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EFFECT OF ADJUNCT CULTURES, SODIUM GLUCONATE, AND RIPENING TEMPERATURE ON LOW-FAT CHEDDAR CHEESE FLAVOR

by

Rebekah M. Lance

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

Dr. Donald J. McMahon Major Professor	Dr. Silvana Martini Committee Member
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UTAH STATE UNIVERSITY
Logan, Utah

2011

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ABSTRACT

Effect of Adjunct Cultures, Sodium Gluconate, and Ripening Temperature on Low-Fat Cheddar Cheese Flavor

by

Rebekah Lance, Master of Science

Utah State University, 2011

Major Professor: Dr. Donald J. McMahon
Department: Nutrition and Food Sciences

Low-fat Cheddar cheese flavor is different from full-fat Cheddar cheese and is not acceptable to many consumers. This 2-part experiment was designed to examine effects adjunct cultures have on low-fat Cheddar cheese flavor as determined through descriptive analysis and consumer feedback.

In Part 1, low-fat (5%) Cheddar cheese was produced in duplicate, using 6 combinations of DVS850, LH32, CR540, CRL431, Emfour, and CR319 bacterial cultures. Due to a previously observed positive effect by sodium gluconate on low-fat cheese flavor, each replicate was split into treatments of 0.0% and 0.8% sodium gluconate. Each of these treatments was then split into ripening temperature treatments: 6°C for 21 ± 1 wk; or 6°C for 3 wk, 10°C for 8 wk, and 6°C for 10 wk. Cheese was tasted first by an informal panel. The 4 treatment combinations for the control cheese and the CR540 (a *Lactococcus lactis* ssp. and *Lactobacillus* ssp. blend) cheese, along with all culture combinations containing sodium gluconate and ripened only at 6°C, were

selected for descriptive analysis. Some statistically significant differences in culture treatment were observed. Sodium gluconate addition had a positive influence on flavor while elevated ripening temperature resulted in undesirable flavor notes. Low-fat (5%) Cheddar cheese with the CR540 adjunct with and without sodium gluconate was evaluated in a consumer taste panel with commercial full-fat (33% fat) and commercial reduced-fat (25% fat) Cheddar cheese. The low-fat cheeses were not significantly different from the commercial reduced-fat, indicating comparable cheese.

Part 2 involved making Cheddar-like cheese with non-Cheddar adjunct cultures, using the same process as Part 1. Sodium gluconate was again added but elevated ripening temperature was not included. Each treatment was also divided into a sodium treatment, full salt (2%) and reduced salt (1.5%). After 2 mo of storage at 6°C, cheese was tasted by an informal panel and found to be bitter because of the starter culture used. A culture was added to the second replicate of the experiment to reduce bitterness. This adjunct was found to be somewhat effective in reducing bitterness but not entirely. Descriptive analysis was performed on the high salt level treatments for both replicates. Some difference was observed among cultures and sodium gluconate treatments; however, no acceptable cheese was produced due to bitterness in both replicates. Sodium treatments were not analyzed.

(102 pages)

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R. M. Lance

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CHAPTER 1

GENERAL INTRODUCTION

Consumers are increasingly conscious of their diet and attempt to make healthy choices (IFIC, 2010). Reducing fat intake, especially saturated fats, reduces risk for heart diseases and cancer (FDA, 2010c,d). Saturated fat is a major component in Cheddar cheese, causing many consumers to limit their consumption of cheese. However, Cheddar cheese is an excellent source of calcium and protein, two nutrients particularly vital to the young, the pregnant, and the elderly. Low-fat Cheddar cheese would provide the nutrients without the drawbacks associated with high fat. However, a lack of characteristic flavor, texture, and appearance of its full-fat counterpart causes low-fat Cheddar cheese to be unacceptable. Health-conscious consumers are still, as yet, unwilling to sacrifice flavor for health (Childs and Drake, 2009).

In full-fat Cheddar cheese, adjunct cultures have long been used to promote flavor development. Starter cultures themselves impart flavor characteristics specific to the cheese manufactured while non-starter lactic acid bacteria (NSLAB) account for many variations in flavor of otherwise identical cheese (El Soda et al., 2000; Crow et al., 2001). Intentionally adding isolated NSLAB as adjunct cultures has altered flavors according to the cheese manufacturer's desired outcome. The same effect might result using adjunct cultures in low-fat Cheddar cheese. However, fat reduction in cheese creates a cascade of consequences. Changing the environment changes the flavor-producing microbial, biochemical, and chemical processes (Singh et al., 2003). Specific adjunct strains would need trial, alteration, and perfection in their use before the desired outcome results.

Nevertheless, adjunct cultures have shown some promise already in reduced-fat cheese (Broadbent et al., 1997; Fenelon et al., 2002). The experiments of this thesis were designed primarily to evaluate the effect adjunct cultures have on overall flavor in current low-fat Cheddar cheese and consumer acceptance of such. The outcome of sodium gluconate addition, ripening temperature, and their effect on overall flavor were analyzed secondarily. Though texture and appearance also influence cheese acceptability to consumers, these subjects will not be addressed in depth in this research.

CHAPTER 2

LITERATURE REVIEW

Diet and Health

Diet plays an important part in development or prevention of non-communicable disease. Dietary calcium is necessary for minimizing risk of osteoporosis (FDA, 2010a) whereas a diet high in total fat has been linked with cancer risk and a diet high in saturated fats has been linked with higher blood cholesterol and increased risk of coronary heart disease (FDA, 2010c,d). Cancer and heart disease were the top two leading causes of death in the U.S. in 2007 (Xu et al., 2010).

Many U.S. consumers do not consume sufficient calcium, a nutrient which dairy products such as cheese can easily provide. However, fat accounts for nearly 75% of the caloric content in cheese (USDA, 2009) and consumers already exceed the government recommended intake for fat in their diet. Despite this, more than half of U. S. consumers surveyed by the International Food Information Council Foundation stated that they were making healthy changes to their diet, with about ¾ of them changing the type of food they eat (IFIC, 2010). Nearly 70% of adults want to reduce their fat consumption (Narasimmon, 2008). Thus, though the calcium in cheese is desirable, full-fat cheese is often seen as a less than optimal product for providing that calcium. Such a predicament would be solved by having quality low-fat Cheddar cheese available with a recognizable full-fat Cheddar cheese flavor profile and functionality.

Low-Fat Cheddar Cheese Flavor

Cheddar cheese flavor is produced by a balance of microbiological processes, enzymatic degradation, and manufacturing manipulation (Banks, 2004; Drake et al., 2010). Lactose, citrate, caseins, and lipids provide substrates which are acted on by the coagulant, indigenous milk enzymes, and microorganism to produce flavor compounds (McSweeney and Sousa, 2000; Yvon and Rijnen, 2001). Of all these metabolic processes, proteolysis is the primary source of flavor compounds in Cheddar cheese. Rennet is responsible for primary proteolysis, cleaving large proteins and producing peptides (Weimer, 2007). These molecules are further degraded by other enzymes and bacteria to small peptides and free amino acids, some of which have basic tastes, such as bitter and umami, and others which serve as precursors to flavor compounds (Adda et al., 1982; Urbach, 1993; Drake et al., 2007).

It follows that low-fat cheese flavor is very different from full-fat cheese flavor (Banks, 2004; Saint-Eve et al., 2009). Low-fat Cheddar cheese is often low in characteristic flavors and lacks overall flavor. Flavors atypical of commercial Cheddar cheese (Mistry, 2001; Drake et al., 2010) including bitterness, astringency, and unclean flavor notes (Banks, 2004) frequently are present. Often, a rosy/burnt flavor Drake et al. (2010) described as being reminiscent of burnt sugar develops. This may also be the meaty off-aroma described by Milo and Reineccius (1997). Research indicates that the major differences between low-fat and full-fat Cheddar cheese flavor are due to changes in biochemistry and the resulting imbalance of flavor compounds (Drake et al., 2010) as well as flavor release.

Nearly every aspect of cheese-making influences flavor (Singh et al., 2003; Drake et al., 2008). A change in one facet will start a domino effect on everything else. Low-fat Cheddar cheese made with the same manufacturing process as full-fat Cheddar cheese is too firm, springy or dry. Consequently, the process is altered to improve moisture retention and thus improve texture. This in turn directs changes in proteolysis, salt-in-moisture, and conditions for enzymatic reactions. These changes in the environment of starter and adjunct cultures likely result in changes in flavor profiles, or flavor inconsistencies (Singh et al., 2003).

Fat intrinsically contributes to the development of traditional Cheddar cheese flavors (Drake et al., 2001; Law, 2001) by providing precursors to flavor compounds. Lipids provide a source of fatty acids which can be converted to flavor compounds such as ketones, esters, lactones, and alcohols (Sandine and Elliker, 1970; McSweeney and Sousa, 2000; Curioni and Bosset, 2002). Some free fatty acids have their own flavor to contribute. In addition to flavor, fat plays a part in the creamy mouthfeel characteristic of Cheddar cheese (Drake and Swanson, 1995), acts as light scattering centers for the typical opacity of cheese (Pastorino et al., 2002), and physically interrupts the protein matrix to prevent it from becoming too stiff and rubbery (Bryant et al., 1995).

Changing the fat content of Cheddar cheese changes one of the fundamental elements of the food. For cheese to be labeled as “low-fat” in the United States, it must have at maximum, 3 g of fat per 50 g reference amount of cheese (FDA, 2009a,b). This would be an 80% reduction in fat, from 33% to 6% fat. When fat is changed this drastically, the fundamental composition of the cheese is altered to replace that loss; the matrix of low-fat cheese is very different from that of full-fat cheese (Drake et al., 2010).

Moisture content is higher (50% in low-fat versus 37% in full-fat), solute concentration such as lactose and mineral availability is altered, and protein content is higher. All of this changes the final flavor profile and may affect flavor detection. Aroma release and olfactory perception are also influenced by fat reduction (Saint-Eve et al., 2009).

Reduction in fat changes the biochemistry of the cheese, as well as flavor perception and flavor release. Cheese can be visualized as a casein protein matrix entrapping pockets of water and fat (Banks and Weimer, 2007). Less fat means fewer hydrophobic portions and fewer interruptions in the protein network. Flavor and aroma compounds are impacted by these phases. Carunchia Whetstine et al. (2006a) found that removing 50% of the fat from an aged Cheddar using a novel extraction process did not remove a significantly affect the aged Cheddar flavor. Therefore, as the volatiles were not in the fat but in the rest of the cheese matrix, flavor perception is not dependent on fat to carry volatiles but on the other components which increase with fat reduction. Urback (1997) indicated that reducing the fat to low-fat level and the resultant change in the protein structure alters the conditions for the enzymatic reactions responsible for producing flavor compounds (Banks and Weimer, 2007). Drake et al. (2010) confirmed that there were large differences in the concentrations of key odorants in cheeses with varying fat levels. These studies seem to illuminate that the biochemistry of ripening is altered and thus the resulting balance of flavor compounds is also altered.

The lack of fat may affect flavor release. Molecular interactions of flavor compounds and volatiles with proteins and fats may affect their perception (Rankin and Berg, 2007). An increase in protein and decrease in fat will change the interactions that take place. As related by Midje et al. (2000) some off-flavor compounds bind at a

hydrophobic/hydrophilic interface, reducing their perception by removing them from the aqueous phase (Rank, 1986). With fat reduction comes a reduced ability to bind these compounds, making them more apparent. Fat affects the perception of bitter compounds, among others, reducing rate release (Drake et al., 2010). Less fat makes them more noticeable because fat is not there to affect their rate of release. Drake et al. (2010) hypothesize that this same mechanism could factor into the release of more flavor compounds than just bitter molecules due to decreased hydrophobicity and increased polarity of low-fat Cheddar cheese as compared to a full-fat cheese. In addition, differences in phase partitioning resulting from fat reduction and the resultant change in salt-in-moisture and manufacturing processes may alter concentration of substrates for flavor compounds. Microbial and enzymatic activity will then change and their flavorful products (Rankin and Berg, 2007). Wendin et al. (2000) showed fat reduction also to reduce the intensity of saltiness, and Saint-Eve et al. (2009) found that perception of salt was more noticeable in low-fat Cheddar cheese. Therefore, it is concluded that both flavor variations due to matrix alterations and compound concentration influence sensory differences.

Though less fat is desirable to those looking for healthy food options, consumers have repeatedly asserted that they are unwilling to accept atypical flavors in low-fat Cheddar cheese (Banks, 2004; Childs and Drake, 2009). Overall flavor must first be modified before low-fat Cheddar cheese gains popularity.

Cheese Microorganisms and Adjunct Use

One method of flavor manipulation is careful selection of the microorganisms used in cheese. Typically lactic acid bacteria are selected as starters and adjuncts that contribute much to flavor profiles and the rate of flavor development (El Soda et al., 2000).

Starter cultures such as *Lactococcus lactis* are the primary drivers of acid during Cheddar cheese manufacture and cause the drop in milk pH from about 6.7 to an acidic final cheese pH of about 5.2 (Adda et al., 1982). Increased acid functions not only to expel whey and promote or inhibit bacterial growth, but also contributes to characteristic flavors by influencing the environment for bacterial and enzymatic processes and their subsequent activity (Broome, 2007; Wilkinson and Kilcawley, 2007; Johnson et al., 2009). Of primary importance for flavor development is the autolytic nature of the starter strain. Hickey et al. (2007) found highly autolytic starter cultures positively influenced flavor by increasing free amino acids whereas poorly autolytic strains promoted off-flavors. This highlights the need for careful strain selection. The starter is essential for overall Cheddar cheese flavor (Lynch et al., 1999). Cheese made without a starter culture in a controlled microbial setting fails to develop flavors characteristic of Cheddar cheese.

Another factor in the cheese microorganism environment is the non starter lactic acid bacteria (NSLAB) which may be incorporated through post-pasteurization contamination. Though low in numbers in young Cheddar cheese, they may increase to as much as 10^7 or 10^8 within the first 3 mo (Coeuret et al., 2003). The effect of NSLAB on Cheddar cheese flavor profiles has been controversial. Hickey et al. (2007) indicated

the effect to be negligible in the first 8 mo in light of the impact the starter strain makes, but others say NSLAB are crucial to proper flavor development (Crow et al., 2001). Whether positively or negatively, these cultures and their lysis play an important role in Cheddar cheese flavor development and need to be managed (Peterson and Marshall, 1990).

Adjunct culture use is one method for controlling NSLAB and improving flavor (Broadbent et al., 2003; Johnson and Lucey, 2006). Adjuncts are microorganisms intentionally added to cheese milk that positively impact cheese sensory quality (El Soda et al., 2000). Adjuncts often were NSLAB isolated from mature cheese and identified as contributing to cheese quality (Fenelon et al., 2002; Di Cagno et al., 2003). Now propagated for flavor enhancement (Drake et al., 1995), adjunct use is common practice in cheese making today (Crow et al., 2001; Johnson and Lucey, 2006). Cultures used as adjuncts should multiply sufficiently to produce the desired benefit to cheese quality, causeing no defects such as off-flavors or gas formation (Di Cagno et al., 2003).

Cheese microflora may act differently in such a changed biological and chemical environment as low-fat Cheddar cheese provides. For instance, NSLAB levels can be lower in low-fat cheese than in full-fat (Fenelon et al., 2000). In such an altered system, adjunct culture addition may be what is needed to impart desireable flavors to low-fat Cheddar cheese.

A logical route to begin flavor acceleration or supplementation with adjunct cultures is by identifying cultures found in good quality Cheddar cheese (Gobbetti et al., 2007). According to O'Sullivan (2007), lactic acid bacteria play the biggest part in cheese manufacture. It is believed that only five genera of LAB actually contribute to

cheese flavor: *Lactococcus, Streptococcus, Lactobacillus, Leuconostoc,* and *Enterococcus*. This being the case, it would make sense to attempt low-fat Cheddar cheese flavor improvement using bacteria from these five commonly used in cheese-making genera. Lactobacilli are prime subjects as most have a long history of use with almost no harmful effects (Bernardeau et al., 2008).

Contribution of adjunct cultures to flavor is generally thought to be due to increased proteolysis and the subsequent increase in small peptides and free amino acids (Lane and Fox, 1996; Fenelon et al., 2002). One study used different *Lactobacillus helveticus* strains with varying autolysis abilities and showed, that autolysis was important to flavor, but that the most autolytic did not receive the highest flavor scores. Thus Cheddar cheese flavor is not firmly correlated with autolysis ability (Kenny et al., 2006), but autolysis of starter or adjunct is an important factor in flavor development (Hannon et al., 2007; Hickey et al., 2007). Other studies have shown lysis of *Lb. helveticus* to contribute to the level of flavor precursors early in ripening, and to the nutty flavor in aged Cheddar cheese (Drake et al., 1996).

Previously, scientists have employed adjunct cultures to impart flavors to full-fat, reduced-fat and lower-fat cheese with varying degrees of success. As one example, much research has been done on various strains of *Lb. helveticus*. *Lb helveticus* was shown to increase consumer acceptance of 13% fat cheese (60% reduced) (Weimer et al., 1997). Johnson et al. (1995; Mistry, 2001) found that attenuated strains of *Lb. helveticus* enhanced overall flavor intensity in 21% fat cheese. However, some adjuncts do not contribute to cheese flavor or cause off-flavors or undesirable tastes, such as bitterness (Broadbent et al., 2003; Kenny et al., 2005; Herreros et al., 2007). Fenelon et al. (2002)

showed that an 18% fat cheese with a *Lb. helveticus* strain as the only adjunct achieved an increase in flavorful low molecular mass peptides and a positive flavor score; however, cheeses made with a blend of *Lb. helveticus* and other cultures were not as favorable.

