# THE INFLUENCE OF ELEVATED TEMPERATURE ON THE CHEMICAL AND SENSORY PROPERTIES OF WHITE CHEDDAR CHEESE

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of EMILY ANN WALSH find it satisfactory and recommend that it be accepted.

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Abstract

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To keep up with consumer demand for aged Cheddar cheese, the dairy industry is tasked with manufacturing quality cheese more quickly. One approach is to reduce production time by utilizing elevated storage temperature during ripening. But while elevated temperatures influence cheese texture and flavor, they also may create an imbalance of flavors, negative changes in texture, and production of off-flavors. However, industry consensus has spurred aging Cheddar cheese at the optimal elevated temperature of 12˚C, to control accelerated aging and reduce these negative outcomes that often result in consumer rejection. Therefore, the objective of this study was to evaluate the chemical and sensory properties of white Cheddar cheese aged at 7.2˚C, 10˚C, or 12.8˚C. Samples were subject to descriptive sensory analysis (n=10), electronic tongue analysis, o-Phthaldialdehyde assay analysis, two-cycle compression, and small amplitude oscillatory shear (SAOS) testing at 2, 5, 8, 10, 11, and 12 months of aging. Additionally, proximate analysis was carried out at month 0 and consumer evaluation (n=120) at 8 and 12 months. Trained panel results showed a loss of cohesiveness and smoothness of mass with higher first-bite fracturability in cheeses aged at elevated temperature, compared to a commercially available reference sample. The electronic tongue data demonstrated high discrimination amongst all samples with discrimination indices ≥ 83%. The instrument could classify samples according to aging month with a validity value of 92.59%. Consumer panel results showed similar overall liking scores for the reference and cheeses aged at 10˚C or 12.8˚C for both 8- and 12-month evaluation. An increase in the degree of proteolysis due to storage time and temperature was observed. Two-cycle compression resulted in a decrease in springiness and cohesiveness as storage time and temperature increased. Solid viscoelastic behavior of the cheese was found using SAOS. Further, many sensory attributes and instrumental parameters, as well as electronic tongue taste analyses and trained panel taste attributes were strongly correlated, illustrating the ability of instrumental analyses to serve as indicators of sensory properties. This study demonstrated elevated storage temperature at 10˚C produce a cheese similar in consumer acceptance to commercial cheese aged for 12 months at 7.2˚C.

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**Dedication**

For my parents, Ann and Joseph Walsh. May this demonstrate my profound respect for your instilment of believing in my inner strength.

# CHAPTER ONE: INTRODUCTION

Change and technological innovation have shaped the dairy industry of the present day. Industrial machinery substituted for human labor has increased the size of facilities as well as spurred a focus on food safety. Automated milking and feeding machinery allowed dairy farms to become more specialized in producing milk (Blayney 2002, Skolrud and others 2007). To sustain demand with fewer farms the amount of milk produced by each cow increased due to farm specialization (Blayney 2002, Skolrud and others 2007). As the number of milk producing farms based in eastern states fell during the 20th century, westward expansion occurred. Milk production in California and southern Idaho grew to sustain the industry and consumer demand (Blayney 2002).

The production of dairy foods has become Washington's second largest agricultural commodity following apples (Neibergs and others 2013). Between 1974 and 2002 Washington saw a 21% decline in the number of farms with four times the cows (Dairy Farmers of Washington 2016). Across 416 farms, over 275,000 cows are used for milk production (Dairy Farmers of Washington 2016). Whatcom county has 108 dairies, Yakima, 67 and Whitman County, one; Knott Dairy in Pullman. (Dairy Farmers of Washington 2016, Skolrud and others 2007). With 90% of all milk produced and processed in WA State, numerous job opportunities exist. Companies within the Pacific Northwest include Darigold Inc., Glanbia Foods Inc., and Tillamook County Creamery Association. The WSU Creamery is home to the well-known aged white Cheddar cheese packaged in a can, more commonly known as

Cougar Gold®. Over 250,000 cans of Cougar Gold® are produced every year for direct sale or mail order. WSU also creates educational opportunities connecting with various campus departments by funding research.

Dr. Norman Shirley Golding discovered how to package and store Cougar Gold® in a can in a project funded by the US government and the American Can Company. Golding attempted to limit the carbon dioxide production by the cultures in the cheese so as the cans did not explode (Marsh 2017). He experimented with countless bacteria cultures including *lactobacillus helveticus* WSU19. This culture aided Golding in packaging Cougar Gold® in a can, and it also influences the sensory properties of this aged cheese.

Aged Cheddar cheese is an increasingly popular dairy product, thus cheese processors require new technology to produce quality cheese with flavors and textures of aged cheese more rapidly. Numerous methods of accelerating maturation time have been studied including the use of adjunct cultures, additional enzymes, and elevated ripening temperature (Kilcawlley and others 2012, Azarnia and others 2006). These methods are employed to reduce ripening time and resources; elevated temperature is the simplest. While Cheddar cheese is normally aged between 4 and 8˚C for 8 – 10 months, the industry consensus of the temperature to reduce maturation time by 60 – 75% is 12˚C (Folkertsma and others 1995, Hannon and others 2005). However, problems associated with an elevated maturation temperature include the development of unbalanced flavors, unpredictable production of off-flavors, and an increased risk of microbiological spoilage (Hannon and others 2005, Marsili 1985). Elevated temperature exerts its influence during ripening by catalyzing the biochemical events of glycolysis, lipolysis, and proteolysis. The macro and microcomponents of cheese are broken down or transformed during these processes, resulting in a weakening curd structure and the production of volatile flavor compounds. These compounds contribute to flavor (aroma and taste) in a complex way. Hundreds of compounds make up Cheddar cheese flavor although any one compound can influence a disproportional balance resulting in poor quality cheese (Mulder 1952).

The advent of quality problems has spurred research using both sensory and instrumental analyses. Elucidating the roles of specific volatile flavor compounds and their quantification using instrumental analyses has yielded insight into the complexity of Cheddar cheese flavor (Wong and others 1975, Weimer and others 1999, Drake and others 2007). Rheological testing has aimed to describe changes in the microstructure of cheese during aging and their effect on quality (Lawrence and others 1987, Rogers and others 2009, Melito and others 2013). Sensory analysis has provided the ability to assess consumer preference and liking, as well as determine the influence of changes made to the cheesemaking and ripening processes on the quality of Cheddar cheese (Muir and others 1995, Drake and others 2001, Hannon and others 2005). During the maturation process, sensory analysis can be used to create attributes profiles of cheeses demonstrating the development and progression of flavor and texture. All are vital to the knowledge of Cheddar cheese and its ripening.

The main objective of this research was to determine the influence of elevated temperature on the chemical and sensory properties of white Cheddar cheese. The first study utilized both instrumental and sensory analyses to understand changes during maturation at elevated storage temperature. The second study employed rheological and sensory analyses to evaluate the influence of elevated temperature on the texture and mechanical properties during aging.

The specific objectives were:

1. To determine the influence of elevated temperature on the consumer acceptance of Cheddar cheese and sensory attributes during cheese maturation. It was hypothesized that elevated aging temperature would result in higher intensities of maturation and aging attributes, as well as the development of an unbalanced flavor leading to consumer rejection at elevated temperatures over a long storage time.
2. To analyze changes in the volatile taste compounds throughout maturation using an electronic tongue and determine correlation to sensory data. It was hypothesized the electronic tongue would discriminate among aged samples.
3. To determine the effect of elevated temperature on the texture and mechanical properties of Cheddar cheese throughout aging. It was hypothesized an increased rate of breakdown of the Cheddar matrix would be observed resulting in a crumblier cheese earlier in maturation.

# CHAPTER TWO: LITERATURE REVIEW

## Economic Importance of Milk and Cheese

Due to consumer demand, dairy products, including fluid milk, cheese, cream, butter, yogurt, ice cream, and whey create a vast market in the United States. With over 200 billion pounds of fluid milk produced nationwide each year since 2012, the United States is the top producer of milk in the world (USDA ERS 2017a). In 2015, approximately $36 billion (USD) in milk sales were recorded from processors, consumers, and manufacturers. In 2010, despite the decrease in fluid milk consumption over the last three decades, the commodity as an export yielded a revenue around $4 billion (IUF 2017, USDA ERS 2016a). The sale of milk as a raw material in other dairy products, specifically cheese, has led to a steady increase in consumption (USDA ERS 2017b, IUF 2017, USDA ERS 2016a). The rising demand has been facilitated, in part, by the diverse selection of cheeses readily available to consumers (USDA ERS 2017b). In the United States, Mozzarella and Cheddar account for the highest consumption per capita based upon variety in 2015, totaling 11.27 and 10.16 pounds, respectively (USDA ERS 2016b). This demand has fueled industry cheesemakers to find technological advances, especially with aged Cheddar cheese, that reduce production time and resources. This review presents the current literature on the composition of Cheddar cheese and how aging affects its chemical and sensory properties.

Economic Impact of Washington's Dairy Industry

The growth and development of the industry in Washington State has greatly impacted the agricultural economy of the state. Following apples, dairy products are Washington's second largest agricultural commodity (Neibergs and others 2013). Washington is ranked tenth, nationally, for total milk production, and in 2015, produced 6.6 billion pounds of milk (Dairy Farmers of Washington 2016, Skolrud and others 2007). Between 2006 and 2011, an economic growth of $491 million (51%) due to an increase in cow herd size was obtained. Washington's dairy industry's profit, totaling $5.2 billion, can be parsed into manufacturing and farming divisions; $2.36 billion and $2.58 billion, respectively (Neibergs and others 2013). In 2011, the number of jobs in the industry totaled around 18,000 with 12,159 on farms and 4,497 within manufacturing companies (Neibergs and others 2013). Cheese manufacturing companies such as the Darigold, Appel Farms, and River Valley Cheese produce an assortment of cheese from Cheddar to Dutch varieties and artisan cheeses. The high quality of these cheeses can be attributed to precise knowledge of the cheesemaking process and the influence of milk composition.

## Cheddar Cheesemaking

Overview

Much of industrial cheesemaking begins with similar processes despite the cheese variety (Singh 2003). Milk is standardized and pasteurized. Addition of starter culture sours milk via lactic acid bacteria (LAB). When the desired acidity is reached, rennet is added. Rennet is a complex of enzymes, chymosin being the major component, which facilitates the coagulation of milk proteins into curd. Curd is separated from the water-soluble portion, whey, and the treatment of that curd is the most influential step in the cheesemaking process, regarding texture (Walstra and others 2006).

Cheddar cheese, specifically, undergoes the Cheddaring process to obtain its unique texture and body. During Cheddaring, the curd is milled, salted, and pressed in hoops to obtain a close texture, further expelling whey (Walstra and others 2006). Cheddar cheese made with raw milk must be aged for at least 60 days at 1.7˚C (35˚F) for safety purposes (U.S. Government Publishing Office 2017). After the various processing steps that induce biochemical changes, milk (pH 6.5 – 6.7) is transformed into cheese (pH 5.0 – 5.2) and ready for ripening or aging (Singh and others 2003). Since many manufacturers attempt to obtain distinct characteristics of their cheese to entice consumers, steps in the Cheddar cheesemaking process may be individualized with varying temperatures, pressures, and amount of additive substances. The basic steps of the process are discussed next.

<u>Pasteurization</u>

Pasteurization using high temperature/short time (HTST) processing is a common practice for modernized cheese makers. This first step in the cheesemaking process holds milk at 72˚C for a minimum of 15 seconds (IDFA 2017) to kill pathogens and spoilage organisms, as well as inactivate enzymes. Pasteurization reduces microbial load and kills pathogens such as *Staphylococcus aureus*, *Listeria* *monocytogenes*, and *Salmonella* species. ensuring a safe product for the consumer (Hutkins 2006b). At pasteurization temperature, the proteins within the milk do not denature, apart from some serum proteins and agglutinin.

The quality of the cheese is influenced by pasteurization as many microflora and enzymes present in raw milk negatively influence the flavor and texture properties become inactive (Hutkins 2006b). On the other hand, some LAB killed with pasteurization no longer produce the flavor-forming substances found in raw milk cheese, which results in desiring a more piquant flavor and character in their cheeses (Mulder 1952). The highest concentrations of volatile compounds when quantified in raw milk cheeses occur at 4 and 6 months. Sensory analysis pinpointed the attributes affected by pasteurization, with intensity ratings of aroma and flavor intensity, acid/sharp aroma, creamy/milky aroma, sulphur/eggy flavor, sour/acid flavor, bitter flavor, being lower in pasteurized compared to raw milk cheeses (Rehman and others 2000). It was Mulder's hope to have the dairy industry pay as much attention to the importance of flavor as they did to safety, thus changing the direction of future research to include strain specificity and influence on flavor (Ayad and others 2000).

<u>Acidification with Starter and Adjunct cultures</u>

The industrialized Cheddar cheesemaking process relies on starter and adjunct cultures that ensure consistent and predictable product attributes after production and ripening. The dairy industry is the main user of bulk cultures. To ensure viable and active organisms of high cell counts, bulk cultures are often added in several steps (Walstra and others 2006). Inoculation of Cheddar cheese with cultures from bulk tanks is 1% (w/w) of milk volume at 30 – 32˚C (Walstra and other 2006). After inoculation the mixture is stirred, allowing the starter cultures to produce lactic acid, souring the milk. Any adjunct cultures or non-starter lactic acid bacteria (NSLAB) added to the cheese milk usually do not influence the acid development, as they do not ferment lactose, but do influence flavor development of the cheese during ripening (Walstra and others 2006).

*Lactic Acid Bacteria*

Dairy starter cultures can be comprised of one or many different strains of LAB, which ferment lactose, forming lactic acid as the main end-product (Walstra and others 2006). These gram-positive, non-spore-forming, acid-tolerant, catalase-negative organisms sour milk anaerobically, but tolerate aerobic conditions (Hutkins 2006a, Walstra and others 2006). Organic carbon is required for these heterotrophic chemoorganotrophs, since they need it as a source of both carbon and energy (Hutkins 2006a). Most LAB are either mesophiles or moderate thermophiles, growing at temperature optima at 30 and 42˚C, respectively. In cheesemaking LAB that engage in metabolizing sugars via the homofermentative pathway are more common. In that pathway lactose is almost exclusively converted (>90%) to lactic acid, while in the heterofermentative pathway >85% is converted to lactic acid with additional products of acetic acid, ethanol, and carbon dioxide.

*Lactic Acid Production*

Acid production by starter cultures influences coagulant (rennet) activity, hydrolysis of protein, and production of $CO_2$ (Walstra and others 2006). A short, crumbly, and brittle texture, not commonly desired by Cheddar manufacturers, occurs when acidification is carried out too quickly by certain thermophilic cultures or the final pH of the cheese is less than 4.95. As lactic acid is produced the pH drops and demineralization of casein influences the texture of the cheese. When casein is demineralized, calcium accompanying negatively-charged casein amino acid residues are displaced by protons and drains with the whey. With less calcium, the cheese holds fat poorly and a brittle texture after aging ensues (Walstra and others 2006).

<u>Coagulation of Cheese Milk</u>

Acidification with starter cultures produces the optimal environment for the coagulation of milk to begin. As the pH of cheese milk decreases toward 5.5, calcium displacement occurs and casein solubility decreases; the casein micelles dissociate into smaller aggregates (Singh and others 2003). With the addition of rennet, the enzyme chymosin transforms the milk into curds. At a basic level, a casein network entraps fat globules, moisture, and calcium to form curd. Casein precipitates under the influence of chymosin, trapping the milkfat within the mass. A gel is formed when casein has reached minimum solubility at its isoelectric point of 4.6. The gel will form within 30 minutes, awaiting cutting and separation from the whey (Hutkins 2006b).

*Rennet Coagulation*

Rennet, first isolated from the stomach lining of calves, facilitates the clotting of cheese milk in two steps. The most important enzyme in this clotting process is chymosin, related to the common stomach enzyme, pepsin (Walstra and others 2006). Chymosin is an endopeptidase that functions to split proteins (κ-casein) into large fragments. In milk, chymosin hydrolyzes κ-casein, cleaving at $Phe^{105} - Met^{106}$, removing the hydrophilic peptides – a glycomacropeptide fraction (Hutkins 2006b) – enabling calcium-mediated precipitation (Walstra and others 2006, Singh and others 2003). Forming bridges between the micelles, calcium ions aid in the development of the curd (Singh and others 2003). The para-κ-casein, molecule with exposed anionic phosphate groups, is stabilized by $Ca^{2+}$ at 30˚C. Held together by electrostatic and hydrophobic interactions within the casein micelle structure, calcium is bound as calcium phosphate (Hutkins 2006b). Not only does rennet coagulate milk, but what is retained in the curd later influences the rate of proteolysis within the cheese during ripening (Walstra and others 2006).

Separation of Curd and Whey

Once the rennet coagulated milk gel has set, the separation of curd and whey can be carried out. The milk gel is cut with wire knives into die-sized curds, enhancing syneresis since the increased surface area allows for easier removal of water (whey). The initial rate of syneresis is influenced by the pH of the curd and as acid development increases from LAB starter culture activity, the pH drops and syneresis is further enhanced. After cutting, the curd is left to heal for ten minutes, and then stirred until the temperature reaches ~38˚C. The heating is done gradually to avoid hardening of the curd surfaces, which influences syneresis. The amount of water removed from the curd later influences the moisture content of the cheese and therefore the texture. The separation of curd and whey continues throughout the future steps in the cheddaring process (Hutkins 2006b, Walstra and others 2006).

Cheddaring

Once the whey is drained, the separated curds begin to mat together. The cheese mass is cut into smaller parts, or loaves. Not only does Cheddaring, a series of rotations, stacks, and flips of loaves during heating, provide time for acid development and pH change within the curds, but it also influences the elasticity of the curd, making it more plastic (Hutkins and others 2006b). As the curds mat together and pores fuse, a water content of 1 – 2%, in the cheese is retained. The stacking and flipping of the loaves squeezes out entrapped air, resulting in a close textured cheese (Walstra and others 2006). The gravitational forces of the stacked loaves enable linearization and stretching of casein. Cheddaring takes about two hours as desired lactic acid levels, 0.40 - 0.60%, must first be reached before continuing to the milling and salting steps of cheesemaking.

Milling, Salting, Hooping, and Pressing

A milling machine, or Cheddar mill, cuts the loaves of curd into smaller (thumb-sized), uniform pieces. The curds have a squeaky, rubbery texture and are bland. A direct and evenly distributed application of salt from 2 – 3% and stirring of the curds further enhances syneresis and suspends the acid production of starter cultures. The salted curd is transferred to hoops, where whey is expelled through perforations when pressed and wrapped with cheese cloth. Curds are pressed under 20 psi for 12 – 16 hours, allowing for a final cheese moisture content around 38% (Hutkins 2006b). All steps in the cheesemaking process influence the development of flavor and texture which occurs during the ripening period.

Ripening

The complex process of ripening, beginning after manufacturing, results in flavor and texture formation, and involves both microbiological and biochemical changes in the curd. Microbiological changes that occur during ripening include starter cultures undergoing lysis and dying, which release lipolytic enzymes into the cheese matrix; non-starter lactic acid bacteria (NSLAB) growing; and secondary microflora or adjunct cultures developing to influence the flavor and aroma of cheese (McSweeney 2004). Cheese ripening is governed by three major biochemical processes; proteolysis, glycolysis, and lipolysis. These three primary events occur during cheese ripening through various pathways and mechanisms. These interrelated events influence both the flavor and texture of aging cheese, but also serve as precursors for secondary events. Precursors, such as fatty acids and amino acids, lead to the production of volatile flavor compounds through secondary reactions. The rubbery, elastic curd will soften during aging when the casein matrix of the curd is disrupted through hydrolysis by various proteolytic enzymes (McSweeney 2004). The amount of time given to the aging process, as well as other factors such as temperature, pH, salt concentration, and moisture content of the cheese all influence the microbiological and biochemical changes that occur during ripening. The various time and temperature regimes employed in ripening and the biochemical processes that occur during ripening are outlined below.

*Glycolysis*

Glycolysis in cheese refers to the metabolism of milk sugar, lactose, and citrate, as well as the metabolized product, lactate. Lactose, metabolized to lactate by LAB during manufacturing, becomes a precursor for metabolism through racemization, microbial, and oxidation pathways (McSweeney 2004). Lactose metabolism with mesophilic starter bacteria ferments to form the L+ isomer of lactate (Singh and others 2003). Racemization of L-lactate results in the formation of D-lactate through oxidation with L-lactate dehydrogenase and reduces pyruvate to D-lactate with D-lactate dehydrogenase. This influences the development of Ca-DL-lactate crystals, commonly found in cheese as white specks (McSweeney 2004). Depending on the type of LAB, lactate can be oxidized to form acetate, ethanol, formate, and $CO_2$, all which influence either the texture or flavor formation in cheese (Fox and others 2000). Citrate is present in Cheddar cheese curd in low levels; 0.2 – 0.5% (McSweeney and others 2004). Citrate-positive ($Cit^+$) strains metabolize citrate to form $CO_2$ and diacetyl. Notably, diacetyl elicits a butter-like flavor and aroma in cheese.

