Sensory characteristics of salt substitute containing L-arginine

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SENSORY CHARACTERISTICS OF SALT SUBSTITUTE CONTAINING L-ARGININE

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

In

The Department of Food Science

by

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B.S., Food Science and Technology, Thammasat University, 2002
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ABSTRACT

Dietary salt restriction is a common approach recommended by physicians in the treatment and prevention of hypertension. Salt substitute is a potential alternative. The most popular salt substitute is KCl, having similar physical properties to NaCl. Because of the higher molecular weight of cations (K⁺), KCl imparts undesired bitterness and metallic aftertaste. L-arginine has been found to have the bitterness-suppression property. Therefore, it may be used in the mixture of salt substitutes.

In the first study, NaCl and four salt substitute solutions consisting of KCl, NaCl, and L-arginine, were developed at 0.5%, 1%, and 1.5% w/v. A discriminative test was performed to determine (1) the effectiveness of L-arginine in masking the bitterness perception of KCl, (2) saltiness perception of mixed salt solutions against NaCl solution, and (3) sensitivity of the simple ranking test vs. the R-index tests for evaluating bitterness and saltiness perception. The differences of saltiness perception of aqueous mixed salt against NaCl solution existed based on the non-parametric Friedman’s test and the R-index test. The samples were not significantly different in terms of bitterness based on both techniques. Therefore, L-arginine could mask the bitterness of KCl.

In study two, eleven formulations of the mixture of NaCl/KCl/L-arginine were developed using a mixture design. The consumer study was performed to determine sensory attributes driving acceptance and to optimize the formulation. Consumers (n=385) evaluated the products, following a balanced incomplete block design. Bitterness was the discriminating attribute. Overall liking was identified as the attribute influencing consumer acceptability. The formulation containing 56-100% NaCl, 0-44% KCl, and 0-5% L-arginine would yield product acceptability score 1.0 unit less than that of NaCl. Consumers were able to discriminate the saltiness and the
bitterness between formulations of salt solutions (100% NaCl vs. 35% NaCl, 65% KCl), using the triangle test with a corrected beta binomial distribution.

L-arginine could partially mask the bitterness of KCl. However, development of the proportion of KCl/NaCl/L-arginine obtained from mixture design, and the application of salt substitute in foods would be worth further study. Moreover, the heat and cold stability of L-arginine in the salt substitutes should be investigated.
CHAPTER 1. INTRODUCTION

The common salt is the chemical compound, sodium chloride – NaCl, approximately consisting of 40% sodium and 60% chloride. The Salt Institute reports that the U.S. food-grade salt sales is higher than 1,500 tons and increasing continuously for almost the past decade. Salt, the most basic ingredient in the kitchen, has many important roles in the food. Not only does salt enhance flavor and act as a preservative but it also provides sensory sensations such as mouthfeel (Pszczola, 2006). Salt occurs naturally in foods in a small amount, but many salt and sodium-containing ingredients are added during processes for various purposes. For the physiological role, sodium is an essential dietary nutrient to regulate the body’s fluid balance, maintain the blood volume and pressure, and transmit nerve impulses.

As many as 65 million Americans or nearly one in three have high blood pressure, one of leading causes of death in the United States and around the world. The risk factors contributing to high blood pressure are age, race, sex, heredity, obesity, and sodium sensitivity. Overconsumption of dietary salt increases blood pressure resulting in strokes, heart attack, kidney disease, and congestive heart failure. Researches have shown that American’s average daily intake of sodium is far higher than 2400 mg, the Daily Reference Value (DRV) recommended by FDA. The higher an individual’s salt intake, the higher an individual’s blood pressure. Accordingly, reduction of dietary sodium intake is advisable to decrease the risk of development of hypertension. The Department of Health and Food Standards Agency recommends that everyone should cut their salt intake from the current amount of 10 to 12 grams of salt a day to 5 to 6 grams a day or less.

Because of those health issues, consumers are more concerned with the amount of salt on their diet as well as are the food manufacturers. Using alternatives such as potassium chloride,
KCl, is one of the most common ways to reduce the sodium content in processed foods. Potassium chloride has physicochemical properties closely resembling NaCl, but it has an unpleasant aftertaste. Mickelsen and others (1977) reported that the solution containing 50% replacement of NaCl by KCl tasted as salty as that with pure NaCl. Ogawa and others (2004) reported that L-arginine could mask the bitterness of various compounds and enhance the saltiness of NaCl. Thus, L-arginine may be used in the mixture of salt substitutes.

The hypothesis studied was that L-arginine would mask the bitterness of KCl in the mixed salt solutions. The objectives were to develop a mixed salt substitute in which more than 50% NaCl is replaced by KCl and to evaluate the effectiveness of L-arginine in masking the bitterness perception in the mixture of salt substitutes.

This thesis is divided into five chapters. Chapter one provides a summarized introduction and discusses this research’s justification. Chapter two presents a literature review with concepts associated with this thesis work. Chapter three presents the evaluation of the effectiveness of L-arginine in masking the bitterness perception of KCl, a salt substitute, using the R-index and the simple ranking tests. Chapter four discusses the sensory optimization of mixed salt solutions. Chapter five consists of a brief summary of all the findings of this research and possible future work. A list of all cited references and appendices containing the survey questionnaires for all consumer studies, research consent forms, SAS codes and other figures are included. Finally, the VITA of the author of this work is provided on the last page of this thesis.
CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

High blood pressure is one of the significant risk factors leading for heart attack, heart disease, strokes, and kidney disease. According to the American Heart Association, more than 60 million or about 1 in 3 American adults have high blood pressure. High blood pressure affects about 2 in 5 African Americans, 1 in 5 Hispanics and Native Americans, and 1 in 6 Asians (CDC 2006). In 2000, approximately 972 million adults in both developed and developing nations were reported to have hypertension, and this number is expected to increase by about 60% to a total of 1.56 billion by 2025 (Kearny and others 2005). High blood pressure caused the death of 52,602 Americans in 2003 (AHA 2006). Furthermore, the American Heart Association (2006) reported that the death rate of hypertension increased by 29.3 percent from 1993 to 2003, and the actual number of death rose by 56.1 percent. Additionally Americans spend more than $15 billion annually on medications for hypertension (AHA 2006). In 2005, the Department of Health and Human Services (DHHS) and the Department of Agriculture (USDA) revealed that nearly all Americans consume substantially more salt than they need. Unfortunately, overconsumption of sodium content causes hypertension. Reducing the sodium dietary intake is one of several ways that people can use to lower their blood pressure, and consuming potassium-rich diet can decrease the effects of NaCl salt on blood pressure as well.

2.2 Salt

Table salt, also known as sodium chloride (NaCl), is an essential component of the human diet. Salt can be found naturally in the environment but mostly found in the ocean with many other minerals. The production of common salt is one of the most ancient and widely distributed industries in the world. Salt is produced from a number of sources worldwide; these
include sea water, deep wells (natural brine, or wet-mined salt) and salt rocks (Amr and Jabay 2004). Mostly in Europe and North America salt are produced by mining while solar evaporation is widely used in Asia, Africa, Australia and South America. The physical and chemical composition of salt produced from the various sources varies widely depending upon the manufacturing techniques, climatic conditions and processes adopted (Mannar and Dunn 1995). The annual world salt production has increased from 10 million tons over the past century to over 200 million tons today. The amount of U.S. food-grade salt sales from 1978-2005 is shown in Figure 1.

![U.S. Food Grade Salt Sales, 1978-2005](image)

**Figure 1:** The Food-Grade Salt Sales in the United States, 1978-2005 (Salt Institute 2006)

### 2.2.1 Properties of Sodium Chloride

Sodium chloride is a chemical compound composed of cationic sodium (Na⁺) and anionic chloride (Cl⁻). For every gram of salt, 39.3% is sodium (Na) and 60.7% is chlorine (Cl). Sodium chloride has 58.44 g/mol of the molar mass, 2.165 for the specific gravity, and 2.16 g/cm³ of the density. For salt with high purity, it is transparent and colorless in the cubic crystalline form. At 25°C its solubility is 35.9 grams in 100 ml of water and it is able to slightly solubilize in alcohol. Sodium chloride starts to melt at 801°C and is boiled at 1465°C (Salt Institute 2006).
Recently edible salt is one of the most used food additives. Being used in a wide range, salt acts in several functions on diet. Basically it is used as a seasoning to make food tasteful and palatable. For thousands of years, salt has been used as a preservative by lowering the water activity (a_w) and limiting the growth of microbiological flora. In the meat processing industry, salt is used as a binding agent as it can extract the salt-soluble protein named myofibrillar; so it binds meat together and reduces cooking losses. Besides, it can be used as texture aids for strengthening gluten in bread dough and controlling the rate of fermentation of yeast-leavened products.

Classic salty taste, one of the four principal tastes, is given by sodium chloride (NaCl) and lithium chloride (LiCl) as well (Lindsay 1996). In the interest of the saltiness perception mechanism, there are many studies investigating on this topic but the actual cause of salty taste has been poorly demonstrated. Deman (1976) reported that the taste of salts is dependent on the nature of both cation and anion. As the molecular weight of either cation or anion or both increases the salts are likely to taste more bitter. According to Reddy and Marth (1991), saltiness is provided by Na⁺ while anions inhibit the taste effect of cations. Moreover, the chloride anion is the least inhibitory since it has no taste of its own (Lindsay 1996). In more complex salts, the original taste of anions, bitterness, is developed and the taste response of cations is inhibited. However, another study in psychophysics by Murphy and others (1981) showed that the saltiness perception does not depend on the molecular weight of cations. The lighter weight anions often produced saltier-tasting salts, while both heavier cations and anions produced more bitter-tasting salts.

2.2.2 Sources of Sodium

Sodium in the diet comes from various sources. It naturally occurs in a small amount
as found in meat, eggs, milk, etc. Mattes and Donelly (1991) revealed the common sources of sodium found in the food supply (Figure 2), showing that about three-fourths of the total sodium intake is derived from salt added by food manufacturers while the natural salt content of food accounts for only about 10% of the total intake and discretionary salt use provides another 5-10% of the total intake.

Figure 2: Sources of Dietary Sodium. Source: Mattes and Donelly (1991).

Likewise, Sanchez-Castillo and others (1987) reported that sources of dietary salt in the British population, where 15% of the total intake was contributed to discretionary sources, i.e., cooking and table salt use and 85% to salt added by manufacturing foods and catering in purchased food. Much of salt consumed is hidden in the processed foods so people do not even know their daily sodium intake. In addition, most of people understand that the main sources of salt are from the kitchen and the table. However, sodium exists in various forms; these include monosodium glutamate (MSG), sodium citrate, sodium alginate, sodium hydroxide, and sodium phosphate. However, it seems interestingly that sodium bicarbonate does not affect blood pressure while sodium chloride does (Luft and others 1991).
Prior to stating the daily salt intake content, it is important to distinguish between salt and sodium. Salt comprises 40% of sodium and 60% of chloride, so the terms ‘salt’ and ‘sodium’ can not be used interchangeably in this case. There are many studies showing that Americans’ average daily sodium intake is higher than 2400 mg, the Daily Reference Value (DRV) recommended by FDA. Ellison (2005) reported that Americans consume an average of 3,400 mg of sodium daily which is up nearly 30% from 2,400 mg in the 1970s as shown in Figure 3.

![Americans' Average Daily Sodium Intake](https://example.com/sodium_intake.png)

**Figure 3:** Americans’ Average Daily Sodium Intake. Source: Ellison (2005).

The dietary guidelines for daily salt intake vary from one country to another country 4.5 g (Singapore) and 10 g of salt (Japan). However, the Institute of Medicine’s Nutrition Review issued revised nutrient recommendations, known as the Dietary Reference Intakes (DRIs), stating that people in all ages should consume sodium less than 1500 mg a day. Unfortunately, excess salt intake has linked to hypertension and several other health effects. Skrabal and others (1981) indicated that salt restriction combined with a high potassium intake helps to prevent hypertension.
2.2.3 Health Effects of Sodium

Sodium is an essential mineral for regulating body fluid balance. When its level is too high, the body retains too much water and the volume of bodily fluids increases. An adult will be able to remove salt from the body through the kidneys into the urine. Kesteloot and Joossen (1988) revealed that dietary cations, such as sodium, calcium, and potassium, are related to the regulation of blood pressure, especially for sodium which has a significant correlation with blood pressure. Many scientists point out that salt intake is linked to high blood pressure, which likely leads to development of heart disease and stroke. The relationship between salt intake and blood pressure is direct and progressive without an apparent threshold (The Dietary Guidelines for Americans, 2005). The higher an individual’s salt intake, the higher an individual’s blood pressure. Accordingly, the reduction of dietary sodium intake is advisable to decrease the risk of development of hypertension. However, salt restriction can be undertaken in free-living hypertensive subjects without any untoward changes in the intake of other nutrients (Korhonen and others 2000).