Other groups used *Brevibacterium linens* to improve consumer acceptance of reduced-fat Cheddar cheese flavor, but results were strain specific (Broadbent et al., 1997; Weimer et al., 1997). *Brevibacterium* has the ability to produce methanethiol, a precursor to volatile sulfur compounds and an important contributor to the Cheddar cheese flavor profile. Often used for surface-ripened cheeses (Camembert, Limburger, Brie, etc), it has been used to improve the flavor of 60% reduced-fat Cheddar cheese (Weimer et al., 1997). *Propionibacterium* is traditionally used in Swiss cheese and others (Emental, Gruyere) and is responsible for many flavors and eye formation. It has been used in a Swiss-Cheddar hybrid cheese with some effect on nutty and sweet flavor attributes (Lawlor et al., 2003). *Leuconostoc* has the ability to metabolize citrate and produce diacetyl (Goff, 2010). It can be used enhance cheeses from Gouda to Gorgonzola, and is used for cultured butter and butter milk (Broome, 2007). These three genera typically not used in Cheddar may have the ability to impart cheese flavors where current low-fat Cheddar cheese starters have failed. Again, careful culture selection is emphasized.

One may not be able to use the same cultures as used in full-fat cheese in lower-fat cheese. Johnson and Chen (1991) found that starters useful in full-fat cheese were not suitable for producing quality low-fat cheese. It is thus essential to investigate the effects of specific strains in the desired cheese system.

Sodium Gluconate Addition

Sodium gluconate (**NaGlu**) is the sodium salt of gluconic acid, and is used in the food industry as a sequestrant, a chelator at alkaline pH, a stabilizer, and a thickener (Ramachandran et al., 2006; FAO and WHO, 2010). There is some indication from previous work that NaGlu improves low-fat Cheddar cheese by minimizing the burnt flavor described by Drake et al. (2010). The mechanism is not yet understood.

Sodium ions mask bitterness (Hayes et al., 2010). Breslin and Beauchamp (1997) found sodium in the form of sodium acetate to not only suppress bitterness, but to also "release sweetness from suppression" due to bitterness. It is possible NaGlu works in a similar way, masking off-flavors and allowing improved perception of typical Cheddar cheese flavors. Keast and Breslin (2002) investigated several cations and anions for minimizing bitterness in pharmaceuticals. They found that sodium was the best cation for inhibiting bitterness and glutamate was one of the best anions. It is known that NaGlu inhibits bitterness (Ramachandran et al., 2006). Keast (2008) experimented with 3 concentrations of caffeine mixed with NaGlu and Zn-lactate. Though the Zn-lactate was superior to NaGlu in decreasing the bitterness of caffeine, he demonstrated that NaGlu did have a significant effect. In low-fat Cheddar cheese, it is possible that NaGlu's major contribution to flavor is a suppression of bitterness.

Accelerated Ripening Through Elevated Temperatures

Appropriate aging of cheese is essential for typical flavor development, a process that is not only time-consuming, but costly (Hannon et al., 2005). Of all the methods

used to accelerate flavor development, employing elevated ripening temperatures is the simplest. It has been shown that a ripening temperature of 13°C accelerated the development of aged Cheddar cheese flavors such as brothy, sulfur, and nutty (Carunchia Whetstine et al., 2006b). Shakeel-Ur-Rehman et al. (2000a,b) found that higher ripening temperatures (8°C vs. 1°C) resulted in higher NSLAB counts, which in turn increased desired volatile compounds and perceived maturity.

In the past, this method was rejected due to concerns of inconsistent products or microbial safety. Low ripening temperatures (1 or 5°C) are proven to aid in producing stable and microbiologically safe cheese, limiting NSLAB growth (Shakeel-Ur-Rehman et al., 2000b; Law, 2001; Azarnia et al., 2006). Current evidence shows that certain elevated ripening temperatures can be used for positive results safely (Hannon et al., 2005). However, microbial safety should be checked at each facility.

Unfortunately, all processes (biochemical, enzymatic reactions) are accelerated at elevated temperatures. Used improperly (too long or too high), elevated ripening temperatures may lead to unbalanced or atypical flavor formation (Hannon et al., 2005; Azarnia et al., 2006). Nevertheless, one study demonstrated that Cheddar cheese ripened at 20°C for 1 wk or at 12°C for 6 wk developed well controlled flavors and was an acceptable method for accelerating ripening by 2 mo (Hannon et al., 2005). Law (2001) concluded that a temperature of 12°C can give acceleration of flavor without texture defects, and thus is useful for reducing maturation time (by up to 60 to 75%).

It has been conjectured that using elevating ripening temperatures for flavor acceleration may not be appropriate for low-fat cheese (Mistry, 2001) because its high moisture may encourage rapid bacterial growth and subsequent off-flavors. However,

there is no reported information on ripening temperature and its impact on flavor development in low-fat cheese

Other Factors

A diet high in sodium is associated with increased incidence of hypertension, which in turn is correlated with increased risk of coronary heart disease and stroke (FDA, 2010b), two of the top 10 leading causes of death in the USA (Xu et al., 2010). Reduction of dietary sodium is logical, but difficult to implement. Salt plays a major role in cheese, from food safety to taste (Johnson et al., 2009). Salt reduction in low-fat cheese with adjunct cultures requires analysis.

Appearance is one sensory characteristic affected by fat reduction. Pastorino et al. (2002) demonstrated that the removal of fat from cheese causes a translucent appearance. One way to improve the color of low-fat cheese is to add a colorant, such as titanium dioxide, for opacity improvement.

Cheese Flavor Analysis

Researchers have employed many techniques to analyze and quantify cheese flavor. Two such techniques are descriptive analysis and affective (consumer) tests (House and Acree, 2002). Though cheese flavor can be complex to decipher, descriptive analysis has been utilized in an effective manner to characterize flavors for many products. A descriptive panel usually consists of a small number of panelists trained to identify and quantify descriptors such as those defined by Drake et al. (2001). The descriptive panel is used as an instrument; their assessments should be precise and reproducible.

If the Spectrum™ method (Meilgaard et al., 2007) is used for training, basic tastes are presented to the panelists (sweet, sour, etc.) for them to become familiar with the flavor concepts and intensity ratings. Cheeses are then presented to the panel which discusses and rates descriptors. When a panel can score consistently and well, they are ready to be used as a tool in cheese evaluation. Ideally, use of a clearly defined descriptive language with food or chemical references allows for results to be used in different experiments and in different laboratories (Drake, 2007). The panel at Utah State University (USU) was trained using a modified version of the Spectrum™ method on the following 20 attributes that they rate on a 15-point scale: bitter, brothy, burnt, buttery, cooked, fishy, fruity, lactone/fatty acid, nutty, metallic, oxidized, pineappley, rancid, rosy/floral, salty, sour, sulfur, sweet, umami, and whey. Like most descriptive panels, the USU panel is not trained to measure overall acceptability in any way.

A consumer panel is also a useful tool. It is part of affective testing used to evaluate consumer preferences, liking, and gather background information, such as purchasing behavior. Samples are presented to untrained panelists and questions are asked, frequently about liking and often using the 9-point hedonic scale. Due to the variability inherent in this kind of testing, a minimum of 50 people need to be recruited (Meilgaard et al., (2007).

Principal Component Analysis is an advanced statistical technique that can be powerful in correlating consumer liking to product characteristics (Meilgaard et al., 2007). It is a method for relating multivariable data by finding the smallest number of unobserved variables that describe the greatest amount of variability in the data. Complex cheese profiles are analyzed for correlation with other cheeses and descriptors.

Using these components, the data for a sample can be charted in two dimensions in relation to the flavor attributes. This chart can then be used to estimate which descriptors correspond to liking or disliking for consumers.

Comparison to Full-Fat Cheddar Cheese Flavor

When analyzing a low-fat Cheddar cheese flavor profile, it is useful to compare with a full-fat Cheddar flavor profile. This is difficult to do as there is no legal definition for a mild Cheddar cheese flavor profile. Cheddar cheese flavor profiles vary widely between manufacturers (Young et al., 2004; Drake et al., 2008) and consumer preference for certain Cheddar cheese flavor profiles can vary just as widely. Still, most consumers indicate flavor to be an important criteria in their food selection (Young et al., 2004; Childs and Drake, 2009; Hayes et al., 2010). It is imperative, therefore, to analyze full-fat Cheddar flavor in order to produce a low-fat Cheddar flavor profile that can approximate it.

In the past, grading or judging as employed in industry was used to evaluate cheese flavor. However, these tests are subjective and identify only quality not specific flavor differences, leading them to be unsuitable for research purposes (Fenelon et al., 2002; Drake et al., 2008). Instead, the approach many have taken for profiling full-fat Cheddar cheese flavor is by developing a language (or a list of labels) through descriptive analysis to describe attributes, and providing a sensory reference for each label when possible. Heisserer and Chambers (1993) analyzed both international and domestic cheese, identifying 30 total attributes classified into the general categories of "dairy-like," "fatty-acid/animal," "fungal," "other aromatics," "mouthfeel," and "basic tastes."

Murray and Delahunty (1999) focused on describing Cheddar cheese, settling on 21 concepts. Some of theirs were very similar to Heisser and Chambers while others varying slightly and some were completely new. Even more recently, Drake et al. (2001) developed another language with references to describe Cheddar cheese. It includes the following flavors and tastes: cooked, whey, diacetyl, lactone, sulfur, brothy, free fatty acid, fruity, nutty, catty, cowy/phenolic, fecal/mothball, rosy/floral, sweet, salty, sour, bitter, and umami.

The goal for developing flavor lexicons with defined references for each attribute is to better communicate regarding Cheddar cheese flavors between researchers, labs, and manufacturers and to overcome personal interpretations of terms that may lead to misrepresentations and false conclusions. However, researchers alter lexicons as they see fit, leading to many, not 1, languages for Cheddar cheese. Even so, these languages have provided groundwork to build upon. Using these languages, researchers have been able to describe flavors that are characteristic of young or aged Cheddar cheese.

Young et al. (2004) addressed Cheddar cheese acceptance of varying ages by North Carolina State University consumers and Oregon State University consumers. They observed that most attributes for Cheddar cheese are found in the 0-4 range on a 15-point scale. A 4 mo milled curd Cheddar received the highest score for overall acceptance, overall color liking, overall flavor liking, and overall texture liking and overall Cheddar cheese intensity. This cheese was characterized by “young” flavors such as cooked/milky, whey, diacetyl, and milkfat/lactone. These flavors were general among the three cheeses aged less than one year, whereas cheeses aged 1 year or more were

characterized more by sulfur, brothy, nutty, fruity, and catty, and were termed "aged" flavors.

Caspia et al. (2006) analyzed 7-mo, 9-mo, and 12-mo Cheddar cheeses with descriptive analysis, and then assessed consumer liking for them. The 9-mo Cheddar cheese was rated the highest for overall liking (7.53 on a 9-pt hedonic scale) and was characterized descriptively by stronger "cooked," "creamy (milkfat)," and "buttery (diacetyl)" flavors. The 12-mo Cheddar cheese was liked least (5.75) and was characterized by bitter, earthy, sulfur, free fatty acid, pungent and prickle bite.

Fruity, free fatty acid (rancid) and bitter are usually considered defects in Cheddar cheese flavor (Caspia et al., 2006; Suriyaphan et al., 2001).

Drake et al. (2010) tracked sensory changes in FF, RF, and LF Cheddar cheese through ripening. They found only milkfat flavor to be different between the full-fat and reduced-fat cheeses, declining with fat reduction. They confirmed that diacetyl (or buttery) is a young/undeveloped flavor as it was unobserved after 6 mo of ripening. At 3 mo, the low-fat cheese had less sulfur than full-fat, more bitter, and a rosy/burnt off-flavor (which was not detected at any of the tested time points in the full-fat cheese) in combination with an overall lack of flavor development.

Using the results of these previous studies, information can be gleaned on what id desirable in a Cheddar cheese flavor profile. Knowing which flavors and tastes are "young" or expected to be in a mild Cheddar cheese, and using a descriptive language similar to the one developed by Drake et al. (2001), it is possible to generalize about flavor changes due to treatments and determine desirable or undesirable effects.

Application to Current Study

Though several studies have been done on adjunct addition to Cheddar cheese with a fat level between 10-33%, and other types of low-fat (5% fat) cheeses, apparently no studies have been done on the effect of adjunct culture addition to the current low-fat (5% fat) Cheddar cheese with descriptive sensory analysis. In this study, cultures from the *Lactococcus* and *Lactobacillus genera* were used in one experiment while cultures from the *Brevibacteriaum, Probionibacterium,* and *Leuconostoc* genera were employed in the second experiment. The former cultures have a history of beneficially contributing to an overall Cheddar flavor profile, while the latter cultures it was hoped would contribute to Cheddar flavor in a way that was as yet untested. Sodium gluconate was included to further determine its effect on overall low-fat Cheddar cheese flavor. Usage of elevated ripening temperature was included in this study to get a full analysis of how adjuncts behave in low-fat Cheddar cheese ripened using industrial practices. Impact was measured by descriptive and affective testing.

HYPOTHESIS AND OBJECTIVES

Hypothesis

Adjunct culture addition will improve overall low-fat Cheddar cheese flavor.

Objective 1

Low-fat Cheddar cheese will be manufactured with adjunct cultures from the *Lactococcus* and *Lactobacillus* genera (commonly used in Cheddar cheese and recommended for low-fat Cheddar cheese use), ripened, and tested for flavor using

descriptive analysis. In addition to adjunct cultures, 2 other factors will be analyzed: sodium gluconate and elevated ripening temperature. Consumer opinion will be ascertained on experimental cheese as compared with commercially available full-fat and reduced-fat Cheddar cheese.

Objective 2

Low-fat Cheddar cheese will be manufactured with a series of cultures not typically used in Cheddar cheese (*Leuconostoc* spp, *Brevibacterium* spp, and *Propionibacterium* spp.), ripened, and tested for flavor using descriptive analysis. Two other factors will be analyzed: sodium gluconate and reduced sodium.

CHAPTER 3

EFFECT OF LACTOCOCCUS AND LACTOBACILLUS ADJUNCT CULTURES, SODIUM GLUCONATE, AND RIPENING TEMPERATURE ON LOW-FAT CHEDDAR CHEESE

ABSTRACT

Various adjunct cultures, including *Lb*. *helveticus* (LH32), *Lc. lactis* ssp. *cremoris/lactis* (CR319), a *Lb. acidophilus* and *Lb*. *helveticus* blend (Emfour), a *Lc. lactis* ssp and *Lb*. ssp blend, and *Lb. paracasei* (CRL431), were used in low-fat (5% fat) Cheddar cheese manufacture. The effect on overall flavor was determined through descriptive analysis. Treatments of sodium gluconate (0% and 0.8%) and accelerated ripening through elevated temperature (6°C and 10°C) were also analyzed. Descriptive analysis showed significant ($P<0.05$) differences in the attributes bitter, buttery, rancid, and sour due to adjunct culture addition, though none were significantly different from the control. A *Lc*. *lactis* ssp. and *Lb*. ssp. adjunct blend (CR540) showed a flavor profile trending toward an arbitrary full-fat Cheddar cheese flavor profile constructed from the average of multiple full-fat Cheddar cheese flavor profiles. Sodium gluconate addition positively affected overall flavor by increasing lactone, sour, sweet and buttery, while decreasing bitter. Elevated ripening temperatures negatively impacted flavor by increasing brothy, salty, sour, and sulfur above and away from the averaged full-fat Cheddar cheese flavor profile. Consumers evaluating cheese made with CR540, ripened at 6°C, with sodium gluconate liked the overall flavor as much as commercially available reduced-fat (25% fat) cheese.

INTRODUCTION

Cheddar cheese flavor is produced by a balance of microbiological processes, enzymatic degradation, and manufacturing manipulation (Banks, 2004; Drake et al., 2010). Lactose, citrate, caseins, and lipids provide substrates which are acted on by the coagulant, indigenous milk enzymes, and microorganism to produce flavor compounds (McSweeney and Sousa, 2000; Yvon and Rijnen, 2001).

Nearly every aspect of cheese-making influences flavor (Singh et al., 2003; Drake et al., 2008). Changes in the environment in which the flavor-producing reactions take place (milk constituents, pH, water activity, ripening) will likely result in changes in Cheddar cheese flavor profiles, or flavor inconsistencies (Singh et al., 2003). A major change in cheese composition must occur for cheese to be labeled as "low-fat" in the United States, having a maximum of 3 g of fat per 50 g reference amount of cheese (FDA, 2009a,b). This would be an 80% reduction in fat, from 33% to 6% fat.

It follows that low-fat cheese flavor is very different from full-fat cheese flavor (Banks, 2004; Saint-Eve et al., 2009). Low-fat Cheddar cheese is often low in characteristic flavors and lacks overall flavor, and may have flavors atypical of commercial Cheddar cheese (Mistry, 2001; Drake et al., 2010) including bitterness, astringency, and unclean flavor notes (Banks, 2004). Low-fat cheese may also develop a rosy/burnt flavor Drake et al. (2010) described as being reminiscent of burnt sugar that is often in low-fat cheese. This may be the meaty off-aroma described by Milo and Reineccius (1997). Both flavor variations due to matrix alterations and compound concentration influence sensory differences. Consumers have repeatedly asserted that

they are unwilling to accept flavors uncharacteristic of Cheddar cheese in low-fat Cheddar cheese (Banks, 2004; Childs and Drake, 2009).

One method for improving flavor is through adjunct culture use (Broadbent et al., 2003; Johnson and Lucey, 2006). Adjuncts are microorganisms intentionally added to cheese milk that positively impact cheese sensory quality (El Soda et al., 2000). Adjunct use is common practice in cheese making today (Crow et al., 2001; Johnson and Lucey, 2006). A logical route to flavor acceleration or supplementation with adjunct cultures is by identifying cultures found in good quality Cheddar cheese (Gobbetti et al., 2007). According to O'Sullivan (2007), lactic acid bacteria play the biggest part in cheese manufacture. It is believed that only five genera of LAB actually contribute to cheese flavor-- *Lactococcus, Streptococcus, Lactobacillus, Leuconostoc,* and *Enterococcus*. These two statements being the case, it would make sense to attempt low-fat Cheddar cheese flavor improvement using bacteria from these five commonly used in cheese-making genera.

