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Sensory Perception of Saltiness and Bitterness in Oil-in-Water Emulsions

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SENSORY PERCEPTION OF SALTINESS AND BITTERNESS IN OIL-IN-WATER EMULSIONS

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ABSTRACT

The objective of this research was to evaluate saltiness and bitterness perception in oil-in-water emulsion systems. For that purpose, three experiments were performed: A sensory threshold study, a descriptive sensory and physical property evaluation, and a psychophysical assessment of taste perception in emulsion systems. Experiment-I: Sensory detection and recognition thresholds of NaCl, caffeine, and KCl in aqueous-solutions vs. oil-in-water emulsions were evaluated. For saltiness recognition thresholds, KCl thresholds were higher compared to those of NaCl. For NaCl and KCl, emulsions did not significantly affect the saltiness recognition threshold compared to that of solutions. The bitterness recognition thresholds of caffeine and KCl in solutions were significantly lower than in emulsions. This study showed that, compared with solutions, emulsions did not significantly affect the saltiness recognition threshold of NaCl and KCl, but exhibited bitterness-suppressing effects on KCl and/or caffeine. Experiment-II: Saltiness and bitterness intensities of NaCl (0.50/0.75/1.00%), KCl (0.50/1.00/1.50%), and caffeine (0.05/0.10/0.15%) in emulsions were evaluated using the Spectrum™ descriptive method (N=16). The type of tastant (NaCl/KCl/caffeine) and its concentration had significant effects on saltiness and/or bitterness. NaCl had higher saltiness intensity compared to KCl. For both NaCl and KCl, increasing oil concentrations increased saltiness in emulsions. Oil did not significantly affect bitterness of caffeine in emulsions. Viscosity largely contributed to overall differences among emulsions. Overall, increasing oil concentrations exhibited saltiness enhancing effects on NaCl and KCl, but oil had a marginal effect on bitterness of caffeine in emulsions. Experiment-III: Saltiness and bitterness intensities of NaCl (0.5-1.0%), KCl (0.5-1.5%), and caffeine (0.05-0.15%) in emulsions were measured using a trained descriptive panel (N=16) and the Electronic-tongue (E-tongue). Linear regression and the Stevens' power law were used to model the taste intensities against the tastant concentrations. For the trained panel, saltiness intensities in emulsions were higher than in

solutions, demonstrating a saltiness-enhancing effect imparted by oil. Bitterness intensities in emulsions were lower compared to those of solutions for caffeine, but they were similar for KCl; this demonstrated that oil suppressed bitterness for caffeine. E-tongue saltiness measurements were corresponding to those of the descriptive data; however, E-tongue bitterness intensities of KCl showed an opposite pattern.

CHAPTER 1.
INTRODUCTION

1.1 Introduction

Throughout the past decades, sodium reduction in foods has been rising rapidly, not only in the United States (US) but around the world. This trend is associated with the increase of major health problems related to hypertension and cardiovascular diseases (CVD). High sodium consumption is a major contributor to high blood pressure which is the leading cause of stroke, coronary heart disease, heart attack, and heart and kidney failures in the US (Appel and others 2011; CDC 2015). Although sodium is vital in cells osmotic balance of the human body (Branen and others 2001), diets in the US have overpassed the recommended daily amounts of sodium. The 2010 Dietary Guidelines for Americans recommend limiting sodium to less than 2,300 mg per day. Individuals with 51 years old and older, and those of any age who are African American or who have hypertension, diabetes, or chronic kidney disease should limit their intake to 1,500 mg of sodium per day. These specific populations account for about half of the total US adult population (CDC 2015). On average, US adults consume more than 3,300 mg of sodium per day. A reduction of 25% in the amount of sodium contained in processed food and food from restaurants could result in an 11% reduction in the daily sodium consumed in the US. This could possibly prevent approximately 28,000 deaths per year and save about $7 billion in health-care related expenses (CDC 2012).

The major source of sodium in foods is common table salt or sodium chloride (NaCl) (He and MacGregor 2010). Salt is commonly used to provide salty taste and to improve flavor of foods. Reducing sodium in diets has proven to be a difficult task since salt, the major contributor of sodium in human diets, not only plays an important role in taste, but also is used for food

preservation, structuring and other purposes (Kilcast and den Ridder 2008). It will be desirable that reduced-sodium products match the original products on all food product attributes and characteristics (Busch and others 2013), particularly the salty taste perception.

Approaches to reduce sodium include stealth sodium reduction, saltiness potentiation, multisensory applications, and the physical modification of salt crystals (Kuo and Lee 2014). Another alternative for sodium reduction is the utilization of salt replacers (ingredients that taste salty but do not contain sodium) such as potassium chloride (Liem and others 2011); however, this alternative has a drawback of imparting bitterness, metallic aftertaste, and off-taste (Sinopoli and Lawless 2012). Moreover, sodium reduction can be accomplished by modification of the food structure, thereby, improving the perception of saltiness (Busch and others 2013). The modification of the food matrix properties has a significant role on the sodium release and saltiness perception (Kuo and Lee 2014). In liquid and semi-solid products, this approach includes modification of certain physical properties including viscosity, overall salt distribution, and the use of inert fillers that concentrate salt in the aqueous phase such in the case of emulsion systems (Busch and others 2013).

1.2 Research Justification

An emulsion is the mixture of two immiscible liquids in which one liquid is dispersed as small spherical droplets (a discontinuous phase) in the other (a continuous phase). Various natural and processed foods consist of either partial or entire emulsions, or have been in an emulsified state during their production (McClement 2005). To our knowledge, most of the work in oils or fats in emulsions is related to the texture and flavor/aroma releases rather than their effect on the perception of basic tastes. Oil affects the taste perception by increasing the viscosity of the foods and affects the diffusion coefficients and retention times of taste substances in the oral cavity (Mela

and others 1994). Thus, sensory perception can be affected by the physical properties of emulsions (Suzuki and others 2014).

Changing the viscosity of food products can alter their taste intensities (Pripp and others 2004; Smith and others 1996). Generally, flavor and taste intensities tend to decrease with increasing viscosity (Malone and others 2003; Pripp and others 2004). Suzuki and others (2014) indicated that the response towards saltiness intensity of NaCl (expressed as a function of the amount of NaCl in the aqueous phase) decreased as an oil phase was introduced in the system. They attributed this decrease in saltiness due to an emulsion dilution effect. Besides, Malone and others (2003) indicated that the perception of saltiness was dependent on the concentration of salt in the aqueous phase, the total aqueous phase volume in the emulsion, and the formation of an oily mouth-coating that reduces the mass transfer of tastant to the taste receptors. However, other studies showed that components of fat may sensitize the sodium taste receptor cells, resulting in higher responses toward sodium (Gilbertson and others 2005).

In case of bitterness perception, Metcalf and Vickers (2002) reported that samples with added fat had less bitter taste and more intense sweet, salty, sour, and umami taste than those with added water. Bitter compounds are hydrophobic and can reside in lipophilic environments; therefore, oil in oil-in-water emulsions may suppress bitterness through a dilution effect of the bitter compound in the water-phase of the emulsions (Metcalf and Vickers 2002). The suppression of bitterness by fat is not universal and depends on the properties of the molecules responsible for the bitter taste; caffeine is more hydrophilic than quinine, so its partitioning into a lipid phase would be expected to be less substantial than that of quinine (Coupland and Hayes 2014). Moreover, Hutchings and Lillford (1988) stated that the oral food processing is a combination of different factors including the degree of structure of the food, the amount of lubrication in the

mouth, and the time of residence in the oral cavity. Simple models of oil-in-water emulsion can be useful to understand effects of emulsion characteristics on taste perception. Effects of emulsion characteristics on the saltiness and bitterness perception are not conclusive, and hence a necessity of studying the effects of oil on the basic taste perception. Findings of this type of research would be useful for understanding the perception of salty and bitter tastes in emulsion systems.

1.3 Research Objectives

Sensory perception is affected by the properties of oil-in-water emulsion systems. No other research has attempted to study comprehensively the effects of oil on the saltiness and bitterness perceptions of NaCl, KCl, and caffeine at threshold and consumer consumption levels. Therefore, the objective of this research was to study the saltiness and bitterness perceptions of oil-in-water emulsion systems. Specific objectives were to: (I) determine the detection and recognition thresholds of salty and bitter tastes in aqueous solutions and oil-in-water emulsion systems, (II) characterize effects of tastant and oil concentrations on the saltiness and bitterness intensities of NaCl, caffeine, and KCl in emulsions, and (III) study the psychophysical effects of oil on saltiness and bitterness perception in emulsion systems and to compare results from a descriptive panel to that from an electronic-tongue.

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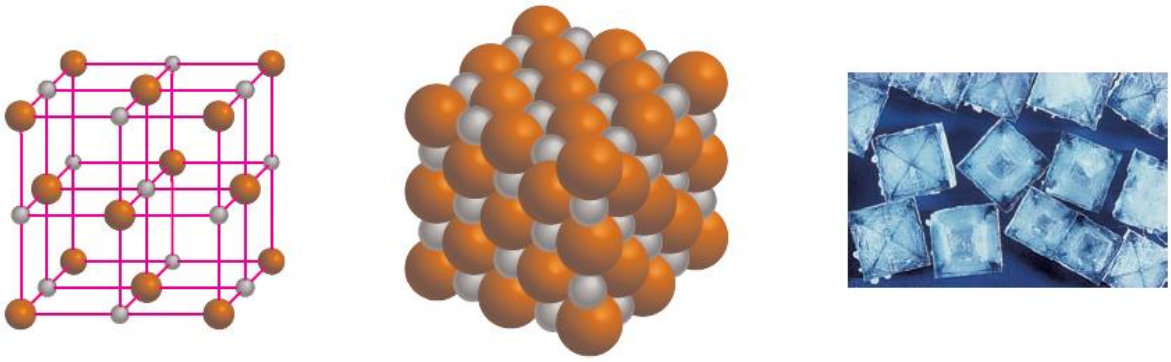
CHAPTER 2.
LITERATURE REVIEW

2.1 Sodium Chloride

Sodium chloride (NaCl) commonly known as table salt is an ionic compound formed by equal number of sodium cations (Na^+) and chlorine anions (Cl^-) that are arranged in a three-dimensional network such that an 1:1 ratio of cations to anions maintains the compound electrically neutral (Figure 2.1; Chang and Overby 2011). Regarding its composition, NaCl has 39.3% of sodium (Na^+) and 60.7% of chlorine (Cl^-), and it is generally found in the physical form of cubic colorless crystals (Figure 2.1) with a molecular weight of 58.44 g mol^{-1}. Sodium Chloride has 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 in aqueous solution ranges from 6.7 to 7.3, and it is water- and glycerin-soluble.



(A) (B) (C)

Figure 2.1 Sodium chloride representations*
*(A) Structure of solid NaCl. (B) In reality, the cations are in contact with the anions. In both (A) and (B), the smaller spheres represent Na^+ ions and the larger spheres represent Cl^- ions. (C) Crystals of NaCl (Source: Chang and Overby 2011).

The solubility of NaCl is 35.9 grams in 100 mL of water at 25 °C and it is slightly soluble in alcohol (Winger and Ren 2008). Sodium chloride is also available in various forms including evaporated salt, rock salt, or solar salt. Sodium chloride crystals are transparent to opaque white crystalline solid of variable particle sizes which, under humidity less than 75%, remain dry but become deliquescent at higher humidity (Branen and others 2001). Factors that affect the physical and chemical composition of salt include the source where salt crystals were obtained (usually by mining and/or solar evaporation), manufacturing techniques, and environmental conditions.