*Lipolysis*

During ripening, fatty acids are liberated when triglycerides, short-chain fatty acids in ruminant milk, are hydrolyzed by lipases. These lipases are found in milk and coagulant as well as adjunct cultures. Resulting cheeses, such as Cheddar, have low lipolytic activity with lipases originating from cheese microflora (McSweeney 2004). Liberated free fatty acids are precursors for secondary events, which produce volatile flavor compounds.

*Proteolysis*

Proteolysis is the most important and complex primary biochemical event to occur during ripening as it influences both texture and flavor of cheese through the hydrolysis of casein. Catalyzed by proteolytic enzymes, such as proteinases and peptidases – native to milk, coagulant, starter and NSLAB, and adjunct cultures – casein is hydrolyzed at various sites to produce a range of peptides and softens the cheese curd. Further breakdown eventually influences the flavor of cheese through the production of various volatile flavor compounds.

*Ripening Time*

Aged from weeks to two years, the internally ripened Cheddar cheese develops flavor and texture over time (Law and others 1979). The optimal ripening time depends on necessary characteristics of the cheese, as well as the rates of biochemical events, which are very much influenced by microflora within the cheese, composition, and environmental conditions. As secondary events occur, free amino and fatty acids are produced as the cheese matrix degrades to form volatile flavor compounds and appropriate texture. Changes in Cheddar cheese aged from three to 12 months have been documented (Muir and others 1996). Sensory character was found to change slowly as aging progressed and after two months, characteristics during early maturation could not be used to predict cheese later in aging. Monitoring the change in sensory characteristics demonstrates the ability of research to create a profile of the cheese throughout its aging time. The 'Cheddar' aroma and flavor increased throughout aging, while the 'creamy' flavor and aroma decreased. Key characteristics that developed, increasing over time, were 'sulphur/eggy', 'fruity', 'acid', 'bitter', and 'mouth-coating' character. Similar results with aged cheeses having higher ratings of "umami", "sulfur", "brothy, and "free fatty acid" attributes have been obtained (Young and others 2004). Conversely, young cheeses were described by "whey", "diacetyl", and "milkfat/lactone". Consumers perceived intense "Cheddar" flavors regardless of the age. Utilizing preference mapping, six segments of consumers preferred different Cheddar cheeses, varying widely in their flavor profiles due to length of time aged. Segment 1 preferred cheese characterized by young flavors and low intensities of brothy and sulfur flavors. Segment 2 preferred the same cheeses as segment 1, but without attribute preferences. Segment 3 preferred young and brothy flavored cheese, while segment 4 liked all cheeses except one characterized by young and low sour taste. Segment 5 disliked young cheeses and segment 6 preferred cheese with aged flavors of nutty, fruity, and sweet, but not brothy (Young and others 2004). This segmentation shows consumers prefer either cheese with young or mature attributes. To reach its desired flavor and texture characteristics, the length of ripening time for aged cheese has proven costly for manufacturers.

*Accelerated Ripening*

To keep up with consumer demand, strides have been taken to reduce production time and resources with accelerated ripening. Various methods have been employed, including elevated ripening temperatures, adjunct cultures, addition of enzymes, and genetically engineered starters. (Azarnia and others 2006, Kilcawley and others 2012). This set of methods pinpoint a diverse and expansive range of possible techniques, although the ease of accomplishing accelerated ripening is hindered by challenges. Given the delicacy of balanced flavor and texture development via primary and secondary biochemical processes, acceleration often leads to an imbalance of flavors or off-flavor development (Marsili 1985), as well as increases the risk of microbiological spoilage (Hannon and others 2005). On the other hand, a faster aging time would also result in more profit for the cheesemaking industry with elevated temperature as the easiest way to achieve reduced aging time.

*Ripening Temperature*

Ripening temperature governs the time required and biochemical process activity during aging. Cheddar cheese is normally aged at low temperatures (4 – 8˚C) compared to other cheese varieties (Azarnia and others 2006). Elevated temperature (9 – 12˚C) can be problematic due to the rates at which proteolysis, glycolysis, and lipolysis occur are increased. The use of extended ripening time at elevated temperature may lead to flavor imbalance due to this catalysis. Using an elevated temperature of 11˚C, the accumulation of propionic acid and free amino acids ensued at significantly faster rates than cheese aged at 6˚C indicating the acceleration of proteolysis and lipolysis (Marsili 1985). The acceleration of these biochemical processes by using elevated temperature has been shown to increase flavor scores (Folkertsma and others 1996). In cheese, following three months of ripening at 16˚C, texture deteriorated and atypical flavor was noted, resulting in poor quality. From this study, the optimal temperature for commercial acceleration of ripening was identified as 12˚C (Folkertsma and others 1996). Atypical flavor with extended maturation at elevated temperature spurred the use of time/temperature combinations in the early stages of ripening to control aging. Utilizing the time/temperature combinations of 20˚C for one week and 12˚C for six weeks, aging was accelerated by 2 months (Hannon and others 2004). Through the differences in aroma and flavor profiles produced by varying temperature, NSLAB were determined to contribute to the perception of maturity through the formation of volatile compounds (Rehman and others 2000). Although the study did not use elevated temperature the cheese samples at 8˚C contained higher levels of volatiles than cheeses aged at 1˚C. The previously completed studies demonstrate the advantages, disadvantages, and influence of elevated temperature on the maturation of Cheddar cheese. Environmental parameters like time and temperature during aging can influence the quality of cheese due to the macro and microcomponents of cheese.

**Macro and Microcomponents of Cheese**

Transforming milk, an oil-in-water emulsion, into cheese is a form of food preservation requiring ten times the amount of milk as the final cheese yield. Milk composition and quality influences the quality of the resulting cheese (Bobbo and others 2017), and thus, before using the milk for cheese production, milk composition is often standardized to reach a desired fat content in the cheese. According to the Code of Federal Regulations (21CFR133.113, 2017), Cheddar cheese cannot contain more than 39% moisture (w/w%), with a minimum of 50% milkfat solids.

Cheddar cheese, a hard, internally ripened cheese, is composed of about 38% water, 31% fat, 28% protein, 2% salt, 1% minerals, and <1% lactose (Hutkins 2006b). Each component will be discussed below, as well as how variations influence the sensory and chemical properties of Cheddar cheese.

<u>Water</u>

Water, quantifiably the largest individual component of cheese, plays several roles in cheese formation and maturation. Water becomes entrapped during curd formation within the casein cheese matrix. Through the duration of the Cheddaring process, whey (water) is expelled from the curd due to its porous nature. Higher moisture retained in the curd results in a cheese with a softer texture (Gunasekaran and others 2003). Water within the curd becomes a medium for many water-soluble compounds that influence the flavor and texture of cheese, including fatty acids, lactose products, bacteria, enzymes and amino acids (Kosikowski and others 1958). Proteolysis is facilitated by the hydrolysis of peptide bonds; as the protein matrix is broken down the “free” water within the curd is reduced resulting in the development of a harder texture with aging (Irudayaraj and others 1999, O’Mahony and others 2005)

Fat

In cheese, fat is found in globular form within the protein network and influences both sensory and rheological properties (Gunasekaran and others 2003). Triglycerides containing fatty acids with varying chain lengths and degrees of saturation compose much of the fat in milk. Milkfat later influences the body, texture, and flavor of the cheese. Fat produces weak points in the protein network (Rogers and others 2009). One study explored the difference in strain weakening properties among full-fat, low-fat, and reduced-fat cheeses. Sensory evaluation found the firmness of cheeses decreased over time, with full fat cheeses losing their springiness consistently during aging. Overall, full fat cheese broke down more during aging, becoming more cohesive and adhesive than low-fat cheeses (Rogers and others 2009).

Protein

Comprising 80% of total proteins, casein is the major protein in milk and thus cheese. The mixture of $\alpha_{s1}$-, $\alpha_{s2}$-, β-, and κ-casein is arranged in micelles in milk. Casein is later cleaved into paracasein and stabilized though calcium mediated precipitation when aggregated by rennet to form curd. The remaining 20% of protein are whey proteins such as β-lactoglobulin and are expelled from the curd during the separation from whey (Hutkins 2006b, Walstra and others 2006). Protein influences the structural rigidity of the cheese curd and throughout aging is broken down influencing both flavor and texture during proteolysis (Kosikowski and others 1958).

Salt

The addition of 2-3% salt creates an environment conducive to microbial and enzymatic activity, thus salt is used to control the ripening of cheese. The salt-to-moisture ratio (S:M) influences microorganisms and enzymes within cheese during ripening. The level is related to how inhibitory the environment is, with a higher ratio obstructing the function of microorganisms and enzymes within the curd. However, if the level is too low, the development of off-flavors may occur when unfavorable microflora become active. The influence of salt on starter culture activity allows manufacturers to control the pH of Cheddar cheese, often resulting in calcium lactate crystal formation (Agarwal and others 2008). To aid in the development of flavor, texture, and quality of Cheddar cheese, the desired S:M is 4.5 – 5.0 (Hutkins 2006b). Salt was found to directly affect the taste of cheese due to enhancement of bitterness, with lower salt levels and suppression of sourness with higher levels. Whereas, higher salt Cheddar cheese at 9 months of age was perceived as being more soft and moist as well as had longer fracture than normal-salt cheeses (Møller and others 2013).

Minerals

Minerals such as calcium, magnesium, and potassium are also present in cheese. Calcium is particularly important since it is present within casein micelles and stabilizes the curd (Hutkins 2006b, Walstra and others 2006). Calcium content of cheese, determined when the curd is separated from whey, is inversely dependent upon pH (Lawrence and others 1987). Due to its presence within cheese structure, calcium influences the moisture content of cheese with lower levels promoting moisture retention of the curd (Upreti and Metzger 2006). Calcium also bolsters the health benefits of cheese. The primary source of dietary calcium, approximately 80%, in the American diet comes from the consumption of dairy products (McCarron and others 1999, Wang and others 2008). An inverse association between dietary calcium intake and the prevalence of hypertension and level of arterial pressure supports the blood pressure-lowering effect of calcium (McCarron and others 1999, McCarron and others 1994).

Lactose

The carbohydrate disaccharide of glucose and galactose make up the reducing milk sugar – lactose – which is converted to lactic acid when starter cultures are added. The metabolism of lactose to lactic acid and other products occurs throughout various pathways depending on the type of Lactic Acid Bacteria (LAB) acidifying the milk (Walstra and others 2006). Cultures commonly utilized in the cheesemaking process include the *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Leuconostoc* species. More specifically, *Lactococcus lactis* ssp *cremoris* and ssp *lactic* are used in Cheddar cheesemaking (Singh and others 2003). Lactose is phosphorylated during its transportation across cell membranes and reduces to its phosphorylated monomer units, galactose and glucose, through hydrolysis by ß-galactosidase. After lactose metabolism, glucose and galactose can follow numerous pathways, creating end products such as L-lactate, D-lactate, and ethanol. Some products influence acidity (lactic acid), while others affect the aroma, flavor, and texture of the cheese during ripening. Higher lactose retention in cheese curd is influenced by the pH at which the cheese was salted, as well as calcium and phosphorus levels (Upreti and Metzger 2006).

**Sensory Methods of Cheese Analysis**

Cheddar cheese sensory properties and their perception have been at the forefront of dairy industry research to help describe changes in cheese with processing or aging, as well as understand the attributes that consumers want in a product. Sensory science coupled with instrumental analysis have assisted researchers with determining the influence of variables acting on Cheddar cheese during the cheesemaking process and maturation period. The perception of macro and microcomponents in cheese, which influence cheese flavor and texture, ultimately affects overall preference and acceptance by consumers.

<u>Flavor and Taste Perception</u>

The sensory perception of flavor itself is where the underlying complexity of Cheddar cheese flavor lies. Flavor is not singular; the mouth perceives tastes (sweet, sour, salty, bitter, and umami), trigeminal responses from chemical feeling factors, and volatile aroma compounds (Croissant and others 2011, Meilgaard and others 2007). The perception of volatile aroma compounds is not perceived solely by the gustatory system, but also by the olfactory system, making flavor a multimodal sensory experience. Detected by the olfactory epithelium, airborne volatile compounds from cheese can be sensed ortho- or retronasally (Croissant and others 2011). Retronasal detection of flavor occurs when volatiles released in the mouth by chewing and swallowing pass through the nasopharyngeal passage to the olfactory epithelium (Meilgaard and others 2007). When the nose is blocked the perception of aroma compounds is limited; only Cheddar cheese's bitterness, saltiness, sweetness, sourness, and umami taste would be perceived. It is the olfactory system that brings the flavor of cheese to life, allowing the nutty, sulfur, and free fatty acid aromatics to contribute to flavor. Because of this, the numerous volatile compounds perceived during mastication of Cheddar cheese have been studied to identify what Cheddar cheese flavor is and where it comes from – see Cheddar Cheese Flavor section.

<u>Texture Perception</u>

Like flavor perception, the perception of texture, or the sensory perception of cheese structure, is multifaceted with sight, touch, and hearing all influencing its properties (Foegeding and others 2007). The first consideration made is how the product looks; consumers eat with their eyes. Next, they use their hands to touch and finally, the surfaces within their mouths during mastication allow them to fully experience the texture of food. Texture is a critical, primary quality attribute based on this sensory experience with a large influence on consumer acceptance preference (Gunasekaran and others 2003). Indeed, research has found a correlation between overall texture liking and overall consumer acceptance (Young and others 2004). Limited to the experienced sensations during mastication, such as the manipulation of a chewed mass as it mixes with saliva by the tongue, texture is difficult to measure using one instrument, technique or sensory attribute (Foegeding and others 2007). Given the individuality and subjectivity of the chewing experience itself, accurate measurement remains challenging (Gunasekaran and others 2003). With sensory science at the forefront, descriptive analysis has attempted to accurately measure this complex human perception.

Development of a Standard Lexicon

The basis of sensory evaluation is the lexicon facilitating the knowledge obtained about the samples being evaluated. Each with their own sensory attribute vocabularies, researchers have studied the intensity of appearance, flavors, tastes, and textures elicited in cheese (Muir and others 1995). To facilitate communication among industry, government, and academia and advance worldwide research on Cheddar cheese flavor, a universal descriptive language for Cheddar cheese was developed (Drake and others 2001). Cheese samples were profiled using a descriptive sensory evaluation panel to develop this lexicon. The 17 validated terms created a standardized language and a universal intensity scale. Additionally, the lexicon was cross-validated by employing sensory panels using the descriptive language in three different locations (Drake and others 2002). Sensory language of texture evaluation includes attributes that relate to temporal aspects of texture and can be parsed into three categories: hand terms, first-bite terms, and chewdown terms (Foegeding and others 2007).

Cheddar Cheese Sensory Attributes

Cheddar cheese must display various body, texture, and flavor sensory attributes to be identified as high quality in the dairy industry. A well-balanced flavor and developed texture directly affects the sensory quality of cheese, which has been used as a basis for grading in the industry following the advent of scorecards by the American Dairy Science Association (ADSA). A good quality product given a high score was determined by a judge knowledgeable in the sensory characteristics of the product, as well as the desired qualities (Partridge and others 2009). The desired characteristics of the "ideal" Cheddar cheese are:

> "(1) a clean, delicate, pleasing aroma and, when cured, a nutty flavor; (2) a firm and springy body, showing smoothness and waxiness (if cured) when worked between the thumb and fingers, and slight curdiness if fresh; (3) a texture that reveals a smoothbore (few or no openings); (4) uniform, translucent color, whether colored or uncolored (when fresh, it may be slightly seamy); and (5) a smooth finish that is clean, well-shaped, uniform in dimensions, and overall size, with a complete, airtight package, and mold free" (Partridge and others 2009).

Cheddar Cheese Flavor

Cheddar cheese flavor is influenced by the combinations of hundreds of volatile and non-volatile compounds which stimulate its perception in the mouth and nose. The idea that a single compound can be identified to constitute Cheddar cheese flavor was reshaped when research explained flavor compounds in cheeses are hard to individually recognize due to their well-balanced proportions (Mulder

1952). The combination of compounds contributes to three classes of taste (flavor and texture); general taste of cheese, typical taste of the kind of cheese, and variation within cheese of the same kind. The blend of these compounds with not one overwhelming the overall characteristic flavor was the advent of the component balance theory (Kosikowski and others 1958). Any deviation from proper balance influences defective flavor.

Volatile compounds result from secondary events of the biochemical processes that occur during cheese ripening: glycolysis, lipolysis, and proteolysis. Lipolysis of triglycerides can lead to the formation of free fatty acids, which are further metabolized to form flavor compounds in cheese. Free fatty acids influence the flavor and quality of cheese by imparting various aromas like sharp, goaty, or rancid (Singh and others 2003). Although fatty acids are essential to the aroma of Cheddar cheese, the exclusion of them from cheese models did not change the overall sensory perception of the food; omitting any of the fatty acids did not change the ratings for ‘Cheddar’ during sensory analysis (House and others 2002). Ethanol produced from glycolysis can interact with fatty acids to produce ethyl esters and impart fruity characteristics, common in Cheddar cheese (McSweeney 2004). Intramolecular esterification facilitates the formation of cyclic compounds, called lactones, from hydroxyacids derived from free fatty acids. In elevated levels, high molecular weight lactones can impart rancid characteristics in Cheddar cheese (Wong and others 1975).

The metabolism of free amino acids, a secondary event which influences the production of volatile compounds and cheese flavor, is the principal contribution of proteolysis. Amino acids can be converted by aminotransferases to α-ketoacids and with the transfer of amino groups, new amino acids are formed (McSweeney 2004). Through this pathway, glutamic acid is formed, which is common source of the umami taste in Cheddar cheese (Drake and others 2007). Methionine catabolism leads to the formation of numerous volatile sulfur compounds, with both methional and methanethiol contributing to the characteristic aroma of cheese (Weimer and others 1999). Despite the wealth of knowledge from research about the mechanisms and characterization of flavor development, further clarification is needed to fully understand the ripening of cheese varieties due to the numerous factors – milk enzymes, residual coagulant, starter LAB, secondary starters, and NSLAB – that catalyze primary and secondary biochemical processes.

Cheddar Cheese Texture

Numerous studies have attempted to elucidate the roles and influence of cheese microstructure and environmental conditions, using both sensory and rheological testing, on the texture of Cheddar cheese from curd development through ripening. Various factors that affect cheese texture were outlined and related to the microstructure to form a full picture of the development of cheese texture (Lawrence and others 1987). Markedly, the events in the first two weeks of ripening were described. The casein network is weakened by proteolytic enzymes, softening the rubbery curd. Proteolytic enzymes, often from residual rennet, hydrolyze the $\alpha_{s1}$-casein fraction into a smaller $\alpha_{s1}$-I peptide. After the first two weeks, the texture changes slowly over time and is largely affected by the pH, salt to moisture ratio, and storage temperature. Spectroscopic methods have been used to investigate texture development of Cheddar cheese by correlating texture profile analysis parameters to key chemical groups (Irudayaraj and others 1999). Cheese hardness and gumminess increased during aging, while springiness and cohesiveness changed slightly. With the knowledge of what affects the development of hardness (fat, moisture, and protein content), it was determined that fat content affected hardness more so than protein.

**Instrumental Methods of Cheese Analysis**

Instrumental analysis has provided the background needed to further understand cheese characteristics. Commonly, instrumentation complements sensory techniques to profile characteristics of cheese, providing insight on the development of flavor and texture attributes. Instrumental analysis has been applied to explore the biochemical processes that occur during ripening. Biochemical metabolites have been identified using high performance liquid chromatography and gas chromatography to find the best predictors of the lipolytic, proteolytic, and glycolytic age of Cheddar cheese (Marsili 1985). The production of propionic and acetic acid best predicted glycolytic age; $C_{10}$, $C_{12}$, $C_{14}$, $C_{16}$, lipolytic age; and leucine, methionine, and glutamic acid foresaw proteolytic age.

The “simplicity” of sensory textural attributes, such as firmness, cohesiveness, hardness, often calls upon fundamental and empirical rheological tests to bolster understanding of sensory texture through mechanical properties. The individuality of texture perception by consumers negates the ability of instrumental analysis to mimic the human sensory process (Foegeding and others 2007). The goal of these measurements is to determine how physical properties relate to the perception of texture. Fundamental rheological tests measure physical properties through the application stresses or strains at a given rate with a defined geometry (Foegeding and others 2007). These tests can be related to microstructural and molecular mechanisms (Foegeding and others 2003). Empirical testing is less limited in its testing parameters, allowing the use with any material, as these tests cannot provide information regarding microstructure. Small and large oscillatory rheology and Texture Profile Analysis are examples of fundamental and empirical testing, respectively. Rheological testing has revealed strain weakening behavior of cheese (Rogers and others 2009). They explained the weak points induced by fat become initiation sites for fracture during aging. Correlations between sensory and rheological behavior to understand structure and breakdown were investigated through the comparison of Cheddar, Mozzarella, and American cheeses (Melito and others 2013). Creep testing showed that Cheddar cheese had a more firm and elastic structure compared to the other cheeses. Firmness and fracturability, defined using sensory analysis, further explained this rigid structure of Cheddar as it was correlated with the non-linear viscoelastic behavior observed through large amplitude oscillatory shear testing.

