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# Effect of Bitterness Blockers and Salt Substitutes on the Quality of Low Sodium White Cheddar Cheese

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# EFFECT OF BITTERNESS BLOCKERS AND SALT SUBSTITUTES ON THE QUALITY OF LOW SODIUM WHITE CHEDDAR CHEESE

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Dedicated to my father Mariano Carabantes and the Carabantes and Ordonez families who have given me their trust and support every day since I started this Journey.

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**ABSTRACT**

Reducing added salt in processed foods is one of the major challenges facing food industry worldwide. Salt (NaCl) replacement in a Cheddar cheese has been successfully achieved up to 25%, which qualifies it as "reduced sodium". However, a low sodium cheddar cheese (less than 140 mg Na/ 50 g cheese) requires at least 50% of salt reduction. Bitterness is one obstacle for development of an acceptable low sodium cheddar cheese. In this study, sensory optimization of low sodium white cheddar cheese was performed. A 3-component mixture design was used: NaCl (30-60%), KCl (45-65%) and glycine (5-10%). A fixed level of adenosine monophosphate (AMP, 500 ppm) was incorporated. A CLT consumer test was run with N = 360 consumers following a BIB design [t = 10, k = 3, r = 9, b = 30, $\lambda = 2$, $E = 0.74$]. One of these ten cheddar cheese formulations served as the control (100% NaCl). Consumers evaluated appearance, odor/aroma, taste, saltiness, chewiness, softness, overall texture, and overall liking using a 9-point hedonic scale after 5 months of ripening. Purchase intent was also evaluated. RSM was constructed from the predicted non-intercept regression model for each sensory attribute. Overlay of contour plots ($\geq$5.5 as a cut-off hedonic score) yielded an optimal formulation range, indicating that up to 60% KCl could be incorporated. The optimal formulation (30% NaCl, 60% KCl and 10% glycine) had mean scores over 6.14 for all sensory attributes. The McNemar test revealed that purchase intent significantly increased in 5 of the 9 formulations after consumers had been informed the products were low-sodium. The substitution of NaCl by KCl and glycine generated differences in the type and amounts of volatile compounds produced. Salt substitution also affected fatty acids degradation. Other physicochemical properties affected by the salt replacement were texture, color and $a_w$; however, these changes although significant, did not affect negatively the sensory acceptance of most the cheese formulations with a high amount of substitution. This study demonstrated that low-sodium (at least 50% salt reduction and less than

140 mg Na/g) cheddar cheeses could be successfully produced without compromising sensory characteristics.

## CHAPTER 1. INTRODUCTION

Cheddar cheese is a massively consumed cheese in the United States of America; it is mostly produced at an industrial level which requires the use of a starter culture to standardize the fermentation process during the ripening process. People, who consume dairy foods, have better overall diets with more nutrients, and have improved bone health, compared to nondairy consumers. However, cheese is also perceived as being high in fat and sodium (Singh et al., 2003). The typical content of sodium in a traditional cheddar cheese is approximately 620 mg of Na / 100g of cheese, contributing to a quarter of the daily recommended value for sodium for a young adult which is 2400 mg/ day.

Salt is a substance essential for life processes and the second most used food additive, which works as a flavor enhancing agent and as a food preservative (Reddy and Marth 1991). The problem related with salt consumption that exceeds the recommended daily intake is the nd high blood pressure which leads to possible heart stroke (CDC, 2009; Chovanian et al., 2000).

The salt reduction approach that better reassembles a full salt cheddar cheese is the use of salt substitution with potassium chloride (KCl) which emulates the clean salty taste of table salt, but KCl imparts bitterness that prevents salt reductions above 25%. The bitter aftertaste perceived is regularly associated with the high molecular weight of KCl and the possible increased proteolysis due to changes in the starter culture.

Most of the studies performed to attempt the substitution of sodium chloride with potassium chloride on cheddar cheese, evaluate 25, 50 and 75% of substitution This study evaluateD substitution levels between 45 and 70% of sodium to find the optimal formulation of NaCl, KCl and Glycine using a mixture design to specifically obtain a low sodium cheddar cheese that requires less than 280 mg Na/ 100g of cheese.

The general objective of this thesis was to evaluate the effect of the use of the salt substitute KCl and the bitterness blocker Glycine on the quality of a white cheddar cheese. The specific objective of this thesis were to evaluate the effect of the salt substitution with KCl and Glycine on, sensory properties and acceptance of ten formulations of cheddar cheese and to perform the sensory optimization of those formulations from the mixture design using Response Surface Methodology. Other physicochemical characteristics were evaluated such as volatiles content, fatty acids profile, color, water activity and Texture Profile Analysis over a period of five months.

This thesis divided on to four chapters. The first chapter is a brief introduction and justification. The second chapter is the literature review, followed by the materials and methods section in the third chapter, and the results and discussion section, in the fourth chapter. The fifth chapter is the conclusion section and the proposed future work. After the references section, the appendices include some extra supporting material. The vita of the author is also provided.

## CHAPTER 2. LITERATURE REVIEW

### 2.1 Cheddar cheese

The production of cheese began thousands of years ago in the Middle East (Scott et al., 1998). Hill and Kethireddipalli (2013) stated that cheese making was introduced into Europe during the period of the Roman Empire, being produced either in monasteries or farms, while the industrialized production of cheese began in the nineteenth century in both Europe and America, rising in the 1930s. Many established varieties were defined by national standards of identity such as the Appellation d'Origine Controˆle´es (AOC) system in France, the Denominazione di Origine Controllata (DOC) in Italy, and the international European Community Protected Designation of Origin (PDO) standards.

According with the Codex Alimentarius (1978) Cheddar is a ripened hard cheese in conformity with the *General Standard for Cheese* (CODEX STAN 283-1978). The body has a near white or ivory through to light yellow or orange color and a firm-textured (when pressed by thumb), smooth and waxy texture. Gas holes are absent, but a few openings and splits are acceptable. The cheese is manufactured and sold with or without rind which may be coated.

For Cheddar ready for consumption, the ripening procedure to develop flavor and body characteristics is normally from 5 weeks at 7–15 °C depending on the extent of maturity required. Alternative ripening conditions (including the addition of ripening enhancing enzymes) may be used, to help the cheese to exhibit similar physical, biochemical and sensory properties as those achieved by the previously stated ripening procedure. Cheddar intended for further processing does not need to exhibit the same extent of ripening when justified through technical and/or trade needs (Codex Alimentarius 1978).

According to Wallstra et al. (1999) cheese making requires maximum control of the processing stems that allow the biochemical transformations affecting composition, yield and

quality of the cheese. The essential step in the making of cheese and yogurt is to induce a clotting reaction in milk, mostly involving the casein proteins. In most cheeses including cheddar, the coagulation of milk is brought about by an enzymatic method, i.e. by the addition of Rennet (Hill and Kethireddipalli 2012).

*Lactococcus lactis ssp. Cremoris* and *lactococcus lactis ssp. Lactis* as a blend form the most common mesophilic and homofermentative culture used in many low-temperature cheese varieties including not only cheddar, but also fresh cheese, American Colby types, Dutch varieties, soft-ripened varieties and others.

Based on the type of culture used to prepare the cheddar cheese, for the one that is characterized as a mesophilic unwashed cheese, the same category of provolone cheese, the coagulation is done by rennet and culture, with a 39% - 52% of moisture in nonfat substance controlled by cooking, curd ripening, rate of acid development and salting. The ripening time of cheddar cheese varies from 1 – 24 months. (Simova and Beshkova, 2007; Slattery et al., 2010)

The clotting of the milk is the first step of the cheddar cheese manufacturing. After that comes the removal of the whey following the syneresis, resulting in a loss of 70 to 90% of the original volume of milk (Walstra *et al*., 1999).

Continuing with the standard process of cheddar manufacturing, acid production is achieved due to the "conversion of lactose into lactic acid by lactic acid bacteria" .The resulting pH of the curd and cheese affects such parameters as syneresis, consistency and ripening. The following processes involve salting and "fusion of the curd grains into a coherent matrix that is easy to handle" (Gutierrez, 2004). "Fusion of curd grains and ripening are typical processing steps of ripened cheese; when these are not carried out; the product is referred to as fresh cheese" (Walstra *et al*., 1999).

The final step is the ripening, where several biochemical, chemical and physical processes take place allowing changes in texture, aroma, flavor and appearance. Those biochemical processes transform the relatively tasteless dairy protein in to a tasty easily digestible cheese with a characteristic flavor (Spreer, 1998).

### 2.1.1 Cheddar cheese marketing

The quality of cheese involves many parameters ranging from compositional, functional, sensory and safety aspects to nutritional, psychological, convenience, process and economic factors (O'Riordan and Delahunty, 2003). According to Fox & Cogan (2000) from the point of view of the cheese manufacturer, the quality of product in terms of attractiveness to the consumer is to a large extent influenced by the sensory and functional properties of cheese.

Research of O'Riordan and Delahunty (2003) stated that commercial cheddar cheese production is mostly characterized by a large degree of automation and economies of scale with modern plants having the capacity to produce up to 30, 000 tons of cheese per year.

According to the IDFA (2010), continuing as a trend that began decades ago in the U.S the production of natural cheese (category that includes cheddar) increased by 3.6% in 2010 to 10.4 billion pounds. While cheddar cheese in 2010 accounted for over 75% of all American-type cheese production, the 3.233 billion pounds produced was only 0.8% more than 2009.

The December's Products Sales Report of the USDA (2012) states that cheddar cheese prices received for US 40 pound blocks averaged $1.92 per pound for the week ending December 1, 2012(table 1). The price per pound decreased 7.7 cents from the previous week.

**Table 1**. Cheddar cheese sales of November 2012.

| **United States** | **3-Nov** | **10-Nov** | **17-Nov** | **24-Nov** | **1-Dec** |
|---|---|---|---|---|---|
| **Weighted Price** (Dollars per pound) | 2.0648 | 2.0783 | 2.0557 | 1.9965 | 1.9197 |
| **Sales** (Pounds) | 12,165,856 | 10,903,254 | 12,119,105 | 11,072,707 | 12,890,602 |

According to Watson (2012), The National Dairy council stated that manufacturers such as “Sargento” had developed reduced sodium cheddar cheeses (25% reduction), the NDC also stated that “Consumer acceptance of these products will be important for future reduction efforts”. These message shows that actual “low sodium” cheddar cheese is not yet available on the market. “Potassium chloride currently costs 90 cents per pound vs. 10 cents per pound of salt. Other potential replacement salts are even more expensive and have less desirable functional characteristics” Watson (2012). This information reveals that the right approach for salt reduction in cheese is still substitution with KCl over other substitutes, although it is still more expensive and imparts undesirable bitterness perception.

**2.2 Table salt**

Salt, a substance essential for life processes, is the second most-used food additive. I is used as a flavoring or flavor enhancing agent, a preservative, or an ingredient for desired functional properties in certain foods (Reddy and Marth, 1991). Food grade salt is a crystalline product consisting predominantly of sodium chloride (NaCl) in amounts higher than 97% on a dry basis (Codex Alimentarius, 1985). The non NaCl remainder should be natural secondary products, which are present in varying amounts depending on the origin and the method of production of the salt, and which are composed mainly of “ calcium, potassium, magnesium and sodium sulphates, carbonates, bromides, and of calcium, potassium, and magnesium chlorides as well”. Natural contaminants can also be present in amounts varying with the origin and the method of production of the salt. Copper (expressed as Cu) shall not exceed 2 mg per portion (Codex Alimentarius, 1985).

Sodium chloride is a chemical compound composed of cationic sodium ($Na^+$) and anionic chloride ($Cl^-$). For every gram of salt, 39.3% is sodium ($Na^+$) and 60.7% is chlorine ($Cl^-$). The

chemical properties NaCl include: atomic weight ($Na^+$) of 22. 989 and an atomic weight ($Cl^-$) of 35.4527 for each of its anions, specific gravity of 2.1 -2.6, eutectic composition of 23.31% NaCl, freezing point of eutectic mixture of -21.12 ℃ (-5.016 °F), and a neutral pH of aqueous solution.

The physical properties of NaCl include: Isometric, cubic crystal form; orthorhombic crystal system with a perfect cleavage at all sides, clear to white color, a refraction index of 1.5442, melting point of 1,465 ℃ (2,669 °F), boiling point of 1,465°C (2,669° F), hardness (Moh's Scale) of 2.5 and a critical humidity at 20 °C (68°F) of 75.3% (The Salt Institute, 2012).

Salt is produced from a number of sources worldwide; these include sea water, deep wells (natural brine, or wet-mined salt) and salt rocks (Amr and Jabay 2004). According to Mannar and Dunn (1995) most of the salt produced in Europe and the U.S comes from mining while solar evaporation is widely use in Asia, Africa, Australia and Latin America. Statistical analyses suggest that 25-50% of the salt intake of Western populations is derived from the discretionary use of cooking and table salt (James et al., 1987).

Salt Institute, 2012).

### 2.2.1 High Blood pressure and Sodium Chloride

According to the National Health and Nutrition Examination Survey or NHANES (1995), 50 million or more Americans have high blood pressure (HBP) requiring some forms of treatments. Also the World Health Report (2002) stated that more than 1 billion individuals had hypertension around the world and approximately 7.1 million deaths per year were due to hypertension.

According to the Center for Disease Control and Prevention (2009), current dietary guidelines for Americans recommend that the general population should consume no more than 2,300 mg of sodium per day (about 1 teaspoon of table salt). Blood pressure can respond to lower sodium intake within weeks. The first aspect to counter attack hypertension is awareness,

and as Chobanian (2003) says, important success has been achieved in the past, in terms meeting the goals of the program. The awareness of hypertension has improved from a level of 51% of Americans from 1980 to 70% in 2000. This corresponds to the reduction in mortality rates due to stroke (Thom, 2000) shown in figure 1.

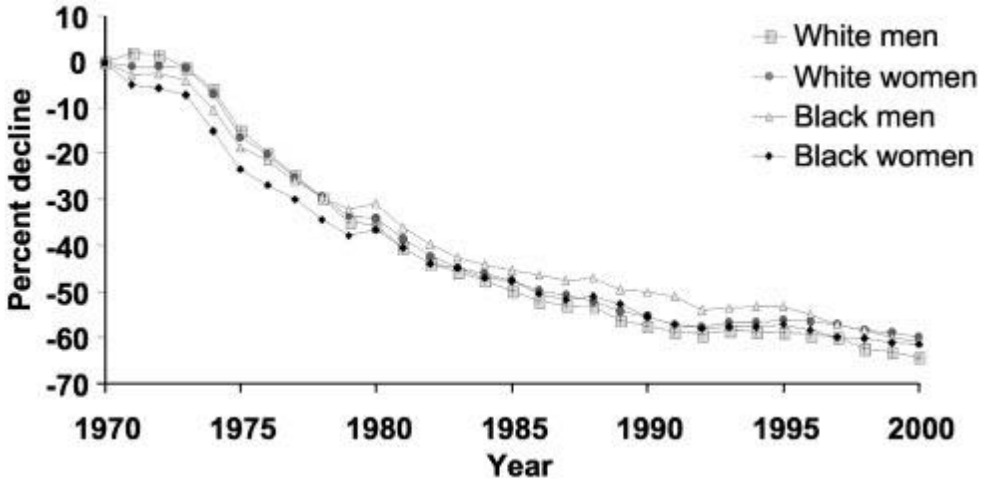



**Figure 1.** Percentile of reduction in mortality rates due to stroke by population type in the US, from 1970 to 2000 (Thom, 2000).

People who have high blood pressure, African Americans, and people older than age 40 are in population groups that should consume no more than 1,500 mg/day. This represents about 70 percent of American adults (CDC, 2009). The prevention and management of hypertension are major public health challenges for the United States (Chobanian, 2003).

According to Sacks et al. (2001) and Chovanian et al. (2000), the most important aspects in lifestyle modifications are the changes in diet so-called "dietary approaches to stop hypertension" (DASH), which includes fruits, vegetables, and low-fat dairy products; it is rich in potassium and calcium content, and the dietary sodium should be reduced to no more than 100mmol per day equivalent to 2.4 g of sodium (Vollmer et al, 2001). Other preventive suggestions include the engagement in regular aerobic activity such as walking at least 30 minutes per day, while reducing alcohol drinking to a minimum (Chobanian et al., 2000).

The lifestyle modifications reduce blood pressure, prevent or delay incidence of hypertension, enhance antihypertensive drug efficacy, and decrease cardiovascular risk. For example, in certain individuals, a 1600 mg of sodium DASH eating plan has blood pressure effects similar to single drug therapy (Sacks et al., 2001).

Reducing sodium intake to 2400 mg can reduce the systolic blood pressure up to 8 mm Hg (Vollmer et al., 2001), which is comparable to the reductions obtained with weigh reduction (He et al., 1997) and sustained physical activity (Whelton et al., 2002). According to Meneely and Battarbee (1976) low sodium diets are therapeutically effective but generally regarded as an impossible or an unnecessary "nuisance". Effective prevention programs must be instituted at as early an age as possible.

The efficacy of a prophylactic/therapeutic low sodium-high potassium diet should be weighed against the uncertain hazards of a lifetime of pill taking. According to Kaplan (2000) and Guiney (2004), in certain developing countries, the amount of sodium ingested by the population can be 10 to 35 times higher than the recommended valued. Reducing salt contents in food has evolved to a trend (Reddy and Marth, 1991). The challenge begins in the practice, since cutting salt from foods negatively affects the characteristics of cheeses and most of other foods (Johnson et al., 2009). Dairy products supply about 11% of the total sodium in the American diet (Demott, 1985)

**2.3 Potassium Chloride**

Many studies describe the replacement of sodium content in cheeses by substituting NaCl with KCl (Fitzgerald and Buckley, 1985; Zorrilla and Rubiolo, 1994; Katsiari et al., 1998; Sihufe et al., 2006; Ayyash et al., 2011), obtaining positive results with minor substitutions (up to 25% of the total amount of salt). According to Lemann et al. (1993), potassium excess over the

recommended dietary intake does not have a negative effect on people with "Na-induced hypertension".

A review of Fletcher (2008) stated that potassium chloride (KCl) can maintain the salty taste of NaCl in foods up to 25% without losses in "palatability"; nevertheless, a residual bitter taste was attributed to the excessive addition of KCl, that consumers may qualify as "unsuitable". According to Gomez et al. (2011), the use of salt substitutes such as KCl is primarily limited nonsalty flavors, specially bitter flavor.

KCl is suitable for a mixture with NaCl, as Breslin (1996) stated that when two flavor elements mix, interference occurs in taste receptor cells or in "taste transduction mechanisms". The bitter elements and the sodium chloride salt often interact, partially removing the bitter taste and enabling the salty taste.

Since long time ago Frank and Mickelsen (1969) suggested that potassium chloride (KCl) is a potentially sodium-free alternative for salt and a common ingredient in salt substitutes. The KCl physical properties make it the best substitute for salt, almost as an "ideal" substitute. The metallic or bitter aftertaste is attributed to the higher molecular weight of cations ($K^+$), which is more evident when large amounts are used (Frank and Mickelson, 1969).

### 2.3.1 Properties of Potassium Chloride

Potassium chloride is composed of 47.55% of the anionic chloride ($Cl^-$) and 52.45% of the cationic potassium ($K^+$), It is very similar to NaCl in appearance to it since both are colorless, transparent cubic crystals with similar refractive indices and resembling in particle sizes (Waimaleongora-Ek, 2006).

KCl is soluble in water and slightly soluble in ethanol. At 25 °C its solubility in water is 35.7 g/100 ml, and solubility in alcohol is 0.25 g / 100 ml. It is insoluble in ether, having an approximate pH of 7, without odor and a melting point of 773 °C. It is arranged as crystals or

crystalline powder or white granular powder or colorless crystals, with saltine taste and odorless, and it has a density of 1.98 (IPCF, 2011)

The most abundant use of potassium chloride is as a fertilizer manufacturing ingredient, since potassium is considered a macronutrient and a limiting factor on plant growth alongside with Nitrogenous and Phosphorous. KCl is found naturally as sylvite, and it can be extracted from sylvinite. It is also extracted from salt water and can be manufactured by crystallization from solution or electrostatic separation from suitable minerals. It is a sub-product in the manufacturing of nitric acid from potassium nitrate and hydrochloric acid (Lorient et al., 1999).

### 2.3.2 Health effects of potassium chloride

According to the USDA (2013), the DRV for potassium is 3500 on a 2000 daily calorie intake. Fruits and vegetables are major sources of dietary potassium. A high potassium diet has been proven to have multiple health benefits including protective role on bone and the $Ca^{+}$ economy preventing osteoporosis and hypertension (Rafferty et al., 2005).

The reduction of sodium in a cheese can help reduce hypertension. According to Ascherio et al., (1998) the potassium intake among US men ranges between 2400 and 2800 mg per day, which is below the recommended values of the USDA. The substitution of salt by potassium chloride in cheese can help supplement that lack of potassium in the diet. Studies of MacGregor (1982) showed that moderate supplementation with potassium (60 mmol/day ingested in tablets) reduced blood pressure by 4% compared with placebo by the 4th week. MacGregor (1982) also emphasizeD that the increase ion potassium intake could be achieved with potassium – based salt substitute and a moderate increase in vegetable and fruit consumption.

Supporting these theories, research from Hiroyasu et al. (1999) concluded that low calcium intake and perhaps low potassium intake, may contribute to increased risk of ischemic stroke in middle –aged American Women.

The supplementation or addition of potassium chloride in foods should be monitored closely and with caution in the elderly, in those with uremia and people under diuretic treatment (Lawson, 1974). Krishna (1990) stated that potassium supplementation lowers blood pressure in hypertensive patients ingesting normal amounts of sodium. Blacks seem more sensitive to the hypotensive effects of potassium, and potassium "depletion" induces sodium retention; however, the hypertensive effects of potassium depletion and hypotensive effects of potassium supplementation are not observed when sodium intake is kept low.

According to Langford (1983), the strong geographic and social class differences in blood pressure may be related to differences in potassium intake or in the ratio of sodium to potassium intake. "Low salt" populations also have high potassium intake. The populations with the least potassium excision are black individuals, which also correlated with higher episodes of hypertension.

**2.4 KCl and NaCl salt substitute mixtures.**

The combination of NaCl and KCl has been used worldwide to reduce the use of 100% salt for salting and preservation purposes in different foods. Fitzgerald (1985) conducted a study where 25% of NaCl could be replaced without affecting negatively the overall flavor of cheddar cheese. This level of salt reduction showed slight reduction in saltiness with very tolerable bitterness. Most of the studies related to replacing NaCl with KCl, use a ratio system to evaluate those replacements using a control (100% salt), 25, 50 and 75% KCl, but not paying much attention to how much sodium can be reduced while keeping the product acceptable.

Cheddar cheese is not the only cheese being studied with the substitution system mentioned above. There is a wide range of maturated and fresh cheeses in which KCl has been used as a salt substitute. Ayyash et al. (2011) found that up to that level of ¼ of substitution, KCl was effective keeping the texture profile similar to the control, while no changes in microstructure among treatments were found up to 50% of substitution.

The lipolysis of feta cheese during storage time was evaluated by Katsiari et al., (2000) showing that the content of free fatty acids was not different among treatments of 25% KCl and NaCl compared against a 100% NaCl control. Katsiari et al. (1997) also suggested that a highly accepted Feta cheese can be done with a 1:1 mixture of NaCl and KCl (feta cheese is a high sodium cheese).

According Breslin and Beauchamp (1995) the degree of average bitterness suppression of KCl was 78% in the presence of high concentrations of NaCl, This theory helps clarify why 50% reduction is more suitable in feta cheese than in cheddar since the latter has much less salt than the former one which is also more humid, allowing the interaction of the ions in solution.

**2.5 Glycine**

Glycine (Gly) is an organic compound categorized as an amino acid, commonly found in proteins. Its chemical formula is $NH_2CH_2COOH$; it is a colorless, sweet-tasting crystalline solid, is the only non-chiral protein genic amino acid, and can be either hydrophilic and hydrophobic since it has a small side chain of only one hydrogen atom (Merk, 1989). The use of glycine as a bitterness blocker has been found mostly in the meat industry (Gou et al., 1996). In combination with KCl, it does not show differences in microbial stability but affecs the sensory properties when the 40% salt substitution mark was reached, and glycine was used up to 30% in some treatments (Gelabert et al., 2003). Taste interactions such as suppression effect, which occurs between bitterness and sweetness can be detected and quantified using a “taste sensor”. Peptides

and amino acids may be classified into several groups according to their own tastes from sensor outputs (Figure 2). For example, bitter-tasting amino acids such as L-tryptophan have response electric patterns similar to a typical bitter substance, quinine. On the other hand Glycine is merely associated with sweetness by itself (Kiyoshi, 2000).

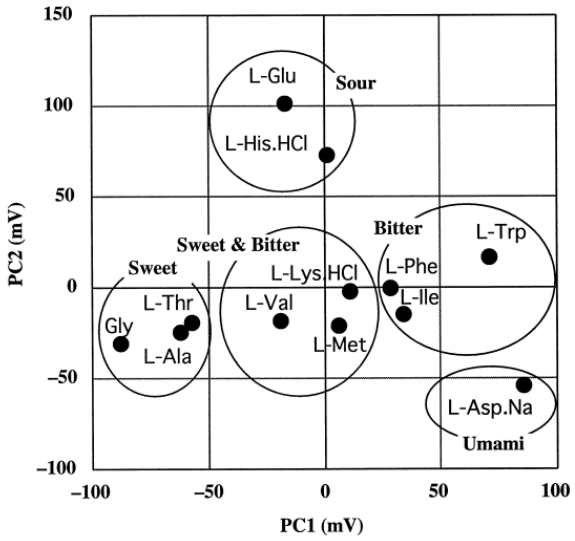



**Figure 2.** Discrimination of taste of amino acids using a lipid multichannel electrode taste sensor (Kiyoshi, 2000).

## CHAPTER 3. MATERIALS AND METHODS

### 3.1 White Cheddar cheese manufacturing

The cheese was manufactured in the dairy processing plant at Louisiana State University, in Baton Rouge, Louisiana. 200 gallons of milk were obtained at the LSU's dairy farm in individual 10 gallon cans, previously washed and sanitized.

Milk was obtained in two separate dates (100 gallons in each date) to manufacture separate batches of cheese. The first batch was fabricated on January 12, 2012 while the second batch was fabricated on January 15, 2012. Ten cheddar cheese treatments were manufactured using nine salt substitute formulations and a control with 100% NaCl (See Table 2 for reference).

The cheese manufacturing procedure was conducted following the methods of Gutierrez (2004). For each batch the milk was stored overnight below 40°F (4.5°C) until the next day for the cheese making. The milk was pasteurized using the system LTLT (Low Temperature, Long Time), a batch pasteurization system in which milk is treated at 145 F$^{o}$ for 30 minutes to eliminate pathogens. After pasteurization, the milk was transferred to the vats using a different set of 10 gallon cans only used to transport pasteurized milk. Once the milk was in the vats, it was reheated to 88°F at an estimated ratio of 1°F every two minutes.

After securing the temperature at 88°F the starter culture was added, mesophilic direct vat set (DVS) from Christian Hansen (Milwaukee, WI) was added at 0.00877 % by weight, containing strains of *Lactococcus lactis spp. Cremoris* and *spp. Lactis.* Immediately after the cheese was inoculated, the cheddar cheese color (annatto) was added at 0.005% by weight. After adding both the color and the starter culture the milk's temperature was maintained for 45 min and stirred. The next step was the addition of calcium chloride food-grade ($CaCl_2$ aqueous solution 32%) from DSM (DSM Food Specialties, Menomonee Falls, WI) at 0.02% w/v.

Agitation was continued for 15 minutes prior to the addition of rennet (CHR Hansen, Milwaukee, WI) at 0.01% w/v. The composition of the rennet was: fermentation produced chymosin, Sodium Chloride (NaCl), Sodium Benzoate and caramel color. For a few seconds the milk was stirred to mix with the rennet, and then allowed to settle for 35 minutes to promote the renneting (coagulation process). Once the time was reached and the curd hardness was appropriate, the curd was cut using curd cutters in three orientations to allow the formation of small squares (approximately 2.5 x 2.5 x 2.5 $cm^3$ = 15.625 $cm^3$ ). After completing the cutting process the curd was allowed to heal for 5 minutes without agitation at 88 F. The next step was the cooking or scalding process requiring a continuous heating from 88°F to 102°F at a rate of 3 °F every 5 minutes in agitation. After the desired temperature was reached, it was held for an hour (cooking process) until the curd precipitated completely, then the whey was drained out of the vat.

The curd was cut in loaves to allow better whey draining, and these loaves were stacked and flipped over every 15 minutes for a more uniform whey separation from the curd. All this process was done while keeping the temperature at 88 °F. The parameter to stop this process was tritratable acidity, once the value of 0.45 was reached; the loaves were not flipped anymore. The milling process was done using a knife to achieve a particle size reduction to 1 x 1 x1 inches.
Ten salt formulations were prepared using the salt substitute formulations described in Table 2:

**Table 2.** Salt replacements and salt formulations for cheese salting

| Salt | NaCl % | KCl % | Glycine % |
|---|---|---|---|
| A | 100 | 0 | 0 |
| B | 45 | 50 | 5 |
| C | 41.25 | 51.25 | 7.5 |
| D | 45 | 45 | 10 |
| E | 37.5 | 57.5 | 5 |
| F | 37.5 | 55 | 7.5 |
| G | 37.5 | 52.5 | 10 |
| H | 30 | 65 | 5 |
| I | 33.75 | 58.75 | 7.5 |
| J | 30 | 60 | 10 |

¢ $X^1$ = Sodium Chloride (NaCl), $X^2$ = Potassium Chloride (KCl), $X^3$ = glycine. Letters A to J Correspond to those in Figure 3.
* The control formulation containing 100% NaCl is not part of the mixture design.

### 3.1.1 Mixture experimental design

The three component extreme vertices mixture design (Cornell 1983) was used with three centroids, two of which were varied to explore interior points in the design (Figure 3, points C and I). In this mixture design, three components, NaCl (X1), KCl (X2), Glycine (X3) ,were the variables comprised in the mixture design (Figure 6 and Table 2). Ingredients were varied to allow the assessment of the effects of each ingredient and the interactions on attribute perception and acceptance. These three factors totalized the amount of salt substitute (sum to 100% or 1.0) used in each product, and the rest of the ingredients on the white cheddar cheese products were constant. The variation of the components was made within the following boundaries: NaCl (30% - 45%), KCl (45% - 65%) and Glycine (2% -10%), as seen in Figure 3 and Table 2.

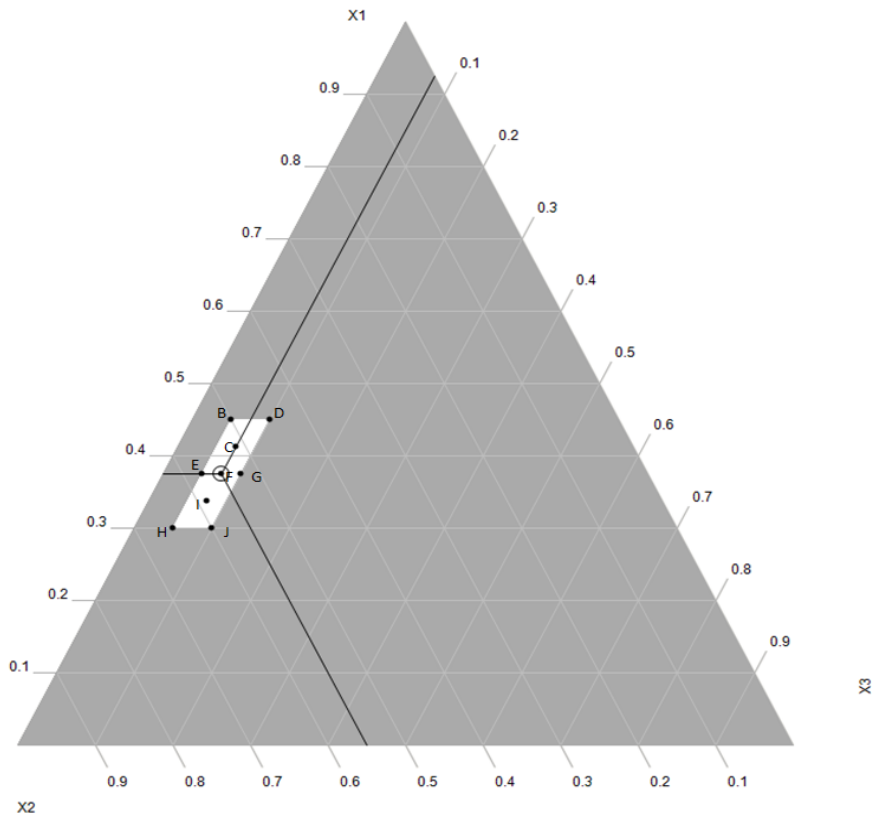



**Figure 3.** The constrained region in the three component coordinate system. X1 = NaCl, X2 = KCl, and X3 = Glycine. Letters within the parallelogram represent the 9 formulations (excluding the control), corresponding to lettering B-J in Table 2.

The amount of total salt or salt substitute used for each treatment was 0.23 % w/w of total milk. Also Adenosine Mono-Phosphate was added at 500 ppm to improve the bitterness blocking power.

After defining the nine formulations that would reduce the amounts of sodium to levels below 140 mg/ 50 g or 280 mg/ 100 g and prior to the samples manufacturing, the formulation with the highest percentage of NaCl (B with 45 % NaCl, 50 % KCl, and 5 % Glycine) was evaluated for sodium content. Inductively Coupled Plasma (ICP) was used to determine if this particular formulation and by consequence the rest of them will meet the requirement for "low sodium" labeling. 2 gal of milk were used to prepare this preliminary study, while the rest of the ingredients and procedures followed the sequence and proportions described on the Cheddar

cheese manufacturing section of the materials and method chapter. The results showed that the cheese prepared with formulation B, had 250.57 ± 17.08 g of Na / 100 g of cheese. These results allowed the further preparation of all formulations.

The salt substitute formulations were added to the curd mixed, and then allowed to rest for 15 min before pressing overnight (12 h.) at 50 psi (cheese clothes were used to contain the curd in the hoops).After pressing, the cheese was removed from the pressing hoops, cut, vacuum packaged, labeled and weighed before storage in the cold room at 3.9 ℃ for ripening during 5 months. Prior to packaging, the cheese blocks were cut into smaller size blocks for individual studies performed as repeated measurements over time (including months 0, 1, 3, and 5).

### 3.2 Analysis

#### 3.2.1 Texture Profile analysis (TPA)

According to Bourne (2002) texture is primarily the response of the tactile senses to physical stimuli that result from contact between some part of the body and the food. The importance of texture in overall acceptability of foods varies widely, depending upon the type of food. Bourne (2002) broke the importance level of texture for foods into three arbitrary groups:

1. Critical. Foods in which texture is a dominant quality characteristic, e.g., meat, potato chips, cornflakes and celery.