2.1.1 Sodium Chloride in Foods

Salt (sodium chloride) is the most commonly used food additive in the food industry worldwide (Heshmati 2014). The most evident role of salt is to make food pleasantly salty; however, salt is a multifunctional ingredient that can also act as a preservative, an agent that provides several favorable functional characteristics of prepared foods, and a food flavor enhancer (DeSimone and others 2013). Salt and other sodium-containing compounds are technologically necessary to prepare foods (Crocco 1982). For instance, salt is mixed into the curd in cheeses, primarily to provide flavor but it also retards growth of undesirable bacteria, helps the growth of desirable flora, controls the rate of lactic acid fermentation, and it is important in the development of flavor, body, and texture during the ripening process (Kaufmann 1968; Reddy and Marth 1991). In processed meats, salt functions as a preservative by lowering water activity and by solubilizing certain muscle proteins to form stable emulsions that can bind moisture and fat (Reddy and Marth 1991). In fermented vegetables such as sauerkraut and cucumber pickles, salt is used for promoting growth of lactic acid bacteria and reducing growth of spoilage microorganisms (Kaufmann 1968). In bakery products, salt is used for enhancing other flavors, regulating alcoholic fermentations, controlling lactic acid fermentations, and strengthening the gluten in bread dough (IFT 1980;

Kaufmann 1968). Salting is also used for other food processes including canning by adding tenderness to peas, lima beans, cucumbers and other vegetables, and curing of meat and fishery products for preservation (Kaufmann 1968).

There are some other compounds different from sodium chloride that contain sodium and possess a broad range of functionalities in the food system. For examples, sodium emulsifiers used in cheese-manufacturing, sodium nitrite and nitrate, sodium phosphates, sodium ascorbate and monosodium glutamate are used for different functions in foods including preservation, acidification, anti-oxidation, emulsification, and as agents to improve color, flavor, body and texture of foods (Reddy and Marth 1991).

2.1.2 Health Concerns of Elevated Sodium Intake

Sodium is required by all mammals, including humans, to maintain blood volume and cellular osmotic pressure, and to form transmissions of nerve impulses (Reddy and Marth 1991). The sodium content of the body is approximately 1.4 g/Kg (Belitz and others 2009), and about 50% of this sodium is located in extracellular body fluids, 10% inside cells, and 40% in bones (Reddy and Marth 1991). In the human body, there are two types of fluid compartments, the extracellular and intracellular spaces which are characterized by a specific composition of cations and anions. The main constituents of the extracellular space are Na^+, Cl^-, Ca^{2+}, and HCO_3^-, and of the intracellular space are K^+, PO_4^{3-}, organic acid ions, Mg^{2+}, and proteins. The concentration of the electrolytes in the extracellular spaces is assumed to be constant; thus, deviations from these ion concentrations caused by external stimuli can generate a fluid shift which is induced by hydrostatic and colloid osmotic forces across the capillary membranes. This fluid shift can assure the reinstatement of the original set-point of the electrolytes concentrations (Heer 2008; Kesteloot

and Joossens 1988). Excessive amounts of sodium and chlorine ions consumed by the human body are excreted by the kidney, maintaining sodium levels within a narrow limit (IFT 1980).

Humans are genetically programmed to consume less than 0.25 g of salt per day in their diets. However, salt intake has been increasing during the past decades due to the consumption of highly salted processed foods (He and MacGregor 2010). The average salt intake in most of the countries around the world is about 9 to 12 g per day with many Asian countries having average consumption of more than 12 g per day. Salt intake in children older than 5 years is usually more than 6 g per day, and this intake increases with age (Brown and others 2009). On an average consumption of 12 g of salt per day, 3 g occurs naturally in foods, 4 to 6 g is added during food processing, and 3.4 to 6.5 g is added during cooking or dining (Woteki and others 1982). According to the 2010 Dietary Guidelines for Americans, sodium consumption for healthy diets should be less than 2,300 mg of sodium per day; however, populations older than 51 years, African Americans, people experiencing high blood pressure and/or diabetes and/or chronic kidney diseases are at risk and they should consume less than 1,500 mg of sodium per day. The Centers for Disease Control and Prevention (CDC 2015) reported that about 90% of Americans consume more sodium (nearly 3,300 mg of sodium per day) than what is recommended for healthy diets. More than 40% of sodium consumed is originated from 10 types of foods including breads and rolls, cold cuts and cured meats, pizza, fresh and processed poultry, soups, sandwiches, cheese, pasta dishes, meat-mixed dishes, and snacks. Of all these, 65% of sodium comes from food bought at retail stores, and about 25% comes from restaurants (CDC 2015).

In several epidemiologic, migration, population-based intervention, genetic, clinical, and experimental studies, excessive dietary salt intake has been linked to increase blood pressure; whereas, a reduction in dietary sodium intake has been documented to lower blood pressure

(Frisoli and others 2012; He and MacGregor 2010). Mechanisms whereby salt raises blood pressure are not fully understood; however, there are some studies showing that individuals who develop high blood pressure have an underlying defect in the kidney's ability to excrete sodium (He and MacGregor 2010). High blood pressure, commonly called hypertension, afflicts 100 million of Americans (nearly 33.33% of the total population), and it can be arbitrarily defined as a blood pressure exceeding 140 mm Hg systolic and 90 mm Hg diastolic (CDC 2015; Reddy and Marth 1991). The mechanisms for salt-induced high blood pressure are that the impaired ability of kidneys to excrete sodium causes sodium and water retention in the body. This leads to a blood volume expansion and the stimulation of several other compensatory mechanisms (He and MacGregor 2010). Excessive accumulation of salt in the body results in increments of the extracellular fluid volume which leads to increased blood volume and arterial pressure (Guyton and Hall 2006). Three major factors contributing to hypertension are the heredity, nutrition, and environment (Reddy and Marth 1991). Postulates of Laragh (1984) indicated that imbalances in the sodium-renin blood-pressure-control-system also contribute to hypertension, and this can be related with the retention of sodium in the body. Besides, Guyton and Hall (2006) explained that increases in extracellular fluid also stimulate the secretory mechanism of the hypothalamic-posterior-pituitary-gland that generates increased quantities of antidiuretic hormone. This causes the kidneys to reabsorb increased quantities of water from the renal tabular fluid before it can be excreted as urine, thereby, diminishing the volume of urine while increasing the extracellular fluid volume.

In conclusion, high sodium consumption is a major contributor to high blood pressure which is a leading cause of stroke, coronary heart disease, heart attack, and heart and kidney failure in the United States (CDC 2015). With high blood pressure, the heart works harder and the high

force of the blood flowing can harm arteries and organs. Excessive workload on the heart leads to early development of congestive heart disease and coronary heart disease. Also, high blood pressure frequently ruptures major blood vessels in the brain causing cerebral damage. High blood pressure can cause multiple hemorrhages in the kidneys producing many areas of renal destruction, and, eventually, kidney failure, uremia, and death (Guyton and Hall 2006). In the US, more than 800,000 people die each year from heart disease, stroke, and other vascular diseases, costing the nation $273 billion in health-care dollars expenses (CDC 2015).

2.1.3 Reducing Sodium Approaches

Busch and others (2013) discussed, in a comprehensive review, the recent approaches to reduce sodium in foods, focusing on the optimization of the food product design that best delivers salt to the sodium receptors cells in the oral cavity. In this review, they stated that three main procedures should be addressed to increase saltiness perception, and thereby, achieving the goal of sodium reduction in foods. These procedures consist of influencing the taste perception by chemical mechanisms, cognitive mechanisms, or modification of the food product structure (Figure 2.2).

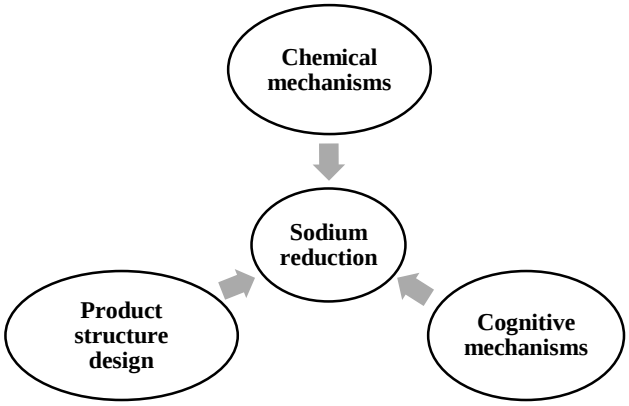


Figure 2.2 Principles for sodium reduction in foods
(Source: Busch and others 2013)

Table 2.1 shows the different principles for sodium reduction and their practical approaches according to Busch and others (2013). For the cognitive approach, one possible alternative is to create a wide-spread consumer need for sodium reduction by using consumer awareness or mandatory regulatory policies (Busch and others 2013); however, it has been proven to be difficult for people to switch to a different diet without creating a dissatisfactory effect on consumers (Karanja and others 2007). Another cognitive approach to reduce sodium intake may include 'stealth reduction' in which the product is reformulated to include the reduction of the salt content in small steps. The goal is to reduce gradually the salt and its taste intensity of foods; therefore, enabling consumers to adapt to the new taste before further salt reduction steps are taken (Dotsch and others 2009). For the chemical stimulation point of view, the reduction of sodium can be achieved by the use of salt replacers (ingredients that taste salty but do not contain sodium) such as potassium chloride (Liem and others 2011); however, this alternative has the drawback of introducing bitterness, metallic aftertaste, and off-taste (Hooge and Chambers 2010). Ingredients that increase umami notes are also used for reducing sodium in foods as the overall flavor profile is rebalanced, and saltiness perception may increase through umami-salt interactions (Keast and Breslin 2002). As another alternative for sodium reduction, Busch and others (2013) stated that the modification of the food product structure by itself can improve the perception of saltiness, thereby, helping the objective of reducing sodium in foods. This approach includes, (1) modifying the dissolution rate of salt crystals through changing their size, shape and morphology, (2) modifying textural properties of the food such as hardness/brittleness that affect the oral breakdown and release of sodium, (3) the use of inert fillers that concentrate salt in the aqueous phase such as in the case of oil-in-water emulsion systems, and (4) the use of inhomogeneous distribution of salt that provides taste contrasts.