Sensory science itself has led to the development of novel techniques and instrumentation for sample analysis. Paired with GC/MS, fatty acids in Cheddar cheese can be identified using the retronasal aroma stimulator. When used in conjunction with descriptive sensory analysis, samples manufactured with the omission of propionic acid had a significant increase in the rating for the 'cream' descriptor. Instrumentation has been developed to mimic human gustation and olfaction; the electronic tongue and electronic nose, respectively. These instruments employ an array of sensors allowing for rapid and efficient classification, recognition, identification, and discrimination of samples (Tudor Kalit and others 2014). More specifically, one type of electronic tongue has an array of potentiometric ion-selective electrodes that detect various ions in liquid samples matrices in comparison to a reference electrode to produce signal data (Ciosek and others 2006). The application of this instrumentation has been utilized by food and pharmaceutical industries and even has legal application in assessing food authenticity (Peris and others 2016). In the dairy industry, the electronic tongue has been used to assess flavor of probiotic fermented milk during storage as well as quantify bitterness of dairy protein hydrolysates (Hruškar and others 2010, Newman and others 2014). The electronic nose has been utilized in combination with descriptive sensory analysis to chemically differentiate aged Cheddar cheese samples (Drake and others 2003). Expanding the application of the electronic tongue to aged Cheddar cheese requires further method development, like its use to assess aging and protein-to-fat ratio (Lipkowitz and others 2017).

# CHAPTER THREE: INFLUENCE OF STORAGETIME AND ELEVATED RIPENING TEMPERATURE ON CHEMICAL AND SENSORY PROPERTIES OF WHITE CHEDDAR CHEESE

## Abstract

Aged cheese is an increasingly popular dairy product. One approach to shorten aging time is to elevate aging temperatures. But while elevated temperatures influence cheese texture and flavor, they may create an imbalance of flavors, negative changes in texture, and production of off-flavors. Therefore, the objective of this study was to evaluate the influence of elevated aging temperature on chemical and sensory properties of aged white Cheddar cheese. White Cheddar cheese was aged at 7.2˚C, 10˚C, or 12.8˚C for 12 months, with samples evaluated at 2, 5, 8, 10, 11, and 12 months with a trained sensory panel (n=10). In addition, an electronic tongue methodology was developed for analysis of non-volatile compounds. Two consumer sensory panels (n=120) assessed the 8-month and 12-month aged cheese for comparison to a commercially available reference sample of the same cheese, aged for 12 months. The trained panel results showed the 2-month cheese were described by milkfat flavor and sweet taste, while the 5-month cheese was described by the attributes of nutty aroma and white color. Results also showed cheese samples at 8, 10, 11, and 12 months developed aged characteristics, such as umami and bitter tastes, brothy aroma, and aged flavor. The consumer panel results showed similar overall liking scores for the reference cheese and cheeses aged at 10˚C or 12.8˚C for both 8- and 12-month evaluation. The electronic tongue data demonstrated high discrimination amongst all samples with discrimination

indices ≥ 83%. The instrument could classify samples according to aging month with a validity value of 92.59%. In conclusion, storage at 10˚C for 8 months accelerated cheese aging, making it more similar to the same cheese aged for 12 months, based on consumer response. Also, the electronic tongue served as a valid method of instrumental analysis for Cheddar cheese samples throughout maturation. This study demonstrated with aged white Cheddar cheese, elevated storage temperature at 10˚C produced a cheese similar in consumer acceptance.

## Introduction

With growing consumer demand for aged Cheddar cheese, manufacturers often experiment with changes to the cheesemaking process and require new technology to produce quality cheese more rapidly. These alterations, including utilizing elevated temperature, enzyme addition, or fat reduction influence the physiochemical and sensory properties of the resulting cheese. While elevated aging temperature can result in a reduction in production time and resources, the risk of microbiological spoilage is increased (Hannon and others 2005). The implementation of elevated temperature reduces refrigeration costs and is the simplest method of accelerating aging (Law 2001).

Ripening temperature governs biochemical process activity during aging. Elevated temperature can be problematic due to the increasing rates of proteolysis, glycolysis, and lipolysis. In addition, the use of extended ripening time at elevated temperature may lead to flavor imbalance due to this catalysis of biochemical processes. Using an elevated temperature of 11˚C, propionic acid and free amino acids increased at faster rates than Cheddar cheese aged at 6˚C, indicating the acceleration of proteolysis and lipolysis (Marsili 1985). The acceleration of these biochemical processes at elevated temperature has been shown to increase flavor intensity in Cheddar cheese (Folkertsma and others 1996). Following three months of ripening at 16˚C, texture deteriorated and atypical flavor was noted, resulting in poor quality (Folkertsma and others 1996). Atypical flavor with extended maturation at elevated temperature spurred the use of time/temperature combinations in the early stages of ripening to control aging. Utilizing the time/temperature combinations of 20˚C for one week and 12˚C for six weeks, aging was accelerated by 2 months (Hannon and others 2004). Through the differences in aroma and flavor profiles produced by varying temperature, non-starter lactic acid bacteria (NSLAB) were determined to contribute to the perception of maturity through the formation of volatile compounds (Rehman and others 2000). These assessments of the influence of elevated temperature (9 – 12˚C) on Cheddar cheese quality commonly employ sensory and instrumental analyses (Croissant and others 2011).

To understand the implications of manufacturing changes on Cheddar cheese characteristics, the electronic tongue (e-tongue) displays potential application in the dairy industry (Lipkowitz and others 2017). Previously this instrument was used to analyze whey hydrolysates (Newman and others 2014) and probiotic fermented milk (Hruškar and others 2009). Development of the e-tongue technology has also allowed for a realistic measurement of the complex human perception of taste (Ross 2009). Specifically, the potentiometric electronic tongue is employed for taste assessment of liquid sample matrices through a set of seven cross selective, ion-selective sensors; sweet, sour, salty, bitter, umami, metallic, and spicy. The e-tongue in the food and pharmaceutical industries has become a tool for rapid classification, recognition, discrimination, and identification of samples and can also be used for quantification purposes (Tudor Kalit and others 2014). While the e-tongue has found applications in the dairy industry, its relationship with sensory evaluation continues to be explored.

Consumer and trained panels are often employed to assess consumer acceptance and changes in cheese characteristics. This is especially important when alterations are made to the cheesemaking process so that the extent of the changes, either positive or negative, can be assessed. To facilitate communication among industry, government, and academia, as well as advance worldwide research on Cheddar cheese flavor, a universal descriptive language for Cheddar cheese was developed (Drake and others 2001). Cheese samples were profiled using a descriptive sensory evaluation panel to develop this lexicon. The 17 validated terms created a standardized language and a universal intensity scale. Additionally, the lexicon was cross-validated by employing sensory panels using the descriptive language in three different locations (Drake and others 2002). Cheddar cheese may also develop unbalanced flavors and off-flavors that can result in consumer rejection when utilizing elevated temperature (Marsili 1985). Sensory science allows for description of how a specific treatment of Cheddar cheese during the manufacturing or ripening process can affect the cheese's profitability.

Considering the challenges of aging cheese at elevated storage temperatures the employment of instrumental, chemical, and sensory analyses bolsters research conclusions, serving as an excellent tool for sample assessment. The purpose of this study was to determine the influence of elevated temperature on consumer acceptance of Cheddar cheese and sensory attributes throughout ripening as well as analyze changes in the volatile taste compounds throughout maturation using an electronic tongue.

**Materials and Methods**

Materials

To produce this cheese, whole milk for cheese production was obtained from Knott Dairy Center (Pullman, WA) and skim milk and cream were purchased from Darigold, Inc. (Seattle WA). Purchased from CHR Hansen (Milwaukee, WI), freeze-dried DV850 cultures were utilized as starter lactic cultures. *Lactobacillus helveticus* WSU19 was utilized as an adjunct culture, provided by the Washington State University Creamery (Pullman, WA). Coagulant, CHY-MAX® Extra, was purchased from CHR Hansen (Milwaukee, WI) and MORTON® Top Flake Topping Salt was purchased from Morton Salt Inc. (Chicago, IL). Sulfuric acid for Babcock Milk Test (CAS 7664-93-9) was purchased from Fisher Scientific (Hampton, NH). For salt determination, chloride meter standard (200mg/L Cl) (Sherwood Scientific Ltd., Cambridge, UK) and combined acid buffer (Nelson-Jameson, Inc. Marshfield, WI) were utilized. The micro Kjeldahl procedure was performed using sodium hydroxide 50 wt% solution (Acros Organics, Thermo Fisher Scientific, Waltham, MA), 0.1N HCl titration solution (Hanna Instruments, Woonsocket, RI), selenized boiling granules (Hengar Co., Thorofare, NJ), cupric sulfate pentahydrate catalyst (J.T. Baker, Phillipsburg, NJ), and boric acid (Sigma St. Louis, MO).

For the preparation of sensory evaluation standards, skim milk, eggs, and heavy whipping cream (Lucerne Foods, Pleasanton, CA), walnuts, canned potatoes, and fresh pineapple (Signature Select, Safeway) were purchased from Safeway (Pleasanton, CA). Black Diamond Mature Reserve Cheddar cheese (Lactalis American Group, Buffalo, NY) was also utilized as a sensory evaluation standard, purchased from Safeway (Pleasanton,CA). Diacetyl, p-cresol, and butyric acid were purchased from Sigma (St. Louis, MO). Taste standards were prepared using quinine sulfate, sucrose, citric acid, mono-sodium glutamate (Sigma, St. Louis, MO) and sodium chloride (Avantor Performance Materials, Inc., Center Valley, PA). Color scales were determined with panel agreement and created using Clark & Kensington and Valspar paint chips purchased from Ace Hardware (Oak Brook, IL). Both the trained and consumer panels utilized Tillamook Sharp White Cheddar as a reference (Tillamook County Creamery Association, Tillamook, OR), purchased from Safeway (Pleasanton,CA). Hydrochloric acid, sodium chloride, and sodium-l-glutamate standards were obtained from α-MOS (Tolouse, France) for electronic tongue analysis. Cheese fat extraction for e-tongue analysis utilized chloroform (VWR International, Radnor, PA) and methanol (J.T. Baker, Phillipsburg, NJ).

Cheese Production and Storage

White Cheddar cheese was provided by the WSU Creamery (Pullman, WA) during three days of production, 60 cans/day, totaling 180 cans. Whole milk (Knott Dairy Farm, Pullman, WA), skim milk, and cream (Darigold, Seattle, WA) were heated in a cheese vat to 32˚C and held for 1hr following inoculation with 0.02% DV850 starter culture and 0.33% *L. helveticus* WSU19. Coagulation ensued with the addition of 0.045% CHY-MAX® Extra (milk rennet) after gentle stirring for 2 min and setting for 30 min. The curd was then cut, allowed to heal, and cooked at 39˚C for 90 min before pumpout onto the Cheddaring table when a titratable acidity (TA) of 0.13 was reached. Whey was drained and the mass was cut into loaves. The loaves were cheddared to facilitate moisture removal by flipping and stacking until a TA of 0.46 was obtained at 37˚C. The curd was milled by hand and salt was added at 0.25% with mixing from the table agitator. Weighed to 12.7 kg, curd was put into hoops and assembled under presses. An initial hour of pressing at 380 kPa allowed for wrapping the now formed mass with cheese cloth for another overnight pressing at 380 kPa. The cheese was sliced and vacuum sealed into steel cans (15.24 cm x 5.40 cm). Seals were checked and cans x-rayed before storage. Cheese from three consecutive days of production were randomly assigned to batch 1, 2, and 3 respectively. Within a given day, cans (n=60) were randomly assigned to specific aging temperature coolers (7.2˚C, 10˚C, and 12.8˚C) equally across days. Over the 12-month aging period, cans from each production day were randomly selected at 2, 5, 8, 10, 11, and 12 months for analysis by chemical, instrumental, and sensory methods.

<u>Moisture Content</u>

Aluminum pans were placed in a BLUE M C-4850-Q forced air oven (Thermal Product Solutions, New Columbia, PA) for 45 min to reach constant mass. Grated cheese samples (5.0 g) were placed on the pans and dried overnight at 100˚C. Triplicate samples from each production day were removed to a desiccator the following day and weighed after reaching room temperature.

Fat Content

The Babcock method (Marth 1978) was used to quantify the fat content of the Cheddar cheese samples in triplicate. In a Babcock bottle, cheese (9 g) and 60˚C distilled water (9 mL) was mixed on a Garver mechanical shaker at maximum speed (Garver Manufacturing Inc., Union City, IN) to form a soft slurry. Sulfuric acid (90 – 93%) was added in two aliquots of 8.75 ml, followed by maximum speed shaking after each, until a dark uniform color was achieved. Samples were then centrifuged using a Garver electrifuge (Garver Manufacturing Inc., Union City, IN) at 60˚C for 5 min at 130 x g. The addition of 60˚C water occurred in two steps; to the "0" mark (> 5 mL) – the beginning of the neck of the bottle – and to the "45 – 50" mark (> 10 mL) preceded centrifugation for 2 min and 1 min, respectively. The bottles were placed in a water bath (60˚C) for 5 min. Red Reader Oil was then used to help read the percent (w/w) fat, as it highlighted the meniscus at the top of the bottle neck. The percent (w/w) fat was obtained by subtracting the number associated with the bottom of the fat in the neck from the number corresponding to the meniscus.

Protein Content

Nitrogen determination was performed using the micro Kjeldahl procedure (AOAC Method 960.52) to quantify the organic nitrogen content of Cheddar cheese using triplicate samples from each production day. Nitrogen to protein calculations used the conversion factor of 6.38 (Rouch and others 2008), with concentration of protein expressed in percent. Briefly, the calculations included the sample weight, mL standardized HCl, [HCl], conversion factor, and percent protein.

Salt Determination

The salt percentage of cheese samples were determined using the Corning Chloride Analyzer 926 (Nelson-Jameson, Inc. Marshfield, WI). The instrument was calibrated using a combined acid buffer to obtain three consecutive readings of 100 ± 3 mg/L before sample analysis. Cheese samples were prepared in triplicate by grating 5.0 g samples into beakers, adding 98.2 g of 150˚F distilled water, and leaching salt (~1.1%) for 20 min. Samples were then homogenized for 30 sec using a Hamilton Beach HMI200 9" immersion blender (Hamilton Beach Brands, Glen Allen, VA) and then filtered through Whatman No. 2 qualitative filter paper (Whatman International Ltd., Maidstone England). Titration with the chloride analyzer resulted in the determination of percentage salt. The salt-to-moisture ratio was then calculated by taking the salt percentage and dividing by percent moisture.

pH Determination

The pH of triplicate cheese samples – as prepared for salt determination, blended with water – was measured using Oakton pH 510 Series Benchtop Meter and the 35805-18 pH probe (Eutech Instruments, Singapore). Samples measurements were made in triplicate.

Cheddar Cheese Preparation for Electronic Tongue

Cheddar Cheese samples were prepared in triplicate using a modified Folch procedure (Folch and others 1951) to extract the fat. Following the addition of 60mL of a 2:1 chloroform:methanol solution, each 3 g sample was homogenized for 1 min at 1600 x g using the Ultra Turrax IKA T18 basic homogenizer (IKA Labortechnik, Staufen, Germany). The probe was rinsed with 10mL chloroform:methanol and 10 mL of MilliQ water was added. Samples sat for 20 min before centrifugation for 30 min at 3000 x g using a Beckman GPR centrifuge (Beckman Coulter, Brea, CA, USA). The top aqueous layer was isolated, filtered through Whatman No. 2 qualitative filter paper (Whatman International Ltd., Maidstone England), and the methanol evaporated using the Büchi Rotavapor (Büchi Labortechnik AG, Flawil, Switzerland) at 85°C for 25 min to a standard volume of 25 mL.

Electronic Tongue Analysis

Following preparation, Cheddar cheese samples were equilibrated to room temperature and filtered using Whatman No. 1 qualitative filter paper (Whatman International Ltd., Maidstone England) into 25 mL electronic tongue analysis beakers. A potentiometric electronic tongue (Astree® II e-tongue unit Alpha MOS) analyzed Cheddar cheese samples for taste attributes (sweetness, saltiness, sourness, bitterness, umami, metallic and spiciness). The e-tongue was equipped with a liquid auto sampler (LS48) and 7 set #5 sensors (sweet, sour, bitter, salty, umami, spicy, and metallic). System preparation entailed conditioning, calibration, and diagnostics. This pre-run system was performed using 25 mL of 0.01M standard solutions, each prepared from 0.1 M hydrochloric acid, sodium chloride, and sodium-l-glutamate. The set of #5 sensors were then left to hydrate overnight in 25 mL reagent grade Milli-Q filtered water. A programmed auto sampler method used the following parameters for sample analysis: delay = 0 s; acquisition time = 120 s; stirring rate = 1, and acquisition period = 1. The sequence alternated between analyzing cheese samples and a 10-sec cleaning of the sensors in 25 mL reagent grade Milli-Q filtered water for 6 loops during data acquisition.

Trained Panel

Panelists (n = 10) were recruited through electronic announcement within the Washington State University community. No previous experience with cheese or sensory evaluation was required for participation. Training, totaling 20 hours, took place for 6 weeks before an evaluation of 2-month old cheese. Prior to the evaluation at the other storage time points of 5, 8, 10, 11, and 12 months, a 1-hr refresher training session was held for panelists to review attributes. Minimal background information was given to panelists about the study to reduce potential bias. The panel was composed of 60% females and 40% males with ages between 23 and 54 years (mean panel age of 29.5). Cheese consumption patterns of panelists was either daily or once to several times per week. The study was approved by the Washington State University Institutional Review Board for human subject participation. Panelists received nonmonetary incentives following all training and formal evaluation sessions.

Panelists received training on correct 15-cm line scale use - anchored at 1.5 cm (low) and 13.5 cm (high) - and evaluation techniques for aroma, flavor, and tastes of cheese. Standards (**Table 3.1**) were presented over 6 weeks of training. Panelist agreement facilitated attribute standard choice and perception of intensity ratings for 11 flavor and aroma and 5 taste attributes. Practice evaluations sessions occurred throughout the training period to validate training in each category by using commercial cheeses. Feedback was provided to panelists during practice sessions, immediately after rating each attribute for all samples, using the Feedback Calibration Method (FCM™); available through *Compusense® Cloud* (Compusense Inc., Guelph, ON, Canada). Additionally, panelists received feedback about performance following data analysis of practice sessions with SenPaq (Qi Statistics Ltd., Berkshire, UK). Refresher sessions, 1-hr in length, the day prior to formal evaluation of cheese at 5, 8, 10, 11, and 12 months also utilized practice tests and panelist feedback with SenPaq.

Instruction, scale presentation, and data collection during formal evaluations was carried out using *Compusense® Cloud* (Compusense Inc., Guelph, ON, Canada). All cheese samples were cut into 1.5 cm cubes with 6 cubes provided to panelists, prepared in 4 oz. soufflé cups with random three digit codes. Samples were removed from refrigeration 1-hr prior to evaluation to equilibrate to room temperature (~23˚C). Panelists were provided with unsalted crackers, green grapes, and water for palate cleansing between cheese samples. Eight cheese samples were evaluated – 4 samples (3 treatment and 1 reference) in replicate- utilizing a randomized serving order, with forced breaks between every sample, except the fourth. A 15-min break after evaluation of the fourth sample was required to prevent sensory fatigue and refresh the palate.

Consumer Panels

Two consumer panels with consumers (n = 120/panel) recruited through electronic announcement within the Washington State University community were conducted to assess consumer liking and preference of aged white Cheddar cheese at 8 and 12 months of aging, as approved by the Institutional Review Board. Consumers were presented with five samples; three temperature treatment cheeses, one-year-old white Cheddar cheese for direct sample comparison (Cougar Gold®), and a commercial medium Cheddar cheese as a reference sample. The panels were composed of 64% female and 36% male with a mean age of 34 years old, ranging from 18 to 68 years old. Consumers were asked to answer demographic questions about race, employment status, consumption of various cheese varieties, frequency of consumption, and factors influencing cheese choice. Liking of samples was indicated by consumers based on various appearance, aroma, flavor, and intensity attributes on a 9-point hedonic or 9-point intensity scale, with extemes of 1 and 9. After the evaluation of each sample, consumers were asked if, based on their previous knowledge and experience, they considered the sample to be Cougar Gold® - consumers could select yes, no, or maybe as an answer. Data collection was carried out using *Compousense® Cloud* (Compusense Inc., Guelph, ON, Canada).

Samples were cut into 1.5 cm cubes and 3 cubes of cheese were provided in 4 oz. soufflé cups with lids. Samples were served at room temperature, labeled with randomized three digit codes. Unsalted crackers, grapes, and water were provided as palate cleansers. Consumers received nonmonetary incentives following the completion of the panels.

Statistical Analyses

Analysis of variance (ANOVA) was conducted on proximate analysis data (pH, fat, moisture, protein and salt) using XLSTAT 18.07 (Addinsoft, New York, NY) to evaluate consistency of samples produced on different days. Significance was established at $p < 0.05$.

Trained panel data were analyzed using XLSTAT 18.07 (Addinsoft, New York, NY) to perform ANOVA, Tukey's HSD, and Principal Components Analysis (PCA). ANOVA was used to assess the main effects of elevated temperature, storage time, and panelist as well as interaction effects of month and temperature on the intensities of appearance, aroma, flavor, and taste attributes of the samples. PCA was used to show relationships among treatments (aging and temperature) and samples over the entire storage time.