2. Important. Texture makes a significant but not a dominant contribution to overall quality, contributing, more or less equally, with flavor and appearance, e.g., most fruits and vegetables, certain cheeses including cheddar, bread, most other cereal-based foods and candy.

3. Minor. Food in which texture makes a negligible contribution to the overall quality; examples are most beverages and thin soups.

The Texture Profile analysis consists of two compression cycles (bites) performed by a "cylindrical specimen" to imitate the grinding action of the jaw. Figure 4 generally illustrates the

texture profile analysis output and the graphical representation of the parameters evaluated. In the "y" axis, the force apply to the food to the probe is measured over the time or deformation (the "x" axis).

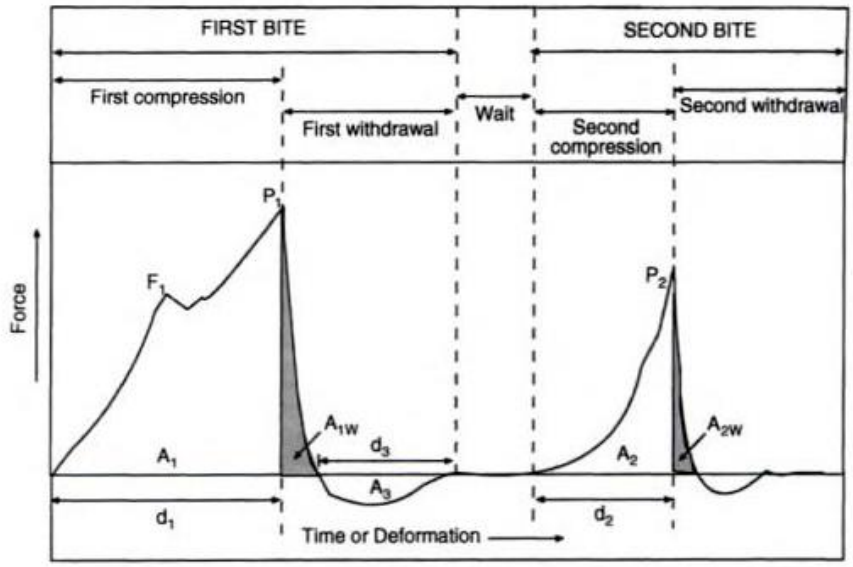



**Figure 4.** Texture Profile analysis output (Bourne, 2002).

The analysis was performed with a texture analyzed Stable Micro Systems model TA.XT.plus (Texture Technologies Corp., New York, USA) with a 2 inches (diameter) compression platen probe. The weight calibration was performed daily with a 2000 g weight standard and the height calibration was set also daily at 47 mm. The set up used in the TPA included: pretest speed of 1 mm/ sec, test speed of 5 mm/sec and posttest speed of 2 mm/sec, the trigger system used "force", and defining 30% of compression deformation. The waiting time between bites (cycles) was 5 seconds.

The cheese samples were cut in cubes of 25 x 25 x 25 mm, vacuum sealed and stored at 4°C overnight to obtain isothermal conditions. The day of the analysis the samples were removed from the refrigerator and immediately analyzed. 3 samples from each duplicate experiment were

analyzed totaling 6 cubes. The parameters evaluated (Table 3) were: hardness, cohesiveness, adhesiveness, gumminess, chewiness, springiness and resilience.

**Table 3.** Texture profile analysis parameter's definition, measurement ant units.

| TPA Term (units) | Definition | Determination |
|---|---|---|
| Hardness (N) | Force necessary to attain a given deformation | Force corresponding to $P_1$ |
| Fracturability (N) | Force at significant break in the curve on the first bite (originally known as "brittleness") | Force corresponding to $F_1$ |
| Cohesiveness (-) | Strength of the internal bonds making up the body of the product | $A_2/A_1$ |
| Adhesiveness (J) | Work necessary to overcome the attractive forces between the surface of the food and surface of other materials with which the food comes to contact | $A_3$ |
| Gumminess (N) | Energy needed to disintegrate a semisolid food until it is ready for swallowing | Hardness*cohesive-ness |
| Chewiness (N) | Energy needed to chew a solid food until it is ready for swallowing | Hardness*cohesive-ness*springiness |
| Springiness (mm) | The distance recovered by the sample during the time between end of first bite and start of second bite (originally known as "elasticity" - rate at which a deformed material goes back to its undeformed condition after the deforming force is removed) | $d_2$ |
| Resilience (-) | Measure of how well a product "fights to regain its original position" | $A_1w/A_1$ |

### 3.2.2 Color

The CIE L*a*b*, or CIELAB, is an approximately uniform color scale. In a uniform, color scale, the differences between points plotted in the color space correspond to visual differences between the colors plotted (Hunter lab, 2009). The CIELAB color space is organized in a cube form, the L* axis runs from top to bottom (Figure 5). The highest value for L* is 100, which represents a perfect reflecting diffuser (white). The lowest value for L* is zero, which

represents black. The a* and b* axes have numerical values from -60 to 60. Positive a* is red. Negative a* is green. Positive b* is yellow. Negative b* is blue (Figure 5).

Samples were cut in cubes (25 x 25 x 25 mm) and then covered with clear cling polyethylene wrap (The Glad Products Company, Oakland, CA) to avoid direct contact of the cheese with the spectrophotometer lens. The color was measured with a handheld spectrophotometer Minolta model CM-508d Series (Osaka, Japan) with a $10^{o}$ standard observer and $D_{65}$ illuminant. The parameters measured were L *, a *, b* (Globalspec, 2011). Prior to each day of analysis the equipment was calibrated with a white standard and blank calibration.

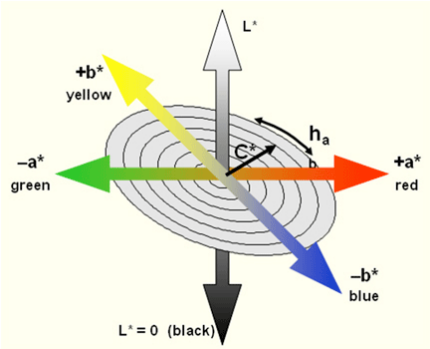



**Figure 5.** Explanatory CIELAB Scale (Globalspec, 2011).

### 3.2.3 Water Activity

Water, as the main component of food and biological materials, plays a predominant role in determining their shape, structure, and physical and chemical properties. It also is a major control component in mass transfer, chemical reactions, and activity of microorganisms (Le Mager, 1986).

Water activity is a measure of how efficiently the water present can take part in a chemical (physical) reaction. If half the water is so tightly bound to a protein molecule that it could not take part in a hydrolysis reaction, the overall water activity would be reduced. Water activity (aw) is defined as: $a_w=p/p_o$., where p and po are the partial pressures of water above the

food and a pure solution under identical conditions respectively (OSU, 2011). Some approximate water activities of foods are given in Table 4 below:

**Table 4.** Water activity ranges for common foods*.

| Water activity range | Food type* |
|---|---|
| 1 - 0.95 | Fresh fruit, meat, milk |
| 0.95 - 9 | cheese |
| 0.9 - 0.85 | Margarine |
| 0.85 - 0.8 | salted meats |
| 0.8 - 0.75 | Jam |
| 0.75 - 0.65 | Nuts |
| 0.65 - 0.60 | Honey |
| 0.5 | Pasta |
| 0.3 | Cookies |
| 0.2 | Dried vegetables, crackers |

*Some examples of food that should be within a $a_w$ range that is not limited to those foods.

The equipment used was the Hygrolab Rotronic 3. Cheese was milled with a knife and filled up to 75% of the volume of 14 mm disposable PS-14 $a_w$ cups. The samples were measured using the standard function of the device which keeps measuring constant values of $a_w$. For each sample $a_w$ was defined at the point where samples were stable in terms of water activity chance in the graph available on the software HW4 (Rotronic, Bassersdorf, Switzerland).

### 3.2.4 Fatty acids profile

In order to measure the fatty acids profile the process involves the extraction and purification of the fat and a derivatization process to convert individual fatty acid compounds into fatty acids methyl esters. The extraction used to obtain the fat from the cheese is described below: 2.5 g of cheddar cheese were mixed with 10 ml of deionized water (0.2 % w/v) and then homogenized manually in a Whirl-Pak bag. Later the entire content was separated and poured into to 4 glass test tubes (2.5 g to each tube).

To each tube, 2 ml of hexane and 1 ml of methanol were added (solvents) to extract the fat from the cheese-water mixture and kept at 60°C for 60 min with agitation in a vortex. The next step was the centrifugation of the tubes at 5000 rpm for 10 min. From each tube, the supernatant was removed (solvents and fat extract) with a glass pipet and mixed in a single, previously weighted tube for each sample. Vacuum was applied during centrifugation to the solvent- fat mix obtained to eliminate the solvents and separate the lipid matrix from the cheese. After finalizing the extraction the tube containing the lipids was weighted in an analytical balance (Fisher Scientific Waltham, MA, USA).

Once the fat was extracted, 50 mg of fat were poured with a glass pipet into a screw cap tube for the derivatization process. To the tube containing the fat, 1 ml of internal standard C17 (200µg/ ml of C17 in hexane) was added, then the tube was capped and sonicated for 2 minutes. The derivatization process included the addition of 2 ml of BC13-methanol and 1 mL 2, 2 dimethoxypropane. (Sigma Aldritch St. Louis, MO, USA). After the tube was capped, samples were heated to 140°C for 30 min allowing the reactions to occur and changing the color of the solution to a dark brown. To stop the derivatization reaction, 1 ml of deionized water was added to each sample and samples were placed on iced water. After the reaction had stopped, 1 ml of hexane was added to extract the fatty acid methyl esters and poured in to a new test tube containing 0.1 g of sodium sulfate ($Na_2SO_4$) at the bottom to remove any possible water. After the fluid made contact with sodium sulfate it was transferred to the GC vials and capped hermetically before storage at -20 °C for future analysis.

The quantification was performed using a gas chromatography Hewlett Packard 580 Series II plus flame ionization detector (FID) and column SP2380, 30 m x 0.25 mm i.d. x 0.25 µm (Supelco, Bellefonate, PA, USA). Helium was used as carrier gas with a flow rate of: 1.2 ml/min. The injection volume was 20uL. The oven temperature was set at 50 °C for the first 3 min. Later, the

temperature was programmed to 250 °C at 4°C/min. All samples used were previously frozen when the ripening process reached a given time (0, 1, 3 or 5 months) for further analysis of fatty acids.

### 3.2.5 Volatile Compounds

The volatiles from the cheese were extracted with the SPME/ head space method, the process is described as follows: To extract the volatiles from the cheese samples, 25g of cheese were milled and placed into a round bottom flask. 250 µl of internal standard (4- methyl –2 pentanone) were added, the flask was capped with a cork and adapted to hold the SPME device. The extraction was held for 30 minutes at 60°C using a water bath.

After the SPME extraction was complete, the GC/MS analysis was set up for the volatiles procedure, using a gas chromatography system (Varian CP-3800 GC, Valnut Creek, CA, USA) with DB-5 column (L 60 m x i.d. 0.25 mm and $d_f$ 0.25 µm thin coating film, Supelco, Bellefonate, PA, USA) and Variance Saturn 2200 with quadruple mass spectrometer (MS). Helium was used as a carrier gas at a flow rate of 1 ml/min. The oven temperature was maintained at 40 °C for 5 min. The temperature was changed to 50 °C at 2.0 °C/min. Later, the temperature was programmed to 200 °C at 10 °C/min. The total time was 30 min. The pressure changes are described as follow: the initial pressure was 0.1 psi and held for 0.2 min. subsequently the pressure was increased. The stable temperatures of the detector and the injector were 250 °C in both cases. The analysis was done from frozen samples.

### 3.2.6 Consumer acceptance test

The sensory quality of the white cheddar cheese was evaluated with a consumer acceptance test. Three hundred and sixty (360) untrained consumers, randomly chosen from Louisiana State University, Baton Rouge Campus, participated in the consumer acceptance test. All the participants had to meet the following criteria: 18 years of age or older, not allergic to

dairy and/or wheat products, and willingness for participation for approximately 7 minutes to complete the survey.

Consumers rated appearance, odor/aroma, taste, saltiness, texture, chewiness softness and overall liking of the product based on the 9-point hedonic scale (1= dislike extremely, 5 = neither like nor dislike, 9= like extremely). Bitterness, off flavor, acceptance, purchase intent, and purchase intent after providing additional information about the sodium reduction of the cheese (low sodium) were evaluated using a binomial (yes/no) scale. Using the Balanced Incomplete Block Design (BIB) plan 11.15 : t = 10, k = 3, r = 9, b = 30, $\lambda = 2$, E = 0.74, Type II. (Cochran 1957), the consumers were presented with 3 out of the 10 cheese formulations. These formulations were randomly coded with a 3 digit number, a total of 108 observations per formulation were evaluated. The consumers were given cubic samples of 1 x 1 x 1cm, placed in lidded transparent 2 oz. containers (Pro Pack, Houston, TX). Room temperature purified water (Nestle Waters, Greenwich, CT) and unsalted saltine crackers (Nabisco, Northfield, IL) were provided for palate cleansing purposes between sample testing. Each costumer received one questionnaire and individual instruction to complete it. Consumers were required read and sign a consent form approved by the Louisiana State University Institutional Review Board.

### 3.2.7 Product optimization

Response Surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It also has important applications in the design, development, and formulation of new products, as well as in the improvement of existing product designs (Myers and Montgomery, 1995).

According to Myers and Montgomery (1995), RSM is mostly applied in industry when particular situations where several “input variables” potentially influence some performance measure of quality characteristic of the product or process. The performance measure is called

response, and most applications will involve more than one response. The input variables are sometimes called independent variables, and are subject to the control of the scientist in an experiment.

The optimization of the white cheddar cheese was done using Response Surface Methodology (RSM) to determine the effect of the salt substitution levels on the response variables and the acceptance of low sodium white cheddar cheese containing salt substitutes and bitterness blockers, Contour graphs were plotted using the prediction models obtained, the graphs represented the combination of the independent factors that were found to affect significantly the acceptance of the formulations. A cutoff value of 5.5 (between neither like nor dislike and like slightly) were selected, meaning that the scores selected within the plots were those equal or greater than 5.5 in a 9 point hedonic scale. The superimposition of the mixture response surface (MRS) plots was used to determine the optimal formulation range. The linear equations without intercept were also generated.

### 3.2.8 Cell recovery an enumeration of *Lactococcus lactis*

Samples of *Lactococcus lactis* frozen at month "0" and month "5" were thawed at 39ºC prior to plating. Samples were sequentially diluted in sterilized peptone water and pour plated (1ml) on M17 agar (0.5% glucose) in duplicate. The plates were incubated anaerobically at 30 ºC for 48 hours. Plates with counts between 25 and 250 colonies were selected to calculate the total colony forming units (CFU) for each treatment at a specific ripening time.

### 3.3 Statistical analysis

For the consumer acceptability test, the analysis performed were one way ANOVA, and Post-Hoc Tukey's Studentized range test ($\alpha = 0.05$) to detect and group the sensory characteristics of the white cheddar cheeses manufactured with the formulations obtained from the mixture design. The MANOVA analysis was done to determine overall differences among

the 10 cheese samples. These analyses were performed using the Statistical Analysis System 9.3 (SAS).

The sensory optimization analysis of the low sodium cheddar cheese formulations was done using the Design Expert Software ® 8.0, the analysis included the generation of the contour profiles for the individual sensory parameters, their respective predictive equations and the superimposition of the contour profiles to determine the optimal formulation range. The analysis of change on purchase intent (the McNemar Test) was performed using both SAS 9.3 and Microsoft Excel.

The physicochemical characteristics of the cheddar cheese treatments were also evaluated using the GLM procedure with one way ANOVA and the Post-Hoc Tukey's Studentized range test ($\alpha = 0.05$) to describe the changes of these characteristics over time for each treatment (months 0, 1, 3 and 5) and comparison of the treatments at a given time. The general null hypothesis ($H_o$) of the study is that no differences existed (for sensory acceptance or physicochemical characteristics) among the treatments defined for the development of the low sodium cheddar cheese. While the alternate hypothesis ($H_a$) is that differences exist (for the same parameters) among the salt substitute treatments.

# CHAPTER 4. RESULTS AND DISCUSSION

## 4.1 Sensory properties of white cheddar cheese

### 4.1.1 Consumer study

The mean score results and their respective standard deviations for appearance, odor/aroma, taste and saltiness are showed in Table 5. The mean scores for texture, softness, chewiness and overall liking are showed in Table 6.

Overall, except for formulation C (41.25% of NaCl, 51.25% of KCl and 7.5 % of Glycine) which had a mean score of 3.79 for taste and 3.84 for overall liking, every formulation, for every attribute had a mean score higher than "4". The analysis of variance showed that, regarding appearance, formulation G was the highest rated treatment ($p < 0.05$). Formulations H (30% of NaCl, 65% of KCl and 5 % of Glycine), I (33.75% of NaCl, 58.75% of KCl and 7.5 % of Glycine), J (30% of NaCl, 60% of KCl and 10% of Glycine) and F (37.5% of NaCl, 55% of KCl and 7.55 % of Glycine) were not significantly different from G (37.5 % NaCl, 52.5% KCl, and 10 % Glycine) but represented the group of formulations that were also not different from the appearance rating of formulation E (significantly lower than G). Formulations B (37.5% of NaCl, 57.5% of KCl and 5 % of Glycine), A (100% of NaCl, 0% of KCl and 0% of Glycine), C (41.25% of NaCl, 51.25% of KCl and 7.5% of Glycine), and D (45% of NaCl, 45% of KCl and 10 % of Glycine) obtained low scores for appearance acceptability ($p< 0.05$).

For the attribute odor, the extreme points of the range of scores where formulation J with a mean score of 6.76 as the highest rated and formulation A was the lowest rated with a mean score of 6.18. The rest of the formulations were not significantly different from one another ($P \geq 0.05$). The highest significant rating ($P < 0.05$) for the attribute taste was the one observed in

formulation J with a mean score of 6.62. Furthermore, formulations F, G, H and I also had scores above 5.5 meaning that they were acceptable when evaluated on a nine point hedonic scale.

Formulation J had also the highest significant rating in saltiness with 6.14, Formulations I H, F ,and G were not significantly different ($P \geq 0.05$) among one another and shared the second highest score for acceptability saltiness rating. The next group included formulations A and E, both of them had saltiness ratings above 5.0 Formulations B and C, were lower rated than 5, tending to be slightly disliked in saltiness.

**Table 5.** Mean Acceptability Scores for Appearance, Odor, Taste and Saltiness.

| Sample | Appearance | Odor | Taste | Saltiness |
|---|---|---|---|---|
| A | $5.98\pm1.62^{def*}$ | $6.18\pm1.56^{c}$ | $4.28\pm2.1^{def}$ | $5.01\pm1.67^{bcd}$ |
| B | $6.42\pm1.56^{cde}$ | $6.49\pm1.46^{abc}$ | $4\pm2.25^{ef}$ | $4.77\pm1.88^{d}$ |
| C | $5.92\pm1.61^{ef}$ | $6.32\pm1.31^{bc}$ | $3.79\pm2.04^{f}$ | $4.72\pm1.76^{d}$ |
| D | $5.68\pm1.87^{f}$ | $6.21\pm1.64^{c}$ | $4.12\pm2.19^{ef}$ | $5.08\pm1.87^{cd}$ |
| E | $6.68\pm1.55^{bc}$ | $6.58\pm1.61^{abc}$ | $4.83\pm2.22^{cde}$ | $5.23\pm1.79^{bcd}$ |
| F | $6.84\pm1.55^{abc}$ | $6.53\pm1.58^{abc}$ | $5.68\pm2.22^{bc}$ | $5.56\pm1.82^{abc}$ |
| G | $7.36\pm1.38^{a}$ | $6.64\pm1.55^{ab}$ | $6.17\pm1.92^{ab}$ | $5.59\pm1.74^{abc}$ |
| H | $7.23\pm1.36^{ab}$ | $6.38\pm1.55^{abc}$ | $5.75\pm1.99^{ab}$ | $5.56\pm1.76^{abc}$ |
| I | $7.04\pm1.54^{abc}$ | $6.45\pm1.54^{abc}$ | $5.84\pm1.96^{ab}$ | $5.66\pm1.72^{ab}$ |
| J | $7.21\pm1.25^{ab}$ | $6.76\pm1.52^{a}$ | $6.62\pm1.72^{a}$ | $6.14\pm1.59^{a}$ |

*Means and standard deviations with the same letters are not significantly different ($P > 0.05$).

Regarding Texture, the formulations with the highest significant rating were G and J with 6.99 and 6.92 respectively; these formulations also have had higher ratings in the previous attributes mentioned (Table 5). Three formulations showed texture acceptability scores lower than "6" including A, C and D, which D being the formulation with the lowest significant score (5.6).

For softness, G was the formulation with the higher score obtaining 6.99, while D was the formulation with the lowest score obtaining 5.93, which was being the only value below 6 (like slightly). Formulations G, H, I were not significantly different from J and all had scores over 6.5. The attribute chewiness showed a tendency: formulations F, G, H, I and J had scores above 6

while the remaining formulations were all below 6. Formulations G and I were significantly higher ($p < 0.05$) than the rest, with mean scores of 6.67 and 6.72 respectively, which showed scores close to the characteristic "like moderately".

Overall liking was one of the most influential attribute of the consumer study. The lowest mean score was 3.84, corresponding to the formulation C. Formulations A, B and D had the mean scores below 5 and were significantly lower ($p < 0.05$) than formulations F, G, H, I and J. From these formulations, J showed the highest score with 6.81 (close to moderate likelihood).

**Table 6.** Mean acceptability scores for Texture, Softness, Chewiness and Overall liking.

| Sample | Texture | Softness | Chewiness | Overall liking |
|---|---|---|---|---|
| A | $5.95\pm1.76^{cd}$ | $6.29\pm1.37^{cbd}$ | $5.92\pm1.68^{bcd}$ | $4.47\pm2.04^{de}$ |
| B | $6.19\pm1.9^{bcd}$ | $6.3\pm1.8^{bcd}$ | $5.75\pm1.94^{cd}$ | $4.19\pm2.14^{de}$ |
| C | $5.64\pm1.78^{d}$ | $6.1\pm1.41^{cd}$ | $5.89\pm1.69^{bcd}$ | $3.84\pm1.97^{e}$ |
| D | $5.6\pm1.61^{d}$ | $5.93\pm1.61^{d}$ | $5.69\pm1.67^{d}$ | $4.16\pm2.04^{de}$ |
| E | $6.21\pm1.77^{bcd}$ | $6.43\pm1.53^{abcd}$ | $5.9\pm1.71^{bcd}$ | $4.99\pm2.15^{cd}$ |
| F | $6.59\pm1.58^{abc}$ | $6.65\pm1.63^{abc}$ | $6.27\pm1.61^{abcd}$ | $5.71\pm2.18^{bc}$ |
| G | $6.99\pm1.51^{a}$ | $6.99\pm1.4^{a}$ | $6.67\pm1.69^{a}$ | $6.07\pm1.93^{ab}$ |
| H | $6.83\pm1.44^{ab}$ | $6.8\pm1.51^{ab}$ | $6.44\pm1.59^{abc}$ | $5.89\pm1.97^{b}$ |
| I | $6.84\pm1.49^{ab}$ | $6.75\pm1.64^{abc}$ | $6.72\pm1.57^{a}$ | $6.06\pm2.16^{ab}$ |
| J | $6.92\pm1.36^{a}$ | $6.89\pm1.45^{ab}$ | $6.57\pm1.47^{ab}$ | $6.81\pm1.41^{a}$ |

*Means and standard deviations with the same letters are not significantly different ($P > 0.05$).

Grummer et al. (2013) reported the use of KCl and flavor enhancers as alternatives for developing low sodium cheddar cheese with the use of a 120-point Labeled affective magnitude scale. In a standard manufacturing method the control (NaCl) received scores of 74 while one of the low sodium formulations received 69, while in an alternate manufacturing procedure the respective scores were 69 and 73, a pattern also seen in this study where the control had lower overall liking scores than a sample formulated with salt substitutes for certain attributes, including overall liking.

### 4.1.2 "Just about right" of saltines, softness, chewiness and bitteness assessment

In nine of the ten formulations evaluated, the majority of the consumers marked that the saltiness as just about right (JAR, Table 7). In formulation C (41.25 % NaCl, 51.25% KCl, and 7.5 % Glycine), the category with the highest percentage was "too weak" with 38.54 % over 35.42% of the category just about right. Formulation J (30 % NaCl, 60 % KCl, and 10 % Glycine) had the highest percentage of JAR responses for saltiness among the ten treatments with 76.8%. Softness and chewiness had very similar frequency percentages in all the formulations. Formulations G (37.5 % NaCl, 52.5% KCl, and 10 % Glycine), J and H (30 % NaCl, 65 % KCl, and 5% Glycine) had the highest percentage of JAR responses for both softness and chewiness, with frequency percentages above or close to 90%.

**Table 7.** Assessment of saltiness, softness and chewiness using just about right "JAR" scale*

| Tr | Saltiness | | | Softness | | | Chewiness | | |
|---|---|---|---|---|---|---|---|---|---|
| | To weak | JAR[ø] | Too strong | Not enough | JAR | Too much | Not enough | JAR | Too much |
| A | 21.82 | 56.21 | 21.97 | 9.85 | 72.73 | 17.42 | 17.97 | 72.66 | 9.38 |
| B | 36.46 | 38.54 | 25 | 13.54 | 68.75 | 17.71 | 22.92 | 67.71 | 9.38 |
| C | 38.54 | 35.42 | 26.04 | 9.38 | 75 | 15.63 | 18.75 | 78.13 | 3.13 |
| D | 36.11 | 42.59 | 21.3 | 9.26 | 76.85 | 13.89 | 21.3 | 73.15 | 5.56 |
| E | 32.71 | 51.4 | 15.89 | 8.41 | 79.44 | 12.14 | 16.82 | 73.83 | 9.34 |
| F | 32.41 | 58.33 | 9.26 | 7.41 | 78.7 | 13.89 | 12.96 | 79.63 | 7.41 |
| G | 33.33 | 60.19 | 6.49 | 4.63 | 92.59 | 2.78 | 6.48 | 91.67 | 1.86 |
| H | 26.85 | 62.04 | 11.12 | 1.85 | 90.74 | 7.41 | 7.41 | 90.74 | 1.86 |
| I | 30.56 | 62.04 | 7.41 | 5.56 | 88.89 | 5.56 | 12.04 | 86.11 | 1.86 |
| J | 12.96 | 76.85 | 10.19 | 2.78 | 91.67 | 5.56 | 8.33 | 89.81 | 1.86 |

*Values represent the percentage of responses obtained by each treatment (Tr) for the attributes Saltiness, softness and chewiness using a just about right scale (JAR[ø]).

The panelists attending the consumer study were also asked if they detected bitterness and off flavor on the cheese treatments followed by a bitterness rating question. Table 8 shows the frequency percentages of the bitterness and off flavor alongside with the percentages corresponding to the categories of bitterness: none, weak, moderate and strong.

Off flavor and bitterness were detected in all treatments. The treatment with the highest off flavor detection was C, in which 82.29 % of the panelists marked its presence, while formulation J only had 26.85% of detection. The extreme ends of the bitterness detection range were observed with the same formulations (C and J); however, the detection of bitterness was higher than off flavor (range of 46.3-90.63). Formulations A (100% NaCl), B (45 % NaCl, 55 % KCl, and 5 % Glycine) and D (45 % NaCl, 45% KCl, and 10 % Glycine) had moderate to strong bitterness, while formulation C had strong to moderate bitterness based on the two most frequent bitterness categories selected. For the rest of the formulations (except E and H) weak bitterness was the most frequent category selected by the panelists. From these cheese treatments, formulation I (33.75 % NaCl, 58.75 % and KCl, 7.5 % Glycine) had the highest percentage of "non bitterness" responses (25.23%) and the third lowest strong bitterness rating (10.28%).

**Table 8.** Bitterness and off flavor assessment

| Tr | Off flavor detection* | bitterness detection* | bitterness rating ᵠ | | | |
|---|---|---|---|---|---|---|
| | (%) | (%) | None | weak | moderate | strong |
| A | 72.73 | 88.64 | 10.3 | 14.85 | 40.91 | 33.94 |
| B | 77.08 | 89.58 | 3 | 14 | 43 | 36 |
| C | 82.29 | 90.63 | 4.17 | 6.25 | 33.33 | 56.25 |
| D | 75.93 | 88.89 | 4.63 | 14.81 | 42.59 | 37.96 |
| E | 64.49 | 78.5 | 8.41 | 28.04 | 28.97 | 34.58 |
| F | 44.44 | 62.04 | 12.04 | 36.11 | 34.26 | 17.59 |
| G | 28.04 | 49.07 | 13.89 | 49.07 | 28.7 | 8.33 |
| H | 41.67 | 62.04 | 15.74 | 30.56 | 36.11 | 17.59 |
| I | 35.19 | 49.53 | 25.23 | 35.51 | 28.97 | 10.28 |
| J | 26.85 | 46.3 | 21.3 | 40.74 | 30.56 | 7.41 |

*Data represent the percentage of panelists who reported the presence of off flavor or bitterness.
ᵠ Data represent the percentage of responses awarded to each category in each treatment (Tr).

### 4.1.3 Overall Product Differences

Multivariate analysis of variance (MANOVA) was performed to determine if all 10 formulations including the control differed considering all sensory attributes evaluated

simultaneously. Since the Wilk's Lambda value P>F, is <0.0001, it could be concluded that difference existed among all ten formulations of salt substitutes used to prepare cheddar cheese when all eight sensory attributes were concurrently compared. This test results can be found on Table 9. The descriptive discriminative analysis (DDA's Table 10) revealed that according with pooled within canonical structurer's in the first dimension (Can 1), overall liking (0.809), overall taste (0.793), appearance (0.606), and texture (0.516) were the sensory attributes significantly contributing to the differences among formulations, while saltiness also contributes in accordance to the second dimension (can 2).

**Table 9.** Multivariate Analysis of Variance.

| Multivariate Statistics and F approximations (S=8 M=0 N=530.5) | | | | | |
|---|---|---|---|---|---|
| **Statistic** | **Value** | **F Value** | **Num DF** | **Den DF** | **Pr>F*** |
| Wilk's Lambda | 0.68933870 | 5.67 | 72 | 6473.6 | <0.0001* |
| Pillai's Trace | 0.33126513 | 5.14 | 72 | 8560 | <0.0001 |
| Hotelling-Lawley Trace | 0.42150491 | 6.21 | 72 | 4344.9 | <0.0001 |
| Roy's Greatest Root | 0.34481901 | 41.00 | 9 | 1070 | <0.0001 |

*P-Value < 0.0001 indicates that a difference exists among all ten formulations

**Table 10.** Canonical Structure's describing group differences among the ten formulations.

| **Pooled With Canonical Structure** | | |
|---|---|---|
| **Variable** | **Can 1** | **Can 2** |
| Appearance | 0.606264* | -0.61990 |
| Odor | 0.132172 | -0.180327 |
| Taste | 0.793401* | 0.394087 |
| Saltiness | 0.411324 | 0.338957 |
| Texture | 0.515946* | -0.088661 |
| Softness | 0.365946 | -0.086542 |
| Chewiness | 0.366449 | 0.219148 |
| Overall liking | 0.809429* | 0.327371 |

* Sensory attributes accountable for the majority of the sensory differences

### 4.1.4 Acceptance and change in probability of purchase intent

Acceptance and the change in probability of purchase intent are described in Tables 11 and 12. Table 11 describes the frequency of affirmative responses given by the panelists in the

consumer study regarding acceptance of the product and purchase intent before and after information about the samples being low sodium was provided. The percentage of acceptance of the treatments ranged from 34.38 % to 88.89 % corresponding to formulations C (41.25 % NaCl, 51.25% KCl, and 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, and 10 % Glycine).

Three formulations (B, C, and D) had lower acceptability than the control, which was considered acceptable by 53.% of the consumers. Besides formulation J, formulations I (33.75 % NaCl, 58.75 % KCl, and 7.5 % Glycine) and G (37.5 % NaCl, 52.5% KCl, and 10 % Glycine) were considered acceptable by more than 80% of the consumers. The purchase intent before additional information was given ranged from 10.42% to 69.44% for formulations C and J, those are the same formulations at the borders of the previous range that describes the percentage of consumers who considered the products acceptable. Formulations B, C and D had less than 20% of purchase intent. After the panelists were informed that the cheese was low sodium, the frequency range increased to 21% - 75.9% which was corresponding to formulations C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) and D (45 % NaCl, 45% KCl, 10 % Glycine).

**Table 11**. Acceptance and purchase intent frequencies obtained from the consumer study.

| Tr | Acceptance* (%) | PI before information ᵠ (%) | PI After information ᵠ (%) |
|---|---|---|---|
| A | 53.03 | 25.76 | --- |
| B | 42.71 | 17.71 | 25 |
| C | 34.38 | 10.42 | 20.83 |
| D | 45.37 | 16.67 | 21.3 |
| E | 61.68 | 38.32 | 42.06 |
| F | 71.3 | 42.59 | 48.15 |
| G | 83.33 | 50.93 | 63.89 |
| H | 77.78 | 46 | 56.48 |
| I | 83.33 | 63.89 | 71.3 |
| J | 88.89 | 69.44 | 75.93 |

*Values represent the percentage of panelist that considered the product “acceptable”
ᵠ Values represent the percentage of panelist that would buy the specific product.
--- Not measured.

In order to conclude if the increase in purchase intent is significant the McNemar test in Table 12 needs to be considered. In order to conclude if the increase in purchase intent is significant the McNemar test in Table 12 needs to be considered. The change in probability of purchase intent was calculated using the McNemar Test. The purchase intent's probabilities were estimated before and after the panelists were informed that the white cheddar cheese was low in sodium, reducing the risk of high blood pressure. The null hypothesis of this study states that the purchase intent probability is equal before ($\pi 1+$) and after ($\pi+_1$) the information about the cheese being low sodium was presented. The alternate hypothesis states that the purchase intent probability is higher after the information about the low sodium characteristic of the cheese was given to the panelists.

Based on the results obtained from the McNemar test shown on Table 12, the probability of higher purchase intent (p-value < 0.05 5） after the consumers were informed that the cheddar cheese was low in sodium,(sodium content lower than 140 mg/ 50 g of sample) was significant in five out of ten formulations. The formulations in which the probability of higher purchase intent was not significantly are: D (45% of NaCl, 45% of KCl and 10 % of Glycine), E (37.5% of NaCl, 57.5% of KCl and 5 % of Glycine), F (37.5% of NaCl, 55% of KCl and 7.55 % of Glycine) and J (30% of NaCl, 60% of KCl and 10% of Glycine).

