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Sensory Characteristics and Optimization of a Low-Sodium Salt Formulation and its Food Application

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SENSORY CHARACTERISTICS AND OPTIMIZATION OF A LOW-SODIUM SALT FORMULATION AND ITS FOOD APPLICATION

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DEDICATION

To my lovely wife Susanna, for her love, trust, tremendous support, encouragement and patience.

To my parents Lavrent and Margarita and my brother Vahan for everything that they have done for me. I am forever thankful.

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ABSTRACT

Excessive consumption of sodium is associated with high blood pressure in people. Reduction of sodium intake and replacement of salt with salt substitutes are an essential component of the primary prevention of hypertension. Potassium chloride is the most widely used salt substitute. However, when used in large amounts, it imparts bitterness and metallic aftertaste. Therefore, bitterness masking agents need to be used in salt substitute formulations. L-Arginine has been reported to have bitterness masking properties.

No research has yet been conducted to investigate the effects of KCl and L-Arginine on the perception of saltiness and bitterness in the mixture of NaCl, KCl, and L-Arginine. To develop acceptable reduced-salt food products, it is critical to understand how consumers perceive about saltiness and bitterness of the salt substitutes, which, will in turn, affect their decisions on product acceptance and purchase intent.

The aim of the present study was to develop an acceptable low-sodium salt mixture by reducing the sodium chloride content and replacing it with potassium chloride and L-Arginine.

The non-parametric R-Index approach was used to evaluate the effectiveness of L-Arginine as a bitterness masking agent in low sodium formulations. The formulations that contained 55% KCl, 35% NaCl and 10% L-Arginine in an aqueous solution at 0.5% w/v, 1.0% w/v and 1.5% w/v were not significantly different in bitterness perception from the control solution.

A response surface methodology was used to optimize and characterize the sensory properties of low sodium formulations in a food system using a chicken broth as a model. Those formulations that contain 57-92% NaCl, 0-35.5% KCl, and 7.5% L-Arginine were as acceptable

as the control formulation indicating that L-Arginine was able to mask the bitterness of salt formulations containing KCl.

The optimized low sodium formulation was compared to existing commercial products using chicken broth as a model. The optimized product was equally accepted for all sensory attributes by consumers (n=200) compared with Morton Table Salt and Morton Lite Salt.

This study demonstrated the potential of using NaCl/KCl/L-Arginine as a low sodium salt mixture by partially replacing NaCl while maintaining desirable sensory characteristics.

CHAPTER 1.

INTRODUCTION

1.1 Introduction

High salt (sodium chloride) intake contributes to development of hypertension (Ball et al., 2002). Early data from animal studies by Tobian (1991) and observational studies in humans by Froment et al. (1979) showed a relation between sodium intake and blood pressure. Short-term trials conducted by Sacks et al. (2001), suggested that reducing sodium intake lowers blood pressure. Law (2000) recommended that reducing sodium intake by 100 mmol/day would decrease stroke mortality by 22% and ischemic heart disease by 16% in Western societies. Loria et al. (2001) recommended that blood pressure can be lowered by decreasing the amount of sodium in the diet among individuals with hypertension. According to Kannel (1996) and Stamler et al. (1993), high blood pressure or hypertension is a risk factor for cardiovascular disease and is highly common in the U.S. population. Therefore, lowering the sodium intake is a necessary constituent of national public health policy (Burt et al., 1995).

As mentioned by Loria et al. (2001), the 1990 Dietary Guidelines Advisory Committee suggested that most Americans consider reducing sodium intakes, given that sodium intakes were well above a safe minimum intake of 500 mg/d. The 1995 Dietary Guidelines also mentioned that an additional health risk related to sodium intake is that high salt intake might increase calcium excretion. The 2000 Dietary Guidelines expanded and stressed this new concern: Eating more salt may increase calcium loss from bone, which suggests a relation between high sodium intake, loss of bone calcium and subsequent increased risk of osteoporosis and bone fractures (Loria et al., 2001). Additionally, a study of 10,000 adults from 32 countries done by Elliott et al. (1989); Intersalt (1988) showed that there was a linear relationship between blood pressure and 24-h urinary sodium excretion levels, and that the increase in blood pressure with age was related to sodium intake.

Statistically, about one fourth of adults have hypertension and one half of adults have higher than optimal blood pressure; therefore, putting them at increased risk for heart disease and stroke. In the U.S., blood pressure increases with age, such that one out of every two Americans older than 60 years has high blood pressure. Some subgroups have even higher frequency of hypertension, for example, 80% of African- American women older than 60 year are hypertensive (Burt et al., 1996). Loria et al. (2001) suggested that, based on evidence from clinical trials among individuals with high blood pressure, hypertension could be prevented through sodium intake reduction. Therefore, recommendations to lower sodium intake are an essential component of the primary prevention of hypertension in the U.S. population. Based on data from Loria et al. (2001), mean dietary sodium intakes among American adolescents and adults between 1988 and 1994 were well above 2400 mg/day, the maximum recommended intake level, which suggests that Americans are unable to judge whether the amount of sodium in their diet is appropriate or not. The majority of sodium intake derives from salt added to processed foods during production (James et al., 1987). However, choosing foods with less sodium content requires that foods available to the population contain less sodium. Hence, the reduction of sodium content and use of salty substances would be an important factor in facilitating the reduction of sodium intake. An approach toward reducing sodium intakes is critical to reducing the prevalence of hypertension and its associated disease risk in the U.S.

1.2 Research Objective

Specific objectives are to: 1) Determine and develop an optimal formulation for NaCl/KCl/L-arginine using the mixture design experiment; 2) Determine consumer perception/sensory discrimination for saltiness and bitterness attributes of the developed low sodium formulation; 3) Determine sensory characteristics of saltiness and bitterness intensities of

the low sodium formulation; 5) Understand the sensorial attributes and acceptability of a low-sodium product, using chicken broth as a model; 6) Compare consumer preferences, acceptance, and purchase intent as well as product sensory characteristics of developed low-sodium formulation against existing low-sodium products using a chicken broth as a model.

1.3 Research Justification

One in three Americans regularly consumes more salt than is recommended. Most salt consumption is derived from processed foods (James et al., 1987). This salt has been identified as a significant risk factor in developing high blood pressure. People with hypertension or high blood pressure are more likely to develop diseases of the heart and blood vessels (Pearson et al., 1982). Therefore, recommendations to lower sodium intake are an essential way to bring blood pressure levels down. A low-salt diet is beneficial for certain people with cardiovascular disease. Loria et al. (2001) recommended that blood pressure can be lowered by reducing the amount of sodium in the diet among individuals with or without hypertension.

One way of lowering sodium content is the use of salt substitutes. However, taste has been a major problem in developing salt substitutes (Pasin et al., 1989). Therefore, it is crucial to modify the formulations by reducing or partially replacing the sodium content and at the same time, maintaining the desirable sensory properties. The use of a healthy salt alternative could be the solution to reducing the prevalence of hypertension and its associated disease risks in the U.S.

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CHAPTER 2.

LITERATURE REVIEW

2.1 Sodium Chloride

Salt occurs naturally in many parts of the world as the mineral halite and as mixed evaporates in salt lakes. Sodium chloride crystals are cubic in form. Table salt consists of tiny cubes tightly bound together through ionic bonding of the sodium and chloride ions. It varies in color from colorless, when pure, to white, gray, or brownish, typical of rock salt (halite). Sodium chloride contains 60.663% elemental chlorine (Cl) and 39.337% sodium (Na). The atomic weight of elemental chlorine is 35.4527 and that of sodium is 22.989768 (Saltinsitiute.org, 2006). Sodium chloride or salt is considered a necessary nutritional ingredient. Salt is the main source of sodium (Na), a key component for every mammalian organism (Danielsa et al., 2004). Sodium plays a critical role as a major electrolyte of the extracellular fluid, maintaining every fluid section in the body. Therefore, the amount of sodium in the bodily fluids must be regulated to ensure of the functioning of various physiological processes in the body such as ion conductance, glomerular filtration, and blood pressure stabilization (Danielsa et al., 2004). Thus, the change in sodium homeostasis can have severe impact on the psychological processes.

Reduced sodium can affect a circulatory collapse while excessive sodium has been linked to hypertension and the risk of cardiovascular disease (Danielsa et al., 2004; Desmond, 2006). It is believed that human beings for at least the past 100,000 years have been "programmed" to eat unprocessed plant and animal foods, that is, foods without complete or partial removal of nutrients and without enrichment with any nutrient component (Karppanen et al., 2006). Thus, the changes in composition of food systems such as processing food could affect the physiological processes of human beings or predispose to pathological conditions. A daily diet, which consists of two-thirds plant food and one-third animal food with the absence of added salt, provides 0.6g of sodium. A daily diet with plant food only provides 0.23g of sodium per day,

while a diet with animal food only without added salt provides 0.8g sodium per day (Karppanen et al., 2006; He et al., 2002; Eaton et al., 1997). Therefore, on the basis of daily diet with the absence of added salt, one can expect that the human body is "programmed" for approximately 1.2g a day of sodium intake per 1000 kkal (Karppanen et al., 2006).

According to Intersalt Cooperative Research Group studies (1998), the average sodium intake in western countries is approximately 3,000 to 4,500 mg/day. The average sodium intake in the United States during the mid-1990s was about 3,500 mg/day at an average energy intake of 10000 kJ (2400 kcal) (Appel et al., 1997). Based on results of Eaton et al. (1997), the sodium level is approximately 600 mg for natural diets without added salt or other sodium compounds. Thus, the average intake of sodium in United States is approximately 5-6 times higher than of natural diets without added salt or sodium compounds.

2.1.1 Sources of Sodium Chloride

As stated by Loria et al. (2001), the Dietary Guidelines identified table salt as a source of sodium and chloride and stated that both nutrients are essential. In the mid-1980s, the Dietary Guidelines stated that sodium is present in certain processed foods, condiments, sauces, pickled foods, salty snacks and sandwich meats. The Dietary Guidelines mentioned that in recent years, most dietary sodium has been added during processing and manufacturing and only small amounts of sodium occur naturally in foods.

2.1.2 Functions of Sodium Chloride in Food Systems

The number of Americans concerned about the amount of sodium in their diet has increased over the past years, due to pervasive information stating that high sodium intake has been identified as a possible contributor to the development of hypertension (Pearson et al., 1982). According to FASEB (1979), the average American consumes about 10-12g of salt a day,

which is equivalent to 3,900-4,000 mg sodium. This sodium intake level, according to Pasin et al. (1989), is 20-25 times greater than the minimum adult requirement. Hence, the recommendation to decrease daily intake of sodium is essential in reducing the prevalence of hypertension and its associated disease risks in the U.S.

Unfortunately the reduction of sodium content from salt in processed products is complicated due to the fact that sodium chloride possesses functions such as shelf life extension, antimicrobial properties, and enhancement of flavor (Pasin et al., 1989). Reduction in flavor results in less consumer acceptability (Bertino et al., 1981). Sodium chloride has significant contribution in functional properties of meat products. Gelabert et al. (2003) showed that NaCl in meat products contributes to fat binding, helps emulsification, increases water-holding capacity and enhances flavor and texture. Therefore, the reduction of salt level results in adverse effects on these properties (Ingram et al., 1967). Fortunately, the replacement of sodium by substitutes could preserve these functions, as reported by (Maurer, 1983).

2.1.3 Functions of Sodium Chloride in Human Body

Sodium is an essential component of every mammalian organism. It is considered to be the primary electrolyte of extracellular fluid (ECF) in the human organism. Sodium plays a key role in maintaining the volume and composition of every fluid compartment in the body, including those within and those that surround and nourish cells such as blood plasma and interstitial fluids (Danielsa et al., 2004). It is important that the amount of sodium in this fluid matrix be controlled to ensure optimal functioning of numerous physiological processes, including ion conductance across cell membranes, underlying neural excitability, glomerular filtration, renal excretion of aqueous waste, and the stability of blood pressure, capillary exchange, and cardiac output. Disorders of sodium balance may have severe consequences: too

little sodium can lead to circulatory collapse, while an excess has been associated with exaggerated vascular reactivity and hypertension (Danielsa et al., 2004).

2.1.4 Absorption of Sodium

Ninety five percent of the ingested salt is absorbed from the gastrointestinal tract (Mervaala, 1995). Massive diarrhea and vomiting or prolonged strenuous exercise with profuse sweating could cause extra-renal loss of salt (Mervaala, 1995). Otherwise, extra-renal loss of salt is minimal, with sweating accounting usually for approximately 1 mmol (0.058 g) and other extra-renal losses for 0.002 to 0.18 g per day. Thus, to preserve the extracellular sodium concentration (≈142 mmol/L) and total body salt content at constant levels, renal salt excretion has to be almost equal to salt intake. Even a small increase in serum sodium concentration after absorption of dietary salt from the gastrointestinal tract, triggers thirst, and causes fluid intake until the normal serum concentration is restored. As an example, Mervaala (1995) showed that a daily excess in salt intake of 8.3 g (3266 mg sodium) must be accompanied by a 1,000 ml increase in water intake each day to maintain the normal extra cellular sodium concentration of 142 mmol/L.

2.1.5 Regulation of Na^+ Metabolism

Simple equilibrium between sodium intake and exertion is required in order to maintain the sodium homeostasis in human body. A healthy human body regulates sodium metabolism using specific mechanisms controlling Na^+ excretion. The most important mechanisms are Glomerular Filtration Rate (GFR) and aldosterone secretion (Verbalis, 2003). GFR depends on many factors such as glomerular plasma flow, the glomerular capillary surface area, the hydrostatic pressure gradient between the glomerular capillaries and Bowman's capsule, and the oncotic pressure produced by the proteins in glomerular capillaries. Approximately 25,000

mmol/day of Na^+ is filtered through the kidneys in healthy adults; therefore, the changes in Glomerular Filtration Rate have an effect on filtered Na^+. However, changes in the filtered load of Na^+ are compensated via a process known as tubuloglomerular feedback (Baylis et al., 1997). According to Verbalis, (2003), as the filtered Na^+ load increases, Na^+ absorption in the proximal tubule also increases, largely compensating for the increased filtered load. An increase in filtered fluid at the glomerulus decreases the hydrostatic pressure and increases the oncotic pressure of the non-filtered fluid delivered to the peritubular capillaries. It, thereby, increases the pressure gradient for re-absorbing the Na^+ which is actively transported from the proximal tubular epithelial cells into the extracellular fluid surrounding the proximal tubule. Although this mechanism dampens the effects of alterations in GFR on renal Na^+ excretion and prevents large changes in urine Na^+ excretion in response to minor changes in GFR, many experimental results indicate that sustained alterations of GFR can significantly modulate renal Na^+ excretion.

The next mechanism that regulates the sodium excretion is adrenal aldosterone secretion. Based on Masilamani et al. (1999), this important factor increases Na^+ re-absorption in the distal nephron by inducing the synthesis and activity of ion channels that affect sodium re-absorption and sodium-potassium exchange in tubular epithelial cells, particularly the epithelial sodium channel (ENaC). Several factors affect adrenal mineralocorticoid secretion. The most important of these factors is angiotensin II, which is formed as the end result of renin secretion from the juxtaglomerular apparatus in response to renal hypoperfusion and high serum K^+ concentrations also stimulate aldosterone secretion (Baylis et al., 1997).

2.1.6 Excessive Na^+ Intake and Hypertension

As discussed above, blood pressure has to be increased or decreased to restore and maintain the salt and water balance in the body. In the case of excessive sodium intake, the body increases blood pressure levels to get rid of excess sodium and water through the pressure-

natriuresis mechanism (Mervaala, 1995; Guyton, 1991). Therefore, there is link between high blood pressure and excessive sodium intake.

High blood pressure is one of the leading causes of death in developed countries (Karppanen et al., 2006). Hypertension generally means systolic blood pressure of greater than 140 millimeters of mercury (mm Hg) or a diastolic blood pressure of greater than 90 mm Hg. Normal blood pressure is a systolic blood pressure below 140 mm Hg and diastolic blood pressure below 90 mm Hg. Hypertension is a public health concern because it is a major risk factor for mortality from coronary heart disease and stroke (U.S. FDA, 2002). Many scientific evidences suggest (Karppanen et al., 2006; Appel et al., 1997; Vaskonen, 2003) a direct relation between excessive sodium intake and high blood pressure and indicate that reducing sodium intake lowers blood pressure and its associated risks in many but not all hypertensive individuals. According to Kearney et al. (2005), the estimated total number of adults with hypertension in 2000 was 972 million (957–987 million), 333 million (329–336 million) in economically developed countries, and 639 million (625–654 million) in economically developing countries. The number of adults with high blood pressure in 2025 was predicted to increase by about 60% to a total of 1.56 billion (1.54–1.58 billion).

Different studies conducted by Intersalt Cooperative Research Group, (Intersalt, 1998) as well as by Law et al. (1991) indicated that in western industrialized countries the average intake of sodium is approximately 3000–4500 mg per day and have shown that blood pressure in various communities increases in a dose-related manner with increasing sodium consumption.

The work conducted by He et al. (2004) showed that there is a some correlation between the reduction in urinary sodium, an indicator of sodium intake, and the reduction in blood pressure.

2.1.7 Current Sodium Intake

According to U.S. Department of Health and Human Services and U.S. Department of Agriculture (2005), the recommended Dietary Reference Intake (DRI) for sodium is 1500 mg/d, while the maximum daily intake is 2500 mg/d and is likely to cause no adverse effects on blood pressure. However, in current diets the average sodium intake is 3000 to 4500 mg/d in various westernized communities, including US. This clearly exceeds even maximum recommended sodium intakes (Intersalt, 1998; Law et al., 1991). In contrary, the recommended intake of potassium for adolescents and adults is 4,700 mg/d, for children 1 to 3 years of age are 3,000 mg/d; for children 4 to 8 years of age are 3,800 mg/d; and for children 9 to 13 years are 4,500 mg/d (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005). The findings from the same study showed that the current average potassium intakes in the United States are very low, only about 43% of the recommended level. Figure 1 (Karppanen et al., 2005; Appel et al., 1997) shows that there is a difference between sodium and potassium intakes for natural and modern diets in US. For natural diets, which consist of unprocessed foods, approximately two-thirds of the energy is derived from plant food and one-third from animal food. The daily intake of sodium in a natural diet is approximately 500 mg that of potassium is about 7400 mg, that of calcium is approximately 1100 mg, and that of magnesium is about 800 mg. By contrast, the modern diet provides different amounts and ratios of sodium, potassium, calcium, and magnesium intakes than the natural diet. In the average US diet, sodium is about 3000 mg a day, which is six-fold as compared to the natural diet. The potassium intake was as low as 1750 mg which is only 24% of the amount provided by the natural diet. Similar trend was observed for the daily intake of calcium, at about 440 mg and magnesium at about

180mg, which are both lower than that of the natural diet by 40% and 23%, respectively (Appel et al., 1997).

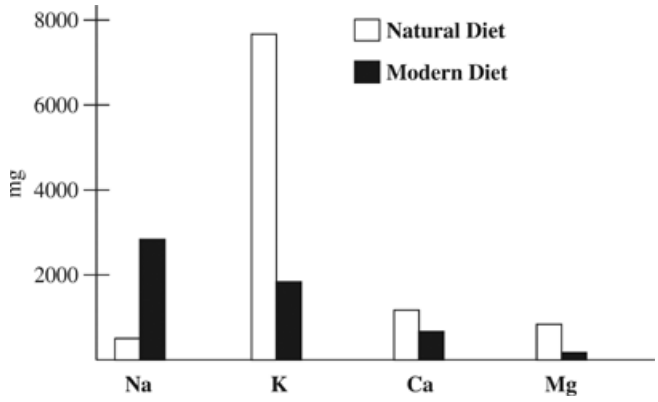

Figure 1: Sodium, Potassium, Calcium and Magnesium Content in Natural and Modern Diets (Average US Diet)
Source: (Appel et al., 1997)

The findings given by Intersalt (1998); Law et al. (1991); Appel et al. (1997); Karppanen et al. (2005) show that the average sodium intake is remarkably higher than the recommended or natural intake, which the body can handle without any difficulties or harm. Even though different hormonal mechanisms such as suppression sodium-retaining renin–angiotensin–aldosterone system can improve the excretion of sodium to some extent, they are not effective enough to match the excessive sodium intake. Therefore, sodium intake reduction is essential in modern diets.

2.1.8 Approaches of Salt Reduction

According to Ruusunen et al. (2005), sodium intake exceeds the nutritional recommendations in many western industrialized countries. Many scientific and epidemiological studies suggest (Karppanen et al., 2006; Ruusunen et al., 2005) that there is a clear link between excessive intake of sodium to hypertension and, consequently, to increased risk of stroke. However, beverage and food companies actively promote high salt intakes and maintain that

there is no scientific justification for any salt reduction at the population level (Godlee, 1996; Salt Institute, 2006; European Salt Producers' Association, 2006). The salt-promotion activities have recently proved highly successful. The use of salt by consumers has increased remarkably. According to Intersalt (1998); Karppanen et al. (2006) in 1998, the total sales of food-grade salt in the United States was as much as 86% higher, and the per capita sales approximately 55% higher than in 1983. Since the late 1990s, the per capita sales of food-grade salt have remained rather constant at a high level. Dietary surveys have also indicated that in 1999 to 2000, salt intakes in the United States were remarkably higher than in the late 1970s (Briefel, 2004). However, consumers seem to be concerned about the harmful effects of excessive sodium intake, there is a tendency by major food companies to reduce sodium in their products (Guardia et al., 2006; Desmond, 2006).

There are different approaches available to reduce sodium intake: stepwise reduction (which assumes that consumers will adapt to a less salty taste), salt replacement, use of salt enhancers, and modification of the physical form of salt (Desmond, 2006). The first approach has been used by many manufacturers, but its use is limited by two major barriers. Firstly, technological limits are frequently encountered resulting from processing, structural and safety issues. Secondly, adverse consumer reaction occurs if the perceived saltiness becomes too low (Phelps et al., 2006). However, apart from lowering the level of salt added to products, the most widely used method is the use of salt substitutes, in particular, potassium chloride (KCl). The most immediate problem encountered was that the use of the replacement does not deliver the clean salty taste of sodium chloride. The bitterness associated with potassium chloride at concentrations needed to deliver saltiness is known to limit its industrial use. Therefore, salt enhancers which themselves do not have a salty taste, but enhance a salty taste when used in

combination with sodium chloride as well as bitterness masking agents, are commonly used in these products in order to deliver the salty taste of sodium chloride. This allows less salt to be added to the products. The third option is optimizing the physical form of salt so that it becomes more taste bioavailable and, therefore, less salt is needed (Desmond, 2006). This last approach of modifying the physical form of salt was based on the hypothesis that the perceived saltiness of salt in the solid form is affected by crystal form and size, and rate of dissolution in the mouth (Phelps et al., 2006).

2.2 Effects of Sodium Reduction and Increased Potassium Intake

The following findings were established by Karppannen et al. (2005); Sacks et al. (2001): Sodium reduction to approximately 40% of the usual level during a control diet, produced a fall of 6.7 mm Hg in systolic blood pressure and 3.5 mm Hg in diastolic blood pressure. A moderate sodium reduction to approximately 67% of the usual level produced a smaller fall in blood pressure. The average fall in systolic blood pressure was 2.1 mm Hg and that in diastolic blood pressure, 1.1 mm Hg. Two other meta-analyses conducted by He et al. (2002); Geleijnse et al. (2003) showed that an approximately 75-mmol-a-day (about 50%) reduction in the intake of sodium lowers blood pressure in both subjects with hypertension and normotensive individuals. In hypertensives, the fall in systolic blood pressure is about 5 mm Hg, and that in diastolic pressure, approximately 3 mmHg. In normotensives, the fall in systolic pressure is approximately 1.3–2 mm Hg and that in diastolic pressure is about 1 mm Hg. Based on findings by Geleijnse et al. (2003); Whelton et al. (1997); Vaskonen (2003), an increase of potassium intake by approximately 1.8–1.9 g a day has proved to lower the blood pressure of hypertensive subjects so that the average fall in systolic blood pressure is approximately 4 mm Hg and that in diastolic pressure is about 2.5 mm Hg. This increase in potassium intake is about 25% of the amount

provided by a 2100 kcal natural diet, and is not sufficient to raise the potassium intake in the US population to the currently recommended level of 4.7 g per day. Several mechanisms, such as increased natriuresis, reduced sympathetic nervous activity, and decreased pressure response to noradrenaline and angiotensin II, seem to be involved in the blood pressure lowering effect of potassium.

2.2.1 Reduction/Substitution of Sodium in Food Products

There is an increasing desire by consumers to lower their sodium intake, due to information in recent years that there is a positive relationship between high sodium and the incidence of hypertension (Kerr et al., 1986; Choi et al., 1994). Taste has been the major difficulty encountered with sodium chloride restriction in food products. Unsalted food products have been less pleasant and less acceptable (Pasin et al., 1989). Therefore, the need arises to modify the food formulations by reducing or partially replacing the sodium content and, at the same time, maintaining the desirable sensory and chemical properties of sodium chloride. Naewbanji et al., (1986) reported that KCl might be possible functional substitute for NaCl in cucumber fermentation brine. The effects of KCl on sensory qualities of fermented cabbage, radish and cucumber have been studied by Park et al. (1986). Choi et al. (1994) indicates that brines containing up to 50% KCl as replacement for sodium chloride has acceptable sensory qualities in kimchi. On the other hand, Gimeno et al. (2001) investigated the use of calcium ascorbate to replace 46% NaCl in dry fermented sausage and concluded that the product had acceptable sensory and physical properties (texture, color etc.). Gou et al. (1996) evaluated the effect of KCl, K-lactate and glycine on the flavor, texture and color characteristics of fermented sausage. They concluded that an acceptable substitution could be achieved in that product. Ball et al. (2002) suggested the use of calcium diglutamate as a possible substitute for NaCl. It could

achieve the similar flavor characteristics at a lower Na concentration in soup. Although a number of salt substitutes have been developed according to Frank et al. (1970), the most commonly used NaCl replacement thus far has been potassium chloride, which has similar physicochemical properties of NaCl and is a good candidate for salt substitute. Though potassium chloride has properties similar to NaCl, it does not taste like NaCl (Pasin et al., 1989).