Table 2.1 Sodium reduction practical approaches

Principles	Approaches	Reference
Chemical stimulation using ingredients that taste salty or otherwise increase the saltiness perception peripherally at/near the receptor	Salt replacer: ingredients that do not contain sodium but taste salty; e.g. KCl, ammonium chloride	Liem and others (2011); Li and others (2009)
	Salt boosters; ingredients that do not taste salty themselves, but make the salt receptor more sensitive	Kilcast and den Ridder (2007); Dotsch and others (2009)
Optimized release of salt from the product structure design to optimize the stimulation of the receptor with regards to saltiness perception	Rate of dissolution of salt crystal from dry products based on crystal size, shape and morphology	Shepherd and others (1989); Kilcast and den Ridder (2007)
	Inert fillers which allows the concentration of salt in the aqueous phase	Goh and others (2010); Malone and Appelqvist (2003)
	Textural effects such as viscosity for liquid foods and hardness/brittleness for solid foods which determine how the food breaks down and subsequently is moved about in the mouth or the rate of release of sodium from the food matrix	Koliandris and others (2010); Ferry and others (2004)
	Structuring agents affecting the physicochemical nature of food systems, such as anionic hydrocolloids and osmolality	Rosett and others (1994); Koliandris and others (2011)
Cognitive mechanisms towards increasing awareness, preference of saltiness levels and perception of saltiness	Increasing consumer awareness of sodium reduction	Webster and others (2011)
	Reduction by stealth, i.e. consumers are getting used to lower saltiness of products	Girgis and others (2003); Bertino and others (1986)
	Perceptual interactions to increase saltiness perception (salt-associated aromas, taste (umami, sourness), textures)	Djordjevic and others (2004); Lawrence and others (2009)
	Contiguity, where lowered salt content in part of the product is unnoticed, as the consumer expects a constant product	Woods and others (2010);
	Mandatory regulatory policies such as nutritional labelling that requires sodium levels of a product to be disclosed	Pietinen and others (2008)

(Source: Busch and others 2013)

In the US, the 21CFR101.61 (CFR 2012) states that the terms “sodium free” or “no sodium” in a food can be made only if the product contains less than 5 mg of sodium per reference amount customarily consumed and/or per labeled serving. A product labeled as “very low sodium” must contain 35 mg or less of sodium per reference amount customarily consumed. A product labeled as “low sodium” must contain 140 mg or less of sodium per reference amount customarily consumed. The term "reduced sodium" may be used in labeling foods when the sodium level is reduced by 25% per reference amount customarily consumed. The terms “unsalted,” “without added salt,” or “no salt added,” may be used when the product is without any added salt during processing.

2.2 Potassium Chloride

Potassium chloride (KCl) is a chemical compound that is colorless or white, cubic and crystalline that closely resembles common salt (sodium chloride). It is soluble in water, alcohol, and alkalis. Potassium chloride occurs pure in nature as the mineral sylvite, and it is found in many minerals, brines, and ocean water. The main use of potassium chloride is in the production of fertilizers; it is also used in chemical manufacturing (Anonymous 2013). Potassium chloride is probably the most common salt substitute used in low- or reduced salt/sodium foods (Desmond 2006). However, potassium chloride has the disadvantage of introducing bitterness, metallic aftertaste and off-taste (Hooge and Chambers 2010; Sinopoli and Lawless 2012). The reason for bitterness perception of potassium chloride is still unclear. One hypothesis explains that the molecular weight of cationic potassium (K^+) is higher than that of cationic sodium (Na^+), and this causes bitterness. Additionally, the receptor sites located on the tongue where saltiness is perceived can readily distinguish potassium from sodium. This difference is physiologically perceived as a difference in bitterness intensity (Murray and Shackelford 1991). In dairy products, mixtures of

sodium chloride and potassium chloride have been used to lowering the total amount of sodium in foods. For instance, by using a mixture of 1:1 sodium chloride-potassium chloride in products such as cheddar, Gouda, and pasteurized process cheeses, acceptable quality in terms of sensory and shelf-life assessments were observed (Karahadian and Lindsay 1984; Lindsay and others 1982; Martens and others 1976). In buttermilk, salt replaced with a 1:1 NaCl:KCl mixture did not significantly affect the flavor scores compared to the standard NaCl buttermilk. This reformulation achieved a 31 % reduction in sodium of the product (Demott and others 1984). In the meat industry, potassium chloride has been used as a salt replacer in addition to some other flavor-enhancer compounds in order to reduce sodium in processed meats (Desmond 2006). In cooked hams, for example, 50% reduction of NaCl with KCl provided a superior protein binding and acceptable sensory scores (Price 1997). In fermented sausages, texture was not significantly affected by replacing NaCl with KCl; however, a bitter taste was detected at 30% level of substitution by a sensory panel (Gou and others 1996). Mixtures of NaCl and KCl have been used successfully in other products such as cereal foods, vegetables, dressings, smoked fish, and fish sauces. All of these products obtained acceptable ratings from sensory panels (Reddy and Marth 1991).

2.3 Other Ingredients Used to Reduce Sodium

Another alternative to reduce sodium is the use of ingredients that increase umami notes in foods (Keast and Breslin 2002). Besides, some other flavor enhancers and masking agents can be used to improve saltines perception. Some of these ingredients are commercially available including yeast extracts, lactates, monosodium glutamate, and nucleotides among others (Desmond 2006). As a strategy of lowering sodium in foods, a salt replacer (usually KCl) is used in combination with one or more flavor enhancer/masking agents to improve saltiness and to mask and/or block bitterness. For instance, the bitter blocker, adenosine 5’-monophosphate (AMP)

works by blocking the activation of the gustducin in taste receptor cells, and thereby, preventing nerve stimulation (McGregor 2004). Yeast autolysates are also used in low salt food preparations, particularly because they mask the metallic flavor of KCl (Desmond 2006). Among various amino acids, arginine has been reported to contribute to the salty taste (Dotsch and others 2009). Combinations of arginine with aspartate have been claimed to be more effective in salt enhancement (Dotsch and others 2009). Lysine, another basic amino acid, is said to provide salt enhancement without the presence of a significant off-taste. Recently, alkyldienamides have also been patented as multimodal enhancers that can elevate both the umami and the salty characters of foods at relatively low levels (1–100 ppm) (Dewis and others 2008).

2.4 Human Perception of Saltiness and Bitterness

In order to understand the differences between sodium chloride and potassium chloride in terms of saltiness perceptions, it is important to analyze the chemical stimulations and the mechanisms of perception in which these compounds are involved. Processes of saltiness and bitterness chemical perceptions are currently under investigation. The lingual taste sensation during food ingestion is a complex convergence of two sensory modalities, (1) the gustatory taste sensation which is the perception of the basic taste modalities including sour, sweet, salty, bitter, and umami by the activation of the taste-bud sensory cells, and (2) the lingual somatosensory sensitivity resulting from temperature tactile stimulation as well as chemical activation of chemosensory receptor on the perigemmal fibers (Hofmann and others 2003).

Taste receptor cells (TRC) are neuroepithelial cell clustered into sensory-end organs called taste buds which contain approximately 50-150 cells including precursor cells, support cells, and receptors cells (Hofmann and others 2003). Taste buds are very small onion-like structures containing receptor cells that act as specific sensors for taste molecules (Figure 2.3). A pore at the

top of the taste bud makes contact with the outside fluid environment in the mouth, and taste molecules are believed to bind to the hair-like cilia near the opening (Lawless and Heymann 2010). When foods or drinks enter the mouth, chemicals from those foods activate the taste receptors. These chemical signals are converted into electrical signals that are transmitted through the seventh, ninth, and tenth cranial afferent nerve fiber to the gustatory processing region of the brain. Moreover, taste is characterized by four separates attributes which are quality (sweet, sour, salty, bitter, and umami), intensity (strength of the taste sensation), temporal (time course of taste perception), and spatial (location of taste sensation) patterns (Liem and others 2011).

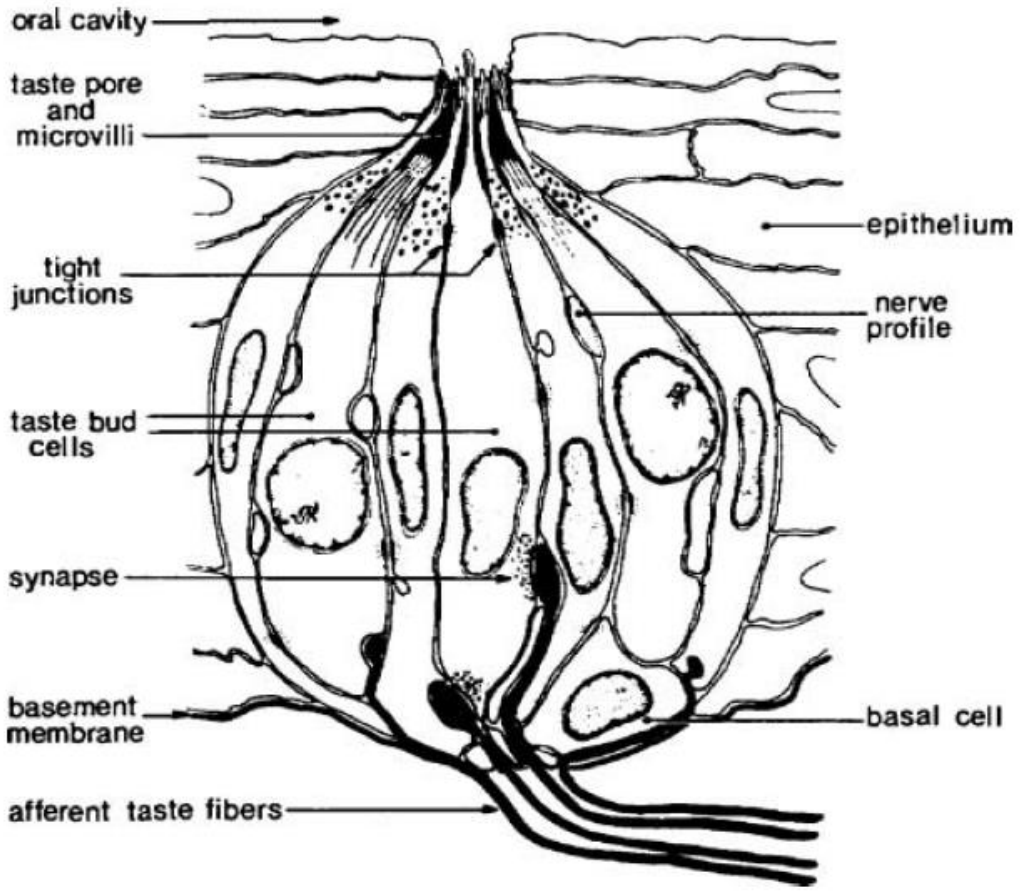


Figure 2.3 Morphology and cellular organization of a typical test bud
(Source: Mistretta 1981)

2.4.1 Saltiness Perception

Salty taste quality is caused by ionized salt, primarily from cations of salt which are detected in the oral space. Taste transduction of simple inorganic salts involves the alternated permeation of the receptor cell membrane by the direct interaction of ions (Na^+ and/or K^+) with particular ion channels that are located in the hair cell membrane of the taste receptor cells (Hofmann and others 2003; Lindemann 1996). In the specific case of sodium, salty taste can be explained by its unique sodium-specific transduction mechanism involving the activation of epithelial sodium channels (ENaCs) on the receptor cells (Hofmann and others 2003; Liem and others 2011). There are two ENaCs subtypes, one that is activated for specific low sodium concentrations, and is believed to be responsible for the appetitive nature of salty taste, and the second ENaCs subtype that is permeable to multiple cations, and is activated at higher sodium concentrations. This subtype is believed to be responsible for the aversive nature of cations in the cell (Liem and others 2011).

Sodium entering the cell is responsible for a cell membrane potential change (an ionic gradient) associated with calcium influx. This is required for binding neurotransmitter vesicles to the cell membrane and releasing neurotransmitter molecules into the synapse to stimulate the associated taste nerve (Lawless and Heymann 2010). The perceived intensity of sodium in an aqueous solution increases as its concentration increases; however, most of the foods are mixtures of different components rather than a singular taste stimulus. The perceived intensity of mixtures may be additive or non-additive resulting in suppression or enhancement outcomes. For instance, lowering the amount of sodium from foods may result in several consequences including loss of saltiness, increased bitterness (due to the effect of sodium as an effective bitterness inhibitor),

slightly decreased sweetness, decreased appetite, and increased aversive bitter taste (Liem and others 2011).

