1
Nutty	0.0	0.0	0.0	0.0	0.0	-0.1	0.0	0.1	0.0
Sour dairy	-1.0	-0.9	-0.7	-1.1	-0.4	-0.4	-1.0*	-1.3*	-0.9
Soapy	0.2	0.2	0.0	0.2*	0.2*	0.3*	0.3	0.0	0.3*
Taste									
Sweet	0.2*	0.2	0.3	0.0	-0.1	0.1	0.3*	0.2	-0.1
Salty	0.8	-0.3	0.7	0.0	-0.3	-1.4*	-0.1	-0.6*	-0.7
Sour	0.2*	-0.6	-0.1*	-0.4	2.4*	1.5*	-0.7	0.1*	1.3*
Bitter	0.9*	0.8	0.9	1.8*	1.1	1.7*	0.8	1.3*	1.2
Umami	-0.1	0.1	-0.2	0.1	0.2	0.3	-0.1	-0.1	0.0
Flavor									
Overall	0.6	-0.3	0.9	1.1	0.3	0.2	-0.5	-0.1	1.0
Diacetyl	1.4*	1.5*	1.3*	1.2	0.4	0.5*	0.2	0.5*	0.8*
Cooked	1.2*	1.2	1.2	0.6	1.0	0.5	0.8	1.9	1.1*
Metallic	0.6*	0.8*	0.7*	1.5	2.2*	2.4*	0.6*	1.2*	1.4*
Milky	-0.7	-0.6	-0.9*	-0.7	-0.4	-0.4	-0.8*	-0.9*	-0.8*
Whey	-0.9*	-0.6*	-0.7*	-0.6	-0.6*	-0.2	-0.9*	-0.7*	0.3
Malty	0.1*	0.1*	0.2*	0.2*	0.0	0.0	0.1*	0.1*	0.2

[1]Treatments: N = sodium chloride; S = sea salt; K = potassium chloride; MK = Modified K; M = magnesium chloride hexahydrate; C = calcium chloride dihydrate

[2]640 mg sodium/100 g sample target

[3]300 mg sodium/100 g sample target

*Indicates the difference is significant ($p < 0.05$)

Table A.4 Continued

	Treatment[1]								
	Full Sodium[2]		Reduced Sodium[3]						
Sensory Attribute	N	S	N/K	N/MK	N/MG	N/C	S/K	S/MK	S/MG
Earthy	0.1	0.0	0.1	0.1*	0.0	0.3	-0.1	0.3	0.3
Moldy	0.2	0.4*	0.3*	0.4*	0.2	0.2	0.2*	0.8*	0.4
Sulfur	1.9*	1.8*	2.3*	1.8*	1.8*	1.9*	1.5*	2.0*	1.9*
Unclean	0.2*	0.3*	0.3	0.6*	-0.5	1.1*	0.1*	0.5*	-0.3
Yeasty	0.5*	0.7*	0.8*	0.7*	0.9*	1.1*	0.8*	0.9*	1.0*
Brothy	0.7*	0.9*	1.3*	0.8*	0.4*	0.4	1.3*	0.9*	1.0*
Pineapple	0.1	0.1	0.0	0.0	0.0	0.0*	0.0	0.1	0.0
Hydrolytic rancidity	-0.2	-0.2	-0.3	-0.2	-0.2	-0.6*	-0.3	-0.4	-0.5*
Fermented	0.1	0.2	0.0	0.1	0.8	1.1	0.1	0.2	0.1
Nutty	0.0	0.1	0.0	0.0*	0.0	0.0	0.1	-0.1	0.1
Sour dairy	-0.5	-0.7	-0.7	-0.6	0.9*	1.7*	-1.0	-1.2	-0.3
Soapy	0.2*	0.7*	0.4	0.5	0.4*	0.8	0.5	0.7*	0.7
Sensation									
Astringency	-0.1	0.2	0.1	-0.2	0.6	0.8	-0.3	0.2	-0.1
Pungent	0.3	0.0	0.2	0.4	0.9	0.8	-0.1*	0.4	0.6
Numbing	-0.1	-0.1	0.1*	-0.1	-0.3	-1.4*	-0.2	0.1	-0.7*
In-Hand Texture									
Firmness	2.3*	2.1*	4.5*	5.6*	1.5	0.8	3.8*	3.8*	5.2*
Springiness	-2.5*	1.9*	-2.3*	-3.5*	-1.8*	0.5	0.0	0.9*	-3.0*
Stickiness	-0.1	-0.9	-0.9	-1.6*	-0.2	-0.4	-1.7*	-1.2*	-0.9
Cohesiveness	1.7*	1.1*	0.7	1.4	2.2	1.5	1.7*	1.6	2.5
Slipperiness	0.1	-4.0	-0.9	0.6	0.4	-0.5	-0.6	-0.5	0.1
First-Bite Texture									
Firmness	-1.0	2.9	0.0	1.0*	-2.4*	-0.1	3.4*	4.2*	1.8
Stickiness	4.8*	0.5	3.4*	1.6	5.3*	3.2*	-0.7	-0.9	2.8
Brittleness	-1.6*	2.0	-0.3	0.8	-2.5*	0.2*	2.6	3.7*	-0.6
Five-Chew Texture									
Firmness	-1.7	2.6	0.2	0.7*	-2.5*	0.0*	2.6	3.0*	1.6
Stickiness	4.7*	1.1*	2.9*	1.7	5.0*	2.0*	-1.5	-0.6*	2.8*
Brittleness	-2.5*	1.6	-0.9*	0.4	-2.9*	-0.8*	2.5	3.0	-0.4
Curdiness	-2.4*	0.0	-0.8	0.0	-3.4*	-1.2	0.8	2.1*	-1.9