For formulations B (45 % NaCl, 55 % KCl, 5 % Glycine), C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), G (37.5 % NaCl, 52.5% KCl, 10 % Glycine), H (30 % NaCl, 65 % KCl, 5% Glycine) and I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine), it can be predicted with 95 % confidence that the probability of purchase intent will increase at least by that value stated by the lower confidence interval and at the most by that value stated by the upper confidence interval (Table 12), e.g., Formulation B will experience an increase of 1.98% to 14.69% of purchase intent after the consumer is aware of the beneficial "low sodium characteristic compared to the

purchase intent when additional information was not given. Cheese salted with formulation G will be the one experiencing the most increase in purchase intent (19.61% at the most).

**Table 12.** Change in purchase intent calculated with McNemar test.

| Formulation | $\chi 2$ | p-value | 95% CI-L* | 95% CI-U* |
|---|---|---|---|---|
| B | 6.231 | 0.0126* | 0.0198 | 0.1469 |
| C | 12.000 | 0.0005* | 0.0518 | 0.1704 |
| D | 3.571 | 0.0588 | -0.0009 | 0.0935 |
| E | 1.600 | 0.2059 | -0.0199 | 0.0940 |
| F | 1.800 | 0.1797 | -0.0249 | 0.1360 |
| G | 8.895 | 0.0029* | 0.0446 | 0.1961 |
| H | 9.000 | 0.0027* | 0.0416 | 0.1806 |
| I | 5.556 | 0.0184* | 0.0176 | 0.1676 |
| J | 2.273 | 0.1317 | -0.0133 | 0.1059 |

* Treatments with p-values marked with *, were for significant purchase intent increase.

### 4.1.5 Sensory Optimization

The Product optimization of the low sodium white cheddar cheese was performed using only the formulations from the three-component mixture to determine the predictive regression models. The predictive models obtained using a restricted regression analysis (not using an intercept), can be found in Table 13. These predictive models were used to plot the mixture response surface (MRS) using the Design Expert software. The optimal formulation was determined by superimposition of all sensory attributes critical to consumer acceptance and purchase intent, as determined by logistic regression analysis. Superimposition was determined by mean acceptance scores of 5.5 and above ( a cutoff value).

**Table 13.** Parameter estimates for variables used in final prediction models for consumer acceptance*.

| Attribute | Predictive model | $R^2$ |
|---|---|---|
| Appearance | $58.76X_1 +37.52X_2+1200.27X_3-153.17X_1X_2-2381.51X_1X_3-1823.4X_2X_3+3086.42X_1X_2X_3$ | 0.672 |
| Odor | $-0.32X_1+1.82X_2+249.24X_3+28.94X_1X_2-309.9X_1X_3-251.18X_2X_3$ | 0.693 |
| Taste | $36.34X_1+29.5X_2+990.66X_3-109.9X_1X_2-1921.13X_1X_3-1464.67X_2X_3+2465.61X_1X_2X_3$ | 0.689 |
| Saltiness | $0.13X_1+7.58X_2+15.04X_3$ | 0.701 |
| texture | $82.1X_1+44.87X_2+918.6X_3-222.75X_1X_2-2199.4X_1X3-1542.09X_2X_3+3456.79X_1X_2X_3$ | 0.713 |
| Softness | $54.66X_1+31.85X_2+678.39X_3-142.98X_1X_2-1566.88X_1X_3-1120.21X_2X_3+2419.75X_1X_2X_3$ | 0.1568 |
| Chewiness | $56.58X_1+33.02X_2+375.86X_3-159.67X_1X_2-1203.08X_1X3-776.05X_2X_3+2426.81X_1X_2X_3$ | 0.0931 |
| Overall liking | $-18.69X_1+1.48X_2+435.64X_3+54.47X_1X_2-511.64X1X_3-437.71X_2X_3$ | 0.532 |

*$X_1$ = % of NaCl, $X_2$ = % of KCl, $X_3$ = % of glycine.

Figures 6 – 13 show the contour profiles generated using the Design Expert software, for the sensory attributes of the white cheddar cheese. The attributes appearance and odor/aroma showed saddle points while the rest of the attributes did not. In each of the figures the areas with a yellow to orange color represent the area containing the formulations with the highest hedonic rating predicted for the restricted parallelogram area.

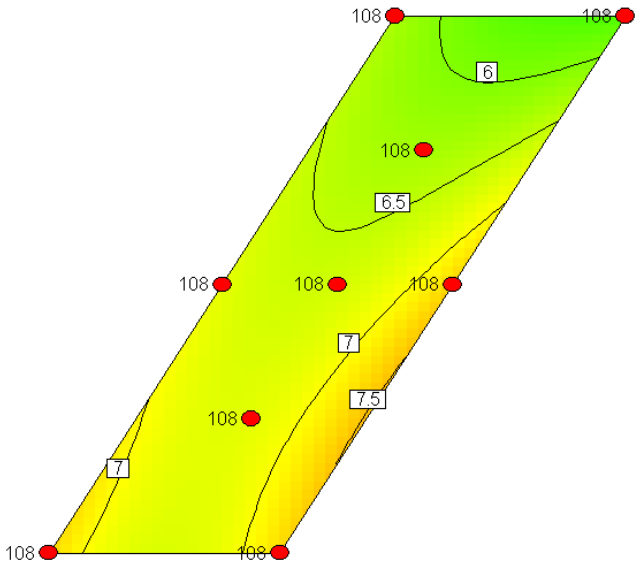



**Figure 6.** Response Surface Methodology (RSM) for appearance, representing mean scores as evaluated by consumers.

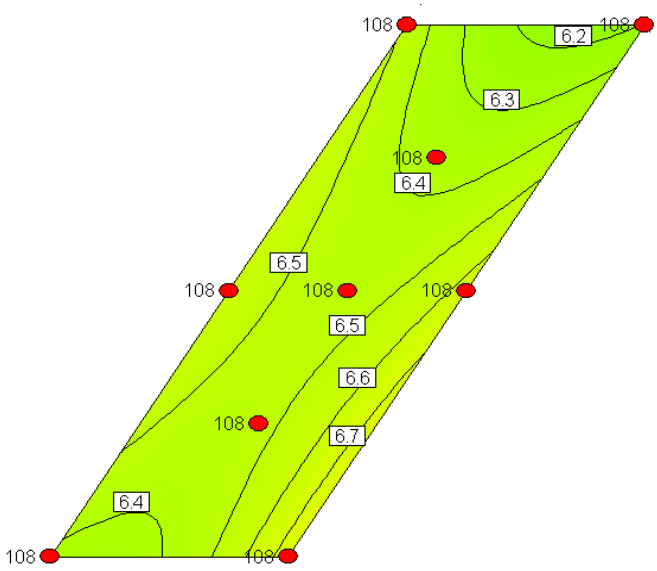



**Figure 7.** Response Surface Methodology (RSM) for odor/ aroma representing mean scores as evaluated by consumers.

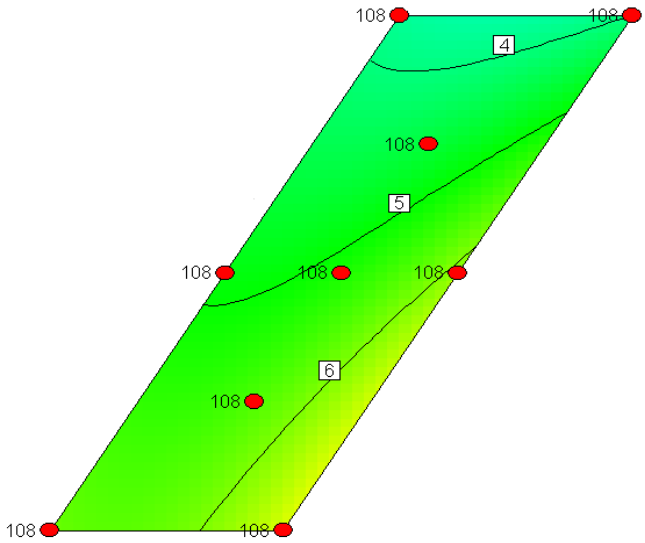



**Figure 8.** Response Surface Methodology (RSM) for taste representing mean scores as evaluated by consumers.

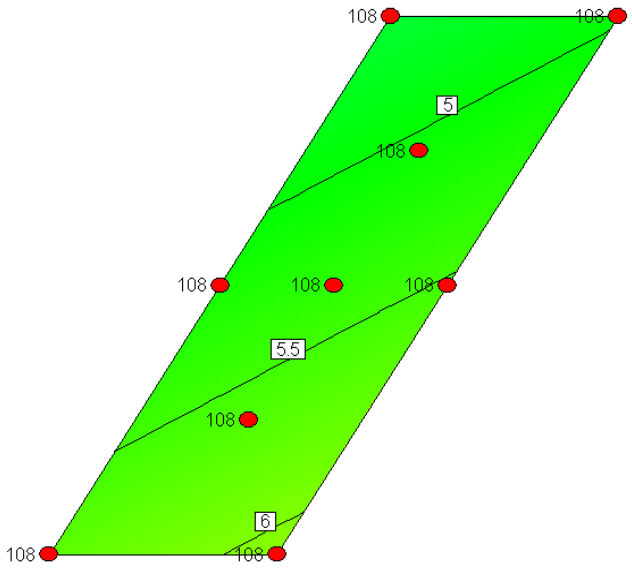



**Figure 9.** Response Surface Methodology (RSM) for saltiness representing mean scores as evaluated by consumers.

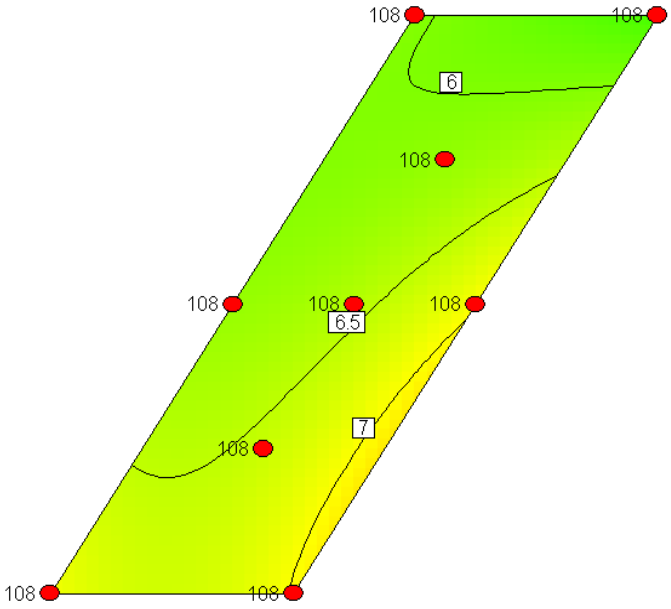



**Figure 10.** Response Surface Methodology (RSM) for texture representing mean scores as evaluated by consumers.

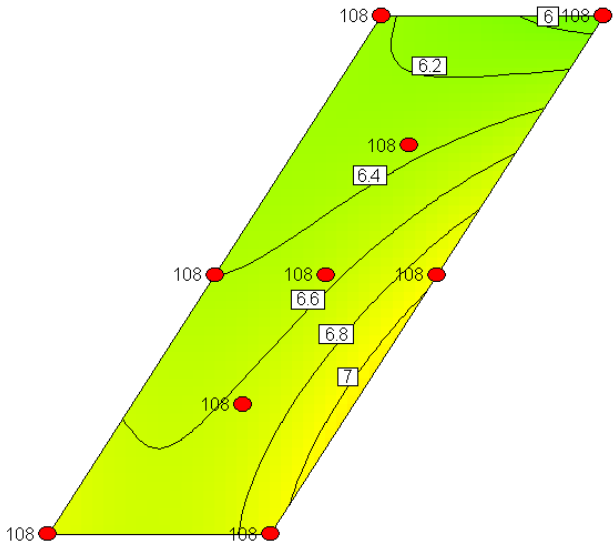



**Figure 11.** Response Surface Methodology (RSM) for softness representing mean scores as evaluated by consumers.

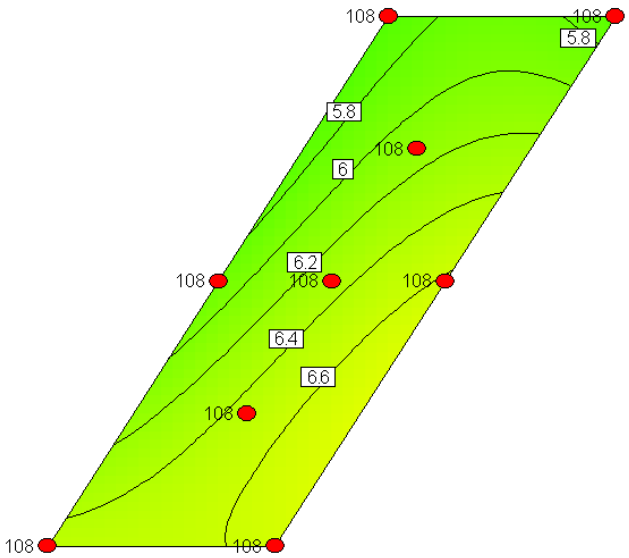



**Figure 12.** Response Surface Methodology (RSM) for chewiness representing mean scores as evaluated by consumers.

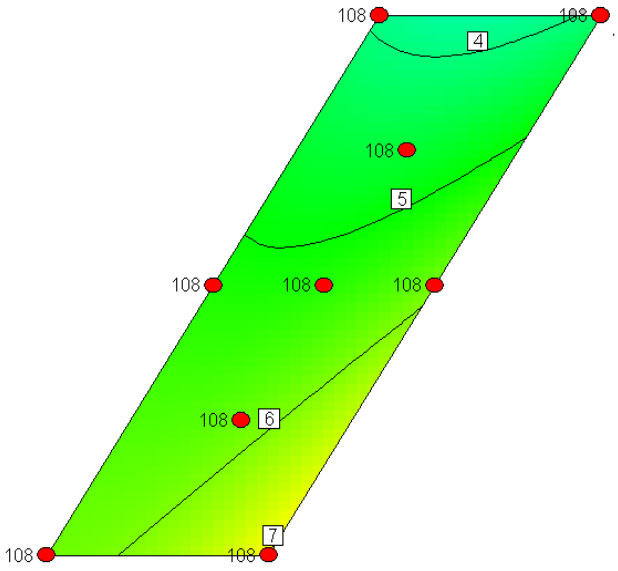



**Figure 13.** Response Surface Methodology (RSM) for overall liking representing mean scores as evaluated by consumers.

The superimposition of the critical attributes at a cutoff point of 5.5 revealed an optimization area highlighted in yellow (Figure 14) in which four formulations of the original set of formulations were contained. The formulations contained in the optimization area are: G (37.5 % NaCl, 52.5% KCl and 10% Gly), H (30 % NaCl, 65% KCl and 5% Gly), I ( 33.65 % NaCl, 58.75% KCl and7.5% Gly) and J (30 % NaCl, 60% KCl and 10% Gly) from these formulations two have 10% of Glycine, one has 7.5% of Glycine and only one has the minimum value of 5% of Glycine.

Using the Design Expert software, the cutoff value was raised to 5.6, 5.7, 5.9 and 6.0. From those superimposition plots, the highest cutoff value with an optimization area containing at least one of the 10 formulations (Figure 15) was 5.9 in which formulation "J" was within the optimal formulation. This shows that to obtain an acceptability rating of at least 5.9 on an 9-point hedonic scale, 10% of Glycine is required and up to 60 % of KCl can be used to substitute NaCl with that level of Glycine.

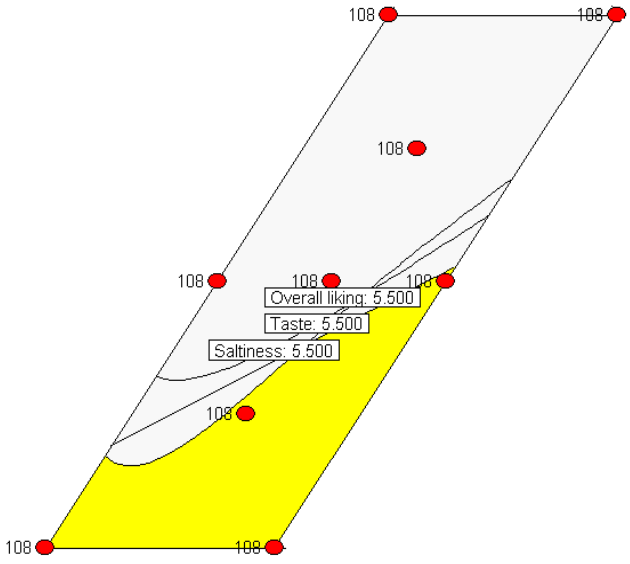



**Figure 14.** Superimposition of critical product attributes for optimal formulation determination with a cutoff value of 5.5.

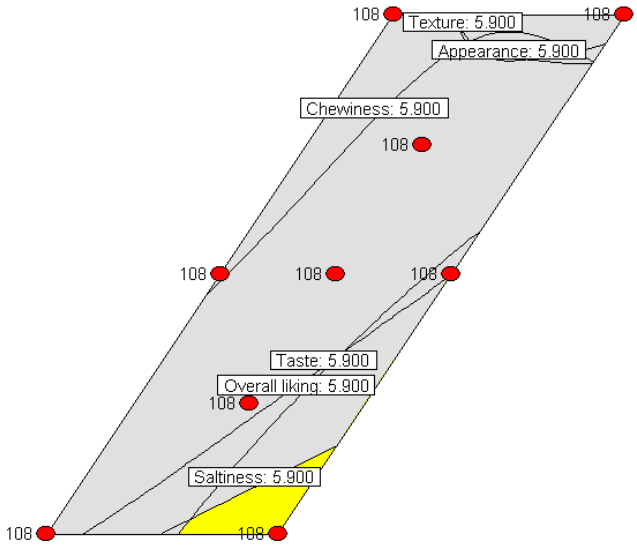



**Figure 15.** Superimposition of critical product attributes for optimal formulation determination with a cutoff value of 5.9.

From the optimal formulation area, it would be interesting to show which /how physicochemical characteristics contribute to sensory acceptance of the low sodium cheddar cheese. The equations to predict the physicochemical characteristics of cheddar cheese based on the salt substitute mixtures are shown in Table 14. The variables evaluated included all the parameters of the Texture Profile Analysis, color analysis (L*, a * and b*), water activity, the two most abundant fatty acids: myristic (C: 14) and palmitic (C: 16) and the two most consistent volatiles: heptanal and benzaldehyde (they were present in every cheese formulation, and in every month analyzed). Only the significant terms of the model are shown for each equation.

The $R^2$ values ranged from 0.32 to 0.79; the equation to predict adhesiveness had the lowest $R^2$ while the one for water had the highest $R^2$ value. An equation to predict the a* value could not be created since no parameter was significant due to non-significant differences among the 10 formulations after five months of storage. Nine of the 16 physicochemical characteristics had $R^2$ values over 0.7. From the TPA only Hardness (0.65) and adhesiveness (0.32) did not have

values over 0.7. From the color analysis, only the ligtness (L*) value was over 0.7 when fitting the linear model. The volatile with the highest $R^2$ was benzaldehide (0.74) while methanetiol and heptanal had 0.61 and 0.54, respectively, showing lower capacity to fit in a linear model. Within the fatty acids, the myristic acid (C: 16) did not reach the 0.7 mark.

**Table 14.** Predictive models for physicochemical properties of White Cheddar cheese based on the optimal formulation range.

| **Attribute** | **Predictive model** $^{\lambda}$ | **$R^2$** |
|---|---|---|
| Hardness | $6374.01X_1+3418.81X_2+23803.5X_3-19831.48X_1+X_2-107756.6X_1X_3-68561.41X_2X_3+258777.78X_1X_2X_3$ | 0.65 |
| Resilience | $1154.89X_1+618.92X_2+6105.96X_3-3566.6X_1X_2-22015.34X_1X_3-13816.45X_2X_3+46429.97X_1X_2X_3$ | 0.70 |
| Cohesion | $29.91X_1+15.44X_2+173.71X_3-90.45X_1X_2-583.33X_1X_3-368.96X_2X_3+1170.79X_1X_2X_3$ | 0.72 |
| Springiness | $-17.31X_1+118.1X_2+259.91X_3$ | 0.72 |
| Gumminess | $5793.541X_1+3167.751X_2+28676.641X_3-18258.041X_1X_2-109163.74X1X_3-71092.11X_2X_3+243661.16X_1X_2X_3$ | 0.73 |
| Chewiness | $4819.34X_1+2690.55X_2+23208.63X_3-15393.16X_1X_2-90834.67X_1X_3-59125.64X_2X_3+206608.07X_1X_2X_3$ | 0.71 |
| Adhesiveness | $-10655.95X_1-4790.62+X_2-98249.59X_3+29101.31X_1X_2+230017.85X_1X_3+164190.89X_2X_3-340681.4X_1X_2X_3$ | 0.32 |
| L* | $106.01X_1+72.56X_2+32.47X_3$ | 0.72 |
| b* | $9.8X_1+25.89X_2+40.07X_3$ | 0.42 |
| $a_w$ | $0.33X_1+0.83X_2+8.03X_3+1.65X_1X_2-6.35X1X_3-9.02X_2X_3$ | 0.79 |
| C:14 | $-15.38+X_1+53.5X_2+61.02X_3$ | 0.48 |
| C:16 | $172.57X_1+85.4X_2-233.02X_3$ | 0.71 |
| Methanetiol | $-0.53X_1+4.04X_2-6.47X_3$ | 0.61 |
| Heptanal | $5.14X_1+24.65X_2-446.77X_3-76.02X_1X_2+717.11X_1X_3+348.81X_2X_3$ | 0.56 |
| Benzaldehide | $195.52X_1+93.41X_2+355.81X_3-581.52X_1X_2-2339.38X_1X_3-1300.9X_2X_3+5733.43X_1X_2X_3$ | 0.74 |

$^{\lambda}$ x1: % of NaCl, x2: % of KCl, x3 = % of Gly

After obtaining the predictive regression models for the physicochemical characteristics of the low sodium white cheddar cheese, the optimal parameters X1 (30% NaCl), X2 (60% KCl) and X3 ( 10% glycine) were used to obtain the estimates of every variable evaluated and the results are shown in the table below:

**Table 15**. Parameter estimations for the optimal sensory salt formulation of low sodium white cheddar cheese.

| Parameter | unit | Estimate |
|---|---|---|
| Hardness | Newton | 85.79 |
| Resilience | % | 32.72 |
| Cohesion | No unit | 0.76 |
| Springiness | % | 91.66 |
| Gumminess | N | 65.39 |
| Chewiness | Newton*second | 56.59 |
| Color | No unit | 78.59 |
| Color | No unit | 3.00 |
| Color | No unit | 22.48 |
| aw | No unit | 0.97 |
| c14 | mg/g | 33.59 |
| c16 | mg/g | 79.71 |
| Methanetiol | RA* | 1.618 |
| Heptanal | RA | 0.413 |
| Benzaldehide | RA | 0.576 |

*RA: Relative abundance of the volatile to the internal standard (4 methyl –2 pentanone).

### 4.2 Volatiles Content

The GC-MS analysis revealed the presence of alcohols, aldehydes, ketones, hydrocarbons, esters, free fatty acids and secondary alcohols, most of which are byproducts of the fatty acid metabolism (Tables 16, 17 and 18). This general groups or volatiles are similar, to a certain extent, to the analysis of regular cheddar cheese performed by Arora et al. (1995). Individually, most of the cheeses prepared with the salt substitute formulations had lesser compounds with methyl or ethyl groups as part of their molecules than the regular cheese analyzed by Arora et al. (1995) and the control of this experiment.

**Table 16.** A list of volatiles found using the head space- SPME / GC-MS method among the 10 formulations of cheddar cheese*

| No | Compound | RT | Reference | No | Compound | RT | Reference |
|---|---|---|---|---|---|---|---|
| 1 | Methanetiol | 4.256 | Sulfurous [b] | 30 | 2-heptanone | 19.794 | gorgonzola |
| 2 | 3-Hydroxy-2-butanone | 4.709 | | 31 | Heptanal | 20.167 | soapy[a] |
| 3 | Methylene Chloride | 5.553 | | 32 | Benzaldehide | 21.91 | almond [c] |
| 4 | 1-hexane, 4-methyl | 6.79 | | 33 | Pentanoic acid | 22.079 | wood[b] |
| 5 | Cyclobutanol | 7.002 | | 34 | hexanoic acid, ethyl ester | 22.1 | goaty[b] |
| 6 | 2,3-butadieone | 7.023 | cheesy[b] | 35 | Cystathionine-diTMS | 22.334 | |
| 7 | 2-butanone | 7.024 | butterscotch[a] | 36 | Bicyclo(3.1.1)heptane, 6,6-dimethyl-2-methylene | 22.573 | |
| 8 | Hexane,2,4- dimethyl | 7.353 | | 37 | Decane | 22.744 | |
| 9 | Acetic acid, anhydride | 7.773 | | 38 | heptane, 5-ethyl-2,2,3-trimethyl- | 23.03 | |
| 10 | methane, oxybis(dichloro- | 8.455 | | 39 | 1- undecyne | 23.245 | |
| 11 | Cyclopentane, methyl- | 8.715 | | 40 | Heptane, 5-ethyl-2,2,3-trimethyl- | 23.275 | |
| 12 | Butanal,3-methyl | 10.036 | Green, malty[b] | 41 | Docosane | 23.31 | |
| 13 | 2-Pentanone | 11.507 | orange peel[a] | 42 | Benzene, 1-methyl-2-(1-methylethyl)- | 23.641 | |
| 14 | 2-dimethyl(trimethylsilyl)siloxytridecane | 11.596 | | 43 | 2-Methyl Propanal | 23.786 | floral[b] |
| 15 | Silane, fluoromethyl- | 12.126 | | 44 | 1-nonene,4,6,8-trimethyl | 23.72 | |
| 16 | Carbamic acid, acetylthio-,O-methyl ester | 12.221 | | 45 | 3-carene | 24.358 | |
| 17 | 2-butanol, 3-methyl- | 12.219 | Cheese, fruity | 46 | Hexane, 3,3-dimethyl- | 24.578 | |
| 18 | Paraldehyde | 12.595 | | 47 | 8- pentadecanone | 24.798 | |
| 19 | Pentane, 1,1'-oxybis | 13.939 | | 48 | 2-nonanone | 24.788 | malty[b] |
| 20 | 2,4,4-Trimethyl-1-hexene | 13.951 | | 49 | Nonanal | 25.104 | grass [b] |
| 21 | Methyl Isobutyl Ketone | 14.097 | | 50 | Butanoic acid, 3-methyl-, 3-methylbutyl ester | 25.05 | cheese[b] |
| 22 | 2-pentanone, 3-methyl | 14.707 | candy[b] | 51 | Phenylethyl Alcohol | 25.617 | roses[b] |
| 23 | Butanoic acid | 16.229 | | 52 | Octadecane, 1-(ethenyloxy)- | 26.917 | |
| 24 | 1,4- (Benzenediol, 2,6 - bis (1,1-dimethylethyl)_ | 16.69 | | 53 | Isopentyl hexanoate | 27.901 | yeasty[b] |
| 25 | 2-methylpropan-1-ol | 17.06 | fruity[b] | 54 | 1-Propene-1-thiol | 28.413 | |
| 26 | Silane, diethylmethyl- | 18.183 | | 55 | Ethlyl butanoante | 28.454 | pleasant |
| 27 | Butanoic acid, 3-methyil, propil ester | 19.176 | | 56 | 2-undecanone | 28.853 | Floral[b] |
| 28 | Benzene, 1,3-dimethyl- | 19.395 | | 57 | 9-octadecanoic acid (Z)-, hexyl ester | 29.211 | |
| 29 | 1-pentanol, 4-methyl- | 19.158 | fresh[b] | 58 | Heptanoic acid, 3-methylbutyl ester | 29.382 | rancid[b] |

*Compounds are listed by retention time (RT). The related aroma cited in the reference is also included.

[a] Odor descriptors detected in cheese using headspace-SPME and GC-MS from Arora et al. (1995).

[b] Odor descriptors detected in cheese using GC- olfactometry from Curioni and Bosset (2002).

**Table 17.** Volatiles Identification in Cheddar cheese treatments during 5 months of ripening*.

| | Tr | A | B | C | D | E | F | G | H | I | J | A | B | C | D | E | F | G | H | I | J | A | B | C | D | E | F | G | H | I | J | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **No** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ |
| 2 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3 | | | | ✓ | | | | | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | |
| 4 | | ✓ | ✓ | ✓ | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | | | | | | | ✓ | ✓ | ✓ | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | ✓ | | | ✓ | | ✓ | | ✓ | |
| 6 | | | | | | | ✓ | | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | ✓ | | | | | | | | | | | | ✓ | | ✓ | | ✓ | | | | | | | | |
| 7 | | | | | | | | ✓ | | | ✓ | | | | | | | | | | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | ✓ | | ✓ | ✓ | ✓ |
| 8 | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | ✓ | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | ✓ | | | | ✓ | ✓ | | | ✓ | | ✓ | | ✓ | ✓ | | ✓ | ✓ |
| 9 | | | | ✓ | | | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | |
| 10 | | | | | | ✓ | | ✓ | ✓ | ✓ | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | ✓ | | ✓ | | | |
| 11 | | ✓ | | | | | | | | ✓ | | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | ✓ | | ✓ | | | | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ | |
| 13 | | | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | | ✓ | | | ✓ | | | | ✓ | | ✓ | ✓ | | | ✓ | | | | | | ✓ | | | | |
| 14 | | ✓ | ✓ | | | | | | | | | ✓ | | | | ✓ | ✓ | | ✓ | ✓ | | | | | | | | ✓ | | ✓ | ✓ | ✓ | | | | | | | | | |
| 15 | | | | ✓ | ✓ | | ✓ | | | | ✓ | | ✓ | ✓ | ✓ | | | | | | | | | ✓ | | ✓ | | | | | | | | | | | ✓ | ✓ | | | | |
| 16 | | | | | | | ✓ | | | | | | | | | | ✓ | | | | | | | | | | ✓ | | | | | | | | | | | | | | |
| 17 | | | | | | | | ✓ | | | | | | | | | ✓ | | | | | | | | | | ✓ | ✓ | ✓ | | | | | | ✓ | | | | | ✓ | | |
| 18 | | | | | | | | | ✓ | ✓ | ✓ | | | | ✓ | | | | | ✓ | | | | ✓ | | | | | | | | | | | | | | | | | |
| 19 | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | ✓ | | | ✓ | | ✓ | | ✓ | ✓ | ✓ | | | ✓ | ✓ | ✓ | | | | | ✓ | | ✓ | ✓ | | | ✓ | |
| 20 | | ✓ | | ✓ | ✓ | ✓ | | | | | | ✓ | ✓ | ✓ | | ✓ | | | ✓ | | ✓ | | | | | | | | | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | | | | | | | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | |
| 22 | | ✓ | | | | | | ✓ | | | | ✓ | | | | | | | | ✓ | ✓ | ✓ | | | | | ✓ | | | | | | | | | | | | | | |
| 23 | | | | ✓ | | ✓ | | ✓ | | | | | | | | ✓ | | | | | ✓ | | | | | | ✓ | | ✓ | | | | | | | | | | | | |
| 24 | | | ✓ | ✓ | | | | ✓ | | | | | | | | | | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | |
| 25 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | ✓ | ✓ | ✓ |
| 26 | | | | | | | | ✓ | ✓ | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | |
| 27 | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 28 | | | ✓ | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | | | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | |
| 29 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | | ✓ | ✓ | ✓ |

*Check marks (✓) represent the presence of a specific volatile in a cheddar cheese treatment (Tr) at a specific ripening month (M). Letters A to J correspond to those in Figure 3 and Table 2. Volatile numbers 1 – 29 correspond to those in Table 16.

**Table 18.** Volatiles Identification in Cheddar cheese treatments during 5 months of ripening*.

| | Tr | A | B | C | D | E | F | G | H | I | J | A | B | C | D | E | F | G | H | I | J | A | B | C | D | E | F | G | H | I | J | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **No** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 30 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 31 | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | ✓ | | | | | | ✓ | ✓ | | ✓ | ✓ | | | | | | ✓ | ✓ | | ✓ | ✓ | | | | | |
| 32 | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | ✓ | | | ✓ | ✓ |
| 33 | | | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 34 | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | | | |
| 35 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | ✓ | ✓ | ✓ | | ✓ | | | | | | | | | | | | | | | ✓ | | | | | | | |
| 36 | | ✓ | | | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | | | ✓ | | | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | ✓ | | | | | | | | | | | | |
| 37 | | | | | | | | | ✓ | | ✓ | | | | ✓ | | | ✓ | | | | | | | | | | | | | | | | | | | | | | |
| 38 | | | | | ✓ | ✓ | | | | | | | | | | | | | | | ✓ | | ✓ | ✓ | | | | | | ✓ | | | | ✓ | | | | | | | ✓ |
| 39 | | | ✓ | ✓ | | | | | | | ✓ | | | ✓ | ✓ | | | ✓ | | | | | | | | | | | | | | | | | | | | | | |
| 40 | | | | | | | | ✓ | | ✓ | | | | | | | | | ✓ | ✓ | | | | | ✓ | | ✓ | | | | | ✓ | | ✓ | | | | | ✓ | ✓ |
| 41 | | ✓ | | ✓ | | ✓ | | | ✓ | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | ✓ | | | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| 42 | | ✓ | | ✓ | | | ✓ | | | | | ✓ | ✓ | | ✓ | ✓ | | ✓ | | | | | | | | | | | | | | | | | | | | | | |
| 43 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 44 | | | | | | | | | ✓ | | | | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ | | | | | | | | ✓ | | | | | ✓ | | | | | | | |
| 45 | | ✓ | | | | | | | ✓ | | | ✓ | | | | | | | | | | | | | | | | | ✓ | | | ✓ | | | | | | | | ✓ | |
| 46 | | | | | ✓ | | | | | | | | | | ✓ | | | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | ✓ | | ✓ |
| 47 | | | ✓ | | | | | | | | | | | | ✓ | | | ✓ | | | | | ✓ | | | | | | | | | | ✓ | | | | | | | | |
| 48 | | | | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | | | ✓ | | ✓ | ✓ | | | | | | | | ✓ | | | ✓ | ✓ | ✓ | | | | | ✓ | | ✓ | | | | |
| 49 | | | | ✓ | | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 50 | | ✓ | | | ✓ | ✓ | | ✓ | | | | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | ✓ | | | | | | | | | | | | | | | |
| 51 | | | | | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 52 | | | ✓ | | | | | | | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 53 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | |
| 54 | | | | ✓ | | | ✓ | | | ✓ | ✓ | | | ✓ | ✓ | | ✓ | ✓ | ✓ | | ✓ | | | | | | | | | | | | | | | | | | | |
| 55 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 56 | | | | | | ✓ | | | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | | | |
| 57 | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | ✓ | | ✓ | | ✓ | | |
| 58 | | ✓ | | | | | | | | | | ✓ | | | | | | | ✓ | | ✓ | | ✓ | | | ✓ | | | ✓ | | ✓ | | | ✓ | | | | | ✓ | | |

*Check marks (✓) represent the presence of a specific volatile.) in a cheddar cheese treatment (Tr) at a specific ripening month (M). Letters A to J correspond to those in Figure 3 and Table 2. Volatile numbers 30 – 58 correspond to those in Table 16.