Consumer sensory evaluation data were first analyzed to determine the consumer liking attributes that describe cheese samples at the various time and temperature combinations using PCA. This was followed by Correspondence analysis (CA) on 8-month consumer panel data to determine the aging temperature that produced sensory characteristics similar to the reference cheese. Both PCA and CA were conducted using XLSTAT Sensory 18.07 (Addinsoft, New York, NY). Consumer liking data was pre-processed for CA by transforming hedonic data to binary data as follows: on the 9-point hedonic scale, "like moderately" = 7, "like very much” = 8, “like extremely” = 9 were converted to 1 while the remaining of the scale (1 to 6) were assigned 0. PCA via AlphaSoft vrs 12 (Alpha MOS, Toulouse, France) was used to create profiles of the e-tongue signal data across storage time. Partial least squares (PLS) regression was performed on electronic tongue data to correlate sensory taste evaluation with electronic tongue data and discriminant analysis (DA) to evaluate classification of cheese samples based on storage time.

## Results and Discussion

### Chemical Analyses

No significant differences were found in moisture content, fat content, protein content, % salt, and pH of cheese samples among batches (**Table 3.2**). The composition upheld Cheddar cheese’s standard of identity (CFR 133.113), requiring a minimum milkfat content of 50% by weight of solids as well as a maximum moisture content of 39%. The values also agree with previous literature proximate analyses values of 37.4% moisture, 33.0% fat, and 25.1% protein (Folkertsma and others 1996).

### Descriptive Sensory Analyses

The effect of time, temperature, and interaction is shown in **Table 3.3**. The largest main effect for all trained panel attributes was panelist. This learning effect is common with trained panels, although more training reduces the effect when panelist’s ability to discriminate individual attributes is enhanced, leading to more precise data (Drake and others 2002). The interaction between month and temperature was significant for only the attributes of nutty and aged flavor, as well as brown color. Nutty and aged flavor are main attributes of Cheddar cheese which develop with time and temperature during ripening. Other color and flavor attributes were significant due to temperature and storage time. Most aroma attributes were significant due to storage time, but not temperature. This indicated aroma attributes were solely influenced by storage time, with flavor and colors influenced by both storage time and temperature.

Based on temperature, Tukey's HSD found significant differences for the cheese samples aged at elevated temperature compared to the normal aging temperature of 7.2˚C (**Table 3.4**). More specifically, cheese aged at 12.8˚C had higher intensity values ($p < 0.05$) than cheese aged at 7.2˚C for the attributes of aged aroma, fruity flavor, nutty flavor, aged flavor, free fatty acid flavor, and all taste attributes except sweetness. Similarly, cheese aged at 10˚C had higher intensity values ($p < 0.05$) for milkfat aroma, diacetyl flavor, aged flavor, and umami taste.

Trained panel evaluation and subsequent PCA (**Figure 3.1**) allowed for characterization of samples throughout the 12-month aging period. PC1, described by the contrasting relationship between milkfat flavor and sweet taste with bitter taste, aged aroma, and brothy flavor, accounted for 42.7% of the variation among samples. PC2 accounted for another 15.4% of variation and was described by the contrasting relationship between nutty flavor and aroma with diacetyl and fruity flavors and aromas. The first two principal components explain a total of 58.1% of sample variation, with another 12.6% of variation accounted for from the third principal component. The texture attributes will be discussed in the following paper (chapter four).

Young cheeses – 2 months in age – were characterized by milkfat flavor and sweet attributes as well as a distinct lack of aged attributes and bitterness. Many of the sensory attributes profiled by trained panelists were included to describe the cheeses during aging thus explaining the low intensity of these aged attributes describing the young cheeses. As aging progressed to 5 months, cheeses began to develop aged characteristics such as nutty aroma and white color. During other aging periods – 8, 10, 11 and 12 months – cheeses were described by various attributes indicating maturity, including free fatty acid (FFA) flavor, sour taste, aged flavor, and brothy aroma. The development of aged attributes in older cheese and the characteristics of young cheese has been explored by other researchers, with the current results are in support of these previous studies (Drake and others 2001, Drake and others 2002).

It is important to note the commercial white Cheddar cheese was equivalent to the 12-month cheese sample aged at 7.2˚C, as those are the normal specifications for the commercial cheese set by the manufacturing facility. This sample is highlighted in **Figure 3.1** with a yellow box. After 8 months of aging, samples aged at elevated temperature were better described by mature attributes – umami and sour tastes, aged and sulfur flavors, and brothy aroma – than the commercial sample. While the commercial sample developed those attributes, it was also described more by smoothness of mass and other texture attributes embodied by the younger aged cheeses. This is a result of elevated temperature increasing the rate of proteolysis and hence flavor development and intensity, as previously described by Folkertsma and others (1996). Cheese aged at elevated temperatures developed higher intensities of the mature attributes and lower intensities of certain texture attributes than the commercial white Cheddar cheese.

<u>Electronic Tongue Analyses</u>

The electronic tongue has been used to assess flavor of probiotic fermented milk during storage as well as quantify bitterness of dairy protein hydrolysates (Hruškar and others 2010, Newman and others 2014). The electronic nose has been utilized in combination with descriptive sensory analysis to chemically differentiate aged (6 – 15 month) Cheddar cheese (Drake and others 2003). The current study utilized the electronic tongue for taste assessment of white Cheddar cheese aged at varying storage times (2, 5, 8, 10, 11, and 12 months) and temperatures (7.2˚C, 10˚C, and 12.8˚C).

When analyzed with PCA, the e-tongue analyses of aged white Cheddar cheese samples had discrimination indices ≥ 83 throughout the 12-month aging period (**Table 3.5**). The discrimination index represents how well the instrument distinguished among samples based upon the instrument's response for sweet, sour, salty, bitter, umami, metallic, and spicy. A high discrimination index (100) indicates great differences between samples, while a poor discrimination index (-100) indicates great similarity between samples. A discrimination index of ≥ 83 suggests the electronic tongue distinguished Cheddar cheese samples with high discrimination and coincides with previously reported electronic tongue analysis of Cheddar cheese (Lipkowitz and others 2017).

Overall, the samples throughout the 12-month aging period displayed groupings by temperature at each month. At 8 months of aging, the samples were separated based on their contrasting relationship between umami and all other sensors (**Figure 3.2**), as with the following 10-month analysis. Elevated temperature samples were mainly described by the metallic sensor at 11 months and by 12 months, samples were described by various sensors.

The most distinct development in cheese taste occurred following 8 months of aging. The grouped samples aged at 12.8˚C were distinctly described by the umami sensor, a change from previous months of aging where these samples were also grouped, but described by multiple sensors (2 months) or the lack of sensors (5 months). This was observed again at 10 months of aging where samples aged at 12.8˚C were described by umami and all other sensors were needed to discriminate samples aged at lower temperatures.

Cheese taste continued to develop during the last two months of the ripening period. The separation of samples based on their response to the metallic sensor was observed at 11 months (**Figure 3.3**), with the samples aged at the highest elevated temperature were described by this sensor (PC1 = 75.35%). Many samples at 11 months were also seen to be described by the sweet sensor on PC2, accounting for 13% of sample variation. Samples at 12 months (**Figure 3.4**) were again described by the contrasting relationship (PC1) between umami and sweetness, bitterness and metallic, with PC2 describing samples with the salty and sour sensors. The 12.8˚C samples were grouped based on their response to the umami sensor, although 10˚C samples were not grouped. Variation of 10˚C samples may be explained by the development of other tastes such as bitterness.

Discriminant analysis of electronic tongue data showed 92.59% correct classification of cheese samples by month (**Table 3.6**). The e-tongue correctly classified all nine cheese samples from 2, 5, and 12 months as belonging to their corresponding months. Misclassification – assigning a sample to a month it did not belong – occurred with 8, 10, and 11 month samples. Misclassified samples were either assigned to the previous month or month after where they would have been correctly classified. A sample misclassified as the month before its actual age could be a sample aged at the lowest temperature, 7.2˚C. Likewise, a sample misclassified as the month after its age could be a sample aged at the highest temperature, 12.8˚C.

Misclassification assigned to the month before or after the actual age of the sample could be explained by elevated temperature's influence on aging. The ability of elevated temperature to accelerate flavor development through the more rapid production of mature flavor attributes (Rehman and others 2000, Hannon and others 2005). For example, the misclassification of two samples aged at 10 months was assigned to 11 months. Given their classification as more mature cheeses (11 months) instead of the actual age of 10 months, the sample likely was aged at elevated temperature.

The influence of storage time on the correct assignation of aging temperature by the electronic tongue was also examined (**Table 3.7**). Correct classification by the electronic tongue varied from 38.9 to 66.7%. The vast changes experienced by cheese during ripening likely explained the electronic tongue's difficulty of correctly assigning aging temperature. For example, the 3 samples aged at 7.2˚C and misclassified as aged at 12.8˚C could be cheese samples from later months of aging. The electronic tongue may have classified the samples at 12.8˚C due to a likeness to 12.8˚C samples from earlier months of aging. The distinct characterization of elevated temperature samples throughout the ripening period from 8- to 12-months by the electronic tongue may support theses misclassifications. The elevated samples developed aged characteristics, such as umami or bitter taste, before the normal aging temperature (7.2˚C) samples. Samples aged at 7.2˚C reached taste development later than those aged at elevated temperature. Therefore, the electronic tongue could be misclassifying samples due to this observed acceleration in taste development, resulting in low (53.70%) validity.

Given the electronic tongue's development to realistically measure the human perception of taste (Ross 2009), its relationship with human sensory evaluation was explored. **Table 3.8** shows correlations between the e-tongue signal and descriptive sensory analysis data. The strength of correlation is indicated by the correlation coefficient, with strong correlations having coefficients > 0.800. A stronger correlation arises from the trained panel being able to identify the comparable intensities of taste attributes as the electronic tongue. In the present study, more strong correlations were prevalent among later aging times (10, 11, and 12 months) aged white Cheddar cheese samples compared to the younger, less aged samples. Increased correlation at later months may indicate that as aging progressed, the cheese developed more distinct tastes allowing ease of evaluation by the trained panel or ability of the e-tongue to distinguish samples. In the early months of cheese ripening, sensory characterization cannot be used to predict character of the final cheese product as sensory character changes slowly during aging (Muir and others 1996). Since temperature is the most important factor governing flavor intensity (Law and others 1979), by the later months of maturation, the cheese had sufficient time to develop its taste profile, yielding ease of evaluation for the trained panel.

Consumer Sensory Evaluation

Consumer demographic data (**Table 3.9**) shows the majority of consumers were white/Caucasian, European American, or Non-Hispanic, consumed Cheddar cheese, had previously consumed Cougar Gold®, and said flavor was the top factor that influenced their cheese choice. Despite 68% and 77% of the consumer panel evaluating the 8- and 12-month aged cheeses, respectively, having consumed Cougar Gold® before, consumer ability to correctly identify a sample as being Cougar Gold® was below expectations. When asked if they considered the reference sample (standard Cougar Gold® that was aged for 12 months at 7.2˚C) to be Cougar Gold®, 22% of consumers at 8 months and 21% at 12 months, correctly identified the sample as being Cougar Gold®. While the majority of the panels at 8 and 12 months (68% and 77%, respectively) had consumed Cougar Gold® before, the question was above their expertise leading to the poor identification of the reference sample.

Aged white Cheddar cheese samples were evaluated by consumers at both 8 and 12 months based on consumer acceptability as well as intensity ratings. Consumers were asked to rate their liking of appearance, aroma, overall flavor, Cheddar cheese flavor, sharpness, aged flavor, overall texture, crumbliness, firmness, and overall liking. Participants were also asked to rate the intensities of Cheddar cheese flavor, sharpness, and aged flavor.

For 8-month consumer assessment of all attributes (**Table 3.10**), the cheeses aged at 10˚C and 12.8˚C were similar to the commercial (12-month) reference ($p < 0.05$), except for aged intensity where consumers rated 10˚C as significantly lower than the reference and overall flavor and Cheddar flavor where 12.8˚C was lower than the reference. However, the cheese aged at 7.2˚C was significantly different ($p < 0.05$) for all attributes except appearance, aroma, and texture, which were similar to the 12-month reference ($p < 0.05$). All other attributes had lower ratings due to the 7.2˚C being aged for 8 months compared to the 12-month reference.

For 12-month consumer assessment (**Table 3.11**), cheeses aged at 10˚C were similar to the 12-month reference ($p < 0.05$) for all attributes. Cheeses aged at 12.8˚C were also similar to the 12-month reference ($p < 0.05$) for all attributes except overall flavor, Cheddar flavor, aged flavor, and overall liking where the 12.8˚C cheeses were rated significantly lower. The cheese aged at 7.2˚C was similar to the reference ($p < 0.05$) for all attributes except Cheddar intensity, sharpness intensity, aged flavor, crumbliness, and firmness. These differences were not expected at 12 months because the 12-month reference and cheese aged at 7.2˚C were comparable samples; both aged for 12 months at 7.2˚C. Overall liking scores placed 10˚C and 7.2˚C as similar to the reference ($p > 0.05$), while 12.8˚C was significantly lower ($p < 0.05$). This change in overall liking compared to the 8-month data may be a result of the development of unbalanced or off-flavors due to aging at of 12.8˚C, a common problem when utilizing elevated aging temperature (Hannon and others 2005). Cheese aged at 10˚C during both 8- and 12-month evaluation, received the greatest overall liking score compared to the 12-month reference, indicating its acceptance by consumers.

Agglomerative hierarchical clustering of consumer data from the 12-month aged cheese sensory panel showed 3 segments of consumers each preferring a different cheese sample (**Figure 3.5**). Cluster 1 had 32 consumers who preferred cheese aged at 12.8˚C with a rating of 7.3, indicating "like moderately" on the 9-point hedonic scale. The 10˚C and 7.2˚C cheese samples had ratings by cluster 1 of 7.1 and 5.9, respectively. Cluster 2 had the fewest number of consumers with 26. These consumers preferred the Cheddar reference rated at 7.3, or "like moderately" over the 7.2˚C, 10˚C, and 12.8˚C with ratings of 6.7, 6.7, and 4.1, respectively. Cluster 3 was the largest cluster with 62 consumers preferring cheese aged at 10˚C with a rating of 7.3, indicating "like moderately". Other samples, 7.2˚C, 12.8˚C, and the Cheddar reference, had respective ratings of 7.2, 7.1, and 4.9.

Other segmentation work has been carried out with Cheddar cheese based on maturity level (Young and others 2004) and demographic location in the United States (Drake and others 2009). Utilizing preference mapping, six segments of consumers preferred different Cheddar cheeses, varying widely in their flavor profiles due to length of time aged (Young and others 2004). Segment 1 preferred cheese characterized by young flavors and low intensities of brothy and sulfur flavors. Segment 2 preferred the same cheeses as segment 1, but without attribute preferences. Segment 3 preferred young and brothy flavored cheese, while segment 4 liked all cheeses except one characterized by young and low sour taste. Segment 5 disliked young cheeses and segment 6 preferred cheese with aged flavors of nutty, fruity, and sweet, but not brothy flavor. Five consumer segments found a distinct distribution of west coast consumers, while east and midwest distribution were similar (Drake and others 2009). Segment 3 contained a larger proportion of west coast consumers who preferred cheese with brothy, nutty, and free fatty acid flavors, as well as sour and salty tastes.  Segments 1 and 4 were driven by their liking of whey flavor and segment 5, milkfat flavor. Segment 2 liked all cheese except those with dominant whey flavor.

While other segmentation work has found more segments of consumers, the current study does share similarities. Most consumers (from clusters 1 and 3) preferred cheese samples aged at elevated temperatures with cluster 1 preferring cheese aged at 12.8˚C and cluster 3 preferring cheese aged at 10˚C.  From trained panel evaluation, these samples are described by mature cheese attributes. They have intensities greater than the Cheddar reference – the sample preferred by cluster 2 – for aged flavor and aroma, bitterness, and sourness. In other words, cluster 2 preferred the Cheddar reference due to its young characteristics. The preference of cheese samples for the 12-month consumer panel can be simplified to consumers liking cheese with mature or young cheese attributes.

Principal component analysis was carried out to determine which of the 8- and 12-month samples were most like the commercial reference cheese aged for 1-year (**Figure 3.6**). The first two principal components, PC1 and PC2, accounted for 84.50% of variation amongst the samples; 50.41% and 34.10% respectively. PC1 can be described by the contrasting relationship between aroma and texture as well as firmness, while PC2 described the contrasting relationship between overall flavor and the intensity attributes of aged, Cheddar sharpness, and Cheddar cheese flavor. Samples aged for 8-months at both elevated temperatures of 10˚C and 12.8˚C and the sample aged for 12-months at 10˚C were most similar to the reference. The samples aged at 10˚C and the reference were best described by the liking attributes of texture, sharpness, and aged flavor, while the sample aged at 12.8˚C was described by the liking attributes of firmness and Cheddar cheese flavor intensity.

Correspondence analysis utilized the 12-month commercial reference as an ideal product to further investigate if elevated temperature influenced the samples at 8 months (**Figure 3.7**). The analysis identified 7 "must have" attributes including sharpness intensity, crumbliness, aged flavor, aged intensity, Cheddar cheese flavor intensity, sharpness, and Cheddar cheese flavor. Those 7 attributes are what a product must have to be like the ideal product. The cheese samples aged at the elevated temperatures were more like the ideal product than the cheese aged at 7.2˚C. Like the ideal product, the elevated temperature samples are described by more mature attributes – Cheddar intensity, aged flavor, sharpness, and crumbliness – than the sample aged at 7.2˚C. Two of three clusters of consumers preferred samples with mature cheese attributes, which further justifies the elevated temperatures embodying attributes necessary to be considered the ideal product.

**Conclusions**

Maturation changes were monitored using chemical, instrumental, and sensory analyses demonstrating the influence of storage time and temperature on the chemical and sensory properties of white Cheddar cheese during a 12-month ripening period. Trained panel evaluation at 2, 5, 8, 10, 11, and 12 months aided in creating a profile of the cheese throughout ripening. Cheeses aged at elevated temperatures (10˚ and 12.8˚C), compared to a commercially available reference aged at 7.2˚C, developed higher intensities of mature sensory attributes such as aged flavor, umami taste, and brothy aroma. Consumer evaluation resulted in similar acceptance of cheese samples aged at 10˚C, as opposed to 12.8˚C, since that elevated temperature produced cheese with similar sensory properties to the commercial reference. The electronic tongue served as a valid and rapid method for cheese evaluation with the ability to predict the age of cheese samples. This study demonstrated that instrumental and sensory methods can be implemented to help control the aging process of white Cheddar cheese.