Scientists have employed adjunct cultures to impart flavors to full-fat and reduced-fat and low-fat cheese with varying degrees of success. Using *Lactobacillus helveticus* as an example, much research has been done on effect various strains have in Cheddar cheese. *Lb. helveticus* was used to improve flavor of 18% fat Cheddar cheese (Fenelon et al., 2002) and shown to increase consumer acceptance of 13% fat cheese (60% reduced) (Weimer et al., 1997). Johnson et al. (1995; Mistry, 2001) found that attenuated strains of *Lb. helveticus* enhanced overall flavor intensity in 21% fat cheese. Fenelon et al. (2002) showed that an 18% fat cheese with a *Lb. helveticus* strain as a single adjunct achieved an increase in flavorful low molecular mass peptides and a

positive flavor score; however, cheeses made with a blend of *Lb. helveticus* and other cultures were not as favorable. Some adjuncts cause off-flavors or undesirable tastes, such as bitterness, or do not contribute to cheese flavor at all (Broadbent et al., 2003; Kenny et al., 2005; Herreros et al., 2007).

One may not be able to use the same cultures as used in full-fat cheese in lower-fat cheese. Johnson and Chen (1991) found that starters useful in full-fat cheese were not suitable for producing quality low-fat cheese. It is thus essential to investigate the effects of specific strains in the desired cheese system.

There is some indication from previous work that sodium gluconate (**NaGlu**) also improves overall low-fat Cheddar cheese flavor by minimizing the burnt flavor described by Drake et al. (2010). The mechanism is not yet understood. Sodium ions mask bitterness (Hayes et al., 2010). Breslin and Beauchamp (1997) found sodium in the form of sodium acetate to not only suppress bitterness, but to also "release sweetness from suppression" due to bitterness. It is possible NaGlu works in a similar way, masking off-flavors and allowing improved perception of typical Cheddar cheese flavors. It is known that NaGlu inhibits bitterness (Ramachandran et al., 2006; Keast, 2008). In low-fat Cheddar cheese, it is possible that NaGlu's major contribution to flavor is suppression of bitterness.

Appropriate aging of cheese is essential for typical flavor development, a process that is not only time-consuming, but costly (Hannon et al., 2005). Of all the methods used to accelerate flavor development, employing elevated ripening temperatures is the simplest. Hannon et al. (2005) demonstrated that Cheddar cheese ripened at 20°C for 1 wk or at 12°C for 6 wk developed well controlled flavors and was an acceptable method

for accelerating ripening by 2 mo. It has been conjectured that using elevating ripening temperature to accelerate flavor development may not be appropriate for low-fat cheese (Mistry, 2001). Its high moisture may encourage rapid bacterial growth and subsequent off-flavors. Nevertheless, its application to low-fat cheese manufacture would be worthwhile to understand if the cheese industry undertakes manufacture of low-fat cheeses. Appearance is another sensory characteristic affected by fat reduction. Pastorino et al. (2002) demonstrated that the removal of fat from cheese causes a translucent appearance. One way to improve the color of low-fat cheese is to add a colorant, such as titanium dioxide, for opacity improvement.

Researchers have employed many techniques to analyze and quantify cheese flavor. Two such techniques are descriptive analysis and affective (consumer) tests (House and Acree, 2002). Though cheese flavor can be complex to decipher, descriptive analysis has been utilized in an effective manner to characterize flavors for many products. The descriptive panel is used as an instrument; their assessments should be precise and reproducible. A consumer panel is useful for evaluating consumer preferences, overall liking, and background information, such as purchasing behavior.

When analyzing a low-fat Cheddar cheese flavor profile, it is necessary to compare with a full-fat Cheddar flavor profile. This is difficult to do as there is no legal definition for a mild Cheddar cheese flavor profile. Cheddar cheese profiles vary widely between manufacturers (Young et al., 2004; Drake et al., 2008) and consumer preference for certain Cheddar cheese flavor profiles can vary just as widely. Still, most consumers indicate flavor to be an important criteria in their food selection (Young et al., 2004; Childs and Drake, 2009; Hayes et al., 2010).

In the past, grading or judging as employed in industry was used to evaluate cheese flavor, but these tests are subjective and unable to precisely identify differences, only quality, leading it to be unsuitable for research purposes (Fenelon et al., 2002; Drake et al., 2008). Instead, Drake et al. (2001) developed a descriptive language with references to describe Cheddar cheese to overcome personal interpretations of terms that may lead to misrepresentations and false conclusions. Fifteen of these attributes are very close to the attributes utilized by the Utah State University (USU) Descriptive Cheese Panel.

Though several studies have been done on adjunct addition to Cheddar cheese with a fat level between 10-33%, and several low-fat (5% fat) cheeses, apparently no studies have been done on the effect of adjunct culture addition to the current low-fat (5% fat) Cheddar cheese with descriptive sensory analysis. The purpose of this study was to determine the effect adjunct culture addition had on low fat cheese flavor, with sodium gluconate and accelerated ripening treatments. Cultures from the *Lactococcus* and *Lactobacillus* genera, having a history of beneficially contributing to an overall Cheddar flavor profile, were employed. Sodium gluconate was included to further determine its effect on overall low-fat Cheddar cheese flavor. Usage of elevated ripening temperature was included in this study to get a full analysis of how adjuncts behave in low-fat Cheddar cheese ripened using industrial practices. Impact was measured by descriptive and affective testing.

MATERIALS AND METHODS

Cultures

All cultures were frozen pellet form from Chr. Hansen Inc. (Milwaukee, WI). Starter bacteria used for each cheese was a blend of *Lactococcus lactis* ssp. *cremoris*/*lactis* (DVS 850) at a rate of 22 g/100 kg milk. Five adjunct cultures from the *Lactococcus* and *Lactobacillus* genera recommended for use in low-fat Cheddar cheese were selected to be used alone or in combination with others as listed in Table 1.

Cheese Manufacture

Raw milk was obtained fresh from the USU Caine Dairy Research and Teaching Center (Wellsville, UT) and transferred to the Gary Haight Richardson Dairy Products Laboratory (Logan, UT). Milk was standardized to a protein to fat ratio of 5, and then pasteurized at 73°C for 16 s. Six different cheeses were made, in duplicate (**Rep 1** and **Rep 2)**. Two cheeses were made each day using Tetra Scherping horizontal cheese vats (Tetra Pak Cheese & Powder Systems Inc., Winsted, MN). Culture combinations were randomized and vat location balanced to adjust for any differences between the vats.

Table 1. Adjunct culture combinations used in conjunction with a *Lc. lactis* ssp. *cremoris*/*lactis* starter culture to make low-fat cheese.

Code	**Adjunct Description**	**Adjunct Usage Level** (g/100kg milk)
Control	Starter only	–
LH32	*Lb. helveticus*	2.2
CRL431	*Lb. paracasei*	11.8
CR540	*Lc. lactis* ssp. and *Lb.* ssp. blend	5.1
Emfour	*Lb. helveticus* and *Lb. acidophilus* blend	9.7
LH32/ CR319	*Lb. helveticus*	2.2
	Lc. lactis ssp. *cremoris*/*lactis*	4.4

As the vat was being filled, warm (23°C) milk was preacidified to pH 6.2 with L-lactic acid (Nelson Jameson, Marshfield, WI) at a 1:16 dilution with distilled water using a peristaltic pump (Masterflex; Cole-Parmer, Vernon Hills, Illinois). Titanium dioxide emulsion (Idacoat White; ROHA, St. Louis, MO) was then added at a rate of 44 g/100 kg milk, and the milk was heated to 32°C with constant agitation at 15 rpm. Starter culture and adjuncts were added, incubated for 30 min, and then additions were made of single strength Annatto cheese color and Maxiren double strength chymosin (~650 International milk clotting units/ml), both from DSM Food Specialties USA Inc. (Eagleville, PA), both at a rate of 7.5 mL/100 kg milk.

After a 20-min coagulation time, curd was cut and healed over a 20-min period. Curd cooking was omitted; temperature remained at the set temperature (32°C) for 20 min with continuous cutting to prevent curd packing on the knives. After a 30-min stir-out period, curds and whey were transferred to a drain table (Kusel Equipment Co., Watertown, WI) where wet acid development continued until a pH of 5.95 was reached. Whey was drained and dry stirring continued until the curd reached a pH of 5.50. A cold water wash (8 to10°C) at a rate of 40 kg/100 kg curd was applied, reducing the curd temperature down to 22°C and drained again.

Curd was salted at a rate of 2.2 kg/100 kg curd in 3 applications each 5 min apart, and then divided into 2 treatments: one with NaGlu added at a rate of 0.8 kg/100 kg in 2 applications, 5 min apart; the other with no NaGlu addition. Then after 5 min, 12.7 kg of curd was filled into hoops and pressed at 0.1 MPa overnight (~18 h) at room temperature (~20°C) yielding 2 blocks of about 10 kg each post-pressing. Cheese was removed the next day, sealed in barrier bags, and ripened. One block from each treatment underwent a

ripening temperature of 6°C, and the other undergoing an accelerated ripening step of 8 wk at 10°C after 3 wk at 6°C. After 11 wk, all cheeses were kept at 6°C. The cheese manufacture and ripening are outlined in Figure 1.

Proximate Analysis

Moisture, pH, salt and fat levels were measured on d 5 ± 1. Moisture content was determined using a SMART Turbo Microwave Moisture/solids Analyzer (CEM Corp., Indian Trail, NC). Between 3 and 4 g of cheese were placed in the microwave analyzer and readings in percent moisture were returned. Tests were performed in triplicate with no deviation greater than 0.50% allowed**.** Cheese pH was determined by adding 20 g of cheese and 10 g of distilled water to a plastic stomacher bag and stomached for 1 min at 260 rpm in a Seward Stomacher 400 (Seward, Riverview, FL). Readings were taken using an Oaklon pH meter (pH 510 series, Vernon Hills, IL). Salt content was analyzed using a 5 g sample of shredded cheese added to 98.2 g distilled water and stomached for 4 min at 260 rpm. After allowing the sample to equilibrate for 30 min, the slurry was filtered using Whatman No. 1 filter paper, and the permeate was analyzed using a Corning Chloride Analyzer 926 (Medfield, MA) in duplicate. Fat was measured using the Babcock method.

Sensory Evaluation

Full-fat Cheddar cheese profiling. As there is no definitive Cheddar cheese flavor profile, it was determined that a general concept of a Cheddar cheese flavor profile needed to be established in order to generalize about the effectiveness of the treatments. From research previously mentioned, it is known that low-fat Cheddar cheese is often

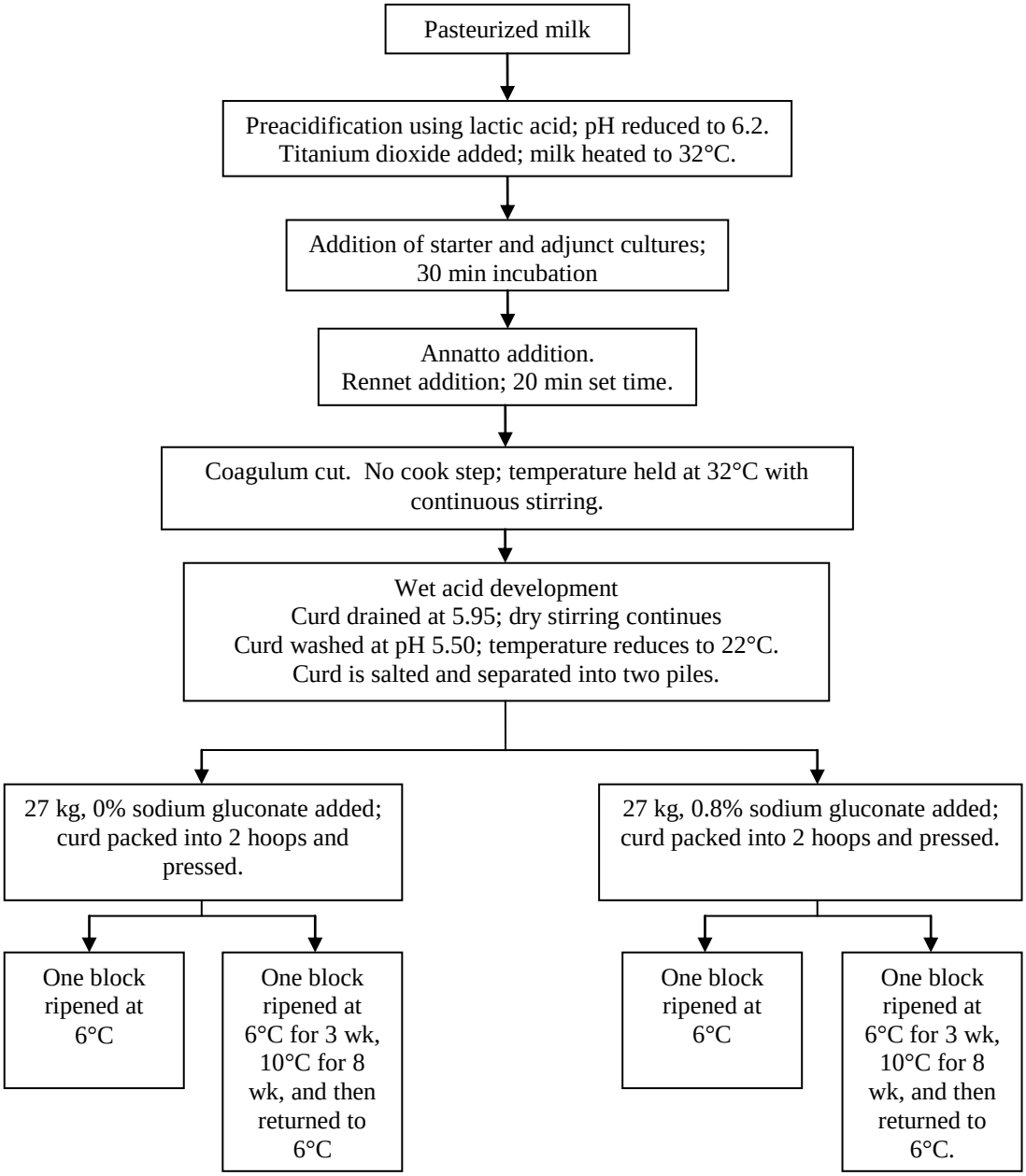


Figure 1. Flow of process steps for making low-fat Cheddar cheese.

low in flavor. An increase in most descriptive analysis attributes would seem to be desired. Mild full-fat Cheddar cheese is often characterized by the terms buttery, milkfat, cooked, and whey (Caspia et al., 2006). Thus it stands to reason that these flavors need to especially be enhanced. It is also known that bitter as well as such attributes as fruity, pineapplely, fishy, and even sulfur can be defects in mild Cheddar cheese if present in too high of quantities which sometimes is the case in low-fat Cheddar cheese. It seems logical that these attributes should either be absent or present to a lesser extent than in the control low-fat cheese. These were some of the changes that were used to identify a more Cheddar-like flavor, along with comparison of the treatments and the control cheese, and to establish positive effects.

In conjunction with these desired changes, a general full-fat Cheddar cheese flavor profile was organized with which to make generalizations about the low-fat Cheddar cheeses made for this experiment. Previous to these experiments, descriptive analysis was performed on several commercial full-fat Cheddar cheeses by the USU Descriptive Cheese Panel. These results were averaged. This gave a basis for a "typical" full-fat Cheddar cheese flavor profile as evaluated by the descriptive panel that would be employed.

Informal Preliminary Tasting. After 3 mo, the 24 blocks of cheese (6 culture treatments, 2 NaGlu treatments, and 2 ripening temperature treatments) from the first replicate (**Rep 1)** were informally evaluated for flavor by 3 experienced cheese scientists. Those treatments with the most Cheddar-like flavor, and those representing a broad range of flavors were identified with the intent that cheeses would be selected for sensory

evaluation based on extent of flavor differences. Trials to be analyzed by descriptive analysis were determined.

Descriptive Analysis. Ten cheese panelists, selected for their ability to differentiate basic tastes and distinguish varying intensity levels, were previously trained using a modified version the Spectrum™ method for the basic tastes bitter, salty, sour, sweet (Meilgaard et al., 2007), and 3 aqueous concentrations of monosodium glutamate (0.7%, 1.4%, 2.8%) to represent 3 levels (5, 9, 13) of "umami" taste on a 15-point scale (Table 19 in Appendix A). After initial training and familiarization with the intensity scale, references of common Cheddar cheese flavors were presented (Table 20 in Appendix A). Cheeses were discussed in terms of these flavors and attributes were clarified. Panelists' responses have previously been monitored for reproducibility between samples, and consistency among the group. Previous to this research, each panelist had more than 100 hrs of training.

Before conducting flavor studies, procedures were approved by the USU Institutional Review Board. Descriptive analysis was performed after wk 18±1 by USU Descriptive Cheese Panel. Panelists evaluated the cheese in 2 panels: the first panel analyzed all treatment variations (with and without NaGu, traditional and elevated ripening temperatures) of the control cheese and CR540 adjunct cheese (8 total cheeses); the second panel included the block of cheese from each culture treatment made with NaGlu and ripened 6°C (6 total cheeses).

Cheese samples were served in 1-cm cube pieces equilibrated to room temperature (~22°C) in covered 60-mL sample cups. Six samples were presented at a time, labeled with 3-digit blinding codes. Distilled water and unsalted crackers were available for

panelists to cleanse their pallets between samples. Panelists rated a number of attributes on a 0 to 15-point scale: bitter, brothy, buttery, burnt, cooked, fishy, fruity, lactone/fatty acid, metallic, nutty, oxidized, pineappley, rancid, rosy/floral, salty, sour, sulfur, sweet, umami, and whey (0 = "no flavor," 15 = "extreme intensity"). They recorded their responses on a computer in individual booths using the Sims2000 software (Sensory Computer Systems, Morristown, NJ). Cheeses were tasted in duplicate.