None of the treatments showed the appearance of hydrogen sulfide, methional or other sulfide compounds which are mostly correlated with the characteristic flavor of Cheddar cheese. This absence of sulfur compounds is also reported by Horwood (1989) and Wood et al. (1994) who proposed the loss of this compounds during maturation or sample extraction. The compounds with the most presence among the cheddar cheese treatments were: methanetiol, 3-hidroxy-2-butanone, 2-methylpropan-1-ol, 1-pentanol, 4-methyl-, 2-heptanone, heptanal, benzaldehide, 2-Methyl Propanal, Isopentyl hexanoate and ethyl butatnoate. All this compound were individually analyzed and compared among treatments and ripening times (Tables 16, 17, and 18).

Certain compounds such as 1 – hexane, 4- methyl were only found after five months of ripening and were present in the control and some of the formulations with lower KCl substitution. Other compounds such as 2,4 – butadienone were found in certain formulations with salt substitutes at month "0", were not found until month 5 or 3 only in treatments that had salt substitutes. Some compounds, e.g., 2-pentanone, 3-methyl, and botanic acid 3-methyl were not found in any formulation at month zero but were found later in the ripening stages. The former one was more characteristic of the control group while the latter one was not found on the formulation with 100% NaCl. The presence of heptatonic acid, 3-methylbutyl ester was observed in some formulations using salt substitutes until month 3 but was not found in month five, while in formulation A (100% NaCl) it was detected for the first time at month 5. Butanoinc acid 3-methyl, propyl ester was a compound found in most of the treatments including the control, but specifically after five months passed. After just three months it was only found in formulations A (100% NaCl), B (45 % NaCl, 55 % KCl, 5 % Glycine) and C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) which were the control or the formulations with the least percentage of substitution with KCl and Glycine. The substitution with KCl and glycine did affect either promoting or suppressing the presence of some compounds.

From the list of volatiles detected using the GC/MS procedure after extraction with SPME –head space method. 10 volatiles were present in most of the treatments and months during ripening. Tables 19-22 show the relative abundance (RA) of every volatile active compound in relationship with the internal standard used (4-methyl – 2-pentanone).

Methanetiol, a compound of natural occurrence in cheese, also known as methyl-mercaptan and responsible for a smell similar to rotten cabbage (Devos, 1996), was identified at a retention time of 4.3 min. For every treatment, the RA of methanetiol significantly increased ($p < 0.05$) at month 5, compared to day 0, except for formulations I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) and J (30%NaCl, 60 % KCl, 10 % Glycine), which had initially significantly higher contents of the compound compared to the rest of the formulations, so that the increase in RA during time observed in these Formulations was not significant. At month five the range of RA varied from 0.131 in formulations A (100 % NaCl), to 2.99 in formulation I (Table 19). From the entire 10 formulations only the control (formulation A) and formulation F (37.5 % NaCl, 55 % KCl, and 7.5 % Glycine) were significantly lower than formulation I, which also was the only formulation from which the previous two were different.

The review of cheese flavors written by Singh and Drake (2003), states that the compound: 3-Hydroxy-2-butanone contributes mainly to young undeveloped flavors such as cooked whey, diacetyl, and milk fat/lactone flavors. 3-hydroxy-2-butanone was identified in the month 0 in all formulations, but formulation B (45 % NaCl, 55 % KCl, 5% Glycine) in which also at months 1 and 3 the compound did not appear. At the beginning of the ripening, this volatile's RA ranged from 0.0529 – 0.8646. Over the time this compound did not significantly increase in nine out of ten formulations, except for G (37.5 % NaCl, 52.5% KCl, 10 % Glycine), a formulation with the highest possible amount of glycine.

**Table 19.** Effect of salt replacement and ripening time on volatiles content*.

| Trt | Mo | Methanetiol | 3-Hydroxy-2-butanone | 2-methylpropan-1-ol |
|---|---|---|---|---|
| A | m0 | $0.035\pm0.02^{bc(C)}$ | $0.05\pm0.04^{cd(A)}$ | $0.0813\pm0.03^{e(A)}$ |
| | m1 | $0.084\pm0.01^{c(B)}$ | $0.08\pm0.05^{b(A)}$ | $0.1624\pm0.0374^{bc(A)}$ |
| | m3 | $0.12\pm0.012^{d(A)}$ | $0.03\pm0.01^{c(A)}$ | $0.1226\pm0.0218^{b(A)}$ |
| | m5 | $0.13\pm0.01^{b(A)}$ | $0.042\pm0.051^{b(A)}$ | $0.14\pm0.12^{ba(A)}$ |
| B | m0 | $0.03\pm0.0089^{c(B)}$ | $0\pm0^{cd(A)}$ | $0.02\pm0.01^{e(B)}$ |
| | m1 | $0.04\pm0.0037^{c(B)}$ | $0\pm0^{b(A)}$ | $0.05\pm0.04^{c(B)}$ |
| | m3 | $0.06\pm0.0067^{d(B)}$ | $0\pm0^{c(A)}$ | $0.04\pm0.01^{b(B)}$ |
| | m5 | $2.03\pm0.53^{ba(A)}$ | $0.821\pm0.96^{b(A)}$ | $2.41\pm0.53^{ba(A)}$ |
| C | m0 | $0.01\pm0.00^{c(B)}$ | $0.07\pm0.04^{cd(A)}$ | $0.20\pm0.02^{de(A)}$ |
| | m1 | $0.021\pm0.03^{c(B)}$ | $0.11\pm0.04^{b(A)}$ | $0.40\pm0.06^{bc(A)}$ |
| | m3 | $0\pm0^{d(B)}$ | $0.16\pm0.07^{bc(A)}$ | $0.66\pm0.08^{b(A)}$ |
| | m5 | $1.06\pm0.31^{ba(A)}$ | $2.75\pm1.52^{a(A)}$ | $4.29\pm4.51^{a(A)}$ |
| D | m0 | $0.11\pm0.09^{bc(C)}$ | $0.15\pm0.02^{cd(A)}$ | $0.24\pm0.15^{de(A)}$ |
| | m1 | $0.61\pm0.19^{bc(B\ )}$ | $0.39\pm0.07^{b(A)}$ | $0.22\pm0.11^{bc(A)}$ |
| | m3 | $0.69\pm0.14^{cd(BA)}$ | $0.46\pm0.073b^{ac(A)}$ | $0.42\pm0.08^{b(A)}$ |
| | m5 | $1.10\pm0.26^{ba(A)}$ | $0.93\pm0.79^{b(A)}$ | $0.51\pm0.16^{ba(A)}$ |
| E | m0 | $0.01\pm0.01^{c(B)}$ | $0.2101\pm0.08^{bcd(A)}$ | $0.11\pm0.05^{e(A)}$ |
| | m1 | $0.03\pm0.05^{1c(B)}$ | $0.32\pm0.19^{b\ (A)}$ | $0.23\pm0.09^{bc(A)}$ |
| | m3 | $0.32\pm0.56^{d(B)}$ | $0.41\pm0.28^{bac(A)}$ | $0.46\pm0.50^{b(A)}$ |
| | m5 | $1.63\pm0.73^{ba(A)}$ | $1.45\pm1.55^{b(A)}$ | $0.02\pm0.02^{ba(A)}$ |
| F | m0 | $0\pm0^{c(B)}$ | $0.43\pm0.09^{abcd(A)}$ | $0\pm0^{e(B)}$ |
| | m1 | $0\pm0^{c(B)}$ | $1.41\pm0.22^{a(A)}$ | $0\pm0^{c(B)}$ |
| | m3 | $0\pm0^{d(B)}$ | $2.98\pm0.33^{ba(A)}$ | $0\pm0^{b(B)}$ |
| | m5 | $0.15\pm0.07^{b(A)}$ | $2.69\pm2.40^{a(A)}$ | $0.27\pm0.06^{ba(A)}$ |
| G | m0 | $0.50\pm0.33^{ab(B)}$ | $0.32\pm0.21^{bcd(B)}$ | $0.8\pm0.131^{a(B)}$ |
| | m1 | $1.16\pm0.25^{ba(B)}$ | $1.43\pm0.52^{a(BA)}$ | $2.14\pm1.01^{a(B)}$ |
| | m3 | $5.10\pm0.18^{a(A)}$ | $2.12\pm0.26^{bac(A)}$ | $2.37\pm0.30^{a(B)}$ |
| | m5 | $1.15\pm0.32^{ba(B)}$ | $0.75\pm0.92^{b(BA)}$ | $1.29\pm0.42^{ba(B)}$ |
| H | m0 | $0.06\pm0.01^{bc(B)}$ | $0.58\pm0.24^{ab(A)}$ | $0.50\pm0.14^{cd(A)}$ |
| | m1 | $0.70\pm0.32^{abc(BA)}$ | $0.88\pm0.29^{ab(A)}$ | $0.46\pm0.10^{bc(A)}$ |
| | m3 | $1.24\pm0.99^{cb(BA)}$ | $1.01\ \pm0.91^{bac(A)}$ | $0.27\pm0.18^{b(A)}$ |
| | m5 | $1.93\pm0.70^{ba(A)}$ | $2.30\pm1.40^{a(A)}$ | $0.91\pm1.29^{ba(A)}$ |
| I | m0 | $0.74\pm0.16^{a(A)}$ | $0.86\pm0.16^{a(A)}$ | $0.77\pm0.12^{ab(A)}$ |
| | m1 | $1.47\pm0.46^{a(A)}$ | $1.39\pm0.57^{a(A)}$ | $1.33\pm0.61^{ab(A)}$ |
| | m3 | $2.56\pm0.83^{b(A)}$ | $3.27\pm2.74^{a(A)}$ | $1.79\pm0.86^{b(A)}$ |
| | m5 | $2.99\pm1.77^{a(A)}$ | $0.76\pm0.35^{b(A)}$ | $2.05\pm0.47^{ba(A)}$ |
| J | m0 | $0.82\pm0.34^{a(A)}$ | $0.46\pm0.30^{abc(B)}$ | $0.68\pm0.20^{bc(A)}$ |
| | m1 | $1.39\pm0.59^{ab(A)}$ | $1.45\pm0.56^{a(BA)}$ | $1.74\pm0.54^{a(A)}$ |
| | m3 | $3.07\pm1.63^{b(A)}$ | $2.36\pm1.25^{bac(A)}$ | $1.86\pm1.55^{b(A)}$ |
| | m5 | $1.77\pm0.41^{ba(A)}$ | $0.36\pm0.24^{b(B)}$ | $0.37\pm0.401^{ba(A)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different ($P > 0.05$)

ϕ Months with the same capitalized letters within inside parenthesis are not significantly different within the same treatment ($P > 0.05$).

**Table 20.** Effect of salt replacement and ripening time on volatiles content.

| Treat | Mo | 1-pentanol, 4-methyl- | 2-heptanone | Heptanal |
|---|---|---|---|---|
| A | m0 | $0.19\pm0.02^{ab(A)}$ | $0.1258\pm0.02^{b(A)}$ | $0.0286\pm0.02^{b(C)}$ |
| | m1 | $0.31\pm0.01^{ab(A)}$ | $0.19\pm0.13^{bc(A)}$ | $0.03\pm0.01^{b(BC)}$ |
| | m3 | $0.47\pm0.11^{a(A)}$ | $0.22\pm0.01^{bc(A)}$ | $0.053\pm0.01^{cb(BA)}$ |
| | m5 | $0.59\pm0.45^{b(A)}$ | $0.23\pm0.16^{c(A)}$ | $0.06\pm0.01^{c(A)}$ |
| B | m0 | $0.32\pm0.13^{a(A)}$ | $0.32\pm0.35^{ab(A)}$ | $0.07\pm0.02^{b(A)}$ |
| | m1 | $0.42\pm0.19^{a(A)}$ | $0.14\pm0.04^{bc(A)}$ | $0.10\pm0.02^{b(A)}$ |
| | m3 | $0.71\pm0.56^{a(A)}$ | $0.28\pm0.06^{bac(A)}$ | $0.12\pm0.0324^{cb(A)}$ |
| | m5 | $0.22\pm0.02^{b(A)}$ | $0.55\pm0.19^{bc(A)}$ | $0.24\pm0.13^{c(A)}$ |
| C | m0 | $0\pm0^{b(A)}$ | $0.10\pm0.02^{b(A)}$ | $0\pm0^{b(A)}$ |
| | m1 | $0\pm0^{c(A)}$ | $0.15\pm0.10^{bc(A)}$ | $0\pm0^{b(A)}$ |
| | m3 | $0\pm0^{a(A)}$ | $0.16\pm0.02^{bc(A)}$ | $0\pm0^{bc(A)}$ |
| | m5 | $0.53\pm0.42^{b(A)}$ | $2.28\pm1.93^{ba(A)}$ | $0.68\pm0.69^{bc(A)}$ |
| D | m0 | $0.21\pm0.27^{ab(A)}$ | $0.10\pm0.06^{b(A)}$ | $0.28\pm0.13^{a(B)}$ |
| | m1 | $0.04\pm0.02^{bc(A)}$ | $0.17\pm0.13^{bc(A)}$ | $0.45\pm0.6^{(B)}$ |
| | m3 | $0.06\pm0.01^{a(A)}$ | $0.42\pm0.01^{bac(A)}$ | $0.65\pm0.25^{a(B)}$ |
| | m5 | $0.062\pm0.01^{b(A)}$ | $0.89\pm0.72^{bc(A)}$ | $1.24\pm0.22^{ba(A)}$ |
| E | m0 | $0.17\pm0.06^{ab(B)}$ | $0.10\pm0.01^{b(A)}$ | $0.34\pm0.13^{a(BA)}$ |
| | m1 | $0.26\pm0.09^{abc(BA)}$ | $0.08\pm0.02^{c(A)}$ | $0.47\pm0.25^{a(BA)}$ |
| | m3 | $0.64\pm0.30^{a(A)}$ | $0.19\pm0.11^{bc(A)}$ | $0.24\pm0.03^{b(B)}$ |
| | m5 | $0.06\pm0.03^{b(B)}$ | $0.19\pm0.11^{c(A)}$ | $0.69\pm0.16^{bc(A)}$ |
| F | m0 | $0\pm0^{b(B)}$ | $0.06\pm0.10^{b(A)}$ | $0\pm0^{b(B)}$ |
| | m1 | $0\pm0^{c(B)}$ | $0.01\pm0.01^{c(A)}$ | $0\pm0^{b(B)}$ |
| | m3 | $0\pm0^{a(B)}$ | $0\pm0^{c(A)}$ | $0\pm0^{c(B)}$ |
| | m5 | $0.30\pm0.02^{b(A)}$ | $0.13\pm0.09^{c(A)}$ | $0.08\pm0.02^{c(A)}$ |
| G | m0 | $0\pm0^{b(B)}$ | $0.52\pm0.26^{ab(A)}$ | $0\pm0^{b(B)}$ |
| | m1 | $0\pm0^{c(B)}$ | $0.77\pm0.26^{a(A)}$ | $0\pm0^{b(B)}$ |
| | m3 | $0\pm0^{a(B)}$ | $0.88\pm0.72^{ba(A)}$ | $0\pm0^{c(B)}$ |
| | m5 | $0.58\pm0.31^{b(A)}$ | $0.15\pm0.09^{c(A)}$ | $0.78\pm0.15^{bc(A)}$ |
| H | m0 | $0.20\pm0.05^{ab(B)}$ | $0.22\pm0.09^{ab(B)}$ | $0\pm0^{b(B)}$ |
| | m1 | $0.21\pm0.09^{abc(B)}$ | $0.33\pm0.10^{bc(B)}$ | $0\pm0^{b(B)}$ |
| | m3 | $0.5254\pm0.62^{a(BA)}$ | $0.41\pm0.18^{bac(B)}$ | $0\pm0^{c(B)}$ |
| | m5 | $2.33\pm2.181^{a(A)}$ | $1.74\pm0.75^{ba(A)}$ | $2.45\pm0.67^{a(A)}$ |
| I | m0 | $0.35\pm0.05^{a(BA)}$ | $0.25\pm0.15^{ab(B)}$ | $0\pm0^{b(B)}$ |
| | m1 | $0.37\pm0.13^{a(BA)}$ | $0.44\pm0.07^{ab(B)}$ | $0\pm0^{b(B)}$ |
| | m3 | $0.23\pm0.08^{a(B)}$ | $0.57\pm0.19^{bac(B)}$ | $0\pm0^{c(B)}$ |
| | m5 | $0.78\pm0.32^{b(A)}$ | $2.93\pm0.16^{a(A)}$ | $2.01\pm1.06^{ba(A)}$ |
| J | m0 | $0.2961\pm0.12^{ab(A)}$ | $0.62\pm0.21^{a(BA)}$ | $0\pm0^{b(B)}$ |
| | m1 | $0.36\pm0.1494^{a(A)}$ | $0.75\pm0.12^{a(BA)}$ | $0\pm0^{b(B)}$ |
| | m3 | $0.43\pm0.237^{a(A)}$ | $1.00\pm0.35^{a(A)}$ | $0\pm0^{c(B)}$ |
| | m5 | $0.63\pm0.48824^{b(A)}$ | $0.29\pm0.08^{bc(B)}$ | $0.12\pm0.03^{c(A)}$ |

* Salt substitute treatments with the same lowercase letters are not significantly different ($P > 0.05$)

ϕ Months with the same capitalized letters within parenthesis are not significantly different within the same treatment ($P > 0.05$).

**Table 21.** Effect of salt replacement and ripening time on volatiles content.

| Treat | Mo | Benzaldehyde | 2-Methyl Propanal | Isopentyl hexanoate |
|---|---|---|---|---|
| **A** | m1 | $0.03\pm0.01^{bc(A)}$ | $0.03\pm0.02^{c(A)}$ | $0.028\pm0.01^{b(A)}$ |
| | m3 | $0.05\pm0.02^{c(A)}$ | $0.046\pm0.03^{c(A)}$ | $0.02\pm0.01^{b(A)}$ |
| | m5 | $0.06\pm0.02^{c(A)}$ | $0.05\pm0.03^{a(A)}$ | $0.02\pm0.01^{cb(A)}$ |
| | m0 | $0.05\pm0.05^{b\,(A)}$ | $0.08\pm0.02^{b\,(A)}$ | $0.03\pm0.04^{c(A)}$ |
| B | m1 | $0.03\pm0.01^{bc(B)}$ | $0.72\pm0.25^{a(B)}$ | $0.02\pm0.01^{b(B)}$ |
| | m3 | $0.06\pm0.04^{c(B)}$ | $0.96\pm0.11^{bac(B)}$ | $0.03\pm0.01^{b(B)}$ |
| | m5 | $0.06\pm0.01^{c(B)}$ | $1.0359\pm0.13^{a(B)}$ | $0.03\pm0.01^{b(B)}$ |
| | m0 | $0.92\pm0.29^{ba(A)}$ | $1.16\pm0.11^{b\,(A)}$ | $0.11\pm0.02^{bc(A)}$ |
| C | m1 | $0.12\pm0.01^{bc(A)}$ | $0.37\pm0.09^{b(B)}$ | $0\pm0^{b(B)}$ |
| | m3 | $0.18\pm0.04^{bc(A)}$ | $0.32\pm0.05^{c(B)}$ | $0\pm0^{b(B)}$ |
| | m5 | $0.2\pm0.03^{c(A)}$ | $1.57\pm0.19^{a(A)}$ | $0\pm0^{c\,(B)}$ |
| | m0 | $1.17\pm0.87^{ba(A)}$ | $0.82\pm0.93^{b\,(B)}$ | $0.46\pm0.26^{a(A)}$ |
| D | m1 | $0\pm0^{c(B)}$ | $0.40\pm0.04^{ab(A)}$ | $0.40\pm0.05^{a(A)}$ |
| | m3 | $0.06\pm0.02^{c(A)}$ | $0.47\pm0.12^{c(A)}$ | $0.38\pm0.16^{a(A)}$ |
| | m5 | $0.07\pm0.01^{c(A)}$ | $0.60\pm0.02^{a(A)}$ | $0.29\pm0.03^{a(A)}$ |
| | m0 | $0.08\pm0.01^{b\,(A)}$ | $0.70\pm0.60^{b\,(A)}$ | $0.41\pm0.21^{ba(A)}$ |
| E | m1 | $0\pm0^{c(B)}$ | $0.17\pm0.06^{bc(A)}$ | $0.025\pm0.01^{b(A)}$ |
| | m3 | $0\pm0^{c(B)}$ | $1.64\pm0.76^{ba(A)}$ | $0.04\pm0.01^{b(A)}$ |
| | m5 | $0\pm0^{c(B)}$ | $1.37\pm1.19^{a(A)}$ | $0.03\pm0.01^{b(A)}$ |
| | m0 | $0.06577\pm0.02^{b\,(A)}$ | $0.55\pm0.60^{b\,(A)}$ | $0.02\pm0.01^{c(A)}$ |
| F | m1 | $0.04\pm0.07^{bc(A)}$ | $0.18\pm0.13^{bc(B)}$ | $0.02\pm0.02^{b(A)}$ |
| | m3 | $0\pm0^{c(A)}$ | $0.86\pm0.23^{bac(A)}$ | $0.03\pm0.02^{b(A)}$ |
| | m5 | $0\pm0^{c(A)}$ | $1.01\pm0.08^{a(A)}$ | $0.020\pm0.0025^{c\,(A)}$ |
| | m0 | $0.01\pm0.01^{b\,(A)}$ | 0.35±0.09b (B) | 0±0c(A) |
| G | m1 | $0\pm0^{c(B)}$ | $0.31\pm0.07^{bc(C)}$ | $0.02\pm0.01^{b(A)}$ |
| | m3 | $0\pm0^{c(B)}$ | $1.64\pm0.19^{ba(BA)}$ | $0.01\pm0.01^{b(A)}$ |
| | m5 | $0\pm0^{c(B)}$ | $2.31\pm0.77^{a(A)}$ | $0.01\pm0.01^{c(A)}$ |
| | m0 | $0.38\pm0.10^{b\,(A)}$ | $0.74\pm0.60^{b(BC)}$ | $0.02\pm0.01^{c(A)}$ |
| H | m1 | $0\pm0^{c(B)}$ | $0.15\pm0.04^{bc(B)}$ | $0\pm0^{b(A)}$ |
| | m3 | $0\pm0^{c(B)}$ | $0.64\pm0.51^{bc(B)}$ | $0\pm0^{b(A)}$ |
| | m5 | $0\pm0^{c(B)}$ | $0.77\pm0.36^{a(B)}$ | $0\pm0^{c\,(A)}$ |
| | m0 | $2.26\pm1.42^{a(A)}$ | $2.01\pm1.32^{a(A)}$ | $0\pm0^{c(A)}$ |
| I | m1 | $0.28\pm0.12^{b(B)}$ | $0.32\pm0.04^{bc(A)}$ | $0\pm0^{b(A)}$ |
| | m3 | $0.35\pm0.13^{b(B\,)}$ | $0.96\pm0.36^{bac(A)}$ | $0\pm0^{b(A)}$ |
| | m5 | $0.76\pm0.23b^{(BA)}$ | $1.36\pm0.75^{a(A)}$ | $0\pm0^{c\,(A)}$ |
| | m0 | $1.26\pm0.29^{ba(A)}$ | $1.46\pm0.28^{ba(A)}$ | $0\pm0^{c(A)}$ |
| J | m1 | $0.74\pm0.24^{a(BA)}$ | $0.36\pm0.18^{bc(BA)}$ | $0\pm0^{b(A)}$ |
| | m3 | $0.91\pm0.19^{a(BA)}$ | $1.72\pm0.33^{a(B)}$ | $0\pm0^{b(A)}$ |
| | m5 | $1.13\pm0.19^{a(A)}$ | $2.72\pm1.12^{a(A)}$ | $0\pm0^{c\,(A)}$ |
| | m0 | $0.54\pm0.23^{b\,(B)}$ | $0.43\pm0.48^{b\,(B)}$ | $0\pm0^{c(A)}$ |

* Salt substitute treatments with the same lowercase letters are not significantly different ($P > 0.05$).

ϕ Months with the same capitalized letters within parenthesis are not significantly different within the same treatment ($P > 0.05$).

**Table 22.** Effect of salt replacement and ripening time on volatiles content.

| Treat | Mo | Ethlyl butanoante |
|---|---|---|
| A | m0 | $0.03\pm0.01^{cd(A)}$ |
| | m1 | $0.03\pm0.01^{c(A)}$ |
| | m3 | $0.02\pm0.01^{b(A)}$ |
| | m5 | $0.02\pm0.02^{b\ (A)}$ |
| B | m0 | $0.016\pm0.02^{cd(B)}$ |
| | m1 | $0.04\pm0.01^{c(B)}$ |
| | m3 | $0.04\pm0.003^{b(B)}$ |
| | m5 | $0.94\pm0.27^{ba(A)}$ |
| C | m0 | $0.16\pm0.07^{abc(A)}$ |
| | m1 | $0.18\pm0.07^{bc(A)}$ |
| | m3 | $0.16\pm0.03^{b(A)}$ |
| | m5 | $1.18\pm0.85^{ba(A)}$ |
| D | m0 | $0.12\pm0.06^{abcd(A)}$ |
| | m1 | $0.02\pm0.01^{c(A)}$ |
| | m3 | $0.25\pm0.14^{b(A)}$ |
| | m5 | $0.20\pm0.15^{b(A)}$ |
| E | m0 | $0.07\pm0.06^{bcd(A)}$ |
| | m1 | $0.04\pm0.02^{c(A)}$ |
| | m3 | $0.12\pm0.14^{b\ (A)}$ |
| | m5 | $0.05\pm0.03^{b(A)}$ |
| F | m0 | $0\pm0^{d(B)}$ |
| | m1 | $0\pm0^{c(B)}$ |
| | m3 | $0\pm0^{b\ (B)}$ |
| | m5 | $0.05\pm0.02^{b(A)}$ |
| G | m0 | $0.23\pm0.03^{a(A)}$ |
| | m1 | $0.25\pm0.15^{bc(A)}$ |
| | m3 | $0.92\pm0.73^{ba(A)}$ |
| | m5 | $0.3\pm0.32^{b(A)}$ |
| H | m0 | $0.24\pm0.08^{a(A)}$ |
| | m1 | $0.40\pm0.33^{bc(A)}$ |
| | m3 | $0.44\pm0.16^{b\ (A)}$ |
| | m5 | $2.64\pm1.10^{a(A)}$ |
| I | m0 | $0.20\pm0.07^{ab(B\ )}$ |
| | m1 | $0.50\pm0.14^{b(BA)}$ |
| | m3 | $0.95\pm0.43^{ba(BA)}$ |
| | m5 | $1.15\pm0.36^{ba(A)}$ |
| J | m0 | $0.13\pm0.07^{abcd(BA)}$ |
| | m1 | $1.28\pm0.30^{a(B)}$ |
| | m3 | $1.80\pm0.84^{a(A)}$ |
| | m5 | $0.49\pm0.24^{b(B)}$ |

* Salt substitute treatments with the same lowercase letters are not significantly different ($P > 0.05$)

ϕ Months with the same capitalized letters within parenthesis are not significantly different within the same treatment ($P > 0.05$)

After five months of ripening significant differences were found between two groups of samples. Formulations A (100% NaCl), B(45 % NaCl, 55 % KCl, 5% Glycine), D (45 % NaCl, 45% KCl, 10 % Glycine), E(37.5 % NaCl, 57.5 % KCl, 5 % Glycine), and J (30 % NaCl, 60 % KCl, 10 % Glycine) had significantly lower levels of 3-Hydroxy-2-butanone than C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine) , G (30 % NaCl, 65 % KCl, 5% Glycine), H and I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine). Regarding the amount of Glycine there was no significant pattern observed, since formulations with either 5, 7.5, or 10 % of glycine were found in the group with lower RA of the compound. Every formulation from the group with the significantly higher amount of the volatile had less than 41.25% of table salt, excluding formulations with 45% and 100% of NaCl.

The next compound quantified was: 2-methylpropan-1-ol, also called: isobutanol, which is produced naturally during the fermentation of carbohydrates and as a byproduct of the decomposition of organic matter (Lide, 2008). In general this compound was present in smaller quantities than the previous two. Over the time the compound only increased significantly in formulation B (45 % NaCl, 55 % KCl, 5% Glycine) and formulation G (37.5 % NaCl, 52.5% KCl, 10 % Glycine), and only at the fifth month at the end of the ripening for both formulations. The only similarity between these two formulations is the KCl content that ranges between 52.5 – 55 %.

At month 0, the RA of 2-methylpropan-1-ol ranged from 0.024 -0.79. The control had the lowest amount of the volatile active compound while formulation G had significantly higher in isobutanol than other formulations. At the end of the ripening time, the RA abnormally raised to 4.29 in formulation C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) while no differences were found among the other nine formulations.

At retention time of 19.158 a compound was identified as 1-pentanol, 4-metyl- (Table 20), this compound is mostly found in Rokpol cheese, a Blue –Veined Cheese (Berezinska et al., 2007). The Relative abundance of this alcohol showed values of 0 in formulation C before ripening and did not reach levels higher than 0.8 except at month 5 of formulation H (30 % NaCl, 65 % KCl, 5% Glycine) , in which the RA was 2.33. Except for formulation H, in which the RA significantly increased since the third month of ripening, no change over time occurred in the cheeses prepared with the other salt substitute formulations.

2-heptanone is a common ketone present in the volatiles of cheddar cheese, but not in amounts as high as sulfide compounds (Arora et al., 1995; Manning and Robinsons, 1973). The Relative Abundance of 2-heptanone significantly increased at month 5 only in formulations H (30 % NaCl, 65 % KCl, 5% Glycine) and I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine), while it decreased in formulation J (30 % NaCl, 60 % KCl, 10 % Glycine). This change in 2-heptanone only occurred at month 5, and the decrease or increase in RA of the compound was exclusively observed in two formulations with only 30% NaCl and one with 33.75% of NaCl.

The initial range of 2-heptanone's RA was 0.10 – 0.61 corresponding to formulations A (100% NaCl) and J (30 % NaCl, 60 % KCl, 10 % Glycine). The formulation J was the only one from which the control and formulation C were different at the beginning of the ripening period. At the ripening Formulation C was significantly higher than other products, except for formulation I, while the control and formulations E and F were lower in RA than the rest.

According to Young (2011), heptanal is an aldehyde volatile compound present in cheddar cheese which increases with higher starter culture activity. Young's research also found that other compounds such as propan-2-one, 2-heptanone, 3-methyl butanal, and benzaldehyde and dimethyl sulfide behaved similarly to heptanal.

The initial heptanal RA range was 0 - 0.33. the "zero" values were observed in formulations C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine), G (37.5 % NaCl, 52.5% KCl, 10 % Glycine), H (30 % NaCl, 65 % KCl, 5% Glycine) , I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine); while the highest amount of heptanal was detected in formulation E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine). Over the time the heptanal produced did not increased significantly in formulations B (45 % NaCl, 55 % KCl, 5% Glycine) and C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine). In other cheese samples (Formulations D, H and I), the RA of heptanal was higher specifically at the fifth month.

Once the ripening process was completed, the range of RA for heptanal was much higher (0.064 – 2.45). From the entire set of formulations, A (control) was the lowest and I the highest. Along with formulation A, formulations F and J had relative abundances lower than 0.12. On the other hand, H (30 % NaCl, 65 % KCl, and 5% Glycine) and D (45 % NaCl, 45% KCl, and 10 % Glycine) joined I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) on the group of cheeses that had relative abundances above 1.2; these two groups of samples are the extreme sides of the range and are different among one another.

Benzaldehide imparts a high sharp, sweet, bitter, almond, and cherry odor (Young, 2011). The initial range of the compound was 0.0- 0.74; the lowest values of the range occurred with formulations E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine), G (37.5 % NaCl, 52.5% KCl, 10 % Glycine) and H (30 % NaCl, 65 % KCl, 5% Glycine), while J (30 % NaCl, 60 % KCl, 10 % Glycine) had the highest significant RA of the initial measurements (Table 21).

The ripening process affected the amount of Benzaldehyde detected in six of the ten treatments. In four of them: B (45 % NaCl, 55 % KCl, 5% Glycine), E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine), G (37.5 % NaCl, 52.5% KCl, 10 % Glycine), and H (30 % NaCl, 65 % KCl,

5% Glycine), the RA increased until the fifth month. Formulation D (45 % NaCl, 45% KCl, and 10 % Glycine) significantly increased in Benzaldehyde since month 1, while formulation J reduced significantly after ripening. Although there was no specific pattern on the change in Benzaldehyde, seven of the nine formulations showed a change, either increasing or decreasing, while the control had a very constant low level of Benzaldehyde (0.03-0.04) measured in relative abundance.

The next compound evaluated was 2-methyl propanal. According to Arora et al. (1995), this methylated aldehyde imparts a floral and sometimes fatty flavor to cheeses. The initial range of RA of this compound was 0.02- 0.72 corresponding to formulations A (100% NaCl) and B (45 % NaCl, 55 % KCl, 5% Glycine). The formulation with the least salt and the most potassium chloride had RA values of only 0.14. Over the time the amount of 2-methyl propanal increased significantly at month 5 in two formulations including: B (45 % NaCl, 55 % KCl, 5% Glycine), and H (30 % NaCl, 65 % KCl, 5% Glycine). 2- methyl propanal was the first compound that originally increased reduced later during the ripening process. In formulations C (41.25 % NaCl, 51.25% KCl, and 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, and 10 % Glycine), the compound significantly increased at the third month and decreased during the analysis performed at the fifth month.

The control did not significantly increased in RA of the compound at month 5 (0.081); the range of the treatments in the mixture design was 0.17- 2.0 corresponding to formulations B (45 % NaCl, 55 % KCl, 5 Glycine) and H (30 % NaCl, 65 % KCl, 5% Glycine), while at the third month, formulation G reached 2.31 in RA but at the end of the ripening it reduced to 0.74.

Isopentyl hexanoate or hexanoic acid was a compound that instead of increasing with time, it reduced specially in the treatments with the highest levels of salt substitution in the cheese formulations (Table 21). The range of the compound was the lowest at the beginning of

the ripening and also at 5 months. The initial range observed was 0.0-0.4; the latest corresponds to formulation D (45 % NaCl, 45% KCl, 10 % Glycine) while samples C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), H (30 % NaCl, 65 % KCl, 5% Glycine), I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine) showed absence of the compound.