The Department of Health and Food Standards Agency recommends that everyone should cut their salt intake from the current amount of 10 to 12 grams of salt a day to 5 to 6 grams a day or less. Moreover, the Dietary Guidelines for Americans (2005) recommends that Americans consume sodium less than 2300 mg, and individuals with hypertension, African-Americans, and middle aged and older adults should consume no more than 1500 mg of sodium daily. These specific groups tend to be more sensitive to sodium than others; for instance, African-Americans have a relatively low potassium intake and a high prevalence of elevated blood pressure. It is likely that genetics affects the salt sensitivity. Luft and others (1991) reported that salt sensitivity is a function of age but is not affected by gender.
2.3 Potassium Chloride (KCl)

To lower blood pressure, commercially prepared foods should be avoided, and a restriction in the use of table salt in cooking and at the table is recommended. Another way to reduce the dietary sodium intake is using a salt substitute. Since sodium chloride has a unique pure salty taste, it may be extremely difficult to determine a comparable salt alternative. These alternatives include halide salts including potassium chloride, magnesium chloride, calcium chloride, ammonium chloride, and lithium chloride. Brandsma (2006) reports that taste enhancers which are able to enhance the taste receptors in the mouth and the throat are also possibly used as salt alternatives. This means that the taste enhancers can provide a pleasurable taste sensation, the umami taste. Umami discovered by Ikeda in 1907 is recognized as the fifth basic taste. This savory taste is associated with glutamate and ribonucleotides, including inosinate and guanylate. Glutamate can be derived from glutamic acid, the non-essential amino acid, so the taste of umami occurs naturally in many foods including meat, fish, vegetables and dairy products (Prescott 2004). In addition, potential ingredients used to reduce sodium in food formulations include yeast extracts, hydrolyzed vegetable protein, monosodium glutamate, disodium inosinate, disodium guanylate, soy sauce, and potassium chloride (Brandsma 2006).

Potassium chloride (KCl) is potentially a sodium-free alternative to salt and a common ingredient in salt substitutes. Potassium chloride is not only a good compound for supplementing of sodium chloride, but its physical properties make it technically an ideal substance for ingredient with ordinary salt (Frank and Mickelson 1969). Because of higher molecular weight of cations (K⁺), KCl has weak and salty flavor and imparts bitterness and metallic aftertaste when the large amount is applied. Therefore, most salt substitutes in the market are usually a mixture of NaCl and other salt substitutes, and the bitterness blockers have been investigated.
2.3.1 Properties of Potassium Chloride

The chemical compound, potassium chloride, is composed of cationic potassium ($K^+$) and anionic chloride ($Cl^-$). Potassium chloride comprises 47.55% of chloride and 52.45% of potassium. The appearance of sodium chloride and potassium chloride is indistinguishable since both salts are colorless, transparent cubic crystals with similar refractive indices and even resembling in particle sizes. The density of KCl (1.99) is similar to that of NaCl (2.16). The solubility in water of both salts is found to be an approximately same value, 35 g in 100 ml but potassium chloride has a property of being more soluble in hot water but less soluble in cold water.

Potassium is the seventh most abundant element in the crust of the earth and the sixth most abundant element in solution in the oceans. It is present in mineral waters and brines, and in various minerals such as carnallite, feldspar, saltpeter, greensand, and sylvite. Potassium is an important constituent of fertile soil and is an essential nutrient for plant growth and in the human diet (Argonne National Laboratory 2005). Potassium chloride can be manufactured industrially by fractional crystallization of carnallite or of solutions from lake brines. It can also be extracted from sylvinitite and salt water. But the quantity present in a given volume of seawater is relatively low compared to sodium. Germany was the main mining of potassium but recently most potassium minerals come from Canada, USA and Chile. Potassium chloride is annually produced in the world around 50 million tons, which is worth approximately $10 billion. The main purpose of using KCl in agriculture is for a fertilizer and for a salt substitute in food processing.

2.3.2 Sources of Potassium

All living cells, both plant and animal, contain the potassium dietary. Fresh foods are the richest sources of potassium, especially fruits, vegetables and beans such as spinach, lettuce,
parsley, broccoli, peas, lima beans, potatoes, citrus fruits, bananas, whole grains, and wheat germ. In addition, potassium can be found in meat, bread, and milk. Most of the potassium is lost while processing or canning; therefore, fresh foods contain much more potassium than sodium. Conversely, most processed foods contain less potassium and more sodium with salt added during the process. Potassium is the primary cation found within the cells. The USDA recommends the DRV for potassium at 3500 mg based on the reference calorie intake of 2000 calories. In 2002, the Centers for Disease Control and Prevention (CDC) reported that Americans had an average potassium intake of 2,723 mg per day from 1988 to 1994. By race, it was found that African-Americans had the least potassium intake which was corresponding to the Dietary Guidelines for Americans in 2005. In an effort to reduce the amount of sodium in salt, it has been reported that potassium is related to the lower level of blood pressure. Many medical researches found that increasing potassium intake can significantly lower the blood pressure. Recently, the National Heart, Lung, and Blood Institute (NHLBI) has published the Dietary Approaches to Stop Hypertension (DASH), i.e., the eating plan features plenty of fruits, vegetables, whole grains, and other foods that are heart healthy and lower in salt/sodium. This guide was designed to help control blood pressure. The DASH diet can reduce blood pressure and risk of heart disease through weight loss, reduced salt intake, moderation in drinking alcohol (for those who drink), and eating foods that are rich in potassium. Replacing common sodium salt by a low sodium, high potassium, high magnesium mineral salt could offer a valuable non-pharmacological approach to lowering blood pressure in older people with mild to moderate hypertension (Geleijnse and others 1994). This is helpful for those with hypertension and African-Americans, since they are sensitive to potassium and consume low-potassium foods.
2.3.3 Health Effects of Potassium

Potassium is an essential dietary constituent, important to both cellular and electrical function. Along with sodium (Na) and chlorine (Cl), potassium (K) is one of the three major electrolytes in the body and functions to maintain cation-anion balance (blood pH). It is important for maintaining a proper osmotic balance within cells, transmitting the nerve impulse, generating the muscle contraction and regulating the heartbeat. Potassium is usually absorbed from the small intestine and excess potassium is excreted through the kidneys (90%) and the gut (10%). The kidney has the function of regulating the amount of supplemental potassium in the body and keeps the blood level steady. However, potassium consumed in excess may be harmful for some people such as subjects with kidney problems. Hyperkalemia occurs when potassium levels elevated exceeds the capacity of kidneys to eliminate or greater than 18 grams orally taken at one time (Wingo and Goldin 2004). Hyperkalemia may develop cardiac arrhythmias or irregular heartbeat condition which can lead to cardiac arrest. However, a result of excessive loss of potassium can cause hypokalemia which can lead to serious muscle weakness, bone fragility, central nervous system changes, decreased heart rate, and even death. Hypokalemia is most commonly caused by the use of diuretics. Diuretics are drugs that increase the excretion of water and salts in the urine. Diuretics are used to treat a number of medical conditions, including hypertension, congestive heart failure, liver disease, and kidney disease. Other common causes of hypokalemia are excessive diarrhea or vomiting, and alcoholism occasionally results in hypokalemia. Therefore, maintaining consistent levels of potassium in the blood and cells is vital to body function. Although using potassium-based salt substitutes is an alternative for people on sodium-restricted diets, it may be hazardous when used in combination with other certain medicines. Thus, it is recommended to check with the physician before using salt substitutes.
2.3.4 Mixtures of KCl and NaCl

NaCl elicits most purely salty taste of all salts. It contributes sweet taste at low concentrations, somewhat sour at mid-range intensities, and a bitter taste in some subjects, and also modifies taste and flavor (Kemp and Beauchamp 1994; Smith and others 1995; Breslin and Beauchamp 1997). However, KCl is responsible for a strong salty taste as well as a weak bitter taste. Over the past two decades, there have been many studies of the replacement of NaCl by KCl and such studies showed that KCl can be used to replace NaCl at up to 50% to remain the similar saltiness perception in the ordinary salt. Kincaid and others (1975) reported that the 1.2% solution of 1:1 mixture of sodium and potassium chlorides tastes as salty as the 1% solution of sodium chloride. On the contrary, the 1:1 mixture of sodium and potassium chlorides has been reported to taste as salty as the NaCl solution alone (Frank and Mickelsen 1969; Mickelsen and others 1977; Duxbury 1986; Best 1989). Breslin and Beauchamp (1997) have suggested that the saltiness perception of NaCl solution significantly increases in the presence of KCl by the summation of the independent salty tastes of NaCl and KCl. In addition, Frank and Mickelsen (1969) and Rosett and others (1995) reported that NaCl can suppress the bitterness perception imparted by KCl. Breslin and Beauchamp (1995) have reported that the degree of average bitterness suppression of KCl was about 78% by high concentrations of NaCl. Likewise, this interaction was explained afterwards by Keast and others (2001) that sodium ions have the bitterness-suppressing ability, but the potassium cation had no bitterness-suppressing ability. Moreover, perceived saltiness decreased when the anion increased in size. The possible mechanism of the suppression of bitter compounds by sodium ion is in Figure 4 (Keast and others 2001).
First, sodium diminishes the receptor’s affinity for the bitterness by forming an ionic shield around parts of G-protein coupled receptors. Then the ion channels/pumps involved in the taste transduction sequence are modulated. Next, sodium may act to stabilize the cellular membrane, thereby limiting a direct access of those compounds through the membrane to intracellular pathways. Finally, sodium may interfere with specific second messenger systems (G-proteins or enzymes) responsible for bitter taste transduction from inside the cell (Keast and others 2001).

In addition, researchers identified a protein called Trpm5 in taste cells that plays a key role in the delivery role in the delivery of bitter taste messages to the brain. This protein in taste cells responds to bitter flavors by converting taste information into signals that are then transmitted to taste nerve cells. The signals are sent to and activate the bitter detection center of
the brain, leading the brain to perceive a bitter taste (Pszczola 2003). Moreover, researchers have discovered more than 20 bitter blockers. All of them are natural substances found in foods such as meat, fish, and milk, i.e., adenosine monophosphate (AMP), L-lysine, L-arginine, etc.

2.4 L-Arginine

According to Breslin and Beauchamp (1995), bitterness is more difficult to suppress as perceived intensity increases. By sodium ions alone, the metallic aftertaste of KCl could not be completely masked when more than 50% replacement of NaCl by KCl is presented at high concentrations. Therefore, the effect of the addition of bitterness inhibitor was investigated in this thesis research. Keast and others (2004) have suggested that there are few known bitterness inhibitors. Sodium (Na⁺) salts have been shown to suppress the bitterness of certain compounds but this effect occurs peripherally (at a receptor/transduction mechanism) rather than cognitively. Ogawa and others (2004) revealed that L-arginine can significantly suppress the bitterness of quinine and the bitterness suppression of L-arginine could be enhanced by the addition of NaCl. With such effects of L-arginine, it was chosen because it elicits a mild salty taste but contributes a slight bitter taste as well.

2.4.1 Properties of L-arginine

L-arginine is not considered as an essential amino acid because under the normal circumstances the body can synthesize sufficient L-arginine from other amino acids obtained from dietary sources to meet physiological demands. However, human infants cannot synthesize it in sufficient amounts to meet their need for growth; it is then called a growth hormone releaser. The Recommended Dietary Allowances (RDAs) of L-arginine is not established. Adversely, if the synthesis of L-arginine is impaired, it may cause stress, and imbalances of other nutrients. The chemical formula of L-arginine is C₆H₁₄N₄O₂, with a molecular weight of 174.2 daltons.
L-arginine has 11.2 for an isoelectric point and 12.5 for pKa. In 100 grams of water at 20°C, its solubility is 14. In solid phase, L-arginine has 1.1 g/cm³ of the density and 244°C of the melting point. The manufacturing process of L-arginine is extraction, fermentation, and synthesis. Based on the physiological properties, L-arginine is helpful in the treatment and prevention of cardiovascular disease including hypertension, and some kidney disorders.

![Guanidinium group]

**Figure 5:** Chemical Structure of L-arginine

Ogawa and others (2004) explained the bitterness-suppressing mechanism of L-arginine by the presence of the guanidinium group which interacts with the sodium channel in the human taste bud, and binding at the receptor site as well. In addition, the effective concentration of the combination of L-arginine and NaCl was reported at a range of 0.05 – 0.15% (w/v). Because of an unpleasant smell, Ogawa and others (2004) recommended the concentration of L-arginine use up to 0.2% (w/v) while the level over 2.0% (w/v) of NaCl was reported to has excessive saltiness. They have also found several interesting properties of L-arginine in blocking bitterness. L-arginine could mask the bitterness of various compounds and enhance the saltiness of NaCl. In addition, the degree of suppression reached by L-arginine and NaCl was greater than that of any of other suppressing agents, including phosphatidic and tannic acids.
CHAPTER 3. EVALUATING THE EFFECTIVENESS OF L-ARGINININE IN MASKING THE BITTERNESS PERCEPTION OF KCL, A SALT SUBSTITUTE, USING THE R-INDEX AND THE SIMPLE RANKING TESTS

3.1 Introduction

Table salt, also known as sodium chloride (NaCl), is an essential component of the human diet. It is usually used as a flavoring agent to make food tasteful and palatable, as a preservative by lowering the water activity \( (a_w) \) and limiting the growth of microbiological flora, and as a binding agent, and used for texture aids. Sodium naturally occurs in a small amount in fresh foods but most of sodium intake is derived from processed foods (The Dietary Guidelines for Americans 2005). Unfortunately, Ellison (2005) reported that Americans consume an average of 3,400 mg of sodium daily which is higher than 2400 mg, the daily reference value (DRV) recommended by FDA. Dietary sodium exists in various forms, not only in sodium chloride but in monosodium glutamate (MSG), sodium alginate, and sodium phosphate. Thus, high-sodium foods do not necessarily taste salty.

The physiological role of sodium is regulation of the body fluid balance and this process is linked to high blood pressure when excess sodium in the body occurs. Hypertension is one of the significant risk factors leading for heart attack, heart disease, strokes, and kidney disease. According to the American Heart Association, more than 60 million or about 1 in 3 American adults have high blood pressure. The higher an individual’s salt intake, the higher an individual’s blood pressure. Accordingly, the reduction of dietary sodium intake is advisable to decrease the risk of development of hypertension. Using salt substitute is an appropriate approach for patients with salt restriction. Potassium chloride may be a good alternative for saltiness of sodium chloride, but it imparts disagreeable bitterness and metallic aftertaste. A 1:1 mixture of sodium and potassium chlorides is perceived as salty as the ordinary salt, and the bitterness perception is
also suppressed by NaCl (Rosett and others 1995). To develop a salt substitute with more than 50% NaCl replacement of KCl, the bitterness-suppressing agent was used in this current study. L-arginine has been found to have the ability to mask bitterness by binding at the receptor site as well as an interaction between the guanidinium side-chain of L-arginine and the sodium channel in the human taste bud (Ogawa and others 2004).

Consequently, the mixtures of KCl/NaCl/L-Arg were developed to evaluate the saltiness and the bitterness perceptions. If consumers can differentiate the bitterness perception among NaCl solution and mixed salt solutions, there would be insignificant suppression of the bitterness of KCl by L-arginine. The objectives of this study were to evaluate (1) the effectiveness of L-arginine in masking the bitterness perception of KCl, (2) saltiness perception of mixed salt (KCl/NaCl/L-arginine) solutions against NaCl solution, (3) sensitivity of the simple ranking test vs. the R-index tests for evaluating bitterness and saltiness perception.

3.1.1 Discriminative Sensory Tests

Discriminative tests should be used when the sensory specialist wants to determine whether two samples are perceptibly different (Lawless and Heymann 1998). According to the limitation of an individual’s perception, it is important to compare the difference of the overall attribute or any specific attributes between existing products and new products when some changes occur. In addition, these techniques should not be used when the differences between samples are subtle; however, these subtle differences make the risk of Type II errors more likely (Lawless and Heymann 1998). Different discriminative tests are classified into overall difference tests and attribute difference tests. Overall difference tests, asking whether any difference at all between samples exists, include tests such as the Triangle, Duo-trio, A-not A, Difference-from-Control, etc (Meilgaard and others 1999). Attribute difference tests, asking about a single or a
few attributes of samples, include tests such as paired comparison, n-AFC, and various types of multiple comparison tests (Meilgaard and others 1999).

There are many general applications of discriminative sensory tests which are (1) to determine whether products differ due to changes in ingredients, processing, packaging, storage, etc., (2) to determine if an overall difference exists, where no specific attribute can be identified as having been affected, (3) to determine whether the difference of a specific attribute of the products exists, (4) to monitor the panelist’s ability to discriminate between test samples, and (5) to select and screen panelists for descriptive analyses.

3.1.2 Signal Detection Theory

Signal Detection Theory (SDT) is the measurement theory that permits the separation of the true observer sensitivity from response bias. The theory of signal detection is most easily derived from a simple experiment in which two levels of a stimulus are to be evaluated. For instance, the background or blank stimulus is called the noise (N), and some weak but higher level of stimulus intensity near threshold, called the signal (S) (Lawless and Heymann 1998). When applying in food sensory tests, the signal can be new, reformulated, improved products and the noise can be a control, existing, or current product produced. In the signal detection matrix as shown in Figure 6, a correct decision made is called “hit” and “correct rejection” when a signal and a noise are presented, respectively. However, when the noise is presented but perceived as a signal, it is called “false alarms”. On the other hand, when the signal is presented but perceived as a noise, it is called “miss” (Lawless and Heymann 1998).

The strategy in signal detection measurement is to allow the criterion to vary and arrange for the judge to make the same judgments at several criterion levels ranging from strict to lax (O’Mahony 1991). Having obtained a set of responses for different criterion levels for replicate
sets of the two food stimuli, there are several indices: $d'$, $P(A)$, and the R-index, which can be used to calculate an index of how sensitive the judge is to the difference (O’Mahony 1991).

**Figure 6:** Signal Detection Matrix. Source: Lawless and Heymann (1998).

**Figure 7:** Signal Detection Scheme. Source: Lawless and Heymann (1998).
The value of sensory difference, $d'$, represents the separation of the means of the two distributions in standard deviation units (Figure 7). It is equal to the $Z$-score for the proportion of hits minus the $Z$ score for the proportion of false alarms (Lawless and Heymann 1998). If the hit rate and false alarm rate are equal, there is then no discrimination of the two levels, and $d'=0$. The higher $d'$ value, the better the discrimination. Unfortunately, several assumptions of this measurement are the limitation. First of all, the theory requires the normal distribution saying that the sensations from signal and noise are normally distributed with equal variance. Variabilities in the signal and noise are also included due to the spontaneous variation in the background level of activity in sensory nerves and other sources (Lawless and Heymann 1998).

3.1.3 ROC Curve-Differing Sensitivities

As mentioned above, $P(A)$ is another signal detection measure and it is exactly related to $d'$. One commonly used method of computing $d'$ is to plot what is called a Receiver Operating Characteristic (ROC) curve (Figure 8). This is a plot of the proportion of times a judge correctly identifies the stronger of the two stimuli in the difference test as being stronger – a hit – versus the proportion of times he incorrectly identifies the weaker of the two stimuli as being stronger – a false alarm (O’Mahony 1991). A point on the graph is produced when a judge is given a criterion level. Several criterion levels will produce several points; once a curve drawn through these points, it is called an ROC curve (O’Mahony 1991). This curve is arch-shaped. However, if the hit rate and false-alarm rates are equal, there would be a straight line or indicating that there is no discrimination and $d'$ is zero. The area under the ROC is one measure of discrimination that does not depend on the exact forms of the signal and noise discriminations. The greater ability of the judge to distinguish the difference between the two foods (the greater $d'$), the taller the arch and the greater the area below the curve (O’Mahony 1991).
3.1.4 The R-Index Approach

The R-index is an estimated probability value of distinguishing between two samples under consideration (O’Mahony 1991). This signal detection index is an alternative measure developed to provide an index of discrimination ability, but without the stringent assumptions entailed by $d'$ – equal and normally distributed variances from signal and noise distributions. R-index is one such measure that converts rating scale performance to an index related to the percentage of area under the ROC (Receiver Operating Characteristic) curve, a measure of discrimination (Lawless and Heymann 1998). An R-index of 100% indicates that the stimulus is perfectly distinguishable conceptually from the prototypical stimulus. If the probability were at the chance level of 50%, then A and B would be perfectly indistinguishable. The higher the R-index value, the greater the degree of distinguishability (O’Mahony 1991).

R-indices have the advantage of being calculated by more than one behavioral technique: rating and ranking (O’Mahony 1986). R-index using rating is used when there are only two samples.
Conversely, when there are multiple food samples, a judge is required to rank the samples to be compared over a set of samples.

There are several advantages of this approach including (1) it is a more powerful parametric statistical analysis, especially when more than two samples are compared, (2) if a judge is considered as a measuring instrument, a large number of judges is not required, and (3) only a few sensitive/accurate judges are needed with a large sample of food tastings. However, this approach also has some disadvantages such as time consuming, more number of food samples required, and the unknown direction of difference in a specific attribute.

3.1.5 Simple Ranking Test

When more than two samples are applied to the discrimination test, ranking is an alternative to the traditional scaling. Meilgaard and others (1991) defined the ranking test as a method of measuring sensory response which the samples are arranged in order of intensity or degree of some specified attribute. Ranking tests are also simple and rapid with little training required, and many samples can be tested at once. In addition, the relative and directional magnitude of difference amid samples is provided when using this test.

3.2 Materials and Methods

3.2.1 Salt Solution Preparation

Four mixed salt solutions of KCl/NaCl/L-Arginine (A, B, C, D) and pure NaCl solution (E, the control), were prepared in the aqueous forms at the concentration of 0.5%, 1% and 1.5% w/v. Four mixed salt solutions were developed from NaCl (20-35%), KCl (55-70%), and L-arginine (10%). The ratio of L-arginine was fixed at 10% of the total mixed salts based on the preliminary study. All ingredients used in this study were food grade. NaCl (Lot No. UC3016) and L-arginine (Lot No. UT0341) purchased from Spectrum Chemical MFG. Corp (Brunswick,
NJ), and KCl (Lot No. K33160635) purchased from EMD Chemicals Inc., (Gibbstown, NJ) were used to prepare salt solutions in the filtered water at 25°C.

**Table 1: Ratio of KCl/NaCl/L-arginine in Mixed Salt Solutions**

<table>
<thead>
<tr>
<th>Sample</th>
<th>%KCl</th>
<th>%NaCl</th>
<th>%L-arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>65</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>60</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>55</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

### 3.2.2 A Simple Ranking Test

Since the purpose of this study was to evaluate the bitterness and saltiness of multiple mixed salt solutions compared to the pure NaCl one, a simple multiple ranking test was conducted. Twenty untrained panelists participated in the ranking test for the concentration of 0.5%, 1%, and 1.5% w/v solution. The panelists were randomly selected from Department of Food Science, Louisiana State University, Baton Rouge campus using the following criteria for recruitment: (1) they had to be at least 18 years of age, (2) they were not allergic to KCl, NaCl, and L-arginine, (3) and they were willing and available for participation and for the completion of the study.

Panelists were presented with five 25-ml samples which were coded according to the ratio of KCl/NaCl/L-arginine as follows: sample A (70/20/10), sample B (65/25/10), sample C (60/30/10), sample D (55/35/10) and sample E (0/100/0). Participants was also provided with room temperature filtered water and unsalted, plain crackers to cleanse their palates between samples. Each panelist ranked the 5 coded samples for saltiness and bitterness on a 5-point scale (1= most intense, 5=least intense) with no tie allowed. Six replications were performed by the same panelists for each level of concentration, i.e., 0.5%, 1%, and 1.5% w/v.
3.2.3 Statistical and Data Analysis Methods

All data were analyzed with a predetermined confidence level of 95% (\( \alpha = 0.05 \)) using the Statistical Analysis Software System, Version 9.1.3 (SAS® Institute, Cary, NC).

3.2.3.1 Friedman’s Test and the Analog to Fisher’s LSD

The ranked data of this study were obtained with the randomized block design; a Friedman-type non-parametric statistics was performed. Dealing with more than two samples, the Friedman’s test is the equivalent of the two-factor, repeated measures ANOVA for ranked data. The non-parametric Friedman test was used to check the hypotheses “the differences of saltiness and/or bitterness exist among four mixed salt solutions and NaCl solution.” The test procedure is to reject the null hypothesis of no sample differences at the \( \alpha \)-level of significance if the T in the equation below exceeds the \( \chi^2 \) distribution with \( t-1 \) degrees of freedom, while \( t \) is number of samples.

\[
T = \left\{ \frac{12}{bt(t+1)} \sum x_{j}^2 \right\} - 3b(t + 1);
\]

where \( b \) = no. of panelists, \( t \) = no. of samples, and \( \sum x_{j}^2 \) = square of the summation of rank sum. If the \( \chi^2 \)-statistic is significant, then a multiple comparison procedure is performed to determine which of the sample pairs differ significantly (Meilgaard and others 1998). The nonparametric analog to Fisher’s LSD (Least Square Difference) for rank sums from a randomized complete block design is:

\[
LSD_{\text{rank}} = z_{\alpha/2} \sqrt{\frac{bt(t+1)}{6}} = t_{\alpha/2,\infty} \sqrt{\frac{bt(t+1)}{6}};
\]

where \( t_{\alpha/2,\infty} \) is 1.96. If the rank sum difference value from 2 samples is greater than the LSD value, then the null hypothesis (i.e., the two samples are same) is rejected. In this study, the non-parametric Friedman’s test and the analog of LSD test were conducted at \( \alpha=0.05 \).
3.2.3.2 R-Index

In the rating procedure, a judge is required to familiarize with the signal (S) and the noise (N). When the difference test is performed, a judge is required to distinguish whether a randomly given sample is S or N. Differences in the level of sureness of the judgment can be provided and there is little effect on the index obtained (O’Mahony 1986). Therefore, the responses can be definitely signal (S), perhaps signal but not sure (S?), definitely noise (N), and perhaps noise but not sure (N?). In addition, the R-index using ranking could be performed when there are more than two samples to compare (Table 2). This technique allows a more powerful parametric statistical analysis. This procedure is a forced-choice test and the R-index can be computed by the formula below. Once a fractional R is provided, it is more recognized to convert it to a percentage.

Table 2: R-Index Response Format for Calculation Procedure

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S4</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td>nS4=a+b+c+d+e</td>
</tr>
<tr>
<td>S3</td>
<td>f</td>
<td>g</td>
<td>h</td>
<td>i</td>
<td>j</td>
<td>nS3=f+g+h+i+j</td>
</tr>
<tr>
<td>S2</td>
<td>k</td>
<td>l</td>
<td>m</td>
<td>n</td>
<td>o</td>
<td>nS2=k+l+m+n+o</td>
</tr>
<tr>
<td>S1</td>
<td>p</td>
<td>q</td>
<td>r</td>
<td>s</td>
<td>t</td>
<td>nS1=p+q+r+s+t</td>
</tr>
<tr>
<td>N</td>
<td>u</td>
<td>v</td>
<td>w</td>
<td>x</td>
<td>y</td>
<td>nN=u+v+w+x+y</td>
</tr>
</tbody>
</table>

\[
\text{R-Index (S_4)} = \frac{[a(v+w+x+y) + b(w+x+y) + c(x+y) + dy] + [1/2 (au+bv+cw+dx+ey)]}{(nS4)(nN)}
\]

In the R-index using a ranking response in this study, samples are presented to the judge as N and S1, S2, S3 and S4. The samples are ranked on a specified attribute which the first place is the most intense and the last place is the least intense. Once the replications are performed, a response matrix can be constructed by using the frequency of each place of each sample. It is necessary to determine whether % R-index is greater by chance (50%) at a given sample size (N) and level of significance (\(\alpha\) level) by compared with the critical values (Bi and O’Mahony 1995).
The null hypothesis of this approach is the % R-index is equal to a chance (50%). If the obtained deviation from 50% is equal to or greater than the value in the table, the null hypothesis is rejected.

3.3 Results and Discussion

3.3.1 Friedman’s Test and the Analog to Fisher’s LSD

Table 3 shows the frequency of rank data and the average rank sum values for saltiness of 0.5%, 1%, and 1.5% w/v solutions. Being stated as the null hypothesis, there were no sample differences in terms of saltiness, at the 0.05 significance level. The null hypothesis is rejected when the T calculated is greater than the critical value. The critical value $\chi^2 (\alpha=0.05, \text{df}=4)$ was equal to 9.39. Using the non-parametric Friedman’s Test, the T-values ($\alpha=0.05$, $b=20$, and $t=5$) for the rank sum of saltiness of three different concentration levels (0.5%, 1%, and 1.5% w/v) were found to have the values of 35.9, 40.1, and 38.2, respectively. Since these values were greater than the critical value, the null hypotheses were rejected. The differences of saltiness among the five samples existed at the concentration of 0.5%, 1%, and 1.5% w/v.

Likewise, the differences in bitterness among the five samples were also determined. The rank response frequency and average rank sums for bitterness of 0.5%, 1%, and 1.5% w/v solutions are presented in Table 4. The T-values based on $\alpha=0.05$, $b=20$, and $t=5$, of the concentration of 0.5%, 1%, and 1.5% were equal to 1.9, 6.2, and 3.4, respectively. It is observed that these values were less than the critical value, $\chi^2_{0.05,4}=9.39$. Therefore, at the 0.05 significance level, the five samples did not differed in terms of bitterness at the concentration of 0.5%, 1%, and 1.5% w/v.
Table 3: Rank Response Frequency and Average Rank Sums for Saltiness

<table>
<thead>
<tr>
<th>Sample</th>
<th>Response Frequency for Ranks</th>
<th>Rank Sum</th>
<th>Average Rank Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1 18 27 30 44</td>
<td>458</td>
<td>76.3</td>
</tr>
<tr>
<td>B</td>
<td>1 33 23 33 30</td>
<td>418</td>
<td>69.7</td>
</tr>
<tr>
<td>C</td>
<td>3 25 38 34 20</td>
<td>403</td>
<td>67.2</td>
</tr>
<tr>
<td>D</td>
<td>5 38 31 21 25</td>
<td>383</td>
<td>63.8</td>
</tr>
<tr>
<td>E</td>
<td>110 6 1 2 1</td>
<td>138</td>
<td>23.0</td>
</tr>
</tbody>
</table>

At the concentration of 1% w/v

<table>
<thead>
<tr>
<th>Sample</th>
<th>Response Frequency for Ranks</th>
<th>Rank Sum</th>
<th>Average Rank Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2 12 22 31 53</td>
<td>481</td>
<td>80.2</td>
</tr>
<tr>
<td>B</td>
<td>2 21 19 43 35</td>
<td>448</td>
<td>74.7</td>
</tr>
<tr>
<td>C</td>
<td>3 21 45 34 17</td>
<td>401</td>
<td>66.8</td>
</tr>
<tr>
<td>D</td>
<td>6 60 29 11 14</td>
<td>327</td>
<td>54.5</td>
</tr>
<tr>
<td>E</td>
<td>107 6 5 1 1</td>
<td>143</td>
<td>23.8</td>
</tr>
</tbody>
</table>

At the concentration of 1.5% w/v

<table>
<thead>
<tr>
<th>Sample</th>
<th>Response Frequency for Ranks</th>
<th>Rank Sum</th>
<th>Average Rank Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4 9 23 30 54</td>
<td>481</td>
<td>80.2</td>
</tr>
<tr>
<td>B</td>
<td>2 17 26 43 32</td>
<td>446</td>
<td>74.3</td>
</tr>
<tr>
<td>C</td>
<td>7 26 40 25 22</td>
<td>389</td>
<td>64.8</td>
</tr>
<tr>
<td>D</td>
<td>3 59 27 20 11</td>
<td>337</td>
<td>56.2</td>
</tr>
<tr>
<td>E</td>
<td>104 9 4 2 1</td>
<td>147</td>
<td>24.5</td>
</tr>
</tbody>
</table>

\(^{a}\text{See Table 1 for formulations}\)
\(^{b}\text{Ranks: 1 = saltiest and 5 = least salty}\)
\(^{c}\text{Rank Sum} = \Sigma(\text{rank*response frequency})\)
\(^{d}\text{Average Rank Sum} = (\text{Rank sum/6}); 6 replications were performed by 20 same panelists.\)
Table 4: Rank Response Frequency and Average Rank Sums for Bitterness

<table>
<thead>
<tr>
<th>Sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Response Frequency for Ranks&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rank Sum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Average Rank Sum&lt;sup&gt;d&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>33</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>B</td>
<td>21</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>D</td>
<td>12</td>
<td>23</td>
<td>42</td>
</tr>
<tr>
<td>E</td>
<td>38</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

At the concentration of 1% w/v

<table>
<thead>
<tr>
<th>Sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Response Frequency for Ranks&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rank Sum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Average Rank Sum&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>41</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>E</td>
<td>37</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

At the concentration of 1.5% w/v

<table>
<thead>
<tr>
<th>Sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Response Frequency for Ranks&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rank Sum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Average Rank Sum&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A</td>
<td>36</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Table 1 for formulations  
<sup>b</sup>Ranks: 1 = most bitter and 5 = least bitter  
<sup>c</sup>Rank Sum = Σ(rank*response frequency)  
<sup>d</sup>Average Rank Sum = (Rank sum/6); 6 replications were performed by 20 same panelists.

Since the \( \chi^2 \)-statistic is significant, it is necessary to determine which of the samples significantly differ. Then the nonparametric analog to Fisher’s LSD for rank sums was performed and \( \text{LSD}_{\text{rank}} \) was calculated based on \( b=20, t=5, \alpha=0.05, t_{\alpha/2,\infty}=1.96 \) which was equal to 19.6. The two-samples rank sum values exceed 19.6 were declared to be significantly different. As shown in Table 5, all of the paired rank sum differences of the NaCl solution compared with those of the mixed salt solutions at the three concentration levels were
significant, and were in the range of 30 to 56. This means that NaCl solution was significantly saltier than the mixed solutions containing KCl, NaCl, and L-arginine, regardless of the concentrations. Conversely, there were no differences in saltiness among aqueous mixed salt solutions at the concentration level of 0.5% w/v. But the pair of sample A-D was significantly different from each other at the concentration level of 1% and 1.5% w/v.

Table 5: Rank Sum Differences of Saltiness

<table>
<thead>
<tr>
<th>Samplea (Rank Sum)</th>
<th>At the concentration of 0.5% w/v</th>
<th>A (66.8)</th>
<th>B (60.2)</th>
<th>C (57.7)</th>
<th>D (54.3)</th>
<th>E (13.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (66.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (60.2)</td>
<td>6.6&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (57.7)</td>
<td>9.1&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (54.3)</td>
<td>12.5&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (13.5)</td>
<td>53.3&lt;sup&gt;S&lt;/sup&gt;</td>
<td>46.7&lt;sup&gt;S&lt;/sup&gt;</td>
<td>44.2&lt;sup&gt;S&lt;/sup&gt;</td>
<td>40.8&lt;sup&gt;S&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samplea (Rank Sum)</th>
<th>At the concentration of 1% w/v</th>
<th>A (70.7)</th>
<th>B (65.2)</th>
<th>C (57.3)</th>
<th>D (45.0)</th>
<th>E (14.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (70.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (65.2)</td>
<td>5.5&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (57.3)</td>
<td>13.4&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (45.0)</td>
<td>25.7&lt;sup&gt;S&lt;/sup&gt;</td>
<td>20.2&lt;sup&gt;S&lt;/sup&gt;</td>
<td>12.3&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (14.3)</td>
<td>56.4&lt;sup&gt;S&lt;/sup&gt;</td>
<td>50.9&lt;sup&gt;S&lt;/sup&gt;</td>
<td>43.0&lt;sup&gt;S&lt;/sup&gt;</td>
<td>30.7&lt;sup&gt;S&lt;/sup&gt;</td>
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</table>

<table>
<thead>
<tr>
<th>Samplea (Rank Sum)</th>
<th>At the concentration of 1.5% w/v</th>
<th>A (70.7)</th>
<th>B (64.8)</th>
<th>C (55.3)</th>
<th>D (46.7)</th>
<th>E (15.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (70.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (64.8)</td>
<td>5.9&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (55.3)</td>
<td>15.4&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>9.5&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D (46.7)</td>
<td>24.0&lt;sup&gt;S&lt;/sup&gt;</td>
<td>18.1&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (15.0)</td>
<td>56.0&lt;sup&gt;S&lt;/sup&gt;</td>
<td>49.8&lt;sup&gt;S&lt;/sup&gt;</td>
<td>40.3&lt;sup&gt;S&lt;/sup&gt;</td>
<td>31.7&lt;sup&gt;S&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values greater than 19.6 signify that the two samples are significantly different from each other.

**Values in parenthesis are the average rank sums for each sample based on 6 replications performed by 20 same panelists.

<sup>a</sup>See Table 1 for formulations
<sup>NS</sup>Not Significantly Different
<sup>S</sup>Significantly Different
Table 6 presents the paired rank sum differences of bitterness. As can be seen, there were no difference values greater than the critical value (19.6) at all three concentration levels. Therefore, it could be concluded that the differences in bitterness among the samples did not exist at the concentration level of 0.5%, 1%, and 1.5% w/v. It is likely that L-arginine could mask the bitterness which is associated with KCl.

**Table 6: Rank Sum Differences of Bitterness**

<table>
<thead>
<tr>
<th>Sampleb (Rank Sum)</th>
<th>At the concentration of 0.5% w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E (57.5)</td>
</tr>
<tr>
<td>E (57.5)</td>
<td></td>
</tr>
<tr>
<td>D (52.3)</td>
<td></td>
</tr>
<tr>
<td>C (50.0)</td>
<td></td>
</tr>
<tr>
<td>B (48.2)</td>
<td></td>
</tr>
<tr>
<td>A (44.5)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampleb (Rank Sum)</th>
<th>At the concentration of 1% w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E (60.2)</td>
</tr>
<tr>
<td>E (60.2)</td>
<td></td>
</tr>
<tr>
<td>D (57.0)</td>
<td></td>
</tr>
<tr>
<td>C (52.7)</td>
<td></td>
</tr>
<tr>
<td>B (41.5)</td>
<td></td>
</tr>
<tr>
<td>A (41.2)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampleb (Rank Sum)</th>
<th>At the concentration of 1.5% w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E (57.0)</td>
</tr>
<tr>
<td>E (57.0)</td>
<td></td>
</tr>
<tr>
<td>D (57.5)</td>
<td></td>
</tr>
<tr>
<td>C (49.0)</td>
<td></td>
</tr>
<tr>
<td>B (46.0)</td>
<td></td>
</tr>
<tr>
<td>A (43.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Values greater than 19.6 signify that the two samples are significantly different from each other. **Values in parenthesis are the average rank sums for each sample based on 6 replications performed by 20 same panelists.

bSee Table 1 for formulations
<sup>NS</sup>Not Significantly Different
<sup>S</sup>Significantly Different
3.3.2 R-Index

Table 7 shows R-indices of saltiness perception of all possible sample pairs at the concentrations of 0.5%, 1%, and 1.5% w/v. The R-critical value for a 2-tailed test, N=20 with \( \alpha = 0.05 \) was 29.50. It is known that NaCl solution is saltier than mixed salt solutions, so an R-index computed which is below the R-critical value will be significant. From the results, it can be observed that panelists were able to distinguish the saltiness perception of the mixed salt solutions from the NaCl solution at the concentration of 0.5%, 1%, and 1.5% w/v. These R-indices lower than the critical value of 29.50 indicates that the four mixed salt solutions are significantly less salty than the NaCl solution. This result substantiates that presented in Table 5 based on the non-parametric Friedman’s Test.

Table 7: R-Indices of Saltiness Perception for the Sample Pairs

<table>
<thead>
<tr>
<th>Samples Compared*</th>
<th>R-Index (%)** at [0.5%] w/v</th>
<th>R-Index (%)** at [1%] w/v</th>
<th>R-Index (%)** at [1.5%] w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-E</td>
<td>3.27</td>
<td>4.04</td>
<td>4.71</td>
</tr>
<tr>
<td>B-E</td>
<td>4.05</td>
<td>4.69</td>
<td>5.13</td>
</tr>
<tr>
<td>C-E</td>
<td>5.13</td>
<td>5.79</td>
<td>8.21</td>
</tr>
<tr>
<td>D-E</td>
<td>6.42</td>
<td>9.71</td>
<td>9.44</td>
</tr>
</tbody>
</table>

*See Table 1 for formulations
**Sample pairs with R-Index value below R-Critical are different from each other.
R-Critical determined for N=20, 2-tailed test, \( \alpha = 0.05 \) is 29.50 (The value below 50 was used because the signal samples had lower saltiness perception than the noise).

The results of R-indices calculation of bitterness perception at 0.5%, 1% and 1.5% w/v solutions are shown in Table 8. Since the mixed salt solutions has more bitter taste than the NaCl solution alone, then any R-index values above the critical values, 70.50, will cause the null hypothesis to be rejected. As can be seen, there were no pairs having the R-index values greater than the critical value. This means that panelists could not differentiate the bitterness perception of the mixed salt solutions from NaCl solution at the concentration of 0.5%, 1%, and 1.5% w/v.
Table 8: R-Indices of Bitterness Perception for the Sample Pairs

<table>
<thead>
<tr>
<th>Samples Compared*</th>
<th>R-Index (%)**</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at [0.5%] w/v</td>
<td>at [1%] w/v</td>
<td>at [1.5%] w/v</td>
</tr>
<tr>
<td>A-E</td>
<td>65.71</td>
<td>69.58***</td>
<td>66.21</td>
</tr>
<tr>
<td>B-E</td>
<td>64.40</td>
<td>68.50</td>
<td>64.75</td>
</tr>
<tr>
<td>C-E</td>
<td>63.04</td>
<td>64.04</td>
<td>62.46</td>
</tr>
<tr>
<td>D-E</td>
<td>62.44</td>
<td>63.17</td>
<td>59.83</td>
</tr>
</tbody>
</table>

*See Table 1 for formulations

**Sample pairs with R-Index value greater than R-Critical are different from each other.

R-Critical determined for N=20, 2-tailed test, α=0.05 is 70.50 (The value above 50 was used because the signal samples had greater bitterness perception than the noise).

***Based on 5 replications, instead of 6, due to missing data

Although Frank and Mickelsen (1969) had a comment that the saltiness of NaCl solutions can be enhanced with KCl solutions above the threshold level, the saltiness of aqueous mixed salts would mostly be associated with NaCl. Therefore, the less mixed salt solutions contain NaCl, the less salty the solutions. From the results, the bitterness of KCl seems to be masked. This is supported by the results of Ball and Meneely (1957) and Frank and Mickelsen (1969) indicating that the unpleasant flavor of KCl is masked in the mixtures of sodium-potassium chlorides. Furthermore, the bitterness perception results were also supported by those of Ogawa and others (2004) reporting that the degree of suppression reached by L-arginine and NaCl was greater than that of any of other suppressing agents.

3.4 Conclusions

Four mixed salt solutions consisting of KCl, NaCl, and L-arginine - A(70/20/10), B(65/35/10), C(60/30/10), and D(55/35/10) – were evaluated in terms of saltiness and bitterness, compared to the NaCl solution (E). It was observed that the mixed salt solutions were significantly less salty than the NaCl solution. Panelists could distinguish the saltiness perception of the four mixed salt solutions from the NaCl solution 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.
Therefore, it would be possible that L-arginine could mask the bitterness which is imparted by KCl.

With Friedman’s test and the Analog to Fisher’s LSD, it was concluded that panelists differently perceived the salty taste of the four mixed salt solutions from the NaCl solution at all three different concentrations (0.5%, 1%, and 1.5% w/v). But at the concentration of 1% w/v, there were additional 2 pairs differently perceived which were A-D and B-D. In terms of bitterness, there were no differences among all samples, regardless of the concentrations.

According to the R-index test, panelists could differentiate the saltiness of aqueous mixed salts from pure NaCl solution at 0.5%, 1%, and 1.5% w/v. For the bitterness perception, subjects could not differentiate the mixed salt solutions and NaCl solution at the three concentrations.

The sensitivities of the simple ranking test using the non-parametric Friedman’s test, and the R-index test were similar when the differences among the mixed salt samples and the NaCl sample were considered.
CHAPTER 4. SENSORY OPTIMIZATION OF SALT SUBSTITUTE CONTAINING L-ARGININE

4.1 Introduction

According to the American Heart Association, more than 65 million American adults age 20 and older suffer from hypertension that can lead to heart disease and stroke, which is, respectively, the first and the third causes of death in the United States. Most of hypertension cases can be controlled with a proper treatment. Doctors and nutritionists have broadly recommended patients to restrict sodium intake for many years. Under FDA's food labeling regulations, the Daily Reference Value (DRV) for sodium is 2,400 mg while Americans consume too much sodium, between 3,000 to 4,000 mg daily. The reduction of dietary sodium intake is a significant approach which can be used to prevent and control high blood pressure since sodium ion is related to the physiological role of regulating body fluid balance.

In recent, consumers have shown their concern and awareness toward a salt-restriction food. Sodium intake is of particular concern to 12% of American consumers, and people between the ages of 56 to 64 are the most sodium-conscious consumers (Shiman 2005). Therefore, those actions lead food manufacturers to reduce salt contents of their products and sodium intake is mostly derived from salt added from food processors. Many salt alternatives have been developed without losing major properties such as salt palatability.

Potassium chloride (KCl) is the most widely-used salt substitute since its properties are very similar to those of sodium chloride (NaCl) such as size, color, solubility, and appearance. Unfortunately, the higher molecular weight of cations (K⁺) causes weak salty flavor of KCl and imparts bitterness and metallic aftertaste when the large amount of KCl is applied. Moreover, there have been many studies showing that L-arginine is able to suppress the bitterness of various compounds. The bitterness-suppressing mechanism is explained by the presence of the
guanidinium group interacting with the sodium channel in the taste bud, and binding at the receptor site (Ogawa and others 2004).

The objectives of this study were to develop and optimize proportion of KCl, NaCl, and L-arginine that is acceptable to consumers and to identify the sensory attributes that would greatly contribute for the success of this product in terms of overall consumer acceptability.

4.2 Materials and Methods

4.2.1 Salt Solution Preparation

Eleven aqueous solutions of L-arginine and sodium-potassium chlorides mixtures were formulated at the concentration of 1% w/v in the filtered water. All ingredients used in this study were food grade. KCl (Lot No. K33160635) purchased from EMD Chemicals Inc. (Gibbstown, NJ), and NaCl (Lot No. UC3016), and L-arginine (Lot No. UT0341) purchased from Spectrum Chemical MFG. Corp (Brunswick, NJ) were used in this study.

Table 9: Eleven Formulations for Mixed Salts

<table>
<thead>
<tr>
<th>Formulation*</th>
<th>%NaCl</th>
<th>%KCl</th>
<th>%L-arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
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<tr>
<td>3</td>
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<tr>
<td>5</td>
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<td>85</td>
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<td>6</td>
<td>40</td>
<td>45</td>
<td>15</td>
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<td>7</td>
<td>85</td>
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<td>9</td>
<td>28</td>
<td>64.5</td>
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</tr>
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<td>10</td>
<td>57</td>
<td>35.5</td>
<td>7.5</td>
</tr>
<tr>
<td>11</td>
<td>92.5</td>
<td>-</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Formulation numbers correspond to the formulations numbers shown in Figure 9.
4.2.2 Mixture Experimental Design

A three component constrained simplex lattice mixture design was used (Cornell, 1983) for the experimental design. The mixture design consisted of NaCl ($X_1$), KCl ($X_2$), and L-arginine ($X_3$). According to fractions of the mixture, the three component proportions ($X_1 + X_2 + X_3$) sum to unity or 1.0 or 100%. Figure 9 and Table 9 summarize the 11 formulations used in this study. The upper and lower boundaries of the component partitions were NaCl (0%-100%), KCl (0-100%), and L-arginine (0-15%).

![Figure 9: The constrained region in the simplex coordinate system; $X_1$ = NaCl, $X_2$ = KCl, and $X_3$ = L-arginine. Numbers 1-11 represent the 11 formulations and correspond to the numbers in Table 9.]

4.2.3 Consumer Acceptance Test

Three hundred and eighty-five untrained consumers participated in the central-location consumer acceptance test. The consumers were randomly chosen from the Louisiana State University campus using the following criteria for recruitment: (1) they were at least 18 years of
age, (2) they were not allergic to NaCl, KCl, and L-arginine, and (3) they were willing and available for participation and for the completion of the survey.

Figure 10: Panelists Evaluating Mixed-Salt Solution Samples

Consumers were presented with two 25-ml samples which were coded following the Balanced Incomplete Block design Plan 11.4a \( (t = 11, k = 2, r = 10, b = 55, \lambda = 1, E = 0.55, \text{Type II}) \) (Cochran and Cox, 1957). This design was chosen because the number of samples was too large for any consumer to evaluate at one time, and for products with bitter substances, up to 2 products are to be served to a panelist (Meilgaard and others 1999), so they were simultaneously presented with 2 out of 11 sample formulations. These formulations were randomly coded with the number 1 to 11 for a total of 70 observations (replications) per formulation.

Consumers were provided with room temperature filtered water and unsalted, plain crackers to cleanse their palates between each sample. Consumers were asked to answer
demographic questions such as age and gender. Each consumer evaluated each sample for acceptability of overall taste, saltiness, bitterness, and overall liking using the 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely). Consumers were asked to rate the intensity of saltiness using the just-about-right scale (JAR) with 3 categories (too weak, just about right, and too strong). Consumers were also asked to rate the bitterness of the samples and indicated how intense of the bitterness was using the JAR scale with 3 categories. The binomial type questions (yes/no) were used to evaluate overall product acceptance, and overall product acceptance after being provided with additional health information about salt substitute.

4.2.4 Triangle Test with Replications

The triangle test was performed to compare NaCl solution and an optimum formulation (i.e., a mixture of 35% NaCl and 65% KCl) in terms of saltiness and bitterness. Ten untrained panelists participated in this discrimination test. The panelists were randomly chosen from the Louisiana State University campus using the following criteria for recruitment: (1) they were at least 18 years of age, (2) they were not allergic to NaCl, KCl, and L-arginine, and (3) they were willing and available for participation and for the completion of the survey.

Panelists were presented with three 25-ml samples which come from two products: NaCl solution and a mixture of 65% KCl and 35% of NaCl. Two of the samples were from the same product and the third was from the other. Panelists were asked to identify which was the odd sample in terms of saltiness as well as bitterness. The aqueous samples were prepared at 1% w/v and coded with 3-digit random numbers. The samples were randomly served with counter balance design. Three replications were performed, and therefore, 30 observations were collected from each attribute (i.e., saltiness and bitterness).
4.2.5 Statistical Data Analysis

All data were analyzed with a predetermined confidence level of 95% ($\alpha = 0.05$) using the Statistical Analysis Software System, Version 9.1.3, 2005 (SAS® Institute, Cary, NC).

4.2.5.1 ANOVA

The analysis of variance (ANOVA) is a statistical technique used to decide which of several effects operating simultaneously on a process are important and what their influence on the results is (Danzert, 1986). Most generally, the analysis of variance is suitable for the study of effects of qualitative factors on a quantitative measurement. The assumptions behind this technique base on the normality of the distribution of the studied variables, variance equality and independence of the errors.

ANOVA was performed in this study to determine whether differences exist among the eleven aqueous mixed salt solutions in terms of acceptability of each sensory attribute as well as overall liking of the products. If the significances among the samples were found, the Tukey’s studentized range test would be performed for post-hoc multiple comparisons.

4.2.5.2 MANOVA and DDA

The multivariate analysis of variance (MANOVA) is an extension of ANOVA methods where the basic principles are the same. MANOVA is used to determine a significant difference of the measurement values between classes. In addition, MANOVA is used in conjunction of with discriminant analysis for data analysis. After MANOVA, descriptive discrimination analysis (DDA) was used to identify the attributes which were responsible for the underlying differences among samples (Huberty 1994).
4.2.5.3 Logistic Regression

Logistic regression is a statistical regression model which is most-commonly used when a dependent variable is dichotomous (yes/no) and the independent variables are quantitative or categorical. This technique can be used to estimate probabilities of given outcomes based on predictor variables. Logistic regression parameters refer to odds and odds ratio. The odds are nonnegative, with value greater than 1.0 when a success is more likely to occur than a failure (Agresti 1996). The odds ratio is defined as the ratio of an event occurring in one group to the odds of it occurring in another group. An odds ratio farther from 1.0 in a given direction represents stronger levels of association. The odds ratio is interpreted as an increase in the odds for a unit increase in the independent variable. In this study, logistic regression analysis was used to predict the product acceptability by using the odds ratio point estimate.

4.2.5.4 McNemar Test

The McNemar test is a test of marginal homogeneity for matched binary responses in a 2 x 2 table. This method is usually used to compare categorical responses for two samples where each sample has the same subjects and the responses are statistically dependent. Methods that treat the two sets of observations as independent samples are inappropriate (Agresti 1996). In this study, the same consumers were categorized in two categories, “before” and “after” condition. The test has a chi-squared distribution with df=1 (Agresti 1996). When the marginal proportions are not homogenous, it results in the change of consumer acceptance with health benefits of the products provided.

In addition to the chi-squared value, a 95% confidence interval (CI) was calculated using marginal sample proportions \((p_{+1} - p_{1+})\), which can be used to estimate the actual differences in the means. The following equation was used to calculate the marginal sample proportions:
\[ p_{ij} = \frac{n_{ij}}{N} \]

where \( n_{ij} \) is the number of consumers making response \( i \) before and response \( j \) after the additional information about health benefits about the salt substitute was provided, and \( N \) represents the total number of consumer responses. The 95% confidence interval for the difference in proportions was calculated using the following formula:

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

where \((p_{+1} + p_{1+})\) represents the difference in proportions between the consumers who answer yes after additional information was provided \((p_{+1})\) and those who answer yes before additional information was provided \((p_{1+})\). The term \( Z_{\alpha/2} \) equals 1.96 and represents the standard normal percentile having a right-tail probability of \( \alpha/2 \). ASE is the estimated standard error for the proportion difference and was calculated using the following equation:

\[ ASE = \left\{ \left[ p_{1+} \left( 1 - p_{+1} \right) + p_{+1} \left( 1 - p_{1+} \right) - 2(p_{11}p_{22} - p_{12}p_{21}) \right] / N \right\}^{1/2} \]

where \( p_{11} \) is the proportion of consumers who would accept the product both before and after the information was provided, \( p_{22} \) is the proportion of consumers who would not accept the product both before and after the information was provided, \( p_{12} \) is the proportion of consumers who would accept the product before but not after, and \( p_{21} \) is the proportion of those who would not accept the product before but would be willing to accept afterwards.

In this study, the McNemar test was used to determine whether a significant change existed in consumer acceptance before and after additional information about health benefits of salt substitute was provided.

**4.2.5.5 Principal Component Analysis (PCA)**

Principal component (PCA), the most commonly used of all multivariate procedures, is a multivariate technique for data reduction. The two main functions of PCA are indicating
relationships among groups of variables in a data set, and showing relationships between objects (Danzert 1986). The data matrix can be visualized as describing a multi-dimensional space with one dimension for each variable, and each sample can be represented as a point in the space. When there are many variables, PCA is proposed for the analysis to reduce the dimensionality of the sample space. PCA proceeds by searching for linear combinations of variables which account for the maximum possible proportion of variance in the original data. If two or more variables are strongly correlated, then the majority of the variance in the data can be explained by drawing a new axis through the centre of the group of observations, so that the sum of squared residual distances is a minimum (Danzert 1986). The remaining proportion of variance in the data can then be explained by constructing a second new axis, orthogonal to the first. However, when the objects form an elliptical group, a principal component can be constructed which explains a large proportion of variance (Danzert 1986).

In this study, PCA was used to illustrate the relationship among sensory attributes, and the relationship between these attributes and the different formulations as illustrated in a product-attribute bi-plot.

4.2.6 Product Optimization

In this study, response surface methodology (RSM) was used in conjunction with the least squared regression analysis to determine the effects of mixed salt formulations on the consumer acceptance of each sensory attribute. Predictive models were used to generate contour plots for overall taste, saltiness, bitterness, and overall liking. The acceptable areas in the mixture triangle were those having scores of 1.0 unit less than that of the NaCl (control) sample on the contour plots. The optimal formulation was determined by superimposing acceptable areas of mixture response surface (MRS) plots.
4.2.7 Beta Binomial Test

Binomial statistics are normally used to analyze data from forced-choice difference tests on condition that all tests come from one person. In small differences between the products, it is necessary to show significance by performing many observations. When it is found that there are no enough panelists available to have the desired number of assessments, each assessor then is allowed to test repeatedly (Kunert and Meyners 1999). However, assessors vary in their sensitivity, and they are not identical. Therefore, the probability of performing the tests correctly is not constant among assessors, and this would account for extra variance which can be described by the beta distribution. The data are spread or dispersed more than expected and this is called overdispersion.

4.3 Results and Discussion

4.3.1 Demographic Information

Demographic information about consumers who participated in this study is presented in Table 10 and Table 11. As can be seen, in a great majority of consumers, the age range of 18-24 years was responsible for 83.64%. About 10% of the consumers were categorized in the 25-34 years of age and nearly 6% of the consumers were 35 years and older. When considering by gender, the proportion of women participants (52.21%) was relatively equal to the number of men (47.79%) as shown in Table 11.

Table 10: Frequency of Consumer Age

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Frequency</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>644</td>
<td>83.64</td>
<td>644</td>
<td>83.64</td>
</tr>
<tr>
<td>25-34</td>
<td>82</td>
<td>10.65</td>
<td>726</td>
<td>94.29</td>
</tr>
<tr>
<td>35-44</td>
<td>14</td>
<td>1.82</td>
<td>740</td>
<td>96.10</td>
</tr>
<tr>
<td>45-54</td>
<td>14</td>
<td>1.82</td>
<td>754</td>
<td>97.92</td>
</tr>
<tr>
<td>over 55</td>
<td>16</td>
<td>2.08</td>
<td>770</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table 11: Frequency of Consumer Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Frequency</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>402</td>
<td>52.21</td>
<td>402</td>
<td>52.21</td>
</tr>
<tr>
<td>Female</td>
<td>368</td>
<td>47.79</td>
<td>770</td>
<td>100.00</td>
</tr>
</tbody>
</table>

4.3.2 Consumer Acceptability

The mean scores and ANOVA results for the acceptability of overall taste, saltiness, bitterness, and overall liking of the eleven formulations of mixed salt solutions are shown in Table 12. It can be observed that all the sensory attributes was rated with a mean score of less than 6.0. Formulation 1 had the highest acceptance scores for overall taste (4.90), saltiness (5.01), bitterness (5.54), and overall liking (5.24). This formulation was the control sample which contained pure sodium chloride. Conversely, formulation 5 received the lowest acceptance scores for overall taste (2.70), saltiness (3.49), bitterness (3.12), and overall liking (2.97). This formulation consisted of 85% KCl and 15% L-arginine. However, formulation 3, (65% KCl and 35% NaCl) was found to have the second highest acceptance scores for overall taste (4.24), saltiness (4.51), bitterness (5.23), and overall liking (4.71). Therefore, this formulation was chosen for the triangle test to compare the difference in terms of saltiness as well as bitterness with formulation 1 (control).

4.3.3 Product Acceptability

Each of the eleven aqueous mixed salt formulations was evaluated separately using a 2-point hedonic scale (yes/no) for consumer acceptance and consumer acceptance after the consumers were informed that the product contained less sodium which negatively caused hypertension. The percent (%) of positive responses can be found in Table 13. The formulation with the highest positive responses of acceptability and acceptability with knowledge of the health effects was formulation 1, the control, which accounts for 75.71% and 72.86%,
respectively. The sample which received the second highest acceptability response was formulation 3 (65% KCl and 35% NaCl). In contrast, formulation 5 (85% KCl and 15% L-arginine) received the lowest positive percentage responses for acceptability. These results agree with the overall liking scores (Table 12) for sample 1, 3, and 5.

Table 12: Mean Consumer Acceptance Scores for Sensory Attributes and Overall liking of Eleven Aqueous Mixed Salt Solutions

<table>
<thead>
<tr>
<th>Formulation Number*</th>
<th>Mean Consumer Acceptance Score</th>
<th>Overall taste</th>
<th>Saltiness</th>
<th>Bitterness</th>
<th>Overall Liking</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.90^A</td>
<td>5.01^A</td>
<td>5.54^A</td>
<td>5.24^A</td>
<td>5.17^A</td>
</tr>
<tr>
<td></td>
<td>(1.87)</td>
<td>(1.81)</td>
<td>(1.61)</td>
<td>(1.71)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.00^ABC</td>
<td>4.09^ABCD</td>
<td>4.94^ABC</td>
<td>4.43^ABC</td>
<td>4.19^ABC</td>
</tr>
<tr>
<td></td>
<td>(1.87)</td>
<td>(1.78)</td>
<td>(2.05)</td>
<td>(1.91)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.24^ABC</td>
<td>4.51^ABC</td>
<td>5.23^AB</td>
<td>4.71^AB</td>
<td>4.18^AB</td>
</tr>
<tr>
<td></td>
<td>(1.88)</td>
<td>(1.78)</td>
<td>(1.84)</td>
<td>(1.84)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.27^CDE</td>
<td>3.76^BCD</td>
<td>3.67^CD</td>
<td>3.54^CDE</td>
<td>3.16^CDE</td>
</tr>
<tr>
<td></td>
<td>(1.40)</td>
<td>(1.52)</td>
<td>(1.80)</td>
<td>(1.60)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.70^E</td>
<td>3.49^D</td>
<td>3.12^D</td>
<td>2.97^E</td>
<td>2.68^E</td>
</tr>
<tr>
<td></td>
<td>(1.61)</td>
<td>(1.89)</td>
<td>(1.84)</td>
<td>(1.68)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.81^BCD</td>
<td>4.20^ABCD</td>
<td>3.74^D</td>
<td>4.07^BCD</td>
<td>3.65^BCD</td>
</tr>
<tr>
<td></td>
<td>(1.81)</td>
<td>(1.86)</td>
<td>(1.91)</td>
<td>(1.90)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.90^BC</td>
<td>4.04^ABCD</td>
<td>3.59^CD</td>
<td>3.96^BCD</td>
<td>3.56^BCD</td>
</tr>
<tr>
<td></td>
<td>(1.95)</td>
<td>(2.15)</td>
<td>(1.90)</td>
<td>(2.01)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.88^DE</td>
<td>3.53^CD</td>
<td>3.13^D</td>
<td>3.09^DE</td>
<td>2.70^DE</td>
</tr>
<tr>
<td></td>
<td>(1.34)</td>
<td>(1.39)</td>
<td>(1.57)</td>
<td>(1.46)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3.77^BCD</td>
<td>4.00^ABCD</td>
<td>3.34^D</td>
<td>3.93^BCD</td>
<td>3.54^BCD</td>
</tr>
<tr>
<td></td>
<td>(1.75)</td>
<td>(1.92)</td>
<td>(1.60)</td>
<td>(1.76)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.36^AB</td>
<td>4.63^A</td>
<td>4.00^BCD</td>
<td>4.49^ABC</td>
<td>4.21^ABC</td>
</tr>
<tr>
<td></td>
<td>(2.13)</td>
<td>(2.17)</td>
<td>(2.21)</td>
<td>(2.21)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3.84^BCD</td>
<td>4.17^ABCD</td>
<td>4.16^ABCD</td>
<td>4.29^ABC</td>
<td>3.81^ABC</td>
</tr>
<tr>
<td></td>
<td>(1.87)</td>
<td>(2.05)</td>
<td>(2.05)</td>
<td>(2.01)</td>
<td></td>
</tr>
</tbody>
</table>

Sample formulations are specified in Table 9. Data are represented as mean and standard deviation values and all values are based on a nine-point hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. Mean scores with the same letter within the same column are not significantly different (p>0.05).

**Numbers in parenthesis represent standard deviation of 70 consumer responses.
Furthermore, formulation 2, containing 65% NaCl and 35% KCl, rated the second highest score (71.43%), while formulation 8, containing 92.5% KCl and 7.5% L-arginine, received the lowest score for acceptance with knowledge of health effects. Therefore, it is likely that when consumers were informed of the health benefits associated with the consumption of a product containing less sodium, they were more willing to accept the product.

Table 13: Positive (Yes) Responses for Product Acceptability of Mixed Salt Solutions

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Acceptability</th>
<th>Acceptability w/ Knowledge of Health Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75.71%</td>
<td>72.86%</td>
</tr>
<tr>
<td>2</td>
<td>64.29%</td>
<td>71.43%</td>
</tr>
<tr>
<td>3</td>
<td>71.43%</td>
<td>68.57%</td>
</tr>
<tr>
<td>4</td>
<td>34.78%</td>
<td>42.03%</td>
</tr>
<tr>
<td>5</td>
<td>30.00%</td>
<td>44.29%</td>
</tr>
<tr>
<td>6</td>
<td>57.14%</td>
<td>67.14%</td>
</tr>
<tr>
<td>7</td>
<td>55.88%</td>
<td>57.35%</td>
</tr>
<tr>
<td>8</td>
<td>30.88%</td>
<td>33.82%</td>
</tr>
<tr>
<td>9</td>
<td>60.00%</td>
<td>68.57%</td>
</tr>
<tr>
<td>10</td>
<td>61.43%</td>
<td>67.14%</td>
</tr>
<tr>
<td>11</td>
<td>64.29%</td>
<td>68.57%</td>
</tr>
</tbody>
</table>

Each formulation was evaluated 70 times.
Sample formulations are specified in Table 9.

4.3.4 Overall Product Differences

The multivariate analysis of variance (MANOVA) was done in order to determine whether the eleven formulations differed considering all the sensory attributes simultaneously. The Wilks’ Lambda p-value of <0.0001 (Table 14) indicates that an overall difference existed among all eleven formulations when all four attributes were considered at the same time. Therefore, descriptive discriminant analysis (DDA) was used to determine attributes underlying differences among the eleven formulations.
According to the pooled within canonical structure in the first dimension (Can 1), it could be concluded that bitterness (canonical correlation = 0.946) and overall liking (0.756), respectively, are the attributes which significantly contributed to overall differences among the eleven mixed salt formulations. In accordance to the second dimension (Can 2), saltiness (0.611) contributed to the overall differences among the eleven formulations (Table 15).

**Table 15: Canonical Structure r’s Describing Group Differences among Mixed Salt Solutions (Based on Pooled Within-Group Variances)**

<table>
<thead>
<tr>
<th>Sensory Attribute</th>
<th>Can 1**</th>
<th>Can 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>0.6230</td>
<td>-0.03</td>
</tr>
<tr>
<td>Saltiness</td>
<td>0.376</td>
<td>0.611</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.946*</td>
<td>0.271</td>
</tr>
<tr>
<td>Overall Liking</td>
<td>0.756*</td>
<td>0.243</td>
</tr>
<tr>
<td>Cum. Variance Explained</td>
<td>73.03%</td>
<td>86.22%</td>
</tr>
</tbody>
</table>

*Indicates sensory attributes which largely account for group differences in first dimension  
**Can = Canonical Structure, Pooled within canonical structure in the first (Can 1) and second (Can 2) dimension.

**4.3.5 Logistic Regression Analysis and Predictive Discriminant Analysis (PDA) for Product Acceptability**

Logistic regression analysis was used in order to correlate the 2-point consumer acceptance scores with the 9-point hedonic scale scores. Table 16 shows the predictive models
that were used to predict consumer acceptability before and after additional information about health benefits of salt substitute was given to the consumers. Both of prediction models were obtained from the intercept and point estimates for each sensory attribute through logistic regression analysis.

Table 16: Full Logistic Regression Models for Predicting Acceptability and Purchase Decisions

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Predictive Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptability</td>
<td>$y = -6.7625 - 0.201(\text{Taste}) + 0.3142(\text{Saltiness}) + 0.4453(\text{Bitterness}) + 1.1219(\text{Overall liking})$</td>
</tr>
<tr>
<td>Acceptability w/ Health benefits</td>
<td>$y = -3.2990 - 0.1248(\text{Taste}) - 0.00034(\text{Saltiness}) + 0.3166(\text{Bitterness}) + 0.7097(\text{Overall liking})$</td>
</tr>
</tbody>
</table>

Based on the logistic regression analysis for consumer acceptance of the salt substitute product, the most influential sensory attributes were determined based on a Wald’s $Pr > \chi^2$ less than $\alpha = 0.05$. Overall liking was the most critical sensory attribute for product acceptability with an odds ratio point estimate of 3.952 (Table 17). Therefore, for every one point increase in overall liking on the 9-point hedonic scale, overall product acceptance will be increased by 295.2%. Subsequently, bitterness and saltiness are the next two important attributes with odds ratio of 2.314 and 2.665, respectively. This means that for every one point increase in bitterness and saltiness scores on the 9-point hedonic scale, overall product acceptance will be increased by 131.4% and 166.5%, respectively. Predictive discriminant analysis (PDA) was used to predict product acceptability. Using PDA (Table 18), product acceptance can be predicted with 83.5% and 80% accuracy based on overall liking and bitterness, respectively. Moreover, with the four-predictor variables (a full model), it could be predicted product acceptability correctly with 83.59%.
Odds ratio estimates were also estimated for consumer acceptance of the salt substitute product with knowledge of health benefits from a less-sodium salt substitute product (Table 17). Overall liking and bitterness, respectively, were found to be the most influential sensory attributes, with the odds ratios of 2.253 and 1.821. Therefore, when the knowledge of product health benefits was informed, product acceptance will increase by 125.3% and 82.1% for every one point increase in overall liking and bitterness scores, respectively, on the 9-point hedonic scale. Using PDA, product acceptance with knowledge of health benefits can be predicted with 77% and 72% accuracy based on overall liking and bitterness, respectively. With all four-predictor variables (a full model), product acceptance with knowledge of health benefits could be predicted with 71.35% accuracy.

Table 17: Probability >χ² and Odds Ratio Point Estimates for Consumer Acceptance of Salt Substitutes

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Pr &gt; χ² (full)</th>
<th>Odds Ratio Estimate (full)</th>
<th>Odds Ratio Estimate (single)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>0.3063</td>
<td>0.818</td>
<td>3.043</td>
</tr>
<tr>
<td>Saltiness</td>
<td>0.0283</td>
<td>1.369</td>
<td>2.665</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.0001</td>
<td>1.561</td>
<td>2.314</td>
</tr>
<tr>
<td>Overall Liking</td>
<td>&lt;.0001</td>
<td>3.071</td>
<td>3.952</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Pr &gt; χ² (full)</th>
<th>Odds Ratio Estimate (full)</th>
<th>Odds Ratio Estimate (single)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>0.4329</td>
<td>0.883</td>
<td>2.041</td>
</tr>
<tr>
<td>Saltiness</td>
<td>0.9977</td>
<td>1.000</td>
<td>1.808</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.0006</td>
<td>1.372</td>
<td>1.821</td>
</tr>
<tr>
<td>Overall Liking</td>
<td>&lt;.0001</td>
<td>2.033</td>
<td>2.253</td>
</tr>
</tbody>
</table>

*Probability values < 0.05 determine which attributes are significant.

**Odds Ratios predict the increase in acceptability and purchase intent due to a one-unit increase in the 9-point hedonic scale.
Table 18: Hit Rate (%) for Product Acceptability

<table>
<thead>
<tr>
<th>Attribute</th>
<th>% Hit Rate</th>
<th>Acceptability</th>
<th>Acceptability w/ Health Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Model (4 variables)</td>
<td>83.59</td>
<td></td>
<td>71.35</td>
</tr>
<tr>
<td>Taste</td>
<td>78.98</td>
<td></td>
<td>71.28</td>
</tr>
<tr>
<td>Saltiness</td>
<td>73.46</td>
<td></td>
<td>71.90</td>
</tr>
<tr>
<td>Bitterness</td>
<td>80.05</td>
<td></td>
<td>72.02</td>
</tr>
<tr>
<td>Overall Liking</td>
<td>83.53</td>
<td></td>
<td>76.86</td>
</tr>
</tbody>
</table>

*Percent hit rate refers to the accuracy with which each of the attributes can be used to predict for the product acceptability.

4.3.6 Change in Probability of Product Acceptability Using the McNemar Test

The McNemar test was performed in order to evaluate the change in the probability of product acceptance of consumers before and after the additional information about health benefits that could be associated with salt substitute consumption (Table 19).

Table 19: Changes in Product Acceptability Probability after Knowledge of the Potential Health Benefits Associated with Product Consumption

<table>
<thead>
<tr>
<th>Formulation</th>
<th>χ²</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.400</td>
<td>0.5271</td>
<td>0.417</td>
</tr>
<tr>
<td>2</td>
<td>1.923</td>
<td>0.1655</td>
<td>0.374</td>
</tr>
<tr>
<td>3</td>
<td>0.286</td>
<td>0.5930</td>
<td>0.307</td>
</tr>
<tr>
<td>4</td>
<td>1.667</td>
<td>0.1967</td>
<td>0.342</td>
</tr>
<tr>
<td>5</td>
<td>7.143</td>
<td>0.0075</td>
<td>0.394</td>
</tr>
<tr>
<td>6</td>
<td>3.267</td>
<td>0.0707</td>
<td>0.353</td>
</tr>
<tr>
<td>7</td>
<td>0.111</td>
<td>0.7389</td>
<td>0.567</td>
</tr>
<tr>
<td>8</td>
<td>0.400</td>
<td>0.5271</td>
<td>0.474</td>
</tr>
<tr>
<td>9</td>
<td>3.000</td>
<td>0.0833</td>
<td>0.443</td>
</tr>
<tr>
<td>10</td>
<td>2.000</td>
<td>0.1573</td>
<td>0.592</td>
</tr>
<tr>
<td>11</td>
<td>0.692</td>
<td>0.4054</td>
<td>0.384</td>
</tr>
</tbody>
</table>

*aAll probabilities calculated by means of the McNemar Test.  
bSample formulations are given in Table 9.

The null hypothesis being tested states that the product acceptance probability is the same before ($\pi_{1+}$) and after ($\pi_{+1}$) the consumer were given the additional information related to health.
benefits of the product or saying that \( \pi_{1+} = \pi_{+1} \). In other words, there is no change in product acceptance after additional information about product is given.

According to the results of the McNemar test, the probability of product acceptability of the salt substitute product after consumers were informed of health benefits of salt substitute is not significant at \( \alpha = 0.05 \) for all 11 formulations with the exception of formulation 5 (p-value = 0.0075) which contained 92.5% KCl and 7.5% L-arginine. This means that for formulation 5, there was an increase product acceptance after additional information was given. It can be predicted with 95% confidence that the probability of consumer acceptability will be increased at least by the value stated by the lower confidence limit and at most by the value stated by the upper limit confidence interval (Table 19). Hence, there will be a consumer acceptability increase of at least by 39.4% and at the most by 76.8% after the information of the product containing less sodium is given. On the whole, product acceptance was not affected by the additional health benefit information provided. This means that the consumer acceptance did not depend on the potential health benefits promoted by the salt substitute containing less sodium except formulation 5. However, the consumers considered overall liking and, particularly, saltiness, as less critical to product acceptance after they had been informed of health benefits of salt substitutes (Table 17).

4.3.7 Principal Component Analysis

The bi-plot expressing the results for principal component analysis was constructed to show the relative positions of the formulations and sensory attributes. Figure 11 shows the results in which four sensory attribute variables (overall taste, saltiness, bitterness, and overall liking) are displayed in the plot of the first two principal components of the product preference data. The end points for the attribute vectors are obtained by projecting the attribute
variables into the product space. Orthogonal projections of the product formulation points on an attribute vector give an approximate ordering of the formulations on the attribute rating.

**Figure 11**: Principal Components Analysis Using a Bi-Plot of Sensory Attributes and products

The taste and the saltiness vectors point straight to the same direction and are about to be overlapped, this means that these two attributes are strongly correlated as well as are correlated to overall liking. Conversely, the taste and the saltiness vector are almost perpendicular with the bitterness vector, meaning that the overall taste and saltiness attributes are not significantly correlated to bitterness, while overall liking is more correlated to bitterness. Moreover, it can be seen that bitterness is the discriminating attribute for the salt substitute products. This result
agrees with the attribute which was found to be responsible for the underlying differences among samples from descriptive discrimination analysis.

According to the relationships between formulations and sensory attributes, it can be seen that formulation 1, 3 and 2 are correlated to bitterness. These formulations ranked the first three highest mean ratings for all sensory attributes and also received comparable acceptability scores: 75.71, 71.43, and 64.29, respectively. On the other hand, formulation 4, 5, and 8 were the least accepted among the 11 formulations. These three formulations (5, 8, and 4) contain no NaCl and are high in KCl and L-arginine with 85:15, 92.5:7.5, and 100:0, respectively. The acceptability scores for formulations 5, 8, and 4, were also comparable: 30.00 (5), 30.88 (8), and 34.78 (4). This means that the combination of KCl and L-arginine would be less accepted when the formulation contained higher L-arginine.

Overall taste, saltiness, and overall liking are found to be correlated to formulation 10 and 11 regarding the same quadrant. These two formulations contain the same amount of 7.5% L-arginine. But formulation 10 contains 57% NaCl and 35.5% KCl, and formulation 11 contains 92.5% NaCl. The acceptability scores of both formulations were comparable: 64.29 (11), and 61.43 (10). Formulation 6, 7, and 9 were not significantly related to any attributes. However, among four sensory attributes, these three formulations received high mean ratings for saltiness and low ratings for bitterness. In addition, their acceptability scores were also comparable: 60 (9), 57.14 (6), and 55.88 (7).

4.3.8 Product Optimization

Product optimization was performed using the three-component mixture design experiment in conjunction with the logistic regression. The predictive models obtained using a restricted regression analysis, without an intercept, are presented in Table 20.
Table 20: Parameter Estimates for Variables Used in the Prediction Models for Consumer Acceptance

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Prediction Model*</th>
<th>Adjusted R-Square**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>3.98<em>x1 + 2.90</em>x2 + 32.58<em>x3 + 0.87</em>(x1<em>x2) - 40.18</em>(x1<em>x3) - 36.80</em>(x2*x3)</td>
<td>0.7951</td>
</tr>
<tr>
<td>Saltiness</td>
<td>4.23<em>x1 + 3.59</em>x2 + 33.66<em>x3 + 0.03</em>(x1<em>x2) - 42.72</em>(x1<em>x3) - 36.93</em>(x2*x3)</td>
<td>0.8090</td>
</tr>
<tr>
<td>Bitterness</td>
<td>5.48<em>x1 + 3.76</em>x2 + 63.70<em>x3 + 1.64</em>(x1<em>x2) - 83.75</em>(x1<em>x3) - 75.27</em>(x2*x3)</td>
<td>0.8252</td>
</tr>
<tr>
<td>Overall Liking</td>
<td>4.62<em>x1 + 3.23</em>x2 + 34.24<em>x3 + 0.45</em>(x1<em>x2) - 46.42</em>(x1<em>x3) - 39.74</em>(x2*x3)</td>
<td>0.8093</td>
</tr>
</tbody>
</table>

*Calculation of parameter estimates based on raw data with no intercept option.

**Calculation of adjusted R-square values is based on reduced regression models for each attribute.

These models were used to plot the mixture response surface (MRS) for each of the sensory attributes as shown in Figure 12. The optimal formulation was determined by superimposing acceptable areas of all of the sensory attributes significant to consumer acceptance, as determined by logistic regression analysis. Since there was no attribute containing a mean score of more than 6.0, superimposition was determined by those mean scores which were 1.0 unit less than that of NaCl (control) sample for all sensory attributes.

The probability greater than chi-square (Pr > χ²) was used in order to determine the critical sensory attributes. If Pr > χ² was less than 0.05, the attribute was then considered significant in terms of consumer acceptance. The Pr > χ² for each sensory attribute is presented in Table 19. Saltiness, bitterness, and overall liking are significant attributes for consumer acceptance. Likewise, when the consumers were given the additional information about product health benefits, bitterness and overall liking are significant. Therefore, the MRS of saltiness, bitterness, and overall liking were used to determine the optimal formulations. The superimposition of the MRS plots as shown in Figure 13 indicates that any formulations with 56-100% NaCl, 0-44% KCl, and 0-5% L-arginine would yield an acceptable product.
Figure 12: Response Surface Methodology (RSM) for Consumer Acceptability of Taste, Saltiness, Bitterness, and Overall liking
4.3.9 Triangle Test with Replications

In this study, each assessor evaluated in triplicate the difference between two samples (formulation 1 and 3) for saltiness and bitterness. Therefore, the triangle test with the beta-binomial model was performed. The difference among two samples exists when the number of correct responses is greater than the critical value.

The minimum number of correct responses ($k = 10$, $n = 3$, and $\gamma = 0.2$) is 17. Panelists detected differences between formulation 1 and formulation 3 with the correct responses of 22 for both attributes. Therefore, the panelists could differentiate the saltiness and bitterness perception between the control (100% NaCl) and formulation 3 (35% NaCl, 65% KCl).
4.4 Conclusions

From this study, the critical sensory attributes driving consumer acceptance of aqueous mixed salt substitutes were recognized. ANOVA and MANOVA results showed significant differences in all four attributes: overall taste, saltiness, bitterness, and overall liking, among the 11 formulations. DDA indicated that bitterness and overall liking were the attributes accountable for the underlying differences among the formulations. LRA showed that overall liking and bitterness were found to be the most two influential attributes in determining consumer acceptance before and after additional information regarding health benefits were informed. The odds ratio point estimate decreased in the presence of health claim, so this claim affected the likelihood of acceptance. In addition, saltiness was no longer critical to product acceptance after consumers were informed of the health benefits of salt substitute. Based on percent hit rate, the product acceptability of a new formulation can be predicted with 83.59% accuracy, and 71.35% accuracy knowing the health benefits of the less-sodium product. PCA using a bi-plot indicated that formulation 5, 8, and 4 were significantly different from all other samples with the lowest acceptance, and had imparted bitter taste which is the most discriminating attribute for the salt substitute. These three formulations contained high L-arginine and KCl, and contained no NaCl. Finally, the superimposition of the MRS plots of saltiness, bitterness, and overall liking revealed the optimal formulation at 56-100% NaCl, 0-44% KCl, and 0-5% L-arginine. The saltiness and bitterness of formulation 3, 65% KCl and 35% NaCl, was significantly different from those of formulation 1 (NaCl) when the triangle test with the beta-binomial model was performed. This study revealed that bitterness and overall liking are the most discriminating sensory attributes, and overall liking is the best produce acceptance predictor. The presence of a health claim did not significantly affect the increase of product acceptability.
CHAPTER 5. SUMMARY AND CONCLUSIONS

A discriminative test by ranking was performed to determine the differences in saltiness and bitterness among NaCl solution and mixed salt solutions containing KCl, NaCl, and L-arginine at 0.5%, 1.0%, and 1.5% w/v concentration. Panelists were able to differentiate the aqueous mixed salts from the NaCl solution when the term of saltiness was considered. More differences in saltiness of samples were found at the concentration of 1% and 1.5% w/v, with few additional significant pairs, A-D and B-D. However, no significant difference in bitterness perception was found between the mixed salt solutions and NaCl solution at three concentration levels when using the non-parametric Friedman’s test and the R-index test. Therefore, it is possible that the presence of L-arginine in the salt substitutes would result in the bitterness-masking effect. The simple ranking test using the non-parametric Friedman’s test and the R-index test detected differences with similar sensitivity when the differences among the mixed salt solutions and the NaCl solution were considered.

The consumer acceptance study was performed to determine sensory attributes driving acceptance and to optimize the formulation of salt substitute containing L-arginine. Eleven formulations of salt substitutes were developed from NaCl (0-100%), KCl (0-100%), and L-arginine (0-15%) following the mixture design. In accordance with the balanced incomplete block design, each consumer (n=385) evaluated 2 of 11 samples for acceptability of overall taste, saltiness, bitterness, and overall liking using a 9-point hedonic scale. This design allowed each formulation to be tested 70 times. Bitterness detection, overall product acceptability before and after being provided with additional health information about salt substitute were evaluated using a binomial (yes/no) scale. The intensity of saltiness was also evaluated using the just-about-right scale (JAR) with 3 categories as well as the intensity of bitterness (if detected). The predictive...
models were obtained using a restricted regression analysis without an intercept.

Superimposition was determined by those mean scores being 1.0 unit less than that of the NaCl (control) sample for all sensory attributes critical to consumer acceptance. Consumers preferred formulation 1 and 3 with the NaCl:KCl:L-arginine ratio of 100:0:0, 35:65:0. The overall liking scores of these two formulations were 5.24 and 4.71, respectively. Both formulations also received the first two highest acceptability score, 75.71% and 71.43%, respectively. With a Wilks’ Lambda p-value of 0.0001, it was concluded that all eleven formulations were different when all four sensory attributes was simultaneously considered. Bitterness was the important attribute responsible for the difference among the eleven formulations. For product acceptability, overall liking and bitterness were the most critical attributes for consumer acceptance. After the consumers had been informed of health benefits associated with less-sodium product, they considered overall liking and, particularly, saltiness, as less critical to product acceptance; and there was only formulation 5 (85% KCl, 15% L-arginine) having a significant change in consumer acceptance.

Superimposition of the optimal response surface areas of saltiness, bitterness, and overall liking indicated that any formulations containing 56-100% NaCl, 0-44% KCl, and 0-5% L-arginine, will yield an acceptable product. Formulation 3 was chosen to be further analyzed for the triangle test. Consumers were able to discriminate the saltiness and the bitterness between the pure salt solution (NaCl) and 35% NaCl, 65% KCl.

A salt substitute containing L-arginine has not been commercially developed. Overall liking was identified as a critical attribute as well as the bitterness of a salt substitute for further formulation improvement. L-arginine could not completely mask the bitterness in the salt-substitute solution. However, development of the proportion of KCl/NaCl/L-arginine obtained
from mixture design, and the application of salt substitute in foods would be worth further study. Moreover, the heat and cold stability of L-arginine in salt substitutes should be investigated.
REFERENCES


APPENDIX A: STUDY 1
a. R-Index Form

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  5 = Least salty
3) No ties please!

Rank Responses

<table>
<thead>
<tr>
<th>Date: _____</th>
<th>1st saltiest</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th least salty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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!

Rank Responses

<table>
<thead>
<tr>
<th>Date: _____</th>
<th>1st most bitter</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th least bitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
b. SAS Code: Wilcoxon Rank Sum Test/Kruskal-Wallis H Test

```sas
data s1;
input Panelist $ First $ Second $ Third $ Fourth $ Fifth $;
datalines;
data saltiness1;
drop First Second Third Fourth Fifth;
set s1;
Rank=1;
Sample=First;
Output;
Rank=2;
Sample=Second;
Output;
Rank=3;
Sample=Third;
Output;
Rank=4;
Sample=Fourth;
Output;
Rank=5;
Sample=Fifth;
Output;
proc sort data=saltiness1;
by panelist sample;
run;
proc npar1way wilcoxon;
class sample;
var rank;
run;
```
APPENDIX B: STUDY 2

a. Research Consent Form

I, _____________________, agree to participate in the research entitled “Optimization and Characterization of Sensory Qualities of a Prototype Salt Substitute Product,” 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 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: _________________________
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 salt solution?

   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 salt solution?

   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 salt solution?

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

6. Do you detect BITTERNESS in this salt solution?

   YES [ ]  NO [ ]

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

   If NO, skip Question #7

7. Is the AFTERTASTE/BITTERNESS of this salt solution 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 salt solution?

   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 salt solution ACCEPTABLE?

   YES [ ]  NO [ ]

10. Is this salt solution ACCEPTABLE knowing that it contains less sodium, which negatively causes high blood pressure?

    YES [ ]  NO [ ]
c. SAS Code: ANOVA, MANOVA, DDA, LRA, and PCA

```
data one;
input panel age gender sample $ X1 X2 X3 taste saltiness JARSalt Bitteryes JARBitter Bitterness Oliking accept accepthealth;
X2(KCl) X3(Arg)/*;
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;
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 accepthealth;
var taste saltiness Bitterness Oliking;
proc discrim crossvalidate pool=test posterr;
class accepthealth;
var taste;
proc discrim crossvalidate pool=test posterr;
class accepthealth;
var saltiness;
proc discrim crossvalidate pool=test posterr;
class accepthealth;
var Bitterness;
proc discrim crossvalidate pool=test posterr;
class accepthealth;
```
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 princomp out = prin;
var taste saltiness Bitterness Oliking;
proc plot;
plot prin2*prin1 = sample;
plot prin2*prin3 = sample;
plot prin3*prin1 = sample;
proc sort; by sample;
proc print; by sample;
var prin1 prin2 prin3;
proc means; by sample;
var prin1 prin2 prin3;
run;

d. SAS Code: McNemar

data one;
input sample $ accept accepthealth Count;
datalines;
proc freq; weight Count;
tables accept*accepthealth/agree;
by sample;
run;

e. SAS Code: Regression Analysis

data one;
input panel age gender sample $ X1 X2 X3 X4 taste saltiness JARSalt Bitteryes JARbitter Bitterness Oliking accept accepthealth;
*x1=NaCl, 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;

f. SAS Code: RSM (sample)

Data taste;
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;
       taste = 0;
       IF (0 LE X1 LE 1) and (0 LE X2 Le 1) and
           (0 LE X3 LE .15) then DO;
       taste= 3.98374*X1+2.90094*X2+32.58467*X3+0.86514*(X1*X2)-
           40.18247*(X1*X3)-36.80429*(x2*x3);
       END;
   OUTPUT;
END;
END;
Run;

Title taste;
Proc Plot;
Plot V1*V2 = taste/ VPOS = 40 HPOS = 60 Contour = 10;
Run;

g. SAS Code: PCA Using Bi-Plot

%Include "biplot.sas";
%Include "equate.sas";
Data one;
Input sample $ taste saltiness bitterness oliking;
datalines;
ODS exclude SimpleStatistics Cov TotalVariance;
Proc princomp data=one cov out=comp1;
  var taste--oliking;
  run;
%Biplot (Data=one,var=taste saltiness bitterness oliking, Id=sample, factype=sym, colors=black blue, symbols=dot none);
quit;
VITA

Pamarin Waimaleongora-Ek was born on October 9th, 1981 in Bangkok, Thailand. In February 2002 she graduated from Thammasat University with a bachelor of science in food science and technology. After receiving her bachelor’s degree, she worked for Seafoods Enterprise Company, Limited, as a research and development supervisor for two and a half years before joining the master’s program in food science at Louisiana State University in 2005. She is now a candidate for the degree of Master of Science from the Department of Food Science at Louisiana State University and Agricultural and Mechanical College, which will be awarded in December 2006.