2.4.2 Bitterness Perception

For an evolutionary perspective, the response to bitter-taste substances has been useful for survival since many toxic compounds are bitter. However, the range of foods available is more varied nowadays. Many bitter foods are not only safe for consumption but contain bitter constitutes that provides nutritional benefits (Naim and others 2002). Bitter compounds in foods cover a vast range of chemical classes including flavonoids, cyanogen glycosides, bitter alkaloids, bitter tasting amino acids, some lipids diols, among others. Some other bitter compounds are formed from taste-less precursors during food processing (enzymatic reactions and some other thermal food processes) (Hofmann and others 2003).

Bitter taste utilizes G protein coupled receptors (GPCR) which are embedded in the cell membrane with seven alpha-helical segments traversing the membrane. These helices pack together to form a ligand binding site. When extracellular ligand binds to the receptor, a conformational change occurs which enables the intracellular segments of the receptor to interact and activate the Guanosine triphosphate (GTP) binding protein (one type of G proteins) which subsequently activates a second messenger (adenylyl cyclase, phosphodiesterase or phospholipase C) inside the cell (Figure 2.4). These messengers modulate the activity of an ion channel causing the cell to depolarize (goes toward neutral) or to hyperpolarize. Resting taste cells have a negative electrical potential. When this potential is raised to a threshold level, ion channels in the cell membrane open and the cell briefly depolarizes (charge goes to neutral), and this triggers the electrical signal in the attached nerve cells (Walters and Roy 1996). The transmembrane domains receptor (T2R) is a type of GPCR which is believed to be responsible for bitter tastes. The T2R

has about 300-330 amino acids and a short extracellular N-terminus. Different T2R may be co-expressed in the same cell, and this may explain why most bitter taste substances are similar in quality and difficult to differentiate (Lawless and Heymann 2010).

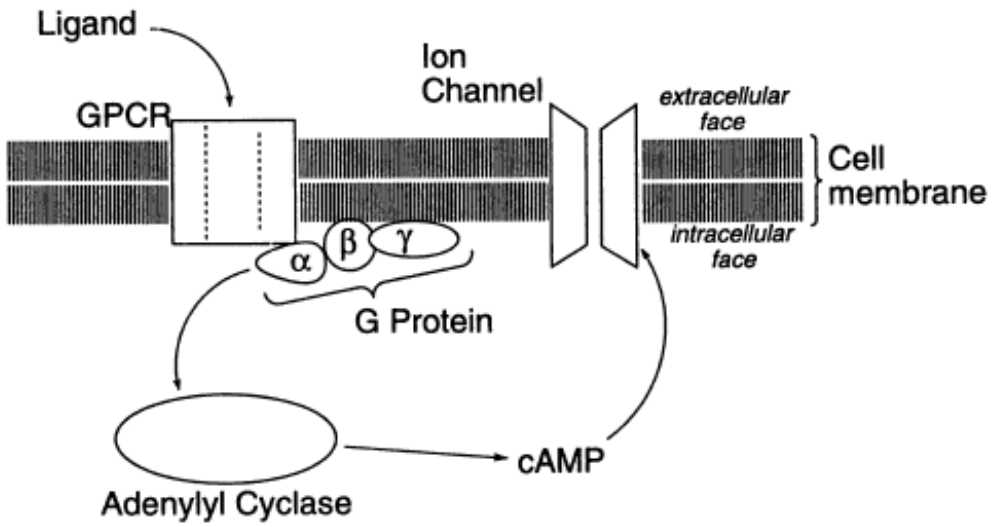


Figure 2.4 G Protein coupled receptor system*
*The second messenger is adenylyl cyclase/cyclic Adenosine monophosphate (cAMP)
(Source: Walters and Roy 1996)

2.5 Emulsions

As stated by Busch and others (2013), another approach for achieving sodium reduction is the modification of the food product structure, thereby, improving the perception of saltiness. This approach includes the modification of certain physical properties of food products. For instance, changes in viscosity and the overall salt distribution in liquid products may change saltiness perception. Moreover, other sodium reduction techniques include the use of inert fillers that concentrate salt in the aqueous phase (such as the case of oil in water emulsions), and the use of inhomogeneous salt distributions that provides taste contrasts. Therefore, the manipulation of emulsion systems could be a potential and viable alternative to reduce sodium in foods. However,

there is limited research performed to understand the relationship between the characteristics of emulsion systems and the human perception of saltiness and bitterness.

Various natural and processed foods consist of either partial or entire emulsions, or have been in an emulsified state during their production. During the last decades, there has been a development of a more rigorous scientific approach and new analytical techniques to understand and characterize emulsion properties. The emulsion science has incorporated disciplines such as sensory science and physiology to correlate the sensorial qualities of food emulsions to their compositions and physicochemical properties (McClement 2005).

2.5.1 Emulsion Definition

An emulsion is defined as a mixture of two immiscible liquids (Figure 2.5) in which one of the liquids is dispersed as small spherical droplets (a discontinuous phase) in the other (a continuous phase). The diameter of the droplets commonly lies between 0.1 and 100 μm. A simple emulsion that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion. Conversely, a system that consists of water droplets dispersed in an oil phase is called a water-in-oil (W/O) emulsion (McClement 2005)

Emulsions can also contain smaller droplets of the continuous phase dispersed within each droplet of the dispersed phase. These systems are called double emulsions or multiple emulsions (Jiao and Burgess 2008; Leal-Calderon and others 2007). In general, emulsion droplets exhibit behaviors of (1) metastable colloids (Brownian motion), (2) reversible phase transitions as a result of droplets interactions, and (3) irreversible transitions that generate disintegration of the emulsion (Leal-Calderon and others 2007). The process of converting two separate immiscible liquids into an emulsion is known as homogenization. This procedure is a unit operation using a class of

processing equipment referred to as homogenizer (Figure 2.6) that is geared towards reducing the droplets size of the dispersed phase (Jochen 2008; McClement 2005).

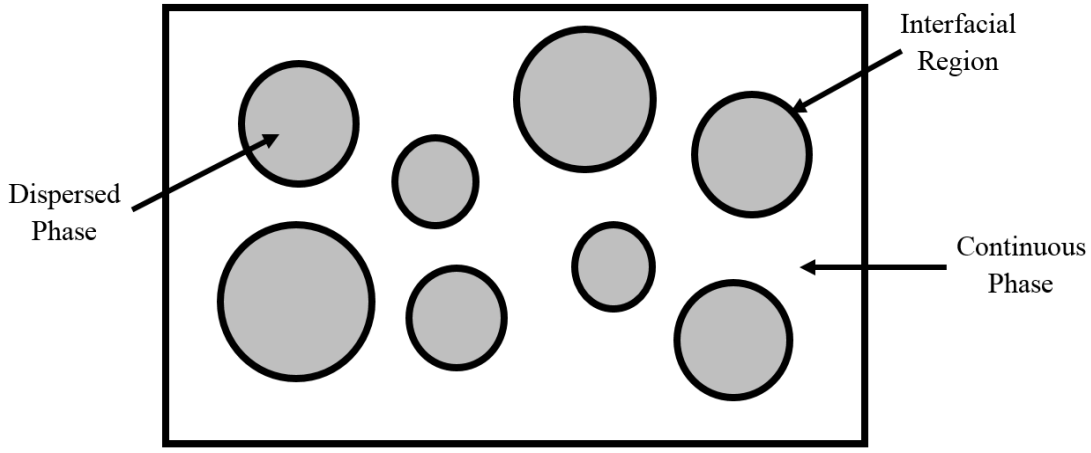


Figure 2.5 A dispersed system that consists of two entirely or partially immiscible liquids*
*The dispersed phase is surrounded by molecules of the continuous phase, and is separated by and interfacial region (Source: McClement 2005).

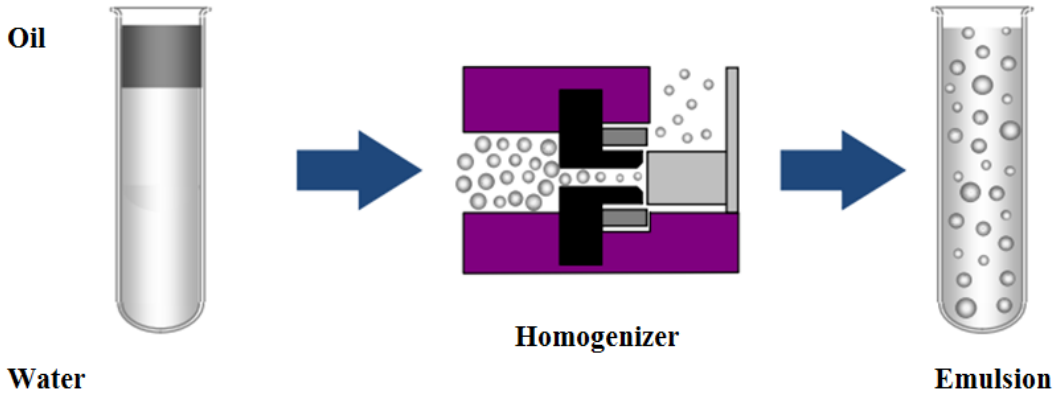


Figure 2.6 Conceptual emulsion formation using a homogenizer
(Source: Jochen 2008)

The parameters that govern the homogenization process are, among others, (1) the energy density that determines the minimum achievable droplet size, (2) the energy efficiency that is referred to the heat loss in the process, and (3) the volume flow rates and product rheology which limit the amount and type of materials that can be homogenized. There are several types of homogenizers including the high speed blender, high pressure homogenizer, colloid mill, high shear dispersers, ultrasonic disruptor, and membrane homogenizers (Jochen 2008).

2.5.2 Emulsifiers and Texture Modifiers

An emulsion is a thermodynamically unstable system in which the two different phases involved (usually oil and water) tend to separate to their original bulk states. In order to retard segregation of phases, a stabilizer can be added to the emulsion which can be either an emulsifier or a texture modifier. An emulsifier is a surface-active amphiphilic molecule that absorbs the surface of freshly formed droplet during homogenization. Emulsifiers are characterized by having a polar and nonpolar region in the same molecule (McClement 2005).

On the other hand, texture modifiers are thickening agents and/or gelling agents that increase the viscosity of the continuous phase of the emulsion which results in retarding the movement of the droplets. The molecules in an emulsion distribute themselves among three regions (droplets, continuous phase, and interphase or stabilizer; Figure 2.5) according to their concentration and polarity. Nonpolar molecules tend to be located in the oil phase, polar molecules in the continuous aqueous phase, and amphiphilic molecules at the interface (McClement 2005).

Emulsifiers act as surface-active compounds in the emulsion lowering the surface or interfacial tension. Mono- and diacylglycerols that contain –OH functional groups are the most extensively used nonionic emulsifiers. Proteins may also be effective surface active compounds due to the existence of lipophilic amino acids such as phenylalanine, leucine, and isoleucine.

Charged proteins stabilize emulsion due to repulsion of like charged droplet. On the other hand, hydrocolloids such as gums and starches have been regarded as thickeners due to their ability to reduce the kinetic motion of droplets that results in a lower rate of flocculation and coalescence (Hasenhuettl 2008).

2.5.3 Emulsion Characteristics

2.5.3.1 Emulsion Capacity

For a food manufacturing point of view, it is important to know the minimum emulsifier amount that can be used to form a stable emulsion. The emulsion capacity (EC) of a water-soluble emulsifier is defined as the maximum amount of oil that can be dispersed into an aqueous solution that contains a specific amount of emulsifier without breaking or inversing the phase (McClement 2005). EC depends of several factors including the type of oil and aqueous solution used, the homogenization process, and more importantly, the type of emulsifier used in the formation of the emulsion.