The only formulations affected over time were: B (which moved from 0.024 to 0.11); and C which went from 0.0 to 0.46 in RA, which was the highest end of the range at the fifth month. The last compound analyzed was ethyl-butanoate which had an initial range of 0.00 - 0.23 (Table 22). The lowest end corresponded to formulation F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine).formulations G (37.5 % NaCl, 52.5% KCl, 10 % Glycine), H (30 % NaCl, 65 % KCl, 5% Glycine) and I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) had the highest initial RA which was also significantly higher in ethyl butanoate than the control . The ripening time did not show any effect on the amount of ethyl-butanoate of the control and formulations C , D, E (all contained more than 37.5 % NaCl) and G. The tendency in the rest of the formulations was that the increase in RA of the compound, from which only formulation F did not have an increase in the same magnitude, with a significant increase from 0.0 to 0.05.

The previous discussion of the results shows that after the five months of ripening time, the production and change of volatiles specially aldehydes, alcohols, acids, esters and ketones were affected by the salt substitution. A definitive trend could not be identified regarding the amount of sodium or substitutes and the increasing or decreasing of certain compounds and not every compound was affected significantly. In the compounds where significant differences were detected among the treatments at month “5”, the control was significantly lower (in relative abundance) than at least one of the cheeses manufactured with a salt substitutes. The formulations that were higher in a specific compound were not necessary the ones with the

highest or lowest amount of substitution with KCl and Gly. All the compounds evaluated are usually present in cheddar cheese or other cheese types as seen in the references provided on the discussion of the individual compounds.

### 4.3 Fatty Acids profile

Prior to the discussion of the change in fatty acids, Table 23 explains the abbreviations, the condensed formulas, common and systematic or scientific names for the fatty acids measured in this study. This table was created using information from Scientific Physics (2010) and Grunstone (1996). The individual and total quantification of saturated and unsaturated fatty acids is shown in Tables 24-29. The compounds in those Tables are identified only with their condensed formula.

**Table 23.** Explanatory table of fatty acids description, common and scientific names and condensed formulas

| **Common name of the Acid** | **Carbon atoms** | **Double bonds** | **Scientific name** | **Condensed** |
|---|---|---|---|---|
| Butyric acid | 4 | 0 | Butanoic acid | C4:0 |
| Caproic Acid | 6 | 0 | Hexanoic acid | C6:0 |
| Caprylic Acid | 8 | 0 | Octanoic acid | C8:0 |
| Capric Acid | 10 | 0 | Decanoic acid | C10:0 |
| Myristic Acid | 14 | 0 | Tetradecanoic acid | C14:0 |
| Palmitic Acid | 16 | 0 | Hexadecanoic acid | C16:0 |
| Palmitoleic Acid | 16 | 1 | 9-Hexadecenoic acid | C16:1 |
| Stearic Acid | 18 | 0 | Octadecanoic acid | C18:0 |
| Alpha-Linolenic Acid | 18 | 3 | 9,12,15-Octadecatrienoic acid | C18:3N9 |
| Gamma-Linonenic Acid | 18 | 3 | 6,9,12-Octadecatrienoic acid | C18:3N6 |
| Eicosadienoic | 20 | 2 | Eicosadienoic | C20:2 |
| Behenic Acid | 22 | 0 | Docosanoic acid | C22:0 |
| Dihomo-gamma-linolenic acid (DGLA) | 20 | 3 | 8,11,14-Eicosatrienoic acid | C20:3N6 |
| **Abbreviation** | | | **Group** | |
| SAFA | | | Saturated Fatty acids | |
| MUFA | | | Mono unsaturated Fatty Acids | |
| PUFA | | | Poly unsaturated Fatty Acids | |
| TOTAL | | | Total Fatty Acids | |

The GC/MS analysis revealed the presence of saturated, monounsaturated and polyunsaturated fatty acids in every formulation analyzed. The adjusted area peaks were used to calculate the amount in mg of fatty acid / gram of cheese (mg/g). The list of compounds and its respective mean, standard deviation and Tukey's grouping for different ripening time within treatments (formulations) are shown on Tables 24 – 28. The amount of fatty acids per category is also shown.

Butyric acid (C6:0) was present in every treatment at any time during the ripening process. The initial range of the fatty acid was 6.62 – 13.89 corresponding to formulations: A (control with 100% NaCl) and formulation I (33.75 % NaCl, 58.75 % KCl, and 7.5 % Glycine); however, no significant differences were found initially among the treatments (Table 24). Overall, during the ripening process, the amount of C6:0 tended to decrease when the fifth month was reached; although, this tendency to decrease was only significant from month 1 (in which against the tendency the amounts of the compound had increased compared to mouth 0. At the end of the ripening the range observed was 4.24 – 13.72 mg C6:0/ g of cheese; nevertheless, no significant difference was observed.

The next saturated fatty acid found was the caprylic acid (C8:0), which was present in amounts that were not different among the treatments(Table 24). The initial range of the compound in mg/g was 13.25 for the control and 22.43 for formulation I. After five months, C8:0 of every single cheese product made with the salt substitute formulations from the mixture design or the control decreased significantly either progressively or suddenly since month 1. The final range (after five months) was 0.69 – 5.16 mg/g, respectively for the control and formulation C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), but as in the previous compound, no differences were found among the final amounts of caprylic acid present in all the formulations.

**Table 24.** Effect of salt replacement and ripening time on the fatty acids profile of cheddar cheese (mg/g).

| Treat | Month | C6:0 | C8:0 | C10:0 | C14:0 |
|---|---|---|---|---|---|
| A | m0 | 6.63±1.15$^{a(A)}$ | 13.25±3.18$^{a(A)}$ | 60.24±7.32$^{a(A)}$ | 99.88±12.71$^{a(A)}$ |
| A | m1 | 3.63±0.5$^{c(A)}$ | 3.63±0.04$^{b(B)}$ | 28.12±0.38$^{ab(B)}$ | 34.42±1.66$^{a(C)}$ |
| A | m3 | 6.95±3.03$^{a(A)}$ | 3.31±1.22$^{a(B)}$ | 20.01±2.12$^{a(C)}$ | 38.73±10.79$^{a(B)}$ |
| A | m5 | 5.53±4.84$^{ab(A)}$ | 0.69±0.61$^{a(B)}$ | 0.84±0.03$^{b(D)}$ | 16.17±12.68$^{b(C)}$ |
| B | m0 | 11.46±7.62$^{a(A)}$ | 13.84±7.95$^{a(A)}$ | 85.59±33.13$^{a(A)}$ | 81.31±10.41$^{ab(A)}$ |
| B | m1 | 21.78±19.19$^{abc(A)}$ | 2.6±2.12$^{b(B)}$ | 5.14±4.22$^{b(B)}$ | 21.8±17.87$^{a(B)}$ |
| B | m3 | 7.96±3.98$^{a(A)}$ | 4.19±0.5$^{a(B)}$ | 0±0$^{B(D)}$ | 28.28±6.45$^{a(B)}$ |
| B | m5 | 7.23±6.36$^{ab(A)}$ | 1.36±1.44$^{a(B)}$ | 1.12±0.09$^{b(B)}$ | 14.49±17.18$^{b(B)}$ |
| C | m0 | 9.42±6.07$^{a(A)}$ | 18.01±1.42$^{a(A)}$ | 55.34±10.68$^{a(A)}$ | 64.41±14.66$^{b(A)}$ |
| C | m1 | 16.69±13.7$^{abc(A)}$ | 1.96±1.41$^{b(B)}$ | 4.41±2.4$^{b(B)}$ | 14.6±10.03$^{a(B)}$ |
| C | m3 | 9.5±1.15$^{a(A)}$ | 3.95±0.54$^{a(B)}$ | 0±0$^{b(B)}$ | 18.15±7.31$^{a(B)}$ |
| C | m5 | 4.24±0.4$^{b(A)}$ | 5.16±3.19$^{a(B)}$ | 0.83±0.15$^{b(B)}$ | 28.26±16.23$^{ab(B)}$ |
| D | m0 | 7.43±0.89$^{a(A)}$ | 16.71±0.52$^{a(A)}$ | 64.93±14.43$^{a(A)}$ | 56.22±4.51$^{bc(A)}$ |
| D | m1 | 15.56±6.89$^{abc(A)}$ | 1.83±0.12$^{b(C)}$ | 24.31±0.25$^{b(B)}$ | 29.18±2.45$^{a(BC)}$ |
| D | m3 | 8.02±3.55$^{a(A)}$ | 3.58±0.54$^{a(B)}$ | 10.07±3.8$^{a(B)}$ | 20.96±6.94$^{a(C)}$ |
| D | m5 | 13.72±1.39$^{a(A)}$ | 1.76±0.13$^{a(C)}$ | 0.81±0.13$^{b(C)}$ | 37.75±1.52$^{ab(B)}$ |
| E | m0 | 8.73±1.49$^{a(B)}$ | 17.22±3.77$^{a(A)}$ | 80.99±10.23$_{a(}^{A)}$ | 22.89±6.29$^{d(A)}$ |
| E | m1 | 35.51±5.98$^{a(A)}$ | 3.25±0.09$^{b(B)}$ | 6.2±0.23$^{ab(B)}$ | 27.19±0.37$^{a(A)}$ |
| E | m3 | 4.05±1.28$^{a(B)}$ | 3.4±0.42$^{a(B)}$ | 11.76±2.86$^{a(B)}$ | 16.71±19.37$^{a(A)}$ |
| E | m5 | 2.92±0.53$^{b(B)}$ | 1.71±0.36$^{a(B)}$ | 0.87±0.12$^{b(C)}$ | 51.87±11.74$^{a(A)}$ |
| F | m0 | 9.96±1.67$^{a(B)}$ | 18.65±6.29$^{a(A)}$ | 84.1±11.42$^{a(A)}$ | 30.41±6.27$^{d(B)}$ |
| F | m1 | 29.86±5.74$^{ba(A)}$ | 3.4±0.33$^{b(B)}$ | 17.04±0.72$^{ab(B)}$ | 17.35±12.59$^{a(AB)}$ |
| F | m3 | 5.37±0.91$^{a(B)}$ | 3.45±0.19$^{a(B)}$ | 9.19±4.21$^{a(B)}$ | 32.23±1.67$^{a(A)}$ |
| F | m5 | 8.06±2.61$^{ab(B)}$ | 1.29±0.39$^{a(B)}$ | 0.89±0.04$^{b(C)}$ | 34.68±4.76$^{ab(A)}$ |
| G | m0 | 12.42±5.33$^{a(B)}$ | 16.45±6.06$^{a(A)}$ | 76.27±13.63$^{a(A)}$ | 31.38±3.24$^{d(C)}$ |
| G | m1 | 26.86±4.5$^{abc(A)}$ | 3.31±0.2$^{b(B)}$ | 13.26±0.7$^{ab(B)}$ | 29.91±1.52$^{a(A)}$ |
| G | m3 | 6.93±0.47$^{a(B)}$ | 3.38±0.2$^{a(B)}$ | 0±0$^{a(B)}$ | 23.54±13.71$^{a(BC)}$ |
| G | m5 | 8.3±2.31$^{ab(B)}$ | 2.88±0.52$^{a(B)}$ | 0.9±0.06$^{b(B)}$ | 33.56±7.53$^{ab(A)}$ |
| H | m0 | 6.85±0.31$^{a(B)}$ | 21.37±0.8$^{a(A)}$ | 63.83±1.7$^{a(A)}$ | 27.42±32.24$^{d(A)}$ |
| H | m1 | 33.35±0.59$^{a(A)}$ | 3.44±0.26$^{b(B)}$ | 5.22±1.17$^{b(B)}$ | 24.22±0.65$^{a(A)}$ |
| H | m3 | 4.66±3.53$^{a(B)}$ | 2.48±1.67$^{b(B)}$ | 0±0$^{a(C)}$ | 25.81±17.32$^{a(A)}$ |
| H | m5 | 4.89±0.19$^{b(B)}$ | 2.89±3.05$^{a(B)}$ | 0.88±0.16$^{b(C)}$ | 36.26±4.38$^{ab(A)}$ |
| I | m0 | 13.89±11.28$^{a(A)}$ | 22.43±3.5$^{a(AB)}$ | 51.88±12.72$^{a(A)}$ | 29.65±4.45$^{d(A)}$ |
| I | m1 | 8.57±1.9$^{bc(A)}$ | 38.63±22.09$^{a(A)}$ | 22.63±4.65$^{a(B)}$ | 33.07±3.7$^{a(A)}$ |
| I | m3 | 4.69±1.17$^{a(A)}$ | 3.27±0.29$^{a(B)}$ | 15.66±2.12$^{a(B)}$ | 31.19±3.85$^{a(A)}$ |
| I | m5 | 8.66±0.61$^{ab(A)}$ | 1.18±0.52$^{a(B)}$ | 1.03±0.32$^{b(C)}$ | 27.15±1.58$^{ab(A)}$ |
| J | m0 | 10.99±1.26$^{a(B)}$ | 16.56±0.47$^{a(A)}$ | 81.68±1.71$^{a(A)}$ | 13.92±8.71$^{d(A)}$ |
| J | m1 | 24.25±3.03$^{abc(A)}$ | 4.63±1.53$^{b(B)}$ | 18.92±3.06$^{a(B)}$ | 20.4±17.62$^{a(A)}$ |
| J | m3 | 4.61±0.78$^{a(C)}$ | 5.12±1.18$^{a(B)}$ | 0±0$^{b(D)}$ | 33.21±4.43$^{a(A)}$ |
| J | m5 | 9.59±0.74$^{ab(B)}$ | 2.14±0.33$^{a(C)}$ | 1.59±0.45$^{a(C)}$ | 37.82±2.25$^{ab(A)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different (Pr > 0.05)

ϕ Months with the same capitalized letter inside parenthesis are not significantly different within the same treatment (Pr > 0.05)

With higher amounts than the previous two fatty acids through a ripening process , capric acid was present in every formulation with an initial range of 51.88 - 85.59 mg/ g (Table 24). This was the first fatty acid in which the control did not occupy the lowest end of the range. Formulations C and F had the lowest and the highest capric acid at month 0. Despite the large range of amounts compared to the previous two compounds, no significant differences existed among the treatments. Over the time a drastic decrease in the amounts of capric acid was observed just after one month, while the decrease became even more evident after five months reaching a final range of capric acid between 0.81 and 1.59, corresponding to formulations D (45 % NaCl, 45% KCl, 10 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine) respectively.

The next compound evaluated was myristic acid, C14:0, the initial range of the compound was high 13.92 – 89.88 in which the control was the highest and formulation J (30 % NaCl, 60 % KCl, 10 % Glycine) the lowest (Table 24). Differences existed among the treatments. The only formulation not different from the control was B (45 % NaCl, 55 % KCl, 5 Glycine) which had 81.31 mg / g. The next formulations were lower than the previous two, but higher than the rest, these formulations were C (41.25 % NaCl, 51.25% KCl, and 7.5 % Glycine) and D (45 % NaCl, 45% KCl, and 10 % Glycine). Over the time the amounts of mytistic acid stabilized between 16.17 and 51.87 mg/g. The control had the maximum decrease (from 99.88 to 16.17 mg/g), while the maximum amount at month 5 was observed with formulation E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine).

The unsaturated fatty acid with the shortest chain, measured was myristoleic acid (C14:1), this compound was present relatively in small amounts compared to the previous four. At month cero the amounts ranged from 0.35 mg/g in formulation B (45 % NaCl, 55 % KCl, 5 Glycine) to 1.37 mg/g in formulation H (30 % NaCl, 65 % KCl, 5% Glycine); none of the formulations were significantly different from the rest. By the fifth month significant changes

compared to the first analysis were not found in nine of the ten formulations. Only formulation J increased significantly in myristoleic acid, occupying the top of the range (0.72 -3.54 mg/g) while the bottom of the range was occupied by formulation B. The amount of the fatty acid in formulation J was significantly higher than the amount in the control, and formulations B and C (41.25 % NaCl, 51.25% KCl, and 7.5 % Glycine).

The next important saturated fatty acid was palmitic acid (C16:0) which was present also in all the cheeses produced. The initial range was 16.49 – 62.41 mg/g corresponding to formulations B and I respectively (Table 25). From the entire set of formulations, the only treatment different was B, since all the rest had amounts above 37 mg/g.

After five months of ripening, seven formulations did not increase or decrease significantly; however, two formulations increased on the palmitic acid's amount, while one decreased. Formulations C and D experienced an increase in C16:0, while I significantly reduced. The control did not experience significant changes over time.

The unsaturated palmitoleic acid (C16:1) was also present in much lower quantities than the saturated acid of 16 carbons (palmitic). The initial range of the fatty acid was 0. 61 – 2.27 mg/g corresponding to formulations B and J. None of the treatments showed any significant difference at the beginning of the ripening time. When comparing the amounts of palmitoleic acid registered during months "0" and "5", no differences were found in nine of the ten formulations, except formulation H, in which the amount of C16:1 was significantly lower at the fifth month. Every formulation experienced an increase in the amounts of the acid at month 1 or 3 but eventually, as said stated previously the amounts decreased again to levels not different from month "0" or lower than that.

**Table 25.** Effect of salt replacement and ripening time on the fatty acids profile of cheddar cheese (mg/g)*.

| Treat | Month | C14:1 | C16:0 | C16:1 | C18:0 |
|---|---|---|---|---|---|
| A | m0 | 0.89±0.2$^{ab(A)}$ | 37.06±8.52$^{ab(B)}$ | 1.45±0.34$^{a(B)}$ | 18.97±4.48$^{a(B)}$ |
| A | m1 | 0±0$^{b(A)}$ | 80.72±5.02$^{a(A)}$ | 4.37±0.46$^{a(A)}$ | 51.32±2.1$^{ab(A)}$ |
| A | m3 | 2.26±3.92$^{a(A)}$ | 75.72±7.64$^{a(A)}$ | 3.38±0.92$^{a(A)}$ | 48.15±3.72$^{ab(A)}$ |
| A | m5 | 1.57±0.75$^{abc(A)}$ | 46.71±6.94$^{a(B)}$ | 1.7±0.27$^{a(B)}$ | 24.32±3.55$^{a(B)}$ |
| B | m0 | 0.35±0.4$^{ab(B)}$ | 16.49±15$^{b(B)}$ | 0.61±0.57$^{a(A)}$ | 8.51±7.72$^{a(A)}$ |
| B | m1 | 0.48±0.41$^{b(B)}$ | 30.71±42.95$^{b(B)}$ | 2.8±2.3$^{a(A)}$ | 31.9±26.03$^{b(AB)}$ |
| B | m3 | 2.52±1.06$^{a(A)}$ | 91.5±5.29$^{a(A)}$ | 3.5±0.65$^{a(A)}$ | 54.6±2.2$^{a(A)}$ |
| B | m5 | 0.72±0.63$^{c(B)}$ | 40.61±26.45$^{a(B)}$ | 1.41±1.26$^{a(A)}$ | 12.18±4.1$^{b(AB)}$ |
| C | m0 | 0.81±0.51$^{ab(A)}$ | 31.13±16.97$^{ab(C)}$ | 0.86±0.91$^{a(B)}$ | 15.79±13.67$^{a(B)}$ |
| C | m1 | 0.5±0.22$^{b(A)}$ | 58.62±15.04$^{ab(AB)}$ | 2.99±0.71$^{a(A)}$ | 44.29±14.22$^{ab(A)}$ |
| C | m3 | 1.87±0.97$^{a(A)}$ | 82.43±6.16$^{a(A)}$ | 3.75±0.87$^{a(A)}$ | 48.32±3.44$^{ab(A)}$ |
| C | m5 | 1.63±0.09$^{abc(A)}$ | 38.42±2.91$^{a(AB)}$ | 1.39±0.1$^{a(B)}$ | 0±0c(B) |
| D | m0 | 1.11±0.1$^{ab(A)}$ | 40.9±2.21$^{ab(C)}$ | 1.61±0.08$^{a(A)}$ | 21.03±1.09$^{a(C)}$ |
| D | m1 | 0.61±0.07$^{b(A)}$ | 61.29±5.77$^{ab(BA)}$ | 2.67±0.05$^{a(A)}$ | 66.89±2.57$^{a(A)}$ |
| D | m3 | 11.73±11.56$^{a(A)}$ | 70.21±2.75$^{a(A)}$ | 2.5±1.11$^{a(A)}$ | 33.5±1.35$^{abc(B)}$ |
| D | m5 | 2.39±0.94$^{abc(A)}$ | 68.46±8.1$^{a(B)}$ | 2.26±0.27$^{a(A)}$ | 0±0$^{c(D)}$ |
| E | m0 | 1.31±0.28$^{a(B)}$ | 46.79±13.4$^{ab(C)}$ | 1.46±1.32$^{a(C)}$ | 18.88±16.67$^{a(BC)}$ |
| E | m1 | 0.75±0.14$^{b(B)}$ | 76.38±2.06$^{ab(BA)}$ | 3.82±0.11$^{a(AB)}$ | 39.45±0.89$^{ab(AB)}$ |
| E | m3 | 2.44±0.22$^{a(A)}$ | 90.18±7.56$^{a(A)}$ | 4.05±0.34$^{a(A)}$ | 49.47±2.98$^{ab(A)}$ |
| E | m5 | 1.09±0.2$^{bc(B)}$ | 54.17±11.08$^{a(BC)}$ | 1.99±0.42$^{a(BC)}$ | 0±0$^{c(C)}$ |
| F | m0 | 1.36±0.5$^{a(A)}$ | 53.15±12.43$^{ab(BC)}$ | 2.28±0.77$^{a(AB)}$ | 28.55±9.12$^{a(B)}$ |
| F | m1 | 0.87±0.34$^{b(A)}$ | 71.56±8.55$^{ab(AB)}$ | 3.61±0.75$^{a(A)}$ | 40.62±4.44$^{ab(AB)}$ |
| F | m3 | 2.27±0.1$^{a(A)}$ | 78.83±5.16$^{a(A)}$ | 3.84±0.03$^{a(A)}$ | 45.08±0.49$^{ab(A)}$ |
| F | m5 | 1.87±1.1$^{abc(A)}$ | 46.44±15.77$^{a(C)}$ | 1.86±0.64$^{a(B)}$ | 0±0$^{c(C)}$ |
| G | m0 | 1.32±0.23$^{a(A)}$ | 52.43±8.08$^{ab(B)}$ | 1.96±0.86$^{a(B)}$ | 25.8±8.31$^{a(B)}$ |
| G | m1 | 0.65±0.02$^{b(A)}$ | 76.56±2.1$^{ab(A)}$ | 3.64±0.37$^{a(A)}$ | 36.29±9.78$^{ab(AB)}$ |
| G | m3 | 2.28±0.22$^{a(A)}$ | 75.09±7.33$^{a(A)}$ | 3.86±0.25$^{a(A)}$ | 46.36±3.6$^{ab(A)}$ |
| G | m5 | 2.16±1.17$^{abc(A)}$ | 62.41±15.25$^{a(B)}$ | 2.25±0.74$^{a(AB)}$ | 0±0$^{c(C)}$ |
| H | m0 | 1.37±0.09$^{ab(B)}$ | 53.15±0.99$^{ab(C)}$ | 2.32±0.04$^{a(B)}$ | 28.35±0.62$^{a(A)}$ |
| H | m1 | 0.48±0.08$^{b(C)}$ | 74.2±0.82$^{ab(B)}$ | 3.65±0.24$^{a(A)}$ | 38.78±0.78$^{ab(A)}$ |
| H | m3 | 2.43±0.17$^{a(A)}$ | 95.59±5.74$^{a(A)}$ | 3.1±2.08$^{a(A)}$ | 49.73±2.96$^{a(A)}$ |
| H | m5 | 1.36±0.5$^{bc(B)}$ | 37.89±1.1$^{a(D)}$ | 1.51±0.21$^{a(C)}$ | 0±0$^{c(B)}$ |
| I | m0 | 1.55±0.27$^{a(A)}$ | 62.26±12.87$^{a\ (A)}$ | 2.7±0.56$^{a(A)}$ | 33.91±7.88$^{a(A)}$ |
| I | m1 | 2.42±0.33$^{a(A)}$ | 69.97±5.87$^{ab(A)}$ | 7.89±5.55$^{a(A)}$ | 34.43±7.35$^{b(A)}$ |
| I | m3 | 1.54±1.34$^{a(A)}$ | 77.56±5.51$^{a(A)}$ | 4.13±0.97$^{a(A)}$ | 24.74±17.65$^{bc(AB)}$ |
| I | m5 | 2.93±0.38$^{ac(A)}$ | 43.52±1.47$^{a(B)}$ | 2.06±0.53$^{a(A)}$ | 0±0$^{c(B)}$ |
| J | m0 | 0.96±0.14$^{ab(B)}$ | 48.13±4.31$^{ab(A)}$ | 2.71±2.77$^{a(A)}$ | 31.42±0.48$^{a(B)}$ |
| J | m1 | 0.75±0.67$^{b(B)}$ | 92.01±31.61$^{a(A)}$ | 5.66±1.63a($^{A)}$ | 53.37±12.16$^{ab(A)}$ |
| J | m3 | 1.87±1.65$^{a(AB)}$ | 82.52±27.61$^{ab(A)}$ | 4.78±1.69$^{a(A)}$ | 17.46±4.85$^{c(B)}$ |
| J | m5 | 3.54±0.09$^{a(A)}$ | 72.67±1.89$^{a(A)}$ | 2.75±0.66$^{a(A)}$ | 0±0$^{c(C)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different (Pr > 0.05)

ϕ Months with the same capitalized letter inside parenthesis are not significantly different within the same treatment (Pr > 0.05)

The final range of palmitoleic acid was 1.39 – 2.75 mg/g in which formulation J (30 % NaCl, 60 % KCl, 10 % Glycine) occupied the top of the range again while the bottom end corresponded to C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine). At the end of the ripening time, no significant differences existed among the treatments.

The stearic acid was the most abundant unsaturated fatty acid present among all the cheese formulations (Table 25). The initial range of the compound in mg/g was: 8.51- 33.91, without significant differences among the treatments at month "0". Formulations B and I were the bottom and the top of the range respectively at month 0. The amounts of the compound increased abnormally after one month of ripening; however in eight of the ten formulations the compound was not found after five months. The compound did not change significantly (comparing months zero and five) in the control, while it reduced but was still detected in formulation B. In the rest of the formulations the compound was not found after five months. The final range of stearic acid was 0.0 – 24.32 mg /g. The 0.0 values corresponded to eight formulations except for B and A. B contained 12.12 mg/g while the control was the top of the range(24.32 mg/g).

The gamma linolenic acid (C18:3n6) was present in relatively low amounts (Table 26). The initial range of the compound was 0.14 – 0.8 mg/g corresponding to treatments C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine) respectively. No significant differences were found between the treatments at month "0", neither at month "1", "3" or "5"; except for treatment B, that increased significantly its amounts of gamma linolenic acid from 0.12 mg/g to 0.26 mg/g. The final range of the fatty acid was 0.12 – 0.67 mg/g from formulations B and J; nevertheless, this difference was not significant among formulations.

The alpha linolenic acid (C18:3n3) initial range was 0.35 – 1.82 mg/ g. corresponding to formulations J (30 % NaCl, 60 % KCl, 10 % Glycine) and G (37.5 % NaCl, 52.5% KCl, 10 %

Glycine). The only treatment significantly different from the rest was formulation J, which was lower in C18:3n3 than formulation G.

Although several treatments increased its amounts of alpha linolenic acid after one or three months, the final amounts (after five months) were different from the initial amounts only in formulation J which maintained that increase over time, while the rest of the formulations reduced its amounts of the compound from month "3" to "5".

Other compounds found in very small quantities were eicosadienoic acid (20:0) and behenic acid (C22:0), neither of them was present in amounts higher than 0.63 mg/g and were mostly present in amounts under 0.1 mg/g. The last compound analyzed was dihomo-gama linolenic acid, which at the fifth month, its amount were not different among the treatments evaluated. The final range of the compound was 0.01- 0.85, corresponding to formulations A (control ) and F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine), respectively (Table 27). When comparing data from month "0" and month "5" no differences were found among the treatments and all the values were below 0.85 mg/g.

The total amounts of saturated fatty acids (SAFA), Mono unsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) are shown in table 27. The total amount of SAFA, initially ranged from 214.09 to 236.53 mg/g corresponding to formulations I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) and A (control with 100% NaCl) respectively, but no differences were found initially.

**Table 26.** Effect of salt replacement and ripening time on the fatty acids profile of cheddar cheese (mg/g).

| Treat | Month | C18:3N6 | C18:3N3 | C20:2 | C22:0 |
|---|---|---|---|---|---|
| A | m0 | 0.25±0.05$^{a(A)}$ | 1.08±0.22$^{ab(A)}$ | 0.01±0.01$^{a(B)}$ | 0±0$^{a(B)}$ |
| A | m1 | 0±0$^{d(A)}$ | 1.85±1.91$^{a(A)}$ | 0±0$^{c(B)}$ | 0.13±0.01$^{b(A)}$ |
| A | m3 | 0.14±0.24$^{b(A)}$ | 1.35±0.4$^{cd(A)}$ | 0.14±0.12$^{ab(A)}$ | 0±0$^{b(B)}$ |
| A | m5 | 0.12±0.2$^{a(A)}$ | 1.18±0.2$^{abc(A)}$ | 0.08±0.07$^{b(AB)}$ | 0±0$^{b(B)}$ |
| B | m0 | 0.12±0.11$^{a(B)}$ | 0.48±0.39$^{ab(B)}$ | 0.05±0.06$^{a(A)}$ | 0.28±0.48$^{a(A)}$ |
| B | m1 | 0±0$^{d(B)}$ | 0.75±0.6$^{a(B)}$ | 0±0$^{c(A)}$ | 0±0$^{b(A)}$ |
| B | m3 | 0.58±0.16$^{b(A)}$ | 2.95±0.92$^{ab(A)}$ | 0±0$^{b(A)}$ | 0±0$^{b(A)}$ |
| B | m5 | 0.26±0.23$^{a(AB)}$ | 0.99±0.89$^{abc(B)}$ | 0.11±0.1$^{b(A)}$ | 0±0$^{b(A)}$ |
| C | m0 | 0.14±0.17$^{a(A)}$ | 0.74±0.69$^{ab(AB)}$ | 0.02±0.02$^{a(A)}$ | 0.02±0.03$^{a(A)}$ |
| C | m1 | 0±0$^{d(A)}$ | 1.51±0.56$^{a(A)}$ | 0.03±0.05b$^{c(A)}$ | 0.15±0.13$^{b(A)}$ |
| C | m3 | 0.36±0.28$^{b(A)}$ | 1.08±0.34$^{d(AB)}$ | 0.06±0.11$^{b(A)}$ | 0±0$^{b(A)}$ |
| C | m5 | 0.24±0.03$^{a(A)}$ | 0±0$^{c(B)}$ | 0.08±0.08$^{b(A)}$ | 0±0$^{b(A)}$ |
| D | m0 | 0.28±0.02$^{a(A)}$ | 1.23±0.12$^{ab(A)}$ | 0.02±0.02$^{a(B)}$ | 0.05±0.04$^{a(A)}$ |
| D | m1 | 0±0$^{d(A)}$ | 1.66±0.16$^{a(A)}$ | 0.13±0.01$^{bc(AB)}$ | 1.37±1$^{a(A)}$ |
| D | m3 | 0.22±0.26$^{ab(A)}$ | 2.06±0.6b$^{cg(A)}$ | 0.07±0.13$^{b(AB)}$ | 0.02±0.03$^{b(A)}$ |
| D | m5 | 0.38±0.15$^{a(A)}$ | 1.79±0.2$^{ab(A)}$ | 0.23±0$^{b(A)}$ | 0.01±0.02$^{b(A)}$ |
| E | m0 | 2.07±3.24$^{a(A)}$ | 0.86±0.88$^{ab(B)}$ | 0.11±0.12$^{a(AB)}$ | 0.03±0.05$^{a(AB)}$ |
| E | m1 | 0±0$^{d(A)}$ | 1.61±0.55$^{a(AB)}$ | 0±0$^{c(B)}$ | 0±0$^{b(B)}$ |
| E | m3 | 0.57±0.03$^{ab(A)}$ | 3.04±0.2$^{ab(A)}$ | 0.23±0.04$^{ab(A)}$ | 0.14±0.05$^{b(A)}$ |
| E | m5 | 0.36±0.05$^{a(A)}$ | 1.6±0.31$^{abc(AB)}$ | 0.13±0.04$^{b(AB)}$ | 0.01±0.02$^{b(AB)}$ |
| F | m0 | 0.36±0.13$^{a(A)}$ | 1.4±0.82$^{ab(A)}$ | 0.03±0.03$^{a(A)}$ | 0.1±0.01$^{a(B)}$ |
| F | m1 | 0.25±0.22$^{cd(A)}$ | 0±0$^{a(B)}$ | 0.63±0.57$^{b(A)}$ | 0±0$^{b(B)}$ |
| F | m3 | 0.56±0.01$^{ab(A)}$ | 2.49±0.22$^{abcd(A)}$ | 0.32±0.13$^{a(A)}$ | 0.12±0.01$^{b(A)}$ |
| F | m5 | 0.31±0.1$^{a(A)}$ | 1.2±0.43$^{abc(BA)}$ | 0.16±0.07$^{b(A)}$ | 0.04±0.01$^{ab(B)}$ |
| G | m0 | 0.3±0.17$^{a(A)}$ | 1.82±0.31$^{a(A)}$ | 0.07±0.07$^{a(AB)}$ | 0.07±0.03$^{a(A)}$ |
| G | m1 | 0.29±0.31$^{bcd(A)}$ | 1.71±0.56a$^{(A)}$ | 0±0$^{c(B)}$ | 0±0$^{b(A)}$ |
| G | m3 | 0.58±0.04$^{ab(A)}$ | 2.59±0.35$^{abc\ (A)}$ | 0.11±0.1$^{ab(AB)}$ | 0.1±0.08$^{b(A)}$ |
| G | m5 | 0.47±0.13$^{a(A)}$ | 1.04±1.47$^{abc(A)}$ | 0.19±0$^{ab(A)}$ | 0.03±0.01$^{ab(A)}$ |
| H | m0 | 0.41±0.03$^{a(A)}$ | 1.3±0.07$^{ab(BC)}$ | 0±0$^{a(A)}$ | 0.1±0.01$^{a(A)}$ |
| H | m1 | 0.9±0.27$^{ab(A)}$ | 1.78±0.38$^{a(AB)}$ | 0±0$^{c(A)}$ | 0±0$^{b(A)}$ |
| H | m3 | 0.46±0.31$^{b(A)}$ | 2.82±0.22$^{ab(A)}$ | 0.08±0.01$^{b(A)}$ | 0.09±0.11$^{b(A)}$ |
| H | m5 | 5.21±8.54$^{a(A)}$ | 0.32±0.55$^{bc(C)}$ | 0.89±0.66$^{a(A)}$ | 0±0$^{b(A)}$ |
| I | m0 | 0.49±0.1$^{a(B)}$ | 1.65±0.42$^{ab(A)}$ | 0.07±0.08$^{a(B)}$ | 0.1±0.03$^{a(A)}$ |
| I | m1 | 1.52±0.37$^{a(A)}$ | 2.1±0.22$^{a(A)}$ | 1.38±0.38$^{a(A)}$ | 1.91±0.72$^{a(A)}$ |
| I | m3 | 0.62±0.16$^{ab(B)}$ | 2.21±0.95$^{abcd(A)}$ | 0.09±0.01$^{ab(B)}$ | 1.11±0.62$^{a(A)}$ |
| I | m5 | 0.38±0.17$^{a(B)}$ | 0.92±0.1b$^{c(A)}$ | 0.21±0.12$^{b(B)}$ | 0.16±0.15$^{a(A)}$ |
| J | m0 | 0.8±0.47$^{a(A)}$ | 0.35±0.1$^{b(AB)}$ | 0.07±0.02$^{a(AB)}$ | 0.07±0.03$^{a(A)}$ |
| J | m1 | 0.72±0.35$^{bc(A)}$ | 1.61±0.42$^{a(B)}$ | 0±0$^{c(B)}$ | 0±0$^{b(A)}$ |
| J | m3 | 1.07±0.2$^{a(A)}$ | 3.35±0.26$^{a(A)}$ | 0.17±0.04$^{ab(A)}$ | 0.48±0.5$^{ab(A)}$ |
| J | m5 | 0.67±0.15$^{a(A)}$ | 2.64±0.65$^{a(C)}$ | 0.14±0.08$^{b(A)}$ | 0.07±0.03$^{ab(A)}$ |