2.2.2. Labeling Requirements of Low Sodium Products

According to US Food and Drug Administration (21CFR101.61), a claim about " low sodium" can be made on the food label provided that the food has a reference amount customarily consumed greater than 30 g or greater than 2 tablespoons and contains 140 mg or less sodium per reference amount customarily consumed. The term " reduced" salt may be used in labeling foods provided that the food contains at least 25 percent less sodium per reference amount customarily consumed than an appropriate reference food. Finally, the term "salt free" may be used on the label or in labeling of foods only if the food is sodium free (21 CFR 101.61).

2.3 Potassium Chloride

Potassium chloride (KCl) is a chemical compound composed of potassium and chlorine. In its pure state, it is odorless, is a white or colorless vitreous crystal, with a crystal structure that cleaves easily in three directions. Potassium chloride is also commonly known as "Muriate of Potash". Potash varies in color from pink or red to white depending on the mining and recovery process used. KCl is also used in medicine, scientific applications, and food processing. Potassium chloride can be found naturally as the mineral sylvyte and in combination with sodium chloride as sylvynite (Lide, 1990). KCl is toxic in excess. The lethal doze LD_{50} is around 2500mg/kg. Regular sodium chloride with similar excessive consumption is about as toxic as potassium chloride. The high usage of potassium chloride can cause cardiac arrest (Lide, 1990).

2.3.1 Usage of Potassium Chloride

Potassium is essential in the human body and oral potassium chloride is the common means to replenish it although it can also be given intravenously. KCl can be used as a salt substitute for food, but due to its weak, bitter, unsalty flavor, it is usually mixed with regular sodium chloride to improve the taste. Medically it is used in the treatment of hypokalemia and associated conditions, for digitalis poisoning, and as an electrolyte replenisher. Overdoses cause hyperkalemia, which can lead to paresthesia, cardiac conduction blocks, fibrillation and also sclerosis (Lide, 1990; Wikipedia, 2007).

2.3.2 Replacement of Sodium Chloride with Potassium Chloride

In the recent decades according to Best (1989); Duxbury (1986), the food industry has used KCl partially as a substitute to sodium chloride. The disadvantage of using KCl alone is that potassium chloride elicits a bitter taste as well as a salty taste (Frank et al., 1969; Bartoshuk, 1980). Therefore, bitterness inhibitors have to be included into food formulations to mask the undesirable taste of potassium chloride. Technically KCl is one of the best substitutes of NaCl, because it has similar physicochemical properties to that of NaCl. Based on observation by Rosett et al. (1995), it is the same in appearance to sodium chloride and can be obtained in similar particle size. Both have close specific gravities (1.99 for KCl and 2.16 for NaCl). The critical humidities at which they absorb water are similar; therefore, they can be protected from caking by the same additives. Frank et al. (1969) noted that they both could readily iodized. However, there were some concerns about the possible vulnerability of certain populations: those with Type I diabetes, chronic renal insufficiency, end stage renal disease, severe heart failure and adrenal insufficiency to high potassium load from these salt substitutes (Desmond, 2006). The US Dietary Guidelines (2005) mentioned the effect that some salt substitutes would have on

certain population groups. However, the guidelines also state that a potassium-rich diet blunts the effects of salt on blood pressure and recommend an intake of 4.7 g potassium/day. According to Ruusunen et al. (2005), the use of mineral salt mixtures is a good way to reduce the sodium content in meat products. The same perceived saltiness can be achieved with salt mixtures at lower sodium content. According to Desmond (2006), some of these mixtures have been commercialized such as Pansalt^{R}. Pansalt^{R} is a patented salt replacer in which almost half of the sodium is removed and replaced with potassium chloride, magnesium sulphate and the essential amino acid L-lysine hydrochloride. According to the manufacturer, the patented usage of the amino acid enhances the saltiness of the salt replacer and masks the taste of potassium and magnesium while increasing the excretion of sodium from the human body. Other commercially available mixtures of NaCl and KCl include Lo salt, Saxa So-low salt and Morton Lite Salt amongst others.

2.3.3 Enhancement of Saltiness by Potassium Chloride

As mentioned above, KCl alone has a bitter as well as a salty taste. Because of their similar physicochemical properties, it is possible to partially substitute sodium chloride with potassium chloride. It is essential to understand the mechanism by which KCl could enhance the salt taste of sodium-reduced food products. It has been showed by Frank et al. (1969) that when KCl [< 0.8% w/v] was added to the distilled water containing 0.1-0.2% w/v NaCl, the subjects reported that the KCl/NaCl mixture was saltier than that sodium chloride alone. In another study, a 50% replacement of sodium chloride by KCl tasted as salty as 100% NaCl alone (Streitelmeier, 1986). Saltiness of NaCl is a function of the state of Na^{+} cations as well as associated with negatively charged anions such as chloride (Cl^{-}) (Price et al., 1977). When tasted alone, NaCl

produces a greater saltiness response than sodium compounds with associated anions larger than Cl^- (Ye et al., 1991).

According to Rosett et al. (1994), food ingredients with large anionic substituents such as the ioninc gums, xanthan and kappa carrageenan, suppressed saltiness as compared to non-ionic gums, locust bean, and guar, in NaCl-gum system. They suggested that the perceived saltiness of NaCl was suppressed by binding of Na^+ to negatively charged groups of ionic gums, as measured by ^{23}NaCl nuclear magnetic resonance spectroscopy. Also, K^+ and Ca^{+2} were associated with increased perceived saltiness. It has been concluded that K^+ and Ca^{+2} interacted with ionic gums in place of Na^+, resulting in a saltier taste. Based on work conducted by Rosett et al. (1995), it has been recommended that salty taste increases with addition of KCl to gum solutions containing an equal weight of NaCl. They suggested that interactions between negatively charged substituents on ionic gums and Na^+ and K^+ affect salty taste. Results of their study show that saltiness was not an additive function of Na^+ and K^+ contents. Enhancement of the salty taste of food systems containing NaCl by potassium chloride as explained by Rosett et al. (1995) is the competitive binding of sodium and potassium ions: K^+ displaces Na^+ on larger negatively charged macromolecules, allowing more Na^+ to remain free for saltiness perception.

2.3.4 Suppression of Bitterness, Taste Enhancers and Masking Agents

The greatest difficulty with lower sodium contained food products has been taste. Usually consumers find unsalted foods less acceptable (Pasin et al., 1989). Although sodium substitution with KCl has been used to develop salt substitutes, bitterness remains a major taste problem. Therefore, bitterness inhibitors have to be included into food formulations to mask the undesirable taste of potassium chloride. One of the methods of blocking the bitterness is the introduction of compounds that perform bitterness blocking properties. It has been found

recently by Keast et al. (2001) that sodium salt itself is able to suppress bitterness. The degree of suppression varies across bitter substances. It has been shown by Keast et al. (2001) that sodium salts substantially suppresses the bitterness of KCl, urea and amiloride, while it was less effective in suppressing the bitterness of quinine and caffeine. Keast et al. (2001) suggested that the bitterness suppression function of sodium anion is due to its chemical properties acting at the peripheral taste level rather than a cognitive effect.

According to Desmond (2006), there are a number of flavor enhancing and masking agents commercially available. These include yeast extracts, lactates, monosodium glutamate and nucleotides among others. As stated by Brandsma (2006), taste enhancers work by activating receptors in the mouth and throat, which helps compensate for the salt reduction. A bitterness blocker that has been approved and received patent protection is adenosine-5'- monophosphate (AMP). AMP works by blocking the activation of the gustducin in taste receptor cells, thereby preventing taste nerve simulation (McGregor, 2004). The other example of a masking agent is Givaudan's new, customized Natural Flavour System which modifies off notes exhibited by KCl and enhances the saltiness overall (Desmond, 2006). Wixon Fontrome produced products such as Magifique Salt-Away or Mimic and claims to mask the bitterness and metallic taste of potassium chloride. Wild Flavors Inc. has introduced SaltTrim. The company claims that this product simultaneously blocks the negative tastes of KCl while keeping the true taste and mouthfeel of salt (Desmond, 2006).

There are other combinations of ingredients such as lysine and succinic acid that have been used as salt substitutes (Turk, 1993) or the use of sodium or potassium lactate with a corresponding reduction in NaCl that tends to maintain certain saltiness while reducing the sodium content in products to some degree (Price, 1997). It has been reported by Riha et al.

(1997) that amino acids are able to enhance the salty taste of sodium chloride. Recent findings by Ogawa et al. (2004) showed that particularly L-arginine could be a potential masking agent in reducing the bitterness of various solutions containing bitter compounds.

2.4 L-Arginine: Biological Properties, Sources and Requirements

L-arginine (2 amino-5-guanidinovaleric acid) is an amino acid present in the proteins of all life forms.

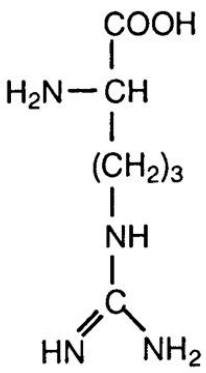

Figure 2: Chemical Structure of L-Arginine
Source: (Humm et al., 1997)

L-arginine is the precursor of nitric oxide, an endogenous messenger molecule involved in a variety of endothelium-mediated physiological effects in the vascular system (Boger et al, 2001). Nitric oxide plays an important role in numerous biological processes ranging from neurotransmission to vasodilatation and inflammation to cell phenotype regulation (Peters et al., 1999). In addition to nitric oxide synthesis, L-arginine is essential for the synthesis of urea, creatine, creatinine, agmatine, and influences hormonal release and the synthesis of pyrimidine bases. This places L-arginine, its precursors and its metabolites at the center of the interaction of different metabolic pathways and interorgan communication (Reyes et al., 1994). As mentioned by Peters et al. (1999), a special feature of L-arginine is that its intake is semi-essential. Under

normal physiological conditions, the human body is able to synthesize sufficient amounts of endogenous L-arginine to maintain whole body L-arginine metabolic homeostasis and dietary L-arginine intake becomes dispensable. However, in conditions of increased demand such as growth, tissue inflammation or wound healing, L-arginine intake may become important. According to Cooper, (1996), dietary arginine could be found in chocolate, wheat germ and flour, buckwheat, granola, oatmeal, dairy products (cottage cheese, ricotta, nonfat dry milk, and skim yogurt), beef, pork, nuts, chicken and turkey light meat, seafood (halibut, lobster, salmon, shrimp, snails, water packed tuna), chick peas, and cooked soybeans. L-arginine has been approved by Food and Drug Administration to be safely used as nutrient added to foods (U.S FDA, 2002). As a food additive, L-arginine can be used in a free, hydrated or anhydrous form or as the hydrochloride, sodium or potassium salts. Although the Recommended Dietary Allowance (RDA) for L-arginine has not been established, according to Food and Drug Administration, the reasonable daily adult intake of L-arginine in food products present in free and combined (as protein) form should not exceed 6.6% by weight of total protein expressed as free amino acid (U.S. FDA, 2002).

In addition to biological properties, L-arginine was reported to mask the bitterness of various compounds and enhance the saltiness of NaCl (Ogawa et al., 2004). These authors reported that L-arginine was successful in reducing the bitterness of various solutions containing bitter compounds. It has been shown that with the usage of L-arginine, the bitterness of quinine was significantly suppressed. The bitterness suppression of L-arginine was enhanced by the addition of NaCl. The study conducted by Ogawa et al. (2004) showed that the degree of suppression reached by L-arginine and NaCl was greater than that of other bitterness suppressing

agents, including phosphatidic acid and tannic acid. Presently the mechanism of bitterness suppression by L-Arginine is unknown.

2.5 Summary

It has been mentioned by various authors (Appel et al., 1997; Law et al., 1991; He et al., 2002; Sacks et al., 2001) that there is a positive relation between sodium intake and hypertension and, consequently, the risk of stroke. Therefore, lowering sodium intake could prevent hypertension. One of the major barriers of lowering salt added to products is consumer reaction when the perceived saltiness becomes too low (Phelps et al., 2006). As mentioned above, currently the average sodium intake is from 3000 to 4500 mg/day which is higher than recommended maximum daily intake of 2500mg/day (Karppannen et al., 2006). In contrary, the average potassium intake in the United States is very low, only about 43% of the recommended level. However, the effective way of lowering sodium in products, and increasing potassium intake, while maintaining a desirable salty taste is by using salt substitute substances and taste enhancers. This approach allows modifying the food formulations by reducing or partially replacing the sodium content and at the same time, maintaining the desirable sensory and chemical properties of sodium chloride. The above mentioned combination of decreasing the sodium and increasing the potassium level in food systems is likely to be effective in the prevention and treatment of blood pressure in US population.

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CHAPTER 3.

A NON-PARAMETRIC R-INDEX APPROACH: A METHOD FOR EVALUATING SALTINESS AND BITTERNESS OF SALT MIXTURES CONTAINING L-ARGININE

3.1 Introduction

There is an increasing desire by consumers to lower their sodium intake, due to information in recent years that there is a positive relationship between high sodium and the incidence of hypertension (Kerr et al., 1986; Choi et al., 1994). Taste has been the major difficulty encountered with sodium chloride restriction in food products (Pasin et al., 1989). Thus, the need arises to modify the food formulations by reducing or partially replacing the sodium content and at the same time maintaining desirable sensory and chemical properties of sodium chloride. KCl alone has a bitter as well as a salty taste. Because of the similar physicochemical properties, it is possible to partially substitute sodium chloride with potassium chloride. However, when used in large amounts, the substitution imparts bitterness and metallic aftertaste. Thus, it is essential to use a bitter masking agent in the salt substitute formulation. It has been recently reported that L-arginine has the ability of masking the bitterness perception of various bitter compounds (Ogawa et., 2004). This study was conducted to evaluate effectiveness of L-arginine in masking the bitterness perception of KCl and to assess the saltiness and bitterness perception of mixed salt (KCl/NaCl/L-arginine) solution against the NaCl solution, using the R-Index approach.

Many traditional sensory difference tests exist to determine whether panelists can detect differences in specific attributes of two or more samples. Commonly used difference tests are triangle, pair comparison, duo-trio, A-Not-A etc. (Amerine et al., 1965). Discriminative sensory tests can be used to determine whether overall difference between products exists due to changes in processing techniques, packaging, and storage conditions. They can also be used to determine whether difference exists in specific attributes of products. However, when the degree of differences between samples is not easily distinguishable, traditional discriminative tests cannot

be used. Alternative approaches are available for determining the degree of difference in confusable samples. These small differences can be measured by using so-called Signal Detection measures which are applicable to the measurements of differences between confusable food stimuli (Green and Swets 1988, O'Mahony 1988). According to Lawless and Heyman (1999), signal detection involves 2 or more levels of stimuli. The noise (N) is a background stimulus, while the signal (S) is a weak but higher level of stimulus near the threshold.

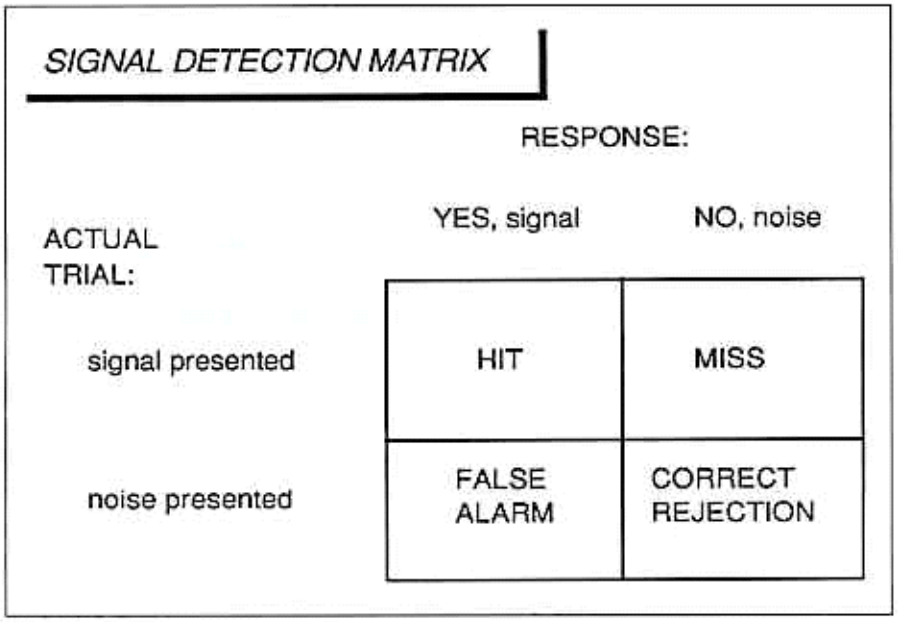

Figure 3: Signal Detection Matrix
Source: Lawless and Heyman (1999)

In the sensory experiments involving food products, the signal can be a new product while the noise can be the control product. Over many different presentations, correct decisions are made when a signal is presented (known as a “hits”) (Figure 3). There are situations when the judge responds incorrectly by responding positively for noise stimuli, thus resulting in a false alarm (Lawless and Heyman 1999). Several assumptions can be made from the signal detection theory. It is assumed that the sensations from both the signal and noise are normally distributed with equal variances. There is variation in the background levels in sensory nerves and other

factors. In addition, the judge will place a stable criterion for judgment of the stimulus once he is familiar with the stimuli (Lawless and Heyman 1999).

According to Lawless and Heyman (1999), d' is the sensory difference between signal and noise stimuli in the signal detection theory (Figure 4).

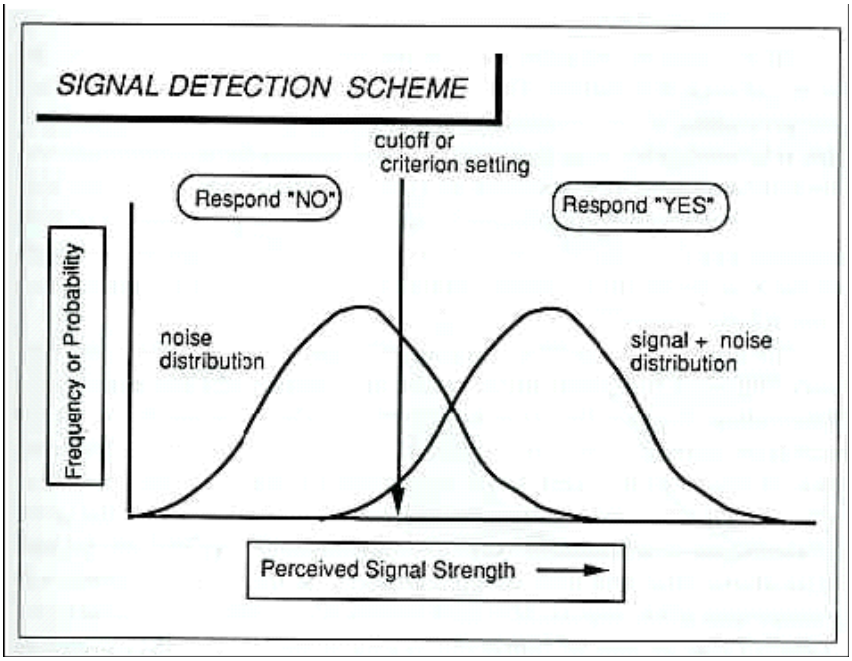

Figure 4: Signal Detection Scheme
Source: Lawless and Heyman (1999)

The d' value is calculated as the difference of the Z-score from the proportion of hits minus the Z-score from the proportion of false alarms (Lawless and Heyman 1999). It represents a separation of the means of the two distributions in units of standard deviation. The value for d' remains approximately constant as each subject's criteria for decision changes. Whenever the hit rate equals the false alarm rate, no discrimination exists between the two levels of stimuli and therefore the panelists are unable to discriminate between the intensities of the stimuli. Lawless and Heyman (1999), stated that an advantage of using the d' value is that it is possible to estimate the sensory differences in specific attributes independently of where the observer sets the

criterion for response. On the other hand, the major disadvantage of the d' value is that it requires a normal distribution in order to be calculated. Some procedures were developed based on the signal detection theory that allowed calculations of differences between samples without having to depend on a normal distribution. A popular method which is based on the signal detection theory is the R-Index approach. The R-Index technique, which was developed by Brown (1974), measures the degree of difference between a control sample, named a 'noise' sample and a comparison sample named a 'signal' sample. The R-Index measures the degree of difference in terms of probability of distinguishing the two samples. The chance value of 0.5 or 50 % signifies no difference between samples, while the value of 1 or 100 % indicates that samples are distinguishable. For samples that are indistinguishable, the R-Index values have a range between 0.5 – 1 or 50 – 100% with higher values showing more discrimination (Cliff et al., 2000).

The R-Index procedure has been used previously by O'Mahony et al. (1979) to detect off-flavors of milk. Robinson et al. (2004) used the R-Index technique to determine the effects of isoflavone content on bitter or astringent tastes. In a study reported by Argaiz et al. (2005), R-Index, as a sensory signal detection method, was used to investigate the temperature dependence of flavor development on a cooked guava beverage. According to Ishii et al. (1992), R-Index values can be obtained by using two methods: rating and ranking. Rating R-Index requires panelists to categorize samples based on how sure the panelist is about categorization. On the other hand, the ranking procedure requires that the samples be ranked along a given dimension. Repeated rankings provide sufficient data for R-Index computation, indicating the degree of perceived difference between two samples. The R-Index method is useful when more than two samples are tested. If panelists are very accurate, then only a few are needed with a number of replications. In addition, the panelists are simply required to indicate whether they feel that the

samples are same or different. However, this method only provides the probability of the judge being able to distinguish between the two samples. In addition, this technique is time consuming and requires more samples and does not provide a direction or magnitude of difference. A recent study conducted by Bi (2006) reported the use of R-Index as a powerful non-parametric test. The author stated the close relation of R-Index to the famous Mann-Whitney U statistics (MWW). Due to a fortunate relationship of these two techniques, it is possible to use MWW statistics to analyze the R-Index data. Bi (2006) mentioned that the motive of using a non parametric R-Index approach in the sensory area is that it is distribution free, more robust, and a measurement index unaffected by the decision criteria and number of categories of ratings data. Finally, R-Index can be related to the Thurstonian model. Thus, this new statistical technique for analyzing the R-Index data developed by Bi (2006) was used in our study to evaluate whether or not L-arginine was effective in masking the bitterness of KCl.

3.2 Materials and Method

3.2.1 Sample Preparation

Food Grade (FCC) NaCl and L-arginine were purchased from Voigt Global Distribution LLC (Kansas City, MO), while FCC grade KCl was obtained from EMD Chemicals INC. (Gibbstown, NJ). The Brita Water Filtration System (Brita Products Company, Oakland, CA) was purchased from a local supermarket.

Table 1: The ratio of KCl/NaCl/L-Arginine in Mixed Salt Solutions

Sample	% KCl	% NaCl	% L-Arginine
A	70	20	10
B	65	25	10
C	60	30	10
D	55	35	10
E	0	100	0

Four mixed salt (KCl/NaCl/L-arginine) aqueous solutions and the control NaCl solution at 0.5 %, 1.0 % and 1.5 % w/v concentrations were prepared (Table 1). The water used for solution preparation was filtered using the Brita Water Filtration System to eliminate the undesirable taste or odor which could have interfered with sensory perception. Each mixed salt solution was poured into 2oz plastic cups and closed with plastic lids. Plastic cups were numbered and kept for further use. All samples were prepared 1 – 2 days before sensory analysis. After each session the remaining samples were discarded.

3.2.2 Panelist Selection and Sensory Evaluation

An untrained panel of 20 people (13 females and 7 males) volunteered for sensory testing. They were students, staff, and faculty from Louisiana State University, Baton Rouge, LA. Each session was conducted at the Sensory Evaluation Laboratory in the Department of Food Science at Louisiana State University. The panelists were briefed about the procedure. The panelists were served with five labeled samples in a random order, and evaluated all samples at room temperature. Each panelist was instructed to take the sample into his/her mouth, swirl it around, and expectorate it into the cups provided. The panelists then rinsed their palate with drinking water after tasting each sample. Unsalted crackers were provided to minimize carryover effects that could be accumulated during the sessions. They were required to take a five-minute break between each testing trial. They evaluated the samples from left to right and ranked the samples in order of saltiness intensity with 1=most intense and 5=least intense. No tie was allowed for the rank score. The response was written by panelist on the special form given to them at the beginning of the session (Appendix A). Six trials were performed by the same panelists for saltiness evaluation during three-day period. A week later the same procedure was

conducted for bitterness evaluation. Data were analyzed using the non-parametric R-Index test according to Bi (2006).

3.3 Data Analysis

In order to obtain the R-Index value, the Mann Whitney U statistics was calculated. Let's assume we have two independent samples, $X_1, X_2 \ldots X_n$ and $Y_1, Y_2 \ldots Y_n$ with sizes m and n from distribution G and H.

The Mann – Whitney U statistic is

$$U = \sum_{i=1}^{m} \sum_{j=1}^{n} \phi\left(X_i, Y_j\right) \qquad \text{Eq. 1}$$

where $\varphi(X_i, Y_j) = 1$, if $X_i < Y_j$, $\varphi(X_i, Y_j) = 1/2$, if $X_i = Y_j$ and $\varphi(X_i, Y_j) = 0$ otherwise. When G and H are continuous, $P(X_i = Y_i) = 0$ according to Bi (2006). For the summarized rating frequency data of our study (Appendix A), the Man-Whitney U statistics can be calculated from the following equation:

$$U = \sum_{j=1}^{k-1} b_i \sum_{j=i+1}^{k} a_j + \sum_{i=1}^{k} a_i b_i / 2 \qquad \text{Eq. 2}$$

Where $a = (a_1, a_2, \ldots a_k)$, $b = (b_1, b_2, \ldots b_k)$ denote the frequency vectors of k-point scale ratings for two independent samples, for example, sample A (control) and sample B (Test), and a_1, b_1 denote the frequencies of samples A and B for the k-th category (Bi, 2006).