Responses were analyzed in a split-split plot design and a randomized block design, using SAS 9.1 and 9.2 (SAS Institute Inc., Cary, NC). The PROC GLM procedure was used with experiment replicate, culture treatment, NaGlu treatment, ripening treatment, panelist, and sample replicate as class factors for determining difference in NaGlu and ripening treatments. Panelist and culture treatment were used as class factors for determining a difference between cultures, using the cheeses ripened at 6°C with NaGlu. One cheese similar in attributes to full-fat cheese (CR540; the same that the preliminary panel concluded was most preferable) was chosen to be compared in a consumer panel with commercial cheeses. Principal Component Analysis (PCA) was conducted on descriptive profiles averaged by sample using the PROC CORR, PROC FACTOR, and PROC SCORE procedures.

Consumer Panel. At week 21±1, a consumer panel with 120 members of the surrounding community was conducted by the USU Sensory Laboratory. Participants were presented with 6 cheese samples (1-cm cubes in a 60-mL sample cup with a lid) each labeled with a three-digit blinding code. Two commercial full-fat cheeses (a mild and a sharp; 33% fat), 2 commercial reduced-fat cheeses (25% fat), and 2 of the low-fat experimental cheeses (adjunct culture CR540 with and without NaGlu ripened at 6°C;

5% fat) were used. Consumers were asked to indicate a preference between the full-fat cheeses, and to rate flavor, appearance, and texture of all samples for liking using a 9-point Hedonic scale (See Figure 5 and 6 in Appendix B for full questionnaire): 1 = "Dislike Extremely," 3 = "Dislike Somewhat," 5 = "Neutral," 7 = "Like somewhat," and 9 = "Like Extremely." Intent to purchase was asked of the 4 lower-fat samples using a 5-point scale: 1 = "Not likely," 2 = "Somewhat not likely," 3 = "Neither likely nor likely," 4 = "Somewhat likely," and 5 = "Likely." Consumers were also asked about usage of full-fat and lower fat cheese, the importance of having lower fat cheese, and background demographic questions. Results were analyzed with SAS 9.2 using panelist, sample and experiment replicate as class variables for the PROC GLM procedure in a randomized block design.

RESULTS AND DISCUSSION

Flavor Profiles for Some Full-Fat Cheese

Included in Tables 2 and 3 are the 20 descriptors evaluated by the descriptive panel for 6 full-fat cheese samples. Of interest is to note that there is quite a bit of variation among commercial cheese, even among those that are all labeled "mild" or "sharp" (see "Range"). This underscores the difficulty in comparing the overall flavor of low-fat Cheddar cheese to full-fat Cheddar—there is no one flavor profile for Cheddar cheese. Thus, for generalizations only, these values have been averaged to provide a full-fat Cheddar cheese flavor profile.

Table 2. Descriptive analysis of commercial full-fat mild Cheddar cheeses

	Brand							
Attribute	White Pine Mild	Kraft Mild	Gossner Mild 1	Old Juniper Mild	Cache Valley Mild	Gossner Mild 2	Average	Range[1]
Bitter	0.2	0.5	1.1	0.3	0.9	0.6	0.6	0.8
Brothy	1.4	0.6	0.9	1.9	1.8	2.7	1.6	2.1
Buttery	1.0	0.8	1.1	1.2	1.4	2.5	1.3	1.7
Burnt	NA[2]	NA	NA	NA	0.1	0.1	0.1	0.0
Cooked	ND[3]	ND	ND	ND	0.1	0.2	0.2	0.2
Fishy	ND	ND	ND	0.1	ND	ND	0.1	0.1
Fruity	ND	0.1	ND	ND	ND	ND	0.1	0.1
Lactone	1.3	2.1	2.1	1.8	2.0	3.0	2.1	1.8
Metallic	ND	ND	ND	ND	ND	ND	0.0	0.0
Nutty	0.3	ND	0.1	0.1	0.4	0.1	0.2	0.4
Oxidized	ND	ND	ND	ND	ND	0.1	0.1	0.1
Pineappley	ND	ND	ND	ND	ND	ND	0.0	0.0
Rosy	ND	ND	ND	ND	ND	ND	0.0	0.0
Rancid	ND	0.1	0.1	ND	0.2	0.1	0.1	0.2
Salty	4.2	3.9	4.4	4.1	5.7	5.3	4.6	1.8
Sour	2.1	2.9	3.2	1.9	4.1	3.4	2.9	2.3
Sulfur	ND	ND	0.2	ND	ND	0.3	0.2	0.3
Sweet	0.8	0.6	0.6	1.7	0.5	1.6	1.0	1.1
Umami	1.0	0.8	0.7	2.1	1.4	1.3	1.2	1.3
Whey	0.4	0.6	ND	0.2	ND	0.3	0.4	0.6

[1]Variation among mild cheeses tested.

[2]NA = Not available.

[3]ND = Not detected.

Table 3. Descriptive analysis of commercial full-fat sharp Cheddar cheeses

	Brand							
Attribute	White Pine Sharp	Kraft Sharp	Gossner Sharp	Old Juniper Sharp	Cache Valley Sharp	Gossner Extra Sharp	Average	Range[1]
Bitter	1.1	0.8	1.1	1.0	1.0	3.2	1.3	2.4
Brothy	1.6	1.3	1.0	1.9	2.7	2.6	1.8	1.7
Buttery	0.4	0.6	0.8	0.6	1.5	1.6	0.9	1.2
Burnt	NA[2]	NA	NA	NA	0.1	1.5	0.8	1.4
Cooked	ND[3]	ND	ND	ND	0.3	0.3	0.3	0.3
Fishy	ND	ND	ND	0.1	ND	ND	0.1	0.1
Fruity	0.3	ND	0.2	ND	ND	ND	0.3	0.3
Lactone	1.2	1.6	2.2	1.3	2.0	3.0	1.9	1.8
Metallic	ND	ND	ND	ND	ND	ND	0.0	0.0
Nutty	0.8	0.1	0.7	1.1	0.5	0.8	0.7	1.0
Oxidized	ND	0.1	ND	ND	ND	0.2	0.1	0.2
Pineappley	0.1	ND	0.2	0.6	ND	0.2	0.2	0.6
Rosy	ND	ND	ND	0.1	ND	ND	0.1	0.1
Rancid	0.8	1.2	0.7	0.7	0.4	1.2	0.8	0.8
Salty	4.6	5.0	5.7	4.8	5.0	5.8	5.1	1.2
Sour	2.7	4.7	4.4	3.3	4.6	4.5	4.0	2.0
Sulfur	ND	0.2	ND	ND	ND	1.2	0.7	1.2
Sweet	0.7	0.3	0.8	0.7	0.9	0.6	0.7	0.5
Umami	2.0	1.4	2.8	2.9	1.7	1.7	2.1	1.5
Whey	0.2	0.2	ND	ND	ND	0.1	0.2	0.2

[1]Variation among sharp cheeses tested.

[2]NA = Not available.

[3]ND = Not detected.

Cheese Composition

Two-way ANOVA was performed to determine any significant differences among the various components (Table 4). None were significant except the moisture content of cheese between NaGlu treatments. Proximate analysis results were pooled to show significance between treatments of this factor and are listed in Table 5. Low-fat composition was achieved (full composition data listed in Table 21 in Appendix B).

Informal Preliminary Tasting

The 3-member panel that tasted all 24 cheeses (6 culture treatments, 2 NaGlu treatments, and 2 ripening temperature treatments) found no outstanding improvement in flavor among the cultures except as relating to those with and without NaGlu. The consensus was that those cheeses with NaGlu and ripened at a low temperature had a

Table 4. ANOVA table for proximate analysis of low-fat Cheddar cheeses

		% SALT			% MOISTURE			% FAT			PH		
SOURCE	DF	MS	Pr > F	Sig	MS	Pr > F	Sig	MS	Pr > F	Sig	MS	Pr > F	Sig
ADJUNCT	5	0.017	0.519	ns[1]	0.896	0.549	ns	0.422	0.767	ns	0.010	0.671	ns[1]
NAGLU	1	0.078	0.067	ns	12.499	0.005	*	0.002	0.965	ns	0.008	0.471	ns
ADJUNCT *NAGLU	3	0.001	0.999	ns	0.093	0.993	ns	0.102	0.985	ns	0.001	0.996	ns
ERROR	12	0.019			1.072			0.835			0.015		

[1]ns = not significant.

Table 5. Mean composition of low-fat Cheddar cheese made with various adjunct cultures

Sodium gluconate content (%)	Mean of 2 replicates			
	pH	Fat content (%)	Moisture content (%)	Salt content (%)
0.8	5.19	5.0	54.5	2.0
0.0	5.15	5.0	53.1	1.9

minimum of the burnt sugar off-flavor. Adjunct culture treatment seemed to have little effect. Yet it was the opinion of a few, including this author, that the CR540 cheese tasted closer to full-fat Cheddar cheese than the control cheese, meaning it had increased desirable flavors (buttery, whey) and less off-flavor (bitterness, burnt). Elevated ripening temperature detracted from overall acceptance, increasing off-flavors (burnt, bitter). Descriptive panels were run accordingly.

Effect of Sodium Gluconate and Ripening Temperature

Taking into account the results of the preliminary testing, only CR540 cheeses were chosen for the analysis of NaGlu and ripening treatments alongside the control. These results were pooled and used to determine the effect of NaGlu and ripening temperature on the low-fat cheese system. Addition of NaGlu had a significant effect on the following flavor and taste attributes as shown in Table 6: bitter ($P = 0.01$), buttery ($P = 0.0004$), lactone ($P = 0.004$), sour ($P = 0.0071$), sweet ($P = 0.0037$), and whey ($P = 0.0024$). Bitter decreased and buttery, lactone, sour, sweet, and whey increased. The NaGlu treatment may have increased the amount or perception of the amount of typical mild Cheddar cheese flavors (buttery, lactone, whey) as it masked bitterness as has been observed in other food or pharmaceutical systems (Breslin and Beauchamp, 1997; Keast and Breslin, 2002; Ramachandran et al., 2006). This corresponds with the informal testing results where the cheeses with NaGlu were considered to be closer in flavor to Cheddar cheese. The gluconate portion may be responsible for providing more sour taste since it is a sour compound and pH is not significantly different between NaGlu treatments.

Table 6. Summary of significant mean-scores from descriptive panel on sodium gluconate treatment using the control cheese and CR540 cheese with culture treatments and ripening treatments combined

	Cheese Sample		Average for full-fat mild (n=6)[1]
Sensory Attribute	0% Sodium gluconate	0.8% Sodium gluconate	
Bitter	1.2[a]	0.9[b]	0.6
Brothy	2.4	2.5	1.6
Buttery	0.7[b]	1.1[a]	1.3
Burnt	2.0	1.8	0.1
Cooked	0.1	0.1	0.1
Fishy	ND	ND	ND
Fruity	ND[2]	ND	ND
Lactone	1.3[b]	1.6[a]	2.1
Metallic	ND	ND	ND
Nutty	0.3	0.2	0.2
Oxidized	ND	ND	ND
Pineappley	ND	ND	ND
Rancid	0.1	0.1	0.1
Rosy/Floral	ND	ND	ND
Salty	5.0	5.2	4.6
Sour	2.8[b]	3.2[a]	2.9
Sulfur	0.3	0.2	0.1
Sweet	0.6[b]	0.9[a]	1.0
Umami	1.4	1.5	1.2
Whey	0.1[b]	0.2[a]	0.2

[a-c]Means within a row with a different letter were different ($\alpha = 0.05$).

[1]Average displayed for comparison only; comparative statistics were not assessed.

[2]ND = Not detected.

There was not a significant difference seen in the burnt descriptor, the one flavor attribute that NaGlu was expected to reduce from the preliminary trial. Reference flavor for this descriptor is still being defined and is not well isolated (Drake et al., 2010).

Ripening temperature also had some effect, with 4 significant descriptors (Table 7): brothy ($P = 0.0086$), salty ($P = 0.0002$), sour ($P = 0.0028$), and sulfur ($P = 0.0039$). All these descriptors were closer to a commercial mild cheese when the cheese was not ripened with elevated temperatures. This supports the theory that elevated ripening temperatures may not be suitable for low-fat cheese (Mistry, 2001) and was expected as previous observation showed that atypical flavors, pinholes, and gas formation may result.

One point to consider is that even though there was a statistically significant difference for the NaGlu and ripening temperature treatments, attribute scores with a difference <0.5 may be considered negligible in terms of practical importance for application. All sensory tests employed must be consulted in order to get a correct picture of the treatment effects. The descriptive panel showed a significant effect in both treatments which is very small and may seem unimportant, but this cannot be known with this information alone as the descriptive panel gives no indication of overall acceptability. The informal panel felt that there was a difference in overall acceptability, with NaGlu addition being more acceptable, and ripening at the elevated temperature being less acceptable. The consumer panel, as will be discussed later, also found cheese with NaGlu to be more acceptable than cheese without NaGlu. Therefore, though the individual difference in one descriptive attribute due to these treatments may be negligible, the combined change due to these treatments does have an impact.

Table 7. Summary of significant mean-scores from descriptive panel on ripening treatment using the control cheese and CR540 cheese with culture treatments and sodium gluconate treatments combined

	Cheese Sample		
Sensory Attribute	6°C	10°C	Average for full-fat mild (n=6)[1]
Bitter	1.0	1.2	0.6
Brothy	2.3[b]	2.6[a]	1.6
Cooked	0.1	0.1	0.1
Fruity	ND[2]	ND	ND
Lactone	1.4	1.5	2.1
Metallic	ND	ND	ND
Nutty	0.2	0.3	0.2
Oxidized	ND	ND	ND
Pineappley	ND	ND	ND
Fishy	ND	ND	ND
Rancid	0.1	0.1	0.1
Rosy/Floral	0.1	ND	ND
Salty	4.9[b]	5.3[a]	4.6
Sour	2.8[b]	3.2[a]	2.9
Sulfur	0.2[b]	0.3[a]	0.1
Sweet	0.7	0.8	1.0
Umami	1.5	1.4	1.2
Whey	0.2	0.1	0.2
Buttery	0.9	0.9	1.3
Burnt	1.7	2.0	0.1

[a-c]Means within a row with a different letter were different ($\alpha = 0.05$).

[1]Average displayed for comparison only; comparative statistics were not assessed.

[2]ND = Not detected.

Effect of Culture Treatment

As a result of the preliminary tasting and the above mentioned descriptive panel, all the cheeses with NaGlu and a low ripening temperature were chosen for a second descriptive panel to analyze the effect of adjunct culture on flavor. Significant effects with no distinct trends were found for 4 descriptors as displayed in Table 8: bitter (P = 0.0005), buttery (P = 0.0244), rancid (P = <0.0001), and sour (P = 0.0044).

Though adjunct culture had a significant effect, the differences detected, as with the panel evaluating NaGlu and ripening treatments, were very small; none were more than 0.8. Again, this may have little practical application. The preliminary panel, as previously stated, detected no great overall difference in flavor among culture combinations. It would seem the combined differences among culture treatments, though significant, were not as consequential as the differences between NaGlu treatments.

Considering this, along with the impressions from the informal preliminary panel, it would seem that these adjunct culture combinations did not contribute positive flavor compounds in the low-fat Cheddar cheese more than those contributed by the starter culture. Their application in Cheddar cheese in the method used in this experiment cannot justified. Usage level increase or other alterations to manufacturing process or strain variety could be tried if attempting to improve flavor with adjuncts. The CR540 cheese approaches the average value of the commercial mild cheeses tested in more attributes than any other culture combination. Though it was not significantly different from the control of the experiment, it exhibits potential for flavor improvement if these

Table 8. Summary of significant mean-scores from descriptive panel on culture treatment using only cheese with sodium gluconate and ripened at 6°C

Sensory Attribute	Cheese Sample						Average for full-fat mild (n=6)[1]
	Control	LH32	CRL431	CR540	Emfour	LH/CR319	
Bitter	0.9^{bc}	1.0^{bc}	1.2^{ab}	0.7^{c}	1.5^{a}	1.1^{ab}	0.6
Brothy	2.3	2.4	2.1	2.3	2.7	2.3	1.6
Buttery	1.3^{a}	0.7^{b}	1.3^{a}	1.0^{ab}	0.9^{ab}	0.9^{ab}	1.3
Burnt	1.8	2.2	2.2	1.4	2.5	2.3	0.1
Cooked	0.1	0.1	0.3	0.1	0.1	0.1	0.1
Fishy	ND[2]	ND	ND	ND	ND	ND	ND
Fruity	ND	ND	ND	ND	ND	ND	ND
Lactone	1.5	1.5	1.5	1.5	1.4	1.5	2.1
Metallic	ND	ND	ND	ND	ND	ND	ND
Nutty	0.2	0.2	0.2	0.1	0.2	0.2	0.2
Oxidized	ND	ND	0.1	ND	ND	ND	ND
Pineappley	ND	ND	0.1	ND	ND	ND	ND
Rancid	0.0^{b}	0.0^{b}	0.4^{a}	0.0^{b}	0.4^{a}	0.0^{b}	0.1
Rosy/Floral	0.1	ND	ND	ND	0.1	ND	ND
Salty	5.1	5.0	5.0	4.9	5.5	5.1	4.6
Sour	3.1^{ab}	3.0^{b}	3.5^{ab}	2.9^{b}	3.6^{a}	3.2^{ab}	2.9
Sulfur	0.2	0.2	0.1	0.1	0.3	0.2	0.1
Sweet	0.8	0.8	1.1	1.1	0.8	0.8	1.0
Umami	1.7	1.5	1.5	1.5	1.5	1.5	1.2
Whey	0.3	0.2	0.2	0.2	0.2	0.2	0.2

[a-c]Means within a row with a different letter are different ($\alpha = 0.05$).