2.5.3.2 Rheology

Emulsions have a wide range of different rheological properties ranging from low viscosity liquid to fairly rigid solids (McClement 2005). Factors that may affect the viscosity and stability of these emulsions are, (1) the dispersed phase volume relative to the emulsion volume, (2) overall pH, (3) ionic strength, and (4) temperature (Demetriades and others 1997). High pressure homogenizations could also affect the rheological properties of different emulsion systems. In an O/W emulsion model consisting of sunflower oil, water, and whey protein concentrate (WPC) as a stabilizer, Floury and others (2000) concluded that increasing the homogenizing pressure can bring the emulsion from a shear-thinning behavior to a Newtonian behavior with a considerable decrease in viscosity.

2.5.3.3 Oil Droplet Size

The emulsion oil droplet size has a strong effect on several physicochemical and sensory properties including shelf-life, appearance, texture, and flavor (McClement 2005). The distribution of these oil droplets can be adjusted by controlling the rate of drop-breakage and coalescence during emulsion formation (Floury and others 2000). The type of emulsification process has an essential role in the formation of oil droplets. Ramisetty and Shyamsunder (2011) studied the oil droplet size distributions of various emulsions and found that emulsions prepared by ultrasonic techniques possessed smaller droplet sizes compared to emulsions prepared by mechanical agitation. Smaller droplet sizes were more thermodynamically stable and were affected by the emulsification time, volume fraction of dispersed phase, viscosity of continuous phase, and concentration of emulsifying agents. Table 2.2 shows the findings of different studies with respect to the increase of input emulsification energy and its relationship to the change in the mean oil droplet size. Input emulsification energy depends on the power density (Watts/mL) and the time of emulsification (seconds). These studies used ultrasonic homogenization for the emulsification. Usually, as the homogenizing energy increases, the oil droplet size decreases (Table 2.2). This phenomenon can affect several parameters of the emulsion such as the creaming velocity, apparent viscosity, and other rheological properties (Chanamai and McClements 2000).

2.5.3.4 Emulsion Stability

Emulsion stability describes the ability of an emulsion to resist changes in its properties during storage. Figure 2.7 shows the instability of emulsions through a variety of physical mechanisms, including creaming, sedimentation, flocculation, coalescence, and phase inversion. Creaming is the upward movement of droplets due to the fact that their density is lower than that of the surrounding continuous phase; whereas, sedimentation is the downwards movement of

droplet due to the fact that they have a higher density than that of the surrounding continuous phase. Droplet flocculation is the process where two or more droplets group together and maintain their individual integrity to form an aggregate. Coalescence is the process where droplets merge together to form a single larger droplet. The phase inversion is the process where an emulsion system changes from an oil-in-water emulsion to a water-in-oil emulsion, or vice versa (McClement 2005)

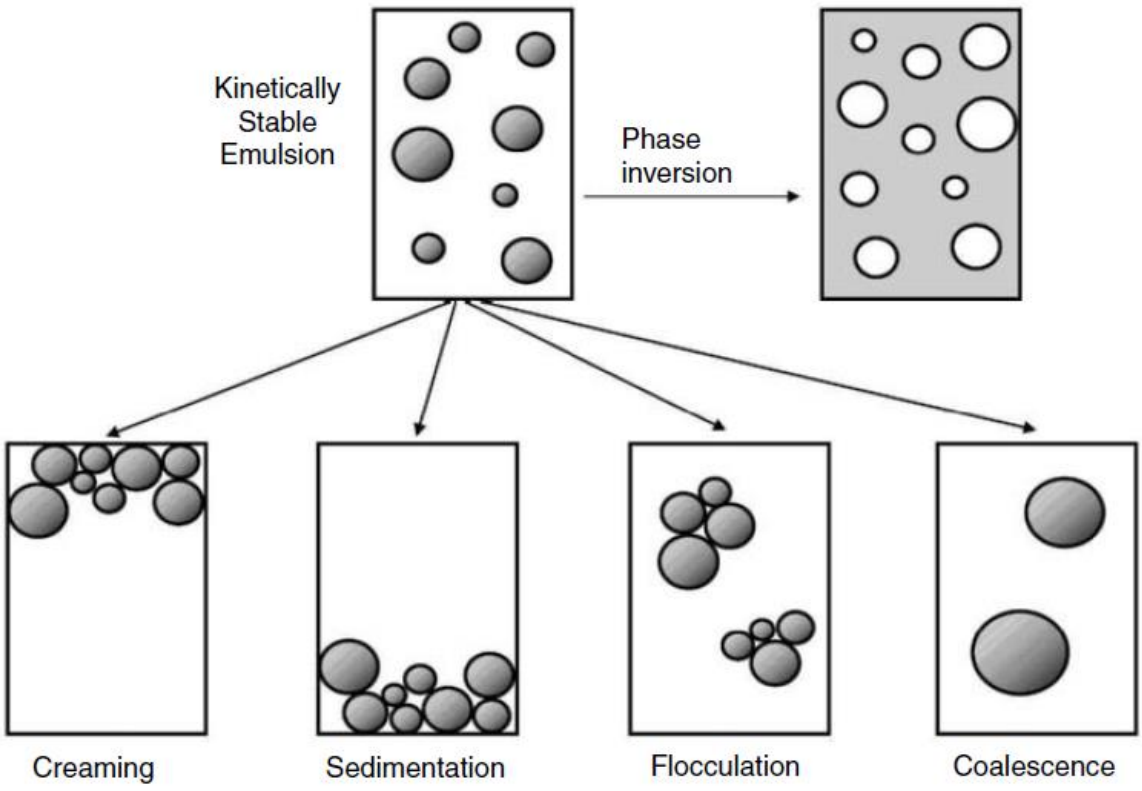


Figure 2.7 Processes of emulsion instability
(Source: McClement 2005)

Table 2.2 Input emulsification energy and its mean oil droplet size

Author*	Energy*	Volume*	Power*	Power Density*	Time*	Mean Droplet Size*
Ramisetty and Shyamsunder (2011)	10,800	60	30	0.500	360	5.00
	14,400	60	30	0.500	480	3.00
Jochen (2008)	1,800	-	-	-	-	2.00
	6,000	-	-	-	-	1.00
Abismail and others (1999)	2,700	80	90	1.125	30	0.10
	3,900	80	130	1.625	30	0.05
Delmas and others (2011)	30,000	-	-	-	-	0.10
	65,000	-	-	-	-	0.05
Cucheval and Chow (2008)	9,000	60	30	0.500	300	0.70
Leong and others (2009)	12,000	40	40	1.000	300	0.10

*Studies used ultrasonic homogenization for the emulsification. Energy in Joules, Volume in mL, Power in Watts, Power Density in Watts/mL, Time in Seconds, and Mean Droplet Size in μm. "-" indicates that values for that parameter were not reported.

2.5.4 Emulsions and Taste Perception

Emulsions comprise a large part of liquid and semi-solid food products. Modifying some emulsion properties such as flavor, fat/oil content, droplet size, and type and concentration of thickening agents may in general affect the sensory perception of emulsions (Vingerhoeds and others 2008). Traditionally, the majority of flavor/taste studies in food emulsions focused on aroma release rather than the effect of fat on taste perception (Malone and others 2003).

Fats/oils, such as medium and long chained fatty acids, appear to have no taste; however, they contribute to texture/mouth-feel, and they interact with other components changing the chemosensory attributes of foods. The influence of oils on taste perception in emulsion systems could be due to a combination of two mechanisms. First, oil can form a barrier between the taste compounds and taste receptors resulting in a decrease in the perceived intensity. Second, oil may increase the concentration of water soluble taste compounds in the aqueous phase creating a more intense taste perception (Metcalf and Vickers 2002). Simpler models of oil in water emulsion are useful to understand the effect of emulsion characteristics on taste. For instance, some studies found that the oil of emulsion systems can suppress sweet and bitter taste whereas others reported that perceived saltiness can increase with increasing oil concentration in emulsion systems (Malone and others 2003; Metcalf and Vickers 2002).

2.5.4.1 Saltiness Perception in Emulsions

The food matrix composition plays a significant role on sodium release and saltiness perception (Kuo and Lee 2014). In emulsion systems, oil does not impart taste by itself but affects taste perception by increasing the viscosity of foods and affecting the diffusion coefficients and retention times of taste substances in the mouth (Mela and others 1994). Therefore, oil mouth-coating can have a suppressive effect on salty taste perception (Lynch and others 1993). Hughes

and others (1997) stated that fat and/or oil, as hydrophobic compounds, act as barriers against sodium migration, hence disfavor its release. Moreover, increasing oil concentration increases the viscosity of the emulsion system and this can lead to a decrease in taste intensity (Moskowitz and Arbie 1970; Malone and others 2003; Pripp and others 2004). Alternatively, Koriyama and others (2002) hypothesized that lipids in emulsions occupy volume but they do not contain NaCl or KCl molecules which are 100% partitioned in the aqueous phase. Therefore, increased perceived intensities are found in emulsions with higher oil concentrations (Kuo and Lee 2014; Koriyama and others 2002). Shamil and others (1992) stated that fat increased saltiness perception in salad cream, and decreased bitterness in Cheddar cheese. Metcalf and Vickers (2002) reported that samples with added fat had less bitter taste and more intense sweet, salty, sour and umami tastes. Thurgood and Martini (2010) reported that oil in emulsion systems increased the perception of umami and saltiness in a threshold study. Gilbertson and others (2005) demonstrated that fat/oil can sensitize the sodium taste receptor cells which can result in higher responses toward sodium. Malone and others (2003) indicated that the perception of saltiness is dependent on the concentration of salt in the aqueous phase, the total aqueous phase volume in the emulsion, and the formation of an oily mouth-coating that reduces the mass transfer of tastants to the taste receptors.

2.5.4.2 Bitterness Perception in Emulsions

In general, most of the bitter molecules are hydrophobic; thus, bitter molecules can be partitioned into the lipid phase, reducing their aqueous concentration, and leading to a decreased perceived bitterness intensity (Coupland and Hayes 2014). Thurgood and Martini (2010) reported that sour and bitter taste intensities were lower in emulsion systems compared to aqueous solutions in a threshold study. Koriyama and others (2006) concluded that tuna oil in emulsion systems

suppressed the bitterness perception of quinine sulfate due to a decreased tastant concentration in the aqueous phase. The suppression of bitterness by fat/oil is not universal and depends on the properties of the molecules responsible for the bitter taste (Coupland and Hayes 2014). Lahtinen (2007) showed that lactose (1% or 2%) in combination with sucrose, glucose or galactose suppressed bitter tastes of NaCl/KCl mixture emulsions. Pripp and others (2004) showed that emulsion systems had a limited effect on bitterness reduction of olive oil phenolic compounds. Keast (2008) stated that as the milk fat content increased from 0% to 4%, the level of caffeine bitterness significantly increased, and he attributed this effect to interactions of the caffeine molecules with milk proteins and carbohydrates.