Table A.5. Mean values for all compositional, chemical, microbial, and texture profile analysis (TPA) measurements through aging (Chapter 5)

		Treatment[1]						
Measurement	Month	N[b]	K[c]	HY[b]	BB[b]	I[b]	G[b]	MK[a]
Moisture, %	0	38.65	37.61	37.70	37.99	38.30	38.38	40.67
	1	38.12	37.55	38.46	38.25	38.79	38.44	40.94
	2	38.28	38.13	38.48	38.24	38.17	37.79	41.13
	3	38.43	37.76	37.82	38.45	38.63	38.55	40.66
	4	38.35	37.65	38.35	37.93	37.63	37.63	41.03
		N[a]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK[b]
pH	0[B]	5.48[w]	5.21[x]	5.20[x]	5.20[x]	5.24[x]	5.23[x]	5.20[x]
	1[B]	5.39[w]	5.19[x]	5.19[x]	5.17[x]	5.18[x]	5.18[x]	5.13[x]
	2[A]	5.53[w]	5.23[x]	5.24[x]	5.23[x]	5.27[x]	5.28[x]	5.18[x]
	3[A]	5.44[w]	5.25[y]	5.32[x]	5.25[y]	5.24[yz]	5.23[yz]	5.19[z]
	4[A]	5.38[w]	5.26[xy]	5.27[xy]	5.29[xy]	5.31[wx]	5.29[xy]	5.22[y]
Water		N	K	HY	BB	I	G	MK
Activity	0[A]	0.967	0.967	0.964	0.965	0.965	0.964	0.968
	1[A]	0.965	0.967	0.968	0.965	0.964	0.965	0.969
	2[A]	0.961	0.965	0.967	0.965	0.965	0.965	0.965
	3[B]	0.962	0.964	0.964	0.963	0.961	0.961	0.965
	4[B]	0.959	0.963	0.961	0.964	0.961	0.961	0.964
WSN[2], %		N[c]	K[cb]	HY[cb]	BB[ab]	I[cb]	G[cb]	MK[a]
total nitrogen	0[E]	8.84	9.70	9.27	9.78	9.83	9.46	10.62
	1[D]	11.29	11.25	11.64	12.76	11.65	11.94	13.01
	2[C]	15.01	14.45	14.49	16.06	14.37	14.38	15.73
	3[B]	14.17	16.58	17.39	17.13	18.17	16.76	18.66
	4[A]	20.15	20.84	20.78	19.84	18.99	19.43	21.63
		N	K	HY	BB	I	G	MK
LAB[3], CFU/g	0[A]	$1.27x10^8$	$2.47x10^8$	$1.64x10^8$	$3.48x10^8$	$1.96x10^8$	$2.88x10^8$	$3.26x10^8$
	1[C]	$2.81x10^7$	$1.48x10^7$	$1.70x10^7$	$1.80x10^7$	$9.45x10^6$	$1.39x10^7$	$1.37x10^7$
	2	NM	NM	NM	NM	NM	NM	NM
	3[B]	$4.64x10^7$	$1.50x10^8$	$1.40x10^8$	$1.13x10^8$	$1.07x10^8$	$1.71x10^8$	$1.20x10^8$
	4[C]	$1.27x10^7$	$3.70x10^7$	$2.09x10^7$	$4.68x10^7$	$3.30x10^7$	$2.05x10^7$	$1.45x10^7$