**Table 3.1.** Standards used in panel training for sensory evaluation (n=10) of white Cheddar cheese samples for appearance, aroma, flavor, and taste attributes.

| Category | Attribute | Preparation |
|---|---|---|
| Appearance | White | Clark & Kensington paint chips Low: CW-C5U Medium: CW-C2U High: CW-C1U |
| | Yellow | Clark & Kensington paint chips Low: 20B-1U High:18B – 4M Valspar paint chip Medium: VR011E |
| | Orange | Clark & Kensington paint chips Low: 16A-1 High: 16A-2 Valspar paint chip Medium: 116Q |
| | Brown | Clark & Kensington paint chips Low: 20A-1U Medium: 20A-2M High: 20A-3M |
| Aroma and Flavor[1] | Cooked | Skim milk heated to 85˚C for 30 minutes |
| | Fruity | Fresh pineapple |
| | Diacetyl | Diacetyl (20 ppm in water) |
| | Sulfur | Boiled egg |
| | Nutty | A: Lightly toasted walnuts F: unsalted wheat thins |
| | Whey | Fresh Cheddar whey |
| | Brothy | Canned potatoes |
| | Milkfat | Heavy cream |
| | Age | Aged Cheddar (≥ 1year) |
| | Cowy | ρ-cresol (160 ppm) |
| | Free Fatty Acid | Butyric acid (20 ppm in water) |
| Taste[1] | Sweet | Sucrose (5% in water) |
| | Sour | Citric acid (0.08% in water) |
| | Salty | Sodium chloride (0.5% in water) |
| | Bitter | Quinine sulfate (0.008% in water) |
| | Umami | Mono-sodium glutamate (1% in water) |

[1]Adapted from Drake and others 2001

**Table 3.2.** Proximate composition of white Cheddar cheese samples obtained from three consecutive production days at month 0, before ripening. No significant differences were found among the sample batches for any of the parameters ($p>0.05$).

| Batch | pH | Fat (%) | Milkfat (%) | Moisture (%) | Total Solids (%) | Protein (%) | S/M (%) |
|---|---|---|---|---|---|---|---|
| 1 | 4.98 ± 0.07 | 34.00 ± 0.33 | 54.76 ± 0.28 | 37.91 ± 0.24 | 62.09 ± 0.24 | 23.56 ± 0.23 | 3.31 ± 0.18 |
| 2 | 4.96 ± 0.07 | 33.72 ± 0.53 | 54.01 ± 0.38 | 37.60 ± 0.23 | 62.40 ± 0.23 | 23.95 ± 0.22 | 2.73 ± 0.04 |
| 3 | 4.95 ± 0.12 | 34.28 ± 0.35 | 54.70 ± 0.27 | 37.33 ± 0.20 | 62.67 ± 0.20 | 23.29 ± 0.41 | 3.14 ± 0.16 |

**Table 3.3.** F ratios from Analysis of Variance of descriptive analysis sensory aroma, color, flavor, and taste parameters for nine white Cheddar cheese samples as evaluated by trained panel (n=10) and analyzed with ANOVA. An * represents a significant difference in a given attribute (row).

| Source of Variation | Month | Temperature | Panelist | Month*Temp |
|---|---|---|---|---|
| df | 5 | 3 | 9 | 15 |
| Aroma | | | | |
| Fruity | 1.19 | 6.30* | 33.86* | 0.97 |
| Sulfur | 7.75* | 2.03 | 32.13* | 1.36 |
| Brothy | 5.26* | 1.38 | 40.47* | 1.02 |
| Milkfat | 10.15* | 2.46 | 20.80* | 1.31 |
| Diacetyl | 8.19* | 0.21 | 38.54* | 0.54 |
| Nutty | 10.66* | 1.68 | 36.67* | 0.49 |
| Age | 18.21* | 11.07* | 17.88* | 1.29 |
| FFA | 1.57 | 2.49 | 50.53* | 0.62 |
| Color | | | | |
| White | 10.73* | 48.00* | 36.16* | 0.36 |
| Yellow | 2.79 | 345.49* | 22.90* | 0.62 |
| Orange | 8.98* | 16.16* | 86.65* | 1.19 |
| Brown | 7.92* | 29.33* | 44.04* | 1.31* |
| Flavor | | | | |
| Fruity | 2.64* | 12.90* | 36.68* | 0.73 |
| Sulfur | 4.94* | 4.94* | 35.65* | 1.64 |
| Brothy | 17.57* | 2.28 | 38.88* | 1.45 |
| Milkfat | 9.87* | 3.28* | 13.57* | 0.98 |
| Diacetyl | 8.17* | 2.67* | 48.23* | 1.06 |
| Nutty | 8.62* | 4.60* | 37.05* | 1.71* |
| Age | 47.17* | 24.82* | 15.88* | 6.68* |
| FFA | 0.67 | 2.75* | 40.27* | 0.80 |
| Taste | | | | |
| Sweet | 9.01* | 14.40* | 27.68* | 0.16 |
| Salty | 5.31* | 5.66* | 4.62* | 1.30 |
| Sour | 3.56* | 4.62* | 25.43* | 1.22 |
| Bitter | 11.53* | 10.08* | 47.06* | 1.22 |
| Umami | 5.94* | 4.10* | 12.74* | 1.51 |

**Table 3.4.** Mean separation of significant aroma, color, flavor, and taste attributes of three Cheddar cheese samples as evaluated by a trained descriptive analysis panel and analyzed using Tukey's HSD. Values are presented as the mean of replicate measurements. Different letters within a row represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Sensory Attribute | 7.2˚C | 10˚C | 12.8˚C | Cheddar Reference |
|---|---|---|---|---|
| Aroma | | | | |
| Fruity | 2.22 ab | 2.50 a | 2.43 a | 1.97 b |
| Sulfur | 1.63 a | 1.58 a | 1.78 a | 1.72 a |
| Brothy | 4.00 a | 4.13 a | 4.25 a | 4.07 a |
| Milkfat | 4.34 ab | 4.55 a | 4.41 ab | 4.19 b |
| Diacetyl | 3.00 a | 3.09 a | 3.06 a | 3.06 a |
| Nutty | 2.85 a | 2.99 a | 3.09 a | 2.91 a |
| Age | 4.33 bc | 4.69 ab | 5.02 a | 4.14 c |
| Free Fatty Acid | 2.48 a | 2.75 a | 2.75 a | 2.56 a |
| Color | | | | |
| White | 3.88 a | 4.07 a | 4.09 a | 1.96 b |
| Yellow | 3.70 b | 3.48 b | 3.55 b | 7.24 a |
| Orange | 1.22 b | 1.15 b | 1.26 b | 1.69 a |
| Brown | 1.77 b | 1.67 b | 1.59 b | 2.51 a |
| Flavor | | | | |
| Fruity | 1.93 bc | 2.14 ab | 2.30 a | 1.64 c |
| Sulfur | 1.61 b | 1.62 b | 1.64 b | 1.93 a |
| Brothy | 4.00 a | 4.29 a | 4.27 a | 4.27 a |
| Milkfat | 4.69 a | 4.61 a | 4.35 a | 4.37 a |
| Diacetyl | 3.10 ab | 3.36 a | 3.21 ab | 3.05 b |
| Nutty | 2.62 b | 2.94 ab | 3.07 a | 2.88 ab |
| Age | 4.07 c | 4.84 b | 5.28 a | 4.37 c |
| Free Fatty Acid | 2.45 b | 2.70 ab | 2.81 a | 2.77 ab |
| Taste | | | | |
| Sweet | 2.83 a | 2.74 a | 2.89 a | 2.13 b |
| Salty | 3.93 b | 4.13 ab | 4.42 a | 4.34 a |
| Sour | 2.58 b | 2.80 ab | 2.95 a | 2.49 b |
| Bitter | 2.55 c | 2.74 bc | 2.95 ab | 3.26 a |
| Umami | 4.42 b | 4.88 a | 4.86 a | 4.76 ab |

**Table 3.5.** Discrimination Indices from PCA of electronic tongue volatile analyses of nine white Cheddar cheese over a 12-month aging period. Data were collapsed across aging temperatures.

| Storage Time (Months) | Discrimination Index |
|---|---|
| 2 | 83 |
| 5 | 82 |
| 8 | 86 |
| 10 | 85 |
| 11 | 84 |
| 12 | 86 |

**Table 3.6.** Confusion matrix classifying samples of white Cheddar cheese over 12-month aging period from discriminate analysis of electronic tongue volatile analyses. Data were collapsed across storage temperature.

| From \ To (Months) | Storage Time as Classified by E-Tongue | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Actual Storage Time | Month 2 | Month 5 | Month 8 | Month 10 | Month 11 | Month 12 | Total | % correct |
| Month 2 | 9 | 0 | 0 | 0 | 0 | 0 | 9 | 100.00% |
| Month 5 | 0 | 9 | 0 | 0 | 0 | 0 | 9 | 100.00% |
| Month 8 | 0 | 1 | 8 | 0 | 0 | 0 | 9 | 88.89% |
| Month 10 | 0 | 0 | 0 | 7 | 2 | 0 | 9 | 77.78% |
| Month 11 | 0 | 0 | 0 | 0 | 8 | 1 | 9 | 88.89% |
| Month 12 | 0 | 0 | 0 | 0 | 0 | 9 | 9 | 100.00% |
| Total | 9 | 10 | 8 | 7 | 10 | 10 | 54 | 92.59% |

**Table 3.7.** Confusion matrix classifying samples of white Cheddar cheese over 12-month aging period from discriminate analysis of electronic tongue volatile analyses. Data were collapsed across storage time.

| From \ To (˚C) | Aging Temperature as Classified by E-Tongue | | | | |
|---|---|---|---|---|---|
| Actual Aging Temperature | 7.2 | 10 | 12.8 | Total | % correct |
| 7.2 | 10 | 5 | 3 | 18 | 55.56% |
| 10 | 5 | 7 | 6 | 18 | 38.89% |
| 12.8 | 2 | 4 | 12 | 18 | 66.67% |
| Total | 17 | 16 | 21 | 54 | 53.70% |

**Table 3.8.** Correlations of electronic tongue volatile analyses and descriptive sensory analyses (n=10) of nine white Cheddar cheese samples aged for 12 months. Data were collapsed across aging temperature.

| Taste | Aging Month | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 5 | 8 | 10 | 11 | 12 |
| Sweet | 0.009 | 0.441 | 0.084 | 0.990 | 0.981 | 0.964 |
| Salty | 0.288 | 0.995 | 0.595 | 0.683 | 0.724 | 0.938 |
| Bitter | 0.990 | 0.008 | 0.687 | 0.998 | 0.651 | 0.977 |
| Sour | 0.458 | 0.005 | 0.208 | 0.918 | 0.714 | 0.621 |
| Umami | 0.191 | 0.155 | 0.073 | 0.570 | 0.935 | 0.951 |

**Table 3.9.** Consumer demographic data collected from panels (n = 120) at 8- and 12-months

| Demographic Question | Selected Answers | 8-month Panel (% composition) | 12-month Panel (% composition) |
|---|---|---|---|
| Gender | Female | 62 | 67 |
| | Male | 38 | 33 |
| Race | White/Caucasian, European American, Non-Hispanic | 79 | 83 |
| | Asian/Asian American | 12 | 14 |
| | Hispanic/Latino American | 7 | 6 |
| Employment Status | Full-time | 50 | 49 |
| | Part-time | 18 | 15 |
| | Student | 43 | 43 |
| Consumption of Cheese Varieties | Cheddar | 95 | 98 |
| | Mozzarella | 89 | 83 |
| | Parmesan | 61 | 68 |
| | Cougar Gold® | 68 | 77 |
| Frequency of Consumption | Once a day | 29 | 30 |
| | 4 – 6 times a week | 33 | 29 |
| | 2 – 3 times a week | 26 | 30 |
| | Once a week | 7 | 8 |
| Factors that Influence Cheese Choice | Flavor | 99 | 98 |
| | Price | 75 | 78 |
| | Texture | 63 | 78 |
| | Availability | 53 | 49 |

**Table 3.10** Mean separation of significant aroma, color, flavor, and texture attributes of three Cheddar cheese samples as evaluated by a consumer panel at 8 months of aging and analyzed using Tukey's HSD. Different letters within a row represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Consumer Liking Parameters | 12-month Reference | 7.2˚C | 10˚C | 12.8˚C |
|---|---|---|---|---|
| Appearance | 7.200 a | 7.350 a | 7.267 a | 7.092 a |
| Aroma | 7.242 a | 7.075 a | 7.150 a | 7.100 a |
| Overall Flavor | 7.550 a | 6.950 b | 7.217 ab | 6.908 b |
| Cheddar Flavor | 7.375 a | 6.750 b | 7.025 ab | 6.883 b |
| Cheddar Intensity | 6.942 a | 5.942 b | 6.492 ab | 6.592 a |
| Sharpness | 7.400 a | 6.417 b | 6.975 a | 6.967 a |
| Sharpness Intensity | 6.842 a | 5.408 b | 6.250 a | 6.625 a |
| Aged Flavor | 7.225 a | 6.592 b | 6.892 ab | 6.992 ab |
| Aged Intensity | 6.875 a | 5.675 c | 6.192 bc | 6.508 ab |
| Texture | 7.133 a | 6.575 a | 6.792 a | 6.942 a |
| Crumbliness | 6.775 a | 6.033 b | 6.375 ab | 6.675 a |
| Firmness | 7.008 a | 6.392 b | 6.925 ab | 6.942 a |
| Overall Liking | 7.458 a | 6.817 b | 7.108 ab | 7.017 ab |

**Table 3.11** Mean separation of significant aroma, color, flavor, and texture attributes of three Cheddar cheese samples as evaluated by a consumer panel at 12 months of aging and analyzed using Tukey's HSD. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Consumer Liking Parameters | 12-month Reference | 7.2˚C | 10˚C | 12.8˚C |
|---|---|---|---|---|
| Appearance | 7.317 a | 7.308 a | 7.250 a | 6.933 a |
| Aroma | 7.225 a | 7.150 a | 7.250 a | 7.092 a |
| Overall Flavor | 7.350 a | 6.967 ab | 7.242 a | 6.633 b |
| Cheddar Flavor | 7.200 a | 6.917 ab | 7.108 ab | 6.650 b |
| Cheddar Intensity | 6.358 ab | 5.967 b | 6.508 a | 6.742 a |
| Sharpness | 7.025 a | 6.658 a | 6.908 a | 6.508 a |
| Sharpness Intensity | 6.425 b | 5.817 c | 6.658 ab | 6.983 a |
| Aged Flavor | 7.033 a | 6.567 ab | 6.833 ab | 6.458 b |
| Aged Intensity | 6.258 b | 5.717 c | 6.658 ab | 6.867 a |
| Texture | 6.983 a | 6.483 a | 6.917 a | 6.708 a |
| Crumbliness | 6.583 a | 5.800 b | 6.475 a | 6.658 a |
| Firmness | 6.975 a | 6.300 b | 6.708 ab | 6.800 ab |
| Overall Liking | 7.233 a | 6.725 ab | 7.092 a | 6.508 b |

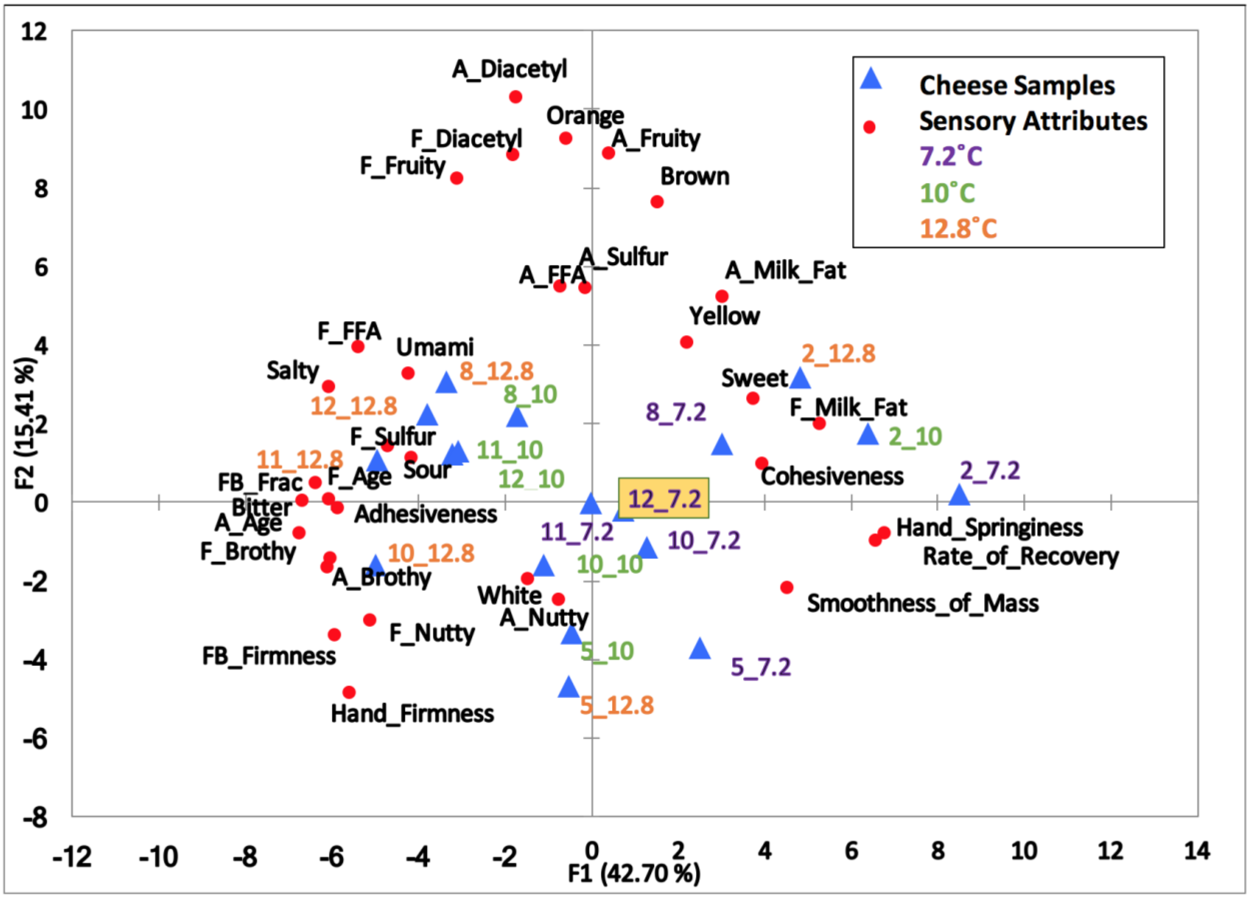


**Figure 3.1.** PCA of descriptive sensory analysis of aroma, color, texture, flavor, and taste by trained panel (n=10) of white Cheddar cheese samples aged at 7.2˚, 10˚, and 12.8˚C, over a 12-month ripening period. Samples are described by their labels with aging month, followed by aging temperature. Attributes were defined by the trained panel, with F describing the flavor attribute and A describing the aroma attribute.

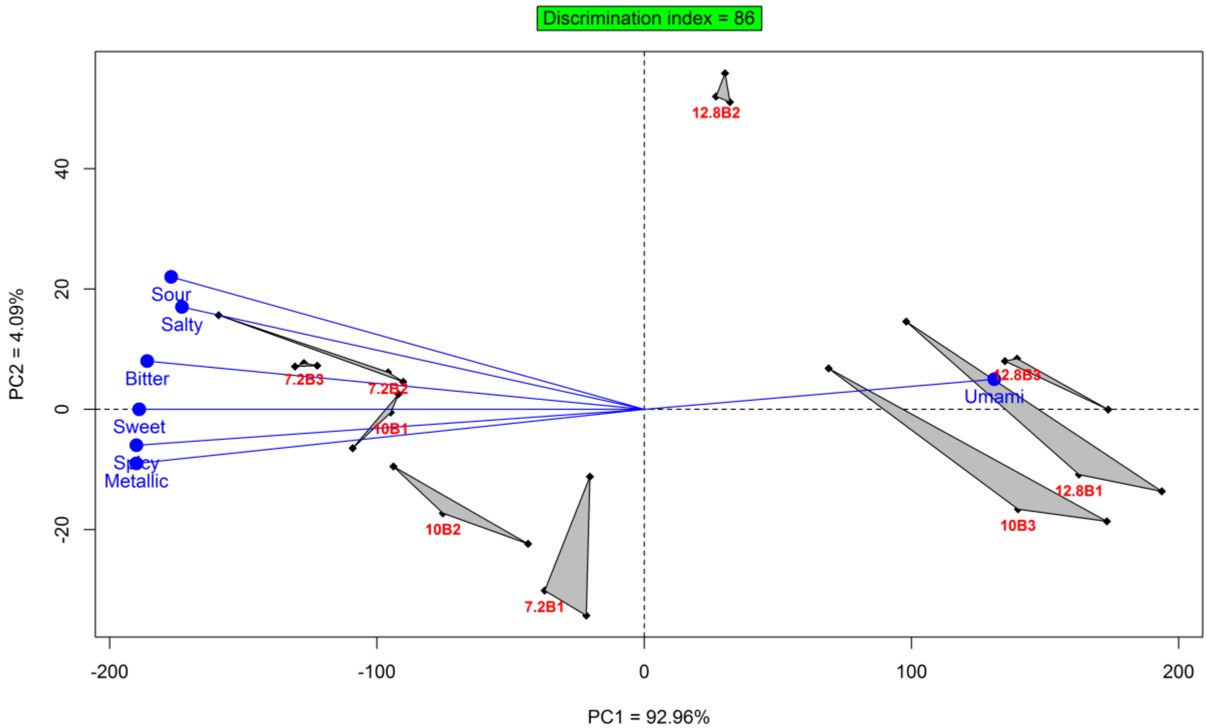


**Figure 3.2.** Electronic tongue volatile analysis using PCA of nine Cheddar cheese samples aged at various ripening temperatures (7.2˚, 10˚, 12.8˚C) for 8 months. Sample labels in red account for the aging temperature and batch (or day of production). Triangles are formed from triplicate measurements of the sample, with the area corresponding to the variation among the measurements.