2.5.5 Properties of Emulsions and Taste Perception

Concentration of fat and/or oil in emulsions can affect the sensory characteristics of food products. Shamil and others (1992) reported that reductions of fat in cheeses can led to an increase in bitterness and astringency with a reduction in saltiness. These increases in bitterness and astringency were assumed to originate from the hydrophobic characteristic of these ingredients, and the resultant increase in their concentrations in the aqueous phase when the fat concentration was reduced. On the other hand, decreases in saltiness intensity when fat levels are reduced are believed to be due to the reduced concentration of salt in the aqueous phase (Metcalf and Vickers 2002). Besides, Wendin and others (1999) reported that a decrease in fat and/or oil content in emulsion systems can lead to a decrease in sour taste due to the reduced concentration of the acid in the aqueous phase. Some other studies found that oil mouth-coating tends to have suppressive effects on taste (Malone and others 2003). Other emulsion characteristics such as the type of oil used, apparent viscosity, and droplet size have shown to have an effect on the sensory perception. Viscosity, in particular, has been proven to have an effect on taste intensities of foods. Some

authors stated that perceived taste intensities can change as a function of viscosity for the majority of taste stimuli, usually, obtaining lower taste intensities as the viscosity of the aqueous solution increases (Christensen 1980; Moskowitz and Arabie 1970; Smith and others 1996). In case of emulsion systems, Vingerhoeds and others (2008) demonstrated that the type of fat and emulsifier used had a significant effect on the textural properties of emulsions. They also stated that droplet size did not affect odor, taste, and aftertaste characteristics. On the other hand, Nakaya and others (2006) found that bitterness intensity was reduced by using tuna oil in emulsion systems with droplet sizes around 1.00 μm. Taking into consideration all of these investigations, there is still a lack of understanding of how the emulsion characteristics and properties may affect the saltiness and bitterness perceptions.

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CHAPTER 3.
OIL-IN-WATER EMULSION EXHIBITS BITTERNESS-SUPPRESSING EFFECTS IN A SENSORY THRESHOLD STUDY

3.1 Introduction

High sodium consumption is a major contributor to high blood pressure which is a leading cause of stroke, coronary heart diseases, heart attack, and kidney failure (CDC 2013). Sodium reduction can be achieved by modification of the food structure, thereby, improving the perception of saltiness (Busch and others 2013). In liquid products, this approach includes modification of certain physical properties including viscosity and overall salt distribution. The use of inert fillers that concentrate salt in the aqueous phase, and the development of products with non-homogeneous distributions of salt can increase the overall perception of saltiness in foods, hence, reducing sodium. Perceived taste intensities can change as a function of viscosity for the majority of taste stimuli; this results in lower taste intensities as the aqueous solution viscosity increases (Moskowitz and Arabie 1970; Christensen 1980; Smith and others 1996).

Various natural and processed foods consist of either partial or entire emulsions, or have been in an emulsified state during their production. An emulsion is a mixture of two immiscible liquids in which one liquid is dispersed as small spherical droplets (discontinuous phase) in the other (the continuous phase). The diameter of droplets usually lies between 0.1 and 100 μm (McClement 2005; Leal-Calderon and others 2007). Ramisetty and Shyamsunder (2011) found that emulsions prepared by ultrasonic systems presented smaller droplet sizes compared to emulsions prepared by mechanical agitation. Smaller droplet sizes are thermodynamically more stable and have different rheological properties. The oil droplet size distribution of an emulsion can be adjusted and has a major effect on several physicochemical and sensory properties including shelf-life, appearance, texture, and flavor (Floury and others 2000; McClement 2005).

Concentration of fat and/or oil can affect sensory characteristics of food products. Several studies reported that oil had suppressive effects on taste (Malone and others 2003). Shamil and others (1992) reported that reductions of fat in cheese can lead to an increase in bitterness and astringency with reductions in saltiness perception. Wendin and others (1999) reported that a decrease in mayonnaise oil content can decrease sourness due to the decreased concentration of acetic acid in the water phase. Modifying some emulsion characteristics including flavor, fat/oil content, viscosity, droplet size, and the type of emulsifier may affect the sensory perception of emulsions (Vingerhoeds and others 2008). Vingerhoeds and others (2008) reported that emulsion droplet size did not have a significant effect on odor, taste, and aftertaste. In contrast, Nakaya and others (2006) reported that bitter taste intensities of tuna oil emulsions with smaller droplet sizes (diameter = 1.00 μm) were lower than that of larger oil droplets (diameter = 5.50 μm). This supported the hypothesis that smaller oil droplets may have a bitterness suppressing effect.

Many studies on emulsion are focused on textural characteristics, and those focused on flavor studies were related to aroma release rather than effects of oil on the taste perception (Malone and others 2003). Oil in emulsions may affect taste perception due to two mechanisms with opposite effects. First, oil can form a barrier between the taste compounds and receptors, hence, decreasing the perceived intensity. Second, oil may increase the concentration of water soluble taste compounds in the aqueous phase, creating a more intense taste perception (Metcalf and Vickers 2002). A simple model of oil-in-water emulsion is useful to understand effects of emulsion characteristics on taste. Some studies found that oil of emulsion systems can suppress sweet and bitter tastes whereas others reported that perceived saltiness can increase with increasing oil concentration in emulsions (Malone and others 2003; Metcalf and Vickers 2002).

There is not a clear understanding of how emulsion characteristics may affect the saltiness and/or bitterness taste perceptions in foods. The thresholds measurements are useful for determining an individual or group mean sensitivity to a given stimulus, including tastants and/or odor compounds (Bi and Ennis 1998; Lawless 2010). Thus, the objective of this research was to evaluate sensory detection and recognition thresholds of NaCl, caffeine, and KCl in aqueous solution vs. oil-in-water emulsion systems. In particular, this study was conducted to demonstrate that oil-in-water emulsions could exhibit bitterness suppressing effects. Additionally, gender effects on detection and recognition (saltiness and bitterness) thresholds were preliminarily assessed.

3.2 Materials and Methods

3.2.1 Panelists

The research protocol for this study was approved (IRB# HE 12-19) by the Louisiana State University Agricultural Center Institutional Review Board. Untrained panelists (Hoehl and others 2013) from a pool of faculty, staff, and students of the Louisiana State University were recruited. Pre-screening was done using the following criteria: availability, health, general product attitudes, sensory awareness, and rating ability. Based on an interview, panelists with taste and smell disorders or kidney/liver problems were excluded from this study.

Panelists were further screened by acuity sensory tests in which they had to demonstrate ability to detect, recognize, and describe sensory characteristics of salty and bitter compounds (NaCl, caffeine, and KCl). Furthermore, they were tested for their ability to evaluate intensities using ranking and rating tests. A panel (N = 15) of 7 males and 8 females with an age range of 20-30 years was selected for this study.

3.2.2 Sample Solutions and Emulsions

3.2.2.1 Solutions Preparation

Sodium chloride and potassium chloride solutions were prepared using NaCl (Morton International, INC., Chicago, IL, USA) and KCl (99% FCC grade, Extracts & Ingredients, LTD., Union, NJ, USA) in odorless and tasteless spring water (Ozarka®, Nestlé Waters North America, Greenwich, CT, USA) at seven concentrations with a fixed ratio of two-fold increments: 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 g in 100 mL (0.86, 1.71, 3.42, 6.84, 13.69, 27.38, and 54.76 mM for NaCl, and 0.67, 1.34, 2.68, 5.37, 10.73, 21.46, 42.92 mM for KCl). Caffeine solutions were prepared using caffeine (caffeine anhydrous 80 mesh, AnMar, Bridgeport, CT, USA) in Ozarka® spring water at seven concentrations with a fixed ratio of two-fold increments: 0.0025, 0.005, 0.01, 0.02, 0.04, 0.08, and 0.16 g in 100 mL (0.13, 0.26, 0.51, 1.03, 2.06, 4.12, and 8.24 mM). Distilled water was not suitable as it may cause a cardboard-like flavor and can introduce a bitter taste (Jellinek 1985). The highest concentration was prepared and diluted to attain the lower concentrations. 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 be distributed over three to four concentration steps (ASTM 2008). The range of concentrations was selected by pretesting in order to ensure that panelists thresholds fell in the range. Each aqueous solution was prepared and stored in 1 L glass bottles and kept at 25 °C for no more than 2 hours. Prior to serving, 25 mL of emulsion was poured into plastic cups with lids that were coded with three-digit random numbers.

3.2.2.2 Emulsions Preparation

To prepare oil in water emulsions, the texture modifier Tic Pretested®Ticaloid®210-S powder (tasteless; gum acacia and xanthan gum; Tic Gums®, Inc., White Marsh, MD, USA) was

used to increase the viscosity of the aqueous phase of the emulsion; it was used at 1% concentration of the total emulsion, and mixed with the aqueous phase. The emulsifier Tandem® 552K (tasteless; a mixture of mono- and di-glycerides, polysorbate, water and propyl gallate) was obtained from Caravan® ingredients (Lenexa, KS, USA); it was used at 1% concentration of the total emulsion and mixed with the oil phase. NaCl, caffeine, or KCl were dissolved in the aqueous portion (water+Tic gum) of the emulsion, and then mixed with canola oil (at 20% by weight of the emulsion; CWP, Cal Western Packaging Corp., Memphis, TN, USA) and the emulsifier to produce ascending concentrations of the tastants (NaCl, caffeine, or KCl) equal to their solution counterparts. Two types of emulsions were prepared. Emulsion 1 (viscosity = 257 cP) was prepared using mechanical stirring (Ika Ultra-Turrax® T18 basic, IKA-Werke GmbH and Co. KG, Staufen, Germany) at approximate 15,000 rpm. Emulsion 2 (viscosity = 59 cP) was prepared using the method for Emulsion 1 but with an additional ultrasonication processing step using a sonicator (Vibracell 750 VCX, Sonics & Materials, Inc. CT, USA) with a total input energy of 25,000 J and an amplitude of wave generated by the probe of 85% (pulse on = 2 s and pulse off = 1 s). The volume used for ultrasonication was 175 mL of the emulsion. Each emulsion was prepared and stored in 1 L glass bottles and kept at 25 °C for no more than 2 hours. Prior to serving, 25 mL of emulsion was poured into plastic cups with lids that were coded with three-digit random numbers.

Two emulsion types were selected for this study due to their significant differences in apparent viscosity. For viscosity measurement, 100 mL of emulsion samples was placed in a 200 mL beaker and viscosity in centipoise (cP) was measured at 20±0.5 °C with a viscometer (model DV-II+, Brookfield Engineering Labs Inc., Middleboro, MA, USA) at 50 rpm using a RV-IV spindle, with data gathered in Wingather V2.1 software (Brookfield Engineering Labs Inc.).

3.2.3 Threshold Measurements Using the Method of Limits (ML)

For each tastant (NaCl, caffeine or KCl), there were seven sets (i.e., seven concentrations) of solution and/or emulsion samples; each set was presented once in the order of increasing concentration. For each set, subjects were presented with three samples, of which two were controls (spring water or emulsion without tastant) and one was the solution and/or emulsion with tastant (NaCl, caffeine, or KCl). Unsalted crackers and spring water were also served for palate cleansing during the test. Two independent replicates (sessions) were performed on different days. A total of 126 sample sets (3 tastants x 3 sample types x 7 sets x 2 replicates) were evaluated over 9 weeks period. In this study, the 3-AFC ascending concentration series method of limits with a slight modification of the ASTM E-679 (ASTM 2008) was applied. The panelists were first asked to select the odd sample (detection threshold) and then further identified specific tastes of the odd sample that exhibited recognizable difference (recognition threshold). The choices of recognizable tastes included four basic tastes (sweet, salty, sour, bitter) and unidentified/water (in case the panelists were unable to identify specific tastes). All threshold evaluations were performed in partitioned booths illuminated with cool, natural, fluorescent lights. Evaluation sessions were conducted at 10:00 am (2 hours before the regular lunch time of panelists), and panelists were advised not to drink, eat, or smoke one hour prior to the test. To avoid biases, panelists did not receive any monetary incentive for participation; however, at the end of the study, all panelists were invited to an appreciation dinner reception, and their contributions were acknowledged. The Compusense *five* (Compusense Inc., Guelph, Canada) computerized data collection system was used to develop the questionnaire, and to collect the data.