[1]Treatments: N = full sodium control (640 mg sodium/100 g cheese target); K = sodium chloride + potassium chloride (220 mg sodium/ 100 g cheese target); HY = K + hydrolyzed vegetable protein/yeast extract blend; BB = K + natural potassium blocker type flavor; I = K + disodium 5' inosinate; G = K + disodium guanylate; MK = sodium chloride + modified potassium chloride (220 mg sodium/ 100 g target)
[2]WSN: Water soluble nitrogen
[3]LAB: Lactic acid bacteria.
NM: No measurement
[a-c]Treatments without a common superscript letter within the same row are significantly different ($p < 0.05$).
[A-D]Months without a common superscript letter within the same measurement are significantly different ($p < 0.05$)
[w-z]Means without a common superscript letter within the same row are significantly different ($p < 0.05$)

Table A.5 Continued

Measurement	Month	Treatment[1] N[b]	K[c]	HY[b]	BB[b]	I[b]	G[b]	MK[a]
TPA								
Hardness, g	1[A]	6315	4895	5290	5690	4595	5185	NM
	2[A]	6300	4995	4400	5195	5115	4770	NM
	3[AB]	5470	5255	5310	5035	3785	4510	NM
	4[B]	5165	4540	4275	4025	4425	4240	NM
		N[a]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK
Springiness	1[A]	0.520	0.350	0.366	0.351	0.367	0.335	NM
	2[AB]	0.436	0.334	0.354	0.315	0.319	0.341	NM
	3[B]	0.384	0.304	0.305	0.308	0.379	0.297	NM
	4[B]	0.430	0.315	0.304	0.342	0.315	0.344	NM
		N	K	HY	BB	I	G	MK
Cohesiveness	1	0.220	0.233	0.236	0.229	0 .245	0.226	NM
	2	0.214	0.225	0.253	0.220	0.219	0.240	NM
	3	0.226	0.209	0.224	0.211	0.276	0.214	NM
	4	0.251	0.214	0.218	0.233	0.229	0.228	NM
Adhesiveness,		N	K	HY	BB	I	G	MK
g x sec	1[B]	20	161	188	181	160	223	NM
	2[B]	67	230	113	184	207	181	NM
	3[A]	223	360	389	351	163	320	NM
	4[A]	111	335	311	230	252	227	NM
		N[a]	K[b]	HY[b]	BB[b]	I[ab]	G[b]	MK
Resilience	1[A]	0.070	0.051	0.054	0.053	0.055	0.048	NM
	2[AB]	0.060	0.047	0.060	0.048	0.047	0.055	NM
	3[BC]	0.052	0.041	0.045	0.041	0.064	0.041	NM
	4[C]	0.064	0.041	0.042	0.047	0.046	0.043	NM
		N[a]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK
Chewiness, g	1[A]	716	400	448	462	413	392	NM
	2[B]	574	374	396	361	355	388	NM
	3[B]	489	330	359	333	405	289	NM
	4[B]	560	306	291	318	316	334	NM

Table A.6. Mean values for all monthly descriptive sensory measurements in Chapter 5 that had significant effects of treatment, time, or both. No interactions between treatment and time were significant (alpha = 0.05)

		Treatment[1]						
Attribute	Month	N	K	HY	BB	I	G	MK
Aroma								
Overall	1[AB]	6.9	6.7	7.1	7.3	7.1	6.8	7.0
	2[B]	6.9	6.8	6.9	6.7	6.6	7.2	6.5
	3[AB]	7.0	7.2	7.3	7.3	7.4	7.1	7.4
	4[A]	7.6	7.4	7.4	7.1	7.7	7.5	7.5
		N	K	HY	BB	I	G	MK
Sulfur	1[B]	1.4	1.3	1.5	1.5	1.3	1.4	1.3
	2[B]	1.4	1.4	1.3	1.3	1.3	1.4	1.5
	3[A]	1.7	1.9	2.2	2.4	2.3	1.9	2.1
	4[A]	2.0	2.1	2.3	2.1	2.0	2.6	2.4
		N	K	HY	BB	I	G	MK
Sour dairy	1[A]	3.8	3.5	3.7	3.7	3.6	3.6	4.0
	2[B]	3.1	3.2	3.3	3.1	2.6	3.3	3.2
	3[AB]	3.1	3.4	3.4	3.2	3.1	3.5	3.8
	4[A]	3.6	3.8	4.0	3.6	3.3	4.0	3.5
Taste		N	K	HY	BB	I	G	MK
Salty	1[B]	3.3	3.2	2.8	3.0	3.3	3.1	3.3
	2[B]	3.3	3.2	3.1	3.2	3.1	3.2	3.0
	3[B]	2.8	2.7	2.9	3.1	3.1	2.8	3.2
	4[A]	3.8	3.2	4.0	4.0	4.1	3.7	4.3
		N[b]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[a]
Sour	1[AB]	2.6	3.3	3.4	3.4	3.5	3.3	3.5
	2[B]	2.3	3.1	3.2	3.2	3.2	3.0	3.2
	3[A]	2.7	3.7	3.9	3.3	3.4	3.5	4.3
	4[A]	2.7	4.1	3.1	4.0	3.3	3.9	3.8