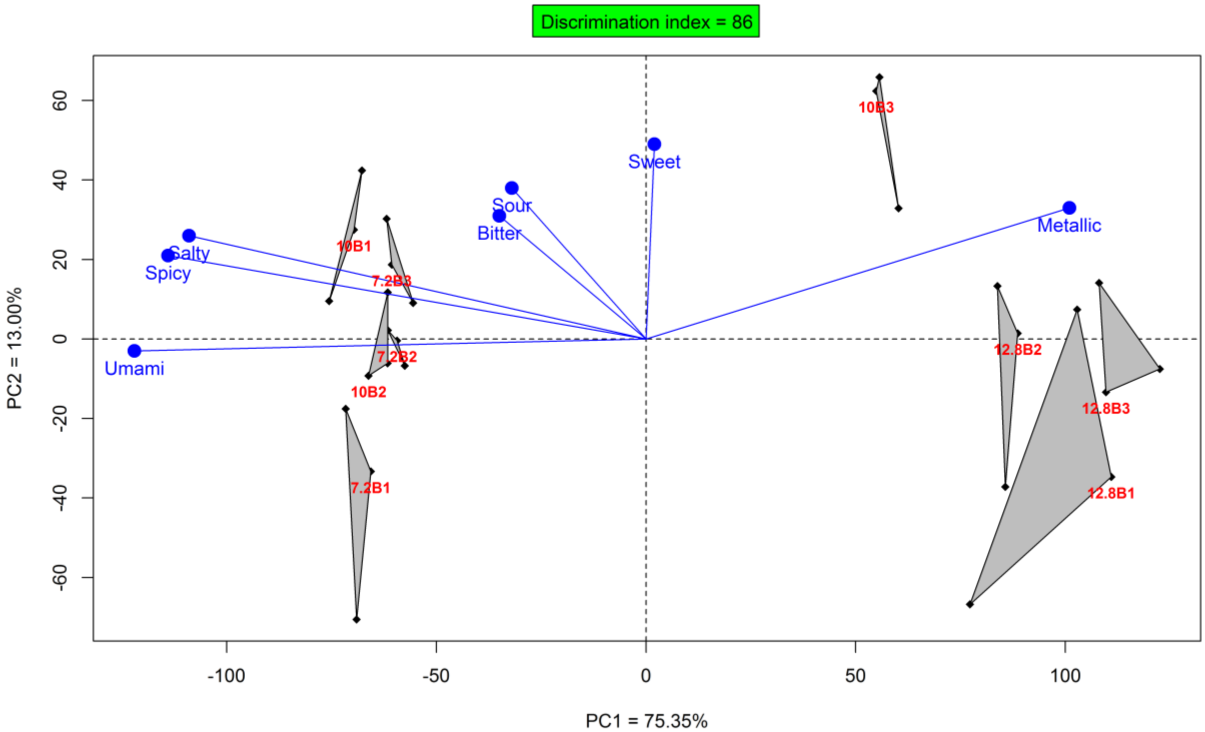


**Figure 3.3.** Electronic tongue volatile analysis using PCA of nine Cheddar cheese samples aged at various ripening temperatures (7.2˚, 10˚, 12.8˚C) for 11 months. Sample labels in red account for the aging temperature and batch (or day of production). Triangles are formed from triplicate measurements of the sample, with the area corresponding to the variation among the measurements.

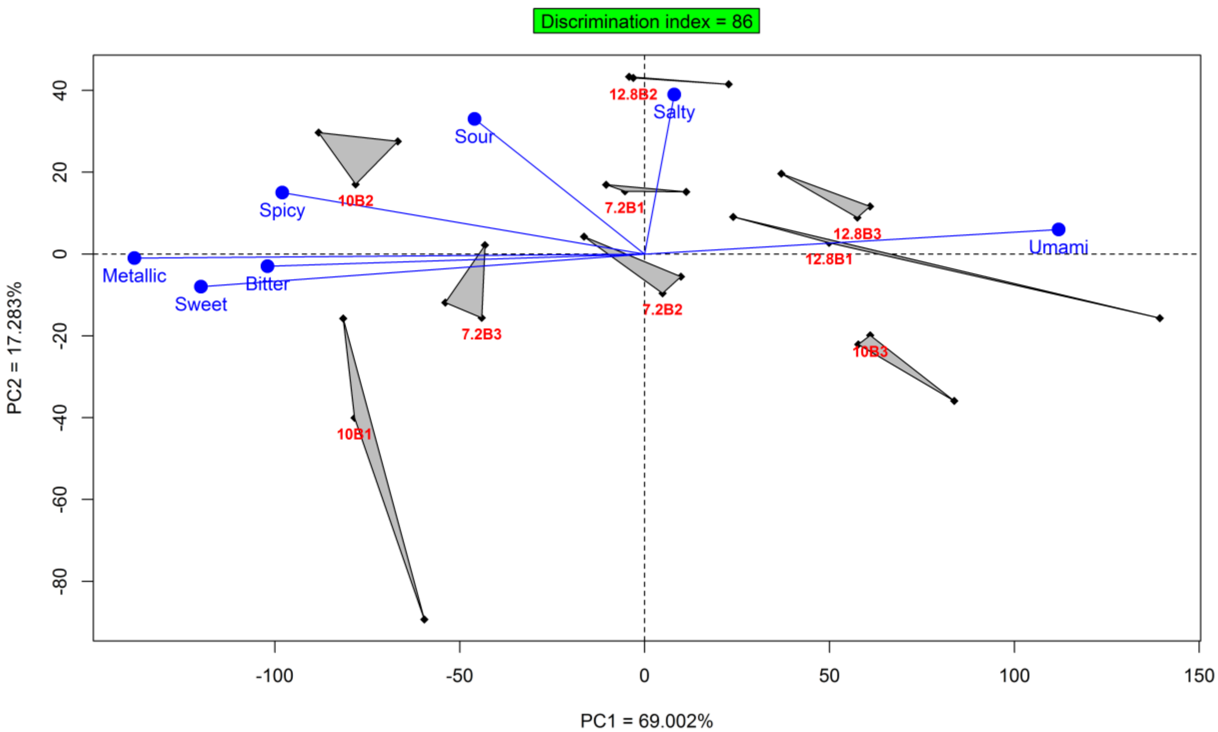


**Figure 3.4.** Electronic tongue volatile analysis using PCA of nine Cheddar cheese samples aged at various ripening temperatures (7.2˚, 10˚, 12.8˚C) for 12 months. Sample labels in red account for the aging temperature and batch (or day of production). Triangles are formed from triplicate measurements of the sample, with the area corresponding to the variation among the measurements.

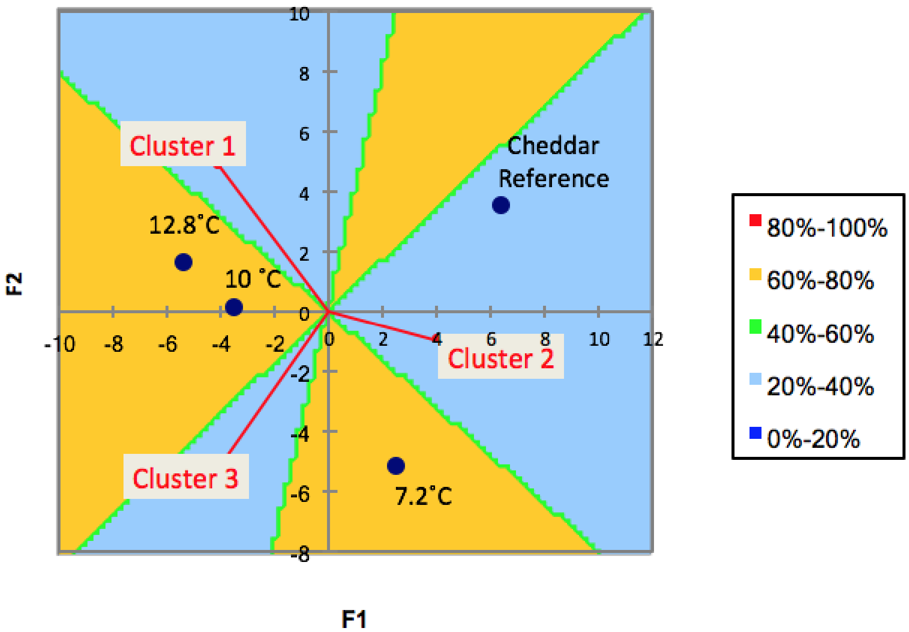


**Figure 3.5.** External preference map of 12-month consumer (n = 120) preferences for the Cheddar cheese samples. Cheddar cheese samples are labelled by a navy-blue circle. Clusters are labelled with red vectors. Shaded areas (percentages of consumers as labelled in the legend) of the contour plot signify which clusters have preferences above average in a given region of the map. The Cheddar reference refers to Tillamook Sharp White Cheddar while the 7.2˚, 10˚, and 12.8˚C refer to the temperature at which the white Cheddar cheese samples were aged.

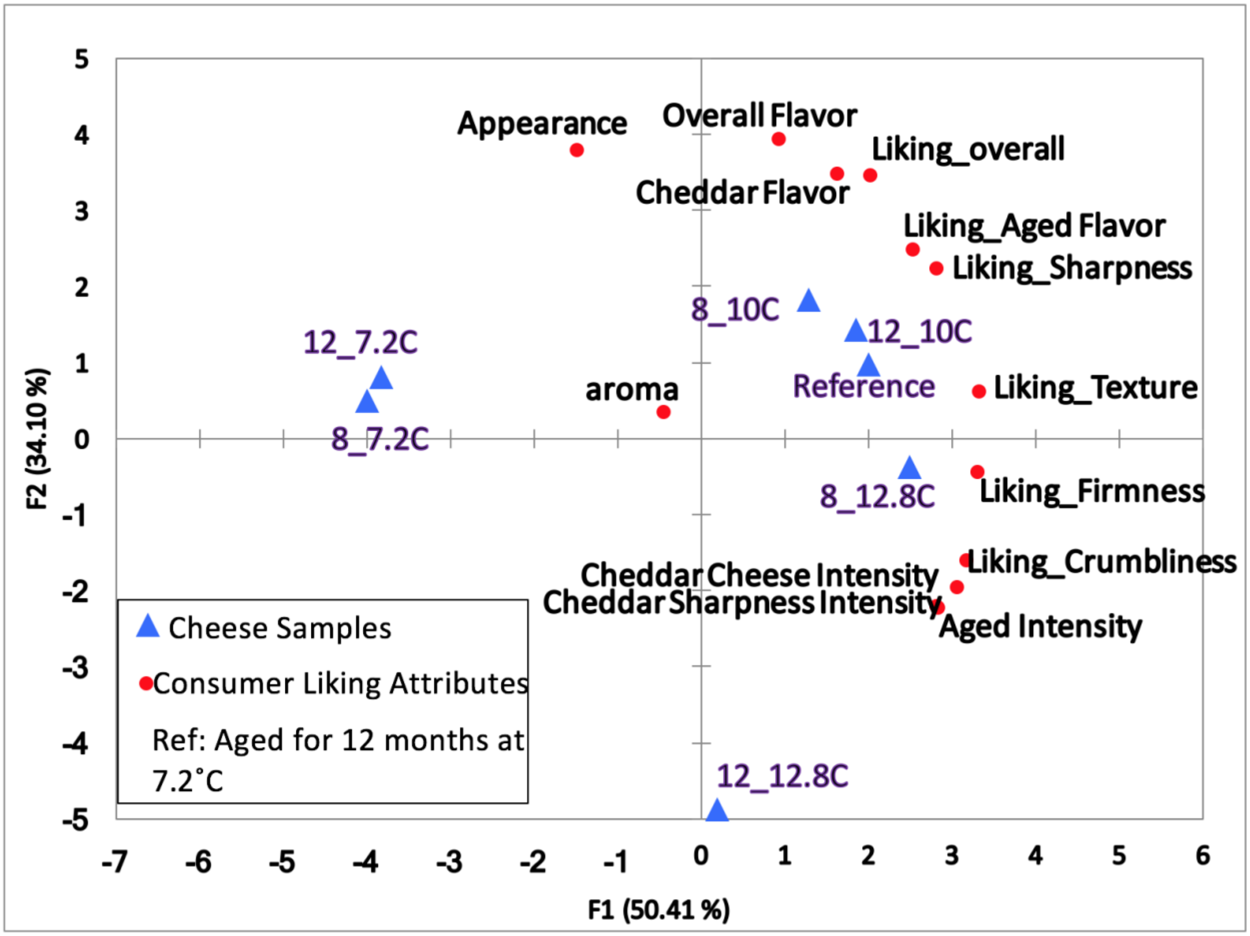


**Figure 3.6.** PCA of consumer panel (n =120) acceptance of various attributes and attribute intensities of white Cheddar cheese samples aged at 7.2˚, 10˚, and 12.8˚C for 8 and 12 months. White Cheddar cheese samples are described by their aging time in months followed by aging temperature.

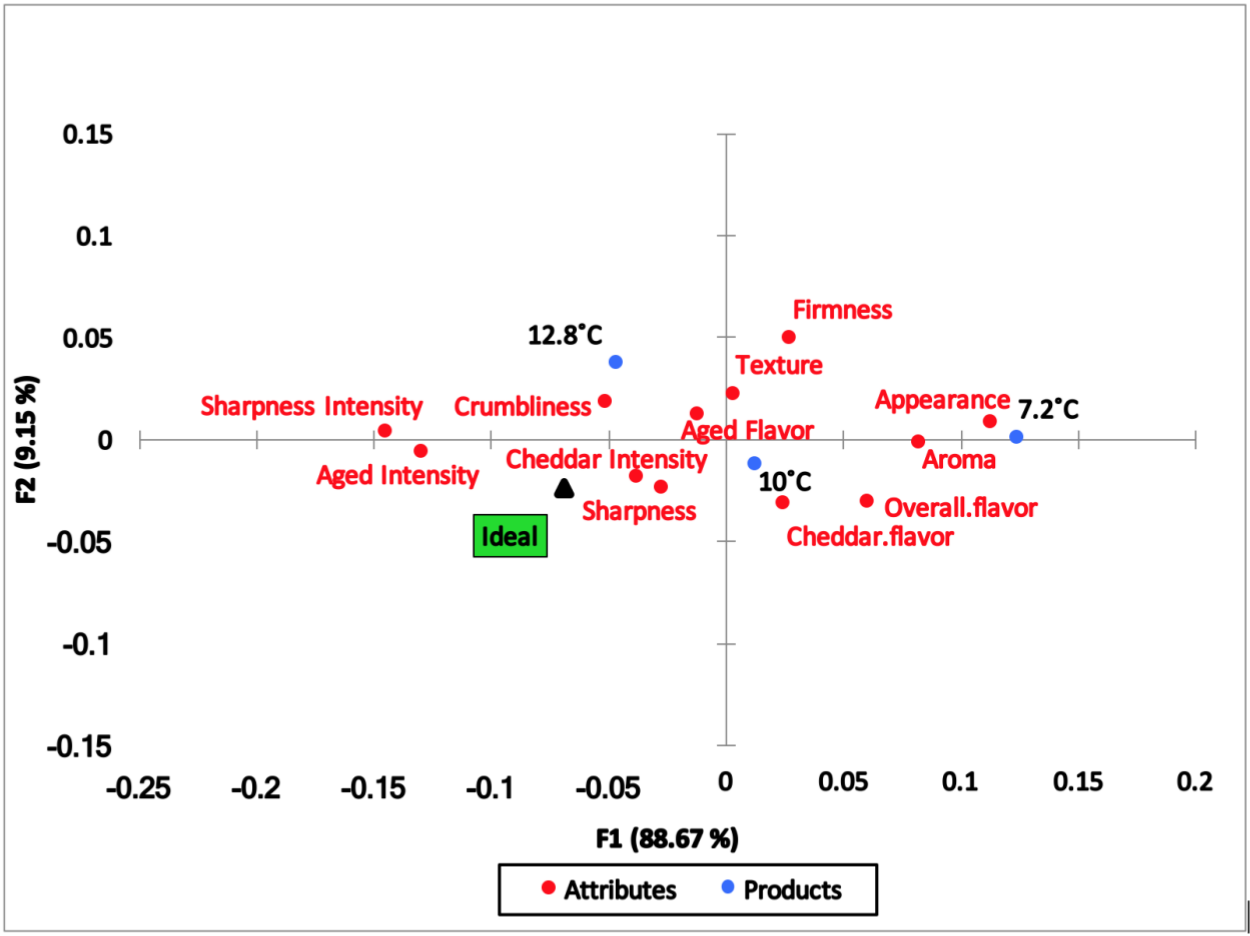


**Figure 3.7.** Correspondence analysis of 8-month consumer panel (n=120) data indicating the ideal product and its "must have" attributes. The sensory attributes determined by the consumers are shown in red. Cheddar cheese samples aged at different temperatures are shown as 7.2˚, 10˚, and 12.8˚C. The consumers' "ideal cheese", as shown in the green box with the black triangle, was the reference sample during consumer panels – aged for 12 months at 7.2˚C.

# CHAPTER FOUR: SENSORY TEXTURE AND RHEOLOGICAL IMPLICATIONS OF WHITE CHEDDAR CHEESE RIPENED WITH ELEVATED STORAGE TEMPERATURE


## Abstract

To keep up with consumer demand for aged Cheddar cheese, the dairy industry is tasked with manufacturing quality cheese more quickly. Elevated aging temperature is a simple approach, but can result in negative changes in texture and an imbalance of flavors. Therefore, the objective of this study was to evaluate the influence of elevated temperature on the sensory and rheological properties of aged white Cheddar cheese. White Cheddar cheese was aged at 7.2˚C, 10˚C, or 12.8˚C for 12 months and were evaluated at 2, 5, 8, 10, 11, and 12 months with a trained sensory panel (n=10). In addition, two cycle compression and small amplitude oscillatory shear (SAOS) were used to monitor mechanical property changes throughout maturation. The trained panel results showed a loss of cohesiveness and smoothness of mass with higher first-bite fracturability in cheeses aged at elevated temperature, compared to a commercially available reference sample aged at 7.2˚C for 12 months ($p<0.05$). Texture degradation was more apparent with cheese aged at elevated temperature due to an observed increased degree of proteolysis. Two-cycle compression results indicated a decrease in springiness and cohesiveness as storage time and temperature increased. The viscoelastic behavior of the cheese was investigated using SAOS and both storage time and temperature resulted in decreased critical stress and strain. Many sensory attributes and instrumental parameters were strongly correlated illustrating the ability of instrumental

analyses to serve as indicators of sensory properties. In conclusion, elevated aging temperature and storage time influenced sensory and rheological properties of white Cheddar cheese samples. This study demonstrated, despite the increased break down of the cheese matrix, white Cheddar cheese aged at 10˚C produced a cheese similar in sensory properties to cheese aged at 7.2˚C.

## Introduction

Cheddar cheese has become the second most popular variety of cheese in the United States following mozzarella (USDA ERS 2016). Given this demand by consumers, manufacturers have searched for alterations to the cheesemaking process to reduce production time and resources. However, production changes such as elevated ripening temperature and reduced storage time often result in consumer rejection due to the development of unbalanced and off-flavors as well as microbiological spoilage (Marsili 1985, Hannon and others 2005). Elevated aging temperature can yield problematic results due to increased glycolysis, lipolysis, and proteolysis that occur during cheese ripening. Most important for texture development is proteolysis, where the protein matrix breaks down, thus reducing the firmness of cheese curd.

The dairy industry has always stressed the importance of texture in cheese. The American Dairy Science Association developed score cards – which include texture as a metric of quality – used by judges to assess product value and potential use (Partridge and others 2009). Modern sensory practices aim to understand the relationship between texture and consumer acceptance, but often require instrumental analysis to elucidate texture further. Cheese texture is a critical, primary quality attribute with a large influence on consumer preference (Gunasekaran and others 2003). Consumer perception of cheese texture is multifaceted, with sight, touch, and hearing all influencing its properties. Consumers ultimately perceive cheese structure, but given the individuality and subjectivity of experiences during mastication, sensory and instrumental assessments of mechanical properties are often combined to elucidate the role of texture.

Research on the development of cheese texture during ripening has sought to clarify the role of proteolysis and factors influencing its rate (Lawrence and others 1987). The impact of elevated ripening temperature on mechanical properties of cheese have been assessed (Folkertsma and others 1996, Joshi and others 2004, Hannon and others 2005) Elevated temperature has been shown to expedite texture development, as Cheddar cheese became more fracturable and less springy as both storage time and temperature were increased (Fedrick and others 1984). Rheological testing has revealed strain weakening behavior of cheese as fat induces weak points in the curd that become initiation sites for fracture during aging (Rogers and others 2009). A reduction in salt content resulted in lower fracture stress and firmness in Cheddar-style cheese which varied in fat content (McCarthy and others 2016). Further elucidation of the influence of elevated temperature on the mechanical properties of cheese and consumer acceptance of texture is vital to the cheese industry, especially Cheddar cheese producers. The current study utilized rheological instrumental analysis and descriptive sensory analysis to determine the influence of storage time and elevated ripening temperature on the physical and sensory properties of white Cheddar cheese.

## Materials and Methods

### Materials

Texture standards for descriptive sensory analysis, purchased at Safeway (Pleasanton, CA) included: Velveeta (Kraft Heinz Foods Company, Chicago, IL), Muenster (Primo Taglio, Lucerne Foods, Inc., Pleasanton, CA), Medium Cheddar (Tillamook County Creamery Association, Tillamook, OR), Parmesan (BelGioioso Cheese Inc., Green Bay, WI), and Feta (Athenos Traditional, Churny Company, Inc., Weyauwega, WI). Also, purchased at Safeway were other panel materials including seedless green grapes, unsalted saltines, hand wipes, and cuspidor. Plastic soufflé cups with lids (SOLO, Lake Forest, IL) were used to hold the cheese samples. Sharp white Cheddar cheese (Tillamook County Creamery Association, Tillamook, OR) was served as a reference for trained panel analysis. Mono- and dibasic potassium phosphate were purchased from J.T. Baker (Phillipsburg, NJ) to make phosphate buffer for peptide extraction. OPA assay materials included sodium dodecyl sulfate, sodium tetraborate, 2-mercaptoethanol, and *o*-phthaldialdehyde (OPA) were purchased from Sigma (St. Louis, MO).

### Cheese Samples

Preparation of cheese samples are outlined in chapter 3. White Cheddar cheese, totaling 180 cans, was provided by the WSU Creamery (Pullman, WA) during three days of production (60 cans/day). Within a given production day, cans (n=60) were randomly assigned, equally across days, to specific aging temperature coolers (7.2˚C, 10˚C, and 12.8˚C). Over the 12-month aging period, cans from each production day were randomly selected at 2, 5, 8, 10, 11, and 12 months for analysis with chemical, physical, and sensory methods described below.