3.2.4 Threshold Data Analysis

3.2.4.1 Individual Best-Estimate Thresholds (BET)

A series of each panelist judgments was tabulated with a sequence containing "0" for an incorrect choice or "+" for a correct choice, which was arranged in the order of judgments of ascending concentrations of NaCl, caffeine, and/or KCl. As the distribution is typically skewed, a geometric mean rather than an arithmetic mean was used to measure the center location of the distribution (ASTM E-679-04; ASTM 2008). Therefore, the best-estimate threshold (BET) concentration for the detection threshold was the geometric mean of the last missed (0) concentration and the next (adjacent) higher concentration (+). 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 individual threshold values from two independent replications.

3.2.4.2 Group Best-Estimate Thresholds (GBET)

For the geometric mean method, the group best-estimate threshold (GBET) was obtained by the arithmetic average of summation of the logarithm with base 10 ($\log_{10}$) of the individual BET values. The $\log_{10}$ standard deviation provided a measure of the group variation (ASTM 2008). The arithmetic average of GBETs of two replicates (group sessions) was reported. This method was used for estimating detection threshold as well as recognition thresholds for saltiness and bitterness.

3.2.5 Statistical Analysis

Analysis of variance (ANOVA) and the *post-hoc* Tukey's Studentized Range (HSD) test were performed at $\alpha = 0.05$ to compare the mean threshold differences between different solutions

and emulsions systems for a given stimulus and threshold test. For an alternative method of analyzing the responses, logistic regression analysis was performed, modelling the panel selection of correct responses (from the 3-AFC test) using the system (solutions over the emulsions 1 and 2) and concentration of the tastant (ascending concentration of NaCl, caffeine, and KCl in the aqueous or emulsion system; continuous variable) as regression variables of the model. All statistical analyses were performed using Statistical Analysis Software® (SAS 2012).

3.3 Results and Discussion

3.3.1 Detection Threshold

The ANOVA table (Table 3.1) summarizes the effects of different taste compounds (NaCl, caffeine, and/or KCl) and systems (solution, emulsion 1, and emulsion 2) on the $\log_{10}$ BET values sorted by the type of threshold test performed (detection, saltiness recognition, and/or bitterness recognition). For the detection $\log_{10}$ BET values, the system effect was significant ($P < 0.05$) but the compound and the compound * system interaction effects were not significant. This indicates that individual detection thresholds of the different compounds tested (NaCl, caffeine, and KCl) were not significantly different ($P \geq 0.05$), but threshold values significantly ($P < 0.05$) varied across the systems (solution vs. emulsions). The group variation expressed as $\log_{10}$ standard deviations for detection thresholds was in the range of 0.24-0.64 (data not shown).

NaCl and KCl detection GBET values (0.0197-0.0286 vs. 0.0215-0.0354 g/100 mL; Figure 3.1) were not significantly different ($P \geq 0.05$) regardless of the system (solution, emulsion 1, and/or emulsion 2). Caffeine detection GBET of water solution was not significantly different ($P \geq 0.05$) from that of emulsion 1 (0.0181 vs.0.0284g/100 mL) but was significantly lower than that of emulsion 2 (0.0516 g/100 mL). No significant differences ($P \geq 0.05$) for caffeine detection GBET were found between emulsions 1 and 2. Generally, detection GBET values (0.0197-0.0516)

were lower than those of saltiness (0.0470-0.1070 g/mL) and bitterness (0.0242-0.1025 g/100 mL) recognition GBET values for all the systems (Figure 3.1).

Variations in the detection threshold values of NaCl, KCl and caffeine in solutions have been reported (Table 3.2). However, the study on the thresholds of KCl is limited. Mojet and others (2001) reported the detection threshold of KCl at 0.034-0.037 g/100 mL for 22 young subjects (19-33 years old).

Table 3.1 ANOVA table of the $\log_{10}$ of the Best Estimate Thresholds (BET's) values for detection, saltiness recognition, and bitterness recognition thresholds

Type III Tests of fixed effects for detection BET's				
Effect[1]	Num DF[2]	Den DF[2]	F Value[2]	Pr > F[2]
Compound	2	237.9	2.71	0.0685
System	2	238.7	10.48	<.0001[3]
Compound*System	4	237.9	2.07	0.0854

Type III Tests of fixed effects for saltiness recognition BET's				
Effect[1]	Num DF[2]	Den DF[2]	F Value[2]	Pr > F[2]
Compound	1	154.2	39.64	<.0001[3]
System	2	154.7	1.82	0.1656
Compound*System	2	154.2	1.55	0.2157

Type III Tests of fixed effects for bitterness recognition BET's				
Effect[1]	Num DF[2]	Den DF[2]	F Value[2]	Pr > F[2]
Compound	1	152.9	11.66	0.0008[3]
System	2	153.7	27.14	<.0001[3]
Compound*System	2	152.9	5.37	0.0056[3]

[1] Three tested compounds (NaCl, Caffeine and KCl) and three systems (solution, emulsion 1 [viscosity = 257 cP] and emulsion 2 [viscosity = 59 cP]). For saltiness recognition BET, only NaCl and KCl were tested. For bitterness recognition BET, only caffeine and KCl were tested. Panelist (N = 15) were considered as a random effect in the model. Two independent replicates were performed.

[2] DF, Degrees of freedom; Num = Numerator; Den = Denominator; F value = Mean square/Mean square error.

[3] Effects were significant when the probability (Pr > F) was < 0.05.

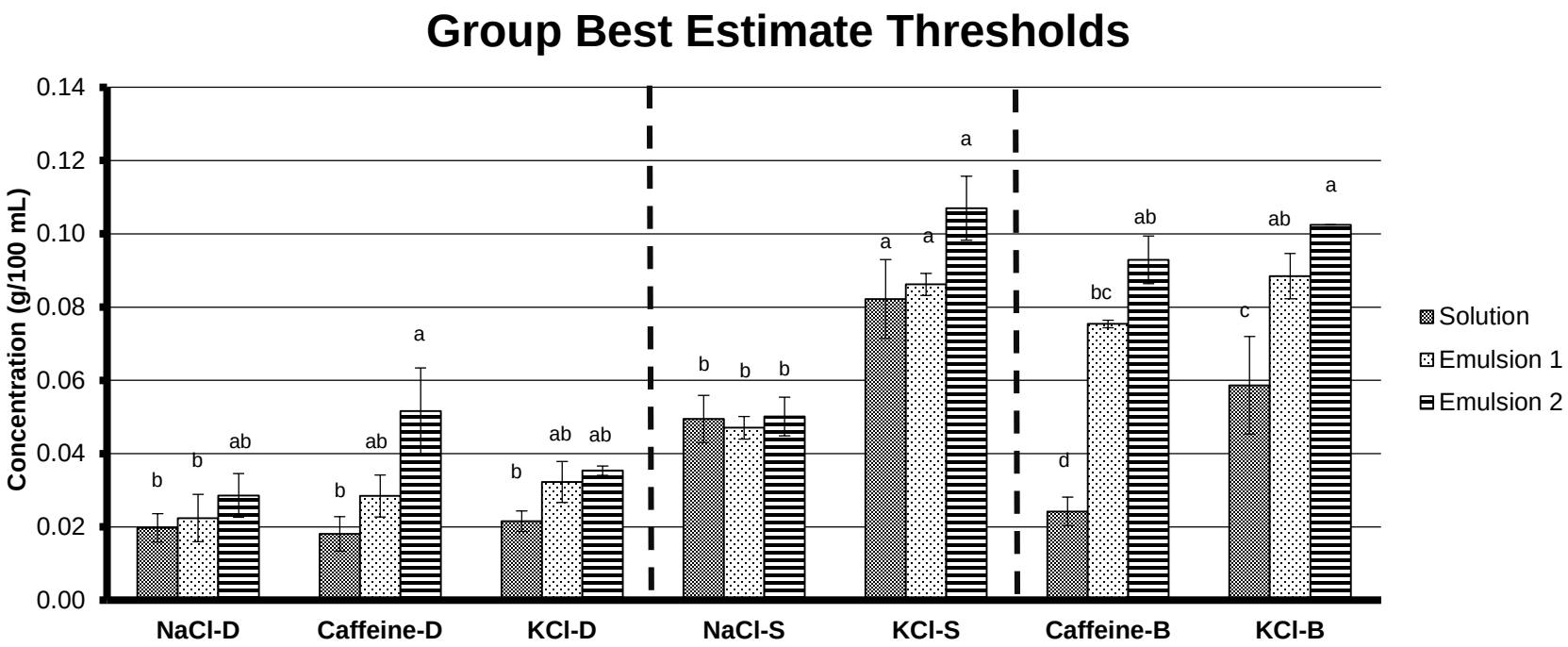

Figure 3.1 Group Best Estimate Thresholds (GBET's in g/100mL) for different threshold types [Detection (D), Saltiness Recognition (S) and Bitterness Recognition (B)] of three compounds (NaCl, caffeine and KCl)

[a-d] Bars with different superscripts on the top indicate significant differences ($P < 0.05$) within each threshold type (D, S, or B) determined by the Tukey's Studentized Range (HSD) test. Each GBET is an average of two values (replicates). Emulsion 1 = viscosity of 257 cP and emulsion 2 = viscosity of 59 cP.

Table 3.2 A comparative table for detection and recognition (saltiness and bitterness) threshold determinations

Authors	Panelist description	System*	Tastant	Type of threshold*	Threshold value (g/100 mL)
Hatae and others (2009)	40 young females	Solution	NaCl	Detection	0.004
Gomez and others (2004)	21 females	Solution	NaCl	Detection	0.038
		Solution	NaCl	Recognition	0.038
Weiffenbach (1995)	69 females (24-82 years old)	Solution	NaCl	Detection	0.018
Gonázlez Viñas and others (1998)	21 tasters	Solution	NaCl	Detection	0.021
		Solution	Caffeine	Detection	0.010
Paulus and Hass (1980)	14 subjects (5 males and 9 females)	Solution (1cP)	NaCl	Detection	0.040
		Solution (1cP)	Caffeine	Detection	0.008
		Solution (100cP)	NaCl	Detection	0.042-0.061
		Solution (100cP)	Caffeine	Detection	0.008-0.010
		Solution (1cP)	NaCl	Recognition	0.082
		Solution (1cP)	Caffeine	Recognition	0.015
		Solution (100cP)	NaCl	Recognition	0.091-0.125
		Solution (100cP)	Caffeine	Recognition	0.016-0.031
Mojet and others (2001)	22 panelists	Solution	NaCl	Detection	0.030-0.033
		Solution	KCl	Detection	0.034-0.037
Thurgood and Martin (2010)	11 panelists (5 males and 6 females; 21-61 years old)	Solution	NaCl	Recognition	0.011
		Emulsion (20%)	NaCl	Recognition	0.022
		Solution	Quinine	Recognition	0.0003
		Emulsion (20%)	Quinine	Recognition	0.0011
The present study	15 panelists (7 males and 8 females; 20-30 years old)	Solution (1cP)	NaCl	Detection	0.020
		Emulsion (257cP)	NaCl	Detection	0.022
		Solution (1cP)	NaCl	Recognition	0.049
		Emulsion (257cP)	NaCl	Recognition	0.047
		Solution (1cP)	Caffeine	Detection	0.018
		Emulsion (257cP)	Caffeine	Detection	0.028
		Solution (1cP)	Caffeine	Recognition	0.024
		Emulsion (257cP)	Caffeine	Recognition	0.075

*****Saltiness recognition threshold for NaCl, and bitterness recognition threshold for caffeine or quinine.

In this study, the KCl detection GBET value in a water solution was 0.0215 g/100 mL. Hatae and others (2009) reported that the NaCl group detection threshold for 40 subjects was 0.004 g/100 mL, which was lower than that (0.018 g/100 mL) for 69 subjects reported by Weiffenbach and others (1995). Gonázlez Viñas and others (1998) estimated group detection threshold values of 0.021 g/100 mL for NaCl and 0.010 g/100 mL for caffeine in solutions using the method of limits (ASTM E-679). Keast and Roper (2007) reported a caffeine detection threshold value of 0.0233 g/100 mL using 33 subjects. Drewnowski (2001) estimated caffeine detection threshold in water solutions at 0.0094 g/100 mL. Paulus and Hass (1980) found that the detection thresholds of 14 subjects were 0.040 and 0.008 g/100 mL, respectively, for NaCl and caffeine in aqueous solutions (viscosity = 1 cP); they further reported that the detection thresholds increased with increased solution viscosity (Table 3.2).