[1]Treatments: N = full sodium control (640 mg sodium/100 g cheese target); K = sodium chloride + potassium chloride (220 mg sodium/ 100 g cheese target); HY = K + hydrolyzed vegetable protein/yeast extract blend; BB = K + natural potassium blocker type flavor; I = K + disodium 5' inosinate; G = K + disodium guanylate; MK = sodium chloride + modified potassium chloride (220 mg sodium/ 100 g target)

[a-c]Treatments without a common superscript letter within the same row are significantly different ($p < 0.05$).

[A-D]Months without a common superscript letter within the same attribute are significantly different ($p < 0.05$)

Table A.6 Continued

		Treatment						
Attribute	Month	N[b]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[a]
Bitter	1[B]	0.4	0.9	0.7	0.9	0.8	0.8	0.9
	2[A]	0.7	1.4	1.3	1.4	1.3	1.3	1.4
	3[A]	1.0	1.4	1.5	1.2	1.1	1.5	1.2
	4[A]	0.8	1.3	1.2	1.2	1.2	1.2	1.3
		N[b]	K[b]	HY[b]	BB[b]	I[a]	G[b]	MK[b]
Umami	1	0.5	0.5	0.5	0.5	1.6	0.8	0.5
	2	0.7	0.7	0.8	0.7	1.1	0.9	0.8
	3	0.5	0.5	0.7	0.7	1.5	0.9	0.6
	4	0.8	0.5	1.0	0.8	1.2	1.0	0.9
Flavor		N[b]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[a]
Overall	1[C]	6.0	6.6	6.6	6.7	7.0	6.7	6.9
	2[C]	6.0	6.7	6.9	6.6	7.0	6.9	6.7
	3[B]	6.5	7.3	7.1	7.1	7.6	7.1	7.8
	4[A]	7.6	7.8	6.9	7.4	7.7	7.7	8.2
		N	K	HY	BB	I	G	MK
Diacetyl	1[B]	2.5	2.9	2.9	3.0	2.6	2.9	2.8
	2[B]	2.9	2.9	3.0	2.8	2.6	2.6	2.9
	3[B]	2.7	3.0	3.1	2.8	2.5	3.0	3.1
	4[A]	3.8	3.9	3.4	3.8	3.4	3.4	3.6
		N	K	HY	BB	I	G	MK
Cooked	1[B]	0.9	1.1	1.3	1.1	1.4	1.2	1.0
	2[AB]	1.4	1.5	1.5	1.4	1.4	1.6	1.5
	3[B]	1.3	1.1	1.3	1.1	1.4	1.0	1.2
	4[A]	1.8	1.7	1.3	1.3	1.6	1.8	1.5
		N	K	HY	BB	I	G	MK
Milky	1[A]	1.2	1.0	0.9	1.1	1.0	1.2	1.1
	2[AB]	1.2	0.6	0.8	1.0	0.8	0.7	1.0
	3[B]	0.7	0.5	0.6	0.5	0.5	0.4	0.4
	4[B]	0.7	0.6	0.6	0.3	0.4	0.5	0.4
		N[b]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[a]
Sulfur	1[C]	1.6	2.0	2.0	1.6	2.1	2.1	2.0
	2[C]	0.9	2.0	2.1	2.1	2.2	2.3	2.2
	3[B]	1.4	2.5	3.1	2.8	2.8	2.6	3.1
	4[A]	2.0	3.6	3.3	3.3	3.2	3.8	3.5