OPA Assay

Preparation of cheese samples was conducted as explained by Ravisankar (2015). Grated cheese (30 g) was suspended in 90 mL of 50mM phosphate buffer (pH 7.0). Using the Ultra Turrax IKA T 18 basic homogenizer (IKA Labortechnik, Staufen, Germany) samples were homogenized for two min at 4500 x g. The samples were then heated to 40˚C and stirred gently for 1 hr. Centrifugation at 3000 x g for 30 min using a Beckman GPR centrifuge (Beckman Coulter, Brea, CA, USA) was used to isolate the top aqueous layer, which was then filtered using Whatman No. 2 qualitative filter paper (Whatman International Ltd., Maidstone England). The pH of the samples was adjusted to 4.6 using 1N HCl and centrifuged again at 10,000 x g for 10 min at 4˚C to precipitate caseins. Polyethersulfone filters (0.45 µm pore size) were used to filter remaining solids. Extracted peptide samples were diluted with PBS (1:35). The OPA assay was then carried out as described by Ravisankar (2015).

Sensory Evaluation

Panelists (n = 10) were recruited to join the trained panel through electronic announcement within the Washington State University community, with no previous experience required for participation. Training, totaling 20 hours, took place for six weeks before an evaluation of 2-month old cheese. Prior to the evaluations at 5, 8, 10, 11, and 12 months, a 1-hr refresher training session was held to allow for panelist review of attributes. To reduce potential bias, minimal background information was provided to panelists about the study. The panel was composed of 60% females and 40% males, with a mean panel age of 29.5 years old (between 23 and 54 years of age). Cheese consumption patterns of panelists was indicated as either once to several times per week or daily. The study was approved by the Washington State University Institutional Review Board for human subject participation with panelists receiving nonmonetary incentives following all training and formal evaluation sessions.

Panelists received training on correct 15-cm line scale use - anchored at 1.5 cm (low) and 13.5 cm (high) - and evaluation techniques for texture of cheese attributes by hand, in mouth, and first-bite. Standards (**Table 4.1**) were presented within the six weeks of training sessions. Feedback was provided to panelists during practice sessions, immediately after rating each attribute for all samples, by using the Feedback Calibration Method, available through *Compusense® at hand 5.6* (Compusense Inc., Guelph, ON, Canada). Additionally, panelists received feedback about performance following data analysis of practice sessions with SenPaq (Qi Statistics Ltd., Berkshire, UK) as well as during refresher sessions, the day prior to formal evaluation of cheese at 5, 8, 10, 11, and 12 months.

Instruction, scale presentation, and data collection during formal evaluations utilized *Compusense® at hand 5.6* (Compusense Inc., Guelph, ON, Canada). All cheese samples were cut into 1.5 cm cubes with 6 total cubes presented in 4 oz. soufflé cups with random three digit codes. Samples were removed from refrigeration 1-hr prior to evaluations to allow for sample equilibration to ambient temperature. Panelists were provided with water, unsalted crackers, and grapes for palate cleansing between cheese samples. Four samples in replicate, totaling eight cheese samples, were evaluated utilizing a randomized serving order, with forced breaks between every sample. A 15-minute break following evaluation of the fourth sample was utilized to minimize panelist sensory fatigue and palate refreshing.

Two-cycle Compression Testing

At each time point, the aged white Cheddar was evaluated using a two-cycle compression test. For this analysis, six replicate samples (22mm x 14.5mm) were obtained from larger wheels of cheese at ambient temperature using a cylindrical cork borer with 14.75mm width. Cores were cut in half to obtain a ~22 mm height. Two-cycle compression testing utilized a TA.XT *plus* Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 50 kg load cell and a 5.08 cm diameter aluminum probe (TA-25). Quintuplicate cheese samples were compressed at 0.83 mm/sec to 75% of their original height with 16.6s rest between compression cycles. Parameters included fracturability, hardness, adhesiveness, resilience springiness, and cohesiveness. Parameters of the test were calculated using Exponent texture analysis software (Version 6, Stable Micro Systems, Godalming, Surrey, UK) with the following rational (Steffe 1996). Fracturability was calculated as the force at the first major drop. The force at the maximum compression during the first of two compressions was calculated as hardness. Adhesiveness was found as area 3 on the force curve or the negative area between compressions. The length of the second compression cycle was calculated as springiness and resilience by dividing the upstroke energy of the first compression by the downstroke energy.

Rheological Testing

Strain sweep data from small amplitude oscillatory shear (SAOS) testing was obtained using the Discovery DHR-3 Hybrid Rheometer and TRIOS Software (TA Instruments, New Castle, DE) in controlled strain mode. Strain sweep testing was utilized to compare the viscoelastic behavior of the samples, rather than a frequency sweep, which would show how the behavior changed. Comparison of the viscoelastic behavior of the samples throughout the 12-month aging period was explored by finding the critical value within the linear viscoelastic region.

A serrated parallel plate (20 mm diameter) system was used to prevent slip. At each storage time point, samples were sliced from cheese wheels using a 3600N commercial slicer (Globe Food Equipment Co., Dayton, OH) at room temperature (22 ± 2˚C). The top plate was lowered onto samples until a normal force of 1.0 N was obtained. Samples were trimmed to fit underneath the geometry before conducting strain sweeps. Due to the briefness of the strain sweep the sample edges were not protected. Before strain sweep data was collected from 0.01 to 100% strain at a frequency of 1 Hz, samples were conditioned for 30 s at 25˚C (Rogers and others 2010). Testing was conducted in triplicate.

Statistical Analyses

Trained panel data were analyzed using XLSTAT Sensory 18.07 (Addinsoft, New York, NY) to perform ANOVA and Principal Components Analysis (PCA). A three-way ANOVA was utilized to assess the main effects of storage time (month), elevated temperature and panelist, as well as the interaction effects of month and temperature, on the intensities of sensory texture attributes of cheese samples. Those same main and interaction effects were applied with ANOVA on the rheological and two-cycle compression data. Significance was established as $p < 0.05$ and mean comparisons computed with Tukey's HSD. To explore the relationship between instrumental mechanical properties and sensory texture attributes through correlation, partial least squares (PLS) regression was employed using XLSTAT Sensory 18.07 (Addinsoft, New York, NY).

**Results and Discussion**

Chemical Analysis

The *o*-phthaldialdehyde assay was developed as a rapid and sensitive fluorescent method for measuring proteolysis of dairy products (Ravisankar 2015). The assay is based on the reaction of OPA and β-mercaptoethanol with primary amines from proteins and their resulting amino acids and peptides (Church and others 1983). The OPA assay results (**Table 4.2**) indicated that the degree of proteolysis increased 3-fold, regardless of ripening temperature, during the 12-month aging period. An increase in the degree of proteolysis was also found with an increase in aging temperature as well. Aging at 12.8˚C accelerated proteolysis 1-fold throughout the ripening period compared to aging at 7.2˚C. Increased proteolysis observed with storage time agree with previously reports (Ravisankar and others

2015). This primary biochemical event softens the cheese curd over time as the casein protein matrix is weakened via hydrolysis.

The acceleration of proteolysis by utilizing elevated temperature has been previously reported with Cheddar cheese (Folkertsma and others 1996). Cheese samples were aged at 8˚C, 12˚C, and 16˚C and proteolysis monitored by measuring water-soluble nitrogen (WSN) content for 9 months. Cheese aged at the elevated temperature of 16˚C had an increased rate of WSN production (2-fold) compared to cheese aged at 8˚C. Additionally, this rate of increased WSN production was decreased when cheese was transferred from elevated temperature to 8˚C. The extent of degradation of $\alpha_{s1}$- and β-caseins was shown to be greater in cheeses aged at elevated temperature yielding validity to the results obtained in this study, as proteolysis was shown to increase with the use of elevated aging temperature. Given the acceleration of proteolysis obtained during aging at elevated temperature, differences in the mechanical properties and sensory texture were expected. Previously, increased proteolysis leading to increased protein breakdown resulted in a more crumbly texture (Drake and others 1999).

Physical Analyses

*Two-cycle Compression*

The increase in degree of proteolysis with storage time lead to the expectation that differences would be observed in the two-cycle compression parameters in aged white Cheddar cheese. ANOVA results showed that storage time influenced all testing parameters, while ripening temperature significantly influenced cohesion, springiness, and chewiness (**Table 4.3**). Mean separation found significant differences ($p<0.05$) among all months for all testing parameters and for temperature, cohesion, springiness, and chewiness. The interaction of month and temperature influenced the parameters of adhesiveness, springiness, and fracturability.

Texture development of Cheddar cheese occurs in phases with rapid change in the first two weeks (Phase 1) and gradual change over the following months (Phase 2) of aging via proteolysis (Lawrence and others 1987). The $\alpha_{s1}$-casein portion is hydrolyzed into the $\alpha_{s1}$-I peptide during Phase 1 and further in Phase 2, along with other casein degradation products: $\alpha_{s1}$-I, β-, and ρ-κ-casein (Irudayaraj and others 1999). Following Phase 1 proteolysis (2 weeks), much of the $\alpha_{s1}$-casein has lost its ability to support the protein matrix. Its cleavage induces a loss of elastic structural elements, influencing fragility of Cheddar cheese (Creamer and others 1982). However, proteolysis itself is not the sole reason for rheological changes during aging, as its rate is affected by numerous manufacturing steps. Moisture content, residual rennet, salt-to-moisture ratio, pH changes during ripening, and ripening temperature all influence the rate of proteolysis (Lawrence and others 1987). With Cheddar being a low moisture cheese, larger changes are observed in the rate of proteolysis because available moisture is bound to casein degradation products. The rate of proteolysis is decreased as water becomes unavailable and change in texture happens slower than in high moisture cheeses (Lawrence and others 1987). Higher rates of proteolysis were seen in Cheddar cheese manufactured with lower salt-to-moisture ratio (Upreti and Metzger 2006). Elevated storage temperature was found to increase the extent of $\alpha_{s1}$-casein degradation over a 70-day ripening period in low moisture Mozzarella cheese (Feeney and others 2001).

The effect of proteolysis on Cheddar cheese's rheological properties have previously been studied. The springiness of Cheddar cheese samples was shown to be greater in younger cheese due to their more elastic properties indicated by yield forces. The yield force was related to the intact $\alpha_{s1}$-Casein in the cheese samples with more present in younger cheeses (Creamer and others 1982). Cheddar cheese showed an increase in hardness following aging for 5 weeks and continued to increase throughout the 8-month ripening period (Irudayaraj and others 1999). Younger cheeses (2 weeks) were found to be more cohesive when utilizing two-cycle compression than older Cheddar cheese (107 weeks) samples (Creamer and others 1982).

Springiness decreased significantly from 2 months to 5 months with no significant differences in the following months. The overall trend of a decrease in springiness as aging time progresses, indicated by the mean separation results (**Table 4.4**), can be explained by proteolysis. As the casein breaks down the structure of the matrix is weakened, reducing the ability of the cheese to recover from enacted stress, and hence reducing the springiness. The 2-month cheese is likely different from all other months of aging because most of Phase 1 protein breakdown has already occurred after the first two weeks of aging and some of

Phase 2 proteolysis has begun (Lawrence and others 1987). Following 5-month evaluation the cheese did not significantly decrease in springiness due to the slower progression of Phase 2 proteolysis and thus matrix weakening, which was occurring by and after the 5-month evaluation period. Therefore, the springiness of cheeses at 2-months was different than all other storage times likely due to the documented phases of proteolysis.

Proteolysis also affected the hardness of the cheese with mean separation results indicating highest hardness in later months of aging; 8-, 10-, 11-, and 12-month samples. Proteolysis is facilitated by the hydrolysis of peptide bonds; as the protein matrix is broken down the “free” water within the curd is reduced resulting in the development of a harder texture with aging (Irudayaraj and others 1999). Moisture was observed in many of the cans during the 8 – 12-month time points. This would change the moisture content of the cheese, although the amount of moisture was less than 5 mL and was not expelled during deformation. Serum release in whey protein/polysaccharide mixed gels has been shown to affect the breakdown of these gels as energy dissipation and fracture properties are changed, requiring correction. Gels with low serum release were perceived as crumbly (van den Berg and others 2008). With no serum release during deformation, only during aging in cans, corrections were not applied to data. However, the change in moisture content may have influenced the perception of sample hardness during later months of aging.

Instrumental cohesiveness decreased throughout aging. Cohesiveness was also influenced by matrix degradation due to proteolysis as available water is decreased resulting in a harder texture, weakening cohesive forces. Weaker cohesive forces within cheese can lead to a more crumbly, less creamy texture during mastication (Møller and others 2013). The cheese pH during manufacturing also plays a major role when describing cohesiveness. With Cheddar cheese, good cohesion occurs around pH 5.2 when conditions are optimal for casein aggregation during manufacturing. Below pH 5.2, cohesion decreases until pH 4.8 where casein micelles lose their paracasein identities due to aggregation and cohesion of the cheese is lost (Lawrence and others 1987, Creamer and others 1998). The overall decrease in cohesion observed from the mean separation results was likely due to the breakdown of the protein with aging, but also because the average pH of the white Cheddar cheese samples – found prior to aging, following manufacturing – was below 5.2, at pH 4.96. pH change over the maturation period should be studied in the future to explore the decrease in cohesiveness. Should the pH decrease, further cohesiveness would be lost.

Mean separation found significant differences between temperatures (**Table 4.5**) for the two-cycle compression parameter of cohesion. The cheeses aged at 7.2˚C had higher cohesiveness values than cheeses aged at 10˚C and 12.8˚C. The increased rate of proteolysis with elevated aging temperature may explain the decrease in cohesiveness. The decrease in chewiness as aging temperature increases are due to their direct relation to springiness, as chewiness is defined as hardness x cohesiveness x springiness, or hardness x ratio of area 2 divided by area 1 x length of compression during the second compression (Steffe 1996).

*Small Amplitude Oscillatory Shear*

Strain sweep parameters, analyzed using ANOVA, indicated significant differences ($p < 0.05$) due to storage time for all parameters and for temperature, storage modulus, loss modulus, and critical strain (**Table 4.6**). SAOS testing parameters were not influenced by the interaction of storage time and temperature.

The strain sweep was implemented to aid in determining the viscoelastic behavior of the cheese samples, which can be explained by the storage and loss moduli. G' is a measurement of the elastic behavior of the sample, or energy storage, while G" is a measurement of the viscous behavior, or energy dissipation in the form of heat (Foegeding and others 2003). Critical stress and strain were obtained by finding the linear viscoelastic region (LVR) with critical strain identified where the complex modulus deviates by 2% from the previous value during the strain sweep (Melito and others 2013). Both G' and G" increased as storage time progressed and as temperature was elevated. The storage modulus across storage time and temperature was larger than the loss modulus within the LVR. This indicates predominately solid viscoelastic behavior. Elevated temperature and storage time both influenced the viscoelastic behavior of the cheese. For both moduli, the differences found among samples were the same throughout storage time with 2-month cheeses differing from all other samples, 5- and 8-months from 10- and 12-month cheeses, and 12-month cheeses differing from all except 10-month cheeses (**Table 4.6**). Regarding temperature, G' and G" were significantly different for all samples (**Table 4.7**). The critical strain decreased with time resulting in less deformation required to cause permanent microstructural damage to the sample. Overall critical stress decreased with a significant difference found at 10 months of aging regarding an increase in stress. Critical stress was not influenced by storage temperature, although critical strain was lower with cheeses aged at 12.8˚C compared to 7.2˚C aging.

Descriptive Sensory Analysis

Storage time, ripening temperature, and panelist influenced (**Table 4.8**) all descriptive sensory analysis attributes except adhesiveness where temperature did not have an effect. A learning effect is common with trained panels, although panelist's ability to discriminate individual attributes is enhanced with more training (Drake and others 2002). The interaction effect of month and temperature caused significant differences in all attributes.

Aging temperature, (**Table 4.9**) influenced all cheese texture attributes except for adhesiveness. More specifically, hand springiness, rate of recovery, smoothness of mass, and cohesiveness decreased as aging temperature increased. Hand firmness, first-bite firmness, and first-bite fracturability also increased with elevated temperature. The same trend was observed regarding storage time (**Table 4.10**), as expected due to the significant interaction effect. The breakdown of the cheese matrix during aging and due to temperature result in these changes.

Trained panel evaluation and subsequent PCA allowed for characterization of samples throughout the 12-month aging period (**Figure 3.1**). PC1, described by the contrasting relationship between hand springiness and rate of recovery with first-bite fracturability, accounted for 42.7% of the variation among samples. PC2 accounted for another 15.4% of variation, and described by the contrasting relationship between hand firmness and first-bite firmness with diacetyl and fruity flavors and aromas (these non-texture attributes were discussed in the previous chapter). The first two principal components explained a total of 58.1% of sample variation, with another 12.7% of variation accounted for from PC3.

Young cheeses were characterized by hand springiness and rate of recovery as well as a distinct lack of hand firmness and first-bite fracturability at 2-months of age. As aging progressed to 5 months, cheeses began to develop aged characteristics such hand and first-bite firmness. During other aging periods – 8, 10, 11 and 12 months – cheeses were described more by first-bite fracturability and first-bite firmness. It is important to note cheese aged at elevated temperature became more adhesive with age, while cheese aged at the normal aging temperature of 7.2˚C retained its cohesiveness. Samples aged at 10˚C, while still described by adhesiveness were closer to the reference sample (12_7.2) than samples aged at 12.8˚C, due to their higher intensities of cohesiveness and smoothness of mass. The samples aged at 12.8˚C had higher intensity values of first-bite fracturability and adhesiveness, setting them apart from the reference sample.

Sensory and Instrumental Correlation

The PLS regression biplot (**Figure 4.1**) shows correlations amongst sensory evaluation and rheological instrumental analysis. General sample trends show the upward progression of samples from 7.2˚C temperatures and short storage time (2, 5, and 8months) to elevated temperatures and longer storage time of 10, 11, and 12 months. The low temperature and short storage time samples were described by critical strain and cohesiveness. Elevated temperature and longer storage time samples were shown to be described by fracturability and hardness.

Correlations of sensory attributes and rheological testing parameters were explored given the following visual groupings seen in **Figure 4.1**. The visual groupings observed were first-bite fracturability, loss modulus, and storage modulus; first-bite fracturability, hand firmness, and adhesiveness; and critical strain, cohesiveness, and smoothness of mass; hand springiness, rate of recovery, gumminess, and cohesion. These groupings give an indication of which sensory and rheological parameters may be correlated.

Correlating sensory and rheological data is complicated due to the difference between the testing. Rheological tests measure physical properties and can be designed to mimic compression during the first several bites, but do not mimic the dynamic perception of sensory texture (Foegeding and others 2003). However, several studies have found correlations between sensory properties and instrumental measurements. Empirical rheological testing was found to predict sensory texture properties in cheese as well as or better than fundamental testing

(Drake and others 1999). Correlations between sensory and rheological behavior to understand structure and breakdown were investigated through the comparison of Cheddar, Mozzarella, and American cheeses. Firmness and fracturability, defined using sensory analysis, explain the rigid structure of Cheddar as it was correlated with the non-linear viscoelastic behavior observed through large amplitude oscillatory shear testing (Melito and others 2013).

The following strong correlations (> 0.80) were found between sensory attributes and rheological instrumental parameters. Sensory hand springiness was negatively correlated with storage modulus ($R^2$ = -0.89) and loss moduli ($R^2$ = -0.90) and positively correlated with instrumental cohesiveness ($R^2$ = 0.84). Rate of recovery was also correlated with those same instrumental parameters, storage modulus ($R^2$ = -0.92), loss modulus ($R^2$ = -0.92), and instrumental cohesiveness ($R^2$ = 0.83). Sensory first-bite fracturabilty was correlated with instrumental cohesiveness ($R^2$ = -0.80) and the storage ($R^2$ = 0.86) and loss moduli ($R^2$ = 0.88). Sensory adhesiveness and instrumental cohesiveness, although not strongly correlated, were correlated (-0.77).

Hand springiness and instrumental cohesiveness both relate to resisting deformation when strain is enacted on the sample. Hand springiness, defined for trained panelists as the total amount of recovery of the sample, is likely to be correlated with instrumental cohesiveness, given that it is a measurement of the sample's ability to stay intact, sticking to itself. This similarity in resisting deformation explains the correlation between hand springiness and cohesiveness.

Likewise, the storage and loss moduli were expected to yield negative correlations with hand springiness, since they are measurements of structural rigidity, either storing or dissipating energy, respectively. The same rational can be used to explain the correlations with rate of recovery, since it is the rate at which the sample recovers to its original shape.

First-bite fracturability, the amount of fracturability of the sample after biting, indicates a sample with high fractruability breaks in multiple places during the first-bite of mastication. A rigid structure, as implied though the correlations with the storage and loss moduli, would likely have a high fracurability. The term was negatively correlated with cohesiveness because a sample with high fracturability would not have the ability to stay intact, as sensory cohesiveness suggests. The negative correlation observed between sensory adhesiveness and instrumental cohesiveness is due to their inherent differences. An adhesive sample sticks to the surfaces of the mouth and teeth, whereas a cohesive sample resists deformation, staying intact during mastication.