NaCl and caffeine detection threshold values reported for the solution system in this study (Figure 3.1) are similar to those reported by Weiffenbach and others (1995), Gonázlez Viñas and others (1998), and Keast and Roper (2007). Contrary to what Paulus and Hass (1980) found regarding the viscosity effect on thresholds, Figure 3.1 shows that the lower viscosity (59 cP) emulsion 2 had higher (but not significant) detection thresholds compared to that of the higher viscosity (257 cP) emulsion 1, for all compounds evaluated. Malone and others (2003) explained that the existence of an oil phase in the oil-in-water emulsion reduces the volume of water in emulsion samples compared to aqueous solution samples. This results in an increase of the perceived taste intensity. However, another perception mechanism could involve the mouth-coating of the taste receptor by the oil phase, which results in a decrease of the perceived taste intensity.

According to the logistic regression analysis, caffeine had higher odds ratio values (solution vs. emulsion 1, and solution vs. emulsion 2) compared to those of NaCl and KCl (1.443-1.838 vs. 1.074-1.353; Table 3.3). This means that the odds of selecting the correct response (the odd sample) in a 3-AFC test was higher in a solution system than in emulsion systems for caffeine. This implies that panelists were more sensitive towards caffeine in the aqueous solution than in emulsion systems.

For NaCl and KCl, the odds ratio (θ) values were not significant (Ho: $\theta = 1$; $P \geq 0.05$), which indicates that the odds of selecting the correct response was indifferent regardless of the system used (solution, emulsion 1, and/or emulsion 2). This denotes that panelists exhibited similar sensitivities towards NaCl and KCl in solution and/or emulsion systems. This finding substantiated the results reported in Figure 3.1. The results from Tables 3.1 and 3.3 and Figure 3.1 collectively indicate that detection thresholds for NaCl and KCl were comparable in solution and/or emulsion systems. However, the emulsion 2 had a higher caffeine detection GBET than the solution.

3.3.2 Saltiness Recognition Threshold

According to ANOVA results for NaCl and KCl saltiness recognition $\log_{10}$ BET values (Table 3.1), the compound effect was significant ($P < 0.05$) but the system and the compound * system interaction effects were not significant ($P \geq 0.05$). This indicates that for each compound, individual saltiness recognition thresholds in the solution and/or emulsion systems were not significantly different; however, threshold values significantly varied across the compounds tested (NaCl vs. KCl). The group variation expressed as $\log_{10}$ standard deviations for saltiness recognition thresholds was in the range of 0.31-0.52 (data not shown) which are higher than the values reported for detection thresholds (0.24-0.64).

Table 3.3 Odds ratio estimates[1] for selecting the correct response (the odd sample) in a 3AFC test for each threshold type (Detection, Saltiness Recognition and Bitterness Recognition) and for each compound (NaCl, caffeine and KCl)

Test	Compound	Odds ratio estimates			
		System (solution vs. emulsion 1)[2]		System (solution vs. emulsion 2)[2]	
		Estimate[1]	$Pr > \chi^2$	Estimate[1]	$Pr > \chi^2$
Detection	NaCl	1.261	0.336	1.353	0.217
	Caffeine	1.443	0.090	1.838[3]	0.005
	KCl	1.074	0.772	1.280	0.317
Saltiness Recognition	NaCl	1.318	0.294	1.541	0.112
	KCl	0.874	0.594	1.739[3]	0.042
Bitterness Recognition	Caffeine	5.988[3]	<.0001	11.494[3]	<.0001
	KCl	1.704[3]	0.035	2.564[3]	0.0004

[1] Based on the logistic regression analysis using systems (solutions and emulsions) and concentration of the tastant as regression variables. The analysis of maximum likelihood estimates was used to obtain the parameter estimates.
[2] Three systems were: solution, emulsion 1 (viscosity = 257 cP) and emulsion 2 (viscosity = 59 cP).
[3] Parameter estimates were considered significant when the probability of the Wald χ^2 was < 0.05.

For NaCl saltiness recognition threshold, the GBET values (0.0471-0.0501 g/100 mL) were not significantly different among the three systems (solution, emulsion 1 and emulsion 2; Figure 3.1). Similar observations were found for KCl saltiness recognition GBET values (0.0822-0.1070 g/100 mL; Figure 3.1). However, for all systems, KCl saltiness recognition GBET values were significantly higher ($P < 0.05$) than those of NaCl. NaCl provides the most pure salty taste of all salts; other salts taste significantly more sour or bitter in addition to salty (Smith and van der Klaauw 1995). The saltiness pureness of NaCl may explain its lower recognition threshold values compared to those of KCl (Figure 3.1).

Gomez and others (2004) reported that the detection and recognition thresholds of NaCl in solution were the same (0.038 g/100 mL); this was not observed in our current study (0.0197 vs. 0.0495 g/100 mL; Figure 3.1). Variations in the saltiness recognition threshold values of NaCl in solutions have been reported (Table 3.2). Paulus and Hass (1980) found that saltiness recognition threshold of NaCl increased with increased solution viscosity from 0.082 g/100 mL in a 1 cP solution to 0.091-0.125 g/100 mL in a 100 cP solution. However, in our study, increased viscosity of emulsion 1 (257 cP) did not significantly increase the saltiness recognition GBET value compared to that of emulsion 2 (59 cP) (Figure 3.1). This is supported by the work of Thurgood and Martin (2010) who reported that NaCl saltiness recognition thresholds in the solution vs. the oil in water emulsion (20% soybean oil) were not significantly different (0.0110 vs. 0.0220 g/100 mL).

The odds ratio values were not significant (Ho: $\theta = 1$; $P \geq 0.05$) for NaCl (Table 3.3), which implies that the odds of selecting the correct response (recognizing the saltiness in the 3-AFC test) was similar regardless of the system used (solution, emulsion 1 and/or emulsion 2). For KCl, the odds ratio value of the solution over emulsion 2 was significant and about 2-fold higher than that

of the solution over emulsion 1 (1.739 vs. 0.874, Table 3.3). This implies that the panelists were less sensitive in recognizing KCl saltiness of emulsion 2 compared to emulsion 1, thus requiring a higher (though not significant as shown in Figure 3.1) concentration of KCl in emulsion 2 to induce saltiness recognition. Results from Tables 3.1 and 3.3 and Figure 3.1 collectively indicate that saltiness recognition threshold values of NaCl or KCl were not affected by the system used (solution, emulsion 1 and/or emulsion 2). However, KCl saltiness recognition threshold values were higher than those of NaCl for all systems used.

3.3.3 Bitterness Recognition Threshold

According to ANOVA (Table 3.1), there were significant effects ($P < 0.05$) of the compound (KCl and/or caffeine), system (solution, emulsion 1, and/or emulsion 2) and the compound * system interaction on the bitterness recognition thresholds. The group variation expressed as $\log_{10}$ standard deviations for bitterness recognition thresholds was in the range of 0.25-0.47 (data not shown). Within each emulsion system, bitterness recognition GBET values of KCl and caffeine were comparable (Figure 3.1). For each compound, bitterness recognition GBET values were not significantly different between emulsions 1 and 2. However, for the solution system, the bitterness recognition GBET value was significantly higher for KCl than for caffeine (0.0586 vs. 0.0242 g/100 mL; Figure 3.1). For both caffeine and KCl, emulsion 2 had slightly higher (but not significant) bitterness recognition GBET values than those of emulsion 1; this implies that a higher viscosity of emulsion (257 cP vs. 59 cP) had no significant effect on bitterness recognition threshold of KCl and/or caffeine, under the conditions of this study. However, Paulus and Hass (1980) reported that bitterness recognition thresholds of caffeine increased with increased solution viscosity (Table 3.2). Although viscosity influences the bitterness perception,

future studies should consider the effects of pH, particle size, microscopy, imaging, and other emulsion characteristics on the threshold values.

Comparing the solution vs. the emulsion systems, both emulsions (1 and 2) had significantly higher bitterness recognition GBET values than those of the solution for both caffeine and KCl (0.0754-0.1025 vs. 0.0242-0.0586 g/100 mL; Figure 3.1); this indicates that emulsion exhibited bitterness-suppressing effects. Some studies reported that oil had suppressive effects on taste (Malone and others 2003). Thurgood and Martin (2010) reported that solution BET values were significantly lower than emulsion BET values for bitterness (quinine) recognition (0.0003 vs. 0.0011 g/100 mL). Moreover, they concluded that lipids can limit the ability of tastants to arrive at and to interact with the taste receptor cells. Nakaya and others (2006) found that bitterness intensities were lower in emulsion with smaller oil droplets.

Caffeine is both water- and oil soluble and can be diluted into lipophilic environments. Tastants partitioned into the oil phase can be less effective at reaching and activating bitter taste receptors (Metcalf and Vickers 2002). On the other hand, for hydrophilic bitter tastants (such the case of KCl), the presence of an oil phase would increase the aqueous phase concentration and the bitter taste. Thus, suppression of bitterness would depend on the properties of the molecules responsible for the bitter taste (Coupland and Hayes 2014).

In this study, all of the odds ratio values were significant (Ho: $\theta = 1$; $P < 0.05$; Table 3.3). Generally, the odds of selecting the correct response (recognizing the bitterness taste in the 3-AFC test) was greater for (the former > the latter) the solution > the emulsions, for caffeine > KCl and for emulsion 1 > emulsion 2. Therefore, this would require the latter to have a higher bitterness recognition threshold. However, according to Figure 3.1, the bitterness recognition GBET value was significantly higher for KCl than for caffeine only in the solution system, and emulsions (1

and 2) had significantly higher bitterness recognition GBET values than those of the solution for both caffeine and KCl. Results from Tables 3.1 and 3.3, and Figure 3.1 collectively indicate that bitterness recognition thresholds of caffeine and KCl were affected by the system used in which emulsions (1 and 2) had higher threshold values than the solution, thus demonstrating bitterness-suppressing effects.

3.3.4 Preliminary Results on Effect of Gender on Sensory Thresholds

A total of 15 panelists (7 males, M and 8 females, F) participated in this study and effects of gender are depicted in Figure 3.2. The detection threshold values for M and F were similar (F = 0.015-0.024 g/100 mL vs. M = 0.024-0.033 g/100 mL for NaCl; F = 0.017-0.066 g/100 mL vs. M = 0.019-0.054 g/100 mL for caffeine; F = 0.020-0.034 g/mL vs. M = 0.