* Salt substitute treatments with the same lowercase letters are not significantly different (Pr > 0.05)

ϕ Months with the same capitalized letters within parenthesis are not significantly different within the same treatment (P > 0.05)

**Table 27.** Effect of salt replacement and ripening time on the fatty acids profile of cheddar cheese (mg/g).

| Treat | Month | C20:3N6 | SAFA | MUFA | PUFA |
|---|---|---|---|---|---|
| A | m0 | 0.26±0.01$^{ab(B)}$ | 236.53±10.75$^{a(A)}$ | 2.34±.56$^{a(B)}$ | 1.59±0.26$^{a(A)}$ |
| A | m1 | 0.63±0.03$^{a(A)}$ | 201.04±10.58$^{a(B)}$ | 4.5±0.39$^{a(A)}$ | 2.61±1.91$^{ab(A)}$ |
| A | m3 | 0±0$^{c(C)}$ | 193.01±7.38$^{ab(B)}$ | 5.64±1.15$^{b(A)}$ | 1.49±0.72$^{b(A)}$ |
| A | m5 | 0.01±0.02$^{a(C)}$ | 97.34±26.9$^{a(C)}$ | 3.27±1.79$^{ab(B)}$ | 1.31±0.53$^{a(A)}$ |
| B | m0 | 0.11±0.1$^{ab(A)}$ | 217.25±39.45$^{a(A)}$ | 1.24±.48$^{a(B)}$ | 0.99±0.66$^{a(B)}$ |
| B | m1 | 0.37±0.3$^{ab(A)}$ | 132.07±86.45$^{a(A)}$ | 3.28±1.28$^{a(BA)}$ | 1.2±0.94$^{b(B)}$ |
| B | m3 | 0±0$^{c(A)}$ | 186.53±9.11$^{ab(A)}$ | 6.02±3.42$^{b(A)}$ | 3.53±1.02$^{a(A)}$ |
| B | m5 | 0±0$^{a(A)}$ | 79.47±50.34$^{a(A)}$ | 2.13±1.01$^{b(B)}$ | 1.25±1.33$^{a(B)}$ |
| C | m0 | 0.18±0.16$^{ab(A)}$ | 194.12±8.75$^{a(A)}$ | 1.69±.37$^{a(B)}$ | 1.08±1.01$^{a(AB)}$ |
| C | m1 | 0.34±0.3$^{ab(A)}$ | 140.6±33.61$^{a(B)}$ | 3.64±0.89$^{a(A)}$ | 2.0±0.93$^{b(A)}$ |
| C | m3 | 0±0$^{c(A)}$ | 162.41±6.$^{51abc(AB)}$ | 5.62±2.85$^{b(A)}$ | 1.44±0.71$^{b(AB)}$ |
| C | m5 | 0±0$^{a(A)}$ | 76.99±20.73$^{a(C)}$ | 3.02±0.41$^{b(AB)}$ | 0.24±0.11$^{a(B)}$ |
| D | m0 | 0.24±0.03$^{ab(A)}$ | 207.24±16.89$^{a(A)}$ | 2.77±0.25$^{a(B)}$ | 1.08±0.18$^{a(A)}$ |
| D | m1 | 0.62±0.09$^{a(A)}$ | 199.19±13.91$^{a(A)}$ | 4.65±1.16$^{a(B)}$ | 3.65±0.26$^{ab(A)}$ |
| D | m3 | 0.02±0.05$^{c(A)}$ | 146.41±21.94$^{cd(B)}$ | 14.25±2.16$^{a(A)}$ | 2.32±1.03$^{ab(AB)}$ |
| D | m5 | 1.3±1.57$^{a(A)}$ | 122.89±6.76$^{a(B)}$ | 4.66±0.23$^{a(B)}$ | 3.48±1.91$^{a(A)}$ |
| E | m0 | 0.01±0.02$^{ab(B)}$ | 195.67±28.47$^{a(A)}$ | 2.8±0.73$^{a(B)}$ | 2.97±4.16$^{a(A)}$ |
| E | m1 | 0.41±0.05$^{ab(A)}$ | 187.98±9.82$^{a(A)}$ | 4.57±1.18$^{a(A)}$ | 2.02±0.6b(A) |
| E | m3 | 0.06±0.06$^{bc(B)}$ | 175.67±14.85$^{abcd(A)}$ | 6.63±3.19$^{b(A)}$ | 3.81±0.34a(A) |
| E | m5 | 0.05±0.04$^{a(B)}$ | 111.82±27.36$^{a(B)}$ | 3.09±0.21$^{b(B)}$ | 2.03±0.41$^{a(A)}$ |
| F | m0 | 0.2±0.2$^{ab(AB)}$ | 224.85±30.67$^{a(A)}$ | 3.74±$^{0.87(B)}$ | 2.06±1.17$^{a(AB)}$ |
| F | m1 | 0.59±0.18$^{a(A)}$ | 180.98±3.87$^{a(A)}$ | 4.48±$^{0.45a(AB)}$ | 0.84±0.96$^{b(B)}$ |
| F | m3 | 0.11±0.01$^{abc(B)}$ | 174.84±6.42$^{abcd(A)}$ | 6.23±$^{1.08b(A)}$ | 3.28±0.34$^{a(A)}$ |
| F | m5 | 0.85±0.24$^{a(A)}$ | 91.52±23.23$^{a(B)}$ | 3.77±$^{1.26a(B)}$ | 2.4±0.80$^{a(AB)}$ |
| G | m0 | 0.32±0.09$^{a(B)}$ | 214.98±12.18$^{a(A)}$ | 3.35±0.45$^{a(B)}$ | 2.51±0.63$^{a(A)}$ |
| G | m1 | 0.6±0.15$^{a(A)}$ | 186.47±4.49$^{a(A)}$ | 4.29±2.17$^{a(AB)}$ | 2.6±1.09$^{ab(A)}$ |
| G | m3 | 0.08±0.06$^{abc(C)}$ | 155.42±6.34$^{bcd(A)}$ | 6.24±2.58$^{a(A)}$ | 3.35±0.50$^{a(A)}$ |
| G | m5 | 0.04±0.06$^{a(C)}$ | 108.52±27.36$^{a(B)}$ | 4.44±2.38$^{ab(AB)}$ | 1.58±1.60$^{a(B)}$ |
| H | m0 | 0.16±0.22$^{ab(B)}$ | 200.92±36.11$^{a(A)}$ | 3.79±1.39$^{a(A)}$ | 1.97±0.3$^{a(A)}$ |
| H | m1 | 0.63±0.15$^{a(A)}$ | 179.21±2.29$^{a(A)}$ | 4.13±1.9$^{a(A)}$ | 3.31±1.8$^{ab(A)}$ |
| H | m3 | 0.06±0.06$^{bc(B)}$ | 178.35±17.$^{65abcd(A)}$ | 5.62±3.49$^{b(A)}$ | 3.43±0.58$^{a(A)}$ |
| H | m5 | 0±0$^{a(B)}$ | 108.07±19.91$^{a(B)}$ | 2.87±2.11$^{ab(A)}$ | 5.53±7.87$^{a(A)}$ |
| I | m0 | 0±0$^{b(A)}$ | 214.09±24.32$^{a(AB)}$ | 4.35±2.99$^{a(A)}$ | 2.24±0.54$^{a(B)}$ |
| I | m1 | 0±0$^{b(A)}$ | 209.09±14.8$^{a(A)}$ | 12.12±5.85$^{a(A)}$ | 5.53±0.82$^{a(A)}$ |
| I | m3 | 0.18±0.13$^{ab\ (A)}$ | 157.2±10.91$^{bcd(B)}$ | 6.78±4.54$^{b(A)}$ | 4.12±1.20$^{a(A)}$ |
| I | m5 | 0.66±0.27$^{a(A)}$ | 87.01±16.87$^{a(C)}$ | 5.15±1.52$^{a\ (A)}$ | 2.12±0.65$^{a(B)}$ |
| J | m0 | 0±0$^{b(B)}$ | 222.84±16.17$^{a(A)}$ | 3.74±2.71$^{b(A)}$ | 1.22±1.54$^{a(B)}$ |
| J | m1 | 0.26±0.2$^{ab(A)}$ | 213.49±34.12$^{a(A)}$ | 6.41±4.81$^{(A)}$ | 2.59±0.92$^{ab(B)}$ |
| J | m3 | 0.21±0.06$^{a\ (B)}$ | 144.51±21.08$^{d(B)}$ | 7.13±3.22b$^{(A)}$ | 5.11±0.52$^{a(A)}$ |
| J | m5 | 0±0$^{a(B)}$ | 123.31±16.07$^{a(B)}$ | 6.36±1.47$^{a(A)}$ | 3.38±0.81$^{a(AB)}$ |

*Salt substitute treatments with the same lowercase letters are not significantly different (P > 0.05)

ϕ Months with the same capitalized letters within parenthesis are not significantly different within the same treatment (P > 0.05)

After five months, SAFA of all the cheese treatments prepared with the salt substitute formulations decreased drastically. These reductions were significant from either months "1", "3" or "5". At the fifth month, the data ranged from 76.96 mg/g in formulation C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) to 122.89 mg/g in formulation D (45 % NaCl, 45% KCl, 10 % Glycine); however, significant differences were not found among the treatments. Formulations D and C had less than 5% of difference in the amounts of Sodium Chloride used to prepare the cheeses.

The amounts of Monounsaturated Fatty Acids (MUFA) found in the treatments were really low compared to the amount of SFA quantified. Initially the data ranged from 1.24 to 4.35 in formulations B (45 % NaCl, 55 % KCl, 5% Glycine) and E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine), and no significant differences were found at month "0". The final amounts of MUFA were not different from the initial amounts in any of the formulations evaluated, neither was in the control. The final range of MUFA was 2.13 – 4.66 mg/g corresponding to formulations B and D (45 % NaCl, 45% KCl, and 10 % Glycine). The initial total range of PUFA was 0.99 – 2.97 mg/g corresponding to from formulations B and E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine), and no differences were found after performing the Tukey's procedure. After five months only one of the formulations showed a decrease in its amounts of total PUFA; this was formulation G (37.5 % NaCl, 52.5% KCl, 10 % Glycine). The final range was 0.24 – 5.53 mg/g for formulations C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine), but differences were not found among the final PUFA amounts at month 5.

The total amount of fatty acids was calculated by sum of the individual compounds (Table 28). The initial range varied from 206.73 to 239.97 mg/g in formulations H (30 % NaCl, 65 % KCl, 5% Glycine) and A (100% NaCl).

Over the ripening time a significant decrease in the total amount of fatty acids was observed in each of the ten formulations, especially because of the reduction in saturated fatty acids, more importantly (C:14 and C:16), the most abundant in the mix. After the five months of ripening, the total fatty acids range was 20.25 – 130.83 from formulations B (45 % NaCl, 55 % KCl, 5% Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine).

According to Banks et al. (1989), the major components present in the cheese fat are saturated fatty acids, specially short chain fatty acids. Their study presents the results in molar percentage alluding difficulties to quantify reproducibly the estimate of various constituents. In their study the major fatty acids present were decanoic, octanoic, hexanoinc and hexadecanoic, which also were the main constituents of the fatty acid profile after five months in all of our treatments; However Banks' results are different in certain ways since hexadecanoic (palmitic) and tetradecanoic (myristic) were not the most abundant as it was in this study.

Concerning the decanoic acid, this compound was highly present at the beginning of the ripening time in the low sodium cheddar cheese (including the control) , but decreased with time, while in Banks' study the final product still had high percentages of decanoic acid. Degradation of the fatty acids could be the main source of differences, since also the data of Bank et al. (1989), also differed from the fatty acids profile of raw milk suggesting that the temperature/ pH conditions reached in the cheddar cheese manufacturing and ripening altered the profile. In Table 29, a comparison between their profiles is shown.

**Table 28.** Effect of salt replacement and ripening time on the fatty acids profile of cheddar cheese (mg/g).

| Treat | Month | Total Fatty Acids |
|---|---|---|
| A | m0 | $239.97 \pm 11.26^{a(A)}$ |
| A | m1 | $208.95 \pm 15.8^{a(B)}$ |
| A | m3 | $200.14 \pm 16.23^{a(B)}$ |
| A | m5 | $98.92 \pm 23.88^{a(C)}$ |
| B | m0 | $219.48 \pm 39.63^{a(A)}$ |
| B | m1 | $118.33 \pm 56.71^{b(B)}$ |
| B | m3 | $196.08 \pm 9.06^{a(A)}$ |
| B | m5 | $80.48 \pm 48.1^{a(B)}$ |
| C | m0 | $196.89 \pm 10.55^{a(A)}$ |
| C | m1 | $146.24 \pm 8.95^{b(B)}$ |
| C | m3 | $169.47 \pm 10.58^{b(B)}$ |
| C | m5 | $80.25 \pm 12.45^{a(C)}$ |
| D | m0 | $211.81 \pm 24.2^{a(A)}$ |
| D | m1 | $207.49 \pm 8.35^{a(A)}$ |
| D | m3 | $162.98 \pm 10.79^{b(B)}$ |
| D | m5 | $130.87 \pm 11.59^{a(C)}$ |
| E | m0 | $201.38 \pm 28.95^{aA)}$ |
| E | m1 | $194.57 \pm 9.07^{a(A)}$ |
| E | m3 | $186.24 \pm 14.16^{ab(A)}$ |
| E | m5 | $116.78 \pm 10.36^{a(B)}$ |
| F | m0 | $230.65 \pm 42.41^{a(A)}$ |
| F | m1 | $185 \pm 10.98^{a(AB)}$ |
| F | m3 | $183.78 \pm 1.56^{ab(B)}$ |
| F | m5 | $97.69 \pm 18.01^{a(C)}$ |
| G | m0 | $220.68 \pm 25.54^{a(A)}$ |
| G | m1 | $193.08 \pm 6.94^{a(A)}$ |
| G | m3 | $165.0 \pm 9.19^{b(B)}$ |
| G | m5 | $114.07 \pm 7.17^{a(C)}$ |
| H | m0 | $206.73 \pm 37.51^{a(A)}$ |
| H | m1 | $186.65 \pm 21.64^{a(A)}$ |
| H | m3 | $187.4 \pm 21.65^{ab(A)}$ |
| H | m5 | $92.1 \pm 15.23^{a(A)}$ |
| I | m0 | $220.68 \pm 34.9^{a(A)}$ |
| I | m1 | $226.43 \pm 26.69^{a(A)}$ |
| I | m3 | $168.1 \pm 24.65^{b(B)}$ |
| I | m5 | $89.02 \pm 6.17^{a(C)}$ |
| J | m0 | $207.73 \pm 18.16^{a(A)}$ |
| J | m1 | $222.58 \pm 10.93^{a(A)}$ |
| J | m3 | $155.33 \pm 12.33^{b(B)}$ |
| J | m5 | $133.69 \pm 14.31^{a(B)}$ |

* Salt substitute treatments with the same lowercase letters are not significantly different ($P > 0.05$)

ϕ Months with the same capitalized letters within parenthesis are not significantly different within the same treatment ($P > 0.05$)

**Table 29.** Reference fatty acids profile (%) of raw milk and cheddar cheese

| | Saturated | | | | | | | | Unsaturated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Carbons | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 16:1 | 18:1 | 18:2 | 18:3 | other |
| Milk | 3 | 2 | 1 | 3 | 4 | 12 | 26 | 11 | 3 | 28 | 2 | 1 | 4 |
| Cheddar | 6.4 | 13 | 18 | 27 | 10 | 7.1 | 5.7 | | | | | | |

Sources: Banks et al., 1989 and Grunstone (1995).

For most of the fatty acids, the results in the low sodium cheddar cheeses (including control) are similar to the raw milk's profile from Grunstone (1995) at month "0" and similar to the cheddar cheese evaluated by Banks (1989).

Fatty acids contribute mostly to the flavor of the cheddar cheese after certain chemical reactions have acted upon them. In undamaged fats, fatty acids are attached to a glycerol molecule forming a triacylglycerol or triglyceride molecule. When a lipase or esterase acts on the bonds between the glycerol and the fatty acids, these are released and become more susceptible to continuing reaction with other agents to produce more volatile compounds that contribute to the flavor of foods. The most important lypolitic agents in cheddar cheese are: milk, rennet, starter culture, secondary starter microorganisms, nonstarter lactic acid bacteria (NSLAB) and, in some cases, exogenous lipase (Collins et al., 2004). The consequent reduction in the original amount of fatty acids in cheese is accompanied with the production of methyl ketones, secondary alcohols, lactones, ethyl esters, aldehydes, acids, and alcohols for which the fatty acids were originally the substrates. Figure 16 shows the possible pathways that lead to the production of flavor compounds from fatty acids in cheese.

The final products originated from the fatty acids proposed by Young (2011) are very similar to the compounds detected by the head space SPTM and GC-MS procedure for volatile compounds described in the previous section. These reactions also help understanding a reduction in some individual and total fatty acids occurring in this study.

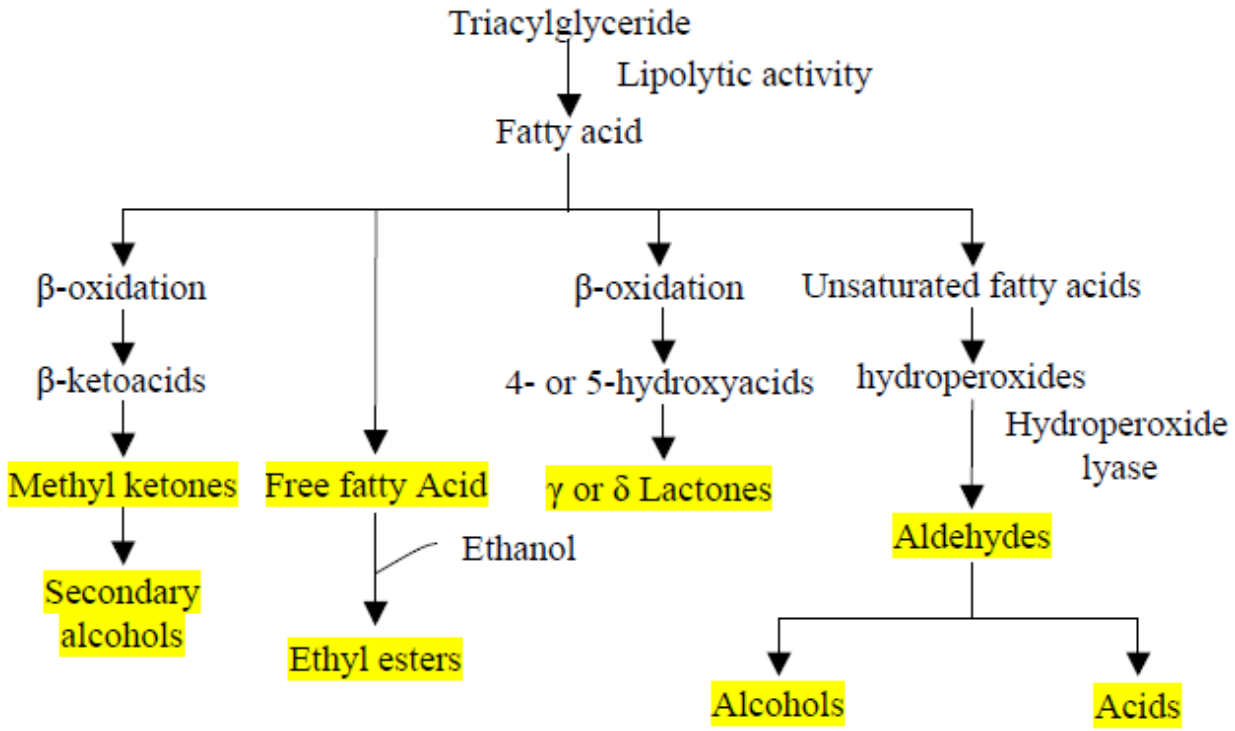



**Figure 16.** Potential pathways of fatty acid flavor development in which highlighted compounds contribute to cheese flavor (Young, 2011)

**4.4 Color**

The color parameters L*, a* and b* were also evaluated at days: 0, 30, 90 and 150 also referred as months: 0, 1, 3 and 5 to describe the ripening time (Figure 17). The initial values of L* had significantly different among several treatments at the beginning of the ripening (month "0").The initial range of L* values was 77.81-85.44 Formulations E, D, and B, C and A were at the top of that range and were significantly more light (white) than the rest of the formulations; these ones have the content of NaCl above 37.5 %.

The cheeses with the lowest L* values were the ones made with formulations J, I,F,G and H; all this formulations contained more than 52.5% of KCl and less than 37.5% of NaCl, while the levels of glycine varied through all the range established (5, 7.5 and 10%).

The differences observed previous to ripening maintained in nine of the ten formulations after one month, and only formulation H became significantly whither than the rest. After five

months of ripening, the control (A, with 100% NaCl) did not change in L* values achieving a final value of 84.78.

Two formulations changed significantly from their initial L* values: H increased in L*, consequently becoming more white, while F became significantly darker, indicated by a reduction in L*values. At the fifth month the range of values was 79.23 - 84.8 corresponding to formulations J and B, respectively. The control (84.78) was only different from formulations F, G, H, I and J which were darker than the control as their particular range of L* values were 79.29 – 80.85.

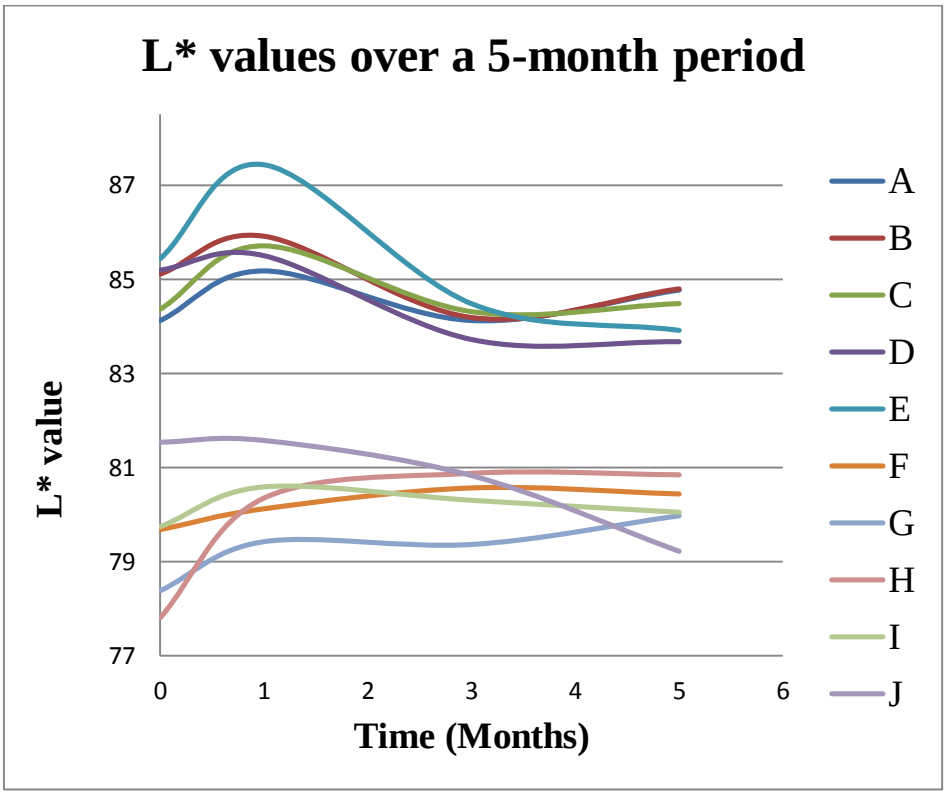



**Figure 17.** Lightness (L*) Values for all 10 Cheeses. The letters A-J correspond to salt substitute formulations.

The a* values that describe the level of green or red that the cheese reflects did not show significant differences at the beginning of the ripening process. The initial range of a* value was 2.68 – 4.77 (appendix D), corresponding to formulations J and B, respectively; however, all the

samples were not different at the start the ripening ($p \geq 0.05$). The ripening time did not have an effect increasing or decreasing of the a* values of the cheddar cheeses made with the 9 salt substitutes; On the Response Surface analysis, an equation to predict the a* values of the cheese could not be created due to the lack of significant parameters. The final range of a* values was 2.5 – 3.03 which shows products tending to reflect wavelengths that are closer to red than to green.

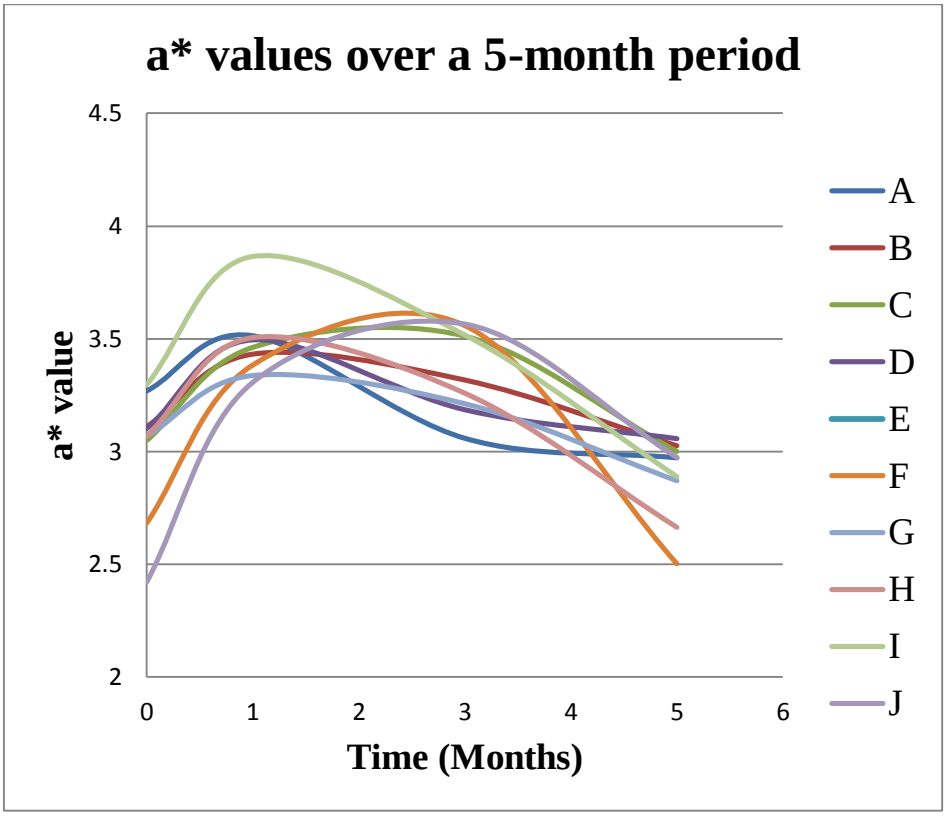



**Figure 18.** Redness (a*) Values for all 10 Cheeses. The letters A-J correspond to salt substitute formulations.

The b* values, that describe color of the cheese between blue and yellow, had an initial range of 18.70 – 19.91, corresponding to formulations C and I respectively; however no difference were found among the treatments at the beginning of the ripening process ($p > 0.05$). The time of ripening affected the b* color of several formulations excluding the control, in every case the b* increased. The only formulations not showing a significant increase in color by the

fifth month of ripening were: B, D, and E. The final range of b* values was 19.17- 22.87. The formulations J, F and I had: the highest values : 22.87, 22.43 and 22.34 respectively and were significantly different from the formulations with more than 41.25% of NaCl.

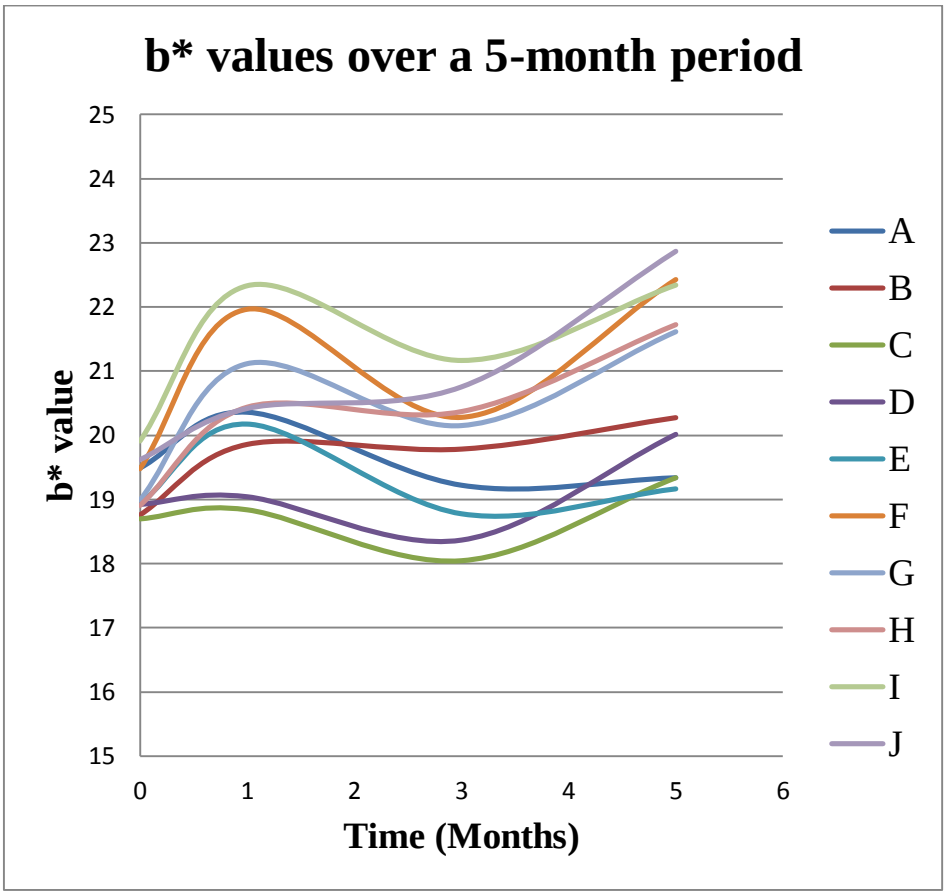



**Figure 19.** Yellowness (b*) Values for all 10 Cheese. The letters A-J correspond to salt substitute formulations.

**4.5 Water Activity.**

Water activity was also measured at the same time color was measured (Figure 20). The initial rage of $a_w$ was 0.971 – 0.996, corresponding to formulations E and H respectively, this initial range of $a_w$ is high compared to the norm, value of cheddar cheese, e.g., close to 0.96. During the ripening time, every cheese product reduced its water activity to a range of values between 0.964 and 0.98. The lowest water activity value corresponded to the control

(formulation A, 100 % NaCl) while the highest water activity corresponded to formulation E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine).

The formulations with the lowest $a_w$ that were not significantly different from the control, were B (45 % NaCl, 55 % KCl, 5% Glycine), C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), D (45 % NaCl, 45% KCl, 10 % Glycine), F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine), and J (30 % NaCl, 60 % KCl, 10 % Glycine). None of these formulations had an $a_w$ higher than 0.971, while the rest of the formulations were significantly higher in $a_w$. The fact that a single formulation with 70% less salt was not different in $a_w$ compared to a formulation with 100% NaCl, is an important step for the preservation of the cheese using KCl and to obtain high quality low sodium cheddar cheese.

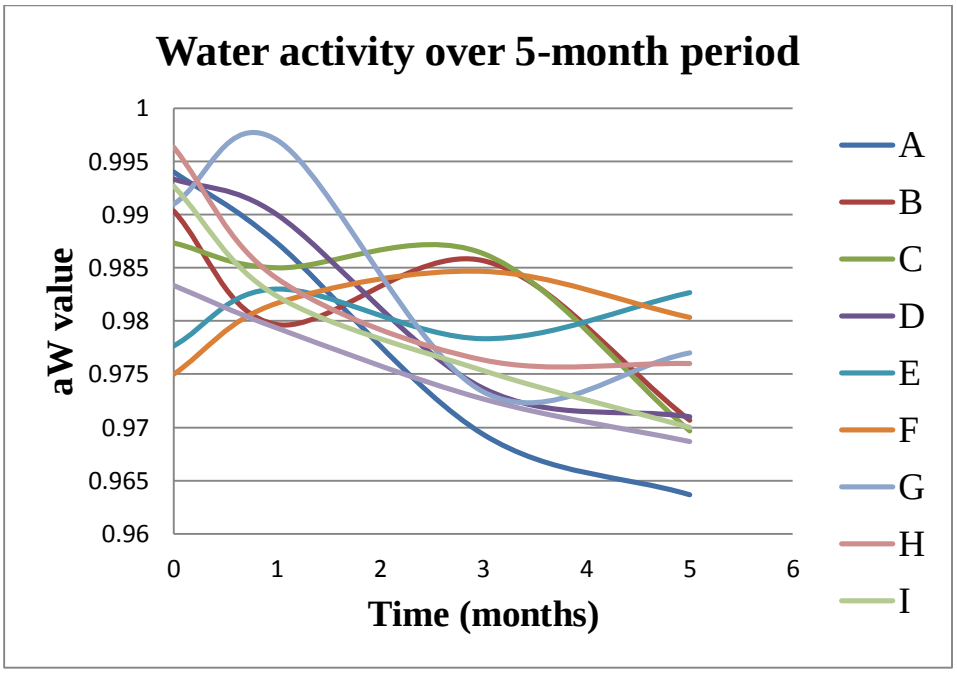



**Figure 20.** Water Activity values for all 10 Cheeses. The letters A-J correspond to salt substitute formulations.

The final $a_w$ value of the control agreed with Grummer et al. (2013), who reported the value above 0.96 for the control while the formulations with KCl were higher than that.