[1]Average displayed for comparison only; comparative statistics were not assessed.

[2]ND = Not detected.

trends toward the full-fat Cheddar flavor profile could be magnified. Adjunct culture use may still be effective when the appropriate culture and manufacturing process are found.

Consumer Panel Results

As the type of adjunct cultures used did not seem to have a significant effect, cheese from one culture combination was chosen to represent all experimental low-fat Cheddar cheese in a consumer panel. Both NaGlu treatments were included.

The full-fat samples were presented as the first 2 samples. Participants rated their preference without knowing which was mild or sharp, and without knowing they were full-fat. This provided some idea as to which flavor profile was favored. In both replicates of the experiment, mild was preferred over sharp (Table 9). This is illustrated better by the data from Rep 2. In Rep 1, the commercial cheese representing mild Cheddar cheese was found to be somewhat sharp, and the cheese representing sharp was found to be somewhat mild. More on this is mentioned with the PCA section. Consumers ranked the overall flavor of full-fat cheeses (33% fat) with the highest ratings, as expected (Table 10). It is remarkable to note that consumers ranked the flavor of one low-fat Cheddar cheese (5% fat, CR540, NaGlu, 6°C ripening) as being liked the same statistically as the commercially produced reduced-fat Cheddar cheese (25% fat). This shows promise for a low-fat cheese to be at least as acceptable as reduced-fat cheese already in production. This could open a market for reduced-fat cheese eaters to be able to consume even less fat while still having a cheese comparable to what they are accustomed. It also allows those consumers who eliminate Cheddar cheese altogether due to reduced-fat cheese still being too high in fat to have a much lower-fat alternative.

Table 9. Data on consumer demographics, Cheddar profile preference, cheese usage level, and importance of lower-fat cheese taken during the consumer panel

Categories	Percentages (Rep1)	Percentages (Rep 2)
Gender	55% Male	54% Male
	45% Female	46% Female
Age	67% 18-25 years	77% 18-25 years
	19% 26-35 years	12% 26-35 years
	5% 36-45 years	4% 36-45 years
	4% 46-55 years	2% 46-55 years
	5% >56 years	5% >56 years
Cheese preference	53% Mild	73% Mild
	47% Sharp	28% Sharp
Cheese usage	0% Never	0% Never
	4% Less than once/month	2% Less than once/month
	7% At least once a month	7% At least once a month
	31% At least once a week	41% At least once a week
	45% About once a day	43% About once a day
	13% More than once a day	7% More than once a day
Lower-fat cheese usage	35% Never	35% Never
	34% Less than once/month	33% Less than once/month
	16% At least once a month	16% At least once a month
	12% At least once a week	13% At least once a week
	3% At least once a day	3% At least once a day
Importance of having lower-fat cheese	31% Not important	32% Not important
	10% Somewhat not important	9% Somewhat not important
	30% Neither not important nor important	22% Neither not important nor important
	23% Somewhat important	27% Somewhat important
	6% Important	10% Important

Table 10. Summary of significant mean-scores for the consumer panel

	Sensory Attribute		
Cheese sample	Flavor	Appearance	Texture
Commercial Mild	7.2[a]	7.0[a]	7.0[a]
Commercial Sharp	6.5[b]	6.6[b]	6.3[b]
Commercial 2% Mild 1	5.6[c]	6.6[b]	5.4[c]
Commercial 2% Mild 2	5.4[c]	6.6[b]	5.2[c]
CR540, 0.8% NaGlu, 6°C	5.4[c]	5.7[c]	5.1[cd]
CR540, 0% NaGlu, 6°C	4.9[d]	5.8[c]	4.8[d]

[a-d]Means within a column with the same letter were not significantly different ($\alpha = 0.05$).

Another noteworthy point is that consumers found a statistically significant difference between the low-fat cheese with NaGlu, and the low-fat cheese made without NaGlu. Thus, although the differences found by the descriptive panel between these two cheeses was small, it was enough of a difference in overall flavor to affect the participants of the consumer panel.

Consumers also ranked appearance and texture of the 6 samples (Table 10). Commercial full-fat cheese is again ranked highest for both attributes. The 2 low-fat experiment cheeses were ranked lowest for appearance, but the averages are not below a 5, indicating that the appearance was generally more liked than disliked. The titanium dioxide prevented the translucency in low-fat cheese that is often repulsive to consumers. Unfortunately, too much was added, giving the cheese the look of a light yellow processed cheese which consumers commented on as being undesirable. One low-fat cheese was ranked not significantly different from reduced-fat cheese in texture. There is still some improvement to be made in these areas. The distribution of responses can be seen in Figure 2.

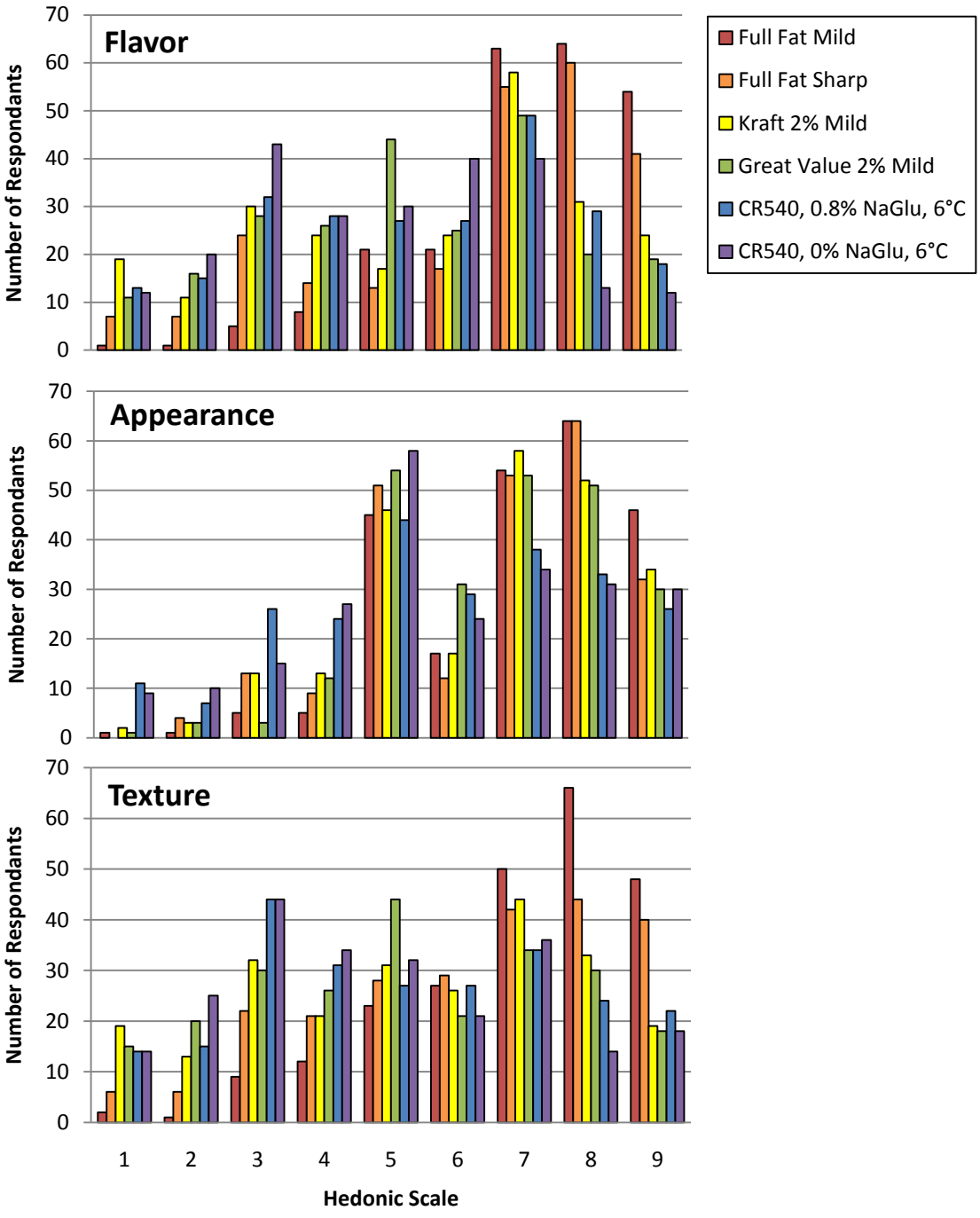


Figure 2. Distribution of responses for liking from the consumer panel. Participants ranked flavor, appearance and texture on a 9-point hedonic scale, 1=dislike extremely, 5=neither like nor dislike, and 9=like extremely. Responses for each replicate were totaled.

For the reduced-fat samples and the low-fat samples, participants in the consumer panel were asked to rate their intent to purchase (Table 11). Though there was a good portion that answered "likely" and "somewhat likely," "not likely" was the most frequent answer for all for samples. This implies that there is still progress to be made on lower fat cheese flavor commercially available and experimentally made.

Demographic questions demonstrated a nearly even split between gender for both replicates (Table 9). The participants were heavily from the 18-25 age-group due to the location of the Sensory Laboratory being on the university campus.

Participants were asked about their cheese consumption (Table 9), the aim being to see how the experimental low-fat cheese fit into the current market of full-fat cheese users. All participants were cheese users with only 31% of them using reduced-fat cheese once a month or more. It would be intriguing to have a future panel consisting of only reduced-fat cheese users to see how the experimental low-fat cheese fits into the current reduced-fat cheese market.

Lastly, consumers were asked to rate the importance in their minds of having cheese with less fat (Table 9). An average of 33% rated it as somewhat important or

Table 11. Intent to purchase reduced-fat and low-fat Cheddar cheese as indicated in the consumer panel

	Kraft 2% Mild Cheddar		Great Value 2% Mild Cheddar		CR540, 0.8% NaGlu, 6°C		CR540, 0% NaGlu, 6°C	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Likely	12%	22%	14%	14%	16%	17%	9%	9%
Somewhat Likely	17%	32%	28%	17%	18%	14%	11%	21%
Neither Likely Nor Likely	16%	18%	18%	17%	19%	28%	21%	27%
Somewhat Not Likely	16%	14%	17%	16%	13%	16%	23%	16%
Not Likely	39%	14%	23%	36%	34%	25%	36%	27%

important, indicating that there is a sizable place for lower fat cheese with expected Cheddar cheese flavor in the market.

Principal Component Analysis

In the principal component biplot of all the cheeses used in this experiment, replicates combined, charted with full-fat commercial mild, medium, and sharp (Figure 3), cheeses are color-coded by type. The two results for the CR540 cheese with NaGlu ripened at 6°C are in turquoise to highlight their difference in position. The principal component biplot represents 52% of the variability (full listing in Figure 7 in Appendix B). There is considerable variability that is not accounted for in this plot. Therefore, it is useful as a visualization of the data and a good tool for increasing understanding, but not for definitive conclusions.

This visual representation of flavors, basic tastes and cheese samples is especially helpful in identifying and describing the differences in these cheeses. Most of the mild full-fat cheeses are in the upper two quadrants, described more by the attributes whey, sweet, lactone, and buttery, which is to be expected for mild Cheddar cheese. The sharp cheeses are mostly in the lower two quadrants, characterized more with descriptors such as nutty, sour, umami, rancid, and bitter, as well as the lactone, but not as much with whey. The reduced-fat cheeses, though mild, are scattered over the graph, some looking like a very flavorful mild, another is medium, and another more sharp. This suggests a lack of consistency in reduced-fat cheese flavor commercially available.

The similarities between the mild and sharp used for the first consumer panel as previously mentioned can be visualized graphically in the PCA biplot (Figure 3). The

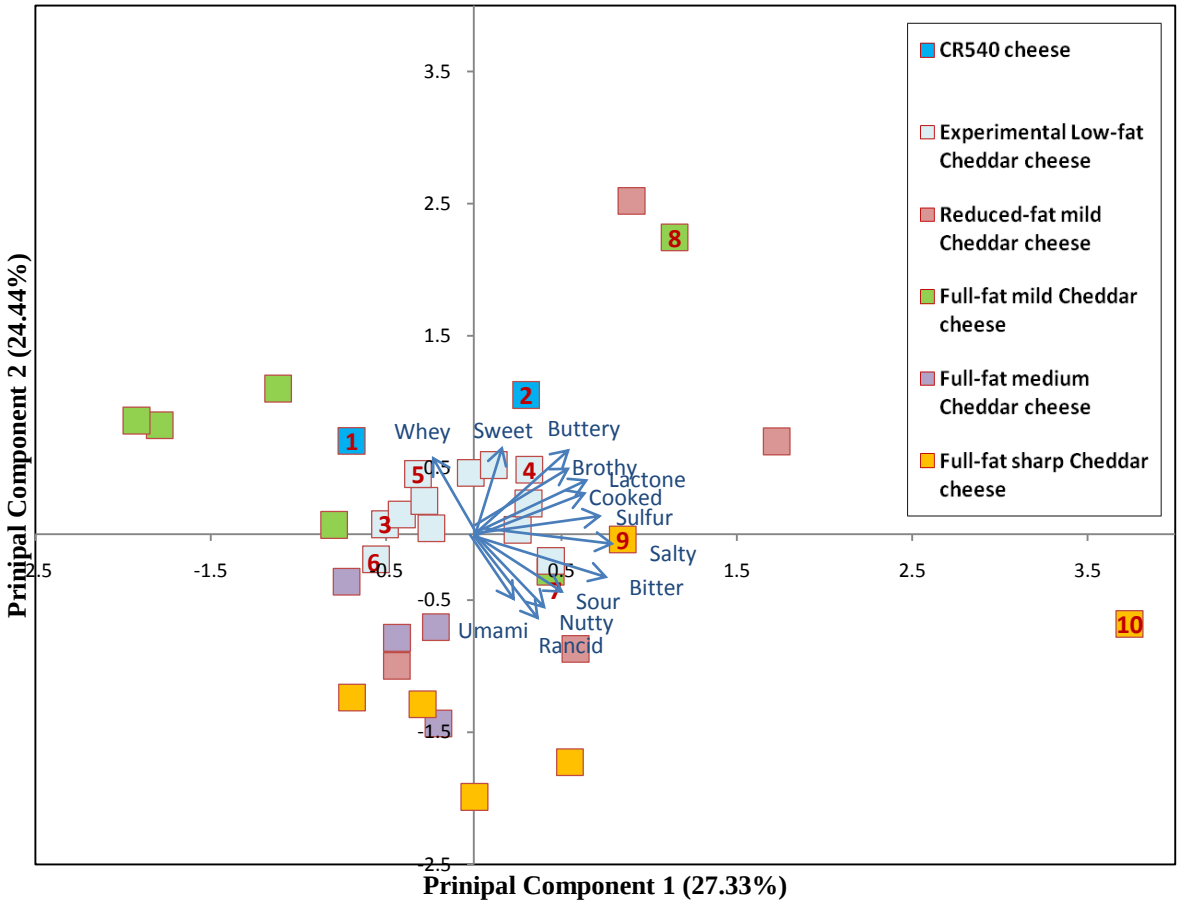


Figure 3. Principal component analysis of all the cheeses used in this experiment charted with full-fat commercial mild, medium, and sharp using descriptive analysis results, replicates averaged by sample. Numbers (culture treatment, sodium gluconate (NaGlu) treatment, ripening treatment): CR540, 0.8% NaGlu, 6°C (1, 2); CR540, 0% NaGlu, 6°C (3, 4); Control, 0.8% NaGlu, 6°C (5); Control, 0% NaGlu, 6°C (6); Mild Cheddar cheeses used in consumer panels (7, 8); Sharp Cheddar cheeses used in consumer panels (9, 10). Blue arrows: attribute trends.

sharp for Rep 1 (#9) and the mild for Rep 1 (#7) are very close together on the chart indicating similar flavor characteristics, while the sharp for Rep 2 (#10) and the mild for Rep 2 (#8) are much further apart, indicating a much greater difference in flavor profile. The same pattern is seen in the PCA biplot of just the cheese used for the consumer panel (Figure 4).

The absence of flavor that characterizes low-fat Cheddar cheese is easily ascertained from Figure 3. Most of the experimental low-fat cheeses congregate around the origin, described more by mild descriptors, but not having enough flavor to match the commercial mild cheeses. It is interesting to note that the low-fat CR540 cheese, with NaGlu, ripened at 6°C, (highlighted in turquoise) is shown as being more flavorful in desirable Cheddar flavors and closer to the full-fat mild Cheddar cheeses than the other low-fat cheeses of the experiment. This validates the results from informal taste panel in that the CR540 cheese seemed closer to full-fat Cheddar cheese and the consumer panel in that low-fat cheese with NaGlu was more acceptable than cheese without.