Due to the relationship of the R-Index and Man-Whitney U statistics, the following equation helps to obtain the R-Index value for rating frequency data:

$$R - \text{Index} = U/mn \qquad \text{Eq. 3}$$

Where m and n are sample size of two independent samples X and Y. U statistics could be calculated using either Eq. 2 or SPSS software (Appendix A).

3.3.1 Testing Sample Difference Using R-Index

The non-parametric R-index approach was used to test whether there were differences in saltines or bitterness perception among five salt formulations (Table 1). The null hypothesis is H_0: $R = 1/2$ and the alternative hypothesis is H_a: $R = 1/2$ for the two-sided test. The two sided test was used because there was no information to indicate which sample was saltier or bitterer in our study. The test statistic is Z, and according to Bi (2006), the following equation is used for the Z test calculation:

$$Z = \frac{R - E(R)_0}{V(R)_0} = \frac{R - 1/2}{\sqrt{(m+n+1)/12mn}} \qquad \text{Eq. 4}$$

Where, $V(R_0) = \sqrt{(m+n+1)/12mn}$, and $E(R)_0 = ½$. The test statistics follows approximately standard normal distribution and approximation is good for $m, n, \geq 8$.

3.3.2 Relationship of R-Index and Thurstonian d'

It has been stated by Bi (2006) that there is a link between the R-Index and Thurstonian d'. The author showed the relationship by the following equation:

$$R = \Phi\left(\frac{\delta}{\sqrt{2}}\right), \text{ or } R = \Phi\left(\frac{d'}{\sqrt{2}}\right), \text{ Then } d' = \sqrt{2}\Phi^{-1}(R) \qquad \text{Eq. 5}$$

where $\Phi^{-1}()$ denotes the quantile of the standard normal distribution. $F(X_0)$ denotes a cumulative distribution function of a bivariate normal distribution with mean vector $(\mu_1, \mu_2) = (0, 0)$ and covariance matrix $V = \begin{pmatrix} 1 & 1/2 \\ 1/2 & 1 \end{pmatrix}$ (Bi, 2006).

Because of the tremendous extent of calculations of R-Index and d' (d-prime), the SPSS statistical software (SPSS Inc., 2007) was used to obtain the Mann Whitney U statistics. The R-Index was calculated using Eq. 3.

3.4 Results and Discussion

Table 2: Analysis of Saltiness and Bitterness Perception of Different Mixed Salt Concentrations Using the Non-Parametric R-Index Approach

	Saltiness Perception			**Bitterness Perception**		
Pairs[b]	**0.5% w/v**	**1% w/v**	**1.5% w/v**	**0.5% w/v**	**1% w/v**	**1.5% w/v**
A – B	0.581[c] 0.024[d] 0.29[e] 2.249[f]	0.574 0.038 0.25 2.078	0.586 0.016 0.32 2.420	**0.541** **0.260** **0.14** **1.125**	**0.516** **0.641** **0.07** **0.466**	**0.541** **0.257** **0.14** **1.135**
A – C	0.616 0.001 0.39 3.227	0.678 0.001 0.66 4.94	0.685 0.001 0.66 5.123	**0.560** **0.096** **0.21** **1.666**	0.630 0.001 0.47 3.591	0.579 0.03 0.36 2.175
A – D	0.644 0.001 0.51 3.988	0.785 0.001 1.1 7.905	0.776 0.001 1.1 7.652	0.585 0.020 0.29 2.332	0.669 0.001 0.62 4.658	0.652 0.001 0.54 4.167
A – E	0.974 0.001 2.66 13.471	0.970 0.001 2.66 13.349	0.959 0.001 2.48 13.034	0.612 0.002 0.39 3.125	0.649 0.001 0.54 4.170	0.601 0.005 0.36 2.838
B – C	**0.530** **0.406** **0.1** **0.831**	0.609 0.002 0.4 3.024	0.617 0.001 0.43 3.242	**0.519** **0.585** **0.07** **0.547**	0.641 0.001 0.51 3.904	**0.538** **0.287** **0.14** **1.064**
B – D	**0.566** **0.067** **0.25** **1.829**	0.738 0.001 0.91 6.482	0.724 0.001 0.82 6.234	0.592 0.01 0.32 2.565	0.684 0.001 0.66 5.092	0.628 0.001 0.47 3.536
B – E	0.975 0.001 2.66 13.5	0.964 0.001 2.47 13.153	0.960 0.001 2.48 12.998	0.590 0.013 0.32 2.498	0.640 0.001 0.51 3.892	0.598 0.006 0.32 2.726
C – D	**0.545** **0.210** **0.14** **1.254**	0.675 0.001 0.62 4.887	0.615 0.001 0.39 3.229	**0.527** **0.444** **0.10** **0.766**	**0.563** **0.076** **0.21** **1.775**	0.603 0.004 0.36 2.850
C – E	0.959 0.001 2.48 13.08	0.953 0.001 2.33 12.870	0.928 0.001 2.1 12.153	0.577 0.032 0.29 2.142	0.600 0.005 0.36 2.778	0.590 0.014 0.32 2.467
D – E	0.947 0.001 2.33 12.78	0.919 0.001 1.99 12.044	0.926 0.001 2.1 12.127	**0.568** **0.06** **0.25** **1.880**	**0.547** **0.196** **0.18** **1.293**	**0.534** **0.348** **0.10** **0.938**

[b]– The letters in each pair correspond to salt formulations in Table 1
[c] – Corresponds to R-Index value, [d] – Corresponds to *p* value
[e] – Corresponds to d prime value, [f] – corresponds to Z value

The d' prime values corresponding to different R-index were obtained from the Eq. 5. It could be also obtained from the corresponding table provided by Bi, 2006 (Appendix A). Z values were calculated using Eq. 4 and corresponding *p* values obtained using SPSS statistical software (SPSS Data editor – Analyze – Non parametric test – 2 independent samples – Mann Whitney Statistics – *p* value) (Appendix A).

The results for the non-parametric R-Index are presented in Table 2. The sensory attributes being tested were saltiness and bitterness. The question of concern was whether there were differences in saltiness and, more importantly, in bitterness intensity among the five salt formulations. The null hypothesis was H_0: $R = 1/2$ and the alternative hypothesis was H_a: $R = 1/2$ for the two-sided test. The decision of whether the two samples were significantly different was based on the R-Index (0.5 means no difference, close to 1.0 means significantly different), *p* value, and *d* ' prime value (close to zero means no difference). The results showed that at a significance level of 0.05, panelists were able to distinguish the saltiness perception of the mixed salt solution containing 70% KCl from others (i.e., A-B, A-C, A-D, and A-E pairs) at the concentration of 0.5% w/v. There was no significant difference for the pair B-C, where the R-Index and corresponding *p* value was 0.530 and 0.460 respectively, for the pair B-D with the R-Index value of 0.566 and *p* value of 0.064, and for the pair C-D with R-Index of 0.545 and *p* value of 0.210. These results showed that when we decreased the sodium chloride proportion from 35 % to 25 %, increased potassium chloride proportion from 55 % to 65 % and kept the L-Arginine proportion constant at 10 %, the saltiness perceptions were not distinguishable for panelists. However, the panelists were able to discriminate the saltiness perception of formulation E (100 % NaCl) from the rest of the formulations. The results showed that the R-index values were 0.974 for pair A-E, 0.975 for pair B-E, 0.959 for pair C-E, and 0.947 for pair

D-E. A similar pattern was obtained when we compared d' -prime values for the above pairs. Table 2 showed that d' prime values were 2.66 for pair A-E, 2.66 for pair B-E, 2.48 for pair C-E, and 2.33 for pair D-E. Therefore, it was concluded that formulation E (100 % NaCl) was perceived different by panelists from the other formulations at 0.5% w/v. When we increased the concentration of each formulation in an aqueous solution to 1.0 % w/v and 1.5 % w/v, the panelists were able to distinguish the saltiness perception of all pairs. The R-index values ranged from 0.574 (pair A-B) to 0.970 (pair A-E), while the d' prime values ranged from 0.25 (pair A-B) up to 2.66 (pair A-E). This indicated that the concentration of the mixed salt substitutes affected saltiness perception. Regardless of the concentrations, the panelists perceived highest differences in saltiness perception between the control (E, 100% NaCl) and those containing 65-70% KCl. This was replicated by the *d* ' prime value of about 2.5, which signifies distinct differentiation (Lawless and Heyman 1999).

The results for the R-Index, *p* value and d' prime of bitterness perception at 0.5 % w/v, 1.0 % w/v and 1.5 % w/v are presented in Table 2. The panelists were not able to differentiate the bitterness perception of pairs A-B, A-C, B-C, C-D and D-E. The R-Index values were 0.541, 0.560, 0.519, 0.527 and 0.568 accordingly. The results showed that an increase of KCl from 60% to 70% at a 0.5% w/v concentration level and a decrease of sodium chloride from 30% to 20% at a fixed 10% L-Arginine yielded salt substitutes with no distinguishable difference in bitterness perception (A-B, A-C, and B-C). Nevertheless, all formulations were still perceived as significantly different from formulation E (NaCl), except formulation D (55% KCl, 35% NaCl and 10% L-Arginine). This means at a 0.5 % w/v concentration level, the NaCl (35%) and L-Arginine (10%) mixture was able to mask the bitterness perception of KCl. When the concentration of each formulation was increased to 1.0% w/v, the bitterness perception was

distinguishable for most of the pairs, except the pair A-B, C-D and D-E. Pair D-E is the most important pair because the bitterness perception of the formulation D (55% KCl, 35% NaCl and 10% L-Arginine) was not distinguishable from formulation E (0% KCl, 100% NaCl and 0% L-Arginine). The R-Index *p* value and d' prime of this pair was 0.547, 0.196 and 0.18, respectively. With further increase of concentration to 1.5% w/v, the pair D-E still showed no significant difference in bitterness perception.

An interesting pattern was observed when all parameters (R-Index, *p* value, d' prime) were compared among three concentrations (0.5% w/v, 1.0% w/v and 1.5% w/v) for the D-E pair. The R-Index value decreased from 0.568 at 0.5% w/v to 0.547 at 1.0% w/v and to 0.534 at 1.5% w/v. A similar trend was observed for *p* value, increasing from 0.06 at 0.5% w/v to 0.196 at 1.0% w/v and to 0.348 at 1.5% w/v, while the d' prime value was decreased from 0.25 at 0.5% w/v to 0.18 at 1.0% w/v and to 0.1 at 1.5% w/v. This indicated that the concentration of the mixed salt substitutes affected bitterness perceptions, i. e., the panelists perceived less bitterness with increased NaCl and L-Arginine in the solutions. This trend can be explained by the fact that L-Arginine was able to mask the bitterness of KCl and NaCl was able to enhance the bitterness suppression of L-Arginine (Table 1). Furthermore, Ogawa et al. (2004) reported that the highest degree of suppression of bitterness by L-Arginine could be achieved with the addition of NaCl.

3.5 Conclusion

Four salt solutions and the control (NaCl) solution were studied. The data for ten possible pairs of formulations were obtained and analyzed. It was observed that panelists were able to distinguish the saltiness perception of mixed salt (KCl/NaCl/L-Arginine) solutions from the NaCl solution. They could discriminate the saltiness perception of all salt formulations from the control NaCl at the concentration of 0.5%, 1%, and 1.5% w/v. For the bitterness perception,

there were no differences at 0.5%, 1% and 1.5% w/v between formulation D (55% KCl, 35% NaCl and 10% L-Arginine) and formulation E (0 % KCl, 100 % NaCl and 0 % L-Arginine). Therefore, L-Arginine and NaCl could synergistically mask the bitterness of potassium chloride in the salt substitutes.

The R-Index is the essential distribution-free statistics test that has been used for sensory research for testing product/attribute effects (Bi, 2006). Currently, the traditional R-index analysis is in use, but because of the recent finding by Bi (2006), the new non-parametric approach was used in this study. Due to the relationship of the R-Index to the Mann-Whitney U statistics and the connection of the R-Index to the Thusrtonian Modeling, we were able to analyze the data for saltiness and bitterness evaluation of a mixed salt solution consisting of KCl, NaCl and L-Arginine.

This new approach showed similar results obtained by Waimaleongora-Ek, (2006). The author conducted a similar study using the traditional R-Index approach. Her findings were comparable to the results obtained from the non-parametric R-Index approach, which shows the effectiveness of this new method suggested by Bi (2006).

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CHAPTER 4.

CONSUMER-ORIENTED SENSORY OPTIMIZATION AND SENSORY CHARACTERISTICS OF A LOW-SODIUM SALT MIXTURE CONTAINING NaCl, KCl AND L-ARGININE

4.1 Study I: Consumer-Oriented Sensory Optimization of a Low-Sodium Salt Containing NaCl, KCl and L-Arginine

4.1.1 Introduction

High sodium chloride intake contributes to the development of hypertension (Ball et al., 2002). Early data from animal studies by Tobian (1991) and observational studies in humans by Froment et al. (1979), showed a relation between sodium intake and blood pressure. Short-term trials conducted by Sacks et al. (2001), suggested that reducing sodium intake lowers blood pressure. Law (2000) recommended that reducing sodium intakes by 100 mmol/day would decrease stroke mortality by 22% and ischemic heart disease by 16 % in Western societies. Loria et al. (2001) recommended that blood pressure can be lowered by reducing the amount of sodium in the diet among individuals with and without hypertension. According to Kannel, (1996); Stamler et al. (1993), hypertension is a risk factor for cardiovascular disease and is highly common in the U.S. population. Therefore, lowering the sodium intake should be a necessary constituent of national public health policy (Burt et al., 1996).

However, taste has been the major difficulty encountered with NaCl restriction in food products. Unsalted food products are less pleasant and less acceptable (Pasin et al., 1989). Therefore, the need arises to modify the food formulations by reducing or partially replacing the sodium content while maintaining the desirable sensory and chemical properties of NaCl. Naewbanij et al. (1986) reported that KCl might be a possible functional substitute for NaCl in cucumber fermentation brine. The effects of KCl on sensory qualities of fermented cabbage, radish and cucumber were studied by Park et al. (1986). Choi et al. (1994) indicated that brines containing up to 50% KCl as a replacement for sodium chloride have acceptable sensory qualities in kimchi. On the other hand, Gimeno et al. (2001) investigated the use of calcium ascorbate to replace 46% NaCl in dry fermented sausage and concluded that the product had

acceptable sensory and physical properties (texture, color, etc.). Gou et al. (1996) evaluated the effect of KCl, K-lactate and glycine on the flavor, texture and color characteristics of fermented sausage. They concluded that a salt substitution could be achieved in that product. Ball et al. (2002) suggested the use of calcium diglutamate as a possible substitute for NaCl in order to lower Na concentration in a soup. They stated that usage of calcium diglutamate could help to achieve the similar flavor characteristics of sodium chloride in a soup.

Although a number of salt substitutes have been developed according to Frank et al. (1970), the most commonly used NaCl replacement thus far has been KCl, which has physicochemical properties similar to NaCl and is a good candidate for salt substitute. Although sodium replacement with KCl has been used to develop salt substitutes, bitterness remains a major taste problem. Thus, bitterness inhibitors have to be included into food formulations to mask undesirable taste of KCl.

In addition to biological properties, L-arginine has been reported to mask the bitterness of various compounds and enhance the saltiness of NaCl (Ogawa et al., 2004). Ogawa et al. (2004) reported that L-arginine was successful in reducing the bitterness of various solutions containing bitter compounds. It has been shown that with a usage of L-arginine, the bitterness of quinine was significantly suppressed. The bitterness suppression of L-arginine was enhanced by the addition of NaCl. The study conducted by Ogawa et al. (2004) showed that the degree of suppression reached by L-arginine and NaCl was greater than that of any of other bitterness suppressing agents, including phosphatidic acid and tannic acid. Thus, development of a healthy salt alternative could be a possible solution for reducing the prevalence of hypertension and its associated disease risk in the U.S. Our previous study (Chapter 3) showed the effectiveness of L-arginine in masking the bitterness of a low-salt formulation. Specifically, panelists were not able

to differentiate the bitterness of formulation D (55% KCl, 35% NaCl and 10% L-Arginine) from the NaCl solution at 0.5%, 1% and 1.5% w/v concentration levels. Therefore, the objectives of this study were 1) to optimize sensory acceptability of the salt mixture of NaCl/KCl/L-Arginine using a mixture design experiment and 2) to develop an acceptable NaCl/KCl/L-arginine low-sodium salt product, using a chicken broth as a model.

4.1.2 Materials and Methods

4.1.2.1 Materials

Food-grade NaCl and L-arginine were purchased from Voigt Global Distribution LLC (Kansas City, MO), while food-grade KCl was obtained from EMD Chemicals INC. (Gibbstown, NJ). The Brita Water Filtration System (Brita Products Company, Oakland, CA) was purchased from a local supermarket. Whole chickens (6) (Piligrim Pride brand name) with the weight of 4.12-4.37lbs were purchased from local Wal-Mart supermarket. All chickens had been cleaned and covered in polyethylene bags prior to purchase.

4.1.2.2 Chicken Broth Preparation

Water used for chicken broth preparation was filtered using the Brita Water Filtration System to eliminate any undesirable taste or odor which could have interfered with sensory perception. All six chickens were thoroughly cleaned before placing them into a 20-gallon stainless steel pot. The filtered water (approximately 45 L) was added to the upper level of the container. They were cooked on an electric stove (Model RBS305PR, Whirlpool Corporation, Benton Harbor, MI) at 300 ^{0}F for 4 h. The chicken broth was regularly stirred and resulting foam was removed every 15 min. The cooked chicken broth was filtered, allowed to cool down, poured into a sanitized plastic container and stored at 4 ^{0}C for the next day consumer test. The cooked chicken meat and bones were discarded.

4.1.2.3 Mixture Design Experiment

In a mixture design experiment, two or more ingredients are mixed or blended together to form a new product. If we are able to control the varying ingredient proportions so that the characteristics of the product depend completely upon the relative percentages of the ingredients in the mixture then, we have a mixture experiment (Cornell, 1983). The proportions of controlled variables could be by weight, by volume, or by mole fractions. The proportions in the system always sum to unity or one. For example, with three ingredients written as X1, X2 and X3, the sum of the proportions will be equal to one:

$$\Sigma X_i = X1 + X2 + X3 = 1.0$$

According to Cornell (1983) a mixture experiment with a three component system can be represented using a triangle (Fig. 5) with the vertices representing the single-component mixtures, where Xi = 1 and Xj = Xk = 0 for i, j, k = 1, 2, and 3 and $i \neq j \neq k$. The vertices of the triangle are denoted by (1, 0, 0), (0, 1, 0), and (0, 0, 1), respectively, for X_i, X_j, and X_k. Any interior points in the triangle represent mixtures that contain all three of the components, and the center (centroid) of the triangle represents a mixture containing equal proportions (1/3, 1/3, 1/3) of each of the three components. Since the component proportions are constrained between zero and one, the experimental region of all possible compositions is (q-1) dimensional simplex, where q is the number of components. For q = 3, the experimental region or simplex is equilateral triangle. To explore the entire simplex region, a special design called a "simplex – lattice" is used (Cornell, 1983). The simplex-lattice design introduced by Scheffe (1958) helps to define points or proportions in a (q-1) dimensional simplex. For three-component blends, three points represent the vertices of the triangle: X1, X2, X3 = (1, 0, 0), (0,1,0) and (0,0,1), while the rest of the points are in the interior of the triangle (Fig. 5).

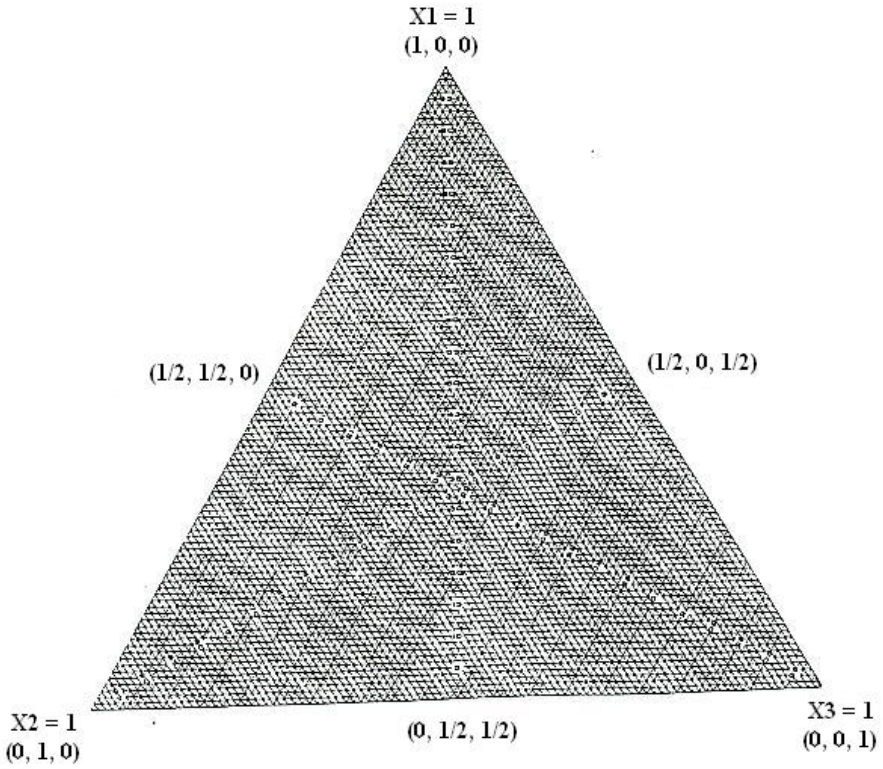

Figure 5: A triangle for Plotting Three Component Systems in the Mixture Experiment
Source: Cornell (1983)

The data collected from a mixture experiment can be modeled using a non-intercept regression analysis. The resulting model is used to generate a contour plot within the triangle. The model will yield predictions of consumer responses for any combinations of the three components involved (Bond, 2004; Cornell, 1983).

4.1.2.4 Selection of Salt Mixture Components

Based on the mixture design experiment, an optimization study was performed using the three-component constrained simplex-lattice mixture design (Cornell 1983). Mixture components, consisting of NaCl (X1), KCl (X2) and L-arginine (X3) were used in the formulations. The proportions of the components were expressed as fractions of the mixture. The salt substitute formulations were prepared using NaCl (0 -100%), KCl (0-100%) and L-Arginine

(0-15%). The sum of the component proportions (X1 + X2 + X3) is equal 1.0 or 100%. The proportions of ingredients in the mixture were established based on the q = 3 simplex-lattice design (Fig. 6) (Cornell, 1983).

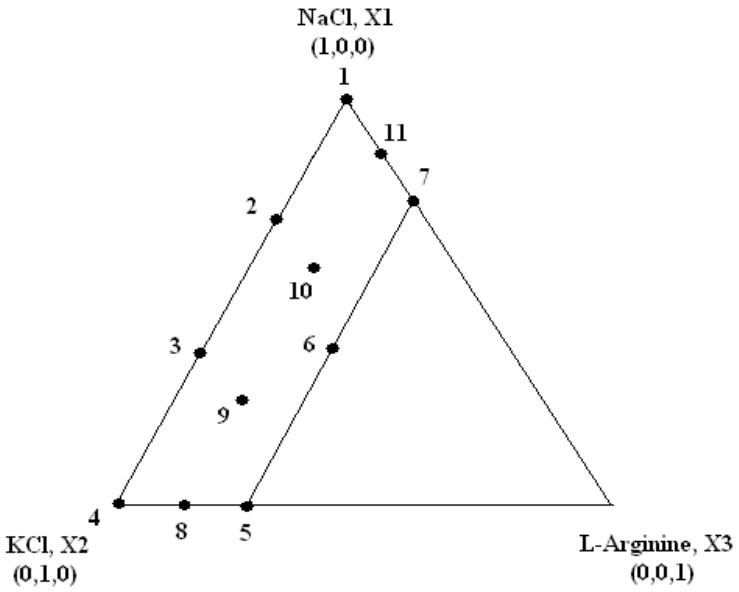

Figure 6: The Constrained Region in the Simplex Coordinate System Defined by the Following Restrictions: $0.0 \leq X1 \leq 1.0$, $0.0 \leq X2 \leq 1.0$ and $0.0 \leq X3 \leq 0.15$. Where X1 = NaCl, X2 = KCl, X3 = L-Arginine. Numbers (1-11) Represent the 11 Formulations and Correspond to the Numbers in Table 3

The proportions of ingredients expressed in weight percentages are shown in Table 3. The percentage of L-Arginine was 0% for formulations 1 – 4, 15% for formulations 5 – 7, and 15% for formulations 8 – 11. Formulation # 1 was the control which consisted of 100% NaCl. Formulations # 2 and # 3 contained 65% NaCl, 35% KCl and 35% NaCl, 65% KCl respectively. Formulation # 4, # 5, and # 8 contained 100%, 85%, and 92.5% KCl respectively, all contained 0% NaCl. Formulation 6 contained 40% NaCl and 45% KCl. Formulation # 7 and # 11 contained 85% and 92.5% NaCl, respectively; both contained 0% KCl. Formulation # 9 contained 28% NaCl and 64.5% KCl. Formulation # 10 contained 57% NaCl and 35.5% KCl. All formulations were applied at 1% w/v to unsalted chicken broth for consumer acceptance test.

Table 3: Salt Mixture Formulations in the Three – Component Constrained Simple Lattice Mixture Design

Formulation[a]	NaCl (%)	KCl (%)	L-Arg (%)
1	100	0	0
2	65	35	0
3	35	65	0
4	0	100	0
5	0	85	15
6	40	45	15
7	85	0	15
8	0	92.5	7.5
9	28	64.5	7.5
10	57	35.5	7.5
11	92.5	0	7.5

[a] Formulation numbers (1 – 11) correspond to the numbers shown in Figure 6.