Additionally, some expected correlations between sensory attributes and instrumental parameters were not strong. Given the elastic property measured through springiness it was expected to correlate with sensory hand springiness as a sample that recovers would have an elastic property to it, although the correlation was $R^2 = 0.55$. Sensory and instrumental cohesiveness resulted in low correlation ($R^2 = 0.47$), as did first-bite fracturability and instrumental fracturability ($R^2 = 0.22$). Saliva plays a vital role during mastication which could justifiably be a reason sensory and instrumental cohesiveness was not more strongly correlated as rheological testing did not utilize saliva. Saliva serves as a protective function in the oral cavity, lubricating its surfaces, influencing texture perception (Vijay and others 2015). The correlations may not have been strong due to the intrinsic problems, such as the subjectivity of mastication, rheological testing faces when attempting to describe the multifaceted sensory perception of texture in food.

Other correlations were found between sensory attributes such as hand springiness with rate of recovery ($R^2$ = 0.98), first-bite fracturability ($R^2$ = -0.90), and sensory adhesiveness ($R^2$ = -0.80). First-bite fracturability was correlated with rate of recovery ($R^2$ = -0.89), first-bite firmness ($R^2$ = 0.81), and smoothness of mass ($R^2$ = -0.81). Hand firmness was correlated with first-bite firmness ($R^2$ = 0.92) and smoothness of mass with sensory cohesiveness ($R^2$ = 0.86). Some instrumental parameters were also correlated; storage modulus with loss modulus ($R^2$ = 0.99), springiness with chewiness ($R^2$ = 0.90), and adhesiveness with critical stress ($R^2$ = -0.83) and hardness ($R^2$ = -0.83). Again, definitions of these sensory attributes and instrumental parameters aid in clarifying these correlations.

**Conclusions**

Utilizing sensory evaluation and rheological testing provided insight as to the effect of storage time and temperature on white Cheddar cheese samples throughout a 12-month ripening period. Changes during maturation were elucidated by employing this combination of methods, and despite the lengthy storage time, correlations between the methods were found. Trained panel evaluation aided in creating a profile of the cheese as it progressed through aging. This evaluation also revealed elevated temperature influenced cheese mostly through loss of cohesiveness and smoothness of mass, as well as the development of higher intensities of first-bite fracturability. Proteolysis expedited by both storage time and elevated aging temperature facilitated the breakdown of the protein matrix resulting in quicker texture degradation.  This was observed with differences found in two-cycle compression and small amplitude oscillatory testing parameters. This study demonstrated that instrumental analysis and sensory evaluation can be employed to aid in elucidating the influence elevated ripening temperature has on texture and mechanical properties of white Cheddar cheese.

**Table 4.1.** Standards used in panel training for sensory evaluation (n=10) of white Cheddar cheese samples for texture. Adapted from Rogers and others 2009.

| Attribute | Evaluation Protocol | Definition | Standard and Assigned Intensity |
|---|---|---|---|
| | | Texture by Hand | |
| Hand Firmness | Press completely through the sample using the thumb and index finger | The amount of force required to completely compress the sample | Velveeta 3<br>Muenster 7<br>Sharp Cheddar 10<br>Parmesan 14 |
| Hand Springiness | Press the sample between the thumb and index finger until it is depressed 30% | The total amount of recovery of the sample | Parmesan 1<br>Velveeta 4<br>Sharp Cheddar 7<br>Muenster 13 |
| Rate of Recovery | Press the sample between the thumb and index finger until it is depressed 30% | The rate at which the sample recovers | Feta 1<br>Velveeta 3<br>Sharp Cheddar 6<br>Muenster 9 |
| | | First-bite Terms | |
| First-bite Firmness | Evaluate the force required to completely bite trhough the sample using molars | The amount of force required to completely bite through the sample | Velveeta 2<br>Muenster 6<br>Sharp Cheddar 9<br>Parmesan 14 |
| First-bite Fracturability | Evaluate the amount of fracturability when completely biting through the sample using molars | The amount of fracturability of the sample after biting | Velveeta 1<br>Sharp Cheddar 5<br>Feta 14 |
| | | Texture in Mouth | |
| Smoothness of Mass | Chew the sample 5 times and evaluate smooth vs grainy surface of the chewed mass | The degree to which the chewed mass surface is smooth | Parmesan 1<br>Feta 3<br>Muenster 8<br>Sharp Cheddar 10<br>Velveeta 14 |
| Cohesiveness | Chew the sample 5 times and evaluate the degree to which the mass holds together | The degree to which the chewed mass holds together | Parmesan 1<br>Feta 3<br>Muenster 9<br>Velveeta 14 |
| Adhesiveness | Chew the sample 5 times and evaluate the degree to which the sample adheres to oral surfaces | The degree to which the chewed sample sticks to the surface of the mouth and teeth | Parmesan 1<br>Muenster 7<br>Sharp Cheddar 10<br>Feta 12<br>Velveeta 14 |

**Table 4.2.** Degree of proteolysis in white Cheddar cheese as storage time (months) increased across temperature. Proteolysis is expressed as a ratio between absorbance from the specified storage time and month 0.

| Month | 7.2˚C | 10˚C | 12.8˚C |
|---|---|---|---|
| 2 | 0.64 | 0.94 | 1.28 |
| 5 | 1.23 | 2.13 | 2.09 |
| 8 | 1.78 | 2.44 | 2.60 |
| 10 | 2.00 | 2.71 | 2.93 |
| 11 | 2.08 | 2.82 | 3.18 |
| 12 | 2.24 | 3.67 | 4.15 |

**Table 4.3.** F ratios from Analysis of Variance of physical analyses parameters for white Cheddar cheese samples aged for 12 months at various ripening temperatures and analyzed with ANOVA. An * represents a significant difference in a given attribute (row).

| Source of Variation | Month | Temperature | Month*Temp |
|---|---|---|---|
| df | 5 | 2 | 10 |
| Two-cycle Compression Parameters | | | |
| Hardness | 6.92* | 1.94 | 0.52 |
| Adhesiveness | 16.63* | 1.85 | 2.57* |
| Resilience | 4.34* | 0.61 | 1.38 |
| Cohesiveness | 14.11* | 14.02* | 0.70 |
| Springiness | 7.16* | 3.80* | 2.32* |
| Chewiness | 29.71* | 42.26* | 7.00* |
| Fracturability | 4.80* | 1.61 | 2.22* |
| SAOS Strain Sweep Parameters | | | |
| Storage Modulus | 19.62* | 20.01* | 1.83 |
| Loss Modulus | 19.77* | 22.65* | 2.03 |
| Phase Angle | 8.94* | 2.79 | 1.19 |
| Critical Strain | 23.45* | 5.17* | 0.62 |
| Critical Stress | 14.81* | 2.97 | 0.99 |

**Table 4.4.** Mean separation of significant two-cycle compression parameters from Cheddar cheese samples based on storage time for 12 months and analyzed using Tukey's HSD. Data was collapsed across storage temperature. Values are presented as the mean of quintuplicate measurements. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Storage Time (Months) | Hardness (N) | Adhesiveness (N.s) | Resilience (%) | Cohesiveness | Springiness | Chewiness | Fracturability (N) |
|---|---|---|---|---|---|---|---|
| 2 | 34.13 c | -0.96 a | 5.91 ab | 0.22 a | 32.47 a | 2.46 a | 20.99 abc |
| 5 | 35.01 bc | -1.47 ab | 7.10 a | 0.19 ab | 23.47 b | 1.55 b | 19.48 c |
| 8 | 44.15 a | -3.52 c | 7.44 a | 0.16 bc | 22.00 b | 1.48 bc | 24.33 a |
| 10 | 42.83 a | -3.26 c | 4.49 ab | 0.13 cd | 24.34 b | 1.37 bc | 23.83 ab |
| 11 | 41.00 ab | -2.51 bc | 2.61 b | 0.12 d | 23.00 b | 1.12 c | 19.90 bc |
| 12 | 40.51 abc | -3.11 c | 7.28 a | 0.14 cd | 23.91 b | 1.37 bc | 22.55 abc |

**Table 4.5.** Mean separation of significant two-cycle compression parameters from Cheddar cheese samples based on storage temperature and analyzed using Tukey's HSD. Data was collapsed across storage time. Values are presented as the mean of quintuplicate measurements. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Temperature (˚C) | Hardness (N) | Adhesiveness (N.s) | Resilience (%) | Cohesiveness | Springiness | Chewiness | Fracturability (N) |
|---|---|---|---|---|---|---|---|
| 7.2 | 40.25 a | -2.70 a | 6.06 a | 0.19 a | 26.09 a | 1.94 a | 21.02 a |
| 10 | 40.72 a | -2.56 a | 5.22 a | 0.15 b | 25.91 a | 1.58 b | 22.69 a |
| 12.8 | 37.84 a | -2.20 a | 6.13 a | 0.14 b | 22.60 a | 1.16 c | 21.83 a |

**Table 4.6.** Mean separation of significant small amplitude oscillatory shear parameters from Cheddar cheese samples based on storage time and analyzed using Tukey's HSD. Data was collapsed across storage temperature. Values are presented as the mean of quintuplicate measurements. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Storage Time (months) | Storage Modulus (kPa) | Loss Modulus (kPa) | Phase Angle (˚) | Critical Strain (%) | Critical Stress (Pa) |
|---|---|---|---|---|---|
| 2 | 33.4 d | 10.4 d | 17.3 a | 0.12 a | 41.4 b |
| 5 | 53.4 c | 16.1 c | 16.8 ab | 0.07 b | 39.7 b |
| 8 | 61.2 c | 18.2 c | 16.8 ab | 0.06 bc | 39.9 b |
| 10 | 80.5 ab | 23.8 ab | 16.5 bc | 0.08 b | 67.1 a |
| 11 | 65.4 bc | 20.0 bc | 17.1 ab | 0.04 c | 27.8 b |
| 12 | 89.9 a | 25.7 a | 15.9 c | 0.04 c | 35.7 b |

**Table 4.7.** Mean separation of significant small amplitude oscillatory shear parameters from Cheddar cheese samples based on storage temperature and analyzed using Tukey's HSD. Data was collapsed across storage time. Values are presented as the mean of quintuplicate measurements. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Storage Temperature (˚C) | Storage Modulus (kPa) | Loss Modulus (kPa) | Phase Angle (˚) | Critical Strain (%) | Critical Stress (Pa) |
|---|---|---|---|---|---|
| 7.2 | 49.0 c | 14.7 c | 16.9 a | 0.08 a | 38.3 a |
| 10 | 65.5 b | 19.2 b | 16.5 a | 0.07 ab | 41.0 a |
| 12.8 | 77.5 a | 23.1 a | 16.7 a | 0.06 b | 46.5 a |

**Table 4.8.** F ratios from Analysis of Variance of descriptive sensory analysis of texture attributes for white Cheddar cheese samples aged for 12 months at various ripening temperatures and analyzed with ANOVA. An * represents a significant difference in a given attribute (row).

| Source of Variation | Month | Temperature | Panelist | Month*Temp |
|---|---|---|---|---|
| df | 5 | 3 | 9 | 15 |
| Hand Springiness | 39.03* | 97.74* | 27.30* | 10.68* |
| Rate of Recovery | 33.35* | 73.05* | 28.97* | 7.57* |
| Hand Firmness | 8.79* | 37.19* | 13.30* | 2.06* |
| First-bite Firmness | 6.05* | 46.60* | 12.49* | 2.06* |
| First-bite Fracturability | 14.14* | 47.50* | 29.99* | 5.77* |
| Smoothness of Mass | 3.31* | 25.00* | 19.61* | 3.61* |
| Cohesiveness | 2.28* | 10.75* | 7.93* | 2.63* |
| Adhesiveness | 11.70* | 2.05 | 16.11* | 2.03* |

**Table 4.9.** Mean separation of significant texture attributes of Cheddar cheese samples based on storage temperature as evaluated by a trained DA panel and analyzed using Tukey's HSD. Data was collapsed across storage time. Values are presented as the mean of replicate measurements. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Storage Temperature (˚C) | Hand Springiness | Rate of Recovery | Hand Firmness | First Bite Firmness | First Bite Fracturability | Smoothness of Mass | Cohesive-ness | Adhesive-ness |
|---|---|---|---|---|---|---|---|---|
| 7.2˚C | 6.39 b | 5.47 b | 7.40 c | 6.48 c | 5.10 c | 9.41 a | 8.80 a | 8.56 a |
| 10˚C | 4.57 c | 3.99 c | 8.07 b | 7.10 b | 6.20 b | 8.89 b | 8.41 ab | 8.95 a |
| 12.8˚C | 3.85 d | 3.33 d | 8.20 b | 7.11 b | 6.91 a | 8.04 c | 8.01 b | 8.93 a |
| Reference | 7.02 a | 6.09 a | 9.36 a | 8.25 a | 5.12 c | 9.10 ab | 8.74 a | 8.90 a |

**Table 4.10.** Mean separation of significant texture attributes of Cheddar cheese samples based on storage time as evaluated by a trained descriptive analysis panel and analyzed using Tukey's HSD. Data was collapsed across aging temperature. Values are presented as the mean of replicate measurements. Different letters within a column represent a significant difference among cheese treatments for a given parameter ($p < 0.05$).

| Storage Time (Months) | Hand Springiness | Rate of Recovery | Hand Firmness | First Bite Firmness | First Bite Fracturability | Smoothness of Mass | Cohesiveness | Adhesiveness |
|---|---|---|---|---|---|---|---|---|
| 2 | 7.51 a | 6.31 a | 7.67 d | 6.74 c | 4.93 d | 8.88 ab | 8.64 a | 7.75 b |
| 5 | 5.74 b | 5.10 b | 8.44 abc | 7.28 ab | 5.43 cd | 9.17 a | 8.65 a | 8.86 a |
| 8 | 5.34 bc | 4.93 b | 7.80 cd | 7.00 bc | 5.70 bc | 9.11 a | 8.72 a | 9.02 a |
| 10 | 5.19 bc | 4.46 b | 8.96 a | 7.68 a | 6.44 a | 8.85 ab | 8.27 a | 9.04 a |
| 11 | 4.90 c | 4.42 b | 8.52 ab | 7.39 ab | 6.37 a | 8.67 ab | 8.29 a | 9.17 a |
| 12 | 4.06 d | 3.07 c | 8.16 bcd | 7.32 ab | 6.12 ab | 8.48 b | 8.36 a | 9.17 a |

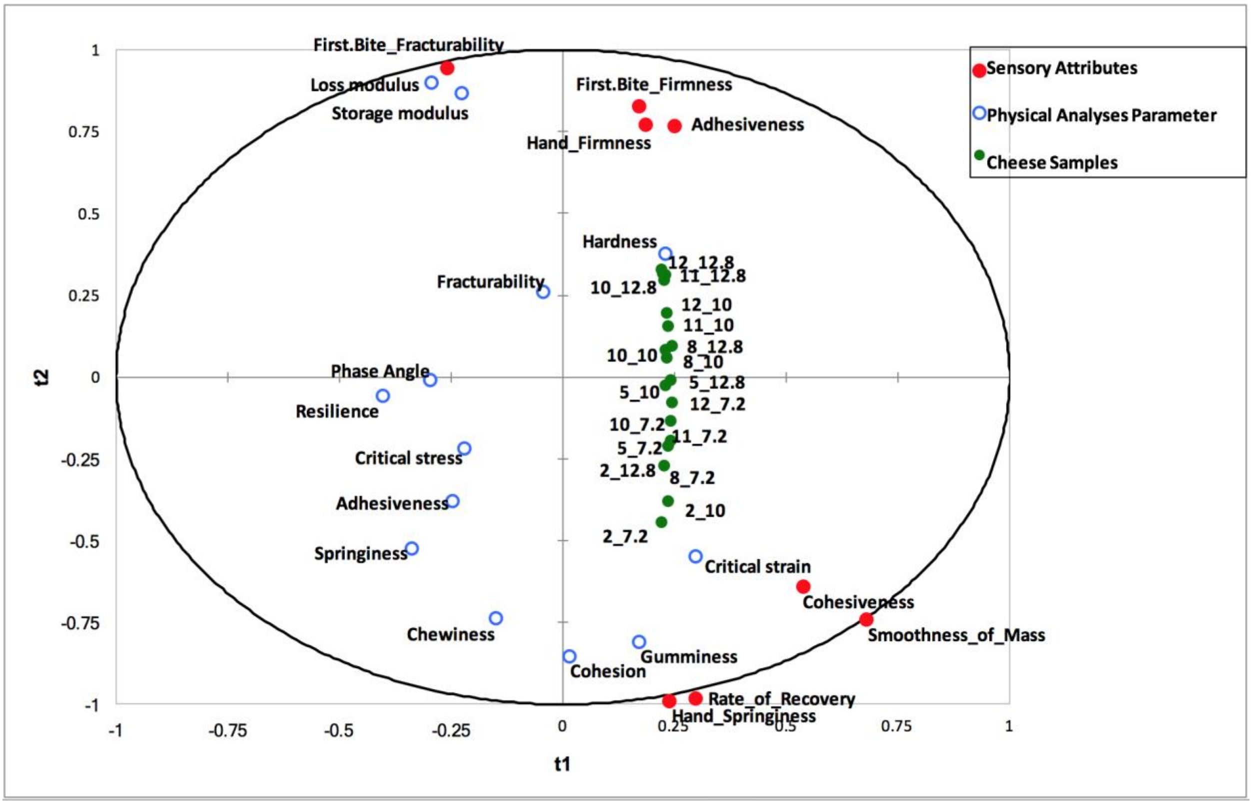


**Figure 4.1.** Partial Least Squares (PLS) regression biplot correlating texture (red dots) evaluated by trained panel (n = 10) to physical analyses parameters (blue dots) for cheese samples (green dots) are described by their storage time (2, 5, 8, 10,11, and 12 months), followed by storage temperature (7.2˚, 10˚, and 12.8˚C).

# CHAPTER FIVE: CONCLUSIONS AND FUTURE WORK

To explore the chemical, sensory, and mechanical properties of white Cheddar cheese aged at elevated temperature two studies were completed. The first, discussed in Chapter III, investigated sensory and chemical changes, induced by elevated storage temperature and storage time, throughout a 12-month ripening period. The second study, discussed in Chapter IV, examined the sensory and rheological implications of utilizing elevated temperature throughout maturation.

Growing consumer demand has required the dairy industry to accelerate production of cheese products. Aged Cheddar cheese requires a lengthy aging period for proper development of desired flavor and texture characteristics. Previous work has found the optimal elevated aging temperature (12˚C) for development without impacting quality of the product negatively. Above 12˚C the risk of microbiological spoilage is increased and off-flavors and deteriorating texture can result in consumer rejection. Despite these potential outcomes this study aimed to reduce the production time required for white Cheddar cheese by implementing elevated aging temperatures of 10˚ and 12.8˚C for comparison with a commercial reference (Cougar Gold®) aged at 7.2˚C. Having the ability to reduce the aging time of their product, may result in a reduction of production costs for the Washington State University Creamery.

The first study led to the following results. The electronic tongue served as a rapid method for cheese evaluation and a valid tool for predicting the age of white Cheddar cheese. Cheese samples, as evaluated by descriptive sensory analysis, were described by various aroma, color, flavor, and taste attributes depending on storage time and temperature. Elevated temperature samples showed increased intensities of mature Cheddar cheese attributes – bitterness, aged flavor, and brothy aroma. White Cheddar cheese aged at 10˚C was found to be most similar to Cougar Gold® based on consumer acceptance. Two of three consumer segments preferred cheese aged at elevated temperature. Employing electronic tongue assessment of non-volatile compounds in conjunction with trained panel analysis, taste correlations were found among later months of aging.

The second study employed sensory and instrumental methods to address the changes in sensory texture and mechanical properties during maturation. Trained panel evaluation demonstrated elevated temperature resulted in the loss of sensory cohesiveness and smoothness of mass, as well as the development of a more brittle texture with increased intensities of first-bite fracturability. Differences in two-cycle compression and small amplitude oscillatory shear were found, which elucidated the accelerated degradation of the cheese matrix catalyzed by proteolysis. Correlations between sensory attributes and rheological instrumental parameters were found.

Despite a difference in the sensory and mechanical properties, white Cheddar cheeses aged at elevated temperature were accepted by consumers. Should the WSU Creamery pursue aging at elevated temperature, 10˚C would be recommended. These studies determined the ripening period of this white Cheddar cheese can be reduced from 12 months to 8 months by utilizing elevated aging temperature.

A vital continuation of this work would be to elucidate the changes in white Cheddar cheese following aging at elevated temperature. Consumers purchase the product and may not consume it immediately. The effect of elevated temperature on the shelf life of the cheese could be investigated with consumer sensory panels. The acceptance of cheese samples following aging at elevated temperature and then storage at refrigeration temperatures would aid in further determining the influence on the chemical and sensory properties of white Cheddar cheese.

Future work may include further investigation into the bitterness developed when utilizing elevated temperature. Microbiological culture alterations to address this undesired characteristic could employ electronic tongue analysis and sensory evaluation. The implications of elevated temperature on cheese with different protein-to-fat ratios could further elucidate casein matrix degradation through sensory texture and mechanical property monitoring during ripening.

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