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# Development and Sensory Characteristics of Salt Substitute Containing Bitterness-Masking Agents

Pamarin Waimaleongora-Ek
*Louisiana State University and Agricultural and Mechanical College*

# DEVELOPMENT AND SENSORY CHARACTERISTICS OF SALT SUBSTITUTE CONTAINING BITTERNESS-MASKING AGENTS

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Pamarin Waimaleongora-Ek
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M.S., Louisiana State University, 2006
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*To my family*

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**ABSTRACT**

This study was conducted to develop a sodium-free salt substitute and assess its sensory characteristics. The efficacy of bitterness-masking agents, adenosine-5'-monophosphate (AMP) and L-arginine (Arg), were studied. However, the effectiveness of AMP in inhibiting bitterness of potassium chloride (KCl) was specifically investigated. The threshold values of KCl with and without AMP were determined using the method of limits and the signal detection method. AMP effectively decreased the bitterness imparted by KCl at a ratio of KCl to AMP of 15:1 when the method of limits was utilized.

To enhance saltiness and inhibit bitterness, Arg was added to KCl/AMP solutions. The optimal ratio of Arg in KCl/AMP at 0.3% was determined using a ranking test. Two methods of data analysis, the Friedman's and the *R*-index ranking tests, provided similar results. The ratio of KCl/Arg/AMP of 15:2:1 was the best proportion among the salt mixtures containing Arg. This proportion was selected to study its sensory characteristics. Using the Spectrum Descriptive Analysis, the sensory characteristics of different salt substitutes were evaluated in triplicate at 0.5% and 1% w/v. Arg and AMP had a synergistic effect in enhancing saltiness in KCl solutions at 0.5% and in inhibiting bitterness at 1.0%. Therefore, the taste qualities of KCl/Arg/AMP (15:2:1) were better than those of KCl.

A consumer study was performed to determine the acceptability of pasta sauces containing different salt substitutes and determine sensory attributes driving acceptance and purchase intent. Consumers evaluated the products following a balanced incomplete block design augmented with control in every treatment. Results indicated that the sauce containing NaCl was the most accepted and vice versa for KCl. Overall liking scores affected product acceptance and purchase intent of all samples except the one containing the commercial salt substitute. The samples that contained salt substitutes were perceived to be bitter and not salty enough which resulted in mean drops of overall liking scores. Consumers' number of positive responses for acceptance and purchase intent of KCl/Arg/AMP (15:2:1) was comparable to those of the commercial salt substitute used in this study. In conclusion, the findings revealed that KCl/Arg/AMP could be a commercial salt substitute.

# CHAPTER 1.
# INTRODUCTION

### 1.1 Introduction

About 74.5 million people in the United States age 20 and older have high blood pressure which is a major risk factor for stroke, congestive heart failure, and heart and kidney diseases. Additionally, high blood pressure was reported as a cause of death for 56,561 people in the United States in 2006. Furthermore, the American Heart Association (2006) reported that the death rate from high blood pressure increased 19.5% from 1996 to 2006, and the actual number of deaths rose by 48.1%. Moreover, Americans spend more than $15 billion annually on medications for hypertension (AHA 2006). The risk factors contributing to the development of hypertension are age, ethnicity, heredity, and dietary factors including excessive sodium intake. The 2005 Dietary Guidelines for Americans recommend limiting sodium to less than 2,400 mg per day but the average daily sodium intake for Americans age 2 years and older is 3,436 mg (CDC 2009).

In November 2007, the U.S. Food and Drug Administration (FDA) held a public hearing regarding sodium in food. The topics included reducing and regulating the sodium amount in food. Moreover, salt also remains under consideration for removal from the Generally Recognized As Safe (GRAS) list. The FDA actions will have an impact on food manufacturers and restaurants. The common sources of sodium are found in the food supply. Moreover, 75% of 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 (Mattes and Donelly 1991). Reducing sodium dietary intake by using a salt substitute is a common way that people without kidney disease can use to restrict their salt intake and decrease the risk for high blood pressure. Furthermore, consuming a potassium-rich diet will help lower the risk of stroke and hypertension (Skrabal and others 1981; NHLBI 2006).

Potassium chloride (KCl) is potentially a sodium-free alternative to salt and a common ingredient in salt substitutes. The appearance of sodium chloride and potassium chloride is indistinguishable since both salts are colorless, transparent cubic crystals with similar refractive indices and even similar in particle sizes. Therefore, potassium chloride is not only a good compound for supplementing sodium chloride, but its physical properties make it technically an ideal substance for an ingredient with ordinary salt (Frank and Mickelson 1969).

The FDA recommends the Daily Value (DV) for potassium at 3,500 mg based on the reference calorie intake of 2,000 calories. In contrast, the current average potassium intake is remarkably lower than the recommended intake level or only about 50% (Karppanen and others 2005). The NHLBI revealed the Dietary Approaches to Stop Hypertension (DASH) eating plan in 2006 which recommends Americans decrease sodium and increase potassium, calcium, and magnesium intakes to reduce elevated blood pressure. 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 those with kidney problems.

**1.2 Research Justification**

Because of the higher molecular weight of the cations ($K^+$), KCl has a weak and salty flavor and imparts bitterness and metallic aftertaste when a large amount is applied. According to Mickelsen and others (1977), the solution containing 50% replacement of NaCl by KCl tasted as salty as that with pure NaCl. Therefore, bitterness-masking agents would be applied in the development of salt substitutes that contain no sodium. The compounds that can reduce or mask the bitter taste of KCl include fumaric acid (Miller 1970), lactose and/or dextrose and cream of tartar (Eisenstadt 1975), potassium phosphate (Mohlenkamp and Hiler 1981), autolyzed yeast

(Shackelford 1981), lysine monohydrochloride (Berglund and Alizadeh 1999), monosodium glutamate (MSG) (Brandsma 2006), adenosine (AMP) and inosine (IMP) monophosphates (Salemme and Barndt 2006), and specific combinations of sulfate-containing and chloride containing salts (Bonorden and others 2003).

Adenosine monophosphate (AMP), a nucleotide compound, has been noted as a potential bitter blocker. It is an endogenous purine nucleotide found in all living organisms. AMP and its mono- and disodium salts are GRAS for use as flavor enhancers in chewing gum, coffee and tea, snack foods, soups and soup mixes, sugar substitutes and salt substitutes at levels ranging from approximately 0.0002 to 0.0008 percent. The use of AMP in food is self-limiting due to its strong, umami-like flavor (USDA 2004). AMP has been known as the first natural compound that can block several bitter tastants (McGregor and Gravina 2005; Salemme and Barndt 2006). The activation of AMP with an *in vitro* assay is that AMP may bind to bitter-responsive taste receptors or interfere with receptor-G protein coupling to serve as naturally occurring taste modifiers (Ming and others 1999). The mechanism of AMP and related compounds in inhibiting bitterness perception is that the compounds inhibited the activation of transducin by bitter tastant-stimulated taste receptors and decreased neuronal stimulation by the tastants (Margolskee and others 2003). Moreover, some types of amino acids such as L-arginine (Arg) have been reported to have the synergistic effect of saltiness perception of NaCl. Accordingly, the hypothesis in this dissertation was that AMP could potentially inhibit the bitterness while Arg could enhance the saltiness of KCl/Arg/AMP mixture.

### 1.3 Research Objectives

With its bitterness inhibiting ability, AMP would be able to decrease the unpleasant bitter taste imparted by KCl and that would make KCl more acceptable. Therefore, this study aimed at developing an acceptable sodium-free salt in which AMP was applied yet maintaining sensory acceptability. Specific objectives were to: (1) evaluate the bitterness-suppression ability of AMP, (2) determine the detection and recognition thresholds of KCl and KCl/AMP, (3) develop and optimize a mixture of salt substitutes with the presence of Arg and AMP, (4) quantify the sensory intensity of sensory attributes (bitterness and saltiness) of the salt substitutes, and (5) determine the acceptability of pasta sauces using the sodium-free salts as a flavoring agent.

The study was carried out with four major phases as follows. The first phase of this study presents the determination of detection and recognition thresholds of KCl with and without the addition of AMP. The second study presents the optimization of the ratio of KCl/Arg/AMP that yields taste quality improvement. The third phase of study presents the descriptive analysis using the Spectrum technique which aimed to quantify the intensity of bitterness and saltiness of different salt mixtures prior to conducting the consumer test. The last phase of this study presents the consumer acceptability of pasta sauces containing different sodium-free salts.

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# CHAPTER 2.
# LITERATURE REVIEW

## 2.1 Sodium Chloride

Sodium chloride, also known as table salt, is a chemical compound consisting of two elemental substances, cationic sodium ($Na^+$) and anionic chloride ($Cl^-$). By weight, it contains 39.3% of sodium (Na) and 60.7% of chlorine (Cl). Pertaining to the physical properties of sodium chloride, it is in the form of cubic colorless crystals or white powder with a molecular weight of 58.44, a specific gravity of 2.165, a density of 2.16 g/cm$^3$, a melting point of 801 °C, and a boiling point of 1413°C. Its pH of aqueous solution ranges from 6.7 to 7.3 and it is water- and glycerin soluble. The solubility is 35.9 g in 100 ml of water at 25 °C and it is able to slightly solubilize in alcohol (Winger and Ren 2008).

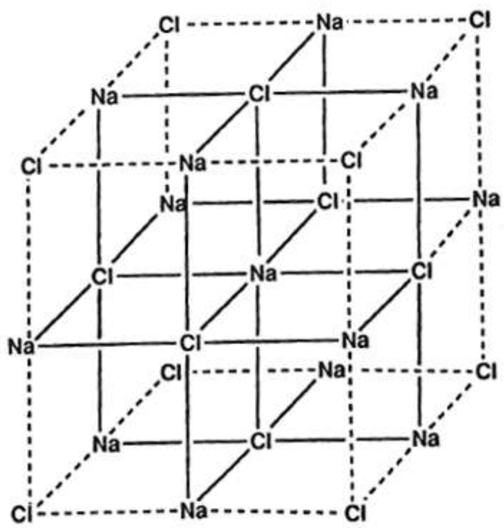

Figure 2.1 Arrangement of sodium and chlorine ions in the sodium chloride crystal.
(Source: Shallenberger 1993).

The octahedral crystalline structure of NaCl is shown in Figure 2.1. Each sodium ion is surrounded by six chlorine ions and each chlorine ion is associated with six sodium ions (Shallenberger 1993). When NaCl is dissolved in water, the sodium and chlorine ions ($Na^+$ and $Cl^-$) are dissociated and hydrated. The hydrated sodium ion and the chlorine ions are coordinated by four oxygen atoms of the water molecules as shown in Figure 2.2.

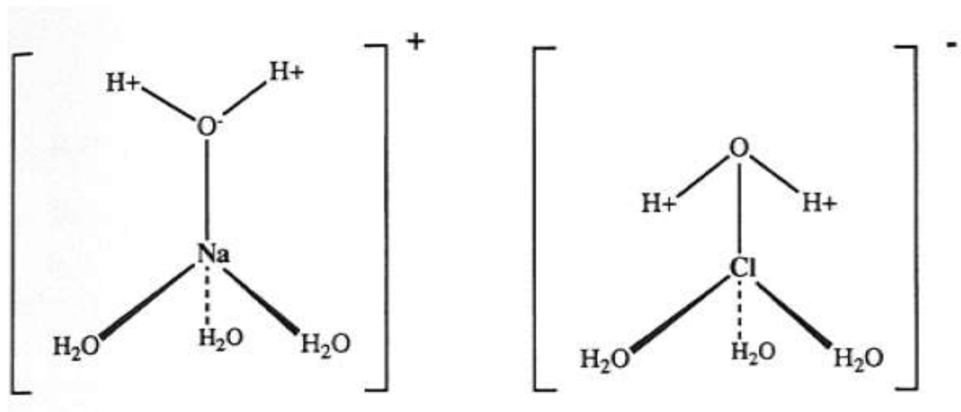


Figure 2.2 Diagrammatic representation of fully hydrated sodium and chlorine ions.
(Source: Shallenberger 1993).

Salt can be found naturally in the environment but mostly found in the ocean with many other minerals. By mining and solar evaporation, salt are produced from various sources including sea water, deep wells, and salt rocks (Amr and Jabay 2004). Sources, manufacturing techniques, and climatic conditions also have an effect on the physical and chemical composition of salt (Mannar and Dunn 1995). Salt is available in different crystal sizes and shapes for different purposes. These include table salt, sea salt, rock salt, kosher salt, and pickling salt.

Table salt is the most common kind of salt that can be found on every table. It is fine-grained, refined rock salts with free-flowing agents (sodium silicoaluminate, calcium phosphate, or magnesium carbonate) and it lacks all trace minerals. With added iodine (sodium iodide, potassium iodine, or iodate), it is called iodized salt. Iodized salt is used to prevent hypothyroidism in areas that lack natural iodine which is naturally present in the ocean. Salt is selected to distribute iodine to consumers because it is cheap, widely consumed, and does not spoil. Sea salt is made from evaporated sea water. Since it is not much refined, this salt still contains traces of other natural minerals such as iron, iodine, calcium, magnesium, and potassium, which give different colors and is more flavorful than traditional table salts. The crystals can be coarse or fine. Rock salt is a non-food salt, it is unrefined and grayish in color. It is large, non-uniform crystals used for making ice cream and de-icing because salt can lower the freezing point of ice. Kosher salt is an additive-free, coarse-grained salt and it is frequently used in the preparation of kosher meats. Pickling salt is fine-grained salt used to make brines for pickles and sauerkraut. This salt is the purest of salts and free of iodine and anti-caking agents which make the brine cloudy.

### 2.1.1 Roles of Salt in Food

Salt is one of the most used food additives. Beyond serving as a seasoning, salt has played many important roles in food and it also played an economic role in history. It is used in a wide range and serves various functions in the diet including a flavoring or flavor enhancing agent to make food tasteful and palatable, a preservative to lower the water activity ($a_w$) and limit the growth of microbiological flora, or an ingredient responsible for desired functional properties in certain products, for example, a binding agent to extract salt-soluble myofibrillar protein (Reddy and Marth 1991).

#### 2.1.1.1 Salty Taste Quality

The primary function of salt in foods is a flavor enhancer. NaCl imparts clean, classic salty taste (Lindsay 1996). Therefore, NaCl represents salty taste quality. Studies have shown that NaCl reduces the sourness of acid, and increases the sweetness of sugar. On the contrary, acids, with the exception of hydrochloric acid, increase the saltiness of NaCl while sugar reduces the saltiness of NaCl (McFarland 1974). The tastes of salts to humans are complex. NaCl is the most purely salty of all salts, but even this stimulus tastes sweet at low concentrations and somewhat sour at mid-range intensities. Other salts taste significantly sour or bitter in addition to salty (Smith and van der Klaauw 1995). NaCl has no taste at 0.009 M and it starts eliciting sweet taste at 0.01 M to 0.03 M. NaCl is salty sweet at 0.04 M and has pure salty taste at 0.5 M (von

Skramlik 1926). The sweetness of dilute NaCl could be explained by the AH-B theory (Lawless 1992).

However, the mechanism of saltiness perception has been poorly demonstrated. Some findings suggested that the salty taste quality was primarily caused by halide ions ($Cl^-$, $Br^-$, $I^-$) because all chlorides are primarily salty (Höber and Kiesow 1898; Kionka and Strätz 1922; von Skramlik 1926). The intensity of salty taste was determined by the sodium cation but other cations give different tastes with varying intensity (Höber and Kiesow 1898; Kionka and Strätz 1922). On the other hand, Beidler (1954) suggested that the saltiness is mainly attributed to the cations where anion is due to the magnitude of the response. Guyton and Hall (1996) stated that the salty taste is elicited by ionized salt, mainly responsible by the cations but lesser by the anions. Nevertheless, some researchers revealed that both cation and anion affect the overall taste quality of salts (Moncrieff 1967; Deman 1976). As the molecular weight of either cation or anion or both increases, the salts are likely to taste more bitter and less salty. In other words, a general rule of thumb is that the smaller the anion and cation, the more saltiness predominates over other tastes (Lawless 1992). 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.

The taste intensity of $Na^+$-containing salt depends on the anions present, and sodium chloride tastes saltier than sodium gluconate at equimolar concentrations (Rehnberg and others

1993; Simon 1992). In general, $Na^+$ influx through an epithelium is directly coupled to the passive diffusion of $Cl^-$ through tight junctions. Substituting $Cl^-$ with less permeable organic anions reduces $Na^+$ influx into taste cells through $Na^+$ channels and results in a reduced nerve response (Simon 1992). Moreover, replacing $Cl^-$ with a larger anion decreased paracellular anionic conductance, leading to more hyperpolarized cells (Elliott and Simon 1990; Simon 1992). This explains why sodium salts with larger anion size elicit the same taste response as NaCl at higher $Na^+$ concentration (Elliott and Simon 1990; Simon 1992). Relative salty taste indices of various substances are shown in Table 2.1.

Table 2.1 Relative salty taste indices of different substances

| Salty Substances | Relative salty taste index | |
|---|---|---|
| | von Skramlik (1926) | Guyton and Hall (1996) |
| NaF | - | 2.0 |
| LiCl | 0.44 | 0.4 |
| NaCl | 1.00 | 1.0 |
| $MgCl_2$ | 0.20 | - |
| KCl | 1.36 | 0.6 |
| $CaCl_2$ | 1.23 | - |
| $NH_4Cl$ | 2.83 | 2.5 |
| NaI | - | 0.35 |
| NaBr | - | 0.4 |

**2.1.1.2 Food Preservation and Other Functional Uses**

In addition to serving as a flavoring agent, salt is often used to preserve foods by lowering the water activity ($a_w$) which is the ratio between the vapor pressure of the food and the vapor pressure of distilled water at a given temperature. When salt is added to a food, the amount of available moisture is reduced and the growth of the microorganisms is inhibited as soon as it is lower than the minimum $a_w$ needed. Most of the pathogenic organisms will not grow below a water activity of 0.90 to 0.92. The reduction of water activity of the food causes plasmolysis in the bacteria cell resulting in death or latency. Some other possible mechanisms of salt inhibition include limiting oxygen solubility to the microbial cell, alteration of pH, toxicity of sodium and chloride ions, loss of magnesium ions, and interference with the cellular enzymes (Ravishankar and Juneja 2000). The addition of sodium chloride in a certain amount affects the shelf-life of food products.

Moreover, salt can also modify or improve texture and improve color and aroma (Gustafson 2008). In processed meats, salt aids in texture modification by solubilizing certain muscle proteins to improve fat binding and emulsification properties, increasing ionic strength of meat systems, increasing water holding capacity, and together with nitrite inhibiting growth of *Clostridium botulinum* (Reddy and Marth 1991). Moreover, salt plays many roles in the natural cheese making process; this includes retarding the undesirable bacteria growth, controlling the rate of the lactic acid fermentation, providing the optimum growth condition for the desired flora, developing satisfactory flavor, body, taste, texture during the ripening process, and helping the formation of the cheeses rind (Reddy and Marth 1991). Like in the cheese making process, salt is added in the fermented vegetable making process to control the microbiological flora, and in bakery products to control the fermentation rate of yeast-leavened products. Moreover, it also enhances other flavors and strengthens the gluten in bread doughs (Reddy and Marth 1991).

However, density, purity, solubility, particle size, mineral content (calcium, magnesium, copper, iron) of salt used in some specific food processing should be considered. Evaporated granulated salt is now produced with 99.99% sodium chloride content. Density and solubility vary according to the granular size of the salt. When the relative humidity is above 75%, salt will take on moisture, and therefore, anticaking agents such as tricalcium phosphate, calcium stearate, and magnesium stearate are needed. However, these substances cause milkiness in the brine.

Particle size of the salt also affects the salt caking. The calcium content in salt should be of concern in the manufacturing process of fruits and vegetables that are high in pectin as calcium binds with the pectin and toughens skins, thereby downgrading the quality. Calcium and magnesium in salt retard the penetration of salt into the fish, resulting in a shelf-life shortening during salting at lower temperatures. Copper and iron residues in salts cause the breakdown of vitamin C and the rancidity in foods high in fat (McFarland 1974).

### 2.1.2 Roles of Salt in Health

#### 2.1.2.1 Sodium/Chloride Intake

Sodium and chloride ions are typically consumed as sodium chloride. The Department of Health and Food Standards Agency recommends that everyone should cut their salt intake from the current amount of 10 to 12 g of salt a day to 5 to 6 g a day or less. Moreover, the 2005 Dietary Guidelines for Americans by the U.S. Department of Health and Human Services and U.S. Department of Agriculture (DHHS/USDA) recommends that Americans consume less than 2,400 mg of sodium. However, individuals with hypertension, diabetes or chronic disease, as well as African-Americans, and middle aged and older adults tend to be more sensitive to the blood pressure-raising effects of sodium chloride than their counterparts, so they should consume no more than 1,500 mg of sodium daily (DHHS/USDA 2005; Institute of medicine 2005). 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 salt sensitivity. Luft and others (1991) reported that salt sensitivity is a function of age but is not affected by gender.

#### 2.1.2.2 Sodium/Chloride Balance

Sodium is the principal cation of the extracellular fluid and 95% of total sodium content of the body is found in extracellular fluids. The major function of sodium is an osmotic determinant in regulating extracellular fluid volume and plasma volume as well as a determinant of the membrane potential of cells and the active transport of molecules across cell membranes (Institute of medicine 2005). In conjunction with sodium, chloride is the principal osmotically active anion in the extracellular fluid and takes a key role in maintaining fluid and electrolyte balance. Chloride is also a component of gastric juice, hydrochloric acid, in the stomach. Sodium and chloride are mostly absorbed in the small intestine. When the level of sodium content 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 under normal sweating conditions, and the amount of sodium excreted is approximately equivalent to the intake amount due to the capacity of the normal human kidney to filter some 25,000 mmol of sodium each day (Valtin and Schafer 1995).

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 (DHHS/USDA 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. Moreover, a reduced salt intake can decrease the risk of developing hypertension in nonhypertensive individuals (Institute of medicine 2005). 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).

### 2.1.3 Adverse Effect of Overconsumption

#### 2.1.3.1 High Blood Pressure

The major adverse effect of increased sodium chloride intake is elevated blood pressure, which has been shown to be an etiologically related risk factor for cardiovascular and renal diseases (Institute of medicine 2005). Generally, blood pressure rises progressively with increased sodium chloride intake. Blood pressure is the force of blood against artery walls. It is measured in millimeters of mercury (mmHg) and recorded as two numbers, systolic pressure over diastolic pressure. Systolic blood pressure is the pressure when the heart beats while pumping blood. Diastolic blood pressure is the pressure when the heart is at rest between beats. High blood pressure (HBP), also called hypertension, is defined as having a systolic blood pressure reading of at least 140 mmHg or diastolic pressure of at least 90 mmHg. Prehypertension is defined as having a systolic blood pressure of 120-139 mmHg or a diastolic blood pressure of 80-119. Normal blood pressure is defined as having a systolic blood pressure of less than 120 mmHg and a diastolic blood pressure of less than 80 mmHg.

Under normal circumstances, the heart and arterial system can supply sufficient blood flow to tissues to maintain adequate local pressure. However, rapid, short-term changes in arterial pressure such as during muscle exercise and other types of stress can be controlled by the nervous system. Conversely, the kidneys play the important role in controlling the long-term arterial pressure. HBP is regularly caused by excessive extracellular fluid volume. The extracellular fluid volume is affected by the balance between intake and output of water and salt because sodium and chloride are the major cation and anion in extracellular fluid. Moreover, salt is not excreted by kidneys as easily as water which is almost rapidly excreted as it is ingested. Therefore, excess accumulation of salt in the body results in increasing the extracellular fluid volume, and small increases in extracellular fluid and blood volume can increase the arterial pressure greatly (Guyton and Hall 1996).

When the body has excess sodium, the osmolality of the body fluids increases and the thirst center is stimulated, making the person drink extra amounts of water to dilute the extracellular salt to a normal concentration. This increases the extracellular fluid volume. Furthermore, the increase in osmolality in the extracellular fluid also stimulates the hypothalamic-posterior pituitary gland secretory mechanism to secrete increased quantities of antidiuretic hormone. The antidiuretic hormone in turn causes the kidneys to reabsorb greatly increased quantities of water from the renal tabular fluid before it is excreted as urine, thereby diminishing the volume of urine while increasing the extracellular fluid volume (Guyton and Hall 1996). The mechanism by which increased extracellular volume elevates arterial pressure is shown in the schema of Figure 2.3.

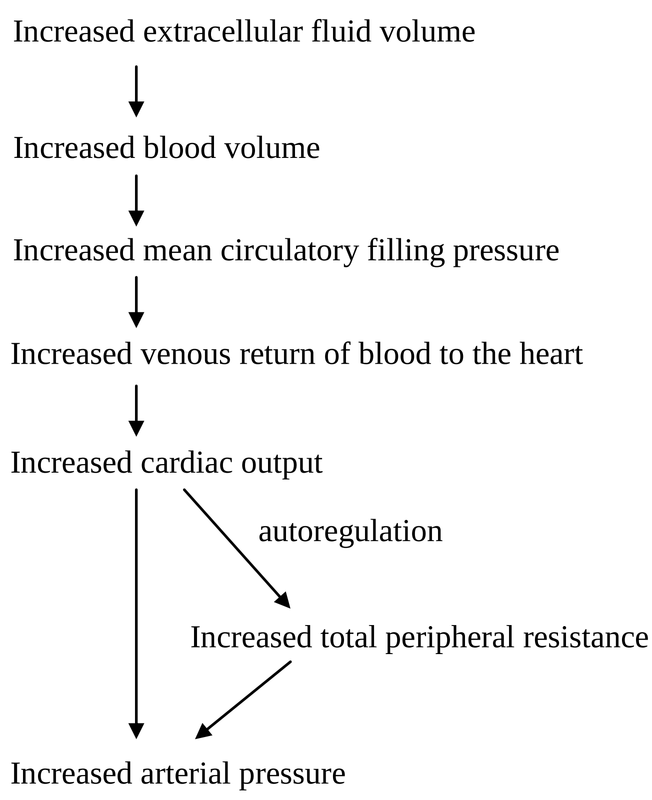


Figure 2.3 Sequential steps by which increased extracellular fluid volume increase the arterial pressure.
(Source: Guyton and Hall 1996).

#### 2.1.3.2 How Does High Blood Pressure (HBP) Affect the Body?

HBP is also called a silent killer since HBP itself usually has no early significant symptoms so that about one fifth (21.3%) of the people with HBP do not know that they have it (CDC 2006). HBP makes the heart work too hard and the high force of the blood flow can harm arteries and organs. HBP has mainly three lethal effects. Firstly, excess workload on the heart leads to early development of congestive heart disease, coronary heart disease, or both, often causing death as a result of a heart attack. Secondly, the high blood pressure frequently ruptures a major blood vessel in the brain, followed by death of major portions of the brain, also called a cerebral infarct or a stroke, and this can cause paralysis, dementia, blindness, or multiple other serious brain disorders. Lastly, HBP almost always causes multiple hemorrhages in the kidneys, producing many areas of renal destruction and, eventually, kidney failure, uremia, and death (Guyton and Hall 1996).

HBP is a major contributor to the global disease burden and is prevalent in the developing countries as well as in the developed countries (Ezzati and others 2002; WHO/INT 2003). HBP is a highly prevalent risk factor for cardiovascular disease and it is also becoming an increasingly common health problem worldwide because of increasing longevity and prevalence of contributing factors such as obesity, physical inactivity, and an unhealthy diet (WHO/INT 2003). Moreover, it is also associated with the risk of stroke and kidney disease (Ostchega and others 2008). According to the American Heart Association (2006), about 74.5 million people in the United States age 20 and older 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 (Kearney and others 2005). High blood pressure caused the death of 56,561 people in the United States in 2006 (AHA 2006). Furthermore, the American Heart Association (2006) reported that the death rate of hypertension increased 19.5% from 1996 to 2006, and the actual number of deaths rose by 48.1%. Additionally, Americans spend more than $15 billion annually on medications for hypertension (AHA 2006). It is estimated that the direct and indirect costs of high blood pressure will be $73.4 billion in the year 2009. 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 a potassium-rich diet can decrease the effects of NaCl salt on blood pressure as well.

#### 2.1.3.3 How to Lower the High Blood Pressure?

The Dietary Guidelines for Americans (2005) stated that changes in lifestyle can prevent or delay the onset of HBP and can lower elevated blood pressure. These changes include reducing salt intake, increasing potassium intake, losing excess body weight, increasing physical activity, and consuming an overall healthful diet. Moreover, increasing intake of fiber and protein as well as mainly minerals such as calcium and magnesium is also recommended by the Dietary Approaches to Stop Hypertension (DASH) eating plan to lower elevated blood pressure (NHLBI 2006). Consuming less salt is often recommended by physicians and nutritionists to reduce the risk of high blood pressure, which also may in turn reduce the risk of heart disease, stroke, congestive heart failure, and kidney disease.

There are several approaches for sodium reduction. However, the primary approach that should be concerned is to determine the minimum level of sodium chloride that can be used in the product without a negative impact on flavor. Once the level is identified, additional sources of sodium can be eliminated or replaced to reach the next level of sodium reduction (Gustafson 2008).

### 2.1.4 Sodium Content and Health Claims

According to the 21CFR101.61 (CFR 2009a), a claim about "sodium free" in a food may be made only if the product contains less than 5 mg of sodium/serving. For a product to be listed as "very low sodium", it must contain 35 mg or less of sodium/serving. The term "sodium reduced" salt may be used in labeling foods when the sodium level must be reduced by 25%. The term "unsalted," "no salt added," or "without added salt", the product must be made without any added salt during processing but may still contain naturally occurring amounts of salt.

According to 21CFR101.74 (CFR 2009b), the following are model health claims that may be used in food labeling to describe the relationship between dietary sodium and high blood pressure: (1) diets low in sodium may reduce the risk of high blood pressure, a disease associated with many factors, (2) development of hypertension or high blood pressure depends on many factors. [This product] can be part of a low sodium, low salt diet that might reduce the risk of hypertension or high blood pressure.

## 2.2 Potassium Chloride

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. Besides reducing sodium content used, another way to reduce the dietary sodium is the use of salt substitutes. Since sodium chloride has a unique clean salty taste, it may be difficult to determine a comparable salt alternative. These alternatives include halide salts including potassium chloride (KCl), lithium chloride (LiCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), and ammonium chloride ($NH_4Cl$). However, lithium chloride is highly toxic; calcium chloride and magnesium chloride are bitter-salty (Kurtz and Fuller 1997; Lawless and others 2003). $NH_4Cl$

has an unpleasant smell and taste, and is potentially unstable at cooking and baking temperatures thus changing the pH value, taste and other properties of food containing it. It also affects the acid-base balance in the blood when ingested (Rood and Tilkian 1984; Kurtz and Fuller 1997). Therefore, only KCl has any feasibility as a NaCl substitute (Kurtz and Fuller 1997).

Potassium chloride (KCl) is potentially a sodium-free alternative to salt and a common ingredient in salt substitutes. It is not only a good compound for supplementing of sodium chloride, but its physical properties make it technically an ideal substance for an ingredient with ordinary salt (Frank and Mickelson 1969). Moreover, the FDA allows for claims that diets rich in potassium will help to lower risk of stroke and high blood pressure (Gustafson 2008). KCl has weak and salty flavor and imparts an off-flavor mostly characterized as bitterness. The reason for bitterness perception with potassium salt and not with sodium salt remains questionable. It is believed that the higher molecular weight of cationic potassium ($K^+$) than cationic sodium ($Na^+$) causes bitterness. Moreover, the receptor sites located on the tongue where saltiness is perceived can readily distinguish potassium from sodium and this difference is physiologically perceived as a difference in bitterness intensity (Murray and Shackelford 1989). To improve taste qualities of potassium chloride, it is necessary to employ additives in salt substitutes to minimize the undesirable flavor. Therefore, most salt substitutes in the market are usually a mixture of NaCl and other salt substitutes, and the bitterness blockers have been investigated.