4.1.2.5 Salted Chicken Broth Preparation

The cooked chicken broth was poured in 500ml beakers, marked with appropriate sample names. One weight percent of each salt mixture formulation and the control was added to each beaker and stirred with a stirring bar until they were totally dissolved. Each sample was then poured into 2 oz plastic cups and closed with plastic lids. The plastic cups were numbered and kept for further use. After each session, the remaining samples were discarded. All samples were prepared one day before the consumer test.

4.1.2.6 Consumer Acceptance Test

The experimental consumer test protocol was approved by the LSU AgCenter Institutional Review Board. Untrained consumers (n = 385) were randomly recruited from the Baton Rouge, LA, area. Criteria for recruitment were that participants were at least 18 years of age, were not allergic to chicken and L-arginine, and were available to participate on scheduled testing days. The central location test for consumer acceptance was conducted for 3 days at the Dairy Store at LSU AgCenter. At the beginning of each session, consumers were asked to

provide demographic information. The Balanced Incomplete Block Design (t = 11, k = 2, r = 10, b = 55, λ = 1, *E* = 0.55, Type II) (Cochran and Cox, 1957) was used in this experiment because it is difficult to evaluate the samples as the number of samples increased (Prinyawiwatkul and others 1997). With this design, each consumer evaluated two out of eleven samples. Prior to evaluation each chicken broth sample was heated in microwave oven (Model RBS305PR, Whirlpool Corporation, Benton Harbor, MI) for 10-15 s. Then each consumer was presented with two coded chicken broth samples in 2 oz plastic cups. These formulations were randomly coded with the number 1 to 11 for a total of 70 observations (replications) per formulation. Water, unsalted crackers, and expectoration cups were provided for consumers during the test to minimize carryover effect.

Consumers were instructed to sip each sample, swirl it with the tongue and then either swallow or expectorate before providing acceptability ratings for sensory attributes. They were told to evaluate each sample for saltiness, bitterness, aftertaste, and overall liking on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely*).* Overall acceptance and purchase intent were evaluated using the binomial (yes/no) scale.

4.1.2.7 Statistical Data Analysis

All analysis was conducted at α = 0.05, using SAS software version 9.1 (SAS Institute. 2003). The analysis of variance (ANOVA) was performed to determine difference in acceptability of each sensory attribute and overall liking of each broth formulation. The Tukey's Honestly Significant Difference (HSD) test was performed for multiple comparisons.

The non-intercept Multiple Regression Analysis (MRA) was performed to predict the acceptability of each sensory attribute and the predictive models were used to plot the mixture response surface for the three-component mixture design experiment. Because of the restriction

of the mixture design, the reduced model was fit. The intercept was set to zero and not included in the model.

Logistic Regression Analysis (LRA) was used to predict acceptance and purchase intent of eleven chicken broth formulations. Logistic regression calculates the probability of success (event) over the probability of failure (non event), and expresses the results in the form of a likelihood or the odds ratio estimate. The odds ratio estimates are a nonnegative number with a value that is greater than 1.0 when a success is more likely to occur than a failure (Agresti, 1996). When odds = 4.0, a success is four times as likely as a failure. When an estimated odds ratio equals 1.0 it means that there is no significant association between the two variables (Agresti, 1996).

The Multivariate Analysis of Variance (MANOVA) was used to further analyze the data in order to identify whether significant differences exist among 11 chicken broth formulations when all four attributes (saltiness, bitterness, aftertaste, and overall liking) were considered simultaneously. Descriptive Discriminant Analysis (DDA) (Huberty, 1994) was conducted to determine discriminating attributes for the underlying differences among the eleven broth samples. Predictive Discriminative Analysis (PDA) (Huberty, 1994) was used to identify sensory attributes critical to overall acceptance and purchase intent. For PDA, hit rate (%) of acceptability was calculated for each of the four sensory attributes. PDA works with classification of products based on several variables simultaneously. It is an analog of a regression analysis. A fitted set of data to a mathematical function will give an observation its highest probability of being assigned to the known correct population while minimizing the probability that the same observation will be misclassified (Resurreccion, 1998). The Principal Component Analysis (PCA) was used to demonstrate any existing relationship among the

sensory attributes (saltiness, bitterness, taste, and overall liking) and the relationship between these attributes and the eleven formulations. The first principal component (PC) covers as much of the variation in the data as possible and the second PC is orthogonal to the first and covers as much of the remaining variation as possible.

The non-parametric McNemar test (Agresti, 1996) was used to determine changes in consumers' acceptance and purchase decision before and after they had been given the information of health benefits of salt substitute. It is a test of marginal homogeneity for matched binary responses and the variation of chi-square distribution with one degree of freedom (Agresti, 1996). The null hypothesis for the McNemar test (Ho: $\pi_{1+} = \pi_{+1}$ or $\pi_{12} = \pi_{21}$) stated whether the difference between the probability of those who answered yes after (π_{1+}) they had been informed about health benefits of salt substitute and the probability of those who answered yes before (π_{+1}) is significant, or whether it is merely by chance.

In order to estimate the actual differences in the means, 95% confidence interval (CI) was calculated using marginal sample proportions ($P_{+1} - P_{1+}$). Marginal sample proportion was calculated using the following formula:

$$p_{ij} = n_{ij}/N$$

where N is the total number of consumer responses, n_{ij} is the number of consumers making decision i before and decision j after the additional information about the health benefits of salt substitute was provided. The following equation was used to obtain 95% confidence interval (CI):

$$(p_{+1} - p_{1+}) \pm Z_{\alpha/2}(ASE)$$

where ($P_{+1} - P_{1+}$) represents the difference in proportions between the consumers who would accept/purchase the product after additional information was provided (P_{+1}) and those who

would also accept/purchase the product before the additional information was provided (P_{1+}). The term $Z_{\alpha/2}$ is the standard normal percentile having a right-tail probability equal to $\alpha/2$. For a 95% CI, $Z_{\alpha/2} = 1.96$. ASE is the estimated standard error for the proportion difference. The following equation was used for calculation:

$$ASE = \{[P_{1+}(1-P_{1+}) + P_{+1}(1-P_{+1}) - 2(P_{11}P_{22}-P_{12}P_{21})]/N\}1/2$$

where P_{11} is the proportion of consumers who would accept/purchase the product before and after additional information was provided, P_{12} is the proportion of those who would accept/purchase before but not after, P_{21} is the proportion of those who would not accept/purchase the product before but would be willing to accept/purchase afterwards, and P_{22} indicates the number of subjects who answered negatively both before and after.

4.1.2.8 Development of Optimal Formulation

Product optimization was performed using the three-component mixture design experiment. The predictive models were obtained using a restricted regression analysis (without intercept) and used to plot the mixture response surface. Based on previous work done by Prinyawiwatkul et al. (1997), predictive models were used to constract contour plots for saltiness, bitterness, aftertaste, and overall liking. The acceptable areas were identified on the contour plots where the consumer ratings > 6.0 on a 9 - point hedonic scale (Prinyawiwatkul et al., 1997). The superimposition of acceptable areas for all four sensory attributes yield the optimal formulation (Palomar et al., 1994).

4.1.3 Results and Discussion

4.1.3.1 Consumer Demographic Information

Out of 385 consumers who participated in this study, 48% were females and 52% were males. The majority of the consumers were distributed among the age of 18 – 34. The remainder were 35 – 44 years of age (2%), 45 – 54 years of age (2%), and over 55 years of age (2%).

4.1.3.2 Consumer Acceptability

Based on sensory acceptability profile (Table 4), all sensory attributes received a mean score of no less than 3.0. Among formulations containing KCl, consumers preferred the saltiness of formulation # 10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) with the highest acceptability score of 5.93. Among formulations containing NaCl, formulation # 7 (85% NaCl and 15% L-Arginine) was most acceptable for saltiness.

Table 4: Mean Consumer Acceptance Scores for Saltiness, Bitterness, Taste and Overall Liking of Eleven Salt Formulations[a]

Formulation number[b]	Saltiness	Bitterness	Taste	Overall Liking
1	5.51 ± 1.89^{ab}	6.16 ± 1.98^{a}	5.99 ± 1.74^{a}	6.03 ± 1.83^{ab}
2	5.53 ± 1.96^{ab}	5.63 ± 2.13^{a}	6.01 ± 1.83^{a}	5.86 ± 1.91^{ab}
3	5.20 ± 1.98^{bc}	5.49 ± 2.03^{a}	5.33 ± 2.03^{a}	5.16 ± 2.00^{bc}
4	3.93 ± 1.69^{d}	3.59 ± 1.83^{b}	3.76 ± 1.69^{b}	3.61 ± 1.87^{d}
5	4.46 ± 1.59^{dc}	4.24 ± 2.02^{b}	3.91 ± 2.03^{b}	4.17 ± 2.13^{dc}
6	5.74 ± 1.57^{ab}	5.99 ± 1.72^{a}	5.90 ± 1.58^{a}	5.90 ± 1.72^{ab}
7	6.30 ± 1.88^{a}	6.0 ± 1.91^{a}	6.34 ± 1.91^{a}	6.26 ± 1.89^{a}
8	4.19 ± 1.97^{dc}	4.26 ± 1.94^{b}	4.00 ± 1.92^{b}	3.83 ± 1.95^{d}
9	5.60 ± 1.92^{ab}	5.46 ± 1.95^{a}	5.44 ± 1.90^{a}	5.54 ± 1.82^{ab}
10	5.93 ± 1.74^{ab}	6.07 ± 1.74^{a}	6.20 ± 1.69^{a}	6.09 ± 1.85^{ab}
11	5.73 ± 2.24^{ab}	5.89 ± 2.15^{a}	6.07 ± 2.20^{a}	6.14 ± 2.18^{ab}

[a] Based on 70 consumer responses and on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Mean values within the same column not followed by the same letters are significantly different ($p < 0.05$).
[b] Formulation numbers correspond to the numbers shown in Fig. 1 and Table 1.

The lowest acceptability scores for saltiness were observed for formulations # 4 (0 % NaCl, 100 % KCl, 0 % L-Arginine) followed by # 8 (0 % NaCl, 92.5 % KCl, 7.5 % L-Arginine), then # 5 (0 % NaCl, 85 % KCl, 15 % L-Arginine). The low acceptability scores observed for formulations # 4, # 5 and # 8 may have been due to bitterness of KCl; these three formulations contained 0% NaCl which could suppress the bitterness of KCl (Keast et al. 2001). Based on Tukey's honestly significant difference (HSD) test results (Table 4), consumer acceptance rating for saltiness showed that there is no significant difference between control formulation # 1 (100

% NaCl, 0 % KCl, 0 % L-Arginine) and formulations # 2, 6, 7, 9, 10, 11. An addition of KCl up to 35.5%, 7.5% L-Arginine and reduction of NaCl up to 57% showed no significant difference in saltiness acceptability among the consumers.

The mean score of bitterness acceptability of the control formulation was 6.16. The bitterness acceptability score was slightly affected by the addition of 35 to 65 % KCl, 7.5 % L-Arginine and the reduction of NaCl up to 57 %. The addition of 85 to 100 % KCl and 15 % L-Arginine adversely affected the bitterness acceptability score by lowering it from 6.16 (formulation # 1) to 3.59 (formulation # 4). The bitterness of salt substitute was most acceptable (score = 6.07) for formulation # 10 (57 % NaCl, 35.5 % KCl, 7.5 % L-Arginine) compared with the control (100% NaCl). This may be associated with the fact that L-Arginine can mask the bitterness perception of KCl (Ogawa and others 2004). The lowest acceptable score was observed for formulation # 4 (0 % NaCl, 100 % KCl, 0 % L-Arginine), followed by # 5 (0 % NaCl, 85 % KCl, 15 % L-Arginine), and # 8 (0 % NaCl, 92.5 % KCl, 7.5 % L-Arginine), which was mostly attributed to bitter taste of KCl.

Mean scores acceptability for taste showed a similar pattern to that of the bitterness acceptability. Among formulations containing KCl, the taste of formulation # 10 containing 57% NaCl, 35.5% KCl and 7.5% L-Arginine received highest acceptability score of 6.20. This was attributed to the property of L-Arginine as well as sodium chloride that synergistically masked the bitterness of KCl (Ogawa and others 2004). The taste of formulation containing from 85 % to 100 % KCl was least acceptable by the consumers receiving a score of 3.76 for formulation # 4 and 3.91 for formulation # 5, respectively. The lowest score received by the consumers was more likely due to the bitter taste of KCl and the absence of NaCl. Although formulation # 5 contains L-Arginine, which is believed to have bitter masking properties, it was not able to mask the

bitterness of KCl by itself. It has been proved by Ogawa et al. (2004) that L-Arginine combined with the NaCl shows more bitterness masking properties. The same trend can be observed in Table 4, where formulation # 10 (57 % NaCl, 35.5 % KCl, and 7.5 % Arginine) received the highest acceptability score whereas formulation # 5 (0 % NaCl, 85 % KCl, 15 % L-Arginine) as well as formulation # 4 (0 % NaCl, 100 % KCl, 0 % L-Arginine) received the lowest scores.

According to Table 4, the mean score for overall liking was influenced by the addition of KCl and L-Arginine. This was evidenced by the wide variation in overall liking scores of all formulations, ranged from 3.83 (Formulation # 8) to 6.26 (Formulation # 7). The mean score for overall liking of the control formulation was 6.03 which was not significantly different from formulation # 10 with the score of 6.09. According to Tukey's test, the formulation # 4, # 5, # 8, all containing 0% NaCl, were significantly different from other formulations, and they received the lowest score of acceptance by the consumers.

4.1.3.3 Overall Product Difference and Discriminating Sensory Attributes

Analysis of variance (ANOVA) and the Tukey's honestly significant difference (HSD) test (Table 4) indicated that differences existed in acceptability of saltiness ($p < 0.0001$), bitterness ($p < 0.0001$), taste ($p < 0.0001$), and overall liking ($p < 0.0001$) among 11 salt formulations. However, to determine if the eleven formulations were different when all four sensory attributes were considered simultaneously, the Multivariate Analysis of Variance (MANOVA) was performed (Table 5).

Based on MANOVA results (the approximate F value of 5.91 and the Wilks' Lambda p value of < 0.0001), it can be concluded that a significant difference existed among all eleven salt substitute formulations when all four sensory attributes were compared simultaneously. Since MANOVA indicated that differences exist among eleven formulations, a Descriptive

Discriminant Analysis (DDA) analysis was performed to identify which sensory attributes accounted for the group difference.

Table 5: Overall Product Difference Analyzed by MANOVA

Test Criteria and F Approximation for the Hypothesis of No Overall Form Effect

S=4 M=2.5 N=376.5

Statistic	Value	F value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.74024384	5.91	40	2864.7	<0.0001
Pillai's Trace	0.27245712	5.54	40	3032	<0.0001
Hotelling-Lawley Trace	0.33403789	6.29	40	2101.8	<0.0001
Roy's Greatest Root	0.27802933	21.07	10	758	<0.0001

Results (Table 6) showed the canonical structure r's (Huberty 1994), an indication for the group differences.

Table 6: Canonical Structure r's Describing Group Differences among Eleven Salt Substitute Formulations[a]

Attribute	**Can 1**	**Can 2**	**Can 3**
Saltiness	0.716	0.557	-0.201
Bitterness	0.827	0.114	0.534
Taste	0.958*	-0.051	-0.224
Overall Liking	0.931*	0.265	-0.100
Cumulative Variance Explained (%)	83.2	90.1	96.0

[a] Based on the pooled within-group variances. Can 1, 2, and 3 refer to the first, second and third canonical discriminant functions, respectively.

* Indicates attributes which accounted for the group differences in the first dimension.

According to the pooled within canonical structure in the first dimension (Can 1), taste (0.958) and overall liking (0.931) were the sensory attributes that significantly contributed to the differences among the eleven formulations. The similar pattern was observed when gender was taken into consideration (Table 7). According to DDA analysis, taste (0.936 for male and 0.923 for female) and overall liking (0.926 for male and 0.864 for female) were the discriminating sensory attributes based on the first canonical dimension. Based on data from Tables 6 and 7 it

can be concluded that the main sensory attributes that largerly accounted for group differences were taste and overall liking.

Table 7: Canonical Structure r's Describing Group Differences among Eleven Salt Substitute Formulations[a] for Male and Female Consumers

Male Consumers (52%)			
Variable	**Can 1**	**Can 2**	**Can 3**
Saltiness	0.857	0.443	-0.057
Bitterness	0.847	-0.102	0.459
Taste	0.936*	-0.263	-0.204
Overall Liking	0.926*	-0.036	-0.246
Cumulative Variance Explained (%)	67.5	85.2	94.5
Female Consumers (48%)			
Variable	**Can 1**	**Can 2**	**Can 3**
Saltiness	0.594	-0.077	0.274
Bitterness	0.747	0.337	-0.421
Taste	0.923*	-0.258	0.091
Overall Liking	0.864	0.254	0.354
Cumulative Variance Explained (%)	83.7	95.3	98.5

[a] Based on the pooled within-group variances. Can 1, 2, and 3 refer to the first, second and third canonical discriminant functions, respectively.
* Indicates attributes which accounted for the group differences in the first dimension.

The Principal Component Analysis (PCA) showed the relationship between eleven formulations and sensory attribute acceptability (Fig. 7). The biplot showed that out of four sensory attributes, taste and overall liking were closely correlated and contributed for group difference among eleven formulations. A similar pattern was obtained from the DDA analysis in the first dimension Can 1 (Table 6). Results from PCA indicate that formulations # 4, # 5, # 8 were positioned distant from control # 1 and other formulations. Based on the following sensory attributes: taste, bitterness, and overall liking, formulation # 1 was highly correlated with formulations # 2, # 6, # 11 and # 10, which means that for the consumers the formulations # 1, # 2, # 6, # 11 and # 10 were not significantly different whereas, formulations # 4, # 5, and # 8 were

negatively correlated with other formulations. Based on the sensory attribute of saltiness, the control formulation was still positively correlated to formulations # 2, # 6, # 11 and # 10.

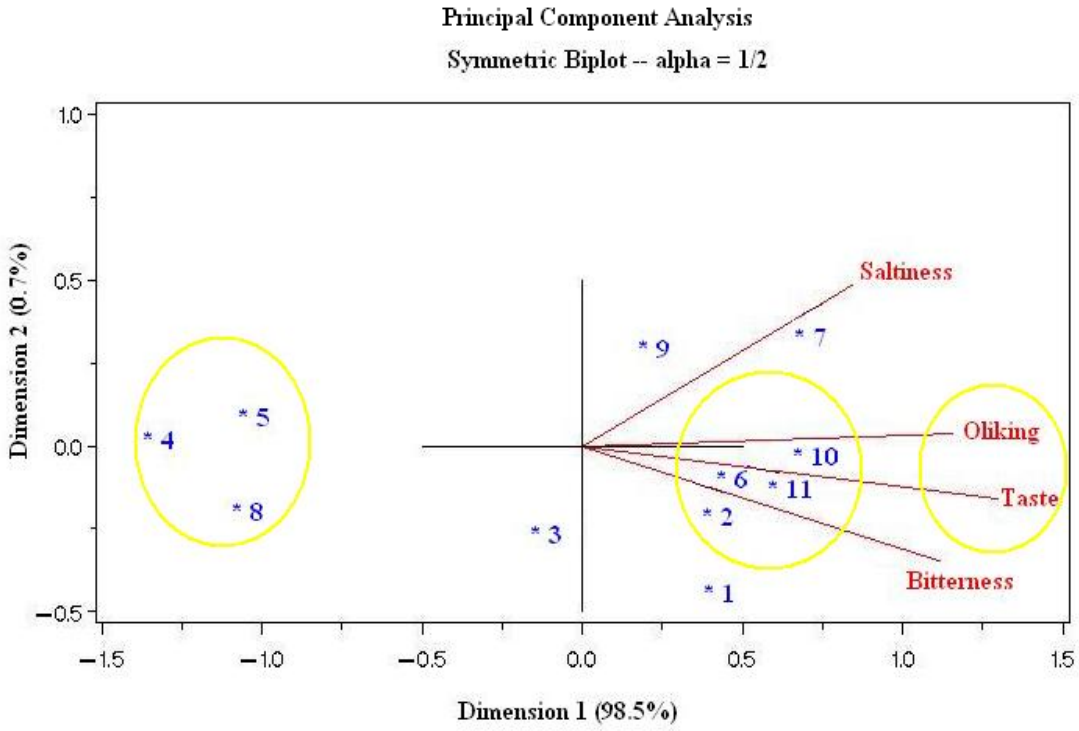

Figure 7: The PCA Product-Attribute Biplot Involving Principal Component1 and Principal Component 2
* Corresponds to eleven salt substitute formulations in Figure 1 and Table 1
Oliking = Overall Liking

4.1.3.4 Sensory Attributes Influencing Overall Acceptance and Purchase Intent

Using Logistic regression Analysis (LRA), we were able to identify the sensory attributes that were critical for overall acceptance and purchase intent of salt formulations and to predict the acceptance and purchase intent based on those attributes. Based on LRA results (Table 8), overall liking, as well as taste, was the most influencing attributes for overall acceptance. The odds ratio of overall liking, considering a full model with four sensory attributes, was 2.048, indicating that the probability of the salt substitute formulation being accepted is 2.048 times

higher than not being accepted with every 1 – unit increase of the overall liking score based on a 9 – point hedonic scale

Table 8: Parameter Estimates, Probability and Odds Ratio Estimates for Predicting Acceptance and Purchase Intent[a] of Salt Substitute Formulations

Variables	Acceptance			Purchase Intent		
	$Pr > \chi^2$ (full model)	Odds Ratio (full model)	Odds Ratio (single-var model)	$Pr > \chi^2$ (full model)	Odds Ratio (full model)	Odds Ratio (single-var. model)
Saltiness	0.0242	1.266	2.790	0.0757	1.185	2.504
Bitterness	0.0040	1.283	2.488	0.0480	1.171	2.185
Taste	<.0001	1.701	3.710	0.0024	1.424	3.129
Overall Liking	<.0001	2.048	3.904	<.0001	2.178	3.517

[a] Based on Logistic Regression Analysis, using full and single variable models with four sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates. Significance of parameter estimates was based on the Wald $\chi 2$ value at $p < 0.05$.

For purchase intent, overall liking and taste were influential attributes with the odds ratio of 2.178 and 1.424, respectively (Table 8). The odds ratio of the taste for acceptance (1.701) was slightly higher than for purchase intent (1.424), indicating that consumers perceived taste as a more influencing factor for overall acceptance, than for purchase intent, whereas consumers perceived overall liking as a more critical attribute to purchase intent than to overall acceptance, with the odds ratio increasing from 2.048 to 2.178. On the other hand, saltines influenced overall acceptance ($p = 0.0242$) but not purchase intent ($p = 0.0757$).

A similar trend was observed when consumers were divided based on gender (Tables 9). According to LRA analysis, for the female consumers, overall liking ($p < 0.0001$) and taste ($p < 0.0017$) were influential attributes for overall acceptance, while overall liking ($p <.0001$) and saltines ($p = 0.0224$) were critical attributes for purchase intent. However, after giving the health benefit information of salt substitute formulations, the overall liking ($p < .0001$) and bitterness (p

< .0.0033) were critical attributes for acceptance, while overall liking ($p < 0.0066$) and saltiness ($p < 0.0002$) were critical attributes for purchase intent.

Table 9: Logistic Regression Analysis for Predicting Acceptance and Purchase Intent of Salt Substitute Formulations for Male and Female Consumers[a]

Male Consumers				
	Acceptance	**Acceptance (after)[b]**	**Purchase Intent**	**Purchase Intent (after)[c]**
Variables	Pr > χ^2	Pr > χ^2	Pr > χ^2	Pr > χ^2
Saltiness	0.1139	0.7594	0.6782	0.9949
Bitterness	0.0461	0.0060	0.2758	0.3179
Taste	0.0045	0.0283	0.0223	0.4847
Oliking	0.0001	0.0504	<0.0001	<0.0001
Female Consumers				
	Acceptance	**Acceptance (after)[b]**	**Purchase Intent**	**Purchase Intent (after)[c]**
Variables	Pr > χ^2	Pr > χ^2	Pr > χ^2	Pr > χ^2
Saltiness	0.0970	0.3612	0.0224	0.0002
Bitterness	0.0391	0.0033	0.1133	0.1558
Taste	0.0017	0.9315	0.1253	0.3351
Oliking	<.0001	<0.0001	<0.0001	0.0066

[a] A full variable model with four sensory attributes were used. The analysis of maximum likelihood estimates was used to obtain parameter estimates (not shown in the table). Significance of parameter estimates was based on the Wald χ^2 value at $p < 0.05$.
[b] Consumers were asked if they would accept the product if it contained salt substitute, which may lower the risk of high blood pressure.
[c] Consumers were asked if they would purchase the product if it contained salt substitute, which may lower the risk of high blood pressure.

For the male consumers, overall liking and taste were influential attributes for overall acceptance (Tables 9) as well as for purchase intent. After giving health benefit information of salt substitute formulations, the critical attributes were taste ($p < 0.0283$) and bitterness ($p < 0.006$) for acceptance, and overall liking only ($p < 0.0001$) for purchase intent. According to Table 9, it is ovious that gender and additional information about heralth benefits affected consumers when they decided upon overall acceptance and purchase intent of these salt substitute formulations. Using predictive discriminative analysis (PDA) and based on four predictor variables, product acceptance and purchase intent (before and after consumers were

informed of the potential benefit of the salt substitute) can be predicted with 88.4%, 82.3%, 84.6%, and 82.3%, respectively (Table 10).

Table 10: Correct Classification (% Hit Rate) for Predicting Acceptance and Purchase Intent[a]

Attribute	% Hit Rate			
	Acceptance (before)	Acceptance (after)[b]	Purchase intent (before)	Purchase intent (after)[c]
Saltiness	81.3	76.0	78.8	77.3
Bitterness	82.2	79.2	76.1	76.9
Taste	88.2	80.7	83.2	81.3
Overall liking	89.2	81.5	84.4	83.4
A full-model with the above four attributes combined	88.4	82.3	84.6	82.3

[a] Based on Predictive Discriminant Analysis. Hit Rate (%) is the correct classification of unknown unit into a group.
[b] Consumers were asked if they would accept the product if it contained a salt substitute, which may lower the risk of high blood pressure.
[c] Consumers were asked if they would purchase the product if it contained a salt substitute, which may lower the risk of high blood pressure.