### 4.6 Texture Profile Analysis

Tables 30 and 31 show the Texture Profile Analysis results at every month of ripening. Freezing was avoided and every analysis was performed the day of specific time was reached (0 day, one month, three months or five months).

#### 4.6.1 Hardness

At zero month of ripening, no differences in hardness were found among the cheeses manufactured with the 10 formulations including the control. The initial range of hardness was 118.12 – 141.32 N corresponding to formulations J (30 % NaCl, 60 % KCl, and 10 % Glycine) and H (30 % NaCl, 65 % KCl, and 5% Glycine) which have very similar composition in the mixture design.

Over the time every single formulation became softer as the Hardness range was reduced to 65.28 – 124.56 N, in which the control had the lowest hardness along with formulation E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine) that obtained 66.34 N. The hardest sample was formulation F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine), tied with formulation I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) with the hardness of 122.51 N. These two formulations were almost twice as hard as the control in instrumental texture at the end of the ripening time and they both had less than 62.5% of sodium; however, formulation E has the same level of salt substitution as formulation F but the difference to the control was barely more than 1N.

#### 4.6.2 Adhesiveness

The initial measurements of adhesiveness (g*sec) showed that no difference existed among the cheeses prepared with the salt substitute formulations. The values are shown as negative values (Table 30); when the absolute value of the sample is higher more force of the attachment was imparted by the cheese.

**Table 30.** Effect of salt replacement and ripening time on the texture profile analysis (TPA) of cheddar cheese.

| Treat | Moth | Hardness (N) | Adhesiveness | Resilience | Cohesiveness |
|---|---|---|---|---|---|
| A | 0 | 111.93±15.43$^{a(A)}$ | -79.72±74.84$^{a(A)}$ | 22.54±0.45$^{b(AB)}$ | 0.55±0.03$^{a(AB)}$ |
| A | 1 | 95.14±0.21$^{ab(AB)}$ | -65.59±15.96$^{a(A)}$ | 23.91±0.4$^{b(A)}$ | 0.59±0.03$^{b(A)}$ |
| A | 3 | 80.87±5.21$^{b(BC)}$ | -87.18±58.85$^{a(A)}$ | 17.29±0.54$^{bc(BC)}$ | 0.44±0.02$^{bc(BC)}$ |
| A | 5 | 65.28±13.97$^{c(C)}$ | -50.63±36.54 $^{a(A)}$ | 16.31±4.5$^{b(C)}$ | 0.39±0.09$^{b(C)}$ |
| B | 0 | 121.34±20.63$^{a(A)}$ | -29.99±26.39$^{a(A)}$ | 22.02±1.47$^{cb(A)}$ | 0.52±0.05$^{cb(A)}$ |
| B | 1 | 104.16±6.16$^{ab(A)}$ | -111.84±80.7$^{a(AB)}$ | 20.28±1.84$^{bc(AB)}$ | 0.46±0.03$^{c(A)}$ |
| B | 3 | 86.07±4.92$^{b(A)}$ | -28.01±15.08$^{a(A)}$ | 18.75±3.2$^{bc(AB)}$ | 0.43±0.05$^{bc(AB)}$ |
| B | 5 | 96.71±21.11$^{abc(A)}$ | -151.4±17.97$^{b(B)}$ | 15.9±1.19$^{b(B)}$ | 0.39±0.02$^{b(B)}$ |
| C | 0 | 116.16±5.27$^{a(A)}$ | -102.15±62.47$^{a(A)}$ | 18.21±1.55$^{cd(A)}$ | 0.43±0.03$^{c(A)}$ |
| C | 1 | 97.73±10.04$^{ab(A)}$ | -64.62±47.51$^{a(A)}$ | 17.64±1.07$^{c(A)}$ | 0.42±0.02$^{c(AA)}$ |
| C | 3 | 92.54±10.63$^{b(A)}$ | -22.68±9.86$^{a(A)}$ | 17.48±1.27$^{cb(A)}$ | 0.43±0.04$^{bc(A)}$ |
| C | 5 | 84.65±18.6$^{bc(A)}$ | -36.47±12.37$^{a(A)}$ | 11.08±0.19$^{b(B)}$) | 0.36±0.01$^{b(A)}$ |
| D | 0 | 119.56±20.4$^{a(A)}$ | -36.28±25.44$^{a(A)}$ | 17.63±2.67$^{d(A)}$ | 0.41±0.11$^{c(A)}$ |
| D | 1 | 116.92±18.24$^{a(A)}$ | -38.78±13.92$^{a(A)}$ | 17.02±0.97$^{c(A)}$ | 0.42±0.02$^{c(A)}$ |
| D | 3 | 80.54±17.1b$^{(A)}$ | -16.05±12.42$^{a(A)}$ | 13.64±1.03$^{c(A)}$ | 0.33±0.02$^{d(A)}$ |
| D | 5 | 82.4±5.9$^{bc(A)}$ | -42.94±0.83$^{a(A)}$ | 17.18±2.94$^{b(A)}$ | 0.36±0.05$^{b(A)}$ |
| E | 0 | 129.98±11.06$^{a(A)}$ | -73.67±21.7$^{a(A)}$ | 18.5±1.41$^{cbd(A)}$ | 0.44±0.04$^{cb(A)}$ |
| E | 1 | 102.72±11.59$^{ab(B)}$ | -111.01±57.16$^{a(A)}$ | 17.67±2.34$^{c(A)}$ | 0.4±0.05$^{a(A)}$ |
| E | 3 | 97.37±6.59$^{ab(B)}$ | -61.43±67.3$^{a(A)}$ | 14.67±3.01$^{bc(A)}$ | 0.34±0.08$^{cd(A)}$ |
| E | 5 | 66.34±11.17$^{c(C)}$ | -26.53±13.32$^{a(A)}$ | 13.79±2.67$^{b(A)}$ | 0.31±0.06$^{bc(A)}$ |
| F | 0 | 131.97±18.44$^{a(A)}$ | -92.29±67.46$^{a(A)}$ | 32.97±1.2$^{a(A)}$ | 0.76±0.02$^{a(A)}$ |
| F | 1 | 111.24±12.64$^{ab(AB)}$ | -48.28±6.56$^{a(A)}$ | 33.35±1.77$^{a(A)}$ | 0.75±0.02$^{a(A)}$ |
| F | 3 | 92.24±7.79$^{b(B)}$ | -42.89±23.24$^{a(A)}$ | 32.19±1.35$^{a(A)}$ | 0.75±0.02$^{a(A)}$ |
| F | 5 | 124.56±14.53$^{a(AB)}$ | -25.83±6.17$^{a(A)}$ | 30.65±1.44$^{a(A)}$ | 0.7±0.02$^{a(A)}$ |
| G | 0 | 139.72±2.09$^{a(A)}$ | -52.34±21.1$^{a(A)}$ | 31.82±1.04$^{a(A)}$ | 0.74±0.01$^{a(AB)}$ |
| G | 1 | 109.79±8.15$^{ab(B)}$ | -95.18±56.26$^{a(A)}$ | 34.07±2.58$^{a(A)}$ | 0.76±0.02$^{a(A)}$ |
| G | 3 | 129.49±19.93$^{a(B)}$ | -128.16±66.87$^{a(A)}$ | 30.03±0.44$^{a(A)}$ | 0.71±0.01$^{a(BC)}$ |
| G | 5 | 113.45±2.08$^{ab(B)}$ | -67.29±68.1$^{ab(A)}$ | 30.16±1.8$^{a(A)}$ | 0.7±0.02$^{a(C)}$ |
| H | 0 | 141.32±1.3$^{a(A)}$ | -64.97±86.88$^{a(B)}$ | 32.76±1.31$^{a(A)}$ | 0.75±0.02$^{a(A)}$ |
| H | 1 | 106.87±5.08$^{ab(A)}$ | -22.23±20.53$^{a(A)}$ | 36.15±1.41$^{a(A)}$ | 0.77±0.02$^{a(A)}$ |
| H | 3 | 109.42±9.09$^{ab(A)}$ | -75.92±57.65$^{a(AB)}$) | 33.56±1.29$^{a(A)}$ | 0.76±0.01$^{a(A)}$ |
| H | 5 | 128.49±12.44$^{a(A)}$ | -42.35±36.15$^{a(AB)}$ | 32.87±1$^{a(A)}$ | 0.73±0.02$^{a(A)}$ |
| I | 0 | 122.51±6.32$^{a(A)}$ | -72.22±67.5$^{a(A)}$ | 35.56±0.21$^{a(A)}$ | 0.79±0.01a($^{A)}$ |
| I | 1 | 87.81±3.34$^{b(C)}$ | -77.2±64.89$^{a(A)}$ | 36.14±1.05$^{a(A)}$ | 0.79±0.01$^{a(A)}$ |
| I | 3 | 94.89±2.93$^{b(C)}$ | -82.43±64.64$^{a(A)}$ | 34.23±1.47$^{a(A)}$ | 0.78±0.01$^{a(A)}$ |
| I | 5 | 107.45±5.59$^{ab(B)}$ | -15.47±14.54$^{a(A)}$ | 33.43±1.01$^{a(A)}$ | 0.74±0.01$^{a(B)}$ |
| J | 0 | 118.13±9.72$^{a(A)}$ | -46.47±14.84$^{a(A)}$ | 34.85±1.23$^{a(A)}$ | 0.79±0.02$^{a(A)}$ |
| J | 1 | 100.25$^{b(A)}$ | -48.16±12.66$^{a(A)}$ | 34.01±1.13$^{a(A)}$ | 0.78±003$^{a(A)}$ |
| J | 2 | 97.23±16.24$^{ab(A)}$ | -56.72±41.98$^{a(A)}$ | 32.61±1.41$^{a(AB)}$ | 0.75±0.02$^{a(AB)}$ |
| J | 3 | 84.51±5.82$^{bc(B)}$ | -38.35±34.74$^{a(A)}$ | 30.5±0.94$^{a(B)}$ | 0.72±0.02$^{a(B)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different (Pr > 0.05).

ϕ Months with the same capitalized letter inside parenthesis are not significantly different within the same treatment (Pr > 0.05). Letters A-J correspond to salt substitute formulations.

At the end of the ripening process, time did not affect the adhesiveness of nine formulations, but only affected formulation B which had the highest adhesiveness at the fifth month (-151.4 g*sec) and it was the only formulation different from other samples. The lowest adhessiveness corresponded to formulation E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine) , with -26.53 g*sec, but this was not different from the rest, except for B (45 % NaCl, 55 % KCl, 5% Glycine).

**4.6.3 Resilience**

IT is the capability of a strained body to recover its size and shape after deformation caused especially by compressive stress, and is measured in percentage (Table 30). The initial range of resilience was 17.63– 35.51. Formulation D (45 % NaCl, 45% KCl, 10 % Glycine) had the lowest resilience while formulation I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) produced the highest. Formulations F (37.5 % NaCl, 55 % KCl, and 7.5 % Glycine), G (37.5 % NaCl, 52.5% KCl, and 10 % Glycine) and J (30 % NaCl, 60 % KCl, and 10 % Glycine) were not significantly different from formulation I, but were significantly higher than the rest including the control; all this formulations obtained initial resilience above 31%.

The ripening time reduced the resilience of the control, and formulations: B (45 % NaCl, 55 % KCl, 5% Glycine), C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine) either at the first, third of fifth month. Formulation B was not significantly different from A, neither at the beginning of the ripening, nor at the end.

**4.6.4 Cohesiveness**

Cohesiveness is a unit-less value between 0 and 1, obtained from the relationship between the areas of the second cycle or “bite” and the first one. The initial cohesion ratio ranged from 0.41 – 0.79, corresponding to formulations D and I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) or J (30 % NaCl, 60 % KCl, 10 % Glycine). The control having a higher cohesion ratio

than them (0.55), was only significantly different from formulations B (45 % NaCl, 55 % KCl, 5% Glycine), C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), D (45 % NaCl, 45% KCl, 10 % Glycine) and E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine), having a higher cohesion ratio than them (0.55). After five months of ripening, the cohesiveness decreased significantly in formulations J, F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine) and B (45 % NaCl, 55 % KCl, 5% Glycine), but in the remaining cheeses the reduction was not significant. The formulations which had the highest cohesiveness at the beginning of the study remained as the highest while the control or formulation A, was significantly lower than those at the top of the range ( the final range was 0.31- 0.73). The very bottom of the range corresponded to formulation E, which was lower than other formulations of cheese in terms of cohesiveness.

**4.6.5 Springiness**

Springiness is the capacity of a product to spring back after a given deformation in the second cycle of the TPA. The initial range of Springiness (%) was 80.72 – 92.62 where the control had the lowest value and formulation I (33.75 % NaCl, 58.75 % KCl, and 7.5 % Glycine) had the highest value but no significant differences were found among the treatments at month zero (Table 31). Over the time the reduction in springiness was noted since only formulations C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine), D (45 % NaCl, 45% KCl, 10 % Glycine) and E (37.5 % NaCl, 57.5 % KCl, 5 % Glycine) were had not significantly different in terms of springiness in either one, three or five months; nevertheless springiness reduced in at least 7%. The final range of springiness was 61.24 – 86.59% in which the lowest was observed in formulation B (45 % NaCl, 55 % KCl, 5% Glycine) which was significantly lower than the rest, while the highest was observed in J (30 % NaCl, 60 % KCl, 10 % Glycine); along with J, formulations H and I had the Highest springiness over time and, even after significant reductions they all had springiness values above 84.53 %

**Table 31.** Effect of salt replacement and ripening time on the texture profile analysis (TPA) of cheddar cheese.

| Treat | Moth | springiness | gumminess | Chewiness |
|---|---|---|---|---|
| A | 0 | 80.78±3.83$^{a(A)}$ | 61.75±7.77$^{bc(A)}$ | 49.69±3.91$^{bc(A)}$ |
| A | 1 | 81.34±3.61$^{ab(A)}$ | 56.53±3.08$^{bc(A)}$ | 46±3.61b$^{c(A)}$ |
| A | 3 | 75±3.02$^{bc(AB)}$ | 35.91±3.32$^{c(B)}$ | 26.93±2.73$^{b(B)}$ |
| A | 5 | 67.1±2.97$^{ab(B)}$ | 26.4±10.34$^{c(B)}$ | 17.91±7.58$^{c(B)}$ |
| B | 0 | 85.43±6.66$^{a(A)}$ | 63.79±15.89$^{bc(A)}$ | 55.13±17.98$^{bc(A)}$ |
| B | 1 | 80.24±3.85$^{ab(A)}$ | 48.16±5.84$^{c(AB)}$ | 38.56±3.92$^{c(AB)}$ |
| B | 3 | 75.45±7.95$^{abc(AB)}$ | 36.63±3.89$^{c(B)}$ | 27.81±5.39$^{b(B)}$ |
| B | 5 | 61.24±4.42$^{b(B)}$ | 37.7±7.46$^{c(B)}$ | 22.93±3.39$^{c(B)}$ |
| C | 0 | 80.03±5.82$^{a(A)}$ | 49.98±1.32$^{c(A)}$ | 40.05±4$^{c(A)}$ |
| C | 1 | 75.94±2.95$^{b(A)}$ | 41.59±5.95$^{c(A)}$ | 31.7±5.66$^{c(A)}$ |
| C | 3 | 75.75±2.13$^{abc(A)}$ | 40.2±7.67$^{c(A)}$ | 30.46±5.94$^{b(AB)}$ |
| C | 5 | 71.04±19.62$^{ab(A)}$ | 21.89±4.3$^{c(B)}$ | 16±7.19$^{c(B)}$ |
| D | 0 | 87.25±9.24$^{a(A)}$ | 48.7±19.74$^{c(A)}$ | 41.57±14.94$^{c(A)}$ |
| D | 1 | 80.49±10.68$^{ab(A)}$ | 48.64±10.23$^{c(A)}$ | 38.65±6.59$^{c(A)}$ |
| D | 3 | 71.68±12.79$^{c(A)}$ | 26.3±6.72$^{c(A)}$ | 19.22±7.56$^{b(A)}$ |
| D | 5 | 70.58±5.11$^{ab(A)}$ | 31.1±0.41$^{c(A)}$ | 16.41±7.73$^{c(A)}$ |
| E | 0 | 90.43±4.5$_{a(A)}$ | 57.77±8.92$_{bc(A)}$ | 52.03±6.53$_{bc(A)}$ |
| E | 1 | 79.53±7.86$_{ab(A)}$ | 41.22±9$_{c(AB)}$ | 33.08±9.82$_{c(AB)}$ |
| E | 3 | 72.79±1.86$_{c(A)}$ | 33.27±10.02$_{c(B)}$ | 24.33±7.94$_{b(BC)}$ |
| E | 5 | 72.45±12.77$_{ab(A)}$ | 19.97±7.22$_{c(B)}$ | 13.93±2.96$_{c(C)}$ |
| F | 0 | 88.59±3.71$_{a(AB)}$ | 99.36±12.04$_{a\ (A)}$ | 88.26±13.76a(A) |
| F | 1 | 90.7±0.27$_{a(A\ )}$ | 83.72±8.11$_{a(AB)}$ | 75.92±7.2$_{a(AB)}$ |
| F | 3 | 88.88±0.72$_{ab(AB)}$ | 68.87±4.63$_{b(B)}$ | 61.22±4.2$_{a(B)}$ |
| F | 5 | 83.41±3.59$_{ab(B)}$ | 87.57±10.83$_{a(AB)}$ | 72.79±5.76$_{a(AB)}$) |
| G | 0 | 90.34±0.7$^{a(A)}$ | 108.09±0.9$^{a(A)}$ | 97.65±0.99$_{a(A)}$ |
| G | 1 | 86.01±3.14$^{ab(AB)}$ | 83.49±7.47$^{a(AB)}$ | 71.87±7.99$_{a(B)}$ |
| G | 3 | 85.4±1.07$^{abc(AB)}$ | 92.42±14.14$^{a(AB)}$ | 78.95±12.39$_{a(AB)}$ |
| G | 5 | 84.29±2.63$^{ab(B)}$ | 79.36±3.37$^{ab(B)}$ | 66.94±4.74$_{ab(B)}$ |
| H | 0 | 90.57±1.66$^{a(A)}$ | 106.57±3.22$^{a(A)}$ | 96.55±4.64$_{a(B)}$ |
| H | 1 | 91.31±1.13$^{a(B)}$ | 82.63±5.43$^{a(B)}$ | 75.41±4.23$_{a(A)}$ |
| H | 3 | 89.21±2.57$^{ab(B)}$ | 83.11±7.23$^{ab(B)}$ | 74.05±4.95$_{a(B)}$ |
| H | 5 | 84.53±2.62$^{a(AB)}$ | 93.92±10.45$^{a(AB)}$ | 79.22±6.43$_{a(B)}$ |
| I | 0 | 92.62±2.3$_{a(A)}$ | 97.01±4.46$_{a(A)}$ | 89.91±6.14$_{a(A)}$ |
| I | 1 | 89.44±1.34$_{ab(AB)}$ | 69.23±3.06$_{ab(C)}$ | 61.95±3.59$_{ab(B)}$ |
| I | 3 | 89.35±1.09$_{ab(AB)}$ | 73.59±3.4$_{ab(BC)}$ | 65.75±2.94$_{a(B)}$ |
| I | 5 | 85.45±2.76$_{a(B)}$ | 79.45±3.85$_{ab(B)}$ | 67.95±5.24$_{ab(B)}$ |
| J | 0 | 91.59±1.56$^{a(A)}$ | 85.79±6.56$^{ab(A)}$ | 78.59±6.43$^{ab(A)}$ |
| J | 1 | 88.12±2.25a(AB) | 81.08±4.62a(A) | 74.77±5.21a(A) |
| J | 2 | 89.78±1.28$^{a(AB)}$ | 72.36±10.4$^{ab(AB)}$ | 64.9±8.62$^{a(AB)}$ |
| J | 3 | 86.58±2.19$^{a(B)}$ | 60.57±2.99$^{b(B)}$ | 52.46±3.22$^{b(B)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different (Pr > 0.05).

ϕ Months with the same capitalized letter inside parenthesis are not significantly different within the same treatment (Pr > 0.05). Letters A-J correspond to salt substitute formulations.

### 4.6.6 Gumminess and Chewiness

Gumminess was affected by the salt substitution; the initial data varied from 48.7 N in formulation D (45 % NaCl, 45% KCl, and 10 % Glycine) to 108.09 N in formulation G (37.5 % NaCl, 52.5% KCl, and 10 % Glycine). Two groups were formed after the Tukey procedure was run; the formulations with higher gumminess were F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine), G, H (30 % NaCl, 65 % KCl, 5% Glycine), I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine), and the rest of the formulations had a lower level of substitution, and included the control (Table 31).

The ripening time reduced the chewiness of every formulation, at either month one, three or five. The Tukey's grouping showed three groups of samples, in which the formulations with the highest chewiness scores at the first month remained as the highest after ripening; however the control (100% NaCl) after ripening formed part of the group with the lowest chewiness along with the rest of the formulations with more than 41.35% of NaCl.

The chewiness of the cheese treatments behaved almost identically at the beginning and at the end of the ripening; also the changes observed during ripening time were similar as in gumminess. When the level of substitution was higher, chewiness and gumminess of the samples were also higher.

The texture of the white cheddar cheese was affected by the sodium substitution as described before. Higher percentages of salt replacement produced harder, more chewy and more cohesive cheeses than the control and treatments with lower percentages of substitution. This difference was appreciated in the consumer study. The harder (TPA) samples receiver higher hedonic ratings than the softer samples (TPA) in both softness and overall texture Acceptability.

After evaluating the texture parameters, some tendencies were found. Over the time, the attributes: hardness, chewiness and gumminess suffered significant reduction in every treatment;

this observation is similar to the tendency shown by O'Mahony et al., (2005) who evaluated the proteolysis and texture of the cheddar cheese not using a texture analyzer, but an Instron. Although their range of data for those attributes is higher than any of the low sodium treatments and our control evaluated (95 – 145 N) after 6 months, a similar parameter of reduction was observed in this study.

O'Mahony's results also showed a reduction in cohesiveness, which is also appreciated in this study; although not in all the treatments. Our control did reduce its cohesiveess significantly, joined by other formulations with lower reduction and one with as much as 60 % reduction. The means of cohesiveness for all the formulations evaluated, decreases with time (significantly or not), compared to O'Mahony's results that show most of the cohesiveness reductions being significant ant month 6 (180 days).

Other parameters of the texture profile analysis were not evaluated by O'Mahony et al., (2005) and are not typically described for cheddar cheese. In conclusion, samples with less sodium were harder, more cohesive, with higher values of chewiness (N) than formulations with lower substitution and less KCl.

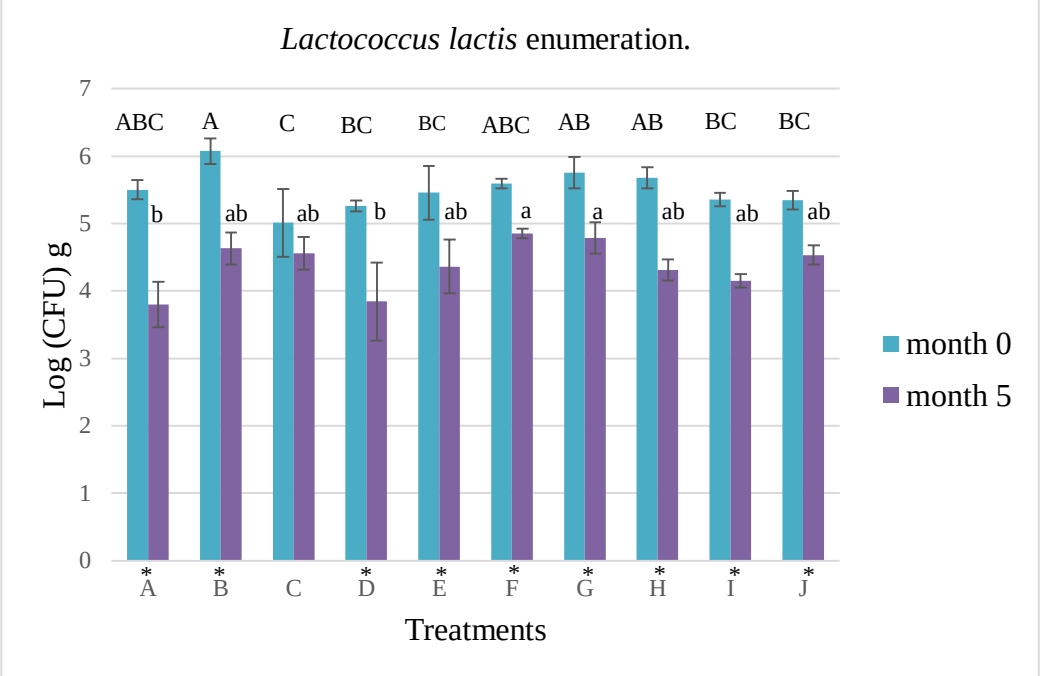
**4.7 Starter Culture enumeration**

According to Fox et al. (2000), *Lactococcus lactis* is a rapid acid producing bacteria with ripening activity and salt sensitivity. It contributes to the formation of cheddar cheese flavors mainly by glycolysis of remnants of lactose and catabolism of lactic acid, catabolism of citrate, lipolysis and catabolism of free fatty acids, and proteolysis and catabolism of amino acids (Young, 2011) and Fox et al. (2000).

The results of the enumeration of *Lactococcus lactis* (both spps. *lactis* and *cremoris*) at month 0 and month 5 are shown in Figure 20. Since the samples were frozen, at both month "0" and month "5", another fresh control sample was manufactured to compare the bacteria counts of

the frozen sample at month "0" with a new sample and evaluate the effects of freezing. Freezing significantly reduced the bacteria counts by 18.18%, (from 6.72±0.25 to 5.50±0.14 log (CFU)/g); however, this reduction is lower than what Carcoba and Rodriguez (2000) reported, revealing that *L. lactis spp. lactis* had a survivability percentage of 44% after freezing without cryoprotectants. Mark and Etzel (1997) reported that *L. lactis spp. cremoris* had survivability rates close to 100% when frozen also at -20ºC. Both studies used specific suspension media containing lactose.

In this study prior to freezing, the cheese samples experienced a "cold shock", since they were kept at 4 ºC for one day for the samples corresponding to month "0" and for five months for the later set of samples. According to Kim et al. (1998), a cold shock of 10ºC for 5 h, improved cell viability of *Lactococcus lactis*.




*Samples marked with the symbol (*) significantly decreased after five months
[A] Samples with different capitalized letters are significantly different (P>0.05) at month zero
[a] Samples with different lowercase letters are significantly different (P>0.05) at month five.
Letters A to J correspond to salt substitute formulations.
**Figure 21.** Enumeration of starter culture bacteria (*Lactococcus lactis*)

At the beginning of the ripening, (month 0 or day 1), the log (CFU)/g of the bacteria ranged from 5.01 to 6.07 corresponding to formulations C (41.25 % NaCl, 51.25% KCl, 7.5 % Glycine) and B (45 % NaCl, 55 % KCl, 5 % Glycine), respectively. Formulation B had significantly higher bacterial counts than four formulations including C, D (45 % NaCl, 45% KCl, 10 % Glycine), I (33.75 % NaCl, 58.75 % KCl, 7.5 % Glycine) and J (30 % NaCl, 60 % KCl, 10 % Glycine); these formulations (D, I and J) were also not different from formulation C.

After five months, the estimated amount of *Lactococcus lactis* significantly decreased in nine formulations including the control. The only sample in which the reduction was not significant was formulation C. The final counts revealed that eight of the nine cheeses made with the salt substitute formulations were significantly higher than the control in log (CFU)/ g. The final range of bacterial counts was 3.80 – 4.85 log (CFU)/ g corresponding to formulations A and F (37.5 % NaCl, 55 % KCl, 7.5 % Glycine). Formulation A along with G had significantly higher counts than the control (A) and formulation D. These two samples, were the only formulations that produced cheeses with *L. lactis* counts below 4 log (Figure 20).

It is reported that substituting NaCl with KCl can cause differences in microbial stability including effects over starter culture and nonstarter lactic acid bacteria (El-Bakry, 2012). The results of this study agree with this statement since during ripening time a difference was evidenced between the cheese made with 100% NaCl and the cheeses containing KCl and Glycine. This pattern in microbial development during the ripening time may contribute to the differences in volatile compounds formation and individual fatty acids degradation previously discussed. The final levels of the bacteria agree with the levels proposed by Beresford and Williams (2004) who suggested that as cheese ripens the counts can be reduced to 4 Log (CFU)/g or $10^4$ CFU/g.

It is important to note that, although, differences exist in starter culture development, volatile contents and fatty acids, and in the sensory acceptance of the products, higher levels of NaCl substitution including formulation J (30% NaCl, 60% KCl and 10% Glycine) had also moderately- high sensory acceptance in most attributes.

According to Fox (1987) the amount of salt influences the post-cheddaring starter activity, controlling the metabolism of lactose and thus the pH of the fresh cheese, which, in turn, affects the rate of maturation and cheese quality. Differences in the amount of *Lactococus lactis* bacteria enumerated after five mounts could induce differences in the maturating rate of the cheddar cheese formulations which could influence the sensory characteristics of the cheese more so than the inherent bitterness of the KCl. The MANOVA analysis showed that overall liking, taste, texture and appearance were the most important sensory attributes contributing to the differences. The review of Singh et al. (2003), remarks that these attributes are also highly influenced by maturation rates and constituent metabolism.

## CHAPTER 6. SUMARY AND CONCLUSIONS

This study included several analysis in order to evaluate the effect of the substitution of common salt (NaCl) in the cheddar cheese formulations with potassium chloride as a salt replacer and Glycine as a bitterness blocker to minimize the wildly known bitterness downside of KCl.

A consumer acceptance study was performed to quantify consumer acceptability and to determine the optimal formulation of a low sodium white cheddar cheese. The study required the manufacturing of ten formulations of cheddar cheese. Every consumer (n = 360) evaluated three of the ten formulations based on a Balanced Incomplete Block Design. The attributes evaluated were: overall appearance, odor/ aroma, saltiness, overall flavor, softness, chewiness, overall texture and overall liking; a 9-point hedonic scale was used to evaluate these attributes. Formulation J (30 % NaCl, 60 % KCl, and 10% Gly) had the highest mean scores in seven of eight sensory attributes. The formulation J did not have a significant increase in purchase intent after additional information about low sodium was given, however other five of the 9 formulations did (excluding the control). The Wilk's Lambda probability ($p < 0.0001$), leads to conclude that a difference existed among all the formulations when all the sensory attributes were simultaneously compared.

The product optimization revealed that cheeses manufactured with salt substitutes containing 30 – 37.5 % of NaCl, 52.5 – 65% of KCl and 5 – 10% of Glycine were acceptable when a cutoff value of 5.5 was set. When the cutoff value was raised to 5.9 the optimal formulation contained 30 % NaCl, 60 % KCl, and 10% Glycine, and we can expect a more acceptable product with those levels of NaCl and KCl but 10% of Glycine is required.

Besides the sensory analysis performed for the white cheddar cheese formulations, certain relevant physicochemical characteristics were also evaluated. The water activity values

experienced a reduction over time until settling on values around 0.96 – 0.97. Most of the formulations of salt substitutes used, produced cheeses with water activity higher than the control.

The color analysis revealed that formulations with less than 37.5 % of NaCl were significantly darker over the time than the rest of the formulations with more salt. The a* values were not affected by salt substitution, neither was by the ripening time. All samples increased in b* values but in three of them the change was not significant (samples with more than 42.25% of NaCl).

The volatiles analysis revealed the presence of alcohols, aldehydes and ketones, which in most cases increased significantly over time. Differences between the formulations and the control were found in several compounds; however, a clear tendency could not be defined. The *L. lactis* enumeration revealed that the control samples had significantly lower counts after five months than two out of the nine salt substitute formulations; nonetheless, the bacterial counts in the control were the lowest among all samples.

The fatty acids profile proved that at the beginning of the ripening time the most common fatty acids in every formulation were capric, myristic and palmitic, while after the ripening time the amounts of capric acid detected were much reduced in every formulation. Differences were found between the control and the cheeses made with the salt substitute formulations, and also among all of them when comparing the individual fatty acids; however the total amount of fatty acids was not different among the treatments at the end of the fifth month.

Formulation J was the only formulation contained in the superimposition of the contour profiles when the cutoff value was raised to 5.9. The effect of the substitution of salt by KCl and Glycine was successfully evaluated. White cheddar cheese, containing up to 70% less salt (60% KCl + 10% glycine) can be acceptable for the consumers.