### 2.2.1 Properties of Potassium Chloride

Potassium chloride comprises 47.55% of chloride and 52.45% of potassium with a molecular of 74.55. 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. Potassium chloride can be manufactured industrially by fractional crystallization of carnallite or of solutions from lake brines. It can also be extracted from sylvinite and salt water. But the quantity present in a given volume of seawater is relatively low compared to sodium. Germany was the main source of mined potassium but recently most potassium minerals come from Canada, USA and Chile. Potassium chloride is annually produced in the world at 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.

In the microbiological aspect, Bidlas and Lambert (2008) reported that, on a molar basis, KCl had an equivalent antimicrobial effect to NaCl on a variety of pathogenic microorganisms including *Aeromonas hydrophila, Enterobacter sakazakii, Shigella flexneri, Yersinia enterocolitica* and 3 strains of *Staphylococcus aureus*.

**2.2.2 Sources of Potassium**

All living cells, both plant and animal, contain the dietary potassium. 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. The FDA recommends the Daily Value (DV) for potassium at 3,500 mg based on the reference calorie intake of 2,000 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 and even a lower intake of 1,500 mg in 1997 (Karppanen and others 2005). By race, it was found that African-Americans had the least potassium intake which was according 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 a lower level of blood pressure. Many medical studies found that increasing potassium intake can significantly lower 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, and high magnesium mineral salt could offer a valuable nonpharmacological 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.

### 2.2.3 Taste of Potassium Chloride

As salt is the icon descriptor for salty taste, the term of salt substitute is mainly focused on the taste quality of a substance that can replace the regular salt. Dzendolet and Meiselman (1967) revealed that KCl exhibits three taste qualities: sweetness, saltiness, and bitterness. At low concentrations, a sweet taste with slight bitterness is exhibited and becomes bitter-sweet taste. As concentration increases, bitterness decreases and saltiness increases.

### 2.2.4 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 those with kidney problems. Hyperkalemia occurs when elevated potassium levels exceed the capacity of kidneys to eliminate or greater than 18 g 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 Taste Modifier

Five fundamental human senses, sight, hearing, smell, taste, and touch, can be classified into two groups based on the stimulation of a sensory receptor. Sight, hearing, and touch are caused by physical forces (light waves, pressure waves, and physical pressure, respectively). Taste and smell are caused by chemical senses (Shallenberger 1993). Sour, salty, sweet, and bitter were supported as the four primary tastes since $19^{th}$ century (Shallenberger 1993). Umami was discovered by Ikeda in 1907 and is recognized as the fifth basic taste. The sour taste is elicited by acids and sour receptors are transmembrane ion channels that admit the protons ($H^+$) liberated by acids into the cell. The intensity of sourness is thus relative to the logarithm of the hydrogen ion concentration. The salty taste is caused by ionized salts, primarily from the cations of the salts. Sodium ions of the sodium salts can directly enter into the cell through the sodium ion channels. The sweet taste is caused by a variety of chemical substances including sugars, glycols, alcohols, aldehydes, ketones, amides, esters, amino acids, some small proteins, sulfonic acids, halogenated acids, and inorganic salts of lead and beryllium. The bitter taste is mostly caused by long-chain organic substances that contain nitrogen and alkaloids (Guyton and Hall 1996). The 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). Threshold values for basic tastes are 0.0009 N HCl for sourness, 0.01 M NaCl for saltiness, 0.01 M sucrose for sweetness, and 0.000008 M quinine for bitterness (Guyton 1996). Although the solution containing 50% replacement of NaCl by KCl tasted as salty as that with pure NaCl (Mickelsen and others 1977), the unpleasant off-notes remain a major problem for total substitution of NaCl with KCl.

Table 2.2 Potential ingredients used to reduce sodium in food formulations

| Ingredient | Form | Composition | Example use level[a] | Characterizing flavor | Color contribution[b] | EU label statement[c] |
|---|---|---|---|---|---|---|
| Yeast extracts | Powder, paste, or liquid | Nucleotides, glutamic acid, other amino acids, peptides, organic acids | 0.1-1.5% | Brothy, available in clean or characterizing flavor (roasted, poultry, etc.) | None, light yellowish to dark brown | Yeast extract, autolyzed yeast extract |
| Hydrolyzed vegetable protein | Powder, paste, or liquid | Glutamic acid, other amino acids | 0.5-1.5% | Bouillon-like, sharp, characteristic HVP, available in characterizing flavor (roasted, poultry, etc.) | Light brown to brown | Hydrolyzed vegetable protein |
| Monosodium glutamate | Crystalline | | 0.5-2% | Savory, mouth-filling, meaty | Colorless | Monosodium glutamate (E621) |
| Disodium inosinate, Disodium guanylate | Crystalline | | 0.05-0.2% | Savory, mouth-filling, meaty | Colorless | Disodium inosinate (E631), Disodium guanylate (E627) |
| Soy Sauce | Liquid or powder | Glutamic acid, other amino acids, peptides, organic acids | 0.5-5% | Fermented, brown, alcoholic | Tan, reddish brown, black | Soy sauce |
| Potassium chloride | Crystalline | | 0.2-0.4% | Salt, bitter | Colorless | Potassium chloride (E508) |

[a]Varies with finished product application.

[b]Depends on usage level and finished product application.

[c]Same as in U.S., except that EU allows the use of either the ingredient name or and "E" number for approved food additives.

(Source: Brandsma 2006).

Bitterness-masking agents and taste enhancers are also possibly incorporated into salt alternatives. Bitterness-masking agents are used to mask the undesirable taste in the development of salt substitutes or sodium-reduced salt in which more than 50% NaCl is replaced by KCl. Maskers are highly flavorful ingredients such as onion, garlic, paprika, red pepper, chili powder, and other spices (Salemme and Barndt 2006). Taste enhancers are employed to improve the salty taste quality. Taste enhancers work by activating taste receptors in the mouth and throat, which helps to compensate for the salt reduction. Taste enhancers particularly elicit the umami taste receptors so that the taste enhancers can provide a pleasurable taste sensation (Brandsma 2006).

Potential ingredients used to reduce sodium in food formulations include yeast extracts, hydrolyzed vegetable protein, monosodium glutamate, disodium innosinate, disodium guanylate, soy sauce, and potassium chloride and their characteristics are shown in Table 2.2 (Brandsma 2006). Some basic salts of acidic amino acids such as L-arginine and L-glutamate are used as a flavor enhancer for people on a sodium restricted diet (O'Hara 1974). In addition, basic amino acids including L-arginine (L-Arg), L-lysine (L-Lys), and L-histidine (L-His) and two acidic amino acids, L-aspartic acid and L-glutamic acid (L-Glu), have been reported to enhance the saltiness of NaCl (O'Hara 1974; Ogawa and others 2004).

Moreover, the compounds that can reduce or mask the bitter taste of KCl include fumaric acid (Miller 1970), lactose and/or dextrose and cream of tartar (Eisenstadt 1975), potassium phosphate (Mohlenkamp and Hiler 1981), autolyzed yeast (Shackelford 1981), lysine monohydrochloride (Berglund and Alizadeh 1999), adenosine (AMP) and inosine (IMP) monophosphates (Salemme and Barndt 2006), and specific combinations of sulfate-containing and chloride containing salts (Bonorden and others 2003). Other known bitterness-masking agents are sweeteners (sucrose, aspartame), tannic acid, phosphatidic acid, and NaCl (Bartoshuk and Seibyl 1982; Breslin and Beauchamp 1995; Keast and Breslin 2002a, 2002b; Keast and others 2004; Ogawa and others 2004).

### 2.3.1 Adenosine Monophosphate

Adenosine monophosphate (AMP), also called adenylic acid or adenosinic acid, is a nucleotide compound composed of adenine, D-ribose, and phosphoric acid. AMP is involved in energy metabolism and nucleotide synthesis. The chemical formula is $C_{10}H_{14}N_5O_7P$ and the chemical structure is shown is Figure 2.4.

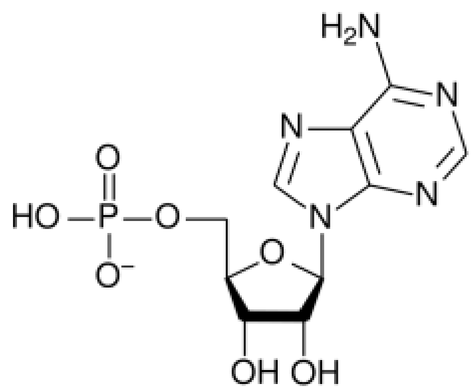


Figure 2.4 Chemical structure of adenosine monophosphate

AMP has been known as the first natural compound that can block several bitter tastants (McGregor and Gravina 2005; Salemme and Barndt 2006). It is an endogenous purine nucleotide found in all living organisms. AMP and its mono- and disodium salts meet Flavor and Extract Manufacturers Association, Generally Recognized as Safe (FEMA GRAS) guidelines for use as flavor enhancers in chewing gum, coffee and tea, snack foods, soups and soup mixes, sugar substitutes and salt substitutes at levels ranging from approximately 0.0002 to 0.0008 percent. The use of AMP in food is self-limiting due to its strong, umami-like flavor (USDA 2004). The activation of AMP with an *in vitro* assay is that AMP may bind to bitter-responsive taste receptors or interfere with receptor-G protein coupling to serve as naturally occurring taste modifiers (Ming and others 1999). The mechanism of AMP and related compounds in inhibiting bitterness perception is that the compounds inhibited the activation of transducin by bitter tastant-stimulated taste receptors and decreased neuronal stimulation by the tastants (Margolskee and others 2003).

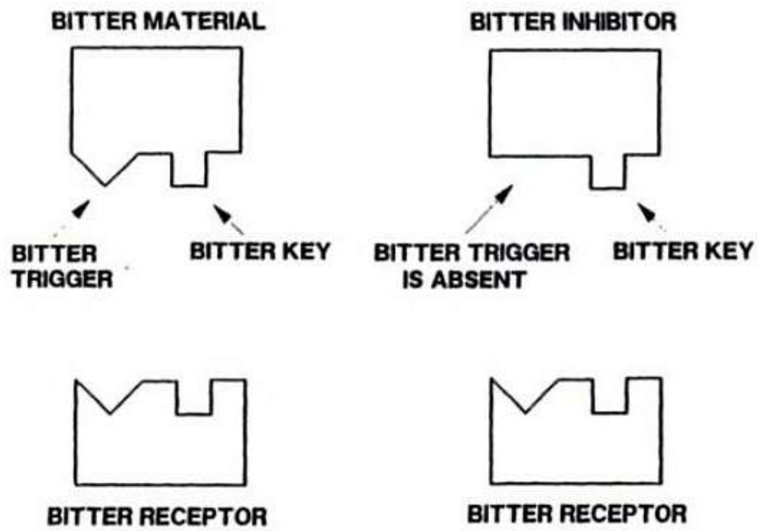


Figure 2.5 Schematic representation of the bitter taste receptor and interactions with a bitter compound and with a bitter inhibitor.

Using a lock-and-key analogy as a model for taste perception, a tastant is like a key that can fit into a receptor, lock. Kurtz and Fuller (1997) developed a model for bitter taste perception based on the assumption of a similarity of bitter and sweet receptors. Figure 2.5 shows the schematic of bitter taste perception with a bitter inhibitor. Tasteless molecules that possess only bitter keys, the molecular characteristics required for binding to a taste receptor, could eliminate the taste of sweet and/or bitter compounds (Kurtz and Fuller 1997).

Salemme and Barndt (2006) have patented bitterness inhibitors for KCl which are taurine, 5'-adenosinic acid (AMP), 5'-inosinic acid (IMP), combinations of AMP and IMP, combinations of AMP, IMP, and 5'-guanylic acid (GMP). A panel of 18 experienced taste testers was conducted for the evaluation of chicken broth samples (50% reduced sodium) containing KCl and various bitterness inhibitors regarding taste qualities and its intensity. Various combinations of taurine (750 ppm), AMP (600 ppm), and IMP (200 ppm) were added. The broth containing KCl and AMP was saltier and less bitter than the broth containing KCl alone. In the beef gravy test, the saltiness and the bitterness scores for the sample containing KCl and AMP were similar to those for the sample containing NaCl.

### 2.3.2 L-Arginine

The chemical formula of L-arginine (Arg) is $C_6H_{14}N_4O_2$, with a molecular weight of 174.2. Arg has an isoelectric point of 11.2 and a p$K_a$ of 12.5. In 100 ml of water at 20°C, its solubility is 14 g. In solid phase, Arg has a density of 1.1 g/cm$^3$ and a melting point of 244°C. Based on the physiological properties, Arg is helpful in the treatment and prevention of cardiovascular disease including hypertension, and some kidney disorders. Arg is not considered as an essential amino acid because under normal circumstances the body can synthesize sufficient Arg 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 Arg is not established. Adversely, if the synthesis of Arg is impaired, it may cause stress, and imbalances of other nutrients.

L-Arginine has a guanide group and is the strongest basic amino acid. The Sakaguchi reaction is used for the determination of arginine. When alpha-naphthol and hypochlorous acid react with the alkali solution, the solution turns red (AminoScience 2009). Schiffman and others (1981) reported that Arg tastes bitter for humans. The recommend use of Arg is up to 0.2% w/v due to an unpleasant smell (Ogawa and others 2004). In the detection threshold studies in rats, the geometric mean threshold ± standard error for Arg was 0.08 ± 0.1 mM and significantly lower than the thresholds for glycine, L-serine, and L-proline (Delay and others 2007).

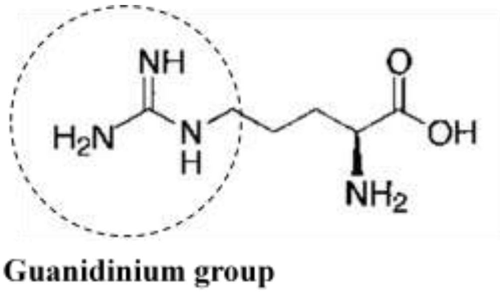


Figure 2.6 Chemical structure of L-arginine

Ogawa and others (2004) revealed that Arg can significantly suppress the bitterness of quinine and the bitterness suppression of Arg could be enhanced by the addition of NaCl. The mechanism of bitterness-suppression of Arg is likely related to the presence of the guanidinium side-chain of Arg (Figure 2.6) which interacts with the sodium channel in the taste bud in humans (Ogawa and others 2004) and catfish (Lipkind and Fozzard 1994; Ogawa and others 1997; Kumazawa and others 1998). As the sodium channel is known to be involved in bitterness perception, a similar interaction between Arg and the sensory receptor may occur in humans (Ogawa and others 2004).

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# CHAPTER 3.
# PRELIMINARY STUDY OF THRESHOLD DETERMINATION

## 3.1 Introduction

Most of Americans consume substantially more salt than they need. Sodium excess of body needs is usually eliminated in the urine processed by the kidney. However, excess sodium intake for a period of time may cause health problems. Salt has been reported to be associated with developing high blood pressure which is one of the significant risk factors leading to strokes, heart and kidney diseases. Salt is not only the source of sodium intake but is the major one. Decreasing salt intake is advisable to reduce the risk of elevated blood pressure. However, salt reduction usually causes changes in taste and texture profiles as the major functions of salt in food are flavor, preservation, and control of texture through maintenance of processability (Phelps and others 2006).

Developing a potential salt replacer is limited since the combined effect of both sodium and chloride gives a unique clean taste of table salt. Potassium chloride (KCl) may be a good alternative for sodium chloride with the similar physical and chemical properties but it imparts undesirable aftertaste. The ratio of salty taste intensity of KCl to NaCl is 0.6 (Guyton and Hall 1996). To make KCl an ideal salt replacer, the salty taste should be increased while the bitter taste should be minimized. Adenosine-5'-monophosphate (AMP) is the first natural compound that has bitterness inhibiting ability (McGregor and Gravina 2005; Salemme and Barndt 2006). Therefore, AMP could be applied in KCl solutions to inhibit bitter taste. In order to demonstrate the taste effects of AMP on KCl, threshold determination of KCl with and without AMP could be assessed.

The detection threshold was defined as the minimum concentration of solution at which a stimulus can be detected but the type of stimulus is not recognized. The recognition threshold was defined as the solution at which the subjects clearly identified the type of stimulus (Lawless and Heymann 1998). The objectives of this study were to (1) approximate the detection threshold values for KCl and KCl/AMP, (2) approximate the recognition threshold values of bitterness and saltiness for KCl and KCl/AMP, and (3) assess the appropriate method for threshold determination.

## 3.2 Materials and Methods

### 3.2.1 Subjects

Instead of trained panelists, untrained panelists were chosen to participate in the study in order to avoid the oversensitivity of the threshold values. The panelists were screened with a basic taste test (Meilgaard and others 2006). They must be able to differentiate the five basic tastes. A panel of twelve people (*N*=12), 3 M and 9 F, an age range of 23-35 years, participated in two testing protocols, the signal detection method and the method of limits, for KCl and KCl/AMP. Two replicates of each testing protocol were performed. Panelists were advised not to drink or eat one hour prior to the test.

### 3.2.2 Sample Solutions

#### 3.2.2.1 Determination of the Threshold of KCl

Sample solutions were prepared with potassium chloride (KCl) in distilled water at seven different concentrations, with a fixed ratio of two-fold increments: 0.035, 0.070, 0.141, 0.281, 0.5625, 1.125 and 2.25 g in 100 ml (4.69, 9.39, 18.91, 37.83, 75.45, 150.90 and 301.81 mM). The concentration scale increased in geometric increments so that any two adjacent concentration steps were separated by a constant factor. The range of concentrations was selected by pretesting in order to ensure that the panelist's threshold fell in the range. The lowest concentration should be two or three concentration steps below the estimated threshold (ASTM E-679 2004). KCl (Spectrum Chemical, Gardena, Calif.) was thoroughly dissolved in distilled water (Wal-Mart Stores, Bentonville, Ark.). The highest concentration was prepared and diluted to attain the lower concentrations. The aqueous solutions were poured in the 25-ml plastic cups and covered with lids. The containers were coded with three-digit random numbers and kept at ambient temperature.

#### 3.2.2.2 Determination of the Threshold of KCl - added AMP

KCl solutions with the addition of AMP were also evaluated. The preparation of KCl/AMP solutions was similar to that of KCl solution but AMP (Zhen-Ao Group, Dalian, China) was added to the most concentrated solution with a ratio of 15:1 (KCl:AMP) before diluting the solution to attain the lower concentrations. For example, there were 2.25 g of KCl and 0.15 g of AMP in 100 ml of the solution at the highest concentration.

### 3.2.3 Threshold Measurements

#### 3.2.3.1 Method of Limits (ML)

Subjects were presented with two controls which were distilled water, and one sample which was the solution. The concentration was presented once each time in order of increasing concentration. Samples were labeled with three-digit random numbers and randomly served with a counter balance design. According to the ASTM E-679 (2004), the procedure entailed a 3-alternative forced choice (3-AFC) method which the panelists' task was to pick the sample that was different from the other two samples in a specified attribute where the overall difference indicated the detection threshold and the specified attribute indicated the recognition threshold. The judgments were completed when the panelist completed the evaluation of all sets of the scale. Unsalted crackers and spring water were also served for cleansing the palate during the test.

#### 3.2.3.2 Signal Detection Rating (SDT) Method

The noise (control) and the five signal samples (0.035, 0.070, 0.141, 0.281, and 0.5625 g in 100 ml) were served at room temperature where the distilled water represented the control and the KCl solutions represented the signal labeled with three-digit random numbers. At the start of evaluation, panelists were asked to rinse their mouths with distilled water and expectorate. Panelists were then instructed to take the whole sample into the mouth all at once, swirl it around for few seconds, expectorate, and complete the task given. Unsalted crackers and spring water were also provided for cleansing the palate between samples. The samples were presented in order of ascending concentrations to prevent sensory fatigue. For this method, subjects were asked to indicate if the unknown sample was "the same as" or "different from" the control in the specified attributes on a continuum of sureness. Therefore, the choices could be same sure, same unsure, different sure, or different unsure. Two sessions of evaluation were held at different times. Saltiness and bitterness were evaluated in the first session. Overall difference was evaluated in the second session. If the sample is different from the control in terms of overall difference, it refers to the detection threshold. Likewise, if the sample is different from the control in terms of a specific perception, it refers to the recognition threshold.

### 3.2.4 Data Analysis

#### 3.2.4.1 Individual Thresholds

- **Method of Limits (ML)**

According to ASTM E-679-04, the geometric mean was then used for detection and recognition thresholds. Since the distribution is typically skewed, a geometric mean rather than an arithmetic mean should be used to measure the location of the distribution. The series of each panelists' judgments was tabulated with a sequence containing "0" for an incorrect choice or "+" for a correct choice arranged in the order of judgments of ascending concentrations of KCl. Then the best-estimate threshold (BET) concentration was the geometric mean of that concentration at which the last missed response (0) occurred and the next higher concentration designated by a "+". The final individual thresholds were obtained by the arithmetic average of the threshold values from two replications. The distributions of individual thresholds and their skewness were then obtained using SAS® software.

• **Signal Detection Rating (SDT) Method**

To calculate the threshold of each panelist, the duplicate data of each panelist were pooled together (*N*=2) and tabulated as frequency counts as shown in Table 3.1 and the *R*-index (%) was then calculated with equation 3.1 (O'Mahony 1992).

Table 3.1 The traditional *R*-index method

| Sample | Judge's Response | | | |
|---|---|---|---|---|
| | Different, sure | Different, unsure | Same, unsure | Same, sure |
| Stimulus | a | b | c | d |
| Control | e | f | g | h |

$$R\text{-index}(\%) = \frac{\left\{[a(f+g+h)+b(g+h)+c(h)]+\left[\frac{1}{2}(ae+bf+cg+dh)\right]\right\}\times 100}{(a+b+c+d)(e+f+g+h)} \quad (3.1)$$

Then KCl concentrations and the *R*-index values were plotted to obtain an equation from the linear trend line. The thresholds were then acquired from this equation at the *R*-index value of 75% which is approximately to a *d'* value of 1, an appropriate level of discrimination to determine threshold values (Robinson and others 2005). The frequency plots of the number of panelists and KCl concentrations were constructed to obtain the threshold distribution by SAS®. Skewness was determined by SAS® to measure the symmetry of a distribution.

**3.2.4.2 Group Thresholds**

• **Method of Limits (ML)**

For the geometric mean method, the population threshold was obtained by the arithmetic average of summation of the logarithm with base 10 of the individual BETs, and the standard deviation $\log_{10}$ provided a measure of the group's variation (ASTM E-679 2004). The arithmetic average thresholds of two replications were reported.

- **Signal Detection Rating (SDT) Method**

For the group threshold estimation, the *R*-index (%) was calculated according to the data of 12 panelists of each replicate (*N*=12). A group threshold was obtained from the concentration of the *R*-index of 75% from the linear regression. The arithmetic average of two replications was then reported as a group threshold for the SDT method.

## 3.3 Results and Discussion

### 3.3.1 Individual Thresholds

The individual threshold distributions using the ML and SDT methods are shown in Figures 3.1 and 3.2 where the Y-axis is the number of observers and the X-axis is the concentration (g/100 ml). As can be seen, the distributions were not normal. Asymmetrical distributions typically occur with the sensory data (ASTM E-1432 2004).

#### 3.3.1.1 Method of Limits

The histograms in Figure 3.1 illustrate that the thresholds for KCl for individuals were more likely normally distributed where the KCl/AMP thresholds were significantly right skewed. The distribution of the detection threshold values for individuals was likely positively skewed with a skewness of 1.31 (Figure 3.1A) and it became more normally distributed for the bitterness and saltiness recognition thresholds with a skewness of 0.14 and 0.18, respectively (Figures 3.1C and 3.1E). The detection and recognition threshold values for KCl were found to be less than 0.035 g/100 ml for one third of the individuals (*N*=12).

More than half of the individuals (*N*=12) had the KCl/AMP threshold values below 0.035 g/100 mg for the detection and the recognition thresholds as the panelists were able to correctly identify the KCl/AMP from spring water. Therefore, the distributions of the detection and recognition thresholds for KCl/AMP were positively skewed with the skewness of 2.67, 2.22, and 2.48, respectively (Figures 3.1B, 3.1D, and 3.1F). The actual threshold values for these individuals were thus not established and this may have also caused inaccurate group thresholds. Therefore, the lowest concentration used in the test should be brought down to be two or three concentration steps below the estimated threshold value (ASTM E-679 2004).

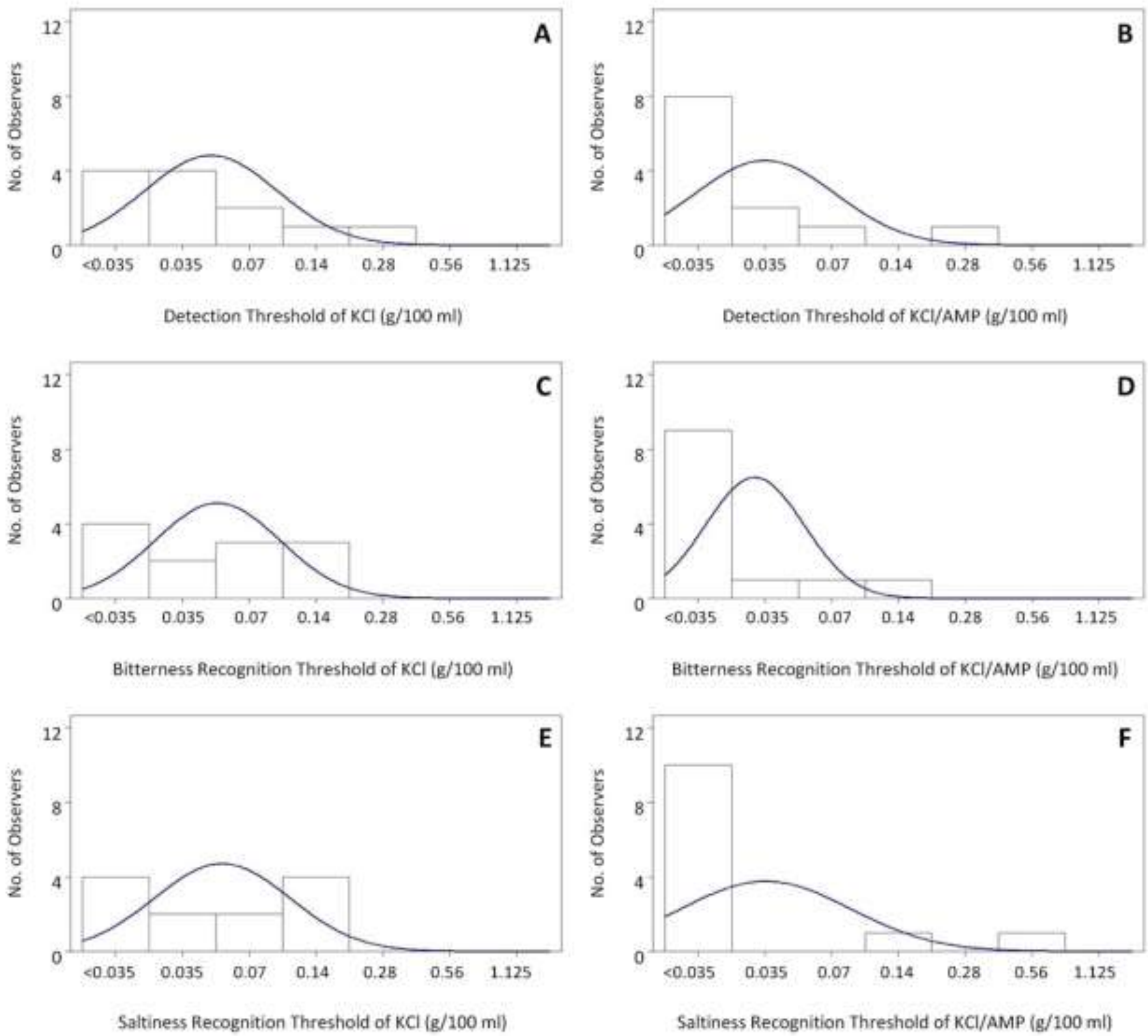


Figure 3.1 Individual threshold distributions of KCl and KCl/AMP using the ML method
(A) detection threshold of KCl, (B) detection threshold of KCl/AMP, (C) bitterness recognition threshold of KCl, (D) bitterness recognition threshold of KCl/AMP, (E) saltiness recognition threshold of KCl, (F) saltiness recognition threshold of KCl/AMP.

### 3.3.1.2 Signal Detection Rating (SDT) Method

The distributions of threshold values for the individuals using the SDT method were nearly normally distributed for the KCl solutions with the skewness of 0.35, -0.71, and 0.17 for the detection, bitterness recognition, and saltiness recognition thresholds as shown in Figures 3.2A, 3.2C, and 3.2E, respectively.

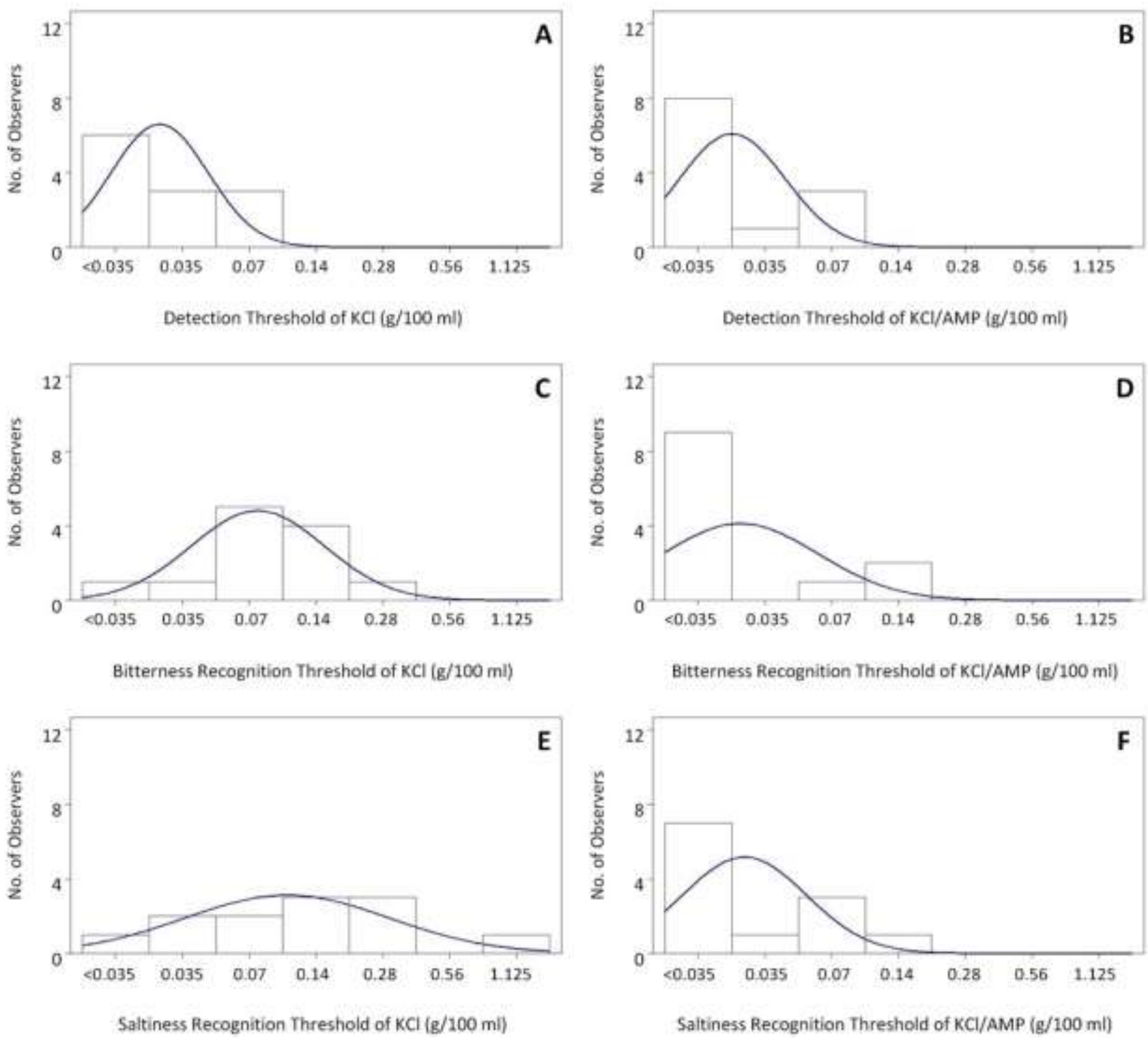


Figure 3.2 Individual threshold distributions of KCl and KCl/AMP using the SDT method
(A) detection threshold of KCl, (B) detection threshold of KCl/AMP, (C) bitterness recognition threshold of KCl, (D) bitterness recognition threshold of KCl/AMP, (E) saltiness recognition threshold of KCl, (F) saltiness recognition threshold of KCl/AMP.