The PCA biplot of only the cheeses used in the consumer panels (Figure 4) represents 72% of the variability. This chart also visually represents the difference in NaGlu treatments. Cheese without NaGlu displays as having higher levels of such attributes as burnt, bitter, metallic, oxidized—flavors and tastes which are defects in Cheddar cheese at too high of a concentration. They are also characterized by sulfur, sour, and whey which can be positive flavor notes. In this depiction, however, they are opposite to the full-fat cheeses. Cheeses with NaGlu are shown as drawing closer to the full-fat mild than the ones without, but they are still characterized by similar flavors. It seems that Cheese with NaGlu has the same flavors as cheese without, but less of the

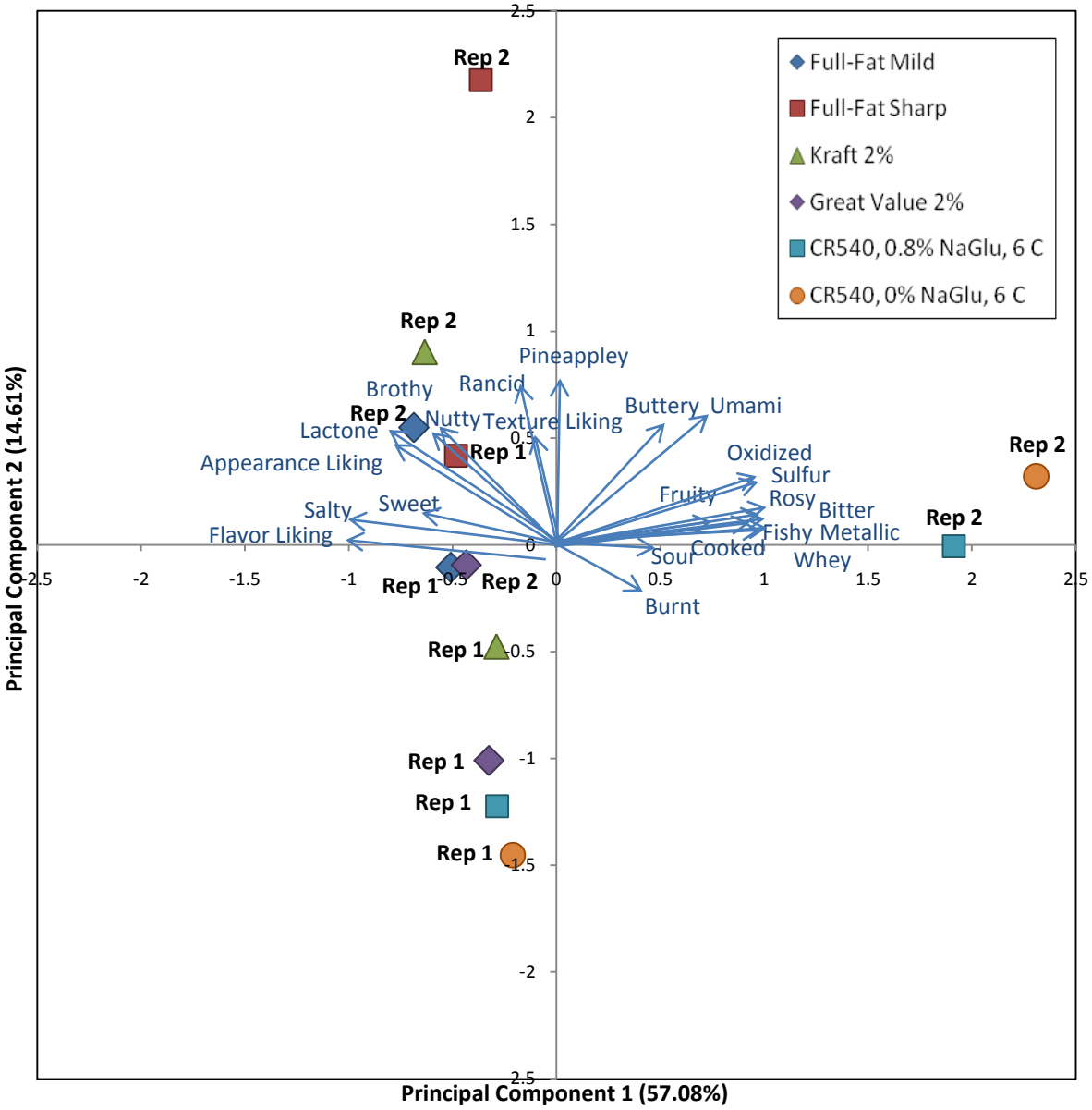


Figure 4. Principal component analysis of only cheeses used in both replicates of the consumer panel. Blue arrows show attribute trends.

off-flavors and more of the desirable flavors are noticed. This again indicates that NaGlu is a useful addition to low-fat Cheddar cheese for overall flavor improvement. Tastes that correlated with flavor liking by consumers were salty and sweet, as well as the lactone flavor.

CONCLUSION

Although some adjunct cultures did positively affect low-fat cheese flavor, specifically CR540, there was no difference statistically from the control cheese. For these cultures in this low-fat cheese system, the flavor contributions need to be magnified if they are to make a meaningful contribution. The trend was positive, indicating that adjunct cultures in low-fat cheese have potential if the right cultures and usage level can be found.

The positive effect of NaGlu on low-fat cheese flavor was confirmed. Consumers preferred low-fat Cheddar cheese with NaGlu over cheese without. Sodium gluconate would be a good addition to low-fat Cheddar cheese. Further research into the mechanism of flavor improvement from NaGlu addition is needed to provide a full understanding of this cheese ingredient and for maximizing its potential.

Elevated ripening temperatures do not appear to be viable for low-fat Cheddar cheese made with these adjuncts. Other accelerated ripening techniques should be investigated.

CHAPTER 4

EFFECT OF LEUCONOSTOC SPP, BREVIBACTERIUM SPP, PROPIONIBACTERIUM SPP. AND LACTOCOCCUS LACTIS SSP. CREMORIS ADJUNCT CULTURES IN LOW-FAT CHEDDAR CHEESE

ABSTRACT

Various adjunct cultures, including *Propionibacterium freudenreichii* (Pal prop), *Leuconostoc mesenteroides* ssp. *cremoris* (100-L), *Brevibacterium linens* (101-V), , were used in low-fat Cheddar cheese manufacture to determine the effect on flavor. Treatments of sodium gluconate (0% and 0.8%) were also analyzed. Reduced sodium treatments (2.0% and 1.5%) were initially included but not analyzed. All cheese was extremely bitter due to starter culture (LL-50) effects. *Lactococcus lactis* ssp. *cremoris* (LL-011) was added to reduce bitterness. Descriptive analysis showed some significant difference in flavor due to the debittering adjunct (LL-011). Despite this, no acceptable cheese was produced because of the overpowering bitterness. Sodium gluconate addition increased brothy, sour, and buttery attributes.

INTRODUCTION

Nearly every aspect of cheese-making influences flavor (Singh et al., 2003; Drake et al., 2008). Cheddar cheese flavor is produced by a balance microbiological processes, enzymatic degradation, and manufacturing manipulation (Banks, 2004; Drake et al.,

2010). Changes in the fat or sodium content will likely result in changes in Cheddar cheese flavor profiles, or flavor inconsistencies (Singh et al., 2003).

It follows that low-fat cheese flavor is very different from full-fat cheese flavor (Banks, 2004; Saint-Eve et al., 2009). Low-fat cheese is often low in characteristic Cheddar cheese flavor and lacks overall flavor, and may have flavors atypical of commercial Cheddar cheese (Drake et al., 2010; Mistry, 2001) including bitterness, astringency, and unclean flavor notes (Banks, 2004). Low-fat cheese may also develop a rosy/burnt flavor Drake et al. (2010) described as being reminiscent of burnt sugar that is often in low-fat cheese. This may also be the meaty off-aroma described by Milo and Reineccius (1997). Acceptability decreases because consumers have repeatedly asserted that they are unwilling to accept atypical flavors in low-fat Cheddar cheese even for the benefit of lower fat or sodium (Banks, 2004; Childs and Drake, 2009).

Reduction in fat not only changes the flavor of the cheese, but may also change flavor perception. Wendin et al. (2000) showed fat reduction to reduce the intensity of saltiness, and Saint-Eve et al. (2009) found that perception of salt changes were more noticeable in low-fat cheese.

One method for improving flavor is through adjunct culture use (Broadbent et al., 2003; Johnson and Lucey, 2006). Adjuncts are microorganisms intentionally added to cheese milk that positively impact cheese sensory quality (El Soda et al., 2000). Adjunct use is common practice in cheese making today (Crow et al., 2001; Johnson and Lucey, 2006) and may be what is needed to impart desirable Cheddar cheese flavors to low-fat cheese. Fenelon et al. (2002) showed that an 18% fat cheese with a *Lactobacillus helveticus* strain as a single adjunct achieved an increase in flavorful low molecular mass

peptides and a positive flavor s core; however, cheeses made with a blend of *Lb. helveticus* and other cultures were not as favorable.

Other groups used *Brevibacterium linens* to improve consumer acceptance of the overall flavor of reduced-fat Cheddar cheese, but results were strain specific (Broadbent et al., 1997; Weimer et al., 1997). Brevibacterium has the ability to produce methanethiol, a precursor to volatile sulfur compounds and an important contributor to the Cheddar cheese flavor profile. Often used for surface-ripened cheeses (Camembert, Limburger, Brie, etc), it has been used to improve the flavor of 60% reduced-fat Cheddar cheese (Weimer et al. 1997). Propionibacterium is traditionally used in Swiss cheese and others (Emental, Gruyere) and is responsible for many flavors and eye formation. It has been used in a Swiss-Cheddar hybrid cheese with some effect on nutty and sweet flavor attributes (Lawlor et al., 2003). Leuconostoc has the ability to metabolize citrate and produce diacetyl (Goff, 2010). It can be used enhance cheeses from Gouda to Gorgonzola, and is used for cultured butter and butter milk (Broome, 2007). These three genera typically not used in Cheddar may have the ability to impart Cheddar cheese flavors where current low-fat Cheddar cheese starters have failed. Again, careful culture selection is emphasized.

One may not be able to use the same cultures as used in full-fat cheese in reduced-fat cheese. Johnson and Chen (1991) found that starters useful in full-fat cheese were not suitable for producing quality low-fat cheese. It is thus essential to investigate the effects of specific strains in the desired cheese system.

Sodium gluconate (**NaGlu**) is the sodium salt of gluconic acid, and is used in the food industry as a sequestrant , a chelator at alkaline pH, a stabilizer, and a thickener

(Ramachandran et al., 2006; FAO and WHO, 2010) . There is some indication from previous work that NaGlu improves low-fat Cheddar cheese by minimizing the burnt flavor described by Drake et al. (2010). The mechanism is not yet understood. Sodium ions mask bitterness (Hayes et al., 2010). Breslin and Beauchamp (1997) found sodium in the form of sodium acetate to not only suppress bitterness, but to also "release sweetness from suppression" due to bitterness. It is possible NaGlu works in a similar way, masking off-flavors and allowing improved detection of typical Cheddar cheese flavors. It is known that NaGlu inhibits bitterness (Ramachandran et al., 2006). Keast (2008) experimented with 3 concentrations of caffeine mixed with NaGlu and Zn-lactate. Thought the Zn-lactate was superior to NaGlu in decreasing the bitterness of caffeine, he demonstrated that NaGlu had a significant effect. In low-fat Cheddar cheese, it is possible that NaGlu's major contribution to flavor is a suppression of bitterness.

A diet high in sodium is associated with increased incidence of hypertension, which in turn is correlated with increased risk of coronary heart disease and stroke (FDA, 2010b), two of the top 10 leading causes of death (Xu et al., 2010). Reduction of dietary sodium is logical, but difficult to implement. Salt plays a major role in cheese, from food safety to taste (Johnson et al., 2009). Salt reduction in low-fat cheese with adjunct cultures requires analysis.

Appearance is one sensory characteristic affected by fat reduction. Pastorino et al. (2002) demonstrated that the removal of fat from cheese causes a translucent appearance. One way to improve the color of low-fat cheese is to add a colorant, such as titanium dioxide, for opacity improvement.

Though several studies have been done on adjunct addition to Cheddar cheese with a fat level between 10-33%, and several low-fat (5% fat) cheeses, apparently no studies have been done on the effect of adjunct culture addition to the current low-fat (5% fat) Cheddar cheese with descriptive sensory analysis. In this study, cultures from the *Brevibacteriaum, Probionibacterium,* and *Leuconostoc* genera were employed. It was hypothesized that these cultures would contribute to Cheddar flavor in a way that was as yet untested. Sodium gluconate was included to further determine its effect on overall low-fat Cheddar cheese flavor. Sodium reduction was included in this study to get a full analysis of how adjuncts behave in reduced-sodium low-fat Cheddar cheese. Impact was measured by descriptive analysis.

MATERIALS AND METHODS

Cultures

DSM Food Specialties USA Inc. (Eagleville, PA) provided all cultures for this project. Cultures in the experiment were used in an exploratory manner determining what kind of effect they might have in Cheddar cheese. Three adjunct cultures chosen for their associated wide range of flavor contributions (nutty, diacetyl, etc) were used in combination with a *Lactococcus lactis* ssp. *lactis/cremoris* blend (LL-50) for the starter in the first replicate (**Rep 1**) (Table 12). A debittering *Lc. lactis* ssp. *cremoris* culture (LL-011) was added in the second replicate (**Rep 2**) at a rate of 4.7 ml/100 kg milk.

Table 12. Adjunct culture combinations used in conjunction with a *Lactococcus lactis* ssp. *cremoris*/lactis starter culture to make low-fat cheese. LL-011, a debittering *Lc. lactis* ssp. *cremoris* was added to the last four

Code	Adjunct description	Usage level
Control (LL-50)	*Starter only*	36.7 g/100 kg milk[1]
Pal prop	*Propionibacterium freudenreichii* (3 strains)	0.88 g/100 kg milk[2]
100-L	*Leuconostoc mesenteroides* ssp. *cremoris*	0.73 g/100 kg milk[1]
101-V	*Brevibacterium linens*	2.2 ml/100 kg milk[3]
Control/LL-011	*Starter*	36.7 g/100 kg milk[1]
	Lc. lactis ssp. *cremoris*	4.7 ml/100 kg milk[3]
Pal prop/LL-011	*Pr. freudenreichii* (3 strains)	0.88 g/100 kg milk[2]
	Lc. lactis ssp. *cremoris*	4.7 ml/100 kg milk[3]
100-L/LL-011	*Leuconostoc mesenteroides* ssp. *cremoris*	0.73 g/100 kg milk[1]
	Lc. lactis ssp. *cremoris*	4.7 ml/100 kg milk[3]
101-V/LL011	*Br. linens*	2.2 ml/100 kg milk[3]
	Lc. lactis ssp. *cremoris*	4.7 ml/100 kg milk[3]

[1]Frozen pellet culture.
[2]Freeze-dried powder culture.
[3]Frozen liquid culture.

Cheese Manufacture

Raw milk was obtained fresh from the Utah State University (**USU)** Caine Dairy Research and Teaching Center (Wellsville, UT) and transferred to the Gary Haight Richardson Dairy Products Laboratory (Logan, UT). Milk was standardized to a protein to fat ratio of 5, and then pasteurized at 73°C for 16 s. Eight different cheeses were made, the second group of 4 being identical to the first 4 except for the addition of a de-bittering culture. Two cheeses were made each day using Tetra Scherping horizontal cheese vats (Tetra Pak Cheese and Powder Systems Inc., Winsted, MN). Culture combinations were randomized and vat location balanced between replicates.

While the vat was being filled, the warm (23°C) milk was preacidified to a pH of 6.20 with L-lactic acid (Nelson Jameson, Marshfield, WI) at a 1:16 dilution with distilled water added using a peristaltic pump (Masterflex; Cole-Parmer, Vernon Hills, Illinois). Titanium dioxide emulsion (Idacoat White, ROHA, St. Louis, MO) was then added at a rate of 50g/100 kg milk, and the milk was heated to 32°C with constant agitation at 15 rpm. Starter culture and adjuncts were added, incubated for 30 min, then additions of single strength Annatto cheese color and Maxiren double strength chymosin (~650 International milk clotting units/ml), both from DSM Food Specialties USA Inc. (Eagleville, PA), were both made at a rate of 7.5 mL/100 kg milk. After a 20-min coagulation time, curd was cut and healed over a 20 min period.

After a 20-min coagulation time, curd was cut and healed over a 20-min period. Curd cooking was omitted; temperature remained at the set temperature (32°C) for 20 min with continuous cutting to prevent curd packing on the knives. After a 30-min stir-out period, curds and whey were transferred to a drain table (Kusel Equipment Co.,

Watertown, WI) where wet acid development continued until a pH of 5.95 was reached. Whey was drained and dry stirring continued until the curd reached a pH of 5.50. A cold water wash (8 to 10°C) at a rate of 40 kg/100 kg curd was applied, reducing the curd temperature down to 22 °C and drained again.

Curd was divided into 4 tubs, 13.6 kg in each. Two tubs each were assigned a sodium treatment, one normal and the other reduced – 2% and 1.5% (Table 13). Each tub was assigned a NaGlu treatment. The 2 sodium levels were adjusted to 1.9% and 1.4% if NaGlu was also added to account for any sodium imparted by the NaGlu. Curd was salted in 3 applications each 5 min apart. Sodium gluconate was then added to designated blocks at a rate of 0.8 kg/100 kg in 2 applications, 5 min apart.
After 5 min, curd was filled into hoops and pressed at a pressure of 0.1 MPa overnight (~18 h) at room temperature (~20°C) yielding 2 blocks of about 10 kg each post-pressing. Cheese was removed the next day, cut into 2-lb blocks, sealed in barrier bags, and stored at 6°C.

Table 13. Description of salt and sodium gluconate (NaGlu) treatments

Block	Salt level
A	2.0 kg salt/100 kg curd 0% NaGlu
B	1.9 kg/100 kg curd 0.8% NaGlu - 0.8 kg/100 kg curd
C	1.5 kg salt /100 kg curd 0% NaGlu
D	1.4 kg salt/100 kg curd 0.8% NaGlu - 0.8 kg/100 kg curd

Proximate Analysis

Moisture, pH, salt and fat levels were measured on d 5 ± 1. Moisture content was determined using a SMART Turbo Microwave Moisture/solids Analyzer (CEM Corp., Indian Trail, NC). Between 3 and 4 g of cheese were placed in the microwave analyzer and readings in percent moisture were returned. Tests were performed in triplicate with no deviation greater than 0.50% allowed**.** Cheese pH was determined by adding 20 g of cheese and 10 g of distilled water to a plastic stomacher bag and stomached for 1 min at 260 rpm in a Seward Stomacher 400 (Seward, Riverview, FL). Readings were taken using an Oaklon pH meter (pH 510 series, Vernon Hills, IL). Salt content was analyzed using a 5 g sample of shredded cheese added to 98.2 g distilled water and stomached for 4 min at 260 rpm. After allowing the sample to equilibrate for 30 min, the slurry was filtered using Whatman No. 1 filter paper, and the permeate analyzed using a Corning Chloride Analyzer 926 (Medfield, MA) in duplicate. Fat was measured using the Babcock method. Results were analyzed using the PROC GLM procedure in SAS 9.2 (SAS Institute Inc., Cary, NC) with culture treatment, NaGlu treatment, and target salt treatment as class factors.