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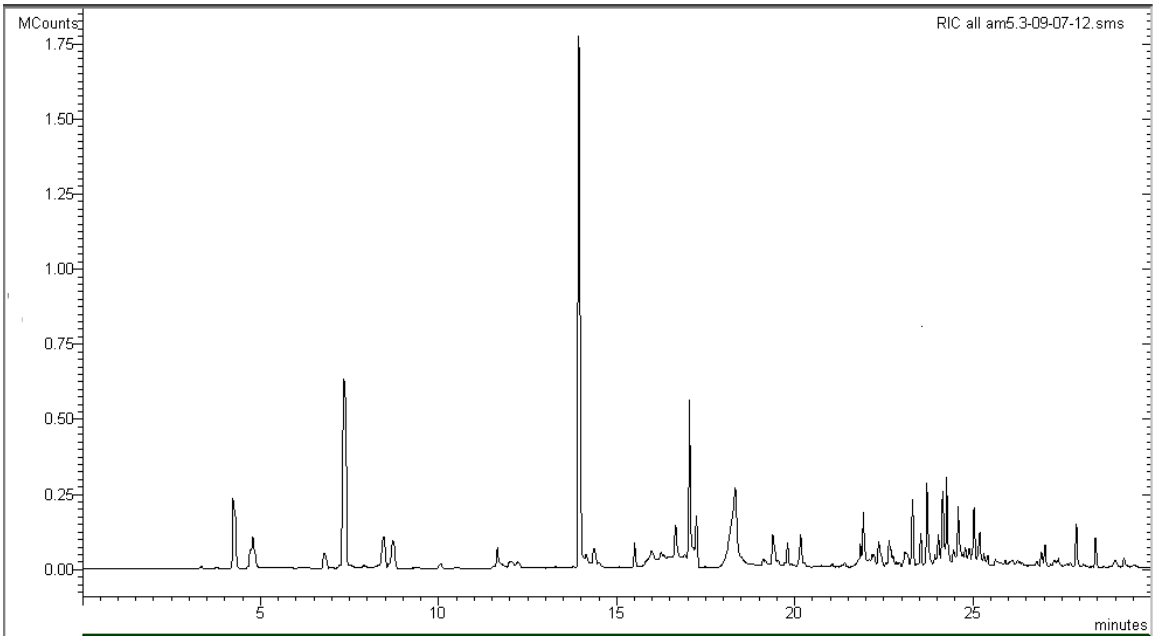
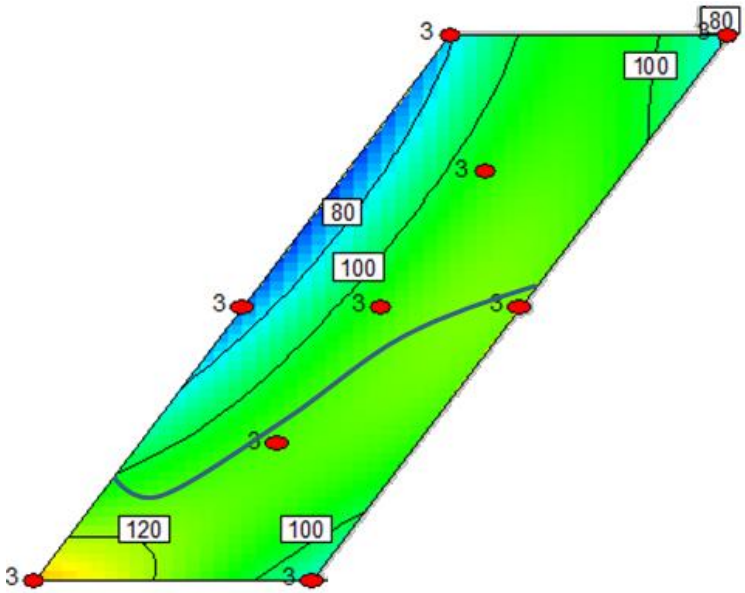
**APPENDIX A. SAMPLE CHROMATOGRAM OF VOLATILES.**



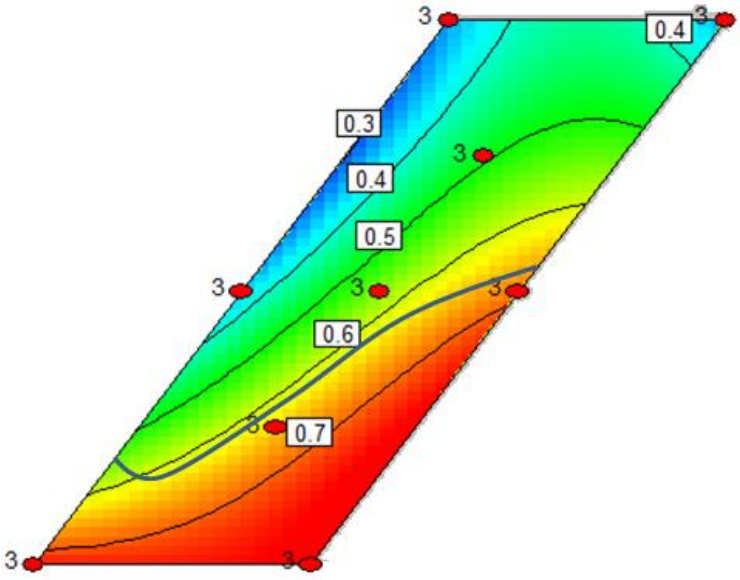

Appendix 1. Sample chromatogram of cheddar cheese volatiles by GC/MS.

## APPENDIX B. CONTOUR PROFILES OF PHYSICOCHEMICAL CHARACTERISTICS

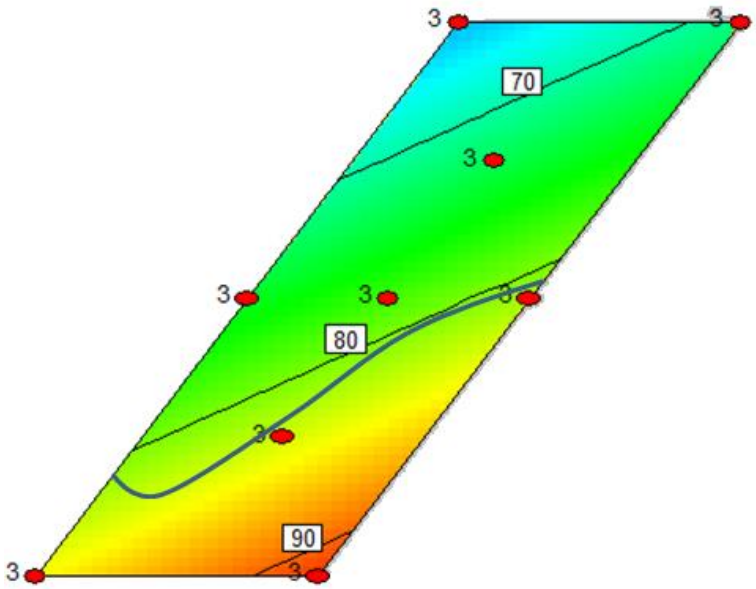
### B.1 Contour profile of hardness (N) of the sensory optimal formulation.



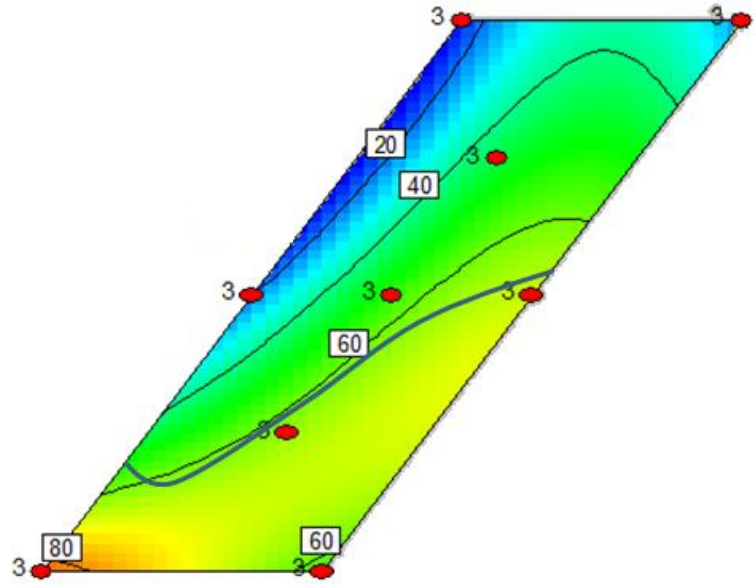

### B.2 Contour profile of Cohesiveness of the sensory optimal formulation.



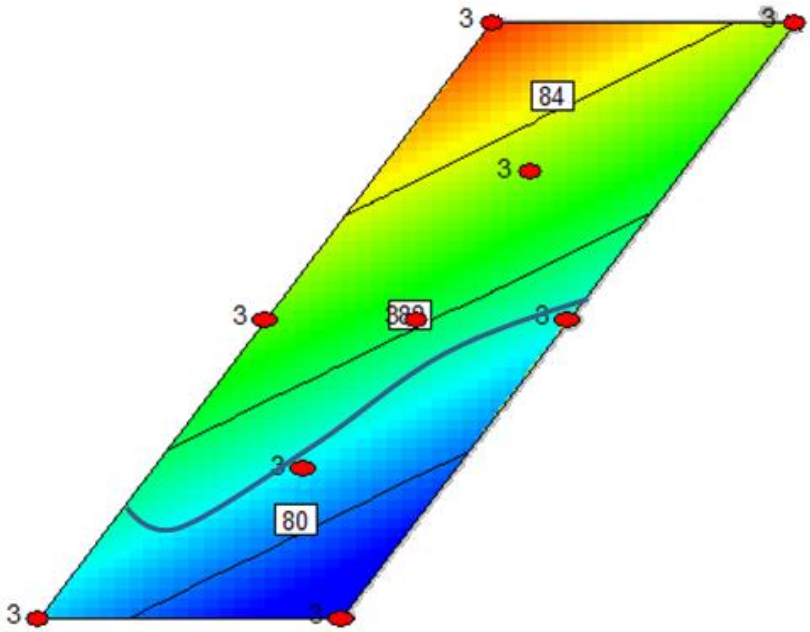
**B.3 Contour profile of springiness (%)of the sensory optimal formulation.**



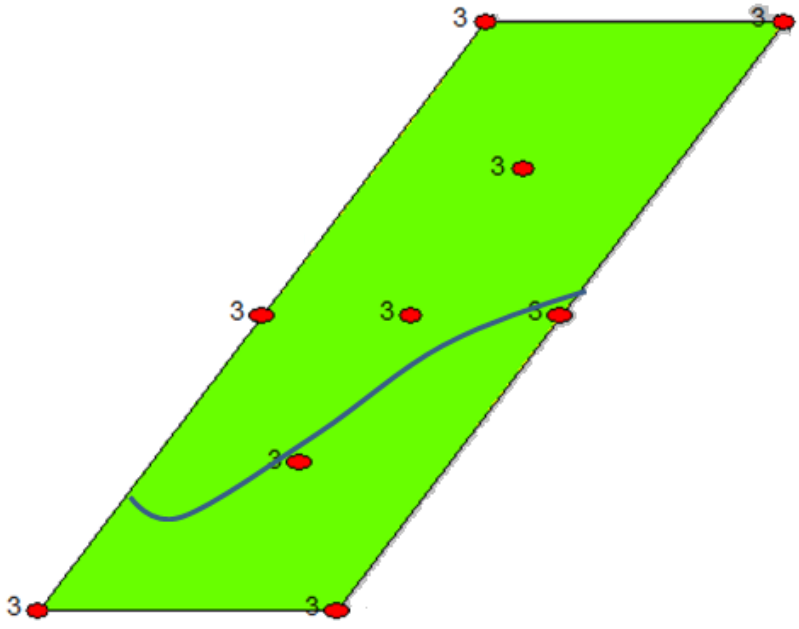

**B.4 Contour profile of chewiness (N) of the sensory optimal formulation.**



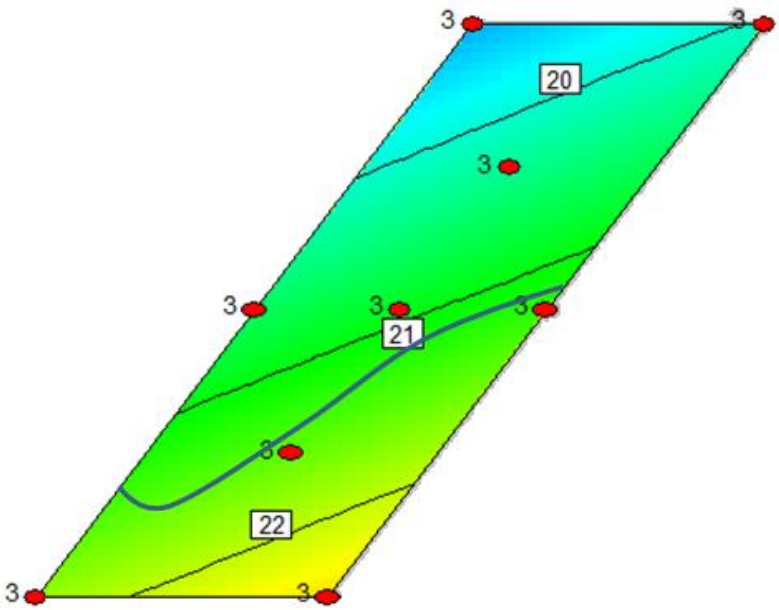
**B.5 Contour profile of L* values of the sensory optimal formulation.**



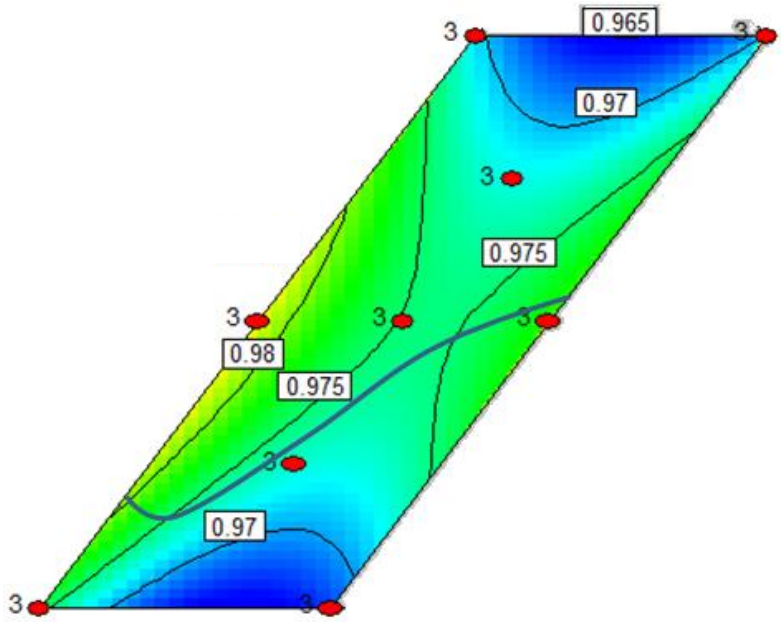

**B.6 Contour profile of a* values of the sensory optimal formulation.**



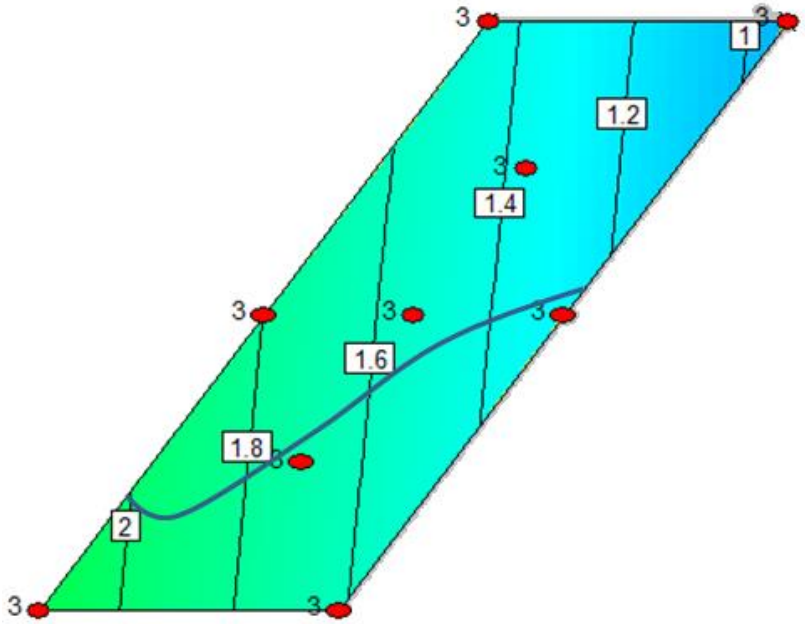
**B.7 Contour profile of b* values of the sensory optimal formulation.**



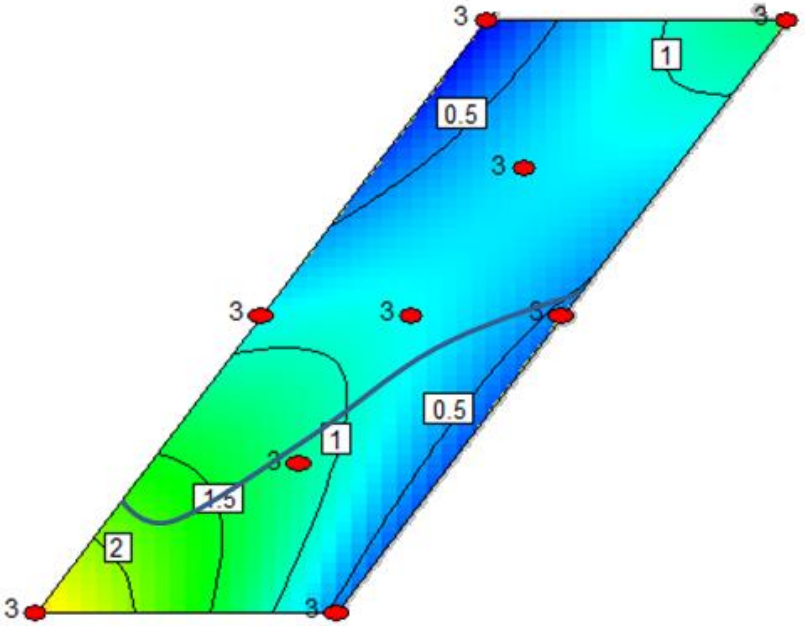

**B.8 Contour profile of Water Activity values of the sensory optimal formulation.**



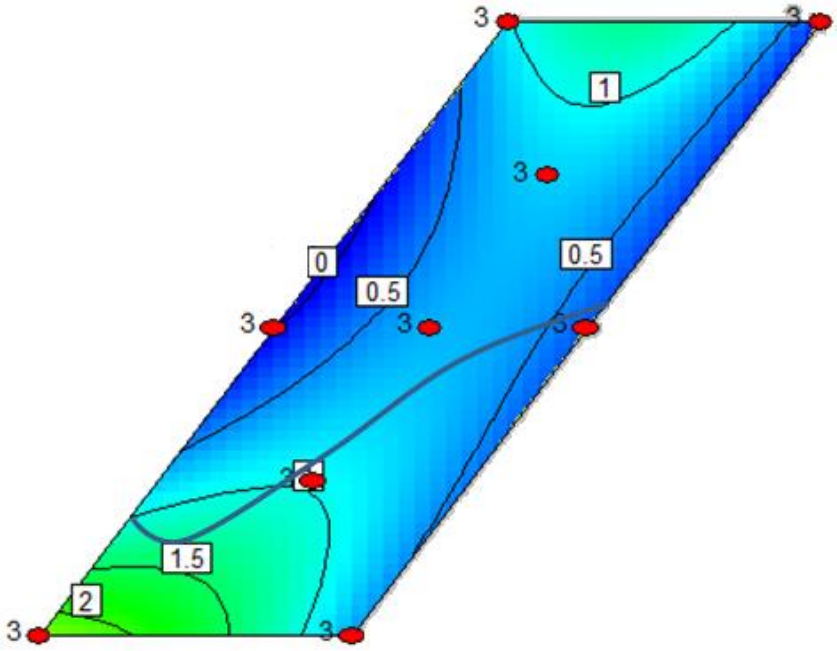
**B.9 Contour profile of methanetiol (relative abundance) of the sensory optimal formulation.**



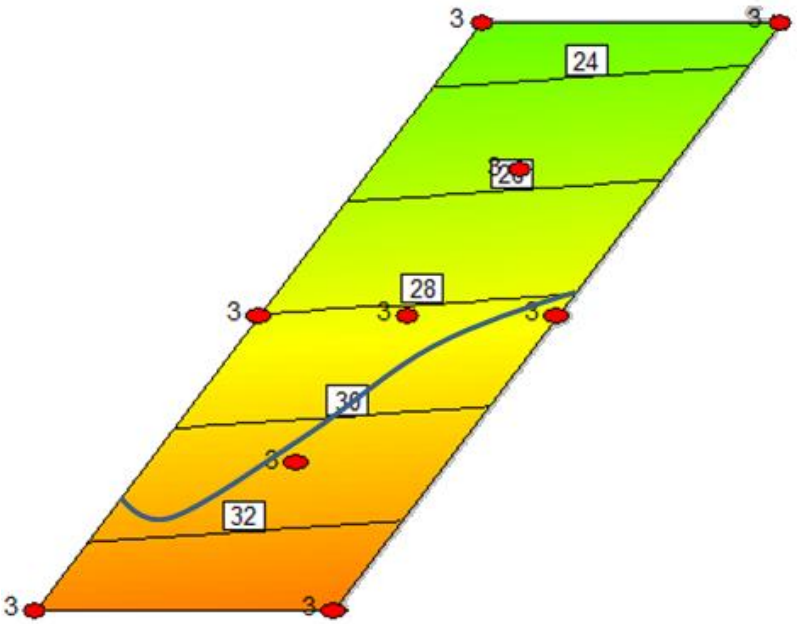

**B.10 Contour profile of heptanal (relative abundance) of the sensory optimal formulation.**



**B.11 Contour profile of benzaldehyde (relative abundance) of the sensory optimal formulation.**




**B.12 Contour profile of myristic acid (mg/g) of the sensory optimal formulation.**



## APPENDIX C. SAS CODES

### C.1 Example of ANOVA and Post-Hoc Tukey's Studentized Range Test

Note: Texture is used as an example to better illustrate the input; nevertheless, other variables were evaluated based on this SAS code.

```
dm 'log;clear;output;clear';
option nonumber;
title1 'texture';
data cheese;
input trata $
 Hardness adhesiveness resilience cohesion springiness gumminess Chewiness
datalines;


;
proc print;
title2 'raw data';
run;
proc sort data=cheese; by trata;
run;
proc means data=cheese n mean stddev min max;
class trata;
var
 Hardness adhesiveness resilience cohesion springiness gumminess Chewiness
;
run;
proc glm;
title2 'anova results using gml';
class trata;
model
 Hardness adhesiveness resilience cohesion springiness gumminess Chewiness
 = trata/ss3;
means trata/ tukey;
run;
```

**C.2 SAS Codes form MANOVA and ANOVA**

```
Title1 'cheese data';
data sensory;
input PANELIST SAMPLE$ appeareance odor taste saltiness texture softness
chewiness overalliking;

Datalines;

proc print;
Title2 'RAW DATA';
run;

proc sort data=sensory; by SAMPLE;
run;

proc means data=sensory N Mean StdDev Min Max;
class SAMPLE;
var appeareance odor taste saltiness texture softness chewiness overalliking;
run;

proc GLM;
Title2 'ANOVA RESULTS';
CLASS SAMPLE PANELIST;
model appeareance odor taste saltiness texture softness chewiness overalliking =
SAMPLE/SS3;
means SAMPLE/tukey;
run;

proc sort; by SAMPLE;


Proc candisc out=outcan mah;
Title2 'MANOVA - OVERALL';
class SAMPLE;
var appeareance odor taste saltiness texture softness chewiness overalliking;
run;


quit;
```

## APPENDIX D. TABLES OF COLOR AND WATTER ACTIVITY

### D.1 Effect of salt substitution on the color of cheddar cheese.

| Trt | Mo | L* | a* | b* |
|---|---|---|---|---|
| A | 0 | 84.13±0.5$^{ab(A)}$ | 3.27±0.13$^{a(AB)}$ | 19.49±0.61$^{a(A)}$ |
| A | 1 | 85.18±1.52$^{b(A}$) | 3.51±0.26$^{a(A)}$ | 20.36±0.83$^{abcd(A)}$ |
| A | 3 | 84.13±0.15$^{a(A)}$ | 3.06±0.24$^{a(B)}$ | 19.22±0.5$^{bcde(A)}$ |
| A | 5 | 84.78±1.16$^{a(A)}$ | 2.98±0.29$^{a(B)}$ | 19.34±1.03$^{d(A)}$ |
| B | 0 | 85.11±1$^{a(AB)}$ | 4.78±4.08$^{a(A )}$ | 18.76±1$^{a(A)}$ |
| B | 1 | 85.92±1.46$^{ab(A)}$ | 3.43±0.29$^{a(A)}$ | 19.86±1.12$^{bcd(A)}$ |
| B | 3 | 84.19±0.92$^{a(B)}$ | 3.32±0.27$^{a(A)}$ | 19.79±0.84$^{abcd(A)}$ |
| B | 5 | 84.8±0.68$^{a(AB)}$ | 3.03±0.16$^{a(A)}$ | 20.28±0.84$^{bcd(A)}$ |
| C | 0 | 84.37±1.2$^{ab(A)}$ | 3.05±0.25$^{a(A)}$ | 18.7±0.48$^{a(AB)}$ |
| C | 1 | 85.71±1.11$^{ab(A)}$ | 3.46±0.4$^{a(A)}$ | 18.84±0.75$^{d(AB)}$ |
| C | 3 | 84.31±0.74$^{a(A)}$ | 3.51±0.38$^{a(A)}$ | 18.05±0.37$^{cde(B)}$ |
| C | 5 | 84.49±0.99$^{a(A)}$ | 3±0.24$^{a(A)}$ | 19.34±0.79$^{d(A)}$ |
| D | 0 | 85.2±0.88$^{a(A)}$ | 3.1±0.12$^{a(A)}$ | 18.92±0.64$^{a(A)}$ |
| D | 1 | 85.5±1.45$^{ab(A)}$ | 3.5±0.36$^{a(A)}$ | 19.04±1.33$^{cd(A)}$ |
| D | 3 | 83.72±0.79$^{a(A)}$ | 3.19±0.49$^{a(A)}$ | 18.37±0.66$^{de(A)}$ |
| D | 5 | 83.68±1.82$^{a(A)}$ | 3.06±0.63$^{a(A)}$ | 19.67±0.86$^{cd(A)}$ |
| E | 0 | 85.44±2.11$^{a(AB)}$ | 3.02±0.23$^{a(B)}$ | 18.94±1.22$^{a(A)}$ |
| E | 1 | 87.43±0.87$^{a(A)}$ | 3.71±0.26$^{a(A)}$ | 20.18±0.78$^{abcd(A)}$ |
| E | 3 | 84.48±0.82$^{a(B)}$ | 3.23±0.3$^{a(B)}$ | 18.78±0.88$^{e(A)}$ |
| E | 5 | 83.92±0.49$^{a(B)}$ | 2.98±0.31$^{a(B)}$ | 19.17±1.17$^{d(A)}$ |
| F | 0 | 79.68±1.75$^{cd(A)}$ | 2.68±0.23$^{a(B)}$ | 19.48±1.23$^{a(B)}$ |
| F | 1 | 80.13±0.52$^{cd(A)}$ | 3.38±0.36$^{a(A)}$ | 21.97±1.8$^{ab(A)}$ |
| F | 3 | 80.57±1.15$^{bc(A)}$ | 3.56±0.7$^{a(A)}$ | 20.28±1.54$^{abc(AB)}$ |
| F | 5 | 80.44±0.81$^{b(A)}$ | 2.5±0.15$^{a(B)}$ | 22.43±0.66a$^{b(A)}$ |
| G | 0 | 78.39±2.1$^{d(A)}$ | 3.07±0.34$^{a(A)}$ | 18.99±1.44$^{a(B)}$ |
| G | 1 | 79.43±0.61$^{d(A)}$ | 3.34±0.32$^{a(A)}$ | 21.12±1.16$^{abc(AB)}$ |
| G | 3 | 79.37±0.53$^{c(A)}$ | 3.21±0.55$^{a(A)}$ | 20.15±0.67$^{abc(AB)}$ |
| G | 5 | 79.98±0.68$^{b(A)}$ | 2.87±0.57$^{a(A)}$ | 21.62±1.84$^{abc(A)}$ |
| H | 0 | 77.81±2.45$^{d(B)}$ | 3.07±0.34$^{a(AB)}$ | 18.91±1.61$^{a(B)}$ |
| H | 1 | 80.36±0.71$^{cd(A)}$ | 3.51±0.23$^{a(A)}$ | 20.44±0.71$^{abcd(AB)}$ |
| H | 3 | 80.88±0.73$^{b(A)}$ | 3.26±0.25$^{a(A)}$ | 20.37±0.54$^{abc(AB)}$ |
| H | 5 | 80.85±1$^{b(A)}$ | 2.66±0.4$^{a(B)}$ | 21.73±1.09$^{abc(A)}$ |
| I | 0 | 79.75±1.27$^{cd(A)}$ | 3.3±0.28$^{a(BC)}$ | 19.91±1.38$^{a(B)}$ |
| I | 1 | 80.59±0.81$^{cd(A)}$ | 3.87±0.29$^{a(A)}$ | 22.33±1.44$^{a(A)}$ |
| I | 3 | 80.31±0.62$^{bc(A)}$ | 3.52±0.33$^{a(AB)}$ | 21.17±1.38$^{a(AB)}$ |
| I | 5 | 80.06±0.56$^{b(A)}$ | 2.89±0.35$^{a(C)}$ | 22.34±1.76$^{ab(A)}$ |
| J | 0 | 81.54±0.65$^{bc(A)}$ | 2.92±0.09$^{a(B)}$ | 19.62±0.83$^{a(B)}$ |
| J | 1 | 81.58±0.42$^{c(A)}$ | 3.31±0.42$^{a(AB)}$ | 20.42±1.17$^{abcd(B)}$ |
| J | 3 | 80.83±0.53$^{b(A)}$ | 3.57±0.32$^{a(A)}$ | 20.76±0.79$^{ab(B)}$ |
| J | 5 | 79.23±0.64$^{b(B)}$ | 2.97±0.27$^{a(B)}$ | 22.87±0.92$^{a(A)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different (P > 0.05).

ϕ Months with the same capitalized letter inside parenthesis are not significantly different within the same treatment (P > 0.05). Letters A-J correspond to salt substitute formulations.

**D.2 Effect of salt substitution on the water activity of cheddar cheese.**

| Trt | Mo | Water Activity |
|---|---|---|
| A | 0 | $0.994 \pm 0.001^{a(A)}$ |
| A | 1 | $0.987 \pm 0.003^{bc(B)}$ |
| A | 3 | $0.969 \pm 0.001^{d(C)}$ |
| A | 5 | $0.964 \pm 0.003^{d(C)}$ |
| B | 0 | $0.99 \pm 0.003^{a(A)}$ |
| B | 1 | $0.98 \pm 0.002^{c(B)}$ |
| B | 3 | $0.986 \pm 0.002^{a(AB)}$ |
| B | 5 | $0.971 \pm 0.003^{bcd(C)}$ |
| C | 0 | $0.987 \pm 0.01^{a(A)}$ |
| C | 1 | $0.985 \pm 0.001^{bc(A)}$ |
| C | 3 | $0.986 \pm 0.002^{a(AB)}$ |
| C | 5 | $0.97 \pm 0.004^{cd(B)}$ |
| D | 0 | $0.993 \pm 0.003^{a(A)}$ |
| D | 1 | $0.99 \pm 0.01^{ab(A)}$ |
| D | 3 | $0.974 \pm 0.002^{cd(B)}$ |
| D | 5 | $0.971 \pm 0.005^{bcd(B)}$ |
| E | 0 | $0.978 \pm 0.003^{a(A)}$ |
| E | 1 | $0.983 \pm 0.005^{bc(A)}$ |
| E | 3 | $0.978 \pm 0.002^{bc(A)}$ |
| E | 5 | $0.980 \pm 0.006^{a(A)}$ |
| F | 0 | $0.975 \pm 0.022^{a(A)}$ |
| F | 1 | $0.982 \pm 0.002^{c(A)}$ |
| F | 3 | $0.985 \pm 0.005^{ab(A)}$ |
| F | 5 | $0.968 \pm 0.003^{cd(B)}$ |
| G | 0 | $0.991 \pm 0.005^{a(A)}$ |
| G | 1 | $0.997 \pm 0.001^{a(A)}$ |
| G | 3 | $0.973 \pm 0.002^{cd(B)}$ |
| G | 5 | $0.977 \pm 0.004^{abc(B)}$ |
| H | 0 | $0.996 \pm 0.002^{a(A)}$ |
| H | 1 | $0.984 \pm 0.005^{bc(B)}$ |
| H | 3 | $0.976 \pm 0.002^{c(B)}$ |
| H | 5 | $0.976 \pm 0.003^{abc(B)}$ |
| I | 0 | $0.993 \pm 0.006^{a(A)}$ |
| I | 1 | $0.982 \pm 0.003^{bc(B)}$ |
| I | 3 | $0.975 \pm 0.001^{cd(BC)}$ |
| I | 5 | $0.97 \pm 0.002^{cd(C)}$ |
| J | 0 | $0.983 \pm 0.007^{a(A)}$ |
| J | 1 | $0.979 \pm 0.003^{c(AB)}$ |
| J | 3 | $0.973 \pm 0.002^{cd(AB)}$ |
| J | 5 | $0.969 \pm 0.002^{cd(B)}$ |

* Salt substitute treatments with the same lowercase letter are not significantly different ($P > 0.05$).

ϕ Months with the same capitalized letter inside parenthesis are not significantly different within the same treatment ($P > 0.05$). Letters A-J correspond to salt substitute formulations.

## APPENDIX E. QUESTIONAIRE USED IN THE CONSUMER STUDY

**Sample #** _____ **Gender:** [ ] Male [ ] Female

**Please evaluate the following attributes of this product.**

1. How would you rate the **overall appearance** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

2. How would you rate the **odor/aroma** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

3. How would you rate the **taste** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

4. How would you rate the **saltiness** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

5. How would you rate the **saltiness** of this product?

[ ] Too weak [ ] Just about right [ ] Too strong

6. Did you detect the **undesirable off-flavor** of this product? YES [ ] NO [ ]

7. Did you detect the **bitterness** of this product? YES [ ] NO [ ]

8. How would you rate the **bitterness** of this product?

[ ] None [ ] Weak [ ] Moderate [ ] Strong

9. How would you rate the **overall texture** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

10. How would you rate the **softness** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

11. How would you rate the **softness** of this product?

[ ] not enough [ ] Just about right [ ] Too much

12. How would you rate the **chewiness** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

13. How would you rate the **chewiness** of this product?

[ ] not enough [ ] Just about right [ ] Too much

14. How would you rate the o**verall liking** of this product?

| Dislike Extremely | Dislike Very much | Dislike Moderately | Dislike Slightly | Neither Like nor Dislike | Like Slightly | Like Moderately | Like Very much | Like Extremely |
|---|---|---|---|---|---|---|---|---|
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

15. Is this product **ACCEPTABLE**? YES [ ] NO [ ]

16. Would you **BUY** this product if it were commercially available? YES [ ] NO [ ]

17. Would you **BUY** this product knowing it is low in sodium? YES [ ] NO [ ]

## APPENDIX F. RESEARCH CONSENT FORM APROVED BY IRB

Committee Action: Exempted √ Not Exempted ____ IRB# HE10-2
Reviewer Michael Keenan Signature Michael Keenan Date 2-9-10

**Research Consent Form**

I, _____, agree to participate in the research entitled "Consumer Acceptance of "White Cheddar cheese" which is being conducted by Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University Agricultural Center, (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. Three hundred and sixty consumers will participate in this research.

The following points have been explained to me:
1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.

2. The reason for the research is to gather information on consumer sensory acceptability of White Cheddar cheese. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.

3. The procedures are as follows: four coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk may be an allergic reaction to **Cheddar cheese and crackers.** However, because it is known to me beforehand that all those foods and ingredients are to be tested, the situation can normally be avoided.

5. The results of this study will be confidential and will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael Keenan of LSU AgCenter at 578-1708.
I agree with the terms above.

_____ _____
Signature of Investigator Signature of Participant
Witness: _____ Date: _____

## VITA

Kennet Carabante was born in Tegucigalpa, Honduras in1988. In 1990 he moved the city of San Marcos de Colon, Choluteca, Honduras where he finished his High school studies in December, 2004. In January, 2005 he enrolled as an undergraduate student in Zamorano University, Honduras where he obtained the degree of Bachelor of Science in Agroindustry Engineering in December, 2008. After graduation he worked for The Honduran Institute of Coffee as a research assistant for three months, before enrolling in the Zamorano Internship Program at Louisiana State University. At this moment he is a candidate for the degree of Master of Science in Food Science at Louisiana State University. He will pursue his Ph.D. degree in Food Science at Louisiana State University.