Although the distributions of the bitterness and saltiness recognition thresholds for KCl were normally distributed, the kurtosis values indicating the peakedness of these distributions were quite small which were 0.48 and 0.05, respectively. These small values revealed the variation in individual thresholds so that the group thresholds were not precisely obtained. Meanwhile, it is clear that the KCl/AMP solutions had positive-skew distributions for all threshold types with the skewness of 1.06, 1.46, and 1.91, respectively (Figures 3.2B, 3.2D, and 3.2F). It seems that all threshold values for individuals decreased with the presence of AMP in KCl solutions. In other words, AMP was more likely to have a taste effect on KCl as it might impart some tastes that the panelists could detect and recognize the taste sensation at a lower concentration. The taste of AMP perceived by panelists may have caused inaccurate threshold values.

### 3.3.2 Group Thresholds

Most of the group threshold values could not be obtained from this study as the threshold values for almost half of the individuals ($N$=12) were not established for both methods. Moreover, a group threshold value from the method of limits could not be obtained from the logistic regression model because there were only two replications. In the method of limits, the responses may be biased because of the method used in the test. Panelists had to pick the odd sample according to the specified attributes but the distilled water was used as control and KCl imparts more than one sensation such as salty and bitter tastes. Therefore, the panelists may correctly pick the odd sample even though they did not perceive that bitter or salty taste was the cause for difference. Consequently, the recognition thresholds would not be accurately established. Moreover, panelists reported that they detected acidic taste in KCl/AMP solutions. In addition, distilled water is not suitable to use in the threshold study as it may cause a cardboard-like flavor and will introduce a bitter taste (Jellinek 1985).

### 3.4 Conclusions

The threshold values obtained from this study may not be accurately established as most of the individual threshold fell below the lowest concentration used in the study where the estimated threshold value should fall in two to three steps above the lowest concentration. Therefore, the lowest concentration used in the tests should be lowered to ensure that the threshold values fall in the middle of the concentration range and a scale step factor should allow the correct responses of panelists to distribute over three to four concentration steps. The number of panelists and replications used in the study should be increased. Odorless and tasteless spring water should be used instead of distilled water.

### 3.5 References


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# CHAPTER 4.
# DETERMINATION OF THRESHOLD SENSITIVITY OF KCL AND KCL/AMP

## 4.1 Introduction

Excessive sodium intake is believed to increase blood pressure. The National Heart, Lung, and Blood Institute (NHLBI 2006) reported that sodium reduction along with the DASH (Dietary Approaches to Stop Hypertension) diet which is rich in potassium, magnesium, calcium, protein, fiber, and low in total and saturated fat is found to lower blood pressure, especially for middle-aged and older individuals, African Americans, and those who already had high blood pressure. Potassium chloride (KCl) has commonly been used in the replacement of NaCl in accordance with their similar chemical and physical properties. Therefore, using KCl as a salt substitute is not only to reduce the amount of sodium intake but also to increase the potassium intake which may help reduce the elevated blood pressure. However, KCl elicits salty taste but also imparts unpleasant bitter taste. Moreover, the taste perception of KCl, bitterness and saltiness, are different from those of NaCl (Nachay 2008). 5'-adenosine monophospate (AMP) has been known as the first natural compound that can block several bitter tastants (McGregor and Gravina 2005; Salemme and Barndt 2006). Incorporated with KCl, AMP may be used to suppress the undesirable bitter sensation. Information on the bitter taste suppression of AMP at threshold levels in salt substitutes is very limited.

Thresholds have been a major concern of sensory scientists for a long time. It is a measure of human sensitivity to a given stimulus (Bi and Ennis 1998). A distinction is often made between the detection threshold, the lowest concentration of a stimulus that can be perceived as different from a baseline stimulus such as water, and the recognition threshold, the minimum concentration of a substance that is detectable and recognizable as a given taste (Lawless 1992). Thresholds have been used for several purposes including being an index of the biological activity in physical units of concentrations and providing useful rules of thumb for formulators and analysts of natural products (Lawless 1992). However, a threshold is not a constant for a given substance but it varies with moods, hunger and satiety, and the time of the biorhythm (Meilgaard and others 2006).

The taste threshold testing is commonly determined using the American Society of Testing and Materials (ASTM) method of ascending limits, known as the three-alternative forced-choice (3-AFC) method of sample presentation in which three samples are presented: two controls and one stimulus. The concentration is presented once each time in order of increasing concentration. However, an alternative tool used for determine threshold testing is signal detection method (Lee and van Hout 2009). Signal detection theory (SDT) was introduced in 1966 by Green and Swets. It is a measurement theory that permits the separation of true observer sensitivity from response bias (Lawless and Heymann 1998). Brown (1974) developed the index of degree of difference denoted as "*R*" which is the area under the receiver operating characteristic (ROC) curve in the signal detection theory. SDT has been applied to basic taste threshold testing: sweet, salt, sour, and bitter (O'Mahony 1972, Robinson and others 2005). The signal detection method has been recommended for taste threshold testing with more accurate threshold values and less sample preparation (Robinson and others 2005). Moreover, the subject's decision process could be observed and statistically modeled (Meilgaard and others 2006). The objectives of this study were to (1) determine the detection and recognition thresholds of bitterness and saltiness of KCl, (2) determine the detection and recognition thresholds of bitterness and saltiness of KCl/AMP, (3) evaluate the bitterness-suppression ability of AMP, and (4) compare the performance of the method of limits and the signal detection method.

## 4.2 Materials and Methods

### 4.2.1 Subjects

To avoid the oversensitivity of the threshold values, untrained panelists were used instead of trained panelists. The panelists were screened with a basic taste test (Meilgaard and others 2006). They must be able to differentiate the five basic tastes. A panel of fifteen people (*N*=15), 5 M and 10 F, an age range of 23-35 years, participated in two testing protocols, the method of limits and the signal detection method, for KCl and KCl/AMP. Panelists were given a warm-up test to familiarize them with the tastes of KCl, as it imparts more than one taste sensation. Three replicates of each testing protocol were performed. Panelists were advised not to drink or eat one hour prior to the test.

**4.2.2 Sample Solutions**

**4.2.2.1 Determination of the Threshold of KCl**

Sample solutions were prepared with potassium chloride (KCl) in spring water at seven different concentrations, with a fixed ratio of two-fold increments: 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 g in 100 ml (1.34, 2.68, 5.37, 10.73, 21.46, 42.92, and 85.85 mM). The concentration scale increased in geometric increments so that any two adjacent concentration steps were separated by a constant factor and this allowed the correct responses of a group of panelists to distribute over three to four concentration steps. The range of concentrations was selected by pretesting in order to ensure that the panelist's threshold fell in the range. The lowest concentration should be two or three concentration steps below the estimated threshold (ASTM E-679 2004).

KCl (Spectrum Chemical, Gardena, Calif.) was thoroughly dissolved in odorless and tasteless Ozarka® spring water (Nestlé Waters North America, Greenwich, Conn.). Distilled water is not suitable as it may cause a cardboard-like flavor and will introduce a bitter taste (Jellinek 1985). The highest concentration was prepared and diluted to attain the lower concentrations. The aqueous solutions were poured in the 25-ml plastic cups with lids. The containers were coded with three-digit random numbers and kept at ambient temperature.

#### 4.2.2.2 Determination of the Threshold of KCl - Added AMP

KCl solutions with the addition of AMP were also evaluated. The preparation of KCl/AMP solutions was similar to that of KCl solutions but AMP (Zhen-Ao Group, Dalian, China) was added to the most concentrated solution with a ratio of 15:1 (KCl:AMP) before diluting the solution to attain the lower concentrations. At the same concentrations, the KCl/AMP solutions had the equivalent amount of KCl to the KCl solutions. For example, there were 0.64 g of KCl and 42.67 mg of AMP in 100 ml of the solution at the highest concentration.

### 4.2.3 Threshold Measurements

#### 4.2.3.1 Method of Limits (ML)

Subjects were presented with three samples, of which two were controls, spring water, and one was the solution. The concentration was presented once each time in order of increasing concentration. There were seven sets in total. Samples were labeled with three-digit random numbers and randomly served with a counter balance design. According to the ASTM E-679 (2004), the procedure entailed a 3-alternative forced choice (3-AFC) method but the instruction was slightly modified in this study. Basically, the instruction of the 3-AFC method is to pick the sample that is different from the other two samples in a specified attribute. In our preliminary study, tasteless spring water was used as control. Therefore, it was likely that panelists picked the odd sample according to the specified attribute asked in the question since they were forced to pick one sample, and KCl imparts few taste sensations. Since the attributes of interest (bitterness and saltiness) were given to the panelists, the recognition thresholds were not accurately established.

For these reasons, in this study the subjects' task was to pick the odd sample (detection threshold) and identify the tastes of the odd sample that exhibit recognizable difference (recognition threshold). The choices of recognizable tastes included four basic tastes (sweet, salty, sour, bitter) and unidentified. The judgments were completed when the panelist completed the evaluation of all sets of the scale. Unsalted crackers and spring water were also served for cleansing palate during the test. Three replicates were preferred.

#### 4.2.3.2 Signal Detection Rating (SDT) Method

Subjects were presented with the noise and the five signal samples at room temperature where the spring water represented the noise or reference labeled as “control” and the KCl solutions (0.01, 0.02, 0.04, 0.08, and 0.16 g/100 ml) represented the signal labeled with three-digit random numbers since the panelists could perceive the taste difference at low concentrations. At the start of evaluation, panelists were asked to rinse their mouths with spring water and expectorate. Panelists were then instructed to take the whole sample into the mouth all at once, swirl it around for few seconds, expectorate, and complete the task given. Unsalted crackers and spring water were also provided for cleansing the palate between samples. To prevent sensory fatigue, the samples were presented in order of ascending concentrations. For this method, subjects were asked to indicate if the unknown sample was “the same as” or “different from” the control in the specified attributes on a continuum of sureness. Therefore, the choices could be same sure, same unsure, different sure, or different unsure. Two sessions of evaluation were held at different times. Saltiness and bitterness were evaluated in the first session. Overall difference was evaluated in the second session. If the sample was different from the control in terms of overall difference, it referred to the detection threshold. Likewise, if the sample was different from the control in terms of a specific perception, it referred to the recognition threshold.

### 4.2.4 Data Analysis

#### 4.2.4.1 Individual Thresholds

• **Method of Limits (ML)**

Two basic statistical methods for estimating thresholds are the parametric and the nonparametric approaches. The parametric approach includes the probit method, the logistic methods, the up-and-down method, and some newly developed methods which are the generalized probit and logit models, the dose-response model based on the beta-binomial distribution. The non-parametric method includes the Spearman-Karber method and the moving average method. Some newly developed non-parametric methods are the trimmed Spearman-Karber method, the Kernel method, and the sigmoidally constrained maximum likelihood estimation method (Bi and Ennis 1998). The logistic model (ASTM E-1432 2004) was used for estimating the detection threshold only due to the limitation of the sensory method used in this study. Since the panelists were asked to pick the odd sample (detection threshold) and identify the tastes of the odd sample that exhibit recognizable difference (recognition threshold), there were no correct or incorrect responses for the recognition thresholds of saltiness and bitterness. Therefore, the logistic model could not be applied to the recognition thresholds. The threshold was obtained at $P$ = ⅔ from the following equation (ASTM E-1432 2004).

$$P = \frac{\frac{1}{3} + e^{K}}{1 + e^{K}} \tag{4.1}$$

where $K = b(t - \log_{10} x)$

$P$ = the proportion of correct response

$b$ = slope

$t$ = the threshold value (in log units)

$x$ = concentration (in g/ 100 ml)

According to ASTM E-679-04, the geometric mean was then used for detection and recognition thresholds. Since the distribution is typically skewed, a geometric mean rather than an arithmetic mean should be used to measure the location of the distribution. The series of each panelists' judgments was tabulated with a sequence containing "0" for an incorrect choice or "+"

for a correct choice arranged in the order of judgments of ascending concentrations of KCl. Then the best-estimate threshold (BET) concentration for the detection threshold was the geometric mean of that concentration at which last missed response (0) occurred and the next higher concentration designated by a "+". If a taste was identified as recognizable difference at these two concentrations, it was also determined as the BET concentration of the recognition threshold of that taste. Otherwise, the BET concentration for the recognition threshold was the geometric mean of the two lowest concentrations at which correct responses occurred and a recognizable taste was identified. The final individual thresholds were obtained by the arithmetic average of the threshold values from three replications. The distributions of individual thresholds were then constructed by SAS®. Skewness was determined by SAS® to measure the symmetry of a distribution.

- **Signal Detection Rating (SDT) Method**

To calculate the threshold of each panelist, the triplicate data of each panelist were pooled together (*N*=3) and tabulated as frequency counts as shown in Table 4.1 and the *R*-index (%) was then calculated with equation 4.2 (O'Mahony 1992).

Table 4.1 The traditional *R*-index method

| Sample | Judge's Response | | | |
|---|---|---|---|---|
| | Different, sure | Different, unsure | Same, unsure | Same, sure |
| Stimulus | a | b | c | d |
| Control | e | f | g | h |

$$R\text{-index}(\%) = \frac{\left\{[a(f+g+h) + b(g+h) + c(h)] + \left[\frac{1}{2}(ae+bf+cg+dh)\right]\right\} \times 100}{(a+b+c+d)(e+f+g+h)} \quad (4.2)$$

Then KCl concentrations and the *R*-index values were plotted to obtain an equation from the linear trend line. The thresholds were then acquired from this equation at the *R*-index value of 75% which is approximately to a *d'* value of 1, an appropriate level of discrimination to determine threshold values (Robinson and others 2005). The frequency plots of the number of panelists and KCl concentrations were constructed to obtain the threshold distributions and their skewness using SAS® software.

#### 4.2.4.2 Group Thresholds

- **Method of Limits (ML)**

The detection threshold obtained by logistic regression model was calculated by the median of the distribution of the individual distribution (ASTM E-1432 2004). For the geometric mean method, the population threshold was obtained by the arithmetic average of summation of the logarithm with base 10 of the individual BETs, and the standard deviation $\log_{10}$ provided a measure of the group's variation (ASTM E-679 2004). The arithmetic average thresholds of three replicates were reported. This method was used for estimating recognition thresholds as well as the detection threshold in order to compare the method of calculation.

- **Signal Detection Rating (SDT) Method**

For the group threshold estimation, the *R*-index (%) was calculated according to the data of 15 panelists of each replicate ($N$=15). A group threshold was obtained from the concentration of the *R*-index of 75% from the linear regression. The arithmetic average of three replicates was then reported as a group threshold for the SDT method.

- **Taste Effect in the Presence of AMP**

Using the Statistical Analysis Software® (SAS 2003), the Student's *t*-test was performed to compare the mean threshold difference between KCl and KCl/AMP at a given concentration at the significance level of 0.05. If the significance exists, there is a taste effect in the presence of AMP in KCl solutions.

- **Effect of Methods Used in the Threshold Determination**

The Student's *t*-test was performed to compare the mean threshold difference between the

ML and SDT methods at a given stimulus and threshold to find any difference in the power of the tests using SAS® software at $\alpha$=0.05.

## 4.3 Results and Discussion

### 4.3.1 Individual Thresholds

The individual threshold distributions using the ML and SDT methods are shown in Figures 4.1 and 4.2 where the Y-axis is the number of observers and the X-axis is the concentration (g/100 ml). As can be seen, the distributions are not normally distributed. The asymmetrical distributions typically occur with the sensory data (ASTM E-1432 2004).

#### 4.3.1.1 Method of Limits

Figure 4.1 shows the distributions of the individual detection and recognition thresholds of KCl and KCl/AMP for 15 panelists obtained by the method of limits test. The distributions of the detection threshold obtained of KCl and KCl/AMP by logistic model and geometric mean were asymmetric where it was bi-modal for KCl (Figures 4.1A and 4.1C) and right-skewed for KCl/AMP (Figures 4.1B and 4.1D). The distribution of bitterness recognition threshold of KCl was nearly normally distributed with a skewness of -0.0525 while KCl/AMP had a bi-modal distribution (Figures 4.1E and 4.1F). Moreover, two panelists did not generate the bitterness recognition threshold of KCl/AMP as they correctly picked the odd sample at the lowest concentration. For the saltiness recognition threshold (Figures 4.1G and 4.1H), the shapes of the distributions closely resembled the normal distribution but slightly skewed to the left with a skewness of -0.70 and -0.32 for KCl and KCl/AMP, respectively.

For the logistic method, the ranges for the detection threshold for KCl and KCl/AMP were 0.007 to 0.11 g/100 ml and 0.012 to 0.15 g/100 ml, respectively. Two individual thresholds of KCl were not obtained from the logistic model as they correctly picked the odd sample at the lowest concentration. Using the geometric mean, the range for the detection threshold of KCl was as the same as that of KCl/AMP which was 0.007 to 0.154 g/100 ml. The individual bitterness recognition thresholds had a wider range than those detection thresholds which were up to 0.33 g/100 ml for both KCl and KCl/AMP. The recognition thresholds of saltiness of KCl ranged from 0.04 to 0.15 g/100 ml. The individual recognition thresholds of saltiness for KCl/AMP were in the range of 0.044 to 0.16 g/100 ml. With the uniformity of the data, these distributions reveal the single modal and sharp peak (Figures 4.1G and 4.1H), indicating that KCl and KCl/AMP start imparting the perceptible salty sensation at this range of concentration.

#### 4.3.1.2 Signal Detection Rating (SDT) Method

The distributions of the individual detection threshold obtained by the *R*-index by a rating method for KCl and KCl/AMP solutions for 15 panelists are shown in Figure 4.2. One can clearly see that the distributions of the detection and recognition thresholds of KCl and KCl/AMP were flat, indicating that these threshold values had high variation. Both KCl and KCl/AMP had the same range of the detection threshold and the bitterness recognition threshold which was <0.01 to >0.16 g/100 ml. The individual recognition thresholds of saltiness of KCl and KCl/AMP ranged from <0.01 to >0.16 g/100 ml and 0.01 to 0.09 g/100 ml, respectively.

In ASTM E-253 (2005), the population threshold is defined as the median or other measure of central tendency of the distribution of detection or recognition thresholds for a specified population. By this definition, estimating many individual thresholds is very difficult and unnecessary (Bi and Ennis 1998). Replicated tests on an individual will not give more precise population threshold estimates as the variation among individuals is the main error source (Bi and Ennis 1998). Therefore, the distributions of individual threshold need not be normalized and the group thresholds were estimated by the rapid method in ASTM E-679 and by logistic method (ASTM E-1432) for detection threshold obtained by the method of limits.

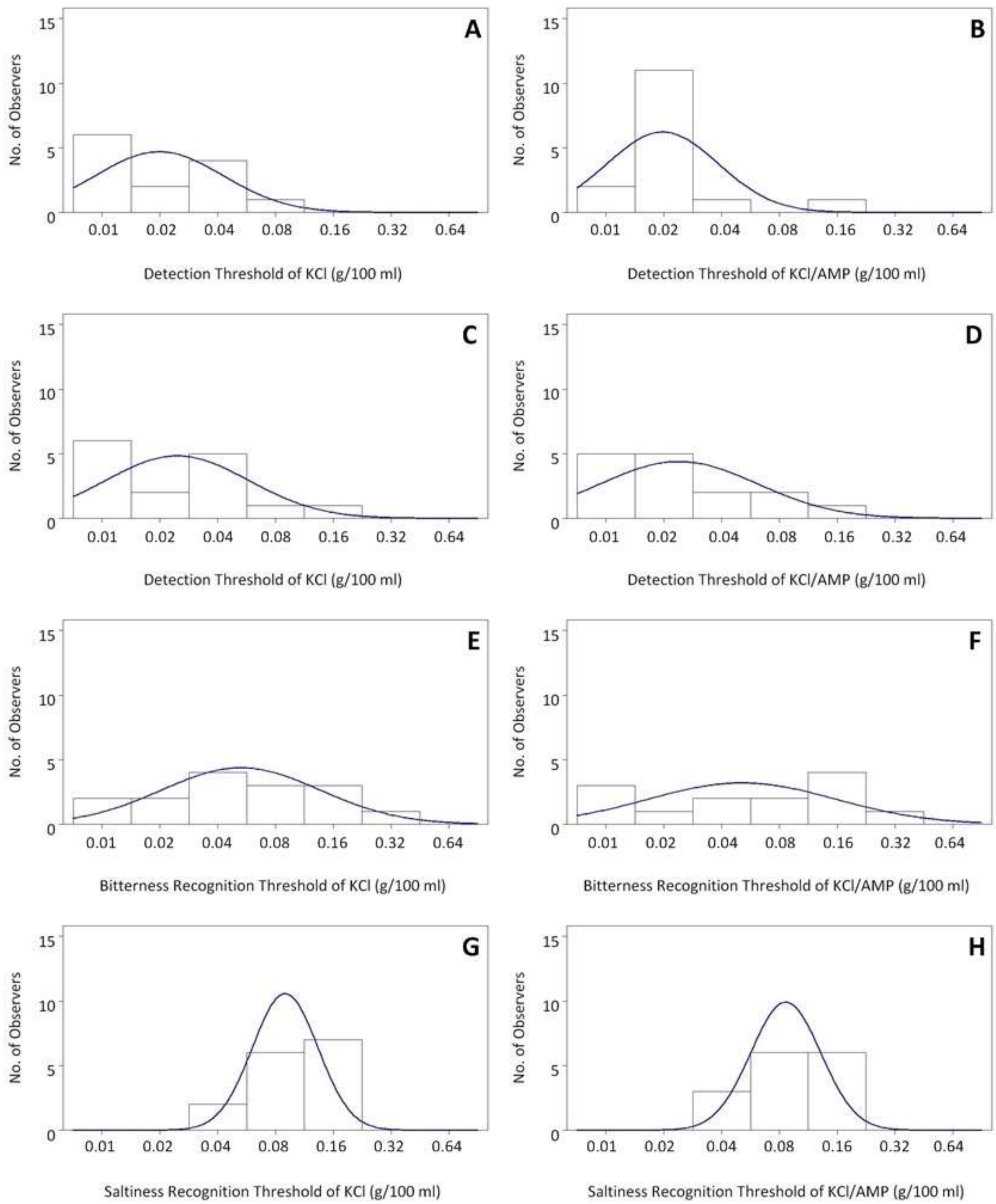


Figure 4.1 Individual threshold distributions of KCl and KCl/AMP using the ML method
(A) detection threshold of KCl, (B) detection threshold of KCl/AMP, (C) detection threshold of KCl, (D) detection threshold of KCl/AMP, (E) bitterness recognition threshold of KCl, (F) bitterness recognition threshold of KCl/AMP, (G) saltiness recognition threshold of KCl, (H) saltiness recognition threshold of KCl/AMP.

*(A)-(B) were obtained from the logistic method; (C)-(H) were obtained from the geometric mean.

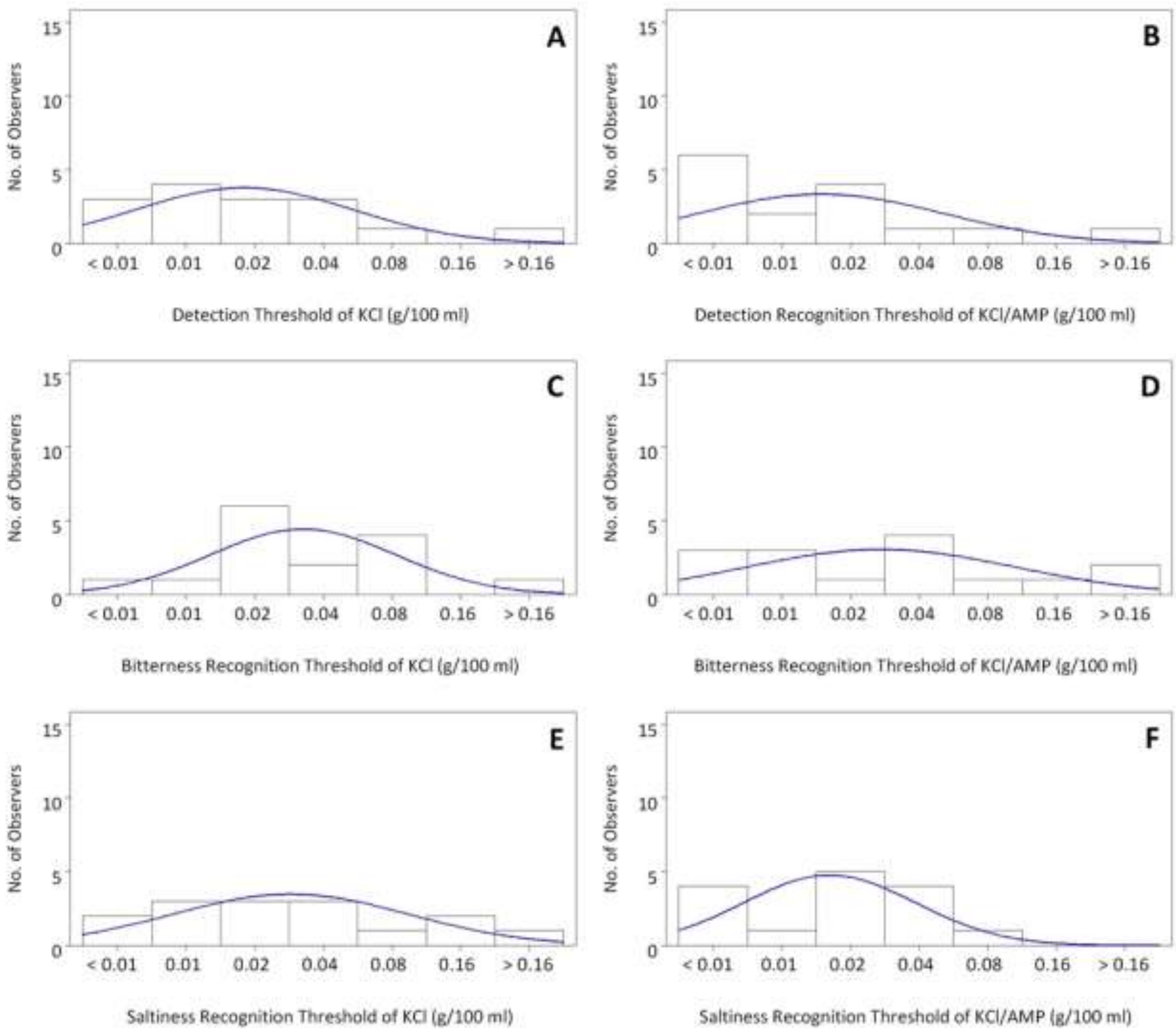


Figure 4.2 Individual threshold distributions of KCl and KCl/AMP using the SDT method. (A) detection threshold of KCl, (B) detection threshold of KCl/AMP, (C) bitterness recognition threshold of KCl, (D) bitterness recognition threshold of KCl/AMP, (E) saltiness recognition threshold of KCl, (F) saltiness recognition threshold of KCl/AMP.

### 4.3.2 Group Thresholds

The population thresholds by the method of limits were calculated by the arithmetic average of summation of the logarithm with base 10 of the individual BETs. Only the detection threshold obtained by the method of limits (ML) was calculated using logistic model. Means and standard deviations of thresholds of KCl and KCl/AMP are shown in Table 4.2. The side-by-side comparisons of threshold of KCl and KCl/AMP are presented in Figure 4.3.

Table 4.2 Population threshold values and standard deviations of KCl and KCl/AMP[a]

| Threshold Type | Solution | ML[b] | SDT[b] |
|---|---|---|---|
| Detection | KCl | 0.0191 ± 0.0039 | 0.0172± 0.0081 |
| | | (0.0144 ± 0.0293) | |
| | KCl/AMP | 0.0169 ± 0.0024 | 0.0174 ± 0.0030 |
| | | (0.0144 ± 0.0353) | |
| Recognition - Bitterness | KCl | 0.0399 ± 0.0057 | 0.0306 ± 0.0094 |
| | KCl/AMP | 0.0604 ± 0.0110 | 0.0310 ± 0.0156 |
| Recognition - Saltiness | KCl | 0.0837 ± 0.0296 | 0.0244 ± 0.0069 |
| | KCl/AMP | 0.0735 ± 0.0153 | 0.0233 ± 0.0043 |

[a]Threshold values (g/100 ml) ± standard deviations were based on 3 replications.
[b]ML = the method of limits using geometric mean. The values in the parenthesis were obtained from the method of limits using logistic regression model (ASTM E-1432). SDT = the signal detection method.

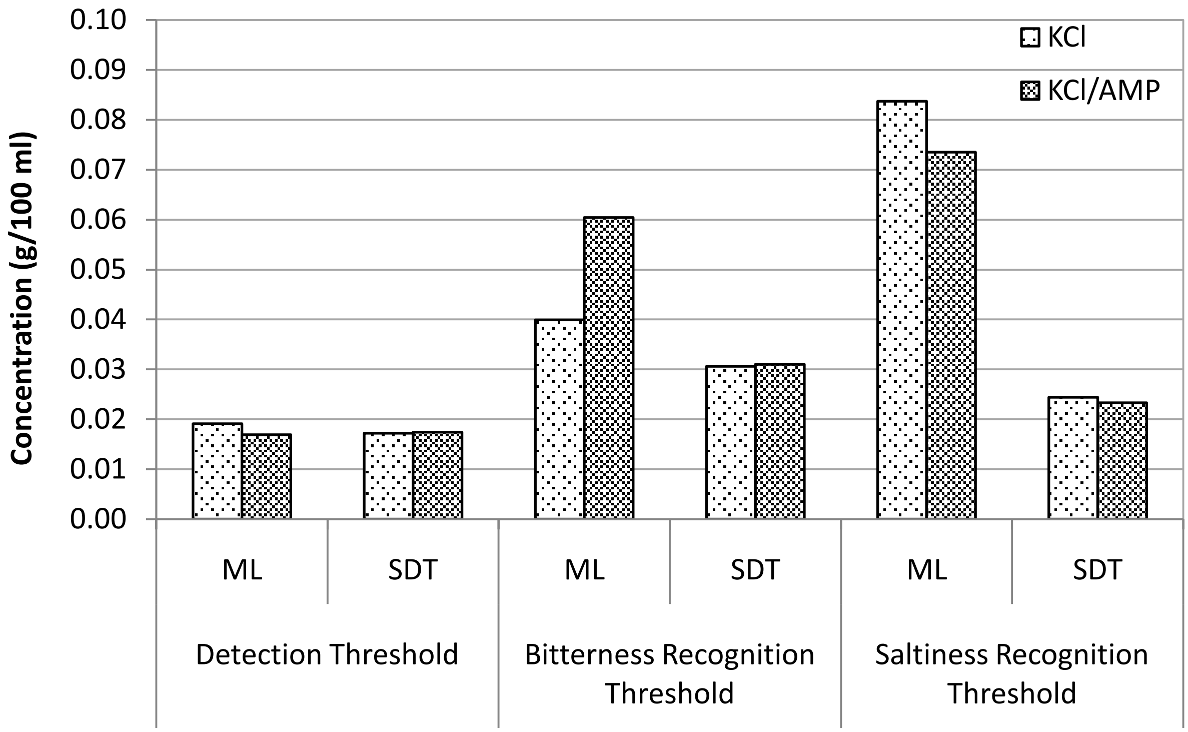


Figure 4.3 Population threshold values of KCl and KCl/AMP.
*ML = the method of limits using geometric mean. SDT = signal detection rating method.