Sensory Evaluation

Informal Preliminary Tasting. After 2 mo, the 16 cheeses underwent an exploratory preliminary evaluation by 4 experienced cheese tasters to determine which trials would be analyzed formally by descriptive analysis.

Descriptive Analysis. Ten cheese panelists, selected for their ability to differentiate basic tastes and distinguish varying intensity levels, were previously trained

using a modified version the Spectrum™ method for the basic tastes bitter, salty, sour, sweet (Meilgaard et al., 2007), and 3 aqueous concentrations of monosodium glutamate (0.7%, 1.4%, 2.8%) to represent 3 levels (5, 9, 13) of "umami" taste on a 15 point scale (see Table 19 in Appendix A). After initial training and familiarization with the intensity scale, references of common Cheddar cheese flavors were presented (Table 20 in Appendix A). Cheeses were discussed in terms of these flavors and attributes were clarified. Panelists' responses have previously been monitored for reproducibility between samples, and consistency among the group. Previous to this research, each panelist had more than 100 hrs of training.

Before conducting flavor studies, procedures were approved by USU Institutional Review Board. Descriptive analysis was performed by USU Descriptive Cheese Panel. Panelists evaluated the high-salt cheeses with and without NaGlu. The 8 samples were randomly divided for presentation into 2 groups of 4, to prevent sensory fatigue.

Cheese samples were served in 1-cm cube pieces equilibrated to room temperature (~22°C) in covered 60-mL sample cups. Each sample was labeled with 3-digit blinding codes. Distilled water and unsalted crackers were available for panelists to cleanse their pallets between samples. Panelists rated a number of attributes on a 0 to 15-point scale: bitter, brothy, buttery, burnt, cooked, fishy, fruity, lactone/fatty acid, metallic, nutty, oxidized, pineappley, rancid, rosy/floral, salty, sour, sulfur, sweet, umami, and whey (0 = "no flavor," 15 = "extreme intensity"). They recorded their responses on a computer in individual booths using the Sims2000 software (Sensory Computer Systems, Morristown, NJ). Cheeses were tasted in duplicate.

Responses were analyzed in a split plot design and a randomized block design using SAS 9.1 and 9.2 (SAS Institute Inc., Cary, NC) with PROC GLM with Panelist, culture treatment, and NaGlu treatment as class factors. No consumer panels were scheduled due to the extreme bitterness of both replicates.

RESULTS AND DISCUSSION

Cheese Composition

Analysis of variance was performed on the data for cheese composition. Sufficiently little variation in cheese composition allowed the experiment to continue (Table 15). Significance for the salt results due to target salt content and sodium gluconate content was expected as these factors were manipulated in order to produce varying salt levels. Moisture was 1.6% higher for sodium gluconate cheese (Table 14). For a full listing of proximate data, see Table 22 in Appendix C. Descriptive testing was undertaken.

Table 14. Pooled mean composition from both replicates of low-fat Cheddar cheese made with various adjunct cultures

Sodium gluconate content (%)	pH	Fat content (%)	Moisture content (%)	Salt content (%)	
				Target	Actual
0.8	5.16	5.4	53.4	1.5	1.3
				2.0	1.7
0.0	5.15	5.5	51.8	1.5	1.3
				2.0	1.7

Table 15. Summary of ANOVA analysis of proximate data for low-fat cheese

		% SALT[3]			% MOISTURE			% FAT			% PH		
SOURCE	DF	Mean Square	Pr > F	Sig	Mean Square	Pr > F	Sig	Mean Square	Pr > F	Sig	Mean Square	Pr > F	Sig
CULTURE	3	0.0020	0.173	ns[1]	3.9450	0.070	ns	3.1153	0.133	ns	0.0215	0.079	ns
NAGLU	1	0.0048	0.048	*	20.0186	0.002	**	0.0903	0.806	ns	0.0009	0.740	ns
CULTURE*NAGLU	3	0.0009	0.474	ns	0.0610	0.987	ns	0.0653	0.987	ns	0.0015	0.902	ns
TARGET[2]	1	1.2364	<.0001	***	0.3763	0.609	ns	0.0028	0.965	ns	0.0058	0.405	ns
CULTURE*TARGET	3	0.0005	0.731	ns	0.0132	0.999	ns	0.2195	0.927	ns	0.0020	0.862	ns
NAGLU*TARGET	1	0.0001	0.788	ns	0.0020	0.971	ns	0.4278	0.594	ns	0.0001	0.922	ns
CULTURE*NAGLU*TARGET	3	0.0028	0.080	ns	0.0049	1.000	ns	0.1611	0.952	ns	0.0001	0.997	ns
ERROR	16	0.0010			1.3849			1.4434			0.0079		

[1]ns = not significant.

[2,3]TARGET refers to the target salt composition. % SALT refers to actual salt content.

Informal Preliminary Tasting of Replicate 1

Cheese from the first replicate (**Rep 1)** was tasted at 2 mo. All cheeses were found to be extremely bitter due to the starter culture used. A second tasting at 3 mo confirmed this. Accordingly, a de-bittering adjunct culture was added to the second replicate (**Rep 2**). No further impression of these first cheeses was made due to the overpowering bitterness.

Informal Preliminary Tasting of Replicate 2

Cheese from Rep 2 was tasted at 3 mo. Bitterness was reduced by the de-bittering culture, but not eliminated in most cheeses. Many other flavors were also present, none of which were found to enhance overall Cheddar cheese flavor. Only the cheeses with a high level of salt for each culture level for both NaGlu treatments were chosen for the descriptive panel.

Descriptive Analysis

Although the adjunct cultures were chosen for the ability to contribute to a wide flavor profile, very little difference was seen between culture treatments. When the data was analyzed as 4 culture profiles with 2 replicates, only one descriptor was statistically significantly different among cultures (Table 16). Cheeses made with *Brevibacterium* and *Leuconostoc* were bitterer than those made with *Propionibacterium* and the control. Other than that, there was no significant difference between cultures, even when analyzed as 8 culture combinations (Table 17), 4 from Rep 1 and 4 from Rep 2 containing the added de-bittering culture. This was most likely due to bitterness, resulting either from insufficient starter or from insufficient starter enzymes to degrade bitter peptides. Bitter

Table 16. Four sample analysis of culture: Summary of significant mean-scores

	Cheese sample			
Attribute	Propionibacterium	Leuconostoc	Brevibacterium	Starter only
Bitter	3.3[c]	4.1[b]	4.7[a]	3.1[c]
Brothy	1.8	1.6	1.6	1.5
Buttery	0.5	0.4	0.5	0.5
Burnt	1.9	1.9	1.8	1.9
Cooked	0.3	0.4	0.3	0.3
Fishy	ND[1]	ND	ND	ND
Fruity	ND	ND	ND	ND
Lactone/Fatty Acid	2.0	1.9	2.0	2.1
Metallic	ND	ND	0.2	ND
Nutty	0.3	0.3	0.4	0.3
Oxidized	0.1	ND	0.1	0.1
Pineappley	ND	ND	ND	0.1
Rancid	0.2	0.3	0.2	0.1
Rosy/Floral	0.4	0.5	0.5	0.3
Salty	4.9	4.9	4.8	4.6
Sour	3.2	3.1	3.2	2.9
Sulfur	0.3	0.4	0.3	0.2
Sweet	0.5	0.6	0.4	0.7
Umami	1.3	1.1	1.2	1.3
Whey	0.1	0.1	0.1	0.1

[a-c]Means within a row with the same letter were not significantly different ($\alpha = 0.05$).

[1]ND = Not detected.

taste may have overwhelmed other flavors, making accurate flavor determination impossible. It is also possible that the adjunct usage level was too low to produce a noticeable difference, or that higher storage temperatures or longer storage times were needed, but the unresolved bitterness from the starter culture negated any adjunct culture flavor contribution.

When data was analyzed as 8 culture combinations (Table 17)—4 from the Rep 1, and the same 4 with the de-bittering culture from Rep 2--significance was only found for several of the descriptors between de-bittering treatments (with and without LL-011). Strangely enough, while the addition of LL-011 decreased bitterness in the *Propinibacteriaum* cheese and the control, it actually increased bitterness for the cheeses with *Leuconostoc* and *Brevibacterium*. Generally, lactone, rosy, sour, umami, and buttery perception decreased while nuttiness, rancidity, and cooked increased except in the control. Though these were statistically significant differences, actual differences were less than 1 point on the 15-point scale. This may be considered a negligible difference in terms of practical application. Unfortunately, no other sensory assessment obtained could confirm or refute this idea as the informal preliminary panel mostly noticed the bitterness and no affective testing was performed. Given the experiences had by the preliminary judges and by the USU Descriptive Cheese Panel, it is likely that any difference in flavor imparted by the adjuncts would be unnoticed by a consumer panel or by any person due to the extremity of the bitter taste. This defect must first be overcome before the effect of the adjunct cultures may be adequately assessed.

Table 17. Eight sample analysis of culture: Summary of significant mean-scores

	Cheese sample							
Attribute	Propioni	Propioni w/LL-012	Leuco-nostoc	Leuco-nostoc w/LL-012	Brevi-bacterium	Brevi-bacterium w/LL-012	Starter Only	Starter Only w/LL-012
Bitter	3.8^{bc}	2.8^{de}	3.6^{bcd}	4.7^{a}	4.4^{ab}	5.1^{a}	3.5^{cd}	2.6^{e}
Brothy	1.6	2.0	1.5	1.9	1.4	1.8	1.3	1.7
Cooked	0.3^{bc}	0.3^{abc}	0.4^{abc}	0.5^{a}	0.2^{c}	0.5^{ab}	0.4^{abc}	0.2^{c}
Fruity	ND[1]	ND	ND	ND	ND	ND	ND	ND
Lactone	2.3^{ab}	1.7^{c}	2.0^{bc}	1.9^{bc}	2.2^{abc}	1.9^{bc}	2.4^{a}	1.8^{bc}
Metallic	ND	ND	ND	ND	ND	0.4	ND	ND
Nutty	0.1^{c}	0.4^{ab}	0.3^{bc}	0.3^{bc}	0.2^{bc}	0.6^{a}	0.3^{bc}	0.3^{bc}
Oxidized	ND	0.3	ND	ND	ND	0.2	ND	0.3
Pine-appley	ND	ND	ND	ND	ND	ND	ND	0.2
Fishy	ND	ND	ND	ND	ND	ND	ND	ND
Rancid	0.1^{b}	0.4^{ab}	ND^{b}	0.6^{a}	ND^{b}	0.4^{ab}	0.1^{b}	ND^{b}
Rosy	0.6^{ab}	0.1^{c}	0.7^{ab}	0.2^{c}	0.8^{a}	0.2^{c}	0.4^{bc}	0.2^{c}
Salty	5.5	4.2	5.1	4.7	4.9	4.6	4.9	4.3
Sour	3.8^{a}	2.5^{c}	3.2^{abc}	2.9^{bc}	3.4^{ab}	2.9^{bc}	3.2^{abc}	2.6^{c}
Sulfur	0.3	0.2	0.1	0.6	0.3	0.3	0.3	0.2
Sweet	0.4	0.7	0.6	0.7	0.3	0.5	0.4	1.0
Umami	1.6^{a}	0.9^{cd}	1.3^{abc}	0.8^{d}	1.3^{abc}	1.1^{bcd}	1.4^{ab}	1.1^{bcd}
Whey	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2
Buttery	0.6^{abc}	0.3^{cd}	0.4^{bcd}	0.4^{bcd}	0.7^{ab}	0.4^{bcd}	0.8^{a}	0.1^{d}
Burnt	2.0	1.8	1.8	2.1	2.0	1.7	1.9	1.9

[a-e]Means within a row with the same letter were not significantly different ($\alpha = 0.05$).

[1]ND = Not detected.

Table 18. Eight sample analysis of sodium gluconate: Summary of significant mean-scores

	Cheese sample	
Attribute	0.8% Sodium gluconate	0% Sodium gluconate
Bitter	3.7	3.9
Brothy	1.7[a]	1.5[b]
Cooked	0.4	0.3
Fruity	ND[1]	ND
Lactone/Fatty Acid	2.0	2.0
Metallic	0.1	ND
Nutty	0.3	0.3
Oxidized	0.1	0.1
Pineappley	ND	ND
Fishy	ND	ND
Rancid	0.2	0.1
Rosy/Floral	0.4	0.5
Salty	4.9	4.7
Sour	3.3[a]	2.9[b]
Sulfur	0.3	0.2
Sweet	0.5	0.6
Umami	1.2	1.2
Whey	0.1	0.1
Buttery	0.5[a]	0.4[b]
Burnt	1.8	1.9

[ab]Means within a column with the same letter were not significantly different ($\alpha = 0.05$).
[1]ND = Not detected.

Significance was observed on account of the NaGlu treatment in 3 attributes (Table 18). Brothy, buttery and sour were greater in the cheese with NaGlu. This demonstrates a good trend, but another analysis should be performed with a different starter culture. Possibly the reason for not noticing a greater significance is, again, the overwhelming nature of the bitterness.

CONCLUSION

Bitterness not eliminated by the starter culture overwhelmed the experiment. The de-bittering culture did improve flavor somewhat in this cheese system in this plant environment. Flavors contributed by the adjunct cultures were difficult to distinguish and not in the direction desired. Bitterness was still too high to be acceptable even with the de-bittering culture. It is the opinion of the author to recommend no further pursuit on these adjuncts used with the LL-50 starter in this particular cheese system.

CHAPTER 5

GENERAL SUMMARY

There was some improvement in flavor due to the adjunct cultures used in this project, though the effect was not different from the flavor imparted by the starter culture and was not sufficient to recommend the use of these adjunct cultures in this cheese formula in addition to the starter culture. However, it has been demonstrated that the overall flavor of low-fat Cheddar cheese with *Lactobacillus* and *Lactococcus* adjuncts (CR540) is as acceptable as reduced-fat Cheddar cheese. This market should be pursued and may expand the users of this product to those who have formerly eliminated Cheddar cheese from their diet.

The positive effect from CR540 and other *Lactobacillus* and *Lactococcus* adjuncts seems to be enhanced by the use of sodium gluconate. Sodium gluconate also increased certain desirable flavor attributes when used with *Propionibacterium, Brevibacterium,* and *Leuconostoc*; however, no acceptable cheese in terms of overall flavor was produced from these adjunct cultures probably due to starter culture and the consequent bitterness. The mechanism through which sodium gluconate minimizes poor flavors in low-fat cheese is yet to be determined.

Elevated ripening temperatures resulted in off-flavor development when used with the *Lactobacillus* and *Lactococcus* adjuncts in this low-fat Cheddar cheese.

Despite the low level of flavor development, there still were trends toward flavor improvement due to the adjunct culture CR540. Potential exists for adjunct cultures in low-fat Cheddar cheese. Perhaps more focus needs to be placed on strains specifically

for low-fat Cheddar cheese that may have no application in full-fat cheese, or strains that have no traditional application in cheese. Sodium gluconate addition in low-fat Cheddar cheese should continue to be pursued.