Table A.6 Continued

		Treatment						
Attribute	Month	N[b]	K[b]	HY[b]	BB[b]	I[a]	G[b]	MK[b]
Brothy	1[B]	0.6	0.4	0.5	0.4	1.5	0.8	0.7
	2[B]	0.9	0.6	0.6	0.6	1.1	0.7	0.5
	3[B]	0.4	0.3	0.3	0.5	2.0	0.5	0.4
	4[A]	1.1	0.8	0.9	0.9	2.1	0.8	0.9
		N[b]	K[a]	HY[a]	BB[a]	I[ab]	G[a]	MK[a]
Sour dairy	1[AB]	3.2	3.8	4.1	4.1	3.5	3.9	4.1
	2[B]	2.7	3.9	3.5	3.5	3.3	3.5	3.6
	3[B]	3.0	3.6	3.8	3.5	3.4	3.9	4.3
	4[A]	3.7	4.2	4.0	4.3	3.9	4.2	5.0
In-Hand Texture		N[a]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[b]
Firmness	1	11.8	11.8	11.5	11.8	11.0	11.4	8.7
	2	11.8	12.1	12.7	11.7	11.2	11.5	8.6
	3	10.3	11.8	11.5	11.5	12.4	11.4	8.9
	4	10.8	11.5	11.9	12.1	11.4	11.8	9.4
		N	K	HY	BB	I	G	MK
Springiness	1[A]	7.2	6.6	6.9	6.8	7.1	6.9	8.0
	2[AB]	6.5	6.5	6.3	6.6	6.9	6.9	7.0
	3[AB]	6.9	6.3	6.7	6.3	6.4	6.3	6.9
	4[B]	6.4	6.7	6.1	6.2	6.2	6.5	7.0
		N[a]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[b]
Cohesiveness	1	13.6	13.0	13.0	13.3	12.9	13.2	11.4
	2	12.8	12.9	13.3	12.6	12.8	12.8	11.8
	3	13.1	13.1	13.4	13.2	13.3	13.1	11.5
	4	12.7	13.0	13.3	12.8	12.9	12.4	11.5
First-Bite Texture		N[a]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[b]
Firmness	1[A]	6.5	5.8	5.6	6.0	5.9	6.2	4
	2[A]	6.2	5.5	6.4	5.6	4.9	5.4	3.8
	3[AB]	5.5	5.9	5.5	5.8	5.5	4.7	3.6
	4[B]	4.4	4.8	5.7	5.1	4.6	4.9	3.8
		N[b]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK[a]
Stickiness	1[D]	8.0	8.6	8.9	8.9	8.4	8.5	10.1
	2[C]	8.8	9.8	10.1	9.8	10.4	9.6	11.1
	3[B]	9.8	10.3	10.7	10.9	10.7	11.1	11.5
	4[A]	10.8	10.6	10.2	11.8	11.9	11.8	12.5

Table A.6 Continued

		Treatment						
Attribute	Month	N[a]	K[ab]	HY[ab]	BB[ab]	I[b]	G[b]	MK[c]
Brittleness	1[A]	4.5	3.7	3.5	3.7	3.4	3.4	2.6
	2[A]	4.3	3.6	3.5	3.3	3.3	3.5	2.3
	3[AB]	3.4	3.3	3.3	3.4	3.4	3.2	2.3
	4[B]	2.9	2.8	3.7	3.3	2.9	2.8	2.1
Five-Chew Texture		N[a]	K[a]	HY[a]	BB[a]	I[a]	G[a]	MK[b]
Firmness	1[A]	8.5	7.7	7.7	8.1	7.6	7.6	5.6
	2[A]	8.1	7.6	8.0	7.4	7.2	7.3	5.4
	3[B]	6.8	6.8	6.7	7	7.2	6.5	5.3
	4[C]	5.7	6.3	6.4	6.3	5.9	6.3	4.4
		N[c]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK[a]
Stickiness	1[D]	8.6	9.3	9.5	8.9	8.9	9.7	10.9
	2[C]	8.5	10.4	10.0	10.2	10.9	10.5	12.1
	3[B]	10.1	11.2	11.3	10.2	10.8	11.3	13.1
	4[A]	11.9	11.9	11.5	12.2	12.1	12.8	13.1
		N[a]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK[c]
Brittleness	1[A]	5.0	4.2	3.8	4.3	4.1	4.0	3.0
	2[B]	4.4	3.7	3.7	3.6	3.4	3.3	2.5
	3[B]	3.6	3.2	3.3	3.7	3.5	2.9	2.4
	4[C]	2.6	2.8	3.6	3.1	2.9	2.8	2.2
		N[a]	K[b]	HY[b]	BB[b]	I[b]	G[b]	MK[c]
Curdiness	1[A]	3.7	3.2	3.2	3.0	3.0	2.8	2.3
	2[B]	3.6	2.9	2.7	2.8	2.6	2.5	2.0
	3[B]	2.7	2.5	2.5	2.6	2.6	2.3	1.9
	4[B]	2.1	2.3	2.7	2.5	2.3	2.4	2.6