- **Method of Limits (ML)**

The detection thresholds of KCl and KCl/AMP by the logistic method were the same value of 0.0144 g/100 ml or 1.93 mM (Table 4.2). The group BET mean for the detection thresholds were 0.0191 g/100 ml or 2.56 mM for KCl and 0.0169 g/100 ml or 2.27 mM for KCl/AMP. The Student's *t* test was performed to determine the difference between the detection thresholds of KCl and KCl/AMP for both the ML and the *R*-index methods at $\alpha$=0.05. No difference was found in the detection thresholds between KCl and KCl/AMP with a *p*-value of 0.4398 (Table 4.3). Threshold values obtained from the logistic model were lower than those obtained from the geometric mean, which is likely to be more accurate as the calculation method in ASTM E-679-04 is a rough method for estimating threshold values. Therefore, the precision of threshold estimations would be assessed by logistic model or other advanced model such as the dose-response model based on the beta-binomial distribution, see Bi and Ennis (1998) for more information.

Table 4.3 Threshold difference between KCl and KCl/AMP[a]

| Threshold | Method | Solution pair | Threshold Difference | SD[c] | *t* | *p*-value |
|---|---|---|---|---|---|---|
| Detection | ML | KCl - KCl/AMP | 0.0023 | 0.0032 | 0.86 | 0.4398 |
| | SDT | KCl - KCl/AMP | -0.0002 | 0.0061 | -0.04 | 0.9683 |
| Recognition - Bitterness | ML | KCl - KCl/AMP | -0.0205 | 0.0088 | -2.86 | 0.0459 |
| | SDT | KCl - KCl/AMP | -0.0004 | 0.0129 | -0.04 | 0.9703 |
| Recognition - Saltiness | ML | KCl - KCl/AMP | 0.0102 | 0.0236 | 0.53 | 0.6253 |
| | SDT | KCl - KCl/AMP | 0.0012 | 0.0034 | 0.25 | 0.8161 |

[a]*p*-value < 0.05 indicates significant difference in solution pair comparison using the Student's *t*-test.
[b]ML = the method of limits using geometric mean (ASTM E-679); SDT = signal detection rating method.
[c]SD = standard deviations.

For the recognition threshold of bitterness, the group BET mean threshold values of KCl were 0.0399 g/100 ml or 5.35 mM, and 0.0604 g/100 ml or 8.10 mM for KCl/AMP (Table 4.2). The value of recognition threshold of bitterness of KCl was significantly higher than that of KCl/AMP about 1.5 times with a *p*-value of 0.0459. With this significance, it would imply that the presence of AMP in KCl solution lowered the bitter taste perception imparted by KCl by raising the threshold from 0.04 g/100 ml to 0.06 g/100 ml. The mechanism of AMP in blocking bitter taste has been explained in previous studies (McGregor and Gravina 2005; Salemme and Barndt 2006). The activation of AMP with an *in vitro* assay is that AMP may bind to bitter-responsive taste receptors or interfere with receptor-G protein coupling to serve as naturally occurring taste modifiers (Ming and others 1999). The mechanism of AMP and related compounds in inhibiting bitterness perception is that the compounds inhibited the activation of transducin by bitter tastant-stimulated taste receptors and decreased neuronal stimulation by the tastants (Margolskee and others 2003).

For the saltiness threshold, the threshold concentrations of KCl and KCl/AMP were 0.0837 g/100 ml (11.23 mM) and 0.0735 g/100 ml (9.86 mM), respectively; and no significant difference between these two stimuli was found with a *p*-value of 0.6253. The salty taste was thus not enhanced by AMP. However, the saltiness recognition thresholds for KCl and KCl/AMP from this method were remarkably higher than the threshold obtained from the SDT method.

- **Signal Detection Rating Method**

The group means of the detection threshold were 0.0172 g/100 ml or 2.31 mM for KCl and 0.0174 g/100 ml or 2.33 mM for KCl/AMP (Table 4.2). However, no difference was found in the detection thresholds between KCl and KCl/AMP at $\alpha$=0.05 with a *p*-value of 0.97 (Table 4.3). For bitterness, the group average threshold of KCl was 0.0306 g/100 ml or 4.10 mM and 0.0310 g/100 ml or 4.16 mM when AMP was added to the KCl solution. The *p*-value of this solution pair from the Student's *t* test was 0.97, meaning that the bitterness of KCl was not inhibited with the presence of AMP in the KCl solution. For the recognition threshold of saltiness in Table 4.2, the averages of group threshold of KCl and KCl/AMP, which are 0.0244 and 0.0233 g/100 ml, or 3.27 and 3.13 mM, respectively; and they were not significantly different with a *p*-value of 0.82 (Table 4.3). From the results of this method, it is likely that the addition of AMP significantly affected neither bitterness nor saltiness of KCl.

Many studies have investigated the thresholds of five basic taste qualities, saltiness, sweetness, sourness, bitterness, and umami taste. The compounds that are often used as the taste stimuli are NaCl for saltiness; sucrose, saccharin, and aspartame for sweetness; HCl, citric acid, and acetic acid for sourness; quinine and caffeine for bitterness; and glutamate and amino acids for umami taste. However, the findings on the threshold values vary largely from one study to another; this would include three major factors; subjects, methods, and tastants. For subjects, age range, gender, health, and number of subjects participated influence the determinations of threshold as well as the experience of subjects with the experimental procedure. Some methods may be more powerful than others and the number of replications is associated with a decrease in variation; hence, these are responsive to the variation of threshold concentrations. The methods used in previous studies include a two-alternative forced choice (2-AFC) method (Weiffanbach and others 1982; Cowart 1989; Stevens and others 1991; Mojet and others 2001), a 3-AFC method (Gonázlez Viñas and others 1998; Robinson and others 2005; Lim and Lawless 2006; Pickering and others 2008), a 4-AFC method (Bartoshuk and others 1986), a triangle method (Schiffman and others 1981), a one-alternative recognition task (Cohen and Gitman 1959), a paired difference test (Cliff and Pickering 2006; Hatae and others 2009), a semi-ascending paired different test (Lundahl and others 1986; Chapman and others 2006), or a signal detection method (O'Mahony 1972; Argaiz and others 2005; Robinson and others 2005; Kappes and others 2006;

Lee and O'Mahony 2007a, 2007b). The concentration range of stimuli used in the study is also a factor of threshold sensitivity since the smaller the concentration step, the more accurate the threshold value. The instructions and the amount of solutions given should also be considered.

Hatae and others (2009) reported that the detection threshold and the recognition threshold of NaCl for 40 young female panelists were 0.719 mM and 0.968 mM, respectively. Gomez and others (2004) found that the detection and recognition thresholds for NaCl had the same value of 6.5mM. Weifenbach and others (1995) reported that the detection threshold of NaCl was 3.12 mM for 69 females (24-82 yr). Guyton and Hall (1996) stated that the recognition threshold of NaCl is 0.01 M and 0.000008 M for quinine. Henkin and others (1992) reported that the detection and recognition thresholds of NaCl were 12 mM and 30 mM for 21 subjects. However, the study on the thresholds of KCl is limited. Mojet and others (2001) investigated on the effect of gender and age on the threshold sensitivity of the basic tastes of 22 young (age 19-33 yr; 11M and 11F) and 21 elderly (age 60-75 yr; 10M and 11F) subjects using the 2-AFC method with 5 ascending concentrations. The age effect was found to be significant but not the gender. The detection thresholds for NaCl were 5.07 and 5.7 mM for young male and young female, respectively. The detection thresholds for KCl were 4.62 and 5.02 mM for young male and young female. Comparing the results, these values are higher than the thresholds obtained in this study by both ML and SDT methods which are 2.56 and 2.31 mM, respectively. As the subjects of the studies were about the same age, the threshold difference would have resulted from use of different methods.

### 4.3.3 Effect of Methods in the Threshold Determination

Comparing the sensitivity of the method of limits and the signal detection method, the Student *t*'s test was performed at $\alpha$=0.05 (Table 4.4). Differences between these two methods were found in the saltiness recognition threshold of KCl and KCl/AMP.

Table 4.4 Comparisons of methods used to determine threshold values of KCl and KCl/AMP[a]

| Threshold | Solution | Method pair[b] | Threshold Difference | SD[a] | $t$[a] | $p$-value[a] |
|---|---|---|---|---|---|---|
| Detection | KCl | ML - SDT | 0.0020 | 0.0064 | 0.38 | 0.7245 |
| | KCl/AMP | ML - SDT | 0.0005 | 0.0027 | -0.23 | 0.8319 |
| Recognition - Bitterness | KCl | ML - SDT | 0.0093 | 0.0078 | 1.46 | 0.2178 |
| | KCl/AMP | ML - SDT | 0.0294 | 0.0135 | 2.66 | 0.0561 |
| Recognition - Saltiness | KCl | ML - SDT | 0.0592 | 0.0215 | 3.37 | 0.0280 |
| | KCl/AMP | ML - SDT | 0.0502 | 0.0112 | 5.47 | 0.0054 |

[a]Threshold values (g/100 ml) and standard deviations (SD) are based on 3 replicates. $P$-value < 0.05 indicates significant difference in method pair comparison using the student's $t$-test.
[b]ML = the method of limits using geometric mean; SDT = signal detection rating method.

As mentioned earlier, the threshold determination is a measure of human sensory sensitivity. In other words, sensory threshold is to distinguish a sensation of a stimulus between imperceptible and perceptible. The models that are currently applied to the discrimination tests are the signal detection theory (SDT) and the Thurstonian model. These two models provide fundamental measures or *d'* which is independent of the measurement method and allows comparison across methods (O'Mahony and Hautus 2008). However, the Thurstonian's approach was mainly developed to determine the sensory difference across a range of stimulus intensities while the signal detection method was primarily established to measure the sensitivity for weak stimuli by separating the true discriminating from response bias (Lawless and Heymann 1998). The response bias or the criteria variation is a major problem in sensory different testing when the difference of two stimuli is small (O'Mahony and Rousseau 2002). The concept of response bias in a judge caused by criterion variation is established in signal detection theory (Green and Swets 1966).

When a sensory test is carried out, three main steps occur: perceiving stimuli/memorizing sensation, performing task, and giving response. Depending on the tasks, the response biases generated in the cognitive process are called the $\beta$-strategy and the $\tau$-criterion (O'Mahony and

Hautus 2008). In a yes/no task, the skimming or *β*-strategy, is the line on the intensity scale that one draws to separate two sensations/stimuli from each other; for example, salty or unsalty, "water" or "stimulus". However, the distance-based or *τ*-criterion, is associated with the comparison of the magnitude of differences; sometimes it is called a sensory yardstick. A length of the yardstick is a maximum difference to consider the difference of two stimuli as 'the same' (O'Mahony and Rousseau 2002; O'Mahony and Hautus 2008). In the method of limits of this study, the *τ*-criterion was applied in the panelists' task of picking the odd sample and the *β*-strategy in the process of stimulus identification. However, the signal detection method allows the judge to arbitrarily select the criterion in judgments varying from strict (*β*) to lax (*τ*) (O'Mahony 1991). A strict criterion is used when the judge is sure there is a difference. Conversely, a lax criterion is applied when the judge is not sure if there is a difference. Such a response scheme with responses graded in terms of sureness has the effect of forcing the judge to use several criteria simultaneously (O'Mahony 1991).

Robinson and others (2005) reported that a lower threshold concentration was obtained using the *R*-index measured by the signal detection rating method. In other words, the SDT method was more sensitive than the forced-choice ascending method of limits (ASTM E-679) since the panelists were exposed to multiple replicates of each stimulus in one session of the signal detection rating method while they had only one replicate per session in the ASTM method. Consequently, the panelists happened to be familiar with the stimulus and that gave a lower threshold. In this study, the SDT method gave a significantly lower threshold concentration than the method of limits for the recognition threshold for saltiness only and the panelists were exposed to the same number of replicates ($N$=3) for each test. However, the ML method is likely to be more accurate than the SDT method because the panelists perceived the difference in bitter taste quality with the presence of AMP which was substantiated by the result from the ML method but not the SDT method.

## 4.4 Conclusions

Developing a salt substitute with an acceptable taste has been a challenge for the food industry. This study demonstrated the ability of 5'-adenosine monophosphate (AMP) in decreasing bitterness imparted by KCl at least 1.5 times at the threshold level using the method of limits. Therefore, AMP is a potential bitterness inhibitor that could be used in cooperation with potassium chloride (KCl). The ASTM method of ascending limits provided more accurate results but it required more sample preparation and likely caused sensory fatigue in the panelists. On a related issue, I believe that the SDT is better but when it follows the new protocol and calculated as *U*/*mn*. The logistic model provides precise threshold estimates. In the future, a larger sample size should be done in order to use a more appropriate approach, a dose-response model based on the beta-binomial distribution.

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# CHAPTER 5.
# SENSORY CHARACTERISTICS OF SALT AND SALT SUBSTITUTES

## 5.1 Study I: Sensory Optimization of Salt Substitutes Containing L-Arginine

### 5.1.1 Introduction

Consumers' demand for low-sodium products has drastically increased for years (Gustafson 2008). In 2007, the U.S. Food and Drug Administration (FDA) held a public hearing regarding sodium in food. The topics included reducing and regulating the sodium amount in food. Moreover, salt also remains under consideration for removal from the "Generally Recognized As Safe" (GRAS) classification under the Food, Drug, and Cosmetics Act. Many scientists agreed that sodium is linked to the risk of high blood pressure and reducing sodium intake is advisable to prevent high blood pressure and decrease the elevated blood pressure. Moreover, the National Heart, Lung, and Blood Institute (NHLBI) revealed the Dietary Approaches to Stop Hypertension (DASH) eating plan in 2006 which recommends that Americans decrease sodium and increase potassium, calcium, and magnesium intakes to reduce elevated blood pressure.

As salt contributes to the major source of sodium intake, common alternatives to reduce sodium contents include decreasing the amount of salt used in foods and use of salt substitutes. Various salts have been determined as substitutes including KCl, $MgCl_2$, $NH_4Cl$, and LiCl (Frodley 1982; Barvieri 1983). In spite of its inherent bitter taste, KCl has been the most widely and successfully used as partial replacement for NaCl (Reddy and Marth 1991). However, factors that could affect sodium reduction strategies include the role of taste in product development, adaptability related to salt taste and sensitivity, and age of intervention. In the development of sodium-free salts, taste qualities have been a challenge for researchers as NaCl has a unique pure salty taste which is affected by both cation $Na^+$ and anion $Cl^-$. Moreover, the smaller the anion and cation, the more saltiness predominates over other tastes (Lawless 1992).

Adenosine-5'-monophosphate (AMP) was revealed to be the first natural compound that can block several bitter tastants (McGregor and Gravina 2005; Salemme and Barndt 2006). The activation of AMP with an *in vitro* assay is that AMP may bind to bitter-responsive taste receptors or interfere with the receptor-G protein coupling to serve as naturally occurring taste modifiers (Ming and others 1999). The mechanism of AMP and related compounds in inhibiting bitterness perception is that the compounds inhibit the activation of transducin by bitter tastant-stimulated taste receptors and decrease neuronal stimulation by the tastants (Margolskee and others 2003). Therefore, the bitter taste of KCl would be inhibited with the presence of AMP. One of the oldest techniques for demonstrating differences in taste potency among substances is the determination of the threshold concentration of a substance (Shallenberger 1993). Ogawa and others (2004) reported that L-arginine (Arg) has bitterness-masking ability by binding at the receptor site as well as an interaction between the guanidinium side-chain of Arg and the sodium channel in the human taste bud. L-arginine was also found to enhance the saltiness of NaCl (Ogawa and others 2004). Therefore, applying Arg in the KCl/AMP mixture may help enhance saltiness and suppressing bitterness. The objective of this study was to determine the optimal amount of Arg in the KCl/AMP solution.

### 5.1.2 Materials and Methods

#### 5.1.2.1 Sample Preparation

From the preliminary test, four different proportions of the mixture of KCl/Arg/AMP, KCl, L-arginine (Arg), and 5'-adenosine monophosphate (AMP), were established. KCl and L-arginine were purchased from Spectrum Chemical, Gardena, Calif. AMP was purchased from Zhen-Ao Group, Dalian, China. All ingredients were food grade. For sample preparation, KCl was prepared at 0.3% w/v in spring water so that all four samples had the same amount of KCl. Then AMP was added to the KCl solutions along with Arg based on the proportion shown in

Table 5.1. These proportions were developed from the preliminary test. The concentration of 0.3% was determined by the concentration above the recognition threshold from the previous study which was approximately 0.08%. However, a sensory test had been conducted using the concentration of 0.1% but no difference was observed among the four samples. The concentration was then increased up to 0.3% and the panelists who had been participated in the threshold study were also included in this test.

Table 5.1 Proportions of the mixed salt substitutes

| Sample | KCl | L-arginine | AMP |
|---|---|---|---|
| A | 15 | 0 | 1 |
| B | 15 | 2 | 1 |
| C | 15 | 4 | 1 |
| D | 15 | 6 | 1 |

All samples and references were prepared in odorless and tasteless Ozarka® spring water (Nestlé Waters North America, Greenwich, Conn.). The aqueous solutions were poured into 25-ml plastic cups with lids. The containers were coded with three-digit random numbers and kept at ambient temperature.

**5.1.2.2 Panelists**

Panelists who participated in this study were categorized into two groups (*N*=53). The first group of panelists was those who were not familiar with the solutions (*N*=38) and the second group was those who participated in the threshold study (*N*=15). One replicate was done for the inexperienced panelists while two replicates were done for the experienced panelists because inexperienced panelists did not perceived any taste difference among the four samples. Therefore, the panelists who participated in the study of threshold determination were included in the test and two replicates were assessed to decrease the variability within individuals. Each panelist was presented with the four samples and they were asked to taste the samples in the order presented from left to right, and then rank the samples in terms of intensity (1 = most intense and 4 = least intense). No tie was allowed for the rank score.

### 5.1.2.3 Data Analysis

#### 5.1.2.3.1 Friedman's Test and the Analog to Fisher's LSD

The Friedman-type nonparametric statistic was performed to analyze the ranked data of this study because the obtained rank data were considered ordinal. 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 "Difference in the intensity of saltiness/bitterness perception exists among four mixed salt solutions." 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 (5.1) below exceeds the $\chi^2$ distribution with $t$-1 degrees of freedom, while $t$ is number of samples.

$$T = \left[\frac{12}{bt(t+1)}\sum_{j=1}^{t} x_{.j}^2\right] - 3b(t+1) \qquad (5.1)$$

where $b$ = number of panelists,
$t$ = number of samples,
$\sum x_{.j}^2$ = summation of the squared 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 2006). The nonparametric analog to Fisher's LSD (Least Square Difference) for rank sums from a randomized complete block design is:

$$\text{LSD}_{\text{rank}} = Z_{\frac{\alpha}{2}}\sqrt{\frac{bt(t+1)}{6}} = t_{\frac{\alpha}{2},\infty}\sqrt{\frac{bt(t+1)}{6}} \qquad (5.2)$$

where $t_{\alpha/2,\infty} = 1.96$

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 nonparametric Friedman's test and the analog of LSD test were conducted at $\alpha$=0.05.

**5.1.2.3.2 *R*-Index**

*R*-Index is a nonparametric statistic that was developed by John Brown in 1974 to study recognition memory. The *R*-index is defined as the probability of distinguishing the two samples involved in the difference test (O'Mahony 1992). *R*-Indices can be computed from a variety of methods appropriate for sensitivity measurements in psychophysics, sensory difference testing, hedonic scaling, preference tests, and for investigating marketing information such as the measurement of consumer concepts (Lee and van Hout 2009). This technique allows a more powerful parametric statistical analysis.

In the *R*-index using a ranking response in this study, the samples (Table 5.1) were presented to the judge as N (Sample A), $S_1$ (Sample B), $S_2$ (Sample C), and $S_3$ (Sample D). The samples were ranked on a specified attribute (i.e., bitterness and saltiness) where 1 = the most intense and 4 = the least intense. Once the replications were performed, a response matrix can be constructed by using the frequency of each place of each sample as shown in Table 5.2. The Wilcoxon rank sum values were calculated using PROC NPAR1WAY (SAS® 9.1 2003) in order to obtain the Mann-Whitney *U* statistic which is related to the *R*-index (Bi 2006).

Table 5.2 Frequencies of ranked data for *R*-index calculation procedure

| Sample | Judge's Response | | | | Sample size |
|---|---|---|---|---|---|
| | $1^{st}$ | $2^{nd}$ | $3^{rd}$ | $4^{th}$ | |
| $S_1$ | a | b | c | d | m |
| N | e | f | g | h | n |

Let $X_1,\ldots X_n$ and $Y_1,\ldots Y_n$ be two independent samples, with sizes *m* and *n* from distribution *G* and *H*. The Mann – Whitney *U* statistic is

$$U = \sum_{i=1}^{m}\sum_{j=1}^{n} \emptyset(X_i, Y_j) \quad (5.3)$$

where $\emptyset(Xi, Yj) = \begin{cases} 1 & \text{if } X_i < Y_j, \\ \frac{1}{2} & \text{if } X_i = Y_j, \\ 0 & \text{otherwise} \end{cases}$

When $G$ and $H$ are continuous, $P(X_i = Y_i) = 0$. For the summarized rating frequency data of this study, the Man-Whitney $U$ statistic can be calculated from the following equation:

$$U = \sum_{j=1}^{k-1} b_i \sum_{j=i+1}^{k} a_j + \sum_{i=1}^{k} a_i b_i / 2 \quad (5.4)$$

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

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

$$R\text{-index} = \frac{U}{mn} \quad (5.5)$$

where $m$ and $n$ are sample size of two independent samples $X$ and $Y$ (Bi 2006).

Once a fractional $R$ is provided, it is more recognized to convert it to a percentage. It is necessary to determine whether $R$-index (%) is greater by chance (50%) at a given sample size ($N$) and level of significance ($\alpha$) by compared with the critical values (Bi and O'Mahony 2007). In this study, the null hypothesis was 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, meaning that the differences in the intensity of saltiness/bitterness perception among four mixed salt solutions exist.

### 5.1.3 Results and Discussion

#### 5.1.3.1 Non-Parametric Friedman's Test

The frequency of rank data and the rank sum values for saltiness and bitterness perception of the 0.3% w/v solutions are shown in Table 5.3 for inexperienced panelists (*N*=38) and in Table 5.4 for experienced panelists (*N*=15).

Table 5.3 Rank response frequency and rank sums for saltiness and bitterness perception for inexperienced panelists (*N*=38)

| Sample[a] | Response Frequency for Ranks[b] | | | | Rank Sum |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| Saltiness Perception | | | | | |
| A | 5 | 14 | 12 | 7 | 97[NS] |
| B | 10 | 11 | 9 | 8 | 91[NS] |
| C | 11 | 8 | 8 | 11 | 95[NS] |
| D | 12 | 5 | 9 | 12 | 97[NS] |
| Bitterness Perception | | | | | |
| A | 13 | 8 | 9 | 8 | 88[NS] |
| B | 8 | 11 | 9 | 10 | 97[NS] |
| C | 6 | 7 | 16 | 9 | 104[NS] |
| D | 11 | 12 | 4 | 11 | 91[NS] |

[a]See Table 5.1 for sample details.
[b]Ranks: 1 = most intense and 4 = least intense; Rank Sum = Σ(rank*response frequency).
[NS]No significant differences ($p > 0.05$).

Being stated as the null hypothesis, the saltiness of at least one sample is less than or equal to that of others at the significance level of 0.05 and the bitterness of at least one sample is more than or equal to that of others at the significance level of 0.05. The null hypothesis is rejected when the *T* calculated is greater than the critical value. For the one-sided test, the critical value $\chi^2$ ($\alpha$=0.05, df=3) was equal to 6.25.

Using the nonparametric Friedman's Test, the *T*-values ($\alpha$=0.05, *b*=38, and *t*=4) for the rank sum of the four different mixed salts at 0.3% w/v for inexperienced panelists were found to have the values of 0.38 and 2.37 for saltiness and bitterness perception, respectively. As these values were not greater than the critical value, the null hypothesis was not rejected. There was no sample that tasted saltier and less bitter than the others among the four samples at the concentration of 0.3% w/v for inexperienced panelists.

Table 5.4 Rank response frequency and rank sums for saltiness and bitterness perception for experienced panelists (*N*=15)

| Sample[a] | Response Frequency for Ranks[b] | | | | Rank Sum |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| | | | Saltiness Perception | | |
| A | 8 | 3 | 11 | 8 | 79 |
| B | 7 | 11 | 6 | 6 | 71 |
| C | 8 | 8 | 6 | 8 | 74 |
| D | 7 | 8 | 7 | 8 | 76 |
| | | | Bitterness Perception | | |
| A | 11 | 4 | 5 | 10 | $74^{AB}$ |
| B | 1 | 9 | 9 | 11 | $90^{B}$ |
| C | 8 | 10 | 10 | 2 | $66^{A}$ |
| D | 10 | 7 | 6 | 7 | $70^{A}$ |

[a]See Table 5.1 for sample details.
[b]Ranks: 1 = most intense and 4 = least intense; Rank Sum = Σ(rank*response frequency). Response frequency was based on 2 replications.
[A-B]Rank sum with different superscripts indicate significant differences ($p < 0.05$).

Due to the limited analysis of replicated ranking test, the results from two replicates of the experienced panelists were analyzed on the basis of the twice number of the panelists (*N*=15) which is 30. This may decrease the variations within panelists as the assumption of the response of an individual consumer is not a constant but a variable (Bi 2007). The *T*-values for saltiness and bitterness ($\alpha$=0.05, $b$=15×2, and $t$=4) of experienced panelists were 0.68 and 6.64, respectively. Therefore, the bitterness of at least one sample tasted different from others. The nonparametric analog to Fisher's LSD for rank sums was then performed and the $LSD_{rank}$ was calculated based on $b$=15×2, $t$=4, $\alpha$=0.05, $t_{\alpha/2,\infty}$=1.96 which was equal to 19.6. The two-sample rank sum values exceeding 19.6 were declared to be significantly different. As shown in Table 5.4, the bitterness rank sum of the sample B was significantly different from samples C and D but not different from A. This means that at the concentration of 0.3% w/v the relationship of the amount of L-arginine in the KCl/AMP mixture and the bitterness inhibition ability was not a linear relationship since the samples C and D seem to be more bitter than samples A and B. Moreover, the experienced panelists were more sensitive than the inexperienced panelists with the significance in the results.

#### 5.1.3.2 *R*-Index

Table 5.5 shows *R*-indices of saltiness and bitterness perception of all possible sample pairs at the concentrations of 0.3% w/v. The *R*-critical value for a 1-tailed test with $\alpha$=0.05 was 61.92 for $N$=15, and 60.65 for $N$=38.

Table 5.5 *R*-indices[a] of saltiness and bitterness perception for the sample pairs

| Sample Pair[b] | Saltiness | | Bitterness | |
|---|---|---|---|---|
| | Inexperienced Panelists | Experienced Panelists | Inexperienced Panelists | Experienced Panelists |
| A-B | 54.43% | 56.94% | 55.99% | 61.67% |
| A-C | 51.32% | 53.89% | 60.39% | 55.67% |
| A-D | 50.48% | 52.50% | 52.04% | 52.67% |
| B-C | 52.42% | 52.22% | 54.81% | **71.89%** |
| B-D | 53.67% | 54.17% | 54.09% | **66.44%** |
| C-D | 51.11% | 51.67% | 58.48% | 52.44% |

[a]For the inexperienced panelists, the *R*-Critical value ($N$=38, 1-tailed test, $\alpha$=0.05) is 60.65. For the experienced panelists, the *R*-Critical value ($N$=30, 1-tailed test, $\alpha$=0.05) is 61.92.
[b]See Table 5.1 for sample details.

From the results, it can be observed that both experienced and inexperienced panelists were not able to distinguish the saltiness perception of the mixed salt solutions at the concentration of 0.3% w/v. These *R*-indices which are lower than the critical value of 60.65

indicate that there was no sample that tastes saltier than others. In other words, L-arginine was not effective in enhancing saltiness of KCl at this concentration. According to bitterness perception, no significant difference was found by the inexperienced panelists. However, differences were found in two sample pairs for the experienced panelists. This result substantiates that presented in Tables 5.3 and 5.4 based on the nonparametric Friedman's Test. Moreover, Powers and Shinholser (1988) reported that training can lower thresholds as much as 1000-fold. Therefore, the experienced panelists were more sensitive than the inexperienced panelists. However, sample B was not significantly different from sample A but different from samples C and D. Hence, sample B is likely to have more potential in inhibiting bitterness with the highest rank sum value. The proportion of KCl/Arg/AMP at 15:2:1 was then selected for the further study of descriptive analysis.

### 5.1.4 Conclusion

At the concentration of 0.3%, the saltiness perception was not enhanced by the addition of arginine. Based on the result of this study, the use of L-arginine in order to improve bitterness-inhibiting in KCl/AMP was effective at the ratio of 15:2:1 (KCl:Arg:AMP). The nonparametric Friedman's test/the analog to Fisher's LSD and the nonparametric *R*-index ranking test provided the same result. Moreover, the panelists who were familiar with samples were likely more sensitive than those who were not. The further study of the salt mixture at higher concentrations or at the level of actual usage should be done.

## 5.2 Study II: Sensory Characteristics of Salt Substitutes Using the Spectrum™ Descriptive Analysis

### 5.2.1 Introduction

The challenge of developing an acceptable salt substitute is minimizing bitter taste and improving salty taste. KCl has been known as a potential salt alternative but its bitter taste is problematic. 5'-adenosine monophosphate (AMP) was found to have the ability to inhibit bitterness of various compounds (McGregor and Gravina 2005; Salemme and Barndt 2006). Therefore, AMP incorporated with KCl may be used to suppress the undesirable bitter sensation. Furthermore, L-arginine (Arg) was found to be able to mask the bitterness of various compounds and enhance the saltiness of NaCl (Ogawa and others 2004). The addition of Arg in a mixture with salt substitute may improve the taste qualities of KCl.

Descriptive analysis is the sensory method by which the attributes of a food or product are identified and quantified using human subjects who have been specifically trained for this purpose. So, this method provides information that cannot be obtained by other analytical methods (ASTM 1992). The use of descriptive analyses is for product maintenance, product improvement, product optimization, and product development. Many descriptive analysis techniques have been developed including the flavor profile method, the texture profile method, the Quantitative Descriptive Analysis (QDA®) method, the Spectrum™ Descriptive Analysis, time-intensity descriptive analysis, free-choice profiling, and flash profiling.

The Spectrum Descriptive Analysis, invented by Gail Civille, is a complete, detailed, and accurate descriptive characterization of a product's sensory attribute profile. The Spectrum method provides the description of the major product sensory categories, the detailed separation and description of each sensory attribute within each major sensory category, the perceived intensity of each attribute, and statistical evaluation of the descriptive data (Muňoz and Civille 1992). The procedures of the Spectrum technique are screening panelists, developing lexicons, training the judges, determining judge reproducibility, evaluating samples, and then analyzing and interpreting the data. Lexicons are the word lists describing perceived attributes associated with the samples and are based on common terminology agreed upon the panelists. The purpose is to make the resulting profiles universally understandable and usable (Meilgaard and others

2006). The unique characteristics of the Spectrum technique include a more technical profile, use of a standardized lexicon of terms, standardized and anchored with multiple reference points (Lawless and Heymann 1998; Meilgaard and others 2006).

The objectives of this phase of the study were to quantify the intensity of each sensory attribute of different mixed salt solutions using the Spectrum descriptive method and to determine the effect of AMP on inhibiting bitterness perception of a salt substitute at an actual usage range.