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APPENDICES

APPENDIX A. TABLES OF ATTRIBUTE REFERENCES USED IN THE TRAINING OF THE UTAH STATE UNIVERSITY DESCRIPTIVE CHEESE PANEL

Table 19. Tastes used in aqueous solutions for descriptive panel training. Each concentration is given an expected value on a 15-point scale

Taste	Definition	Concentration of reference	Scale
Bitter	Basic taste brought out by caffeine	0.05% caffeine	2
		0.08% caffeine	5
		0.15% caffeine	10
Sour	Basic taste brought out by acids	0.05% citric acid	2
		0.08% citric acid	5
		0.15% citric acid	10
Salty	Basic taste brought out by salts	0.2% NaCl	2.5
		0.35% NaCl	5
		0.5% NaCl	8.5
Sweet	Basic tastes brought out by sugars	2% sucrose	2
		5% sucrose	5
		10% sucrose	10
Umami	Basic taste brought out by monosodium glutamate (MSG)	0.7% MSG	5
		1.4% MSG	9
		2.8% MSG	13

(Meilgaard et al., 2007; Curtis Maughan, private communication, 2011)

Table 20. Attribute references used by the Utah State University Descriptive Cheese Panel; references evaluated in skim milk

Attribute	Reference
Brothy	canned chicken or potato broth, room temp
Burnt	low-fat cheese with blackened sugar flavor
Cooked	skim milk heated to 85-90 deg C for at least 30 min
Diacetyl/Buttery	Unsalted butter in skim
Fishy	fish oil supplement
Fruity	Kroger fruit cocktail; 1/2 apple juice concentrate, 1/2 peach/white grape juice
Lactone/Fatty Acid	Store-bought heavy cream; whole milk
Metallic	Ferrous sulfate, 0.08g / 1000ml water
Nutty	walnuts & hazelnuts; smell wheat germ and wheat thins
Oxidized	0.2 ml 1% copper sulfate soln to 1 qt past. Non-homogenized milk, let sit 48 hrs
Pineappley	chilled canned pineapple chunks; pineapple juice concentrate
Rancid	0.1% butyric acid
Rosy/Floral	0.0215g 2-phenethylamine to 1073.99g skim
Sulfur	8 x 10^-7 or 0.8 ppm H2S in water; poached egg yolk & corresponding filtered solution
Whey	Fresh Cheddar cheese whey

APPENDIX B. OBJECTIVE 1 EXTENDED TABLES & FIGURES

Table 21. Composition of all low-fat Cheddar cheeses made with *Lactococcus* and *Lactobacillus* adjunct cultures

Adjunct code	Rep	Sodium gluconate content (%)	pH	Fat content (%)	Moisture content (%)	Salt content (%)
Control	1	0	5.13	6.0	51.82	1.85
Control	1	0.8	5.14	5.5	53.43	1.96
Control	2	0	5.22	5.5	54.61	1.92
Control	2	0.8	5.32	5.0	55.30	2.03
LH32	1	0	4.99	5.5	53.79	1.87
LH32	1	0.8	5.02	5.5	55.59	1.88
LH32	2	0	5.24	4.0	52.25	1.84
LH32	2	0.8	5.23	4.0	53.64	2.16
CRL431	1	0	5.12	5.5	53.12	1.80
CRL431	1	0.8	5.14	5.5	54.58	1.96
CRL431	2	0	5.19	5.0	52.09	2.27
CRL431	2	0.8	5.19	5.0	53.55	2.28
CR540	1	0	5.05	5.5	52.82	1.87
CR540	1	0.8	5.13	5.5	54.28	1.97
CR540	2	0	5.35	4.0	53.78	1.92
CR540	2	0.8	5.43	4.0	55.01	2.01
Emfour	1	0	5.14	5.8	52.23	1.83
Emfour	1	0.8	5.14	5.5	54.31	1.95
Emfour	2	0	5.07	3.5	52.44	1.81
Emfour	2	0.8	5.09	4.0	54.27	1.93
LH32/CR319	1	0	5.12	5.5	53.12	1.80
LH32/CR319	1	0.8	5.14	5.5	54.58	1.96
LH32/CR319	2	0	5.18	4.0	54.92	1.99
LH32/CR319	2	0.8	5.28	5.0	55.77	2.05
Mean			5.17	5.0	53.80	1.95
Std. Dev.			0.1032	0.7413	1.1480	0.1316

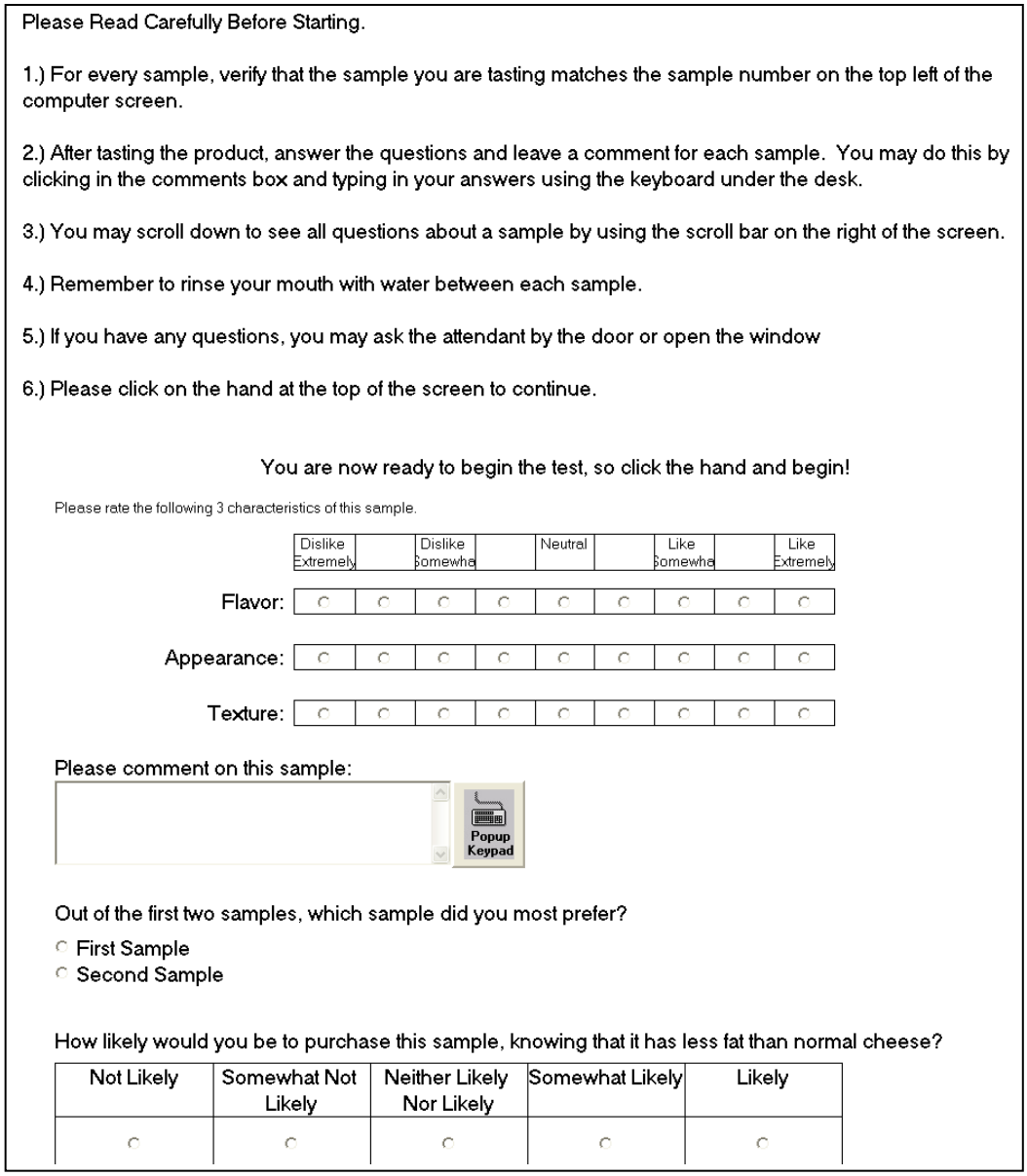
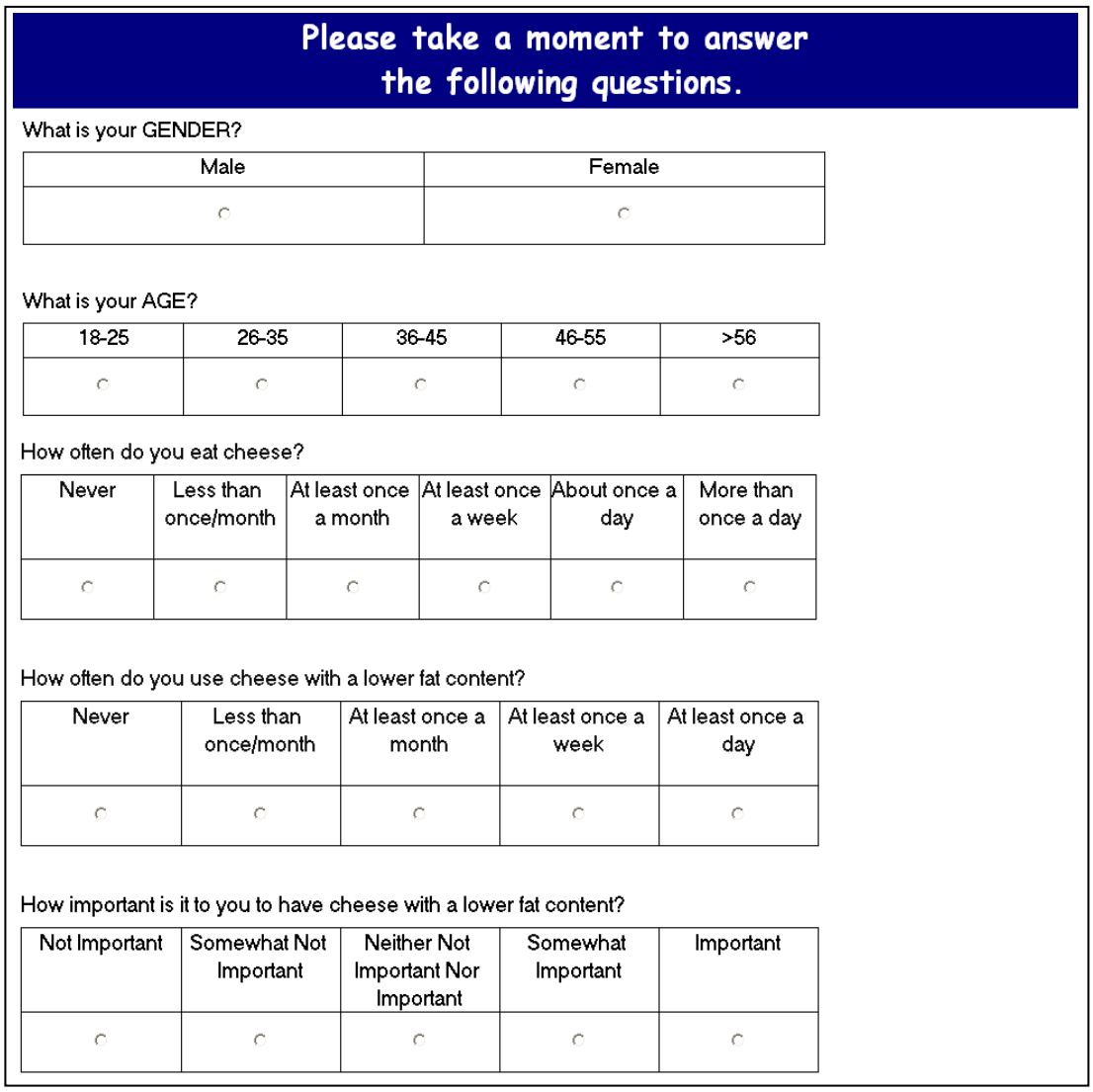


Figure 5. First half of consumer panel questionnaire. This involved evaluating the samples.



Please take a moment to answer the following questions.

What is your GENDER?

Male	Female
○	○

What is your AGE?

18-25	26-35	36-45	46-55	>56
○	○	○	○	○

How often do you eat cheese?

Never	Less than once/month	At least once a month	At least once a week	About once a day	More than once a day
○	○	○	○	○	○

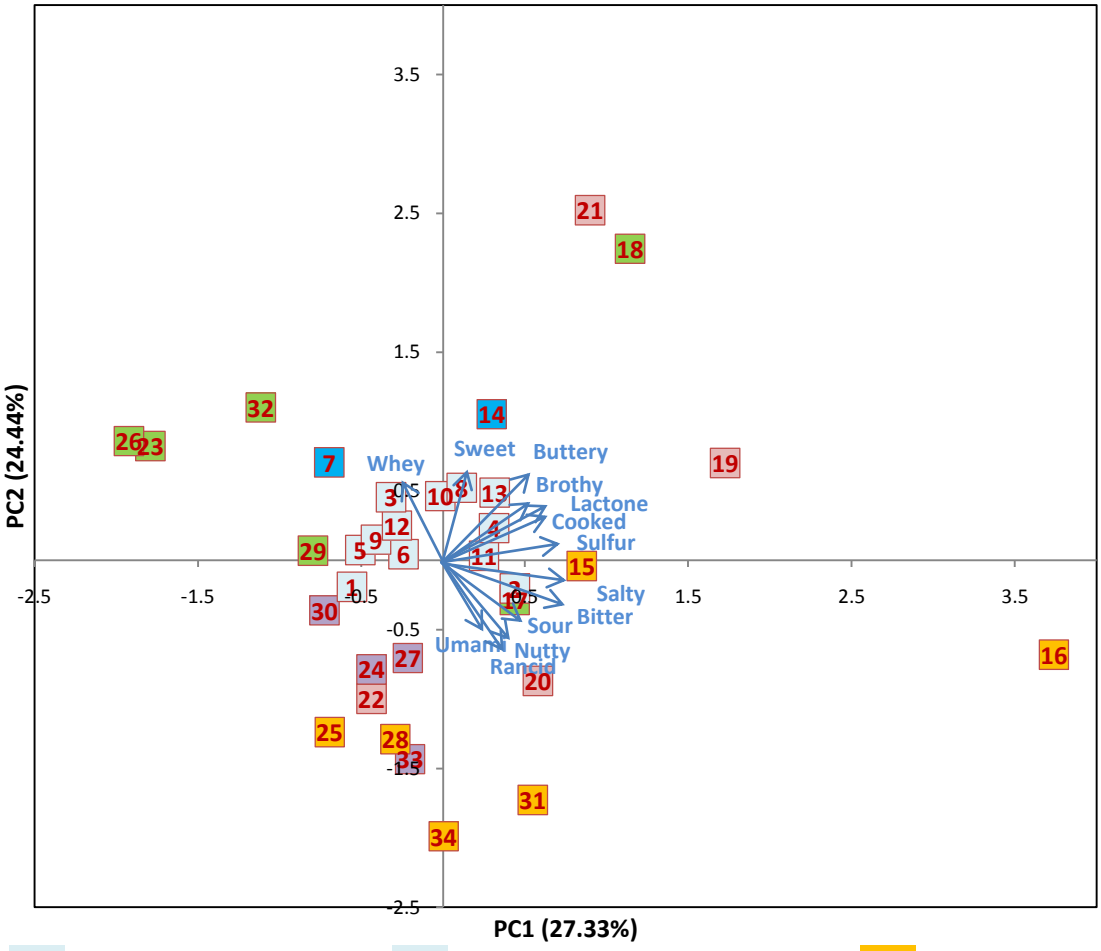
How often do you use cheese with a lower fat content?

Never	Less than once/month	At least once a month	At least once a week	At least once a day
○	○	○	○	○

How important is it to you to have cheese with a lower fat content?

Not Important	Somewhat Not Important	Neither Not Important Nor Important	Somewhat Important	Important
○	○	○	○	○

Figure 6. Second half of consumer panel questionnaire. This involved demographic questions.



1	Control 0% 6°C	13	Consumer Panel CR540 0% 6°C	25	White Pine Sharp
2	Control 0% 10°C	14	Consumer Panel CR540 NaGlu 6°C	26	Kraft Mild
3	Control NaGlu 6°C	15	Consumer Panel CV Sharp	27	Kraft Med
4	Control NaGlu 10°C	16	Consumer Panel Gossner Sharp	28	Kraft Sharp
5	CR540 0% 6°C	17	Consumer PanelCV Mild	29	Gossner Mild
6	CR540 0% 10°C	18	Consumer Panel Gossner Mild	30	Gossner Med
7	CR540 NaGlu 6°C	19	Kraft 2% Mild2	31	Gossner Sharp
8	CR540 NaGlu 10°C	20	Kraft 2% Mild 1	32	Old Juniper Mild
9	LH32 NaGlu 6°C	21	Great Value 2% Mild2	33	Old Juniper Med
10	CRL431 NaGlu 6°C	22	Great Value 2% Mild 1	34	Old Juniper Sharp
11	Emfour NaGlu 6°C	23	White Pine Mild		
12	CR319 & LH32 NaGlu 6°C	24	White Pine Med		

Figure 7. Labeled principal component analysis of low-fat, reduced-fat, and full-fat commercial and experimental cheese, replicates combined. Squares: ■ (turquoise) = CR540 low-fat cheese; ■ (sky blue) = low-fat cheese; ■ (green) = commercial full-fat mild; ■ (red) = commercial reduced-fat mild; ■ (purple) = commercial full-fat medium; ■ (orange) = commercial full-fat sharp. Blue arrows: attribute trends.

APPENDIX C. OBJECTIVE 2 EXTENDED TABLES

Table 22. Composition of all low-fat Cheddar cheeses made with non-Cheddar type adjunct cultures

Culture Set	NaGlu (%)	Rep[1]	% Salt Target	% Salt Actual	% Moisture	% Fat	pH
Control	0.8	1	1.4	1.29	54.19	5.0	5.20
	0.8	2	1.4	1.29	51.06	7.0	5.20
	0	1	1.5	1.32	52.36	5.0	5.17
	0	2	1.5	1.33	50.06	7.5	5.20
	0.8	1	1.9	1.73	53.87	5.0	5.19
	0.8	2	1.9	1.69	50.98	6.5	5.25
	0	1	2.0	1.73	52.15	5.0	5.20
	0	2	2.0	1.68	49.69	7.0	5.20
Propionibacterium	0.8	1	1.4	1.28	54.10	4.0	5.21
	0.8	2	1.4	1.29	52.11	6.5	5.22
	0	1	1.5	1.32	52.49	4.0	5.17
	0	2	1.5	1.28	50.78	6.5	5.17
	0.8	1	1.9	1.67	53.70	4.5	5.20
	0.8	2	1.9	1.64	51.91	6.5	5.20
	0	1	2.0	1.73	52.30	5.0	5.17
	0	2	2.0	1.76	50.63	7.0	5.17
Leuconostoc	0.8	1	1.4	1.31	54.11	5.0	5.21
	0.8	2	1.4	1.29	54.51	5.0	4.88
	0	1	1.5	1.21	52.39	4.5	5.20
	0	2	1.5	1.33	52.81	4.0	4.96
	0.8	1	1.9	1.63	53.65	4.5	5.20
	0.8	2	1.9	1.65	54.40	4.0	5.04
	0	1	2.0	1.65	52.32	4.5	5.18
	0	2	2.0	1.70	52.30	5.0	5.10
Brevibacterium	0.8	1	1.4	1.25	53.69	5.0	5.17
	0.8	2	1.4	1.26	54.31	6.5	5.04
	0	1	1.5	1.31	52.15	5.0	5.13
	0	2	1.5	1.33	52.26	6.5	5.07
	0.8	1	1.9	1.72	53.75	5.0	5.22
	0.8	2	1.9	1.65	53.96	6.3	5.07
	0	1	2.0	1.65	51.76	5.0	5.16
	0	2	2.0	1.70	52.54	6.5	5.08
				Average	52.60	5.4	5.15
				STDEV	1.327	1.051	0.082

[1]Replicate 2 had an additional culture – LL-011 – for debittering.