Table 11: Correct Classification (% Hit Rate) for Predicting Acceptance and Purchase Intent for Male and Female Consumers[a]

Attribute	Male Consumers % Hit Rate			
	Acceptance (before)	Acceptance (after)[b]	Purchase intent (before)	Purchase intent (after)[c]
Saltiness	81.2	76.2	77.7	74.5
Bitterness	77.7	81.7	75.7	73
Taste	86.2	83.2	82.7	78
Overall liking	89.5	81.4	84.5	81.2
A full-model with the above four attributes combined	88.7	83	84.2	79.7
Attribute	**Female Consumers % Hit Rate**			
	Acceptance (before)	Acceptance (after)[b]	Purchase intent (before)	Purchase intent (after)[c]
Saltiness	81.3	75.6	80.0	80.3
Bitterness	80	76.8	76.5	75.7
Taste	87	78.1	83.8	81.6
Overall liking	88.9	81.6	84.3	83.5
A full-model with the above four attributes combined	87.8	81.6	84.6	83.8

[a, b, c –] Same as in Table 10

4.1.3.5 The McNemar Test for Tracking Changes in Probability of Overall Acceptance and Purchase Intent

In order to evaluate if changes in probabilities occur before and after additional information about the health benefit of the salt substitute was given to the consumers, the McNemar test was performed. The results from the McNemar test (Table 12) show that the probability of overall acceptance of salt substitute formulations after giving health benefit information to consumers was significant at $\alpha = 0.05$ for all formulations, except for formulation # 7 (85 % NaCl, 0 % KCl, and 15 % L-Arginine), formulation # 3 (35% NaCl, 65% KCl, and 0% L-Arginine), and formulation # 11 (92.5 % NaCl, % KCl, 7.5% L-Arginine).

Table 12: Acceptance and Purchase Intent Changes Analyzed by the McNemar Test[a]

	Acceptance		Purchase Intent	
Formulations[b]	**p-value**	**95% CI**	**p-value**	**95%**
1	0.0082	54.1 – 91.1	0.0082	63.3 – 93.2
2	0.0027	17.9 – 62.5	0.0016	52.9 – 86.2
3	0.0707	28.9 – 71.5	0.0082	66.0 – 93.7
4	<.0001	36.8 – 71.8	0.0009	34.6 – 77.4
5	0.0005	50.5 – 82.8	0.0001	38.1 – 73.7
6	0.0339	31.8 – 83.1	0.0114	46.6 – 84.5
7	0.1797	49.0 – 94.9	0.0588	56.8 – 92.0
8	0.0076	32.2 – 70.4	0.0005	42.9 – 79.7
9	0.0047	55.2 – 89.7	0.0003	46.4 – 80.0
10	0.0455	61.3 – 98.6	0.0114	50.4 – 85.7
11	0.3173	86.5 – 100.0	0.0253	67.7 – 97.0

[a] the test follows a Chi-Square distribution with df = 1.
[b] Formulation numbers correspond to those in Table 1 and Figure 1.

For example, we can predict with 95% confidence interval that the probability of overall acceptance would be increased by at least 61% and at most 98% for formulation # 10 (57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine) after consumers are informed of the potential benefit of the salt substitute product. However, the probability of purchase intent of salt substitute formulations, after giving health benefit information to consumers, was significant at $\alpha = 0.05$ for all formulations, except formulation # 7 (85 % NaCl, 0 % KCl, and 15 % L-Arginine).

We can predict with 95% confidence interval that the probability of purchase intent would be increased by at least 50% and at most 85% for formulation # 10 (57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine) after consumers are informed of the potential benefit of the salt substitute product. Overall, it can be concluded that consumers' willingness to accept this particular product and their purchase intent depends on the health benefit information of the salt substitute product.

4.1.3.6 Product Optimization

Product optimization was performed using the three-component mixture design experiment in conjunction with the multiple regression analysis. Based on previous work done by Prinyawiwatkul and others (1997), Mixture Response Surface methodology (MRS) was used to obtain an optimal formulation range.

Table 13: Multiple Regression Models (No Intercept) for Predicting Mixture Response Surface of Sensory Attributes of the Salt Substitute Formulations

Attribute	Regression Equation[b]	Adjusted R^{2a}
Saltiness	Y = 5.40430*X1 + 3.93921*X2 - 3.23419*X3 + 3.26245*(X1*X2) + 16.36292*(X1*X3) + 12.42951*(X2*X3)	0.89
Bitterness	Y = 5.91844*X1 + 3.65964*X2 - 7.35575*X3 + 3.73844*(X1*X2) + 16.61626*(X1*X3) + 18.80022*(X2*X3)	0.88
Taste	Y = 5.88072*X1 + 3.73371*X2 - 7.19807*X3 + 4.08100*(X1*X2) + 18.83564*(X1*X3) + 5.00147*(X2*X3)	0.89
Overall Liking	Y = 5.93133*X1 + 3.53045*X2 - 6.76282*X3 + 3.81007*(X1*X2) + 17.60579*(X1*X3) + 17.33946*(X2*X3)	0.88

[a]Adjusted R^2 was calculated based on reduced regression models for each attribute
[b] X1 = NaCl, X2 = KCl, X3 = L-Arginine

A predictive model (Table 13) was obtained by using a restricted regression analysis (without intercept) and used to generate the mixture response surface (MRS) for each of the four sensory attributes studied (Fig. 8). Acceptability scores of each sensory attribute decreased with increased KCl content (Fig. 8). Areas of each sensory attribute within the MRS plots having a score equal to or greater than 6.0 were selected for optimization. Superimposing acceptable areas

of contour plots of all sensory attributes revealed the optimal formulation range (Fig. 9). The superimposition of the selected areas of MRS plot (shaded area) indicated that any formulation, containing 57-92% NaCl, 0-35.5% KCl, and 7.5-15% L-Arginine, will yield an acceptable salt substitute product that could be accepted by the consumers.

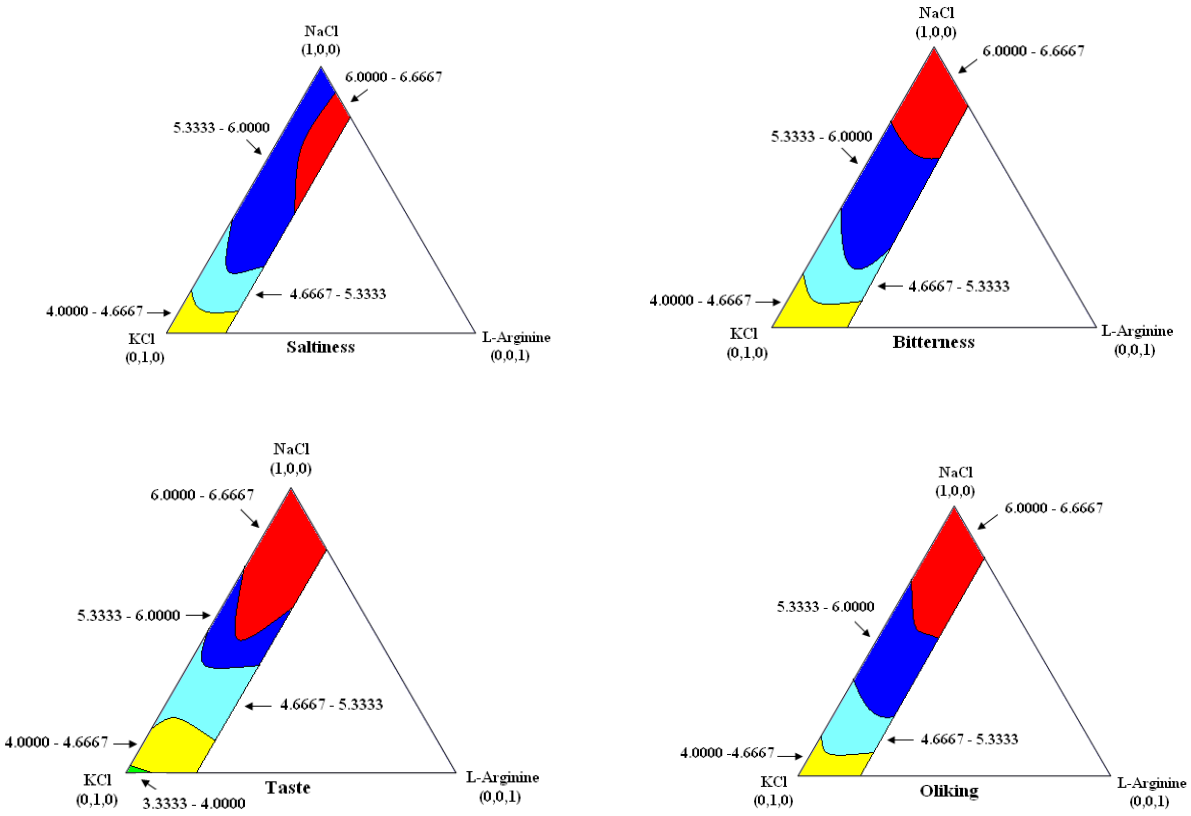

Figure 8: Mixture Response Surface (MRS) for Predicted Acceptability Values (Based On a 9-Point Hedonic Scale) of Saltiness, Bitterness, Taste, and Overall Liking

4.1.4 Conclusion

The main purpose of this study was to develop a salt substitute product with partially replaced KCl and with added L-Arginine and to determine its optimal formulation range. Development of a salt substitute could be a solution to reducing the prevalence of hypertension and its associated disease risk in the U.S. Taste and overall liking of a chicken broth (used as a model) containing salt substitute product were more influential for overall acceptance and

purchase intent than saltines and bitterness. Mixture Response Surface methodology identified, through the superimposition of acceptable areas of contour plots of all sensory attributes, that those formulations that contain 57-92% NaCl, 0-35.5% KCl, and 7.5-15% L-Arginine were as acceptable as the control formulation and would yield an acceptable product.

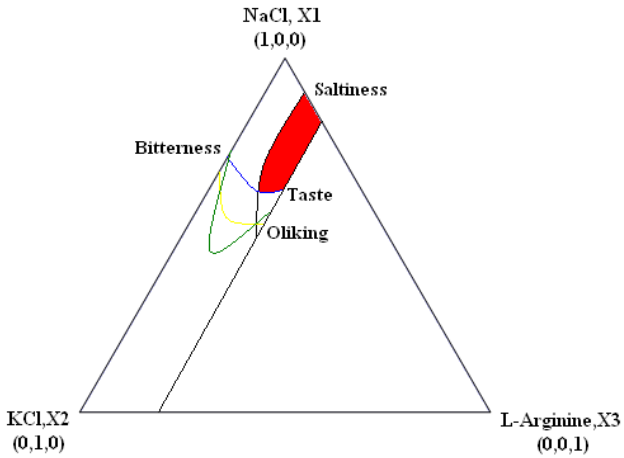

Figure 9: Superimposition of Sensory Attributes to Attain Optimal Formulation Range (Shaded Region) That Would Yield Salt Substitute with Acceptable Sensory Qualities (Score ≥ 6.0 On a 9 – Point Hedonic Scale)

4.1.5 References

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4.2 Study II: Sensory Discrimination Test for Optimized Low Sodium Salt Formulation Containing L-Arginine

4.2.1 Introduction

Discrimination tests are used in sensory research to determine whether two samples are different. If the differences between samples are too large, discrimination tests are not useful. Sensory discrimination tests are designed for determining the presence or absence of sensory attributes between very similar, confusable samples (Jean-Marc Dessirier ,1998). Discrimination tests are usually conducted when there are only two samples need to be tested. It is possible to perform multiple difference tests on more than two products, but results are not statistically reliable. There is a range of discrimination tests available including triangle tests, duo-trio, paired comparison, n-alternative forced-choice tests (Lawless and Heyman, 1999).

In the traditional triangle test, three samples are presented at once to the panelists. Two samples are from the same formulation, while the third is from the different formulation. Each panelist is required to indicate which sample is the odd sample and which two samples are similar. The null hypothesis states that the probability of making correct selection when there is no difference between two test samples is one in three (H_o: P=1/3). The alternative hypothesis states that the probability of making a correct decision when there is perceptible difference between samples will be larger than one in three (H_a: P>1/3) (Lawless and Heyman, 1999). This test is one-tailed and it has six possible serving combinations (AAB, ABA, BAA, BBA, BAB, and ABB). The number of panelists/judgments is important in order to gain reliable results from the triangle test. Usually 20 to 40 panelists are used in the triangle test. On the other hand, the similarity triangle test testing requires 50 to 100 panelists (Meilgaard et al., 1999).

Unfortunately, the number of judges available is often limited, therefore, the number of judgments may be increased by having panelists evaluate each sample more than once during the

session. The binomial test has been used to analyze the data gathered from the triangle test with replications when judges' responses have fallen into two categories. For example, in the triangle test, when panelists were asked to indicate which sample is odd, the response was either correct or incorrect. The binomial model makes two assumptions 1) responses are independent and 2) judges are identical. This model takes into account the variance between the samples, but it is unable to account for the variation among the subjects/judges. The variance between the subjects can be explained by a beta-distribution, which is known as overdispersion and is measured by γ (gamma) (Liggett et al., 2005). When $\gamma = 0$, there is no overdispersion and the binomial model can be used, while $\gamma = 1$ indicates there is an overdispersion and the beta-binomial model is favored. In contrast to the binomial model, the beta-binomial model can account for variation both between samples as well as between judges (Liggett et al., 2005). Although the use of the beta-binomial model is not well known in sensory research, the application of this model has been realized by Rosett et al. (1995). Recently, the beta-binomial model has been used in sensory preference of electrostatically coated potato chips (Ratanatriwong et al., 2003), and in the sensory quality of cabbage (Radovich et al., 2004).

The objective of this study was to determine whether the low-sodium salt formulation differed from the control (100% NaCl) using the replicated triangle test.

4.2.2 Materials and Methods

4.2.2.1 Materials

Food Grade (FCC) NaCl, KCl, and L-arginine were used in this experiment. Food-grade NaCl and L-arginine were purchased from Voigt Global Distribution, LLC (Kansas City, MO), while food-grade KCl was obtained from EMD Chemicals, INC. (Gibbstown, NJ). The Brita

Water Filtration System (Brita Products Company, Oakland, CA), plastic cups, and usalted crackers were purchased from a local supermarket.

4.2.2.2 Sample Preparation

Samples were prepared 2 h prior to evaluation for two consecutive days. The proportion of each ingredient was determined from the three-component mixture design experiment conducted in the previous study (Chapter 4, Study I). The Mixture Response Surface methodology identified, through the superimposition of acceptable areas of contour plots of all sensory attributes, that the formulation that contains 57-92% NaCl, 0-35.5 % KCl, and 7.5-15 % L-Arginine was as acceptable as the control formulation (100 % NaCl, 0 % KCl and 0 % L-Arginine) and would yield an acceptable product. Based on this finding and an effort to maximize KCl as a replacement for NaCl, 57% w/v NaCl, 35.5% w/v KCl and 7.5% w/v L-Arginine was used in the sample preparation. Each ingredient was dissolved in filtered water and distributed in 2 oz three-digit coded plastic cups. All remaining samples were discarded after evaluation.

4.2.2.3 Procedure

LSU AgCenter Institutional Review Board approved experimental consumer test procedures and methods. The panelists were recruited from Louisiana State University, Baton Rouge, LA. The criteria to recruit the untrained panelists were their willingness to participate in this experiment and no allergic reaction to NaCl, KCl, and L-Arginine. The sensory panel consisted of 16 judges: seven females and nine males. Each panelist received a ballot (See Appendix B) with written instructions regarding the experimental procedure. The following instructions were stated in the ballot: “You will be presented with three sets of coded samples. For each set, two samples are identical and one is different (or odd). You must pick or identify the odd sample. Please take a 5-minute break between each set of samples”.

Judges evaluated the samples in individual partitioned booths in the sensory evaluation lab. The first three sets of samples (two same and one different) were given to the judges, and they were allowed to taste the samples with no time limit. Panelists were told to evaluate the samples from left to right for saltiness/bitterness perception only and to indicate which sample was the odd sample. Each panelist was asked to sip each sample, swirl it with the tongue and expectorate. Filtered, room temperature water, unsalted crackers and expectoration cups were provided for consumers to minimize any possible carryover effects. Each session was replicated three times. Replication was applied to the test for overdispersion and improvement of the test power (Dacremont et al., 1997; Ennis et al., 1998; Radovich et al., 2004). To minimize fatigue, five-minute breaks occurred between sessions. Presentation order of samples was counterbalanced within and across the panelists.

4.2.2.4 Data Analysis

Panelist variability was measured by calculating overdispersion or γ (gamma**)**. The gamma was estimated, based on the formula reported by Bi (2006).

$$\gamma = \frac{1}{n-1}\left[\frac{nS}{\mu(1-\mu)k} - 1\right]$$

Where n = the number of replications per panelist, μ = the mean probability of correct choice response, k = the number of judges, $S = \sum_{i=1}^{k}(p_i - \mu)^2$ and p_i = the number of correct responses in the i_{th} trial. Given the parameter estimates for the beta-binomial model, we can easily obtain critical values and compare them to our correct choice responses at α = 0.05 significance level. If the critical value is larger than correct choice responses, we would conclude that panelists were not able to detect difference for a given attribute. If the critical value is

smaller than the correct choice responses we would conclude that panelists were able to detect the difference for a given attribute (Bi 2006).

4.2.3 Results and Discussion

Each panelist evaluated in triplicate the saltiness and bitterness attributes for the control (NaCl) and formulation # 10 (57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine). Cumulatively, from the triplicate triangle test, there were 25 correct responses for saltiness evaluation and 24 correct responses for bitterness evaluation (Table 14). In order to determine if panelists could detect the difference between two samples, the beta binomial model was applied.

Table 14: Correct Responses for Saltiness and Bitterness Perception by Panelists in a Triplicate Triangle Test

	Saltiness	**Bitterness**
Panelist	**x**[a]	**x**[a]
1	1	0
2	2	0
3	1	1
4	2	1
5	2	3
6	1	1
7	3	2
8	3	3
9	2	2
10	3	1
11	2	2
12	0	3
13	2	0
14	0	3
15	1	2
16	0	0
∑	**25**	**24**

[a] - x is the number of correct responses from each panelist from 3 trials

In order to conclude whether a difference exists between the two samples, we needed to compare the minimum number of choice responses at $\alpha = 0.05$ level to the critical value (Bi, 2006). The critical values could be easily obtained, given the parameters of beta-binomial model: μ and γ (panelist variability, overdispersion or gamma). According to Bi (20060, there are two

main techniques to estimate the parameters in the beta-binomial distribution: moment estimate and maximum likelihood estimate. We considered the moment estimate technique with an equal number of replications or observations in the trial. For the data in Table 14, n = 3, k = 16, the following are moment calculations for estimates of μ and γ for saltiness evaluation.

$$\mu = \frac{\sum_{i=1}^{k} p_i}{k} = \frac{1/3 + 2/3 + \cdots + 1/3 + 0/3}{16} = 0.521$$

$$S = \sum_{i=1}^{k} (p_i - \mu)^2 = (1/3 - 0.521)^2 + (2/3 - 0.521)^2 + \cdots + (1/3 - 0.521)^2 + (0/3 - 0.521)^2 = 1.76$$

$$\gamma = \frac{1}{n-1}\left[\frac{nS}{\mu(1-\mu)k} - 1\right] = \frac{1}{3-1}\left[\frac{3 \times 1.76}{0.521 \times (1 - 0.521) \times 16} - 1\right] \approx 0.2$$

Using Table 7A.2 from Bi (2006), we obtained the critical value or the minimum number of correct responses (Table 15). The minimum number of correct responses with n = 3, k = 16 and γ = 0.2 is 25. Because the correct choice response for saltiness was 25 and the obtained critical value was equal to the correct choice response value, we could conclude that panelists were able to detect differences between control and formulation # 10 (Table 2). Therefore, the panelist could differentiate the saltiness perception between the control sample and formulation # 10 (57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine).

In order evaluate the bitterness perception by panelists, the same calculation was conducted. For the data in Table 14, n = 3, k = 16, the following are moment calculations for estimates of μ and γ for bitterness evaluation based on the formulae provided by Bi (2006).

$$\mu = \frac{\sum_{i=1}^{k} p_i}{k} = \frac{0/3 + 0/3 + \cdots + 2/3 + 0/3}{16} = 0.5$$

$$S = \sum_{i=1}^{k} (p_i - \mu)^2 = (0/3 - 0.5)^2 + (0/3 - 0.5)^2 + \cdots + (2/3 - 0.5)^2 + (0/3 - 0.5)^2 = 2.22$$

$$\gamma = \frac{1}{n-1}\left[\frac{nS}{\mu(1-\mu)k} - 1\right] = \frac{1}{3-1}\left[\frac{3 \times 2.22}{0.5 \times (1-0.5) \times 16} - 1\right] \approx 0.3$$

Using again Table 7A.2 from Bi (2006), we can obtain the critical value or the minimum number of correct responses (Table 15). The minimum number of correct responses with n = 3, k = 16 and γ = 0.3 is 25. Because the correct choice response for bitterness was 24, and the obtained critical value is larger than the correct choice response value, we could conclude that panelists were not able to detect differences between the control and formulation # 10. Therefore, the panelist could not differentiate the bitterness perception between the control sample (NaCl) and formulation # 10 (57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine).

Table 15: Summary of Statistics for the Replicated Triangle Test using the Beta-Binomial Model

Parameters	**BB[a] Triangle Test for Saltiness**	**BB Triangle Test for Bitterness**
n (number of judges)	16	16
k (number of replication)	3	3
α level	0.05	0.05
γ (gamma)	0.2	0.3
Critical value[b]	25	25
Number of correct responses	25	24
Detect difference?	Yes	No

[a] – BB corresponds to beta-binomial model
[b] – From Table 7A-2 (Bi 2006)

4.2.4 Conclusion

In order to evaluate whether our optimized product (formulation # 10: 57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine) was different from the control (NaCl) based on saltiness and bitterness perception, the replicated triangle test with the beta-binomial model was used. This particular discrimination technique is more reliable because this model accounts for variations both between samples as well as across judges (Liggett et al., 2005). Results from Table 15 showed that judges were able to differentiate the saltiness perception of the control and test

samples using the beta-binomial triangle test. On the other hand, they could not differentiate the bitterness perception between the control and test samples. The next step was to evaluate/characterize the saltiness and bitterness perception of optimized salt mixture (formulation # 10: 57 % NaCl, 35.5 % KCl, and 7.5 % L-Arginine) using Spectrum Descriptive Methodology.

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4.3 Study III: Sensory Descriptive Characteristics of the Optimized Low-Sodium Salt Formulation Containing L-Arginine

4.3.1 Introduction

Descriptive analysis is one of the most essential techniques in sensory evaluation. Descriptive tests are used to evaluate sensory properties such as flavor, aroma, taste, and texture of foods and beverages and various types of non-food materials. Various descriptive methods were used to obtain information in the marketplace using sensory mapping for possible development of new products, to understand consumer responses to product sensory attributes, and to maintain quality characteristics of products (Gacula, 1997). In order to gain valuable information, several factors are considered such as training and experience of the panelists, skill of panel leader, and sensory execution. Panelists must be trained and be able to describe the perceived sensory characteristics of a test samples. The panel leader has a critical role in the whole process of descriptive analysis. He/she must be able to establish, maintain, and motivate the sensory panel. Correct sensory execution depends on choices of reference standards, test design, conduction of the test, and analysis of data (Gacula, 1997).

Several descriptive analyses has been developed and applied in recent decades. The Flavor Profile technique is used to describe the perceived aroma and flavor attributes of the product. The Texture Profile method is used to obtain a description of textural parameters of food (Meilgaard et., 1999). The Quantitative Descriptive Analysis (QDA) uses panelists as measuring instruments, and their ability to express their perceptions of a product. This particular technique includes the complete listing of sensory attributes, their order of occurrence, relative intensity of each attribute, and statistical analysis of the responses (Stone et al., 1993). The Free Choice Profile method differs from the other descriptive techniques. Panelists are not extensively trained, are allowed to evaluate product in different ways and can create their own list of

descriptors. The other feature of the Free Choice Profile is the statistical analysis of data. The data are usually analyzed using the generalized procrustes analysis.

The Spectrum™ Descriptive Analysis is a complete, detailed and accurate method used to obtain the description of a product's sensory attributes. This descriptive characterization provides information on the perceived sensory attributes, the levels of the intensities of each attribute, and a statistical evaluation of the descriptive data (Muñoz et al., 1992). The unique characteristic of the Spectrum approach is that panelists do not generate a panel-specific vocabulary to describe sensory attributes of products but that they use a standardized lexicon of terms (Civille et al., 1996). The Spectrum™ Descriptive Analysis provides the tools to design a descriptive procedure for a given product. The principal tools are the reference, scaling procedure, and the methods of panel traking.

The aim of the Spectrum™ method is to choose the most practical system (given the product in question), the overall sensory program, the specific project objectives in developing panel, and the desired level of statistical treatment of data (Meilgaard et al., 1999). The Spectrum™ technique may be applied to numerous applications such as food products, beverages, personal and home care items, and other products (Muñoz et al., 1992). The Spectrum ™ method tends to be universal, which means that results obtained from the performance of a particular Spectrum ™ analysis may be reproducible and get similar results, provided that the experiment is correctly done under identical conditions.

This study was aimed to determine the detailed description of each sensory attribute, to evaluate the perceived intensity of each sensory characteristics of our created low-sodium product, and to indicate how, in sensory dimension, the sodium chloride is different from this low-sodium formulation.

4.3.2 Materials and Methods

4.3.2.1 Sample Description

Eleven low salt formulations (Figure 6; table 3) evaluated by Spectrum Descriptive Analysis. Each formulation was generated from the mixture design experiment conducted in the previous study (Chapter 4, Study I). Each sample contained different proportions of NaCl, KCl, and L-Arginine. Food-grade NaCl and L-arginine were purchased from Voigt Global Distribution LLC (Kansas City, MO), while food-grade KCl was obtained from EMD Chemicals INC (Gibbstown, NJ). Samples were prepared every week one hour prior to evaluation. Each sample mixture was dissolved in filtered water and distributed in 2 oz three-digit coded plastic cups. All remaining samples were discarded after evaluation.

4.3.2.2 Panel Selection

A total of twenty panelists were selected from Louisiana State University, Baton Rouge, LA. Selection criteria were based on availability, health, interest in research, and rating ability. They all participated in the screening process. Screening process consisted of a series of acuity tests to investigate panelists' ability to recognize, describe, and rate the basic tastes in solutions. Participants were to be able to identify two basic tastes for this study: saltiness and bitterness. In addition they were to be able to evaluate a series of solutions and correctly rate their intensities (Meilgaard et al., 1999). After successfull completion of screening, 12 panelists were selected for the subsequent training program.

4.3.2.3 Panel Training

The training program helped panelists to identify, describe, and discriminate the sensory characteristics of products following the Spectrum ™ method. In this study, the training program consisted of two parts: general orientation and practice sessions. During the general orientation

session, panelists were given detailed explanation about the Spectrum™ descriptive sensory methodology. During the next session, various samples were reviewed and a preliminary lexicon was developed. Several sessions were devoted for group meetings for selection of reference standards and development of terminology. Individual training on the developed lexicon was conducted at the following session. For the next eight sessions, panelists were trained to quantify perceived intensities and to use intensity references. Two basic tastes (bitterness and saltiness) were used for references. Caffeine solution in water was used for bitterness intensity reference. Four caffeine solutions in water were prepared, which corresponded to four referene points on 15-cm scale. Reference point 2 corresponds to 0.05% caffeine solution, reference point 5 corresponds to 0.08% caffeine solutions, reference point 10 corresponds to 0.15% caffeine solution, and reference point 15 corresponds to 0.20% caffeine solution (Meilgaard et al., 1999). On the other hand NaCl solutions in water were prepared for saltiness intensity references. Reference point 8.5 corresponds to 0.5% NaCl solution, reference point 15 corresponds to 0.7% NaCl solution, reference point 18 corresponds to 1.0% NaCl solution, and reference point 22 corresponds to 1.4% NaCl solution (Kwan, 2004). Once panelists had been trained, several products were given to them to evaluate. These exercises allowed panelists to apply developed concepts and terminology. Total training time was 15 h. Then, two sessions of individual sample evaluation were completed to collect data for statistical analysis.

4.3.2.4 Product Evaluation

Product evaluation was conducted in the sensory laboratory in the Department of Food Science at Louisianan State University. During two sessions, trained panelists evaluated eleven test samples for saltiness and bitterness in individual partitioned sensory booths using the developed terminology. The panelists were instructed to test the samples and asked to rinse their

palate with filtered water between samples and to use unsalted crackers to eliminate carryover and adaptation. Intensities of bitterness were recorded on the 15-cm line scale, where zero indicated the absence of intensity, and fifteen corresponded to an extreme intensity (Meilgaard et al., 1999). Intensities of the saltiness were recorded on the 22-cm line scale, where zero indicated the absence of intensity, and twenty-two corresponded to an extreme intensity. A 22-cm scale was used for saltiness intensity evaluation because the panelists perceived the samples to be saltier from our intensity at a 15-cm point.Therefore, new reference samples were prepared following the 22-cm reference scale used (Kwan, 2004). Overall, panelists performed one replication for each sensory attribute (saltiness, bitterness) for all eleven formulations.

4.3.2.5 Data Analysis

The data were analyzed using univariate and multivariate statistical analysis at an α level of 0.05. An analysis of Variance (ANOVA, proc mixed, SAS version 9.1, 2006) was performed to determine significant effects on the attribute intensities for the eleven test samples. The Tukey's adjustment post-hoc test was then performed to study individual significant differences among the eleven test samples. The Principal Component Analysis was used to evaluate attributes and attribute-sample relationship.

4.3.3 Results and Discussion

4.3.3.1 Analysis of Variance (ANOVA)

The data for the intensities of saltiness and bitterness for all eleven samples were analyzed using analysis of variance (SAS Institute Inc., 2003) to determine if there were significant differences in the judgments. Table 16 shows the means, standard deviations and Pr > F values for the intensities of saltiness and bitterness evaluated for each of the eleven formulations. Saltiness perception ($P < 0.0002$) and bitterness perception ($P < 0.0001$) showed

significant differences in intensity among eleven samples. Tukey's Honestly Significant Difference (HSD) test illustrated that the saltiness intensities of formulations # 1, 2, 3, 6, 7, 9 and 10 were not significantly different from one another, while samples # 4, 5, 8 were significantly different from formulation # 11. The lowest intensity scores for saltiness were observed for formulations # 4, 5, 8. The significantly different intensities and lowest saltiness intensity scores of formulations # 4 (0% NaCl, 100% KCl, 0% L-Arginine), # 5 (0% NaCl, 85% KCl, 15% L-Arginine) and # 8 (0% NaCl, 92.5% KCl, 7.5% L-Arginine) was due to absence of NaCl.

Table 16: Means, Standard Deviations and Analysis of Variance for Saltiness and Bitterness Intensities of Eleven Low Sodium Product Formulations[a]

Sample[b]	Saltiness	Bitterness
1	9.67 ± 5.35^{ab}	0.33 ± 0.53^{c}
2	9.05 ± 5.64^{ab}	0.62 ± 0.87^{c}
3	6.58 ± 5.11^{ab}	2.19 ± 1.73^{cb}
4	4.53 ± 3.26^{b}	4.47 ± 3.01^{ab}
5	4.64 ± 3.77^{b}	5.50 ± 3.66^{a}
6	6.51 ± 5.38^{ab}	2.23 ± 1.89^{cb}
7	8.47 ± 5.06^{ab}	1.93 ± 1.68^{cb}
8	3.10 ± 2.82^{b}	6.33 ± 4.48^{a}
9	7.28 ± 5.77^{ab}	2.23 ± 2.00^{cb}
10	9.65 ± 5.18^{ab}	0.93 ± 1.45^{c}
11	12.46 ± 5.85^{a}	0.73 ± 0.92^{c}
Pr>F	0.0002	0.0001

[a] - Mean values within the same column not followed by the same letter are significantly different ($p < 0.05$).
[b]- Sample numbers (1-11) correspond to those in Figure 1 and Table 1.

Higher intensity scores were observed for formulations # 1 (100% NaCl, 0% KCl, 0% L-Arginine), # 2 (65% NaCl, 35% KCl, 0% L-Arginine) # 10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine), while the highest score was observed for formulation # 11 (92.5% NaCl, 0% KCl, 7.5% L-Arginine) which can be explained due to an increased amount of NaCl, a reduced amount of KCl, and an addition of L-Arginine. Table 16 shows that the saltiness intensity score of formulation #10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) was the closest to the control

formulation # 1 (100% NaCl, 0% KCl, 0% L-Arginine). This suggests that addition of KCl up to 35.5%, and 7.5% L-Arginine and reduction of NaCl up to 57% imparted no significant differences in saltiness intensity compared to the control sample (100% NaCl, 0% KCl, 0% L-Arginine).

Regarding bitterness intensity, there was no significant difference observed for formulations # 1, 2, 10 and # 11. Samples # 4, 5, 8 were significantly different from the samples # 1, 2, 10, and 11. The three highest intensity scores for bittereness were observed for formulations # 4, 5, 8. It may be associated with the fact that formulations # 4 (0% NaCl, 100% KCl, 0% L-Arginine), # 5 (0% NaCl, 85% KCl, 15% L-Arginine) and # 8 (0% NaCl, 92.5% KCl, 7.5% L-Arginine) contain the highest amount of potassium chloride and no sodium chloride. In contrary, the lowest bitterness intensity was observed for formulations # 1 (100% NaCl, 0% KCl, 0% L-Arginine), # 2 (65% NaCl, 35% KCl, 0% L-Arginine) # 10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) and # 11 (92.5% NaCl, 0% KCl, 7.5% L-Arginine). This may be due to an increased amount of sodium chloride and a decreased amount of KCl. As for formulation #10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) and # 11 (92.5% NaCl, 0% KCl, 7.5% L-Arginine), it could be explained that L-Arginine along with NaCl synergistically masked the bitterness perception of KCl (Ogawa et al., 2004).

The trends for saltiness and bitterness intensity scores were similar to those for sensory acceptability profile (Table 4). Based on sensory acceptability profile (Table 4), consumers preferred formulation # 10 (57 % NaCl, 35.5 % KCl, 7.5 % L-Arginine) and # 1 (100 % NaCl, 0 % KCl, 0 % L-Arginine) with the highest acceptability score of 5.93 and 5.51, respectively. Regarding consumer acceptance rating for saltiness (Table 4), Tukey's Honestly Significant Difference (HSD) test showed that there was no significant difference between the control

formulation # 1 (100 % NaCl, 0 % KCl, 0 % L-Arginine) and formulations # 2, 6, 7, 9, 10, 11. On the other hand, formulations # 4, 5, and 8 were perceived as significantly different from most of the formulations by the consumers.

A similar trend was observed for bitterness intensities and acceptability scores (Table 16 and 4). The lowest acceptable score was received for formulation # 4 (0% NaCl, 100% KCl, 0% L-Arginine), # 5 (0% NaCl, 85% KCl, 15% L-Arginine) and # 8 (0% NaCl, 92.5% KCl, 7.5% L-Arginine), which was mostly due to the bitter taste of KCl. Among the extended formulations, the bitterness was most acceptable (score = 6.07) for formulation # 10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine). Based on similar patterns for intensity and acceptability scores, it could be concluded that consumers liking of formulations # 10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) and # 1 (100% NaCl, 0% KCl, 0% L-Arginine) could be associated with the close descriptive intensity scores. Whereas, the low acceptability scores of formulations # 4 (0% NaCl, 100% KCl, 0% L-Arginine), # 5 (0% NaCl, 85% KCl, 15% L-Arginine) and # 8 (0% NaCl, 92.5% KCl, 7.5% L-Arginine) could be connected with the higher intensity of bitterness and lower intensity of saltiness.

4.3.3.2 Principal Component Analysis (PCA)

Principal Component Analysis (SAS Institute Inc., 2003) was conducted to study attribute-sample relationships. The Principal Component Analysis (PCA) showed the relationship between eleven formulations and sensory attribute intensity (Fig. 10). The attribute-sample relationships were explained by the first and second principal components, which explained 95.7% and 4.3% of the variability, respectively. The biplot showed that formulations # 4 (0 % NaCl, 100 % KCl, 0 % L-Arginine), # 5 (0 % NaCl, 85 % KCl, 15 % L-Arginine) and #

8 (0 % NaCl, 92.5 % KCl, 7.5 % L-Arginine) were positioned distant from control # 1 (100 % NaCl, 0 % KCl, 0 % L-Arginine) and the rest of the formulations.

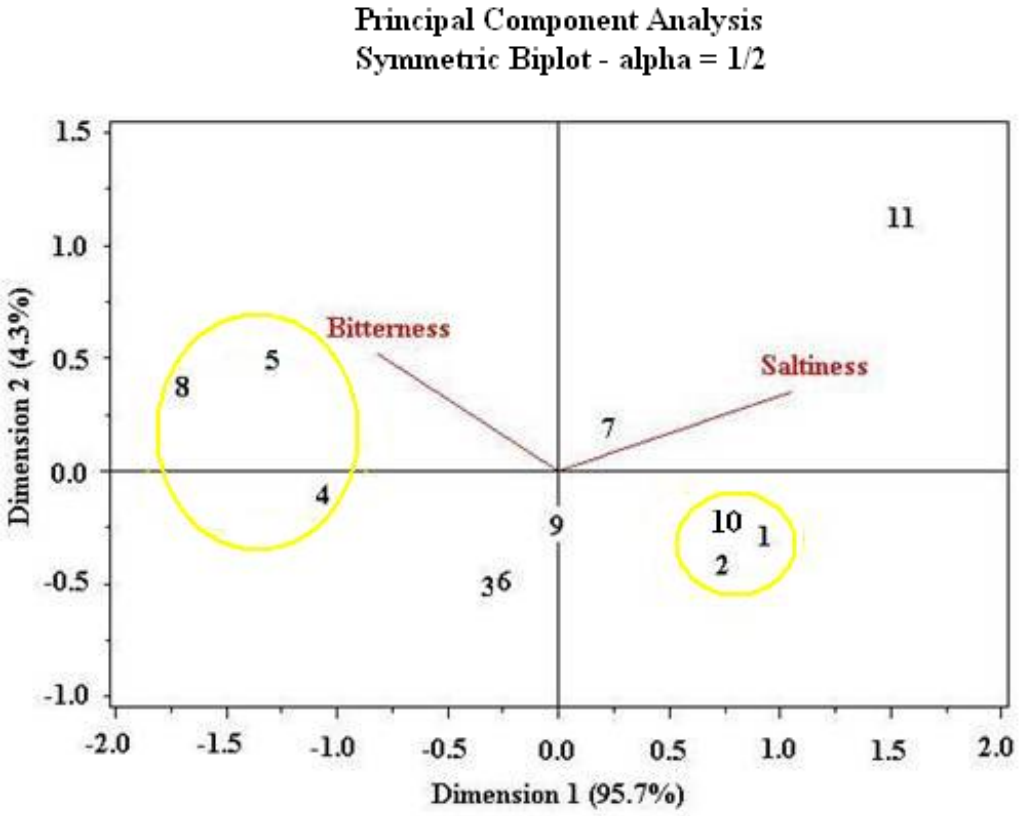

Figure 10: The Product-Attribute Biplot of Descriptive Sensory Attributes Involving Principal Component1 and Principal Component 2.
[a] Numbers (1-11) correspond to eleven formulations in Figure 1 and table 1.

Based on the sensory attribute of saltiness, control formulation # 1 was positively correlated with formulations # 2 (65 % NaCl, 35 % KCl, 0 % L-Arginine) and # 10 (57 % NaCl, 35.5 % KCl, 7.5 % L-Arginine). Based on the sensory attribute of bitterness, formulations # 4, 5, 8 were closely correlated to each other. Similar pattern was observed in the relationship between eleven formulations and sensory attribute acceptability (Fig. 7). Based on Fig. 7, formulations # 4, # 5 and # 8 were positioned distant from formulation # 1 and the rest of the formulations, while formulation # 1 was highly correlated with formulations # 2 # 6, # 11 and # 10. These

similar patterns between descriptive analysis (Fig. 10) and acceptability profile (Table 4) indicate that the higher intensity of saltiness and a lower intensity of bitterness yielded higher acceptability scores, whereas a lower intensity of saltiness and a higher intensity of bitterness yielded a lower acceptability scores (Ttables 16 and 4).

4.3.4 Conclusion

Descriptive Sensory Analysis of the control and ten low-sodium formulations showed that they were different among one another. Saltiness and bitterness were discriminating attributes. The saltiness intensity score of formulation #10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) was the closest to the control formulation # 1 (100% NaCl, 0% KCl, 0% L-Arginine). Regarding bitterness intensity, there was no significant difference for formulations # 1, 2, 10 and # 11. Samples # 4, 5, 8 were significantly different from formulations # 1, 2, 10, and 11. The attribute-sample relationships (Fig. 10) showed correlation between formulations # 1, 2, 10 according to the first and second principal components. The similar patterns observed for sensory acceptability profile (Table 4 and Figure 7) and sensory descriptive profile (table 16 and Figure 10) indicated that consumer rated acceptability of saltiness and bitterness based on their intensity. They generally accepted the formulations with low bitterness intensity.

4.3.5 References

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CHAPTER 5.

EVALUATION OF CONSUMER SENSORY CHARACTERISTICS OF OPTIMIZED LOW SODIUM MIXTURE VS. COMMERCIAL SALT REDUCED PRODUCTS

5.1 Introduction

High sodium chloride intake contributes to the development of hypertension (Ball et al., 2002). Various studies conducted by Loria et al. (2001); Sacks et al. (2001) showed that reducing sodium intake by 100 mmol/day would decrease stroke mortality by 22% and ischemic heart disease by 16 % in Western societies. They recommended that blood pressure can be lowered by reducing the amount of sodium in the diet among individuals with and without hypertension. Due to information in recent years that there is a positive relationship between high sodium intake and the incidence of hypertension, consumers have been paying more attention to reducing sodium intake in their diets. Based on a recent trend by consumers to lower sodium in their diets, food industries have begun to reduce sodium content in their products. In recent decades, the food industry has used KCl to partially or fully substitute NaCl (Best, 1989; Duxbury, 1986). The disadvantage of using KCl alone is that KCl elicits a bitter taste as well as a salty taste (Frank et al., 1970; Bartoshuk, 1980). Therefore, bitterness inhibitors have to be included into food formulations to mask the undesirable taste of KCl.

According to Desmond (2006), some of these mixtures have been commercialized such as Pansalt^{R}. Pansalt^{R} is a patented salt replacer where almost half of the sodium is removed and replaced with potassium chloride, magnesium sulphate and the essential amino acid L-lysine hydrochloride. Other commercially available mixtures of NaCl and KCl include Lo Salt, Saxa So-Low salt and Morton Lite Salt, among others. Some commercial mixtures such as Morton Salt Substitute and No-Salt, fully, replace NaCl with KCl.

In our previous studies we successfully optimized the salt mixture of NaCl/KCl/L-arginine (Chapter 4). Based on results of Chapter 4, the new prototype low-sodium formulation was created. Consumer acceptance test showed that this prototype product containing 57% NaCl,

35.5% KCl, and 7.5% L-Arginine was as acceptable as the control (100% NaCl, 0% KCl, and 0% L-Arginine) formulation.

The objectives of this study were to conduct a consumer affective test in order to understand consumer acceptance, and purchase intent and to compare consumer perceptions of optimized NaCl/KCl/L-Arginine mixture against commercially available low salt/substitute products.

5.2 Materials and Methods

Food-grade NaCl and L-arginine were purchased from Voigt Global Distribution, LLC (Kansas City, MO), while food-grade KCl was obtained from EMD Chemicals, INC. (Gibbstown, NJ). The Brita Water Filtration System (Brita Products Company, Oakland, CA) was purchased from a local supermarket. Five whole chickens (Piligrim Pride brand name) with the weight ranging from 4.9 to 6.0 pounds were purchased from the local Wal-Mart supermarket. All chickens were cleaned and kept in polyethylene bags at the time of purchase.

5.2.1 Preparation of Chicken Broth

The water used for chicken broth preparation was filtered using the Brita Water Filtration System to eliminate the undesirable taste or odor of water which could have interfered with sensory perception. All five chickens were thoroughly cleaned before placed in a 20-gallon stainless steel pot. The filtered water (approximately 40 L) was added to reach the upper level of the container. Cooking was conducted with an electric stove (Model RBS305PR, Whirlpool Corporation, Benton Harbor, MI) at 300 ^{0}F for 4h. The chicken broth was regularly stirred, and the foam was removed every 15 min. The cooked chicken broth was filtered, allowed to cool down, poured into a sanitized plastic container and stored at 4 ^{0}C before test. The cooked chicken meat and bones were discarded.

5.2.2 Sample Preparation and Experimental Design

All ingredients used to prepare the salt substitute formulations are listed in Table 17. Chicken broth samples with the four salt mixtures were prepared. The cooked chicken broth was poured in 500 ml beakers, and marked with appropriate sample names. One percent by weight of each salt substitute in chicken broth and control (Table 17) was added to each beaker and stirred with a stirring bar until it was totally dissolved.

Table 17: List of the Salt Substitute Samples Used in this Experiment

Sample	Sample name*	Composition
346	Control (Morton Table Salt)	NaCl, Calcium Silicate
593	Test (Optimized product)	NaCl, KCl, L-arginine
738	Morton Lite Salt	NaCl, KCl, Calcium Silicate, Magnesium Carbonate, Dextrose, Potassium Iodide
165	Morton Salt Substiute	KCl, Fumaric Acid, Tricalcium phosphate, monocalcium phopshate

* Control, Morton Lite Salt and Morton Salt Substitute are products of Morton International Inc., Chicago, IL

Each sample was then poured into a 2-oz plastic cup and covered with plastic lid. Plastic cups were numbered and kept for further use. After each session, the remaining samples were discarded. All samples were prepared on a day before the consumer test.

The experimental consumer test protocol was approved by the LSU AgCenter Institutional Review Board. Untrained consumers (n = 200) were randomly recruited from the Louisiana State University campus, Baton Rouge, LA. Criteria for recruitment were that consumers were to be at least 18 years of age, were not allergic to chicken, or L-arginine, and were available to participate on scheduled testing days. The central location test for consumer

acceptance was conducted for one day at the LSU Dairy Store. Consumers were asked to provide demographic information at the beginning of the session.

The Randomized Block Design was used in this study. Samples were randomized using Proc. Plan procedure in SAS software version 9.1 (SAS Institute. 2003) (See Appendix D). Prior to evaluation, each chicken broth sample was heated in a microwave oven (General Electric Company, Louisville, KY) for 10 – 15 s. Then each consumer was presented with four coded chicken broth samples in 2 oz plastic cups. Water, unsalted crackers, and expectoration cups were provided for consumers during the test to minimize carryover and adaptation effects. Consumers were instructed to sip each sample, swirl it with the tongue, and then either swallow or expectorate before providing acceptability ratings for sensory attributes. They were told to evaluate each sample for saltiness, bitterness, taste and overall liking on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely*)*.Consumers also rated saltiness and bitterness intensify using a 3-point Just About Right (JAR) scale, where 1 = not enough, 2 = JAR, and 3 = too strong (Stone et al., 1993). Overall acceptance and purchase intent were evaluated using the binomial (yes/no) scale.

5.2.3 Statistical Data Analysis

All analysis was conducted at $\alpha = 0.05$, using SAS software version 9.1 (SAS Institute. 2003). The analysis of variance (ANOVA) was performed to determine difference in acceptability of each sensory attribute and overall liking of each product formulation. The Tukey's Honestly Significant Difference (HSD) test was performed for multiple comparisons.

The Logistic Regression Analysis (LRA) was used to predict acceptance and purchase intent of four chicken broth products. Logistic regression calculates the probability of success (event) over the probability of failure (non event), and expresses the results in the form of a

likelihood or the odds ratio estimate. The odds ratio estimates are a nonnegative number with a value that is greater than 1.0 when a success is more likely to occur than a failure (Agresti, 1996). When odds = 4.0, a success is four times as likely as a failure. When an estimated odds ratio equals 1.0, it means that there is no significant association between the two variables (Agresti, 1996).

The Multivariate Analysis of Variance (MANOVA) was used to further analyze the data in order to identify whether significant differences exist between four chicken broth formulations when all four attributes were compared simultaneously. Descriptive Discriminant Analysis (DDA) (Huberty, 1994) was conducted to determine the discriminating attributes for the underlying differences among the four samples. Predictive Discriminative Analysis (PDA) (Huberty, 1994) was used to identify sensory attributes critical to overall acceptance and purchase intent. For PDA, the hit rate (%) of acceptability was calculated for each of the four sensory attributes. PDA works with classification of products based on several variables simultaneously. It is an analog of a regression analysis. A fitted set of data to a mathematical function will give an observation its highest probability of being assigned to the known correct population whereas minimizing the probability that the same observation will be misclassified (Resurreccion, 1998).

Principal Component Analysis (PCA) was used to demonstrate any existing relationship among the sensory attributes (saltiness, bitterness, taste, and overall liking) and the relationship between these attributes and the four samples. The first principal component (PC) covers as much of the variation in the data as possible, and the second PC is orthogonal to the first and covers as much of the remaining variation as possible. The non-parametric McNemar test (Agresti, 1996) was used to determine changes in consumers' acceptance and purchases decision

before and after they had been given the information of health benefits of a salt substitute. It is a test of marginal homogeneity for matched binary responses and the variation of chi square distribution with one degree of freedom (Agresti, 1996). The null hypothesis for the McNemar test (Ho: $\pi_{1+} = \pi_{+1}$ or $\pi_{12} = \pi_{21}$) stated whether the difference between the probability of those who answered yes after (π_{1+}) they had been informed about health benefits of salt substitute and the probability of those who answered yes before (π_{+1}) is significant, or whether it was merely by chance.

In order to estimate the actual differences in the means, 95% confidence interval (CI) was calculated using marginal sample proportions ($P_{+1} - P_{1+}$). Marginal sample proportion was calculated using the following formula:

$$P_{ij} = n_{ij}/N$$

Where N is the total number of consumer responses, n_{ij} is the number of consumers making decision i before and decision j after the additional information about the health benefits of salt substitute was provided. The following equation was used to obtain 95% confidence interval (CI):

$$(P_{+1} - P_{1+}) \pm Z_{\alpha/2}(ASE)$$

Where ($P_{+1} - P_{1+}$) represents the difference in proportions between the consumers who would accept/purchase the product after additional information was provided (P_{+1}) and those who would also accept/purchase the product before the additional information was provided (P_{1+}). The term $Z_{\alpha/2}$ is the standard normal percentile having a right-tail probability equal to $\alpha/2$. For a 95% CI, $Z_{\alpha/2} = 1.96$. ASE is the estimated standard error for the proportion difference, and the following equation was used for calculation:

$$ASE = \{[P_{1+}(1-P_{1+}) + P_{+1}(1-P_{+1}) - 2(P_{11}P_{22}-P_{12}P_{21})]/N\}1/2$$

where P_{11} is the proportion of consumers who would accept/purchase the product before and after additional information was provided, P_{12} is the proportion of those who would accept/purchase before but not after, P_{21} is the proportion of those who would not accept/purchase the product before but would be willing to accept/purchase afterwards, and P_{22} indicates the number of subjects who answered negatively prior to and after additional information had been given to consumers.

The just about right (JAR) data were analyzed using the Stuart-Maxwell and the McNemar tests (Fleiss et al., 1971; Stone et al., 1993). The Stuart-Maxwell is a test for homogeneity for matched products in which there are more than 2 scale categories (1 = not enough, 2 = just about right, 3 = too strong). It is used to determine if there is a significant difference in the distribution of responses for the products. If there is a significant difference, the data matrix can be collapsed into a series of matrices (2 x 2) and the McNemar test is then used to determine individual scale categories for which differences are significant (Fleiss et al., 1971; Stone et al., 1993). For a 3-categroy classification, the following is the Stuart-Maxwell statistics:

$$\chi^2 = \frac{\overline{n}_{23}d_1^2 + \overline{n}_{13}d_2^2 + \overline{n}_{12}d_3^2}{2\left[\overline{n}_{12}\overline{n}_{13} + \overline{n}_{12}\overline{n}_{23} + \overline{n}_{13}\overline{n}_{23}\right]}$$

Where: $\overline{n}_{ij} = \frac{n_{ij} + n_{ji}}{2}$

n_{ij} = number of matched pair at row i column j, n_{ji} = number of matched pair at row j column i
$n_{1.}$ = number of paired responses in row 1, $n_{.1}$ number of paired responses in column 1
$d_1 = (n_{1.} - n_{.1})$, $d_2 = (n_{2.} - n_{.2})$, $d_3 = (n_{3.} - n_{.3})$

The calculated chi-square value from the Stuart-Maxwell statistics was compared with the chi-square table at df = (k-1) = 3 – 1 = 2. If the distributions of responses from the two products are different, the categories can be combined and the McNemar test can be used:

$$\chi^2 = \frac{\left[(b-c)-1\right]^2}{b+c}$$

where b and c correspond to responses of combined scales for both products. The calculated chi square value from the McNemar statistics was compared with chi square table at df = (k-1) = 3 – 1 = 2.

5.3 Results and Discussion

5.3.1 Consumer Demographic Information

Out of 200 consumers who participated in this study, 55.5 % were males and 45.5 % were females. The age of consumers ranged from the majority of 18 – 34 years old to 35 – 44 years old (16 %), 45 – 54 years old (1 %) and over 55 years old (1 %).

5.3.2 Consumer Acceptability

Based on results from ANOVA (Table 18), all sensory attributes for samples 346 (control), 593 (Test), 738 (Morton Lite Salt) received a mean score of greater than 5.3. The mean score for all sensory attributes was less than 3.5 for sample 165 (Morton Salt Substitute). This might be explained by the bitter/metallic taste of the product. Regarding the saltiness acceptability, samples 346 (Control), 593 (Test), and 738 (Morton Lite Salt) were perceived as significantly different from sample 165 (Morton Salt Substitute) by the consumers. Consumers equally preferred the samples 346 (Control) and 593 (Test) with the acceptability score of 5.83 and 5.62, respectively. The lowest acceptability score for saltiness was observed for sample 165 (Morton Salt Substitute), which may be due to the bitterness of KCl and the absence of a bitterness inhibitor in the formulation.

A similar trend was observed for the bitterness acceptability by consumers. Among all samples, the bitterness acceptability scores were higher for samples 346 (Control) and 593 (Test), with the acceptability score of 6.09 and 5.86, respectively. The Tukey's Honestly Significant Difference (HSD) test results for bitterness acceptability showed no significant

difference for control and Test samples (Table 18). This might be due to the presence of L-Arginine in the Test sample (57% NaCl, 35.5% KCl, and 7.5% L-Arginine) that helped mask the bitterness perception of KCl. L-arginine was reported to mask the bitterness of various compounds and enhance the saltiness of NaCl (Ogawa et al., 2004). The lowest acceptable score for bitterness perception was observed for sample 165 (Morton Salt Substitute). Samples 738 (Morton Lite Salt) showed no significant difference from the sample 593 (Test).

Table 18: Mean Consumer Acceptability Scores for Saltiness, Bitterness, Taste and Overall Liking of Four Salt Substitute Samples*

Samples	Saltiness	Bitterness	Taste	Overall Liking
346	5.83 ± 2.02^{a}	6.09 ± 1.96^{a}	5.99 ± 1.96^{a}	6.13 ± 1.92^{a}
593	5.62 ± 1.85^{ab}	5.86 ± 1.79^{ab}	5.88 ± 1.80^{a}	5.81 ± 1.84^{ab}
165	3.14 ± 1.82^{c}	2.71 ± 1.78^{c}	2.69 ± 1.79^{c}	2.72 ± 1.68^{c}
738	5.32 ± 1.79^{b}	5.49 ± 1.79^{b}	5.60 ± 1.84^{a}	5.54 ± 1.83^{b}

* Based on 200 consumer responses and on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Mean values within the same column not followed by the same letters are significantly different ($p < 0.05$). See Table 17 for sample descriptions.

Mean scores concerning taste and overall liking showed similar patterns to that of the saltiness and bitterness perception. The acceptability scores of taste and overall liking for the control and test samples showed no significant difference, with a mean score of 5.99 and 5.88 for taste and 6.13 and 5.81 for saltiness, respectively. This was attributed to the synergistic property of L-Arginine as well as NaCl in masking the bitterness of KCl (Ogawa et al. 2004). The acceptability of taste for sample 738 (Morton Lite Salt) was also not significantly different from the control and test samples. The lowest scores for taste and overall liking of 2.69 and 2.72, respectively, were observed for sample 165 (Morton Salt Substitute).

According to ANOVA (Table 18), the sensory acceptability profile for 593 (Test) sample showed no significant difference from 346 (Control) sample or sample 738 (Morton Lite Salt).

The high mean scores of sample 593 (Test) for all sensory attributes were the results of the presence of L-Arginine and KCl in the optimized test product.

5.3.3 Overall Product Differences

Analysis of variance (ANOVA) and the Tukey's Honestly Significant Difference (HSD) test (Table 18) indicated that differences existed in acceptability of saltiness, bitterness, taste, and overall liking among the four samples.

Table 19: Overall Product Difference Analyzed by MANOVA

Test Criteria and F Approximation for the Hypothesis of No Overall Form Effect S=3 M=0 N=395.5					
Statistic	Value	F value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.597895	37.52	12	2098.4	< 0.0001
Pillai's Trace	0.406086	31.11	12	2385	< 0.0001
Hotelling-Lawley Trace	0.665879	43.96	12	1383.4	< 0.0001
Roy's Greatest Root	0.655732	130.33	4	795	< 0.0001

To determine if the four samples were different when all four sensory attributes were considered simultaneously, the Multivariate Analysis of Variance (MANOVA) was performed. Based on MANOVA results (the approximate F value of 37.52 and the Wilks' Lambda *p* value of < 0.0001), it can be concluded that significant differences existed among four samples when all four sensory attributes where compared simultaneously (Table 19).

5.3.4 Discriminating Sensory Attributes

Since the Multivariate Analysis of Variance (MANOVA) indicated that differences existed among four samples (Table 19), Descriptive Discriminant Analysis (DDA) was performed to identify which sensory attributes were accounted for the group differences. Results (Table 20) showed the canonical structure r's (Huberty 1994), which accounted for the group differences. According to the pooled within canonical structure in the first dimension (Can 1),

saltiness (0.912), bitterness (0.908) and overall liking (0.926) were the sensory attributes that significantly contributed to the differences among the four samples.

Table 20: Canonical Structure r's Describing Group Differences among Four Samples [a]

Attribute	Can 1	Can 2	Can 3
Taste	0.711	- 0.261	- 0.070
Saltiness	0.912*	0.180	0.280
Bitterness	0.908*	0.133	0.330
Overall Liking	0.926*	0.197	- 0.243
Cumulative Variance Explained (%)	98.48	99.82	100

[a] based on the pooled within-group variances. Can 1, 2, and 3 refer to the first, second and third canonical discriminant functions, respectively.
* Indicates attributes which accounted for the group differences in the first dimension.

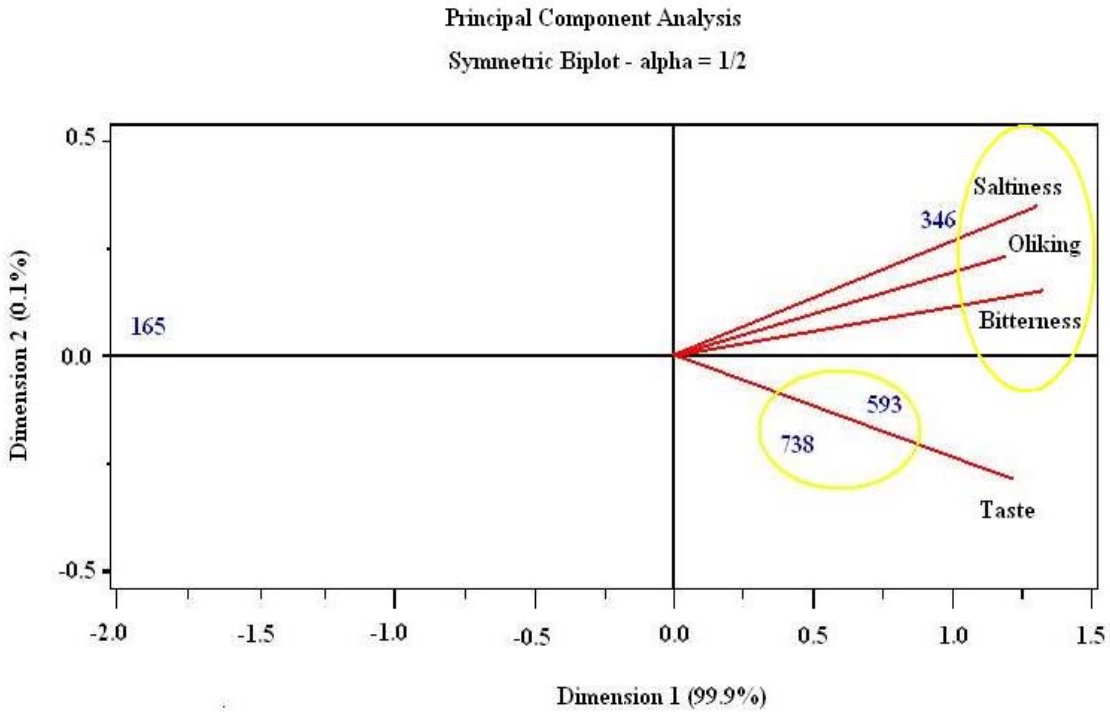

Figure 11: The Product-Attribute Biplot Involving Principal Component1 and Principal Component 2
Oliking = Overall Liking
Sample 165 Corresponds to Morton Salt Substitute; Sample 346 Corresponds to Morton Table Salt; Sample 593 Corresponds to Test sample; Sample 738 Corresponds to Morton Lite Salt.

The Principal Component Analysis (PCA) showed the existing relationship between sensory attributes (saltiness, bitterness, taste, and overall liking) and the relationship between four samples and these sensory attributes (Fig. 11). The biplot showed that out of four sensory attributes, saltiness, bitterness and overall liking were closely correlated and contributed to group differences among the four samples. A similar trend was observed from the DDA analysis in the first dimension, Can 1 (Table 20). The Principal Component Analysis (PCA) showed that sample 165 (Morton Salt Substitute) was positioned distant from sample 346 (Control) and the rest of the samples. Based on taste acceptability, samples 593 (Test) and sample 738 (Morton Lite Salt) were highly correlated. Based on saltiness, bitterness and overall liking, sample 593 (Test) was more positively correlated to the Control than sample 738 (Morton Lite Salt).

5.3.5 Sensory Attributes Influencing Overall Acceptance and Purchase Intent

Logistic Regression Analysis (LRA) results of consumer acceptance and purchase intent before and after health benefits of salt substitute given to consumeras are Table 21. We were able to identify the sensory attributes that were critical for overall acceptance and purchase intent of salt substitute samples and the control and to predict the acceptance and purchase intent based on those attributes. Based on LRA results (Table 21), overall liking and saltiness was the most influential attributes for overall acceptance. The odds ratio estimate of overall liking, considering a full model, with four sensory attributes, was 1.630, indicating that for every 1 point increase in the overall liking score on a 9-point hedonic scale, acceptance of the product will increase by 63%. Similar to overall liking, the odds ratio of saltiness was 1.64, again implying that for every point increase in this attribute, the acceptance will increase by 64 %. For purchase intent, the overall liking and taste was influential attributes with the odds ratio of 2.057 and 1.495, respectively (Table 21).

Table 21: Parameter Estimates, Probability and Odds Ratio Estimates for Predicting Acceptance and Purchase Intent[a] of Salt Substitute Formulations

Variables	**Acceptance**			**Purchase Intent**		
	$Pr > \chi^2$ (full model)	Odds Ratio (full model) model)	Odds Ratio (single-var	$Pr > \chi^2$ (full model)	Odds Ratio (full model) model)	Odds Ratio (single-var.
Saltiness	<.0001	1.640	3.252	0.0240	1.028	2.578
Bitterness	0.003	1.348	2.882	0.2763	1.098	2.231
Taste	0.0044	1.454	3.839	0.001	1.495	3.152
Overall Liking	<.0001	1.630	4.575	<.0001	2.057	3.499
Variables	**Acceptance (health)[b]**			**Purchase Intent (health)[c]**		
	$Pr > \chi^2$ (full model)	Odds Ratio (full model) model)	Odds Ratio (single-var	$Pr > \chi^2$ (full model)	Odds Ratio (full model) model)	Odds Ratio (single-var.
Saltiness	<.0001	1.451	2.601	0.0317	1.211	2.385
Bitterness	<.0001	1.561	2.707	0.0028	1.268	2.321
Taste	0.1011	1.204	2.657	0.0079	1.339	2.666
Overall Liking	0.0037	1.419	2.845	<.0001	1.642	2.850

[a] Based on LRA, using full and single variable models with four sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates. Significance of parameter estimates was based on the Wald $\chi 2$ value at $p < 0.05$.
[b] Consumers were asked if they would accept the product if it contained salt substitute, which may lower the risk of high blood pressure.
[c] Consumers were asked if they would purchase the product if it contained salt substitute, which may lower the risk of high blood pressure.

The odds ratio estimates for these two attributes indicates that for every 1 point increase in overall liking and taste on a 9 – point hedonic scale, purchase intent will increase by 105.7% and 49.5%, respectively. The odds ratio of the overall liking for acceptance and purchase intent was higher among all attributes, indicating that consumers perceived overall liking as a more influential factor for acceptance and purchase intent. Whereas consumers perceived saltiness as a more critical attribute for acceptance than for purchase intent with the odds ratio decreasing from 1.64 to 1.028. When consumers were asked if they would accept the product if it contained a salt substitute, which may lower the risk of high blood pressure, saltiness and bitterness were the

most critical attributes for acceptance with the odds ratio of 1.45 and 1.56, respectively. The odds ratio estimates in this case indicate that for every 1 point increase in saltiness and bitterness on a 9 – point hedonic scale, the acceptance will increase by 45% and 56%, respectively. When the consumers were asked if they would purchase the product if it contained a salt substitute, which may lower the risk of high blood pressure, the overall liking was the only most influential attribute. The odds ratio for overall liking was 1.642, meaning that purchase intent will increase by 64 % with every one point increase in overall liking score.

Table 22: Correct Classification (% Hit Rate) for Predicting Acceptance and Purchase Intent[a]

Attribute	**% Hit Rate**			
	Acceptance (before)	**Acceptance (after)**[b]	**Purchase intent (before)**	**Purchase intent (after)**[c]
Saltiness	85	80.8	82.3	79.8
Bitterness	84.8	83.2	78.2	79.5
Taste	88.7	83.8	83.2	83.8
Overall liking	90.6	85.2	84.7	84.2
A full-model with the above four attributes combined	90.4	87.1	84.7	84.5

[a] Based on Predictive Discriminant Analysis. Hit Rate (%) is the correct classification of unknown unit into a group.
[b] Consumers were asked if they would accept the product if it contained a salt substitute, which may lower the risk of high blood pressure.
[c] Consumers were asked if they would purchase the product if it contained a salt substitute, which may lower the risk of high blood pressure.

Based on % hit rate from Predictive Discriminative Analysis (PDA), product acceptance and purchase intent (before and after consumers were informed of the potential health benefit of the salt substitute) was predicted (Table 22). Results indicated that acceptability of the product could be generally predicted by overall liking (90.6%), taste (88.7%), saltiness (85.0%), and bitterness (84.8%). For acceptance after consumers were informed of the potential benefit of the salt substitute, the most critical factor was overall liking with the % hit rate of 85.2 %. For prediction of purchase intent and purchase intent after consumers were informed of the potential

benefit of the salt substitute, the influential attribute was, again, overall liking with the % hit rate of 84.2 -84.7%.

5.3.6 The McNemar Test for Tracking Changes in Probability of Overall Acceptance and Purchase Intent

In order to evaluate if changes in probabilities occur before and after additional information about the health benefit of the salt substitute was given to the consumers, the McNemar test was performed. The null hypothesis for McNemar test (Ho: $\pi_{1+} = \pi_{+1}$ or $\pi_{12} = \pi_{21}$) stated whether the difference between the probability of those who answered yes after (π_{1+}) they had been informed about health benefits of salt substitute and the probability of those who answered yes before (π_{+1}) was significant, or whether it was merely by chance. The results from the McNemar test (Table 23) show that the probability of overall acceptance of salt substitute formulations after giving health benefit information to consumers was significant at $\alpha = 0.05$ only for sample 165 (Morton Salt Substitute) with p-value = 0.0002. We can predict with 95% confidence interval that the probability of overall acceptance would be increased by at least 45% and at most 72% for the sample 165 (Morton Salt Substitute) after consumers were informed of the potential benefit of the salt substitute product. For the Control, Test and Morton Lite salt samples, there was no change for overall acceptance; this means consumers equally accepted these samples before and after being informed about health benefits of salt substitutes.

However, the probability of purchase intent after consumers were given health benefit information was significant at $\alpha = 0.05$ for all samples. For example, we can predict with 95% confidence interval that probability of purchase intent would be increased by at least 61% and at most 80% for the sample 593 (Test) after consumers were informed of the potential benefit of the salt substitute product. Even though the probability of overall acceptance was not significant at α

= 0.05 for (Control, Test and Morton Lite Salt), the consumers were more willing to purchase the product given the health benefit information of the salt substitute.

Table 23: Acceptance and Purchase Intent Changes Analyzed by the McNemar Test[a]

McNemar Test for Acceptance			
Sample[b]	**χ^2**	**p-value**	**95% CI for acceptance**
Control	1	0.3173	65.3 – 87.3
Test	0	1.000	66.8 – 87.9
Morton Salt Subst.	14.3	0.0002	45 – 71.8
Morton Lite Salt	3.24	0.071	55.1 – 78.8
McNemar Test for Purchase Intent			
Sample[b]	**χ^2**	**p-value**	**95% CI for acceptance**
Control	15.2	<.0001	69.1 – 87.4
Test	24.1	<.0001	60.9 – 80.3
Morton Salt Subst.	15.0	0.0001	55.5 – 83.7
Morton Lite Salt	30.1	<.0001	56.4 – 76

[a] the test follows a Chi-Square distribution with df = 1.

Overall, it can be concluded that consumers' willingness to accept this particular product and their purchase intent both depend on the health benefit information of the salt substitute.

5.3.7 Comparisons of Saltiness and Bitterness Intensity

Since the saltiness and bitterness attributes were critical in this study, we compared the intensity of saltiness and bitterness perception (obtained from the JAR scale). The saltiness intensity of the following pairs was compared: 593/346, 593/738, 593/165, 346/738, 346/165, and 738/165. The Stuart-Maxwell statistic equation for the three-category classification was used to determine if there was a significant difference in the distribution of responses for the products. The χ^2 value was calculated and compared to the critical χ^2 value = 5.99 at df = 2 and $\alpha = 0.05$. The results (Table 24) showed that there was a significant difference in the distribution of responses for all pairs except 593 vs. 738. Therefore, the data matrix was collapsed into two categories (too salty and other) and the McNemar test was used to determine individual scale categories for which differences were significant (Fleiss et al., 1971; Stone et al., 1993).

Table 24: Comparisons of Saltiness and Bitterness of Product Pairs Using the McNemar Test[a]

Product pairs	Stuart-Maxwell χ^2	χ^2 values for saltiness	χ^2 values for bitterness
593 vs. 346[c]	45.1	15.75	–
593 vs. 738	0.57	–	–
593 vs. 165	61.3	12.85	34.02
346 vs. 738	48.3	13.1	–
346 vs. 165	28.9	0.11[b]	20.3
738 vs. 165	157.5	11.04	44.02

– no significant difference in the distribution of responses (categories were not collapsed)
[a] – the critical χ^2 value = 5.99 at df = 2 and $\alpha = 0.05$
[b] – no significant difference between pair 346 vs. 165
[c]165 – Morton Salt Substitute
346 – Morton Table Salt
593 – Test sample
738 – Morton Lite Salt

Results (Table 24) showed that for five product pairs (593/346, 593/165, 346/738, 346/165, and 738/165), the former product was saltier than the latter product. For example, for the pair 593/346, product 593 (Test sample) was saltier than product 346 (Control). This could be due to increased saltiness effect of both KCl and L-Arginine on NaCl. There was no significant difference in saltiness perception between product 346 (Control) and product 165 (Morton Salt Substitute). Similar statistical analyses were conducted for bitterness intensity evaluation for the same pairs: 593/346, 593/738, 593/165, 346/738, 346/165 and 738/165. The Stuart-Maxwell statistic equation for three-category classification was used to determine if there was a significant difference in the distribution of responses for the products. The results showed that for the pairs 593/165, 346/165 and 738/165, sample 165 (Morton Salt Substitute) was more bitter than samples 593 (Test), 738 (Morton Lite Salt) and 346 (Control or Morton Table Salt). However, for the pairs 593/346, 593/738 and 346/738, the Stuart-Maxwell statistic showed no significant difference in the distribution of responses for these pairs; therefore, the categories were not combined, and no further analyses were conducted.

5.4 Conclusion

The main objective of this study was to understand the sensory characteristics of optimized low sodium salt and compare these characteristics with those of commercially available low sodium/salt substitute products. Two hundred consumers participated in this study. Four samples and four attributes were evaluated. Consumers preferred the samples 346 (Control) and 593 (Test) with the highest acceptability scores for saltiness, bitterness, overall liking and taste. The control sample showed no significant difference in all four sensory attributes from the test sample. The lowest acceptability score for all attributes was observed for sample 165 (Morton Salt Substitute). The acceptability scores of taste, saltiness, bitterness and overall liking for the control (sample 346) were not significantly different from those of sample 593 (Test). According to descriptive discriminate analysis, saltiness, bitterness, and overall liking were the influential attributes that significantly contributed to the differences among the four samples. A similar trend was observed from Principal Component Analysis (PCA). The biplot showed that saltiness, bitterness, and overall liking were closely correlated and were influential to the difference among the four samples. Overall liking and saltiness were critical attributes for overall acceptance, while taste and overall liking were critical for purchase intent. When consumers were given information about health benefits of salt substitute products, saltiness, taste and overall liking were influential for overall acceptance and purchase intent. Based on the McNemar test, the probability of overall acceptance was not significant at $\alpha = 0.05$ for (Control, Test and Morton Lite Salt) but the consumers were more willing to purchase the products after given the health benefit information of a salt substitute. A comparison of saltiness intensity between four products shows that sample 593(Test) was saltier compared to the rest of the samples. Based on bitterness intensity comparison results, sample 165 (Morton Salt Substitute) was bitterer than

other three samples. For the pairs 593/346, 593/738, and 346/738, the Stuart-Maxwell statistic showed no significant difference in the distribution of responses for these pairs. Overall it can be concluded that optimized salt mixture (57% NaCl, 35.5% KCl, and 7.5% L-Arginine) compared with Control (Morton Table Salt) and sample 738 (Morton Lite Salt) was equally accepted for all sensory attributes and that sample 165 (Morton Salt Substitute) was unacceptable by consumers. Finally, after knowing the health benefits of salt substitute, consumers were still accepting and willing to purchase the optimized product.

5.5 References

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CHAPTER 6.

SUMMARY AND CONCLUSIONS

The excessive consumption of NaCl has been identified as a significant risk factor in developing high blood pressure. People with hypertension are more likely to develop diseases of the heart and the vascular system. Reducing sodium intake is one of the ways to fight against hypertension. One way of lowering sodium content is through the use of low salt products or salt substitutes. However, taste has been a major problem in developing acceptable salt substitutes. Therefore, it is crucial to modify the food formulations by reducing or partially replacing the sodium content, and at the same time, maintaining the desirable sensory and chemical properties of NaCl.

The aim of the present study was to develop an acceptable formulation of a low-sodium salt mixture by reducing the NaCl content and replacing it with KCl and L-Arginine. L-Arginine has been reported to have bitterness masking property. Therefore, it has been used in development of low sodium mixture formulations.

The non-parametric R-Index approach was used to analyze the data for saltiness and bitterness evaluation of a mixed salt solutions consisting of KCl, NaCl and L-Arginine. It was observed that panelists were able to distinguish the saltiness perception of mixed salt solutions from a NaCl solution. For the bitterness perception, there were no differences at 0.5%, 1% and 1.5% w/v between formulation D (55 % KCl, 35 % NaCl and 10 % L-Arginine) and formulation E (0% KCl, 100% NaCl and 0 % L-Arginine). Therefore, it would be possible that L-Arginine, with the addition of sodium chloride, could mask the bitterness of potassium chloride.

An optimization study was conducted to develop and characterize low sodium formulation in a food system. The Mixture Response Surface methodology identified, through superimposition of acceptable areas of contour plots of all sensory attributes, that those

formulations that contained 57-92% NaCl, 0-35.5% KCl, and 7.5% L-Arginine were as acceptable as the control formulation and would yield an acceptable product.

Spectrum Descriptive Analysis was conducted to determine the detailed description of each sensory attribute, to evaluate the perceived intensity of each sensory characteristic of low sodium formulations, and to indicate how, in the sensory dimension, the NaCl is different from the best acceptable optimized formulation. The saltiness intensity score of formulation #10 (57% NaCl, 35.5% KCl, 7.5% L-Arginine) was closest to that of the control formulation # 1 (100% NaCl, 0% KCl, 0% L-Arginine). Regarding bitterness, there was no significant difference in intensity for formulations # 1, 2, 10 and # 11. Samples # 4, 5, 8 were significantly different from the rest of the samples.

A Beta-Binomial analysis showed that judges were able to differentiate the saltiness perception but could not differentiate the bitterness perception between the control and the developed salt substitute samples.

A Consumer affective test was conducted in order to understand consumer acceptance and their purchase intent of the optimized NaCl/KCl/L-arginine mixture against commercially available low salt/substitute products. It was concluded that optimized salt mixture (57% NaCl, 35.5% KCl, and 7.5% L-Arginine) was equally acceptable to Control (Morton Table Salt) and sample 738 (Morton Lite Salt) for all sensory attributes. After giving the health benefit information of salt substitute to the consumers, they were still accepting and willing to purchase the optimized product.

Overall, this study demonstrated the potential of NaCl/KCl/L-Arginine as a low-sodium salt mixture by partially replacing NaCl while maintaining desirable sensory characteristics. The use of a healthy salt alternative could be a solution to reducing the prevalence of hypertension

and its associated disease risk in the U.S. However, the application of developed low salt mixture to different food systems should be further investigated. Moreover, the effect of anti-caking agents should be examined. Furthermore, the effect of crystallization of the salt substitute mixture (NaCl, KCl, L-Arginine) on saltiness and bitterness perception needs to be further investigated. The combination of L-Arginine with other bitter masking agents is in need of further study.

APPENDIX A. CHAPTER 3

a. Form for R-index

Name: **Gender:**

Part I: Saltiness Evaluation

Note:

1) You will be presented with the 5 labeled samples in random order.

2) Please evaluate them from left to right and rank the samples in order of saltiness intensity

with 1 = Saltiest and 5 = Least salty

3) No ties please!

Date: _____

	1st Saltiest	2nd	3rd	4th	5th Least salty
Sample					

Part II: Bitterness Evaluation

Note:

1) You will be presented with the 5 labeled samples in random order.

2) Please evaluate them from left to right and rank the samples in order of bitterness intensity

with 1 = Most bitter 5 = Least bitter

3) No ties please!

Date: _____

	1st Most Bitter	2nd	3rd	4th	5th Least bitter
Sample					

b. d prime values corresponding to different R-Index values

Linking R-Index with Thurstonian d-prime										
R	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.5	0.000	0.035	0.071	0.106	0.142	0.178	0.214	0.249	0.286	0.322
0.6	0.358	0.395	0.432	0.469	0.507	0.545	0.583	0.622	0.661	0.701
0.7	0.742	0.783	0.824	0.867	0.910	0.954	0.999	1.045	1.092	1.140
0.8	1.190	1.242	1.295	1.349	1.406	1.466	1.528	1.593	1.662	1.735
0.9	1.812	1.896	1.987	2.087	2.199	2.326	2.476	2.660	2.904	3.290

Source: Bi, (2006)

c. Coefficient estimation of variance of d prime from R-Index values

R	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.5	12.556	12.574	12.598	12.638	12.694	12.766	12.856	12.963	13.089	13.234
0.6	13.399	13.586	13.796	14.029	14.289	14.578	14.897	15.250	15.639	16.069
0.7	16.544	17.069	17.650	18.294	19.009	19.806	20.695	21.691	22.812	24.079
0.8	25.517	27.159	29.047	31.232	33.783	36.790	40.371	44.691	49.980	56.565
0.9	64.936	75.842	90.491	110.939	140.942	188.024	269.327	431.993	853.127	2815.56

Source: Bi, (2006)

d. Rank Response Frequency for Saltiness

	Frequencies of Ranking data at 0.5 % w/v concentration				
Sample[a]	**1**[b]	**2**	**3**	**4**	**5**
A	1	18	27	30	44
B	1	33	23	33	30
C	3	25	38	34	20
D	5	38	31	21	25
E	110	6	1	2	1
	Frequencies of Ranking data at 1.0 % w/v concentration				
Sample	**1**	**2**	**3**	**4**	**5**
A	2	12	22	31	53
B	2	21	19	43	35
C	3	21	45	34	17
D	6	60	29	11	14
E	107	6	5	1	1
	Frequencies of Ranking data at 1.5 % w/v concentration				
Sample	**1**	**2**	**3**	**4**	**5**
A	4	9	23	30	54
B	2	17	26	43	32
C	7	26	40	25	22
D	3	59	27	20	11
E	104	9	4	2	1

[a] – See table 1 for formulations

[b] - 1 = saltiest and 5 = least salty

e. Example of output for Mann-Whitney Statistics, *p* value and Z value for pair of A-C (1.0% bitterness)

```
NPAR TESTS
  /M-W= Intensity   BY Treatment(0 1)
  /MISSING ANALYSIS.
```

NPar Tests

[DataSet3] E:\Disso\R-index\MWstat\1.0% bitterness\C-E.sav

Mann-Whitney Test

```
NPAR TESTS
  /M-W= Intensity   BY Treatment(0 1)
  /MISSING ANALYSIS.
```

Ranks

	Treatment	N	Mean Rank	Sum of Ranks
Intensity	Control	120	108.40	13008.50
	Treatment	120	132.60	15911.50
	Total	240		

```
NPAR TESTS
  /M-W= Intensity   BY Treatment(0 1)
  /MISSING ANALYSIS.
```

Test Statistics(a)

	Intensity
Mann-Whitney U	5748.500
Wilcoxon W	13008.500
Z	-2.778
Asymp. Sig. (2-tailed)	.005

a Grouping Variable: Treatment

f. Rank Response Frequency for Bitterness

	Frequencies of Ranking data at 0.5 % w/v concentration				
Sample[a]	**1**[b]	**2**	**3**	**4**	**5**
A	33	28	17	26	16
B	21	30	26	28	15
C	16	32	27	29	16
D	12	23	42	28	15
E	38	7	8	9	58
	Frequencies of Ranking data at 1.0 % w/v concentration				
Sample	**1**	**2**	**3**	**4**	**5**
A	41	24	23	14	18
B	25	46	20	16	13
C	10	23	43	32	12
D	7	20	30	53	10
E	37	7	4	5	67
	Frequencies of Ranking data at 1.5 % w/v concentration				
Sample	**1**	**2**	**3**	**4**	**5**
A	36	32	16	13	23
B	25	27	32	22	14
C	13	31	35	34	7
D	6	24	31	40	19
E	40	6	6	11	57

[a] – See table 1 for formulations

[b] - 1 = most bitter and 5 = least bitter

APPENDIX B. CHAPTER 4

a. Research Consent Form

I, _____, agree to participate in the research entitled "Optimization and Characterization of Sensory Qualities of Chicken Soup Containing Salt Substitute" which is being conducted by Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University, phone number (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 eighty five consumers will participate in this research. For this particular research, about 15 min participation will be required for each consumer.

The following points have been explained to me:
1. In any case, it is my responsibility to report prior participation to the investigators any allergies I may have.
2. The reason for the research is to gather information on consumer sensory acceptability of a salt substitute from sodium chloride, potassium chloride and L-arginine. 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: Two 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 which can be envisioned is the allergic reaction toward chicken, NaCl (regular salt), KCl and L-Arginine (amino acid). Individuals who have kidney problem should not participate in this study.
5. The results of this study 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 investigators 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. David Morrison, Assistant Vice Chancellor of LSU AgCenter at 578-8236. I agree with the terms above.

_____ _____
Signature of Investigator Signature of Participant

Date: _____ Witness: _____

SAMPLE #

b. Sample Survey Form

1. What is your age group? (Please check one)

18-24 years____ 25-34 years____ 35-44 years____ 45-54 years____ Over 55 years____

2. What is your gender? Male_____ Female_____

3. How would you rate the **OVERALL TASTE** of this chicken broth?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

4. How would you rate the **SALTINESS** of this chicken broth?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

5. How would you rate the **SALTINESS** of this chicken broth?

[ ] Too Weak [ ] Just About Right [ ] Too Strong

6. Do you detect **BITTERNESS** in this chicken broth? YES [ ] NO [ ]

If YES, is it [ ] Weak [ ] Moderate [ ] Strong

7. Is the **AFTERTASTE (Such as bitterness and metallic)** of this chicken broth acceptable?

Not Accepted Extremely	Not Accepted Very much	Not Accepted Moderately	Not Accepted Slightly	Undecided	Accepted Slightly	Accepted Moderately	Accepted Very much	Accepted Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

8. How would you rate the **OVERALL LIKING** of chicken broth?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

9. Is this chicken soup **ACCEPTABLE**? YES [ ] NO [ ]

10. Is this chicken broth **ACCEPTABLE** knowing that it contains salt substitute, which **DOES NOT CAUSE HIGH BLOOD PRESSURE?** YES [ ] NO [ ]

11. Would you purchase this chicken broth? YES [ ] NO [ ]

12. Would you purchase this chicken broth knowing that it contains salt substitute, which **DOES NOT CAUSE HIGH BLOOD PRESSURE?** YES [ ] NO [ ]

c. SAS Code for Chapter 4 (ANOVA, MANOVA, PDA, DDA, LRA)

```
dm 'log;clear;output;clear';
data one;
input panel age    gender   sample $ X1 X2 X3          taste      saltiness
JARSalt Bitteryes          JARBitter          Bitterness          Oliking
accept    acceptheath buy buyhealth;
*/X1(NaCl)X2(KCl)X3(Arg)/*;
datalines;
proc freq;
tables age          gender;
proc sort; by sample;
proc freq;by sample;
tables JARSalt  Bitteryes  JARBitter  accept acceptheath;
tables gender Bitteryes*JARBitter accept*acceptheath buy*buyhealth;
proc freq;
tables accept*acceptheath/agree;by sample;
run;
proc freq;
tables buy*buyhealth/agree;by sample;
run;
proc means mean std n maxdec=2;by sample;
var  taste          saltiness Bitterness Oliking;
proc anova;
class sample;
model taste         saltiness Bitterness          Oliking = sample;
means sample/tukey lines;
proc candisc out=outcan mah;
class sample;
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class accept;
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class accept;
var taste;
proc discrim crossvalidate pool=test posterr;
class accept;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class accept;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class accept;
var Oliking;
```

```
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var taste saltiness Bitterness Oliking;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var taste;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var Oliking;
proc discrim crossvalidate pool=test posterr;
class buy;
var taste saltiness Bitterness Oliking;
proc discrim crossvalidate pool=test posterr;
class buy;
var taste;
proc discrim crossvalidate pool=test posterr;
class buy;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class buy;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class buy;
var Oliking;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var taste saltiness Bitterness Oliking;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var taste;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var Oliking;
proc logistic data = one;
model accept = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model accept = taste/ ctable;
proc logistic data = one;
model accept = saltiness/ ctable;
proc logistic data = one;
model accept = Bitterness/ ctable;
proc logistic data = one;
model accept = Oliking/ ctable;
proc logistic data = one;
```

```
model accepthealth = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model accepthealth = taste/ ctable;
proc logistic data = one;
model accepthealth = saltiness/ ctable;
proc logistic data = one;
model accepthealth = Bitterness/ ctable;
proc logistic data = one;
model accepthealth = Oliking/ ctable;
proc logistic data = one;
model buy = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model buy = taste/ ctable;
proc logistic data = one;
model buy = saltiness/ ctable;
proc logistic data = one;
model buy = Bitterness/ ctable;
proc logistic data = one;
model buy = Oliking/ ctable;
proc logistic data = one;
model buyhealth = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model buyhealth = taste/ ctable;
proc logistic data = one;
model buyhealth = saltiness/ ctable;
proc logistic data = one;
model buyhealth = Bitterness/ ctable;
proc logistic data = one;
model buyhealth = Oliking/ ctable;
```

d. SAS Code for Chapter 4 (Regression)

```
dm 'log;clear;output;clear';
data one;
input panel age    gender   sample $ X1 X2 X3          taste      saltiness
JARSalt Bitteryes          JARBitter          Bitterness          Oliking
accept   accepthealth buy buyhealth;
*// x1 = NaC       X2 = KCl          X3 = Arg //*;
x4 = x1*x2;
x5 = x1*x3;
x6 = x2*x3;
datalines;
proc reg;
model  taste       saltiness Bitterness          Oliking  = x1 x2 x3 x4 x5 x6/noint ;
run;
```

e. SAS Code for Chapter 4 (RSM Mixture Experiment)

```
Data;

DO V1 = -0.45 to 0.90 by 0.05;
        DO V2 = -0.8 to 0.15 by 0.001;
                X1 = (SQRT (6)*V1+1)/3;
                X2 = (1-X1-SQRT(2)*V2)/2;
                X3 = 1-X1-X2;
```

```
                Oliking = 0;
                IF (0 LE X1 LE 1) and (0 LE X2 Le 1) and
                        (0 LE X3 LE .15) then DO;
                Oliking  =   5.93133*X1+  3.53045*X2-6.76282*X3+ 3.81007*(X1*X2)+
                                                        17.60579*(X1*X3)+ 17.33946*(x2*x3);
                END;
                OUTPUT;
                END;
                END;
                Run;
Proc Plot;
Plot V1*V2 = Oliking/ VPOS = 40 HPOS = 60 Contour = 10;
Run;
```

f. SAS Code for Chapter 4 (PCA Biplot)

```
Title1 "Salt PCA";

Data Salt;
 Length sample $2;
 Input sample taste saltiness Bitterness Oliking;
 /*
Variables are:
taste (x1)
saltiness (x2)
Bitterness (x3)
Oliking (x4)

        sample    taste   saltiness  Bitterness    Oliking
 */
Datalines;

Title2 "Basic Principal Components Solution";
Proc Princomp Data=Salt Cov Out=Order;
 Var taste saltiness Bitterness Oliking;
Run;
Proc Sort Data=Order;
 By Prin1;
Run;
Proc Print Data=Orders;
 Var Person taste saltiness Bitterness Oliking Prin1 Prin2;
Run;
%include "biplot.sas";
%include "equate.sas";
GOptions HText=1 HTitle=1 FText=Swiss FTitle=Swiss NoPrompt;
Title3 "Symmetric Biplot -- alpha=1/2";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=sample,FacType=SYM);
Title3 "GH Biplot -- Alpha=0";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=Person,FacType=GH,scale=0.01);
Title3 "JK Biplot = Principal Components - Alpha=1";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=sample,FacType=JK,Scale=24);
Title2 "Analysis of Consumer and Attributes";
Title3 "Symmetric Biplot -- alpha=1/2";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=sample,FacType=SYM,Scale=0.5);
```

g. Ballot for Triangle Test using Beta-Binomial Model

Name: _____ **Gender:** _____

Procedure:

1. You will be presented with 3 sets of three coded samples.
2. For each set, two samples are identical and one is different (or odd).
3. You must pick or identify the odd sample.
4. Please take a 5-minute break between each set of samples.

Part I: SALTINESS

- Evaluate each set from left to right for the **SALTINESS ONLY**, then select the odd sample.

Samples	Which is the odd sample?
478-964-841	
988-524-437	
263-651-847	

Part II: BITTERNESS

- Evaluate each set from left to right for the **BITTERNESS ONLY**, then select the odd sample.

Samples	Which is the odd sample?
635-742-328	
244-560-891	
628-112-715	

APPENDIX C. DESCRIPTIVE ANALYSIS

a. Consent form for descriptive analysis

I, _____, agree to participate in the research entitled "Sensory Evaluation of a Prototype Salt Substitute Product", which is being conducted by Witoon Prinyawiwatkul, Professor of the Department of Food Science, phone number (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. 12 consumers will participate in this research. For this particular research, about 20-30 min. participation will be required for each consumer.

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

2. The reason for the research is to gather information on consumer sensory acceptability of a salt substitute from sodium chloride, potassium chloride and L-arginine. 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: 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 which can be envisioned is the allergic reaction toward NaCl (regular salt), KCl and L-Arginine (amino acid). Individuals who have kidney problem should not participate in this study.

5. The results of this study 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 my questions have been answered. I understand that additional questions regarding the study should be directed to investigators listed above. In addition, I understand that research at Louisiana State University, which involves human participation, is carried out under the oversight of the Institutional Review Board for Human Research Subject Protection. Questions or problems regarding these activities should be addressed to Dr. David Morrison (225)578-8236. I agree with the terms above and acknowledge

I have been given a copy of the consent form.

_____ _____
Signature of Investigator Signature of Participant

Witness:_____ Date: _____

b. Questionnaire for panelist screening

Name: _____ Date:_____

Phone Nº: _____ email: _____

Screening Part I:

Match each solution to one of the perceived tastes (salty, or bitter)

Taste: Write down the solution number

Salty _____

Bitter _____

Screening Part II:

1. Rank the saltiness intensity of the solutions from the least salty to the saltiest. Write down the solution numbers on the space below.

_____ _____ _____ _____

Least salty Saltiest

2. Rank the bitterness intensity of the solutions from the least bitter to the most bitter. Write down the solution numbers on the space below.

_____ _____ _____ _____

Least bitter the most bitter

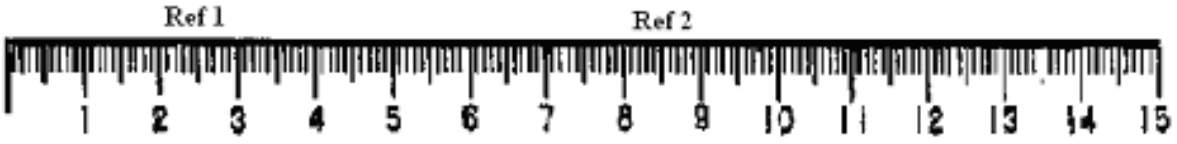
Screening Part III:

1) Taste each reference sample: Ref 1 and Ref 2
2) Taste unknown sample
3) Rank the intensity of unknown sample on 15 cm scale

Bitterness

Saltiness

1) Taste each reference sample: Ref 1 and Ref 2
2) Taste unknown sample
3) Rank the intensity of unknown sample on 15 cm scale



c. Ballot for Bitterness Intensity Evaluation Sample # 1

BITTERNESS INTENSITY EVALUATION

Name: _____

Date: _____

SALTINESS INTENSITY EVALUATION

Sample #

Date:

Name:

133

d. Ballot for Saltiness Intensity Evaluation

APPENDIX D. CHAPTER 5

a. Research Consent Form

I, _____, agree to participate in the research entitled "Optimization and Characterization of Sensory Qualities of Chicken Soup Containing Salt Substitute" which is being conducted by Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University, phone number (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 eighty five consumers will participate in this research. For this particular research, about 15 min participation will be required for each consumer.

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

2. The reason for the research is to gather information on consumer sensory acceptability of a salt substitute from sodium chloride, potassium chloride and L-arginine. 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 which can be envisioned is the allergic reaction toward chicken, NaCl (regular salt), KCl and L-Arginine (amino acid). **Individuals who have kidney problem should not participate in this study.**

5. The results of this study 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 investigators 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. David Morrison, Associate Vice Chancellor of LSU AgCenter at 578-8236. I agree with the terms above.

_____ _____
Signature of Investigator Signature of Participant

Date: _____ Witness: _____

b. Questionnaire for Chapter 5

SAMPLE

1. What is your age group?

18-24 years____ 25-34 years____ 35-44 years____ 45-54 years____ Over 55 years____

2. What is your gender? Male_____ Female_____

3. How would you rate the **OVERALL TASTE** of this chicken soup?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

4. How would you rate the **SALTINESS** of this chicken soup?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

5. How would you rate the **SALTINESS** of this chicken soup?

[ ] Too Weak [ ] Just About Right [ ] Too Strong

6. Do you detect **BITTERNESS** in this chicken soup? YES [ ] NO [ ]

If YES, is it [ ] Weak [ ] Moderate [ ] Strong

7. Is the **AFTERTASTE (Such as bitterness and metallic)** of this chicken soup acceptable?

Not Accepted Extremely	Not Accepted Very much	Not Accepted Moderately	Not Accepted Slightly	Undecided	Accepted Slightly	Accepted Moderately	Accepted Very much	Accepted Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

8. How would you rate the **OVERALL LIKING** of chicken soup?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

9. Is this chicken soup **ACCEPTABLE**? YES [ ] NO [ ]

10. Is this chicken soup **ACCEPTABLE** knowing that it contains salt substitute, which **DOES NOT CAUSE HIGH BLOOD PRESSURE?** YES [ ] NO [ ]

11. Would you purchase this chicken soup? YES [ ] NO [ ]

12. Would you purchase this chicken soup knowing that it contains salt substitute, which **DOES NOT CAUSE HIGH BLOOD PRESSURE?** YES [ ] NO [ ]

c. SAS Code for Randomization

```
title 'All Permutations of 1,2,3,4';
  proc plan seed=60359;
    factors    Subject  = 20
               Order    = 4  ordered;
    treatments Stimulus = 4  perm;
    output out=Psych;
  proc sort data=Psych out=Psych;
    by Subject Order;
  proc tabulate formchar='          ' noseps;
    class Subject Order;
    var Stimulus;
    table Subject, Order*(Stimulus*f=8.)*sum=' ' / rts=9;
        run;
```

d. SAS Code for (ANOVA, MANOVA, PDA, DDA, LRA)

```
dm 'log;clear;output;clear';
data one;
input panel age    gender   sample taste saltiness
JARSalt Bitteryes          JARBitter          Bitterness          Oliking
accept   accepthealth buy buyhealth;
datalines;
proc freq;
tables age            gender;
proc sort; by sample;
proc freq;by sample;
tables JARSalt  Bitteryes  JARBitter  accept accepthealth;
tables gender Bitteryes*JARBitter accept*accepthealth buy*buyhealth;
proc freq;
tables accept*accepthealth/agree;by sample;
run;
proc freq;
tables buy*buyhealth/agree;by sample;
run;
proc means mean std n maxdec=2;by sample;
var  taste            saltiness Bitterness Oliking;
proc anova;
class sample;
model taste         saltiness Bitterness          Oliking = sample;
means sample/tukey lines;
proc candisc out=outcan mah;
class sample;
```

```
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class accept;
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class accept;
var taste;
proc discrim crossvalidate pool=test posterr;
class accept;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class accept;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class accept;
var Oliking;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var taste;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class acceptheath;
var Oliking;
proc discrim crossvalidate pool=test posterr;
class buy;
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class buy;
var taste;
proc discrim crossvalidate pool=test posterr;
class buy;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class buy;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class buy;
var Oliking;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var taste saltiness Bitterness          Oliking;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var taste;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var saltiness;
proc discrim crossvalidate pool=test posterr;
```

```
class buyhealth;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class buyhealth;
var Oliking;
proc logistic data = one;
model accept = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model accept = taste/ ctable;
proc logistic data = one;
model accept = saltiness/ ctable;
proc logistic data = one;
model accept = Bitterness/ ctable;
proc logistic data = one;
model accept = Oliking/ ctable;
proc logistic data = one;
model accepthealth = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model accepthealth = taste/ ctable;
proc logistic data = one;
model accepthealth = saltiness/ ctable;
proc logistic data = one;
model accepthealth = Bitterness/ ctable;
proc logistic data = one;
model accepthealth = Oliking/ ctable;
proc logistic data = one;
model buy = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model buy = taste/ ctable;
proc logistic data = one;
model buy = saltiness/ ctable;
proc logistic data = one;
model buy = Bitterness/ ctable;
proc logistic data = one;
model buy = Oliking/ ctable;
proc logistic data = one;
model buyhealth = taste saltiness Bitterness Oliking/ ctable;
proc logistic data = one;
model buyhealth = taste/ ctable;
proc logistic data = one;
model buyhealth = saltiness/ ctable;
proc logistic data = one;
model buyhealth = Bitterness/ ctable;
proc logistic data = one;
model buyhealth = Oliking/ ctable;
```

e. SAS Code for PCA

```
Data Salt;
 Length sample $2;
 Input sample taste saltiness Bitterness Oliking;
 /*
Variables are:
taste (x1)
saltiness (x2)
Bitterness (x3)
```

```
Oliking (x4)
        sample    taste   saltiness  Bitterness    Oliking
 */
Datalines;
Title2 "Basic Principal Components Solution";
Proc Princomp Data=Salt Cov Out=Order;
 Var taste saltiness Bitterness Oliking;
Run;

Proc Sort Data=Order;
 By Prin1;
Run;
Proc Print Data=Salt;
 Var Person taste saltiness Bitterness Oliking Prin1 Prin2;
Run;
%include "biplot.sas";
%include "equate.sas";
GOptions HText=1 HTitle=1 FText=Swiss FTitle=Swiss NoPrompt;
Title2 "Analysis of Consumer Characteristics";
Title3 "Symmetric Biplot -- alpha=1/2";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=sample,FacType=SYM);
Title3 "GH Biplot -- Alpha=0";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=Person,FacType=GH,scale=0.01);
Title3 "JK Biplot = Principal Components - Alpha=1";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=sample,FacType=JK,Scale=24);
Title3 "Symmetric Biplot -- alpha=1/2";
%Biplot(Data=Salt,Var=taste saltiness Bitterness Oliking,Id=sample,FacType=SYM,Scale=0.5);
```

VITA

Armen Khachatryan was born 1976 in Yerevan, Armenia. He earned his medical degree in 1999 from Yerevan State Medical University, Yerevan, Armenia. In January 2000, he moved to Baton Rouge, Louisiana, where he graduated from Louisiana State University. He received his Bachelor of Science degree from the Department of Food Science.

In August 2003, he successfully completed Master of Science degree in the Department of Food Science at Louisiana State University and immediately began working for a Doctor of Philosophy degree in the Department of Food Science at Louisiana State University, Baton Rouge, LA and he is a candidate for that degree.