### 5.2.2 Materials and Methods

#### 5.2.2.1 Samples and References

The reference samples used in this study were NaCl and caffeine for saltiness and bitterness perception, respectively. Five different salt solutions and their mixture proportions were used as test samples including NaCl, KCl, KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Salemme and Barndt (2006) reported that the amount of KCl added to a composition will vary depending on the amount of perceived saltiness desired and other compounds present in the composition; for example, KCl may be present at a concentration between about 0.5% to 1.5%, preferably about 0.9% of the food or beverage. Therefore, test samples in the evaluation session were prepared at 0.5% and 1% w/v. To compare the taste intensities of the samples, the salt mixtures were prepared on a basis of KCl weight so that KCl, KCl/Arg, KCl/AMP, and KCl/Arg/AMP had the same amount of KCl. Then AMP or Arg was added to the solutions in the specified proportion to the amount of KCl. KCl, NaCl, Arg, and caffeine were purchased from Spectrum Chemical, Gardena, Calif.). AMP was purchased from Zhen-Ao Group, Dalian, China). All ingredients were food grade. Samples and references were freshly prepared with odor- and tasteless Ozarka® spring water (Nestlé Waters North America, Greenwich, Conn.).

#### 5.2.2.2 Panel Selection

Panelists from Louisiana State University were recruited and pre-screened using the following criteria: availability, health, general product attitudes and sensory awareness, and rating ability. Next, judges must be qualified for the screening phase with the acuity tests in which they had to be able to detect and describe sensory characteristics and rate their intensities using matching, ranking, and rating tests. Finally, twelve judges were selected to participate in the training process.

#### 5.2.2.3 Panel Training

The training program was required for panelists to be able to discriminate, describe, and quantify the sensory characteristics of products following the Spectrum method. The main purposes of training were to expose the panel to the underlying dimensions of sensory attributes to ensure accurate evaluation of the characteristics and to provide a similar frame of reference in terminology and scaling among all participants. The training program consisted of two parts, general orientation and practice sessions. The general orientation included a detailed explanation about the descriptive sensory methodology, a review of samples, and panel training to use intensity references and scales. The practice sessions included a review of the sample references and evaluation procedures, and the product evaluation and discussion of results.

Two basic tastes (bitterness and saltiness) were used as reference samples. Caffeine solutions in water were used as a bitterness reference. Two caffeine solutions were prepared, which corresponded to two reference points on 15-cm scale. The caffeine concentrations of 0.05% and 0.08% corresponded to the intensity scale values (0-15) of 2 and 5, respectively (Meilgaard and others 2006). For saltiness perception, NaCl solutions in water were used as a saltiness reference. There were four reference points for saltiness intensities. The reference points of 8.5, 15, 18, and 22 correspond to NaCl concentrations of 0.5%, 0.7%, 1%, and 1.4% as shown in Table 5.6. Once panelists had been trained, the test samples were given to them to evaluate. These exercises allowed panelists to apply developed concepts and terminology. The total training time was 15 h or until they had less than 10% of error of the intensity scale values. Then, two sessions of the training program had been completed and the product evaluation was performed to collect data for statistical analysis.

Table 5.6 Taste lexicon for salt

| Attribute | Definition[a] | Attribute | Intensity | |
|---|---|---|---|---|
| Salty | The basic taste, perceived on the tongue, stimulated by sodium salt, especially sodium chloride. | Sodium chloride solutions in spring water. | 0.5% | 8.5 |
| | | | 0.7% | 15 |
| | | | 1.0% | 18 |
| | | | 1.4% | 22 |
| Bitter | The basic taste, perceived on the tongue stimulated by substances such as quinine, caffeine, and certain other alkaloids. | Caffeine solutions in spring water. | 0.05% | 2 |
| | | | 0.10% | 5 |

(Source: Lee and others 2008; Kwan 2004)

#### 5.2.2.4 Product Evaluation

Trained panelists evaluated test samples in individual booths. Unsalted, plain crackers and water were provided to cleanse the palate during the evaluation. A15-cm anchored scale was used to measure the intensities of the bitterness perception where 0 = none, 15 = extreme. A 22-cm anchored scale was used to measure the intensities of the saltiness perception where 0 = none, 22 = extreme (Kwan 2004). Three replications of each sample were performed for both saltiness and bitterness perception. Individual scores were collected and analyzed statistically.

#### 5.2.2.5 Data Analysis

Data were analyzed using univariate and multivariate data analysis techniques at $\alpha$=0.05. Multivariate analysis of variance (MANOVA) was conducted to determine the overall difference among the test samples and the saltiness and bitterness intensities. If significance existed, an analysis of variance (ANOVA) was then performed to determine if differences of the taste intensities exist among samples. If the significances among the samples had been found, the Tukey's studentized range test was then performed for post-hoc multiple comparisons. Moreover, the single linkage hierarchical clustering algorithm, using Euclidean distances, was used to identify clusters of salts based on bitterness and saltiness intensities. Subsequently, principal component analysis (PCA) was performed to correlate the relationship of sensory attributes and samples as illustrated in a product-attribute bi-plot (Lipkovich and Smith 2001).

**5.2.3 Results and Discussion**

**5.2.3.1 Multivariate Analysis of Variance**

Table 5.7 shows multivariate statistics for the saltiness and bitterness intensities among five different salts. The *p*-value of Wilks' Lambda of less than 0.001 indicates that an overall difference existed among five salts when the taste intensities at different concentrations were considered simultaneously.

Table 5.7 Multivariate statistics and *F* approximations

| Statistic | Value | F Value | Num DF | Den DF | Pr > F |
|---|---|---|---|---|---|
| Wilks' Lambda | 0.4397 | 22.10 | 8 | 348 | <.0001 |
| Pillai's Trace | 0.5761 | 17.70 | 8 | 350 | <.0001 |
| Hotelling-Lawley Trace | 1.2387 | 26.85 | 8 | 246.26 | <.0001 |
| Roy's Greatest Root | 1.2091 | 52.90 | 4 | 175 | <.0001 |

**5.2.3.2 Analysis of Variance**

ANOVA was performed to determine whether differences of bitterness/saltiness intensities exist among all five salt solutions using the Statistical Analysis Software® (SAS 2003). Table 5.8 shows the mean values, standard deviations, and *p*-values for the intensities of saltiness and bitterness of the five salt solutions. For saltiness, the *p*-values were less than 0.0001 meaning that the saltiness intensities of salts were significantly different from each other at a given concentration (0.5% and 1%). Likewise, the bitterness intensities of salt solutions were significantly different from each other at a given concentration (0.5% and 1%) with $p < 0.0001$.

Table 5.8 Means ± standard deviations and ANOVA *p*-values for saltiness and bitterness intensities of five salt solutions at 0.5% and 1% w/v

| Salt Solution[a] | Saltiness | | Bitterness | |
|---|---|---|---|---|
| | 0.5% | 1 % | 0.5% | 1% |
| NaCl | 8.04 ± 0.52[A] | 17.38 ± 0.89[A] | 0.02 ± 0.07[B] | 0.08 ± 0.28[C] |
| KCl | 2.72 ± 1.84[C] | 8.06 ± 3.99[B] | 1.07 ± 0.60[A] | 2.61 ± 2.28[A] |
| KCl/Arg | 3.59 ± 2.13[BC] | 9.74 ± 4.42[B] | 1.02 ± 0.64[A] | 2.06 ± 1.79[AB] |
| KCl/AMP | 3.41 ± 2.21[BC] | 7.69 ± 4.02[B] | 0.89 ± 0.56[A] | 1.94 ± 1.54[AB] |
| KCl/Arg/AMP | 4.20 ± 2.15[B] | 10.16 ± 5.00[B] | 0.75 ± 0.45[A] | 1.53 ± 1.26[B] |
| *Pr* > F | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

[a]The proportions of salt solutions were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1).
[A-C]Means within the same column with different letters are significantly different ($p < 0.05$).

As the concentration increases, the bitterness and saltiness intensities increased. Regardless of concentrations, NaCl was the saltiest and the least bitter solution and it is significantly different from other sodium-free salt solutions. On the contrary, KCl was the least salty and the most bitter solution. When either L-arginine or AMP had been added in the KCl solution, the saltiness perception was slightly enhanced and the bitterness perception decreased but not significantly different from the KCl solution. However, the salty quality was significantly intensified and the bitter quality was lowered when both L-arginine and AMP had been presented in the KCl solution at 0.5% and 1%, respectively. Therefore, L-arginine is more likely responsible for saltiness enhancement and AMP is more related to bitterness inhibition. Moreover, L-arginine and AMP seem to have synergistic effect when they are used together.

#### 5.2.3.3 Cluster Analysis

The results achieved by the cluster analysis (CA) of five salts considering bitterness and saltiness intensities at the concentration of 0.5% and 1% are presented as a dendrogram in Figure 5.1. Three clusters of groups were observed; (1) NaCl, (2) KCl and KCl/AMP, (3) KCl/Arg and KCl/Arg/AMP. These results disclosed that there were differences among salts in the taste intensities. KCl/Arg and KCl/Arg/AMP had the smallest inter-cluster distance indicated by the vertical line linking them. Next, KCl and KCl/AMP had the next smallest inter-cluster distance, so they are joined. Subsequently, NaCl and the other four salt substitutes had the biggest inter-cluster distance. Considered by groups, sodium salt and L-arginine are the substances underlying differences among salts in terms of bitterness and saltiness intensities.

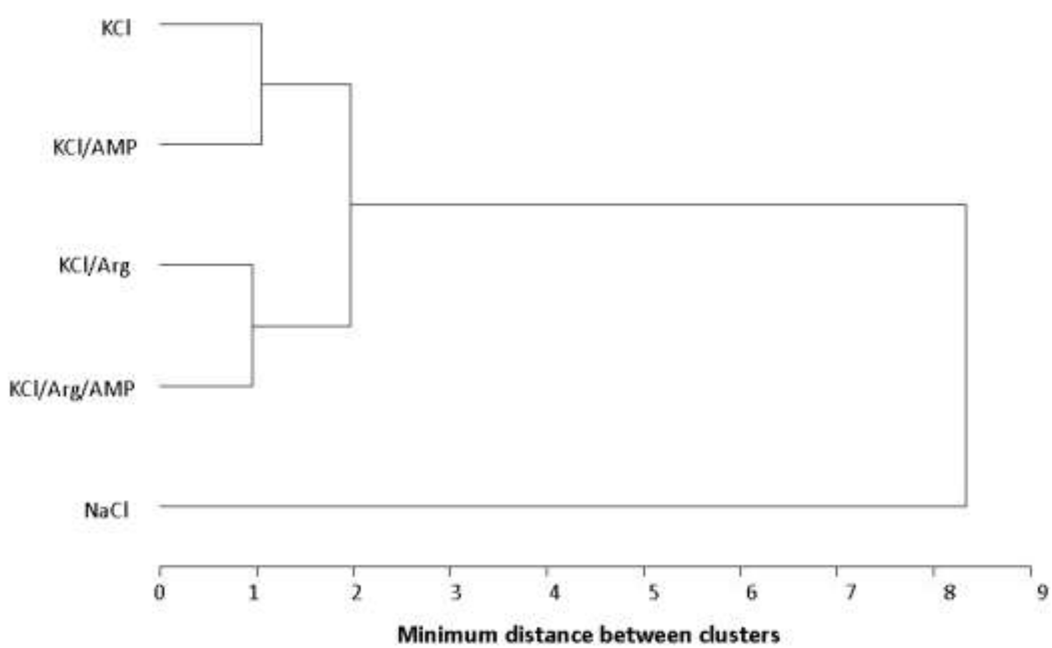


Figure 5.1 Dendrogram from cluster analysis of different salts considering bitterness and saltiness intensities.

#### 5.2.3.4 Principal Component Analysis

The bi-plot expressing the results for principal component analysis was constructed to show the relative positions of the salts and sensory attributes. Figure 5.2 shows the results in which bitterness and saltiness perception at the concentrations of 0.5% and 1% are displayed in the plot of the first two principal components of the product characterization data. The first 2 PC of the PCA explains 99.8% of total variability in the salt samples. This percentage of total variability is relatively high.

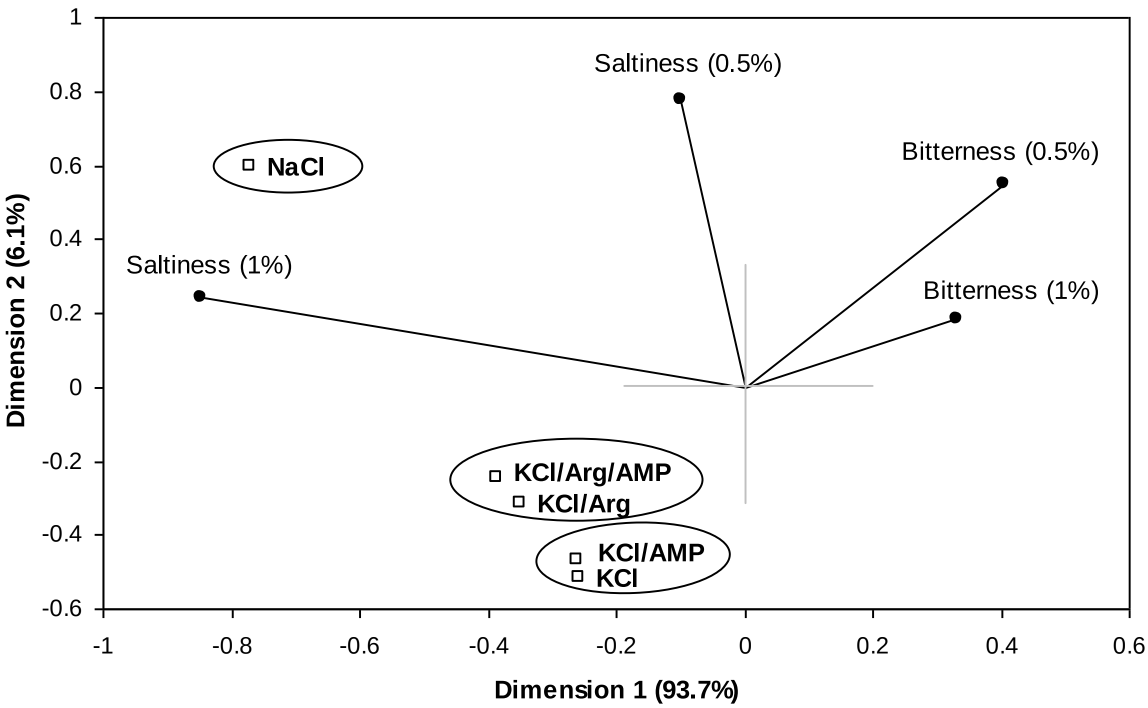


Figure 5.2 Principal component analysis using a bi-plot for salts and taste intensities.

As expected, the bitterness (0.5% and 1%) vectors point straight to the same direction as well as the saltiness (0.5% and 1%) vectors. Conversely, at the concentration of 0.5%, the saltiness vector is almost perpendicular with the bitterness vector, meaning that these two attributes are not significantly correlated. According to the relationship between salts and sensory attributes, it can be seen that NaCl is found to be associated with the saltiness perception, especially at the concentration of 1%. Potassium salts are related among them. This result also agrees with the result from the cluster analysis in that salts are classified into three groups. The potassium salts that contained Arg are likely more associated with saltiness than those without Arg. In other words, the saltiness perception was intensified with the presence of Arg in KCl solutions.

### 5.2.4 Conclusions

L-arginine and 5'-adenosine monophosphate had a synergistic effect not only in inhibiting undesirable bitterness but also enhancing saltiness in KCl solutions. The further studies would be the application of this sodium-free salt mixture in food systems and the effect of use of the salt on consumer acceptability compared to the regular salt and the commercial salt substitute in the market.

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# CHAPTER 6.
# CONSUMER ACCEPTABILITY OF PASTA SAUCES CONTAINING SALT SUBSTITUTES

## 6.1 Introduction

The awareness of health risks associated with food ingredients has drastically increased for recent years. Not only consumers and food industries but the medical community has been alerted on sodium content in foods since an excess sodium consumption may increase the chance of developing hypertension which can lead to other health problems. Recently, the Center for Science in the Public Interest (CSPI) and the American Medical Association (AMA) requested the Food and Drug Administration (FDA) to regulate the sodium amount in foods by reviewing and changing the status of sodium from being current Generally Recognized As Safe (GRAS) to being restricted as a food additive. Moreover, the reduction of Daily Value of sodium from 2,400 mg to 1,500 mg has also been under consideration. The 2005 Dietary Guidelines for Americans recommend limiting sodium daily intake to 2,400 mg for young people and 1,500 mg for African-Americans, and middle-aged and elderly people. However, Americans consume sodium at two to three times higher than the recommended amount and Americans' sodium daily intake steadily increases.

Besides reducing the amount of salt used, the most-common approach to lower sodium contents in diets is the use of salt substitutes. Many studies have investigated substitutes for NaCl but it seems no other salts can replace NaCl in terms of taste quality and acceptability. The saltiness of NaCl is unique, pure, and clean as it is believed that both cation and anion are associated with the overall taste quality of salts (Moncrieff 1967; Deman 1976; Murphy and others 1981). The smaller the anion and cation, the more saltiness predominates over other tastes (Lawless 1992). NaCl solutions at low concentrations elicit sweetness and it provides saltiness with increasing concentration (Shallenberger 1993). However, KCl has been the most widely and successfully used for partial replacement for NaCl regardless of the bitter taste (Reddy and Marth 1991). To develop a sodium-free salt, bitterness blockers would be added to decrease the bitterness or metallic off notes of KCl by blocking the bitterness receptors. Adenosine-5'-monophosphate (AMP) has been reported to be able to block several bitter tastants (McGregor and Gravina 2005; Salemme and Barndt 2006). The mechanism of AMP and related compounds in inhibiting bitterness perception is that the compounds inhibited the activation of transducin by bitter tastant-stimulated taste receptors and decreased neuronal stimulation by the tastants (Margolskee and others 2003). Moreover, L-arginine (Arg) 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 Arg and the sodium channel in the human taste bud (Ogawa and others 2004). With these properties, AMP and Arg would be used to synergistically improve the taste qualities of KCl.

Pasta sauces in the market contain approximately 25% or 600 mg of the sodium content of the Daily Value per serving. With its high sodium content, the pasta sauce product would be an ideal food model for the evaluation of salt substitutes. Reformulating the products may result in changes of consumer acceptance and consumers expect products in similar taste, and flavor of those familiar products (Rosett and others 1995; Lawless and Heymann 1998). Therefore, it is necessary to evaluate consumer acceptability of the products containing salt substitutes. The objectives of this study were to determine consumer acceptability of pastas served with the sauces containing a salt substitute and identify the sensory attributes that would greatly contribute to the success of this product in terms of overall consumer acceptability.

## 6.2 Materials and Methods

### 6.2.1 Sample Preparation

Pasta sauces were used as a food model system to investigate the consumer acceptability of salt substitute with bitterness blockers. Ingredients and the pasta sauce formula are provided in Table 6.1. Five different types of salt were used, including NaCl, KCl, KCl/AMP (15:1), KCl/Arg/AMP (15:2:1), and a commercial salt substitute. Salemme and Barndt (2006) reported that the amount of KCl added to a composition will vary depending on the amount of perceived saltiness desired and other compounds present in the composition; for example, KCl may be present at a concentration between 0.5% to 1.5%. Therefore, the concentration of salt added in the sauce was 1% by weight. The salt mixtures were prepared on a basis of KCl weight at the concentration of 1% so that the treatments of KCl, KCl/AMP, and KCl/Arg/AMP had the same quantity of KCl with the addition of arginine and AMP. KCl and Arg were purchased from Spectrum Chemical, Gardena, Calif. AMP was purchased from Zhen-Ao Group, Dalian, China. The salts used in this study were FCC-graded NaCl, KCl, Arg, and AMP. All ingredients other than salts used in the recipe were salt-free. Sauces were prepared the day before the consumer test was conducted.

Table 6.1 Ingredients and the pasta sauce formula

| Ingredients | Source | % By Weight |
|---|---|---|
| Tomato Paste | ConAgra Foods, Omaha, Neb. | 30.52% |
| Water | Nestlé Waters North America, Greenwich, Conn. | 26.92% |
| Fresh onion | Local supermarket, Baton Rouge, La. | 12.56% |
| Fresh bell pepper | Local supermarket, Baton Rouge, La. | 12.56% |
| Fresh tomato | Local supermarket, Baton Rouge, La. | 12.56% |
| Unsalted butter | Wal-Mart Stores, Bentonville, Ark. | 2.69% |
| Salt* | | 1.00% |
| Sugar | Domino Foods, Yonkers, N.Y. | 0.90% |
| Mixed Italian seasoning | Magic Seasoning Blends, Harahan, La. | 0.19% |
| Garlic powder | McCormick & Co., Hunt Valley, Md. | 0.05% |
| Onion powder | Adams Extract & Spice, Gonzales, Texas. | 0.05% |

*Salt – varied by treatments (NaCl, KCl, KCl/AMP, KCl/Arg/AMP, and commercial salt substitute).

Before the test was started, the sauces were brought to a light boil on the stovetop. Salt was then added according to the sauce weight. Five samples were prepared with different combinations of salt: NaCl, KCl, KCl/AMP, KCl/Arg/AMP, and a commercial salt substitute. The commercial salt substitute (Chicago, Ill.) contained KCl, fumaric acid, tricalcium phosphate, monocalcium phosphate. The ratios of salt mixtures (KCl/AMP and KCl/Arg/AMP) were determined from the first two studies. If there was a bitterness blocker in the salt mixture, the amount of the blocker was associated with the weight of sauces instead. Therefore, all four non-commercial salts had the same amount of primary salty tastant (NaCl or KCl). However, as the percentage of KCl in the commercial salt substitute was unknown, the amount of KCl was determined as the amount of commercial salt substitute.

The sauces, once mixed with the salt, were placed into a slow cooker that was set on the "warm" setting. At the "warm" setting, the product was hold at a temperature of 55 °C. Bowtie shaped pastas (Luxury Pasta, Kansas City, Mo.) were used in this study since it was easy to put in the serving cups and its size fit the cups well. The bowties were cooked in boiling water for 14 minutes according to package instructions with no salt added. Two cooked bowties were placed into a 2-oz plastic cup. Approximately 15 g of sauces were then placed on top of the pasta. Preliminary testing established that this amount of sauce and pasta was appropriate. The cups were covered with lids with 3-digit code labels. Samples were presented to consumers according to the balanced incomplete block (BIB) design.

**6.2.2 Experimental Design**

In this study, interest lies in comparing salt substitutes with original table salt rather than among salt substitutes. Therefore, a balanced incomplete block design augmented with a control treatment in every block was used. The balanced incomplete block design with $t + 1 = 5$, $k + 1 = 3$, $r = 3$, $b = 6$, $\lambda = 1$ (Gacula and others 2008) was employed due to the number of samples were 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 2006). So, each consumer was presented with two samples and one control sample (Table 6.2). The control sample was assessed 204 times while others were evaluated 102 times. The order of samples was counter-balanced within and across judges.

Table 6.2 Balanced incomplete block design augmented with control in every block

| Blocks | Control | KCl | KCl/AMP | KCl/Arg/AMP | Commercial SS | Design Parameters |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| 1 | × | × | × | | | $t + 1 = 5$ |
| 2 | × | | | × | × | $k + 1 = 3$ |
| 3 | × | × | | × | | $r = 3$ |
| 4 | × | | × | | × | $b = 6$ |
| 5 | × | × | | | × | $p = 1$ |
| 6 | × | | × | × | | $N = bp(k + 1) = 18$ |

$t$ = number of treatments;
$k$ = number of treatments received per block;
$r$ = number of replications of each treatment;
$b$ = number of blocks (panelists);
$\lambda$ = number of blocks in which pair of treatments are compared;
$p$ = number of repetitions;
$N$ = number of total samples.
(Source: Gacula and others 2008).

### 6.2.3 Consumer Test

A total of two hundred and four consumers ($N$=204) participated in the central-location consumer acceptance test. Participants were students, faculty, and staff randomly chosen from the Louisiana State University campus. The participants must meet the following criteria for the recruitment: (1) they had to be at least 18 years of age, (2) they were not allergic to butter, wheat, tomato, bell pepper, onion, spices, regular salt, potassium chloride, nucleotide, and amino acid

(3) and they were willing and available for participation and for the completion of the study. Individuals who had kidney problem were asked to not participate in this study.

Each participant was presented with three samples. A glass of water was also served to each subject to cleanse the palate between samples. They were asked to answer demographic questions such as gender and age. Consumers rated pastas with the sauces for appearance, flavor, taste, saltiness, and overall acceptability on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely. The saltiness intensity of the sauce was rated on a 3-point just-about-right (JAR) scale where 1 = not salty enough, 2 = just about right, and 3 = too salty. The bitterness detection was also rated on a yes/no scale along with the acceptance if the bitter taste was detected where 0 = no, 1 = yes but acceptable, and 2 = yes but unacceptable. Moreover, the bitterness intensity was also rated on a 3-point scale only if the bitterness was detected where 1 = weak, 2 = moderate, and 3 = strong. The binomial type questions (yes/no) were used to evaluate overall product acceptance and purchase intent. Product acceptance and purchase intent after the claim of no added sodium which does not cause high blood pressure had been given to consumers were also asked.

**6.2.4 Statistical Analysis**

Data were analyzed at $\alpha$=0.05 using SAS® software (SAS 2003). The statistical methods used to analyze the data were nonparametric, univariate and multivariate techniques as follows.

**6.2.4.1 Multivariate Analysis of Variance and Descriptive Discriminant Analysis**

Multivariate analysis of variance (MANOVA) is an extension of analysis of variance (ANOVA) where the basic principles are the same. MANOVA is used to determine a significant difference of the measurement values between classes. MANOVA has advantages over multiple ANOVAs as performing multiple ANOVAs may increase the overall Type I error rate (Stevens 1986) and the collinearity among the variables is not taken into account (Lawless and Heymann

1998). In addition, MANOVA is used in conjunction with discriminant analysis. Descriptive discriminant analysis (DDA) or canonical variate analysis is the mean separation techniques for MANOVA (Lawless and Heymann 1998). It is used to identify the attributes which were responsible for the underlying differences among samples (Huberty 1994). MANOVA was conducted to determine the overall difference among the test samples and sensory attributes. DDA was applied to identify sensory attributes that most contributing to the overall difference among the samples.

**6.2.4.2 Analysis of Variance**

Analysis of Variance (ANOVA) is a statistical technique used to determine 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. For theoretical validity of this technique, one assumes that observations follow the normal distribution and that the error terms are independently distributed (Gacula and others 2008). ANOVA was performed in this study to determine whether differences over the samples exist in terms of acceptability of each sensory attribute as well as overall liking of the products. As the balanced incomplete block design augmented with a control had been used in this study, the effects of repetitions, blocks within repetitions and treatments were also taken into account in the *F*-statistic calculation (Table 6.3; Gacula 2003; Gacula and others 2008). If the significances among the samples were found, the confidence interval would be calculated for testing the significance of pairwise comparisons of means. Statistical significance is declared when zero is not included in the interval (Gacula 2003).

$$d - Z_{\propto/2}SE \le D \le d - Z_{1-\propto/2}SE \qquad (6.1)$$

where $d$ = estimated difference between means;

$Z_{\alpha/2}$, $Z_{1-\alpha/2}$ = normal deviates from the standard normal distribution;

$D$ = value of the difference between means under the null hypothesis;

and $SE$ = standard error.

As shown in Table 6.2, the control treatment in the intramodel is designated treatment 1, and the other $t$ treatments are numbered 2, 3,.., ($t$ +1) for statistical analysis. $X_{i..}$, $X_{.j.}$ denote the total of the observations for the $i^{th}$ treatment, the $j^{th}$ block, and the $m^{th}$ repetition, respectively; $i$ = 1,…, $t$ +1; $j$ = 1,.., $b$; $m$ = 1,…, $p$. $B_{(i)}$ denotes the total of all blocks having the $i^{th}$ treatment. $Q_i$ is defined as follows:

$$(k+1)Q_i = (k+1)X_i - B_{(i)}, \qquad i = 1, \dots, t+1. \qquad (6.2)$$

The intrablock estimates of the treatment effects are given by

$$t_1 = (k+1)Q_i/bk, \qquad (6.3)$$

$$t_i = (k+1)(Q_i + Q_1/t)/(rk+\lambda), \qquad i = 2, \dots, t+1. \qquad (6.4)$$

Table 6.3 ANOVA table for the BIB design augmented with control treatment in every block

| Source of Variance | DF | SS |
|---|---|---|
| Total | $N-1$ | $SSto = \sum_i \sum_j \sum_m X_{ijm}^2 - CF$ |
| Repetitions | $p-1$ | $SSp = \frac{\sum_m R_m^2}{b(k+1)} - CF$ |
| Blocks (Unadjusted for Treatment) within Repetitions | $p(b-1)$ | $SSbl{:}p = \frac{\sum_j X_{.j}^2}{(k+1)} - SSp - CF$ |
| Treatments (Adjusted for Blocks) | $t$ | $SStr_{adj} = \sum_i \hat{t_i} Q_i$ |
| Error | $N - pb + p + t$ | $SSe = SSto - SSp - SSbl{:}p - SStr_{adj}$ |

- $R_m$ = the total of the $m^{th}$ repetition;
- $CF = (\Sigma R_m)^2/N$;
- $F$ statistic = $MStr_{adj}/MS_e$;
- See Table 6.2 for details of other parameters.

For comparisons, the estimate of the standard errors can be calculated from the following formulas. For the control treatments,

$$SE(t_1) = \sqrt{(k+1)MSe/bk}. \tag{6.5}$$

For the other effects,

$$SE(t_i) = \sqrt{(k+1)(t+1)^2 MSe/tr(kt-1)}, \tag{6.6}$$

$$SE(t_i - t_1) = \sqrt{(k+1)(k+t-2)MSe/r(kt-1)}, \tag{6.7}$$

$$SE(t_i - t_j) = \sqrt{2(k+1)(t-1)MSe/r(kt-1)}, \tag{6.8}$$

where $i \neq j = 2, \ldots, t$, and *MSe* is the mean square error from analysis of variance, Table 6.3. The estimates of the treatment means are

$$\bar{X}_i = \frac{\sum X_i}{N-1} + t_i, \qquad i = 1, \ldots, t+1 \tag{6.9}$$

**6.2.4.3 McNemar's Test**

The McNemar's test is a test of marginal homogeneity for matched binary responses in a 2×2 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 changes of consumer acceptance/purchase intent when sodium claim has been provided to the consumers.

In addition to the chi-squared value, a 95% confidence interval (C.I.) was calculated using marginal sample proportions $(p_{+1} - p_{1+})$ for estimating the actual differences in the means. The following equation was used to calculate the marginal sample proportions:

$$p_{ij} = \frac{n_{ij}}{N} \tag{6.10}$$

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:

$$(p_{+1} - p_{1+}) \mp Z_{\frac{\alpha}{2}}(ASE) \qquad (6.11)$$

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 $Z_{\frac{\alpha}{2}}$ equals 1.96 and represents the standard normal percentile having a right-tail probability of $\frac{\alpha}{2}$. ASE is the estimated standard error for the proportion difference and was calculated using the following equation:

$$ASE = \sqrt{\frac{p_{1+}(1-p_{1+})+p_{+1}(1-p_{+1})-2(p_{11}p_{22}-p_{12}p_{21})}{N}} \qquad (6.12)$$

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's test was used to determine whether a significant change existed in consumer acceptance and purchase intent before and after additional information of no-sodium claim was provided.

#### 6.2.4.4 Signal-to-Noise Ratio

Signal-to-noise ratio (SNR) is a measure of variability as a deviation from the target value. In this ratio, the estimate of treatment effect or product effect is maximized, and the estimate of variability (noise effect) is minimized (Gacula and others 2008). The SNR statistic can be applied to the just-about-right (JAR) scale where the target value is the middle category of the scale because the JAR scale category value is the signal and the others are noise. For the JAR of saltiness, a 3-point scale was used, so the target value was 2 or just-about-right. Meanwhile, the SNR statistic can also be employed to the intensity rating scale of bitterness which the signal was the non-bitter category value; thus, the target value was 0. The average SNRs for a product for saltiness and bitterness can be obtained by the following formulas:

$$SNR_{saltiness} = \frac{\sum_{i=1}^{n}[-10\times\log((X_i-2)^2+k)]}{n} \tag{6.13}$$

$$SNR_{bitterness} = \frac{\sum_{i=1}^{n}[-10\times\log(X_i{}^2+k)]}{n} \tag{6.14}$$

where $i$ = panelists = 1, 2,…, $n$; $X_i$ = the response of the $i^{th}$ panelist on the JAR rating scale for saltiness and on the intensity rating scale for bitterness, respectively. The constant $k$ is used to avoid taking the logarithm of zero, which is undefined. It can range from 0.1 to 0.5 and the constant value of 0.25 was used in this study (Gacula and others 2008).

**6.2.4.5 Bootstrapping Penalty Analysis**

The intent of penalty analysis is to determine the effect of specific attributes being at a non-optimal level (i.e., scores above or below the JAR score) on the hedonic level of a product (Meullenet and others 2007). Hedonic scores are calculated according to each of the *n*-categories of the JAR scale for any specific attribute, which is usually 3 or 5, and the percentage of consumers represented in each of the groups are calculated as well. A minimum of 70% of the responses in the JAR group is suggested to claim an optimal level of a specific attribute of a product. Furthermore, a minimum of 20% of the responses is required in the “too weak” or “too strong” categories to conclude that an attribute is not at its optimal level. The penalties are calculated by subtracting the mean hedonic score for the JAR group to the mean of any non-JAR category (“too weak” or “too strong”).

$$\text{Penalty} = \bar{X}_{JAR} - \bar{X}_{non\ JAR} \quad (6.15)$$

The bootstrap method is a computer-based Monte Carlo simulation technique for estimating standard errors, confidence intervals, biases, and prediction errors (Efron 1982). This alternative technique allows ones to perform a *t*-test of the overall hedonic scores comparing scores of a non-JAR and a JAR group for samples of unequal sizes and variances when the number of responses is small (Meullenet and others 2007).

**6.2.4.6 Logistic Regression**

Logistic regression is a statistical regression model which is commonly used when a dependent variable is dichotomous (yes/no) and the independent variables are quantitative or categorical. It can be used to predict the estimated probability that one event will (success) or will not occur (failure) based on a number of independent variables (Hair and others, 1998). Results from logistic regression can be interpreted either using estimated probability or estimated odds ratio. The odds are a ratio of two probabilities, the probability an event occurs ($p$) divided by the probability the event does not occur ($1 - p$). So, when an event has probability ($p$), the odds of an event are $\frac{p}{1-p}$. The odds ratio is defined as the ratio of an event occurring in one group to the odds of it occurring in another group. The odds ratio is interpreted as an increase in the odds for a unit increase in the independent variable. The odds are nonnegative, with value greater than 1.0 when a success is more likely to occur than a failure (Agresti 1996). An odds ratio farther from 1.0 in a given direction represents stronger levels of association.

In this study, all sensory attributes were included in the logistic models to predict the product acceptability and purchase intent using the odds ratios. However, a model with several predictors is likely to have multicollinearity, strong correlations among predictors, that makes it seems that no one variable is important when all the others are in the model (Agresti 1996).

Hence, the stepwise selection method was also performed along with the model. Using the stepwise selection method, parameters are estimated for effects forced into the model, as in the forward selection steps. Once a significant effect is in the model, the effect is checked for significance again. If it becomes non-significant, it will be removed from the model as in the backward elimination steps. These steps depend on the significance levels of the score chi-square statistic for entering an effect into the model in the forward method and the Wald chi-square for removing an effect from the model in a backward elimination step. The stepwise selection process terminates if no further effect can be added to the model or if the effect just entered into the model is the only effect removed in the subsequent backward elimination (SAS 2003).

#### 6.2.4.7 Principal Component Analysis

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 center 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 (Lipkovich and Smith 2002).

**6.3 Results and Discussion**

**6.3.1 Demographic Information**

Approximately three-fourths of participants were in the age range of 18-24 years which was a great majority of undergraduate students as the test was conducted on the university campus. About 20% of the consumers were categorized in the 25-34 years of age and nearly 5% of the consumers were 35 years and older. When considering by gender, the proportion of female participants (48.04%) was relatively equal to the number of men (51.96%).

**6.3.2 Overall Product Differences**

MANOVA was employed in order to determine whether differences in the acceptance for five pasta sauces using different types of salt existed considering all the attributes simultaneously. The Wilks' lambda was used in assessing the influence of all sensory attributes at the same time. The *p*-value of <0.0001 indicates that product acceptance for all samples significantly differed across the four sensory attributes and overall liking (Table 6.4).

Table 6.4 Multivariate statistics and *F* approximations

| Statistic | Value | F Value | Numerator DF | Denominator DF | *Pr > F* |
|---|---|---|---|---|---|
| Wilks' Lambda | 0.6750 | 12.36 | 20 | 1964.4 | <.0001 |
| Pillai's Trace | 0.3329 | 10.80 | 20 | 2380 | <.0001 |
| Hotelling-Lawley Trace | 0.4699 | 13.88 | 20 | 1295 | <.0001 |
| Roy's Greatest Root | 0.4442 | 52.86 | 5 | 595 | <.0001 |

Since the significant difference was found in MANOVA, DDA was then performed to determine attributes underlying differences among the samples. According to the pooled within canonical structure in the first dimension (Can 1), the acceptances for overall liking (canonical correlation = 0.956), overall taste (0.948), flavor (0.859), and saltiness (0.732) largely contributed to overall differences in acceptance among the pasta sauces resulting in the cumulative variance explained of 94.52% (Table 6.5).

Table 6.5 Canonical structure *r*'s describing group differences among pasta sauces using different salts

| Variable | Can 1* | Can 2 |
|---|---|---|
| Appearance | 0.1215 | 0.1478 |
| Flavor | **0.8592** | -0.3738 |
| Taste | **0.9481** | -0.0623 |
| Saltiness | **0.7315** | 0.4849 |
| Overall liking | **0.9561** | 0.0545 |
| Cum. Variance Explained | 94.52% | 98.71% |

Bold values indicate sensory attributes which largely account for group differences.
*Can = Canonical structure, pooled within canonical structure in the first (Can 1) and second (Can 2) dimensions.

### 6.3.3 Consumer Acceptability

Table 6.6 provides the mean acceptability scores and *p*-values of ANOVA results for overall appearance, flavor, taste, saltiness, and overall liking of the pasta sauces containing different salts. In addition to the effect of treatments as typically evaluated by the normal ANOVA model, the effects of repetitions, blocks within repetitions were also included in the calculation for the ANOVA model of the balanced incomplete block design augmented with control treatment in every block. Differences among the samples containing different types of salt were observed in the acceptability for appearance, flavor, taste, saltiness, and overall liking with the *p*-values of less than 0.05, meaning that at least two samples were different from each other for all attributes. Comparing the results from this model and the normal ANOVA model, the significant difference in liking scores for sample appearance was only found when the mean was adjusted. Besides the appearance acceptability, both methods of calculation provided the same results.

Table 6.6 Consumer liking scores for pasta sauces

| Treatment* | Appearance | Flavor | Taste | Saltiness | Overall liking |
|---|---|---|---|---|---|
| NaCl | $7.10^{A} \pm 1.25$ | $7.85^{A} \pm 1.40$ | $7.99^{A} \pm 1.44$ | $7.21^{A} \pm 1.53$ | $7.92^{A} \pm 1.43$ |
| KCl | $6.65^{B} \pm 1.47$ | $3.90^{C} \pm 2.20$ | $3.58^{C} \pm 2.21$ | $4.25^{C} \pm 1.87$ | $3.58^{C} \pm 2.15$ |
| KCl/AMP | $6.91^{A} \pm 1.39$ | $4.99^{B} \pm 2.01$ | $4.57^{B} \pm 2.12$ | $4.72^{B} \pm 1.80$ | $4.41^{B} \pm 1.92$ |
| KCl/Arg/AMP | $6.94^{A} \pm 1.41$ | $5.37^{B} \pm 2.02$ | $4.98^{B} \pm 2.12$ | $4.89^{B} \pm 1.91$ | $4.79^{B} \pm 2.02$ |
| Commercial SS | $7.04^{A} \pm 1.23$ | $5.10^{B} \pm 2.14$ | $4.77^{B} \pm 2.20$ | $4.79^{B} \pm 2.12$ | $4.54^{B} \pm 2.18$ |
| F statistic | 4.85 | 139.69 | 176.64 | 85.20 | 193.32 |
| Pr > F | 0.0008 | <.0001 | <.0001 | <.0001 | <.0001 |

*The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute.

A-C Adjusted means within the same column with different letters are significantly different ($p < 0.05$). Data are represented as adjusted mean ± standard deviation values of 204 consumer responses for NaCl and 102 consumer responses for the rest. The adjusted means were obtained from Equation 6.9 and the standard deviations were calculated by the normal method.

All values are based on a nine-point hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

The 95% confidence interval was then constructed to test the significance of pairwise comparisons of means. The null hypothesis of pairwise comparisons was that the mean difference of a sample pair equals zero ($H_0$: $D = 0$). If the 95% confidence interval contains zero, then the mean difference is not significant. The pasta sauce containing regular salt (NaCl) significantly received the highest liking scores for appearance (7.10), flavor (7.85), taste (7.99), saltiness (7.21), and overall liking (7.92). On the contrary, the sauce with KCl obtained the lowest acceptability scores for appearance (6.65), flavor (3.90), taste (3.58), saltiness (4.25), and overall liking (3.58). However, the samples containing the commercial salt substitute and the sodium-free salts with AMP (KCl/AMP and KCl/Arg/AMP) were not significantly different from each other in the consumer acceptability in flavor, taste, saltiness, and overall liking. An observable trend of liking scores for flavor, taste, saltiness, and overall liking was found with the highest scores of the sample containing NaCl followed by KCl/Arg/AMP, the commercial salt substitute, KCl/AMP, and KCl, respectively.

### 6.3.4 Product Acceptance and Purchase Intent

The samples were separately evaluated using a dichotomous scale (yes/no) for product acceptance and purchase intent with and without the claim of no added sodium which does not cause hypertension. The percent (%) of positive responses of product acceptance and purchase intent can be found in Tables 6.7 and 6.8, respectively.

Table 6.7 Positive (yes) responses and changes in probabilities of product acceptance

| Treatment[a] | Acceptance | Acceptance with Na Claim | Δ[b] | 95% C.I.[c] | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| NaCl | 94.61% | - | - | - | - | - |
| KCl | 40.82% | 60.20% | 19.38% | 0.1070 | 0.2808 | <0.0001 |
| KCl/AMP | 54.90% | 69.61% | 14.71% | 0.0783 | 0.2158 | 0.0001 |
| KCl/Arg/AMP | 63.00% | 74.00% | 11.00% | 0.0487 | 0.1713 | 0.0009 |
| Commercial SS | 51.49% | 67.33% | 15.84% | 0.0872 | 0.2296 | <0.0001 |

[a]The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute. NaCl was evaluated 204 times while other treatments were evaluated 102 times.
[b]Δ = the difference value between positive responses of acceptance with and without Na claim.
[c]C.I. = confidence interval.

Approximately 95% of participants concurred that the sauce containing regular salt was acceptable. However, no matter if the sodium claim had been informed; the sauce with KCl alone received the lowest positive responses for product acceptance which were responsible for 40.82% and 60.20%, respectively. In a comparison among the sodium-free pasta sauces, the products containing KCl/Arg/AMP and KCl/AMP received the highest affirmative responses for the product acceptance with the agreement of 63% and 55% of participants and the agreements increased to 74% and 70% after the sodium claim had been informed, respectively. These results also suggest that the sauces with KCl/Arg/AMP and KCl/AMP were more acceptable than the one with the commercial salt substitute. Furthermore, there was a similar observable trend in product acceptance before and after the sodium claim had been given that the most acceptable sauce was the sample with KCl/Arg/AMP followed by the ones with KCl/AMP, the commercial salt substitute, and KCl, respectively.

Participants were also asked if they would accept the product knowing that it had no sodium added which does not cause high blood pressure. However, the sample containing NaCl were not evaluated for this query. The affirmative responses for the product acceptability for the four samples revealed that an additional 11% to 19% of participants were more likely to accept the product. These significant increases suggested that additional information given caused an increase ($p < 0.05$) in product acceptance. With the no-added sodium claim, the sauce with KCl was associated with the greatest increase in product acceptance with a 19.38% increase in participants' agreement, where the sauce containing KCl/Arg/AMP had the least acceptance change with only 11%. Moreover, it can be predicted with 95% confidence that the probability of consumer acceptability will be increased at least by 4.87% and at most by 17.13% for the pasta containing KCl/Arg/AMP, and at least by 10.7% and at the most by 28.08% for the sauce containing KCl after the information of the no added sodium claim was given.

The affirmative responses and the changes in probabilities of purchase intent with and without the claim of no-added sodium given are shown in Table 6.8. Like the product acceptance, the sample that contained table salt had the highest positive response of purchase intent with 78.33%, saying that they would buy the product, and the sample with KCl had the lowest purchase intent with the positive response of 24.51%. The other three samples, KCl/AMP, KCl/Arg/AMP, and the commercial salt substitute, had comparable probabilities of purchase intent of approximately 31-33% before the additional information had been given, and about half of participants would buy the products after the no-added sodium claim had been informed. When comparing the purchase intent among the sodium-free pasta sauces, most participants were willing to buy the one containing KCl/AMP with 33.33% and 51.96% for before and after the sodium claim had been given, respectively.

Table 6.8 Positive (yes) responses and changes in probabilities of purchase intent

| Treatment[a] | Purchase Intent | Purchase Intent with Na Claim | Δ[b] | 95% C.I.[c] | | p-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| NaCl | 78.33% | - | - | - | - | - |
| KCl | 24.51% | 38.24% | 13.73% | 0.0705 | 0.2040 | 0.0002 |
| KCl/AMP | 33.33% | 51.96% | 18.63% | 0.1107 | 0.2618 | <0.0001 |
| KCl/Arg/AMP | 31.37% | 48.04% | 16.67% | 0.0943 | 0.2390 | <0.0001 |
| Commercial SS* | 31.37% | 50.00% | 18.63% | 0.1107 | 0.2618 | <0.0001 |

[a]The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute. NaCl was evaluated 204 times while other treatments were evaluated 102 times.
[b]Δ = the difference value between positive responses of acceptance with and without Na claim.
[c]95% C.I. = 95% confidence interval.

After participants had been informed about the claim of no added sodium which does not cause high blood pressure, they were significantly more willing to buy all four products with *p*-values $< 0.05$. For purchase intent, increases in change ranged from 13.73% to 18.63% when the additional information of product claims had been given. The sample which contained KCl received the smallest increase in changes in purchase intent with a value of 13.73% while the product with KCl/AMP had the greatest increase in purchase intent. According to the 95% confidence interval, it is possibly that the purchasing willingness of participants for KCl/Arg/AMP will be higher by at least 9.43% and at most 23.90% after the information of the no-added sodium claim is given. Consequently, when the participants were informed about the additional information of the product claim, they were more willing to accept and buy the products. In other words, additional information of the products had significant positive effects of the product acceptability and purchase intent.

### 6.3.5 Taste Intensities and Acceptability

The frequency of taste intensity of saltiness on the JAR scale is shown in Table 6.9. Only about 10% of participants described the saltiness intensity of all samples as too salty. Almost 80% of participants said that the sample added with table salt had just-about-right saltiness intensity and about half of participants suggested that the samples with sodium-free salts were not salty enough. Moreover, approximately 40% of responses stated that the saltiness of the sauces containing KCl/Arg/AMP and the commercial salt substitute were just about right. About 30% responded as just-about-right for the saltiness of the sauces with added KCl and KCl/AMP. In other words, NaCl is saltier than the other 4 salt substitutes. These findings substantiate the results from the descriptive analysis in Chapter 5.

Table 6.9 Frequency distribution of the JAR scores for saltiness of pasta sauces

| Treatment* | Saltiness JAR | | |
|---|---|---|---|
| | Not salty enough | Just-about-right | Too salty |
| NaCl | 15.27% | 78.82% | 5.91% |
| KCl | 57.84% | 33.33% | 8.82% |
| KCl/AMP | 56.86% | 29.41% | 13.73% |
| KCl/Arg/AMP | 53.00% | 42.00% | 5.00% |
| Commercial salt substitute | 50.00% | 42.86% | 7.14% |

*The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). NaCl was evaluated 204 times while other treatments were evaluated 102 times.

The signal-to-noise ratios (SNR) of each product were plotted against saltiness, overall taste, and overall liking on the 9-point hedonic scale as shown in Figure 6.1. As can be seen, the sauce containing NaCl was significantly different from others in saltiness, overall taste, and overall liking on a 9-point hedonic scale. The saltiness SNR values are 4.54, 1.36, 1.09, 1.97, and 2.03 for the product containing NaCl, KCl, KCl/AMP, KCl/Arg/AMP, and the commercial salt substitute, respectively.

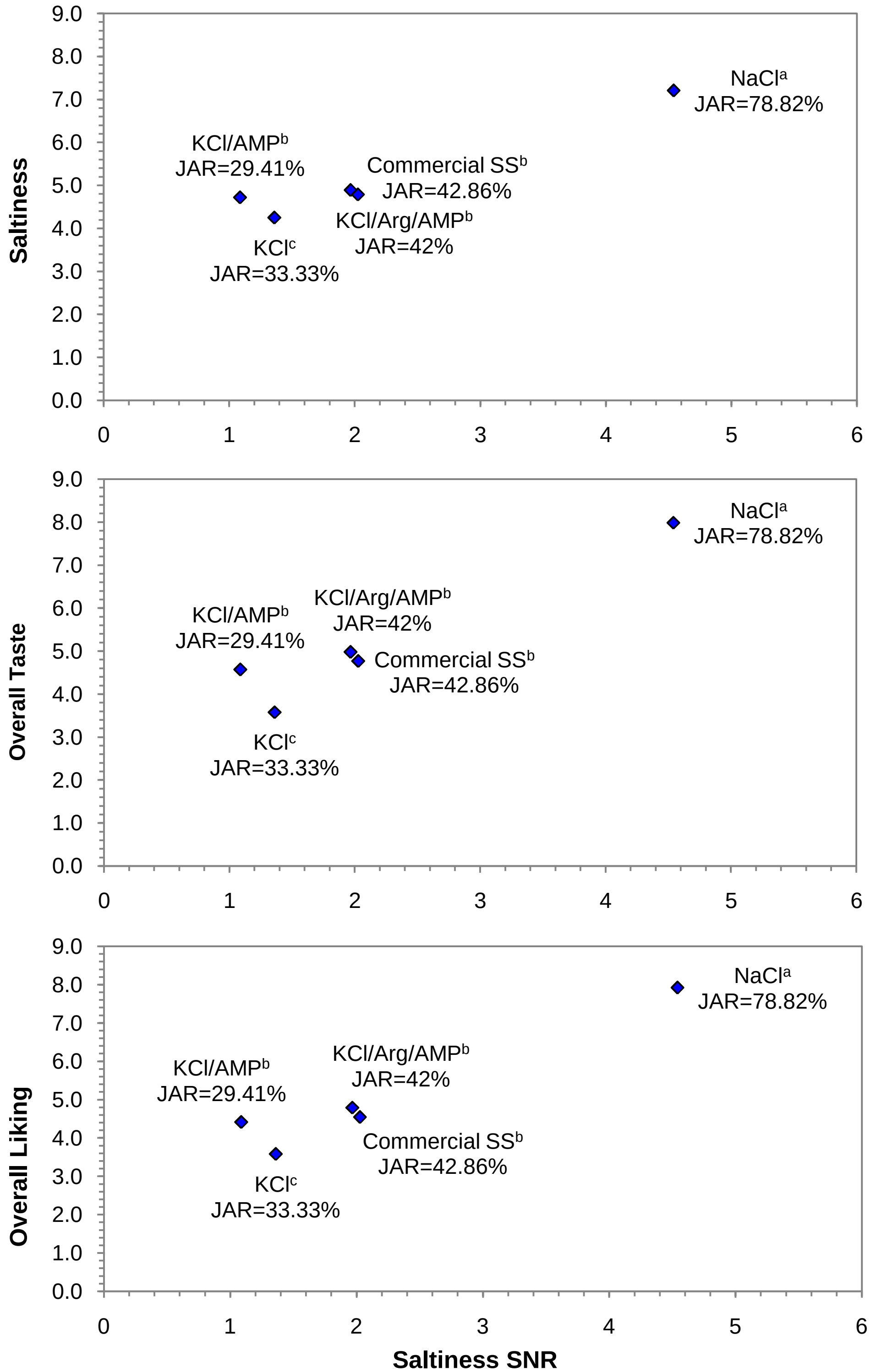


Figure 6.1 Plots of products in the space of sensory acceptability means against the saltiness SNR.

*JAR=just-about-right saltiness corresponding to the number of responses.

[a-b]Products with different lower letters were significantly different ($p < 0.05$) for the acceptability variable. The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute.

Means of liking scores of saltiness, overall taste, and overall liking for each sample were very similar (Table 6.6); thus, the plots graphically look the same. With a high value of saltiness SNR, the saltiness intensity of the sample containing NaCl was scored as just-about-right. This result suggested that the saltiness intensity on a JAR scale of the products is positively correlated with saltiness, overall taste, and overall liking on the hedonic scale.

Table 6.10 shows the frequency distribution of the responses for bitterness intensity and acceptability of pasta sauces. Because the bitterness is an unpleasant sensory attribute in pasta sauces, the JAR scale cannot be used for this attribute and an intensity rating scale was used instead (0 = none, 1 = weak, 2 = moderate, and 3 = strong).

Table 6.10 Frequency distribution of bitterness intensity and acceptability of pasta sauces

| Treatment[a] | Bitterness Intensity | | | | | | |
|---|---|---|---|---|---|---|---|
| | None | Weak | | Moderate | | Strong | |
| | | Acc[b] | Unacc[c] | Acc[b] | Unacc[c] | Acc[b] | Unacc[c] |
| NaCl | 65.26% | 18.42% | 1.05% | 11.58% | 2.63% | 0.53% | 0.53% |
| KCl | 18.07% | 18.07% | 1.20% | 14.46% | 15.66% | 1.20% | 31.34% |
| KCl/AMP | 28.40% | 22.73% | 4.55% | 12.50% | 12.50% | 2.27% | 17.05% |
| KCl/Arg/AMP | 31.46% | 29.21% | 4.49% | 15.74% | 6.74% | 1.12% | 11.24% |
| Commercial Salt Substitute | 30.00% | 17.78% | 2.22% | 20.00% | 8.89% | 1.11% | 20.00% |

[a]The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). NaCl was evaluated 204 times while other treatments were evaluated 102 times.
[b]Acc – The bitterness/aftertaste in the product is acceptable on a yes/no scale.
[c]Unacc – The bitterness/aftertaste in the product is unacceptable on a yes/no scale.

About 65% of responses found no bitterness/aftertaste in the sauce containing table salt where only 18% of responses reported no bitterness detection in the sauce containing KCl and about 30% for the ones containing KCl, KCl/AMP, and the commercial salt substitute. About 35% of respondents indicated some degrees of bitterness/aftertaste in the control sample. However, it is unlikely that the bitterness/aftertaste came from NaCl. There are a few potential causes of this incident. Firstly, the bitterness/aftertaste was given by spices in the seasoning mix which consisted of garlic, onion, crushed red pepper, thyme, basil, oregano. Secondly, the psychological errors of leading questions may occur. Lastly, the buildup of bitterness/aftertaste from the no-sodium salt samples was uncontrolled if panelists did not cleanse their palates thoroughly between samples. However, Johnson and Vickers (2004) found that no differences in the effectiveness of these palate-cleansing strategies for their ability to control adaptation and build-up of bitterness in cream cheese samples containing different amounts of caffeine. They also suggested that any effectiveness of a palate cleansing strategy is more likely due to a mental adjustment that prepares one to focus on the next sample than to any physical cleaning.

Generally, if the bitterness/aftertaste in the sample had been rated as weak, the taste was likely to be acceptable since the responses for acceptable were higher than unacceptable, regardless of samples. But if it was rated as strong, the taste was more associated with being unacceptable for all samples except the sample containing NaCl which had the probability of being acceptable equal to being unacceptable. Among the sauces containing salt substitutes, the sauce containing KCl/Arg/AMP seems to be the least bitter sample which the data were more distributed on the left side of the distribution and vice versa for the sample with KCl. Comparing the samples containing the commercial salt substitute and KCl/Arg/AMP, both samples had about the same number of responses for the absence of bitterness detection but the one with KCl/Arg/AMP was judged in the weak category for 33.3% which was higher than the one with the commercial salt substitute, 20%. Moreover, the number of responses in the moderate and strong categories for the sample with the commercial salt substitute was higher than the one that contained KCl/Arg/AMP.

Figure 6.2 illustrates the plots of the signal-to-noise ratios (SNR) of bitterness intensity against the overall taste and overall liking on the 9-point hedonic for each product. Like the plots of saltiness SNR, the sample containing NaCl was significantly different from others.

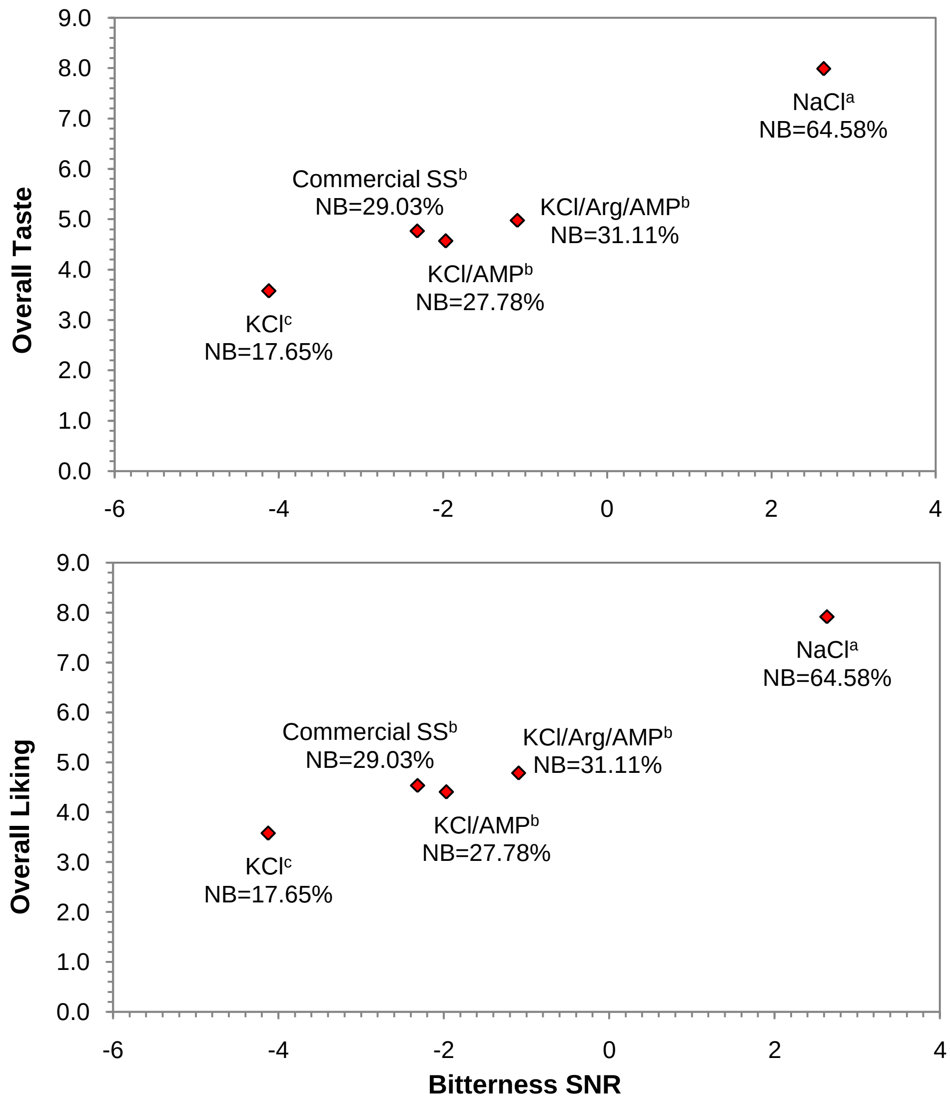


Figure 6.2 Plots of products in the space of sensory acceptability means against the bitterness SNR.

*NB = No bitter taste corresponding to the number of responses.

[a-b]Products with different lower letters were significantly different ($p < 0.05$) for the acceptability variable. The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute.

The bitterness SNR values for the samples containing NaCl, KCl, KCl/AMP, KCl/Arg/AMP, and the commercial salt substitute are 2.63, -4.12, -1.97, -1.10, and -2.32, respectively. These values suggested that the sample that was most associated with bitterness/aftertaste was KCl followed by the commercial salt substitute, KCl/AMP, KCl/Arg/AMP, and NaCl, respectively. Since the bitterness intensity of the sauce containing

NaCl was scored to zero with a high value of bitterness SNR, the bitterness intensity of the products was negatively correlated with overall taste and overall liking on the hedonic scale. This result may imply that AMP could help block the bitter taste in KCl and this blocking effect was better with the addition of L-arginine.

As stated earlier, a minimum of 70% of the responses in the JAR group is required to bring out a conclusion of the optimal level. Therefore, the NaCl sample had a favorable level of saltiness intensity with approximately 80% of the saltiness JAR responses. For bitterness, there were 65% of the responses for the no detection of bitterness intensity. But if the responses for the bitterness in the 'weak' category along with the 'acceptable' group were taken into account, the responses of bitterness for the NaCl sample will be greater than 70%. Hence, only the salt substitute samples were assessed for the penalty analysis, especially the category of 'not salty enough' for saltiness intensity, and each level for bitterness intensity.

Table 6.11 shows the mean drops of saltiness, overall taste, and overall liking on the hedonic scale when the saltiness and bitterness intensities were not at the ideal level. The mean drops in the first row of each sample were obtained from the bootstrap resampling method which a number of replications ($N$=1000) had been assessed and the Student's $t$ test was then performed to test if the mean drop was equal to zero. The mean drops in the second row of each sample were obtained from the averages of the raw data; therefore, the statistical hypothesis testing could not be done with the one replication of data. As can be seen in Table 6.11, the liking scores of saltiness, overall taste, and overall liking significantly dropped when the saltiness intensities were rated as "not salty enough" or the bitter taste was perceived. However, the mean drops in taste and overall liking were found to be statistically significant only if the bitterness was judged as strong for the pasta sauce containing the commercial salt substitute.

Table 6.11 Mean drops of the saltiness, taste, and overall liking of the penalty analysis

| Treatment | Saltiness | Taste | | | | Overall Liking | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $Salty_{NE}$ | $Salty_{NE}$ | $Bitter_{weak}$ | $Bitter_{moderate}$ | $Bitter_{strong}$ | $Salty_{NE}$ | $Bitter_{weak}$ | $Bitter_{moderate}$ | $Bitter_{strong}$ |
| KCl | -2.44[A] | -2.34 | 0.32 | -1.41 | -2.53 | -2.11 | **0.22** | -0.54 | -2.64 |
| | -2.20[B] | -2.28 | 0.45 | -1.24 | -2.72 | -2.04 | 0.44 | -0.63 | -2.53 |
| KCl/AMP | -2.43 | -1.90 | -0.65 | -1.96 | -2.77 | -1.84 | -0.57 | -1.73 | -2.70 |
| | -2.64 | -1.82 | -0.69 | -1.81 | -2.80 | -1.88 | -0.57 | -1.65 | -2.88 |
| KCl/Arg/AMP | -2.32 | -2.37 | -1.01 | -1.62 | -4.23 | -2.08 | -1.31 | -1.47 | -4.54 |
| | -2.32 | -2.34 | -1.08 | -1.62 | -4.29 | -1.99 | -1.32 | -1.60 | -4.49 |
| Commercial salt substitute | -3.11 | -1.96 | **0.30** | **-0.03** | -3.08 | -2.12 | **0.00** | **-0.07** | -3.22 |
| | -2.97 | -1.91 | 0.17 | -0.02 | -3.04 | -2.23 | -0.01 | 0.02 | -3.17 |

[A]The numbers in the first row of each treatment indicate mean drops that were calculated from the bootstrap resampling method ($n$ = 1000). Bold values indicate no significant difference from zero ($p > 0.05$).
[B] The numbers in the second row of each treatment indicate mean drops that were calculated from the non-resampling method.
- $Salty_{NE}$ = saltiness intensity is not enough based on the JAR scale. Mean drop of $salty_{NE}$ of a sample was a difference of scores of an attribute on the 9-point hedonic scale which the saltiness was rated as "just-about-right" and "not salty enough" on the JAR scale.
- $Bitter_{weak}$, = weak bitter taste; $Bitter_{moderate}$ = moderate bitter taste; $Bitter_{strong}$ = strong bitter taste.
Mean drop of $bitter_{weak}$/$bitter_{moderate}$/$bitter_{strong}$ of a sample was a difference of scores of an attribute on the 9-point hedonic scale which the bitterness intensity was rated as none and weak, moderate, or strong on the rating scale.

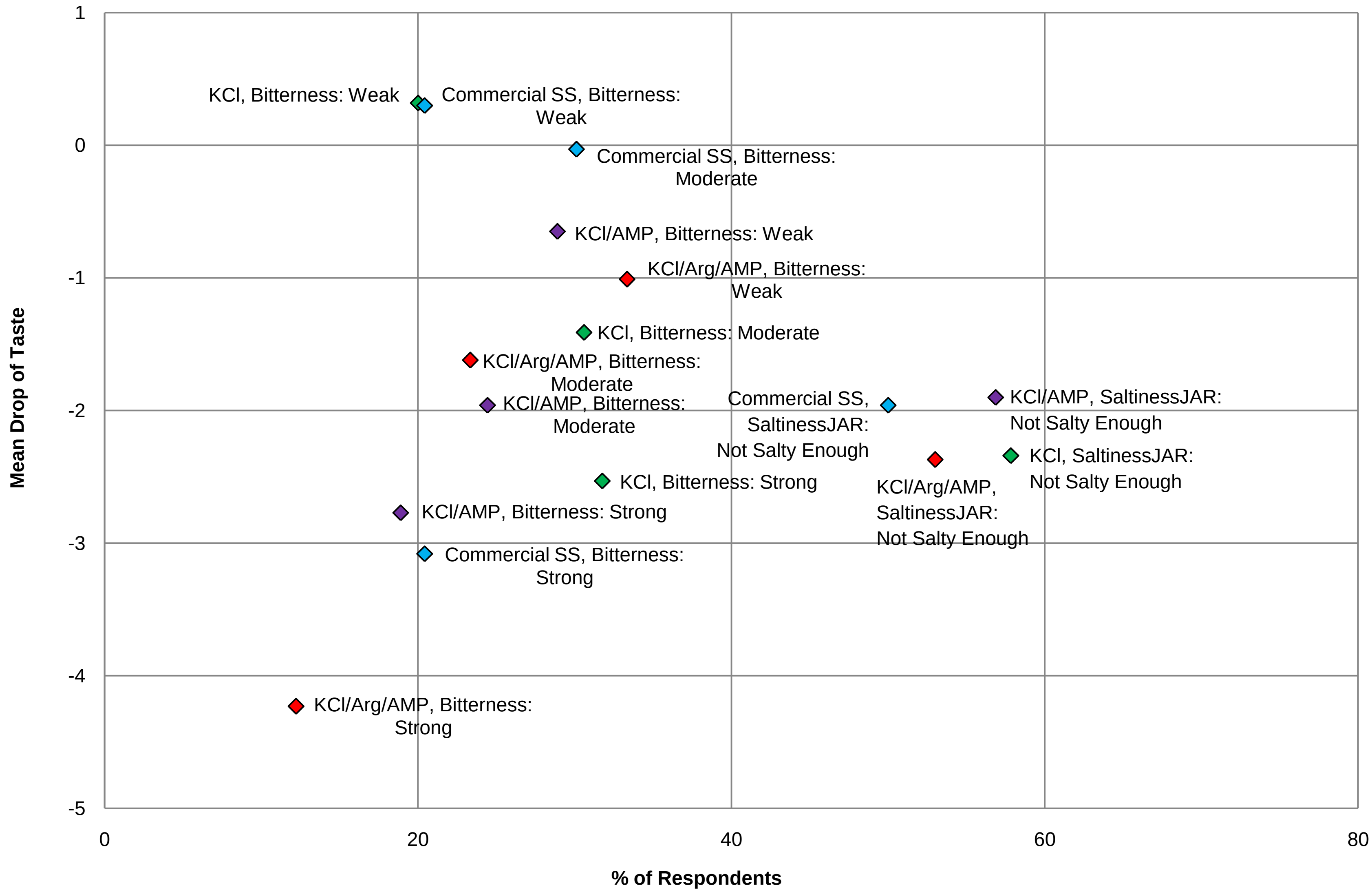


Figure 6.3 Scatter plots for the penalty analysis of overall taste against % responses for the JAR scale
*The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute.

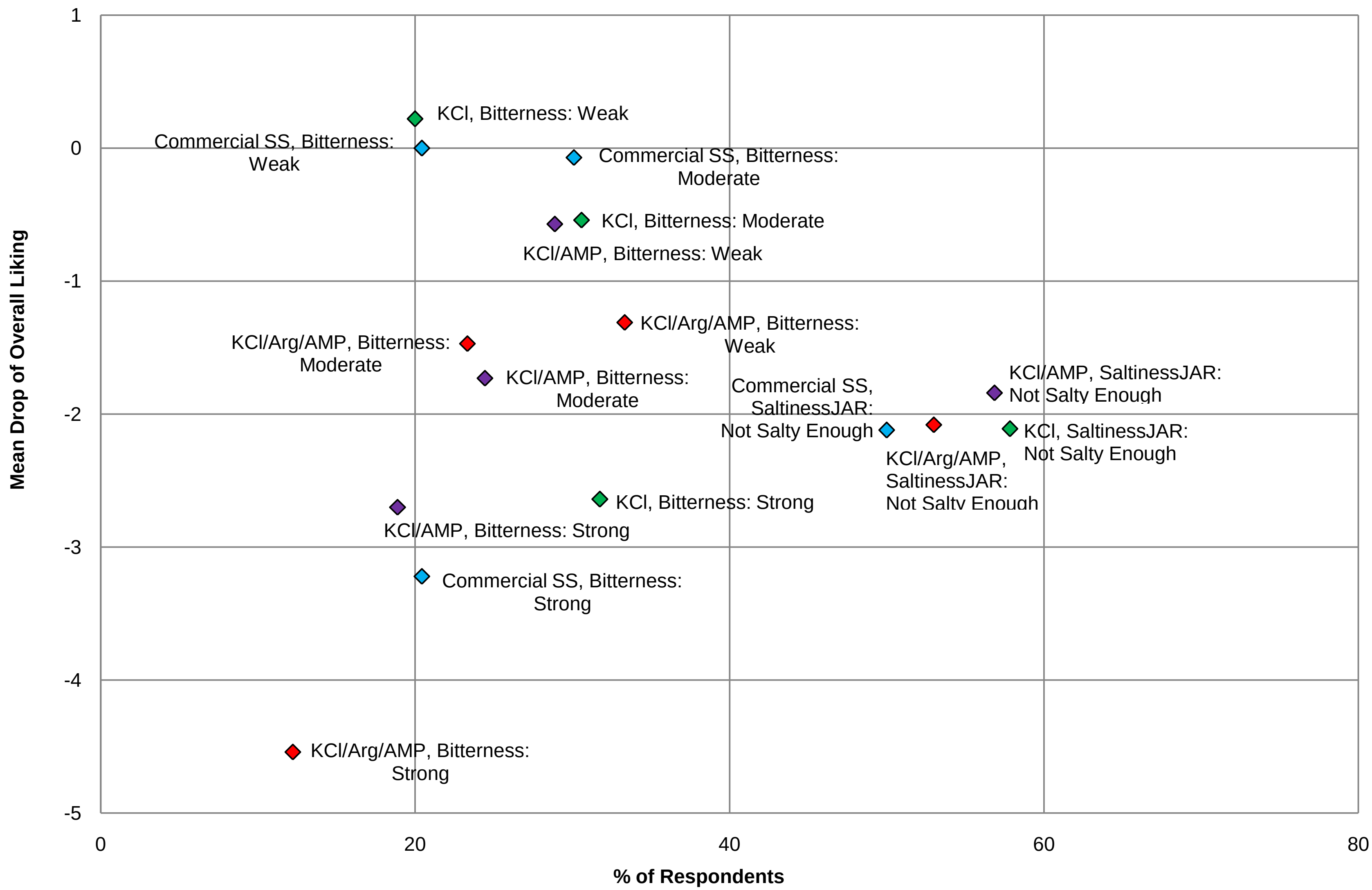


Figure 6.4 Scatter plots for the penalty analysis of overall liking against % responses for the JAR scale
*The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute.

When the saltiness intensity had been rated as not salty enough, the average means of saltiness, taste and overall liking on the 9-point hedonic scale dropped about 2 points. When the bitterness intensity was found as weak, moderate, and strong, the liking scores decreased at least 0.5, 1, and 2 points, respectively. The results from penalty analysis were also illustrated in the scatter plots of the mean drop of a sensory attribute (Y-axis) against the number of respondents (X-axis) as shown in Figures 6.3 and 6.4.

### 6.3.6 Predicting Probabilities of Acceptance and Purchase Intent

Logistic regression analysis (LRA) along with the stepwise selection method was used to determine the importance of each attribute in predicting probabilities of acceptance and purchase intent of the sauce containing different types of salt. Using the significance level of 0.05, only appearance, overall liking, and bitterness intensity were the effects remained in the acceptance model, regardless of treatments, entailing that these attributes mainly affected the overall product acceptance while overall liking, bitterness intensity, and acceptance were critical attributes corresponding to the purchase intent (Table 6.12).

Table 6.12 Critical sensory attributes for acceptance and purchase intent of pasta sauces

| Effect* | DF | Acceptance | | Purchase Intent | |
|---|---|---|---|---|---|
| | | Wald $\chi^2$ | Pr > $\chi^2$ | Wald $\chi^2$ | Pr > $\chi^2$ |
| Appearance | 1 | 4.8625 | 0.0274 | - | - |
| Overall liking | 1 | 103.6856 | <.0001 | 88.2002 | <.0001 |
| Bitterness | 3 | 8.8159 | 0.0318 | 16.0684 | 0.0011 |
| Acceptance | 1 | - | - | 8.3209 | 0.0039 |

*Wald tests of individual effect with $p < 0.05$ indicate statistical significance.

For model fitting, the saltiness and bitterness intensities and product acceptance were considered as categorical variables where liking scores on the hedonic scale were considered as continuous variables since the models were not fit so well when the liking scores were treated as categorical variables. The categories of "just-about-right" of saltiness intensity, "none" of bitterness intensity, and "no" of acceptance were used as dummy variables.

Table 6.13 Full logistic regression models for predicting the probabilities of acceptance and purchase intent for each sample

| Treatment[a] | Predictive Model[b] |
|---|---|
| NaCl | $Y_{Buy} = -11.0505 + 0.8405X_{T} + 0.9467X_{O} - 0.2735X_{S1} - 0.6049X_{S2}$ |
| KCl | $Y_{Accept} = -6.5712 + 1.4539X_{O}$ |
| | $Y_{Buy} = -7.3894 + 1.1894X_{O}$ |
| KCl/AMP | $Y_{Accept} = -4.7276 + 1.0169X_{O} + 0.4714X_{B1} - 0.3485X_{B2} – 1.6748X_{B3}$ |
| | $Y_{Buy} = -8.7932 + 1.4264X_{O} + 0.9687X_{Acc}$ |
| KCl/Arg/AMP | $Y_{Accept} = -7.4334 + 1.8624X_{O}$ |
| | $Y_{Buy} = -10.2234 + 1.724X_{O}$ |
| Commercial salt substitute | $Y_{Accept} = -6.9406 + 1.4222X_{T}$ |
| | $Y_{Buy} = -8.9136 + 1.2734X_{T} + 1.1988X_{Acc}$ |

[a]The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1).
[b]The predictors in the models include taste ($X_{T}$), overall liking ($X_{O}$), saltiness*not salty enough* ($X_{S1}$), saltiness*too salty* ($X_{S2}$), bitterness*weak* ($X_{B1}$), bitterness*moderate* ($X_{B2}$), bitterness*strong* ($X_{B3}$), and acceptance ($X_{Acc}$). No predictive model for acceptance for NaCl since most of the consumers (94.61%) said it was acceptable.

Considering the type of salts used in the pasta sauce, at the significance level of 0.05, overall liking was the only attribute that was significant in most of the models which seems to lack of meaningfulness. To include more effects in the model, the significance levels for entering and remaining effects were then increased to 0.10. The models for predicting the probabilities of acceptance and purchase intent of each sample are described in Table 6.13 where $Y$ corresponds to the $\log\left(\frac{\pi_{yes}}{1-\pi_{yes}}\right)$ and $\pi_{yes}$ represents the probability of accepting or buying the product while $(1 – \pi_{yes})$ indicates the probability of not accepting or not buying the product. The odds ratios which were computed by raising $e$ to the power of the logistic coefficients are represented in Table 6.14.

Table 6.14 Odds ratio estimates and *p*-values from logistic regression model for predicting the probabilities of acceptance and purchase intent of pasta sauces containing different salts[a]

| Acceptance | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Attribute | NaCl | | KCl | | KCl/AMP | | KCl/Arg/AMP | | Commercial SS | |
| | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ |
| Taste | - | - | - | - | - | - | - | - | 24.10 | **<.0001** |
| Overall liking | - | - | 4.28 | **<.0001** | 2.76 | **<.0001** | 6.44 | **<.0001** | - | - |
| $\text{Bitterness}_{weak}$ | - | - | - | - | 0.34 | 0.3619 | - | - | - | - |
| $\text{Bitterness}_{moderate}$ | - | - | - | - | 0.15 | 0.5168 | - | - | - | - |
| $\text{Bitterness}_{strong}$ | - | - | - | - | 0.04 | **0.0466** | - | - | - | - |

| Purchase Intent | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Attribute | NaCl | | KCl | | KCl/AMP | | KCl/Arg/AMP | | Commercial SS | |
| | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ | Odds Ratio | Pr > $\chi^2$ |
| Taste | 2.32 | **0.0107** | - | - | - | - | - | - | 3.57 | **0.0004** |
| Overall liking | 2.58 | **0.0085** | 3.29 | **<.0001** | 4.16 | **<.0001** | 5.61 | **<.0001** | - | - |
| $\text{Saltiness}_{not\ salty\ enough}$ | 0.32 | 0.5410 | - | - | - | - | - | - | - | - |
| $\text{Saltiness}_{too\ salty}$ | 0.23 | 0.2737 | - | - | - | - | - | - | - | - |
| $\text{Acceptance}_{yes}$ | - | - | - | - | 6.94 | 0.0564 | - | - | 11.00 | 0.0604 |

[a]The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute. NaCl was evaluated 204 times while other treatments were evaluated 102 times.
[b]Bold values indicate sensory attributes that largely contribute to the product acceptability/purchase intent.

For instance, the odds ratio for overall liking in the acceptance model for the sauce containing KCl is $e^{1.4539}$ or equals 4.28. This means that for every one point increase in overall liking scores on the 9-point hedonic scale, the probability of accepting the sauce containing KCl to the probability of not accepting the sauce is 4.28 times higher. Overall liking and bitterness intensity, especially when bitter taste was found to be strong, were important in determining acceptability of the sample containing KCl/AMP with the odds ratios of 2.76 and 0.04. In other words, the probability of accepting the product increased 2.76 times higher when the overall liking score increased one point on the 9-point hedonic score and the probability of decreased 25 times when the consumers said the bitter taste was strong. Moreover, an increase in the overall liking score was also critical in the probability of product acceptance for the sample containing KCl/Arg/AMP with the odds ratio of 6.44. The liking score for taste was significantly important for consumers in product acceptance for the sample containing the commercial salt substitute with the estimated odds ratio of 24.10.

Overall liking was a critical attribute for predicting the probability of purchase intent for all samples except the one containing the commercial salt substitute. The odds estimates for overall liking for the samples containing NaCl, KCl, KCl/AMP, and KCl/Arg/AMP were 2.58, 3.29, 4.16, and 5.61, respectively. Therefore, the higher the scores of overall liking, the higher the probability of buying such product. Moreover, the liking scores for taste were also associated with the probability of purchase intent of the products containing table salt and the commercial salt substitute with the odds ratio estimates of 2.32 and 3.57, respectively. The saltiness intensity was significant only in the purchase intent for the sauce containing table salt. When consumers said the product was not salty enough, the probability of purchasing the product decreased by 3.125 times. Likewise, when consumers said the product was too salty, the probability of buying the product decreased by 4.35 times. Finally, acceptability was very important to determine if consumers would purchase the sauces that contained KCl/AMP and the commercial salt substitute, with estimated odds ratios of 6.94 and 11 times higher for those who accepted the product compared to those who did not accept. The probability of buying a product increased when the product was considered as acceptable.

### 6.3.7 Internal Preference Mapping

Based on the results obtained from DDA, principal component analysis (PCA) was performed with all three primary sensory attributes included to visualize the acceptability of sensory attributes using macro add-in for Microsoft Excel (Lipkovich and Smith 2002). Individual scores for acceptability of flavor, taste, and overall liking for the sauces containing different types of salt were assessed. Factor loading scores for PCA of the acceptability scores of flavor, taste and overall liking are shown in Table 6.15. In general, the acceptability scores of three attributes were positively correlated with PC1. The acceptability scores of flavor obtained a high positive loading score for PC2. Then PC2 could separate the acceptability scores of flavor from the ones of taste and overall liking.

Table 6.15 Factor loading scores for PCA of acceptability of pasta sauces containing five different salts

| Attribute | PC1 | PC2 | PC3 |
|---|---|---|---|
| Flavor | 0.57 | 0.80 | 0.17 |
| Taste | 0.58 | -0.25 | -0.77 |
| Overall liking | 0.58 | -0.54 | 0.61 |

The PCA bi-plot illustrated was used to arrive at conclusions about consumers' acceptability for sensory attributes of pasta sauces containing five different salts (Figure 6.5). For both the covariance and the correlation options, 99.99% of the total variance could be explained by the first two principal components where the first and second components are explained by 99.77% and 0.22%, respectively.

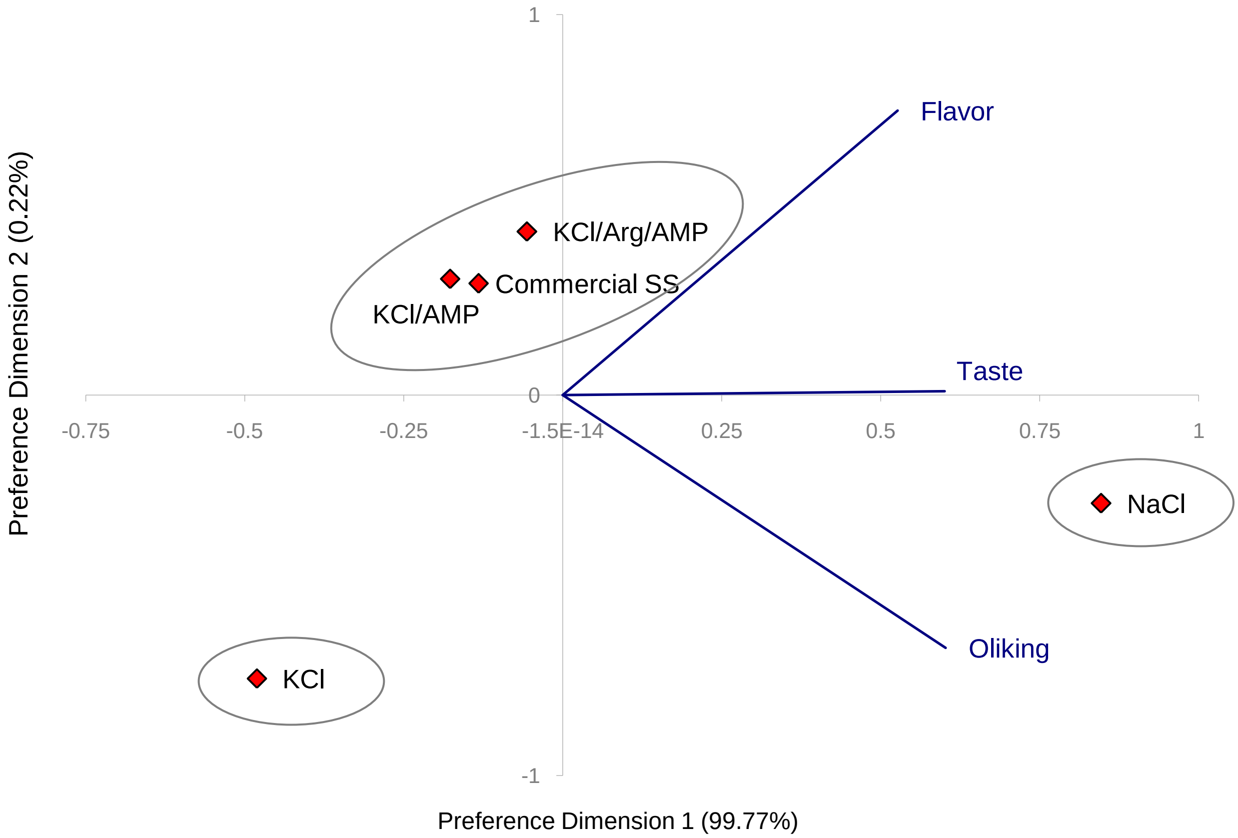


Figure 6.5 Internal preference mapping of the pasta sauces containing different salts
*The proportions of salt mixture were KCl/Arg (15:2), KCl/AMP (15:1), and KCl/Arg/AMP (15:2:1). Commercial SS is the commercial salt substitute.

The vector of the acceptability scores of overall taste was in between the vectors of flavor and overall liking, meaning that the acceptability scores of the overall taste and flavor were likely more correlated to each other as well as the scores of overall taste and overall liking while the scores of overall liking was less likely correlated with the scores of flavor. The sample that was located close to any other samples on the biplots would indicate similarity. Visual inspection of the biplots would suggest the sauces containing KCl/AMP, KCl/Arg/AMP with the commercial salt substitute being the closest where the samples containing NaCl and KCl were isolated from each other. This is similar to the pairwise comparison of the means in the ANOVA result. If one were to recommend a salt substitute, the sauce with KCl/Arg/AMP would appear to be the most favorable one at least under the conditions from this study.

## 6.4 Conclusions

Different salt substitutes added in the pasta sauce caused significant differences in the consumer responses toward sensory attributes. Flavor, taste, and overall liking were discriminating attributes for overall product difference. As expected, the sauce containing NaCl was the most accepted followed by the sauces containing KCl/Arg/AMP, KCl/AMP, and the commercial salt substitute, and the one with KCl was the least accepted. Acceptance and purchase intent of the sauces containing KCl and KCl/Arg/AMP would be predicted by the overall liking scores. Acceptance of the sample containing KCl/AMP was mainly affected by overall liking and bitterness intensity Acceptance of the sample containing the commercial salt substitute was contributed to the liking scores for taste. Purchase intent of the sauce containing NaCl was influenced by the liking scores for taste, overall liking, and saltiness intensity. However, overall liking scores and overall product acceptability affected purchase intent for the sauce that contained KCl/AMP where the liking scores for taste and acceptability influenced the probability of buying the sauce that contained the commercial salt substitute. The overall liking score decreased approximately by two points when saltiness intensity was reported as not salty enough and the score decreased up to 4.5 points when the bitter taste was found to be strong. KCl/Arg/AMP could be commercialized with the similar acceptance scores to the commercial salt substitute along with the similar positive responses of product acceptance and purchase intent.

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# CHAPTER 7.
# SUMMARY AND CONCLUSIONS

Consumer awareness of health risks associated with consumption of food ingredients including dietary sodium has drastically increased over recent years. Excess sodium intake may cause high blood pressure which is linked to other health problems such as strokes, heart and kidney diseases. Salt restriction is advisable to prevent high blood pressure and decrease elevated blood pressure. In addition, consuming a potassium-rich diet helps lower the risk of stroke and hypertension. As table salt is the major source of sodium in our diets, use of salt substitutes is a practical approach to reduce sodium content in the diet as an alternative to decreasing the amount of salt used in foods. Potassium chloride (KCl) is one of the most widely-used salt substitutes but its undesirable bitter taste remains a problem. The anion and cation size of salts affects their taste qualities, especially saltiness and bitterness. Effects of the sodium cation and the chloride anion generate the unique clean salty taste of NaCl and it seems that there is no salt replacer that can give such taste. Therefore, taste qualities have been a challenge for researchers in the development of sodium-free salts.

The use of bitterness-inhibiting agents incorporated into salt substitute is undergoing extensive investigations. The compounds that have been used to inhibit the bitter taste of KCl include fumaric acid, lactose and/or dextrose and cream of tartar, potassium phosphate, autolyzed yeast, lysine monohydrochloride, monosodium glutamate (MSG), adenosine (AMP) and inosine (IMP) monophosphates, and specific combinations of sulfate-containing and chloride-containing salts. Adenosine-5'-monophosphate (AMP) is the first natural compound that has been found to be able to block several bitter tastants. However, limited studies have done on the potential of AMP in inhibiting bitterness, the sensory characteristics of AMP in KCl solutions, and the consumer acceptability of a food model using a salt substitute with AMP. These aspects are crucial in developing a potential sodium-free salt and were a focus of this research. Additionally, L-arginine (Arg), was also assessed to improve salty and bitter taste qualities of salt substitutes.

The aim of the present study was to develop an acceptable sodium-free salt in which AMP and Arg were applied without compromising desirable taste qualities. The current study addressed the potential of AMP in inhibiting bitterness by evaluating threshold values of KCl/AMP compared to those of KCl. Results revealed that AMP exhibited the ability of inhibiting bitterness of KCl with the recognition threshold value of 1.5 times less in KCl/AMP than in KCl using the method of limits. The salty taste of KCl was not affected by the presence of AMP. The ASTM method of ascending limits provided more accurate results but it required more sample preparation and likely caused sensory fatigue in the panelists.

With the aim of enhancing saltiness and inhibiting bitterness of KCl, Arg was added and the optimal ratio of Arg in KCl/AMP was determined at 0.3% using a ranking test. A ratio of KCl/Arg/AMP of 15:2:1 was the best proportion among the salts that contained Arg. Using the Spectrum Descriptive Analysis, KCl/Arg/AMP (15:2:1) was significantly saltier and less bitter than KCl. Arg and AMP had a synergistic effect not only in inhibiting undesirable bitterness but also enhancing saltiness in the 0.5% and 1% KCl solutions.

Regarding consumer acceptability, the pasta sauce containing NaCl was the most accepted where the pasta sauce containing KCl was the least accepted. Overall liking scores affected overall product acceptance and purchase intent for all samples except the one that contained the commercial salt substitute. The pasta sauces that were sodium-free were found to be bitter and not salty enough which resulted in mean drops of overall liking scores. Further studies should include other saltiness enhancers or compounds that can enhance umami taste. Saltiness or savoriness may improve the overall taste quality of products. In conclusion, the findings of the bitterness-inhibiting ability of AMP in KCl solutions revealed that KCl/Arg/AMP (15:2:1) could potentially be a commercial salt substitute.

## APPENDIX A: PRELIMINARY STUDY OF THRESHOLD DETERMINATION IN CHAPTER 3

Name: ........................................................................................ Date: ..............................

NOTE:

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

---

**Part I. Familiarizing with the tastes.**

| | | |
|---|---|---|
| Sample **A** | – | no salty and bitter tastes |
| Samples **B** and **C** | – | salty taste |
| Samples **D** and **E** | – | bitter taste |

**Part II. Circle the taste(s) that you perceived.**

| | | | | | | |
|---|---|---|---|---|---|---|
| **312** | Sweet | Salty | Sour | Bitter | Unidentified | No Taste |
| **740** | Sweet | Salty | Sour | Bitter | Unidentified | No Taste |
| **189** | Sweet | Salty | Sour | Bitter | Unidentified | No Taste |
| **635** | Sweet | Salty | Sour | Bitter | Unidentified | No Taste |

Name: ........................................................................................ Date: ..............................

**INSTRUCTION:**

1) Please rate the following attribute of 6 samples, compared with the control solution.
2) Your answer can be “DIFFERENT FROM” or “SAME AS” the attribute evaluated of the CONTROL.
3) You also have the options of "I am sure" or "I am not sure" about the answer given.

**NOTE:**

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

---

| **BITTERNESS** | | | | |
|---|---|---|---|---|
| **Sample** | **Different** | | **Same** | |
| | **Sure** | **Not sure** | **Not Sure** | **Sure** |
| 024 | | | | |
| 301 | | | | |
| 102 | | | | |
| 404 | | | | |
| 908 | | | | |
| 516 | | | | |

| **SALTINESS** | | | | |
|---|---|---|---|---|
| **Sample** | **Different** | | **Same** | |
| | **Sure** | **Not sure** | **Not Sure** | **Sure** |
| 030 | | | | |
| 105 | | | | |
| 221 | | | | |
| 403 | | | | |
| 850 | | | | |
| 160 | | | | |

Name: ................................................................................ Date: ....................................

**INSTRUCTION:**

1) Please rate the following attribute of 6 samples, compared with the control solution.
2) Your answer can be "DIFFERENT  FROM" or "SAME AS" the attribute evaluated of the CONTROL.
3) You also have the options of "I am sure" or "I am not sure" about the answer given.

**NOTE:**

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

---

| **OVERALL** | | | | |
|---|---|---|---|---|
| **Sample** | **Different** | | **Same** | |
| | **Sure** | **Not sure** | **Not Sure** | **Sure** |
| 024 | | | | |
| 301 | | | | |
| 102 | | | | |
| 404 | | | | |
| 908 | | | | |
| 516 | | | | |

Name: ..........................................................................................................................................  Date: ...................................

**INSTRUCTION:**

1) Taste the samples from left to right. Two samples are identical; one is different.
2) Select the **ODD/DIFFERENT** sample.
3) Identify the taste(s) of the odd sample that exhibits recognizable difference, **only if you perceived**.
   Otherwise, circle "unidentified".

**NOTE:**

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

| Set of 3 Samples | Which is the odd sample? | Circle the taste(s) which exhibits the difference | Remarks |
|---|---|---|---|
| **415 – 850 – 175** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **311 – 462 – 768** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **330 – 166 – 092** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **723 – 655 – 841** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **007 – 754 – 486** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **168 – 039 – 215** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **047 – 206 – 480** | | Sweet – Salty – Sour – Bitter - Unidentified | |

## APPENDIX B: THRESHOLD DETERMINATION IN CHAPTER 4

### a. Questionnaire

**INSTRUCTION:**

1) Please rate the following attribute of 6 samples, compared with the control solution.
2) Your answer can be "DIFFERENT FROM" or "SAME AS" the attribute evaluated of the CONTROL.
3) You also have the options of "I am sure" or "I am not sure" about the answer given.

**NOTE:**

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

---

| BITTERNESS | | | | |
|---|---|---|---|---|
| **Sample** | **Different** | | **Same** | |
| | **Sure** | **Not sure** | **Not Sure** | **Sure** |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

| SALTINESS | | | | |
|---|---|---|---|---|
| **Sample** | **Different** | | **Same** | |
| | **Sure** | **Not sure** | **Not Sure** | **Sure** |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Name:**.................................................................................... **Date:**......................

**INSTRUCTION:**

1) Please rate the following attribute of 6 samples, compared with the control solution.
2) Your answer can be “DIFFERENT FROM” or “SAME AS” the attribute evaluated of the CONTROL.
3) You also have the options of "I am sure" or "I am not sure" about the answer given.

**NOTE:**

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

---

| **OVERALL** | | | | |
|---|---|---|---|---|
| **Sample** | **Different** | | **Same** | |
| | **Sure** | **Not sure** | **Not Sure** | **Sure** |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Name:** ........................................................................................................................ **Date:** ..............................

**INSTRUCTION:**

1) Taste the samples from left to right. Two samples are identical; one is different.
2) Select the **ODD/DIFFERENT** sample.
3) Identify the taste(s) of the odd sample that exhibits recognizable difference, **only if you perceived**.
   Otherwise, circle "unidentified".

**NOTE:**

1) Take the whole sample into the mouth.
2) Swirl it for 2-3 seconds.
3) Expectorate and answer the question.
4) Rinse your mouth with water between samples.

| Set of 3 Samples | Which is the odd sample? | Circle the taste(s) which exhibits the difference | Remarks |
|---|---|---|---|
| **175 – 281 – 054** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **463 – 121 – 356** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **141 – 260 – 329** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **811 – 750 – 162** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **182 – 509 – 031** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **504 – 128 – 423** | | Sweet – Salty – Sour – Bitter - Unidentified | |
| **223 – 315 – 420** | | Sweet – Salty – Sour – Bitter - Unidentified | |

**b. Sample Calculation of the Group Best-Estimate Threshold of KCl for the Method of Limits**

| Panelists | Judgments (concentration increase --> ) | | | | | | | Best-Estimate Threshold (BET) | |
|---|---|---|---|---|---|---|---|---|---|
| | **0.01** | **0.02** | **0.04** | **0.08** | **0.16** | **0.32** | **0.64** | **BET** | **$log_{10}$BET** |
| 1 | 0 | + | + | + | + | + | + | 0.0141 | -1.849 |
| 2 | + | + | + | + | + | + | + | 0.0071 | -2.151 |
| 3 | + | + | + | + | + | + | + | 0.0071 | -2.151 |
| 4 | 0 | 0 | + | + | + | + | + | 0.0283 | -1.548 |
| 5 | 0 | + | + | + | + | + | + | 0.0141 | -1.849 |
| 6 | + | + | + | + | + | + | + | 0.0071 | -2.151 |
| 7 | + | 0 | + | + | + | + | + | 0.0283 | -1.548 |
| 8 | + | + | + | + | + | + | + | 0.0071 | -2.151 |
| 9 | 0 | 0 | 0 | + | + | + | + | 0.0566 | -1.247 |
| 10 | 0 | + | + | + | + | + | | 0.0141 | -1.849 |
| 11 | 0 | 0 | + | + | + | + | + | 0.0283 | -1.548 |
| 12 | + | 0 | + | + | + | + | + | 0.0283 | -1.548 |
| 13 | + | + | + | + | + | + | + | 0.0071 | -2.151 |
| 14 | 0 | + | + | 0 | + | + | + | 0.1131 | -0.946 |
| 15 | 0 | 0 | 0 | + | + | + | + | 0.0566 | -1.247 |
| | | | | | | | | $\sum log_{10}$ → | -25.936 |
| **Group BET geometric mean** | | | | | | | | 0.0187 ← | -1.729 |
| **Standard deviation** | | | | | | | | | 0.391 |

”0” indicates that the panelist selected the wrong sample of the set of three.

“+” indicates that the panelist selected the correct sample.

**c. Scale Conversion for the Frequency Distribution**

$$Y = \ln(x)/\ln 2 + c$$

where X = concentration (g/100 ml),
Y = concentration in the logarithm scale,
C = a constant such that the most dilute solutions used in the tests correspond to 1,
and ln 2 was used because the dilution factor per step was 2.

So, $Y = 1.4427*\ln(x) + 7.6439$

**d. SAS Code: Individual Threshold Distribution**

```
data one;
/* xkcl= threshold of KCl in g/100 ml; xkclamp= threshold of KCl/AMP in g/100 ml*/
input xkcl xkclamp;
/* log (x) without base in SAS = ln (x)*/
kcl=1.442*log(xkcl)+6.643;
kclamp=1.442*log(xkclamp)+6.643;
label kcl='Detection Threshold of KCl';
label kclamp='Detection Threshold of KCl/AMP';
cards;
inserted data;
run;
proc univariate;
histogram kcl / midpoints= 0 to 6 by 1
                normal(color=blue w=3)
                font = calibri
                height = 6
                vscale = count
                vaxis = 0 to 15 by 5
                vaxislabel='No. of Observers';
run;
proc univariate;
histogram kclamp /midpoints= 0 to 6 by 1
                normal(color=blue w=3)
                font = calibri
                height = 6
                vscale = count
                vaxis = 0 to 15 by 5
                vaxislabel='No. of Observers';
run;
```

**e. SAS Code: Logistic Model**

```
data IndThreshold;
input panelist x c;
p=c/3;
cards;
inserted data;
run;
proc sort;
by panelist;
proc nlin data= IndThreshold  best=5;
by panelist;
parms B=-10 to 10 by 0.5;
T=-10 to 10 by 0.5;
K = B*(T-log10(x));
E = exp(K);
N = (1/3 + E);
D = (1 + E);
MODEL P=N/D;

data one;
input xkcl;
kcl=1.442*log(xkcl)+6.643;
label kcl='Detection Threshold of KCl;
cards;
inserted data;
run;
proc univariate;
histogram kcl / midpoints= 0 to 6 by 1
                    normal(color=blue w=3)
                    font = calibri
                    height = 6
                    vscale = count
                    vaxis = 0 to 15 by 5
                   vaxislabel='No. of Observers';
run;
```

## APPENDIX C: RANKING TEST IN CHAPTER 5.1

Name: .................................................... Date: ..........................

Note: 1) You will be presented with the 4 labeled samples in random order.

2) Please taste the samples in the order presented, from left to right.

3) Rank the samples for intensity. No ties allowed!

**I: Saltiness Evaluation**

- Rank the solutions in a descending order of saltiness

_________ > _________ > _________ > ___________

Saltiest ... Least salty

**II: Bitterness Evaluation**

- Rank the solutions in a descending order of bitterness

___________ > _________ > _________ > ___________

Most bitter ... Least bitter

# APPENDIX D: DESCRIPTIVE ANALYSIS IN CHAPTER 5.2

## a. Research Consent Form

I, _________________________, agree to participate in the research entitled "Sensory Evaluation of a Prototype Salt Substitute Product", which is being conducted by Witoon Prinyawiwatkul, Professor of the Department of Food Science, phone number (225)-578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. 12 consumers will participate in this research. For this particular research, about 10-15 min. participation per session will be required for each subject.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.
2. The reason for the research is to gather information on descriptive sensory characteristics of caffeine and a salt substitute (sodium chloride, potassium chloride, L-arginine, and 5'-adenosine monophosphate (AMP)). The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.
3. The procedures are as follows: Coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risk: The only risk which can be envisioned is the allergic reaction toward NaCl (regular salt), KCl, L-Arginine (amino acid), caffeine, and AMP (nucleotide). Individuals who have kidney problem should not participate in this study.
5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

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

I have been given a copy of the consent form.

_______________________________ _________________________________

Signature of Investigator Signature of Participant

Witness:________________________ Date: _________________________________

**b. Questionnaire**

Name: ______________________________ Date:_______________

**Part I:** Match each solution to one of the perceived tastes (salty or bitter)

Salty ______________________________

Bitter ______________________________

**Part II:** Ranking the intensity of samples

***Saltiness:*** _____ _____ _____ _____

Least salty Saltiest

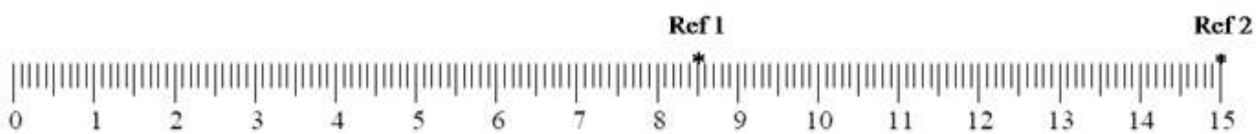
***Bitterness:*** _____ _____ _____ _____

Least bitter Most bitter

**Part III:** Rating the intensity of unknown samples on a 15-cm scale

1. Taste each reference sample: Ref 1 and Ref 2
2. Taste unknown sample
3. Rate the intensity of unknown sample on a 15-cm scale

***Saltiness:***




***Bitterness:***

**BITTERNESS**


**SALTINESS**

## SALTINESS INTENSITY EVALUATION

Please rate the intensity of unknown samples on a 22-cm scale

**Sample# A**


**Sample# B**


**Sample# C**


**Sample# D**


**Sample# E**

## BITTERNESS INTENSITY EVALUATION

Please rate the intensity of unknown samples on a 15-cm scale

**Sample# A**


**Sample# B**


**Sample# C**


**Sample# D**


**Sample# E**

**c. SAS Code: ANOVA**

```
data DA;
input panelist  sample $ S1 S2 B1  B2;
cards;
inserted data;
proc means mean std n;
by sample;
proc anova;
class sample;
model  S1 S2 B1 B2 = sample;
means sample /tukey;
run;
```

**d. SAS Code: Cluster Analysis**

```
data intensities;
input salt $ x1-x4;
label    x1 = "0.5% Saltiness"
         x2 = "1% Saltiness"
         x3 = "0.5% Bitterness"
         x4 = "1% Bitterness";
cards;
inserted data;
proc sort data=intensities;
by salt;
proc distance data=intensities(Obs=5) out=distances  method=euclid;
var interval(x1-x4);
id salt;
proc print data=distances;
id salt;
run;
proc cluster data=distances(Type=distance) outtree=tree method=single noeigen nonorm;
id salt;
proc tree data=tree horizontal;
run;
```

# APPENDIX E: CONSUMER STUDY IN CHAPTER 6

## a. Research Consent Form

I, ______________________, agree to participate in the research entitled "Consumer Acceptance of Pasta" which is being conducted by Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University Agcenter, 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. Two hundred and four consumers will participate in this research. For this particular research, about 15-minute 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 product. 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: Three 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 butter, wheat, tomato, bell pepper, onion, spices, regular salt, potassium chloride, nucleotide, and 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

Witness: ______________________ Date: ______________________________

## b. Questionnaire for the Control Sample

**Age group** [ ] 18-24 yrs [ ] 25-34 yrs [ ] 35-44 yrs [ ] 45-54 yrs [ ] over 55 yrs

**Gender** [ ] Male [ ] Female

**Please evaluate the following attributes of the pasta sauce.**

1. How would you rate the **APPEARANCE** of this pasta sauce?

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

2. How would you rate the **OVERALL TASTE** of this pasta sauce?

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

3. How would you rate the **OVERALL FLAVOR** of this pasta sauce?

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

4. How would you rate the **SALTINESS** in this pasta sauce?

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

5. How would you rate the **SALTINESS** of this pasta sauce?

[ ] Not salty enough [ ] Just about right [ ] Too salty

6. Did you detect **BITTERNESS/AFTERTASTE** in this pasta sauce?

[ ] YES, but acceptable [ ] YES, but unacceptable [ ] NO

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

7. How would you rate the **OVERALL LIKING** of this pasta sauce?

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

8. Is this pasta sauce **ACCEPTABLE**? [ ] YES [ ] NO

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

## c. Questionnaire for Other Samples

**Age group** [ ] 18-24 yrs [ ] 25-34 yrs [ ] 35-44 yrs [ ] 45-54 yrs [ ] over 55 yrs

**Gender** [ ] Male [ ] Female

**Please evaluate the following attributes of the pasta sauce.**

1. How would you rate the **APPEARANCE** of this pasta sauce?

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

2. How would you rate the **OVERALL TASTE** of this pasta sauce?

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

3. How would you rate the **OVERALL FLAVOR** of this pasta sauce?

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

4. How would you rate the **SALTINESS** in this pasta sauce?

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

5. How would you rate the **SALTINESS** of this pasta sauce?

[ ] Not salty enough [ ] Just about right [ ] Too salty

6. Did you detect **BITTERNESS/AFTERTASTE** in this pasta sauce?

[ ] YES, but acceptable [ ] YES, but unacceptable [ ] NO

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

7. How would you rate the **OVERALL LIKING** of this pasta sauce?

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

8. Is this pasta sauce **ACCEPTABLE**? [ ] YES [ ] NO

9. Is this pasta sauce **ACCEPTABLE** knowing that it has <u>**NO SODIUM ADDED**</u>, which does not cause high blood pressure? [ ] YES [ ] NO

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

11. Would you **BUY** this product knowing that it <u>**NO SODIUM ADDED**</u>, which does not cause high blood pressure? [ ] YES [ ] NO

**d. Distribution of Consumer Age and Gender**

| Frequency<br>Percent<br>Row percent<br>Column percent | Age Group | | | | | Total |
|---|---|---|---|---|---|---|
| | 18-24 yr | 25-34 yr | 35-44 yr | 45-54 yr | over 55 yr | |
| Male | 76 | 25 | 4 | 1 | 0 | 106 |
| | 37.25 | 12.25 | 1.96 | 0.49 | 0 | 51.96 |
| | 71.7 | 23.58 | 3.77 | 0.94 | 0 | |
| | 49.03 | 62.5 | 100 | 33.33 | 0 | |
| Female | 79 | 15 | 0 | 2 | 2 | 98 |
| | 38.73 | 7.35 | 0 | 0.98 | 0.98 | 48.04 |
| | 80.61 | 15.31 | 0 | 2.04 | 2.04 | |
| | 50.97 | 37.5 | 0 | 66.67 | 100 | |
| Total | 155 | 40 | 3 | 3 | 2 | 204 |
| | 75.98 | 19.61 | 1.96 | 1.47 | 0.98 | 100 |

**e. ANOVA Tables for Consumer Liking Scores of Each Attributes**

| Appearance | DF | SS | MS | F |
|---|---|---|---|---|
| Total | 611 | 1096.06 | 1.79 | |
| Repetitions | 33 | 105.61 | 3.20 | |
| Blocks within repetitions | 170 | 699.11 | 4.11 | |
| Treatments (adjusted) | 4 | 13.36 | 3.34 | 4.85 |
| Error | 404 | 277.98 | 0.69 | |

| Flavor | DF | SS | MS | F |
|---|---|---|---|---|
| Total | 611 | 2869.67 | 4.70 | |
| Repetitions | 33 | 174.28 | 5.28 | |
| Blocks within repetitions | 170 | 989.39 | 5.82 | |
| Treatments (adjusted) | 4 | 990.12 | 247.53 | 139.69 |
| Error | 404 | 715.88 | 1.77 | |

| Taste | DF | SS | MS | F |
|---|---|---|---|---|
| Total | 611 | 3235.72 | 5.30 | |
| Repetitions | 33 | 191.39 | 5.80 | |
| Blocks within repetitions | 170 | 1005.00 | 5.91 | |
| Treatments (adjusted) | 4 | 1297.47 | 324.37 | 176.64 |
| Error | 404 | 741.86 | 1.84 | |

| Saltiness | DF | SS | MS | F |
|---|---|---|---|---|
| Total | 611 | 2519.47 | 4.12 | |
| Repetitions | 33 | 155.75 | 4.72 | |
| Blocks within repetitions | 170 | 924.39 | 5.44 | |
| Treatments (adjusted) | 4 | 658.61 | 164.65 | 85.20 |
| Error | 404 | 780.72 | 1.93 | |

| Overall Liking | DF | SS | MS | F |
|---|---|---|---|---|
| Total | 611 | 3217.67 | 5.27 | |
| Repetitions | 33 | 180.83 | 5.48 | |
| Blocks within repetitions | 170 | 1024.17 | 6.02 | |
| Treatments (adjusted) | 4 | 1321.98 | 330.50 | 193.32 |
| Error | 404 | 690.69 | 1.71 | |

**f. SAS Code: *P*-values of *F* Approximations**

```
data ftests;
*aa = df numerator; bb = df denominator; Number = F-value;
input aa bb Number;
*Probf(X,a,b) = Prob(F(a,b) <= X), not the P-value;
Pvalue = 1-probf(Number,aa,bb);
datalines;
inserted data;
proc print;
run;
```

**g. Mean Differences and 95% Confidence Intervals of Sample Pairs for the Consumer Liking Scores of Each Attributes**

| Appearance | D[a] | L, 95% C.I.[b] | U, 95% C.I.[b] |
|---|---|---|---|
| NaCl-KCl | **0.46** | 0.247 | 0.669 |
| NaCl-KCl/AMP | 0.20 | -0.013 | 0.408 |
| NaCl-KCl/Arg/AMP | 0.16 | -0.047 | 0.164 |
| NaCl-Commercial salt substitute | 0.06 | -0.148 | 0.274 |
| KCl-KCl/AMP | **-0.26** | -0.519 | -0.002 |
| KCl-KCl/Arg/AMP | **-0.29** | -0.552 | -0.036 |
| KCl- Commercial salt substitute | **-0.39** | -0.653 | -0.137 |
| KCl/AMP-KCl/Arg/AMP | -0.03 | -0.292 | 0.225 |
| KCl/AMP-Commercial salt substitute | -0.13 | -0.393 | 0.124 |
| KCl/Arg/AMP-Commercial salt substitute | -0.10 | -0.359 | 0.157 |

[a]D – Mean difference of sample pairs.
[b]95% C.I. – 95% confidence interval; L = lower bound; U = upper bound.
[c]Bold values indicate significant difference as their 95% confidence interval do not contain zero ($p < 0.05$).

| Flavor | D[a] | L, 95% C.I.[b] | U, 95% C.I.[b] |
|---|---|---|---|
| NaCl-KCl | **3.95** | 3.615 | 4.292 |
| NaCl-KCl/AMP | **2.86** | 2.519 | 3.195 |
| NaCl-KCl/Arg/AMP | **2.48** | 2.145 | 2.483 |
| NaCl-Commercial salt substitute | **2.75** | 2.414 | 3.090 |
| KCl-KCl/AMP | **-1.10** | -1.511 | -0.682 |
| KCl-KCl/Arg/AMP | **-1.47** | -1.885 | -1.056 |
| KCl-Commercial salt substitute | **-1.20** | -1.616 | -0.787 |
| KCl/AMP-KCl/Arg/AMP | -0.37 | -0.788 | 0.040 |
| KCl/AMP-Commercial salt substitute | -0.11 | -0.519 | 0.309 |
| KCl/Arg/AMP-Commercial salt substitute | 0.27 | -0.145 | 0.683 |

[a]D – Mean difference of sample pairs.
[b]95% C.I. – 95% confidence interval; L = lower bound; U = upper bound.
[c]Bold values indicate significant difference as their 95% confidence interval do not contain zero ($p < 0.05$).

| Taste | D[a] | L, 95% C.I.[b] | U, 95% C.I.[b] |
|---|---|---|---|
| NaCl-KCl | **4.41** | 4.065 | 4.753 |
| NaCl-KCl/AMP | **3.41** | 3.069 | 3.757 |
| NaCl-KCl/Arg/AMP | **3.01** | 2.661 | 3.006 |
| NaCl-Commercial salt substitute | **3.22** | 2.876 | 3.564 |
| KCl-KCl/AMP | **-1.00** | -1.418 | -0.574 |
| KCl-KCl/Arg/AMP | **-1.40** | -1.825 | -0.982 |
| KCl-Commercial salt substitute | **-1.19** | -1.611 | -0.767 |
| KCl/AMP-KCl/Arg/AMP | -0.41 | -0.829 | 0.014 |
| KCl/AMP-Commercial salt substitute | -0.19 | -0.615 | 0.228 |
| KCl/Arg/AMP-Commercial salt substitute | 0.21 | -0.207 | 0.636 |

[a]D – Mean difference of sample pairs.

[b]95% C.I. – 95% confidence interval; L = lower bound; U = upper bound.

[c]Bold values indicate significant difference as their 95% confidence interval do not contain zero ($p < 0.05$).

| Saltiness | D[a] | L, 95% C.I.[b] | U, 95% C.I.[b] |
|---|---|---|---|
| NaCl-KCl | **2.96** | 2.602 | 3.308 |
| NaCl-KCl/AMP | **2.48** | 2.131 | 2.838 |
| NaCl-KCl/Arg/AMP | **2.32** | 1.967 | 2.321 |
| NaCl-Commercial salt substitute | **2.42** | 2.068 | 2.775 |
| KCl-KCl/AMP | **-0.47** | -0.903 | -0.038 |
| KCl-KCl/Arg/AMP | **-0.63** | -1.067 | -0.202 |
| KCl-Commercial salt substitute | **-0.53** | -0.966 | -0.101 |
| KCl/AMP-KCl/Arg/AMP | -0.16 | -0.596 | 0.269 |
| KCl/AMP-Commercial salt substitute | -0.06 | -0.496 | 0.370 |
| KCl/Arg/AMP-Commercial salt substitute | 0.10 | -0.332 | 0.533 |

[a]D – Mean difference of sample pairs.

[b]95% C.I. – 95% confidence interval; L = lower bound; U = upper bound.

[c]Bold values indicate significant difference as their 95% confidence interval do not contain zero ($p < 0.05$).

| Overall Liking | D[a] | L, 95% C.I.[b] | U, 95% C.I.[b] |
|---|---|---|---|
| NaCl-KCl | **4.34** | 4.006 | 4.671 |
| NaCl-KCl/AMP | **3.50** | 3.170 | 3.835 |
| NaCl-KCl/Arg/AMP | **3.12** | 2.792 | 3.124 |
| NaCl-Commercial salt substitute | **3.38** | 3.044 | 3.709 |
| KCl-KCl/AMP | **-0.84** | -1.243 | -0.429 |
| KCl-KCl/Arg/AMP | **-1.21** | -1.621 | -0.807 |
| KCl-Commercial salt substitute | **-0.96** | -1.369 | -0.555 |
| KCl/AMP-KCl/Arg/AMP | -0.38 | -0.785 | 0.029 |
| KCl/AMP-Commercial salt substitute | -0.13 | -0.533 | 0.281 |
| KCl/Arg/AMP-Commercial salt substitute | 0.25 | -0.155 | 0.659 |

[a]D – Mean difference of sample pairs.
[b]95% C.I. – 95% confidence interval; L = lower bound; U = upper bound.
[c]Bold values indicate significant difference as their 95% confidence interval do not contain zero ($p < 0.05$).

**h. SAS Code: MANOVA, DDA**

```
data one;
input Panelist  Sample $ Age $  Gender $ Appearance Flavor Taste Saltiness Oliking;
cards;
inserted data;
proc freq;
tables gender*age;
proc sort; by sample;
proc means mean std n maxdec=2;
by sample;
var  Appearance--Oliking;
proc candisc out=outcan mah;
class sample;
var Appearance--Oliking;
run;
```

**i. SAS Code: McNemar's Test**

```
data one;
input Panelist  Sample $ SJAR  Bitter  bDetect Accept AccNHBP Buy BuyNHBP;
if bDetect='0' then bitter='0';
```

```
cards;
inserted data;
proc sort; by sample;
proc freq; by sample;
          tables Accept AccNHBP Buy BuyNHBP SJAR;
tables Accept*AccNHBP/agree;
tables Buy*BuyNHBP/agree;
tables Bitter*bDetect;
run;
```

**j. SAS Code: Signal-to-Noise Ratio**

```
data one;
input  Panelist  Sample $ Taste Saltiness Oliking SJAR bDetect Bitter;
if bDetect='0' then Bitter='0';
k = 0.25;
sSNR= -10*log10((SJAR-2)**2 + k);
bSNR= -10*log10((Bitter)**2 + k);
cards;
inserted data;
proc sort;
by sample;
proc means mean n maxdec=2;
by sample;
var  Taste--Oliking sSNR bSNR;
run;
```

**k. SAS Code: Penalty Analysis for Non-Resampling Data**

```
data meandrop;
Input Panelist  Sample $ Taste Saltiness Oliking SJAR bDetect Bitter;
if bDetect='0' then Bitter='0';
cards;
inserted data;
run;
data meandrop1;
drop taste saltiness oliking sJAR bitter;
set meandrop;
if sJAR=1 then SS1=Saltiness;
if sJAR=2 then SS2=Saltiness;
if sJAR=3 then SS3=Saltiness;
if sJAR=1 then TS1=Taste;
if sJAR=2 then TS2=Taste;
if sJAR=3 then TS3=Taste;
if sJAR=1 then OS1=Oliking;
```

```
if sJAR=2 then OS2=Oliking;
if sJAR=3 then OS3=Oliking;
if bitter=0 then TB0=Taste;
if bitter=1 then TB1=Taste;
if bitter=2 then TB2=Taste;
if bitter=3 then TB3=Taste;
if bitter=0 then OB0=Oliking;
if bitter=1 then OB1=Oliking;
if bitter=2 then OB2=Oliking;
if bitter=3 then OB3=Oliking;
run;
proc sort; by sample;
proc print data=meandrop1 noobs; run;
proc means data=meandrop1;
class sample;
var OS1 OS2 OS3 TS1 TS2 TS3 SS1 SS2 SS3 TB0 TB1 TB2 TB3 OB0 OB1 OB2 OB3;
output out=means1 mean(SS1 SS2 SS3 TS1 TS2 TS3 OS1 OS2 OS3 TB0 TB1 TB2 TB3 OB0
OB1 OB2 OB3) = SS1 SS2 SS3 TS1 TS2 TS3 OS1 OS2 OS3 TB0 TB1 TB2 TB3 OB0 OB1
OB2 OB3;
run;
proc print data=means1 noobs; run;
data means2;
        set means1;
        if _type_ = 1;
        meandropSS1 = round(SS1 - SS2,.01);
        meandropSS3 = round(SS3 - SS2,.01);
        meandropTS1 = round(TS1 - TS2,.01);
        meandropTS3 = round(TS3 - TS2,.01);
        meandropOS1 = round(OS1 - OS2,.01);
        meandropOS3 = round(OS3 - OS2,.01);
        meandropOB1 = round(OB1 - OB0,.01);
        meandropOB2 = round(OB2 - OB0,.01);
        meandropOB3 = round(OB3 - OB0,.01);
        meandropTB1 = round(TB1 - TB0,.01);
        meandropTB2 = round(TB2 - TB0,.01);
        meandropTB3 = round(TB3 - TB0,.01);
run;
proc print data=means2 noobs;
var sample meandropSS1 meandropSS3 meandropTS1 meandropTS3 meandropOS1
meandropOS3;
run;
proc print data=means2 noobs;
var sample meandropTB1 meandropTB2 meandropTB3 meandropOB1 meandropOB2
meandropOB3;
run;
```

**l. SAS Code: Penalty Analysis for Resampling Data**

**Creating the Bootstrap Data in MS Excel**

1. Pair hedonic scores ($X_1$) and JAR scores ($X_2$) in the form of ($X_1$, $X_2$). Use the data of a non-JAR (too weak or too strong) with the data of JAR.
2. Select the original data set.
3. Create a formula.
   - For Excel 2007, go to "Formulas tab" → "Define Name" under the Defined Names section.
   - For Excel 2003, go to menu "Insert" → "Name" → "Define"
4. Define a formula name; for example, '*Sample1*'.
5. Click a blank cell and type exactly below. In this example type it in D1.
   =INDEX(*Sample1*,ROWS(*Sample1*)*RAND()+1,COLUMNS(*Sample1*)*RAND()+1)
6. It will give a bootstrap data.
7. Then create more bootstrap data by copying the formula across columns and rows. In this example, a data set with 250 observations was created.
8. Copy the data set of bootstrap data then paste special as values in a new worksheet (in A1) to convert from formulas to the actual numbers. Notice that these numbers are different from those shown in the original data sheet.
9. Save the worksheet in *.prn (Menu → Save as → Other Formats → Save as Type: Formatted Text (Space Limited) → Enter a filename →Save → OK to save only the active sheet → Yes to keep the "prn" format. Exit from Excel.

```
*Read prn file output from Excel;
data penalty;
infile 'prn file location' ;
input F1 $ F2 $ F3 $ F4 $ F5 $ F6 $  F7 $ F8 $ F9 $ F10 $;
run;
*This step slices and dices the character variables in the form (X1,X2) into separate single-digit numbers X1 and X2;
data penalty2 (keep= rep Hedonic JAR);
set penalty;
*Create an array to hold the ten Hedonic-JAR pairs (one per rep);
array boot{10} F1 - F10;
do i=1 to 10;
*Set the rep to i (1 through 10) so that means can be calculated by rep;
rep = i;
*Create Hedonic and JAR by slicing and dicing the character variable into digits and converting to numeric;
Hedonic = input(substr(boot{i},2,1),1.);
JAR = input(substr(boot{i},4,1),1.);
output;
end;
run;
proc print data=penalty2;
```

```
run;

data penalty3;
drop hedonic JAR;
set penalty2;
if JAR=1 then x1=Hedonic;
if JAR=2 then x2=Hedonic;
proc print data=penalty3;
run;

*Calculate the means for x1 and x2 by replication;
proc means data = penalty3 maxdec=2;
class rep;
var x1 x2;
output out = means1 mean(x1 x2) = x1 x2;
run;
proc print data = means1 round;
run;

* Keep only the means for each replication, not the overall mean, and calculate the meandrop;
data means2 (keep = meandrop);
set means1;
if _type_ = 1;
meandrop = round(x1 - x2,.01);
run;
proc print data = means2;
run;
proc means data = means2 mean t prt maxdec=2;
var meandrop;
run;
```

**m. SAS Code: Logistic Regression**

```
*Critical sensory attributes for acceptability and purchase intent regardless of sample;
data one;
input   Panelist  Sample $ Age  Gender Appearance Flavor Taste Saltiness Oliking SJAR  Bitter
bDetect Accept  Buy   BuyNHBP;
if bDetect=0 then bitter=0;
cards;
inserted data;
run;
title 'Acceptance - All Samples';
proc logistic data=one desc;
class sJAR (ref='2') bitter(ref=first);
model accept=Appearance Flavor Taste Saltiness Oliking SJAR BITTER /link=logit
selection=stepwise;
run;
```

```
title 'Purchase Intent - All Samples';
proc logistic data=buy desc;
class sJAR (ref='2') bitter(ref=first) accept (ref=first);
model buy= Appearance Flavor Taste Saltiness Oliking SJAR BITTER accept /link=glogit
selection=stepwise;
run;
```

**Predictive models for acceptability and purchase intent;*

```
ods html file='filename.html';
ods graphics on;
data B;
set one (where=(sample='B'));
Title 'Acceptance - KCl';
proc logistic data=B desc;
class sJAR (ref='2') bitter(ref=first);
model accept= Appearance Flavor Taste Saltiness Oliking SJAR BITTER /link=glogit
selection=stepwise sle=0.1 sls=0.1 outr=AccB;
run;
Title 'Buy - KCl';
proc logistic data=B desc;
class sJAR (ref='2') bitter(ref=first) accept (ref=first);
model buy= Appearance Flavor Taste Saltiness Oliking SJAR BITTER accept /link=glogit
selection=stepwise sle=0.1 sls=0.1 outr=BuyB ;
run;
ods graphics off;
ods html close;
quit;
```

**n. SAS Code: Principal Component Analysis - Biplot**

```
data one;
proc princomp data=pasta out=prin;
var Flavor Taste 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;
run;
proc means; by sample;
var prin1 prin2 prin3;
%plotit(data=prin,labelvar=sample,plotvars=Prin2 Prin1, color=black, colors=blue);
%plotit(data=prin,labelvar=sample,plotvars=Prin3 Prin1, color=black, colors=blue);
%plotit(data=prin,labelvar=sample,plotvars=Prin3 Prin2, color=black, colors=blue);
run;
```

```
*Bi-plot
data one;
input sample$ Flavor Taste Oliking;
cards;
inserted data;
%Include "biplot.sas";
%Include "equate.sas";
%Biplot (Data=one,var=Flavor Taste Oliking, Id=sample, factype=sym, colors=black blue,
symbols=dot none);
quit;
run;
```

# VITA

Pamarin Waimaleongora-Ek was born in October, 1981, in Bangkok, Thailand. She attended in Thammasat University and earned a Bachelor of Science degree in food science in 2002. In June of 2002, she began her career as a research and development supervisor at Seafoods Enterprise (SFE) Company, Limited, Thailand. Working at SFE proved to be a career-defining experience that directed her to return back to graduate study to obtain her graduate degrees in food science specializing in sensory science. She left SFE in December 2004 and came to the U.S. to join the graduate program at Louisiana State University in January 2005. She received a master's degree in food science in December 2006. She continued to work on her doctorate in food science with a minor in experimental statistics, which she expects to receive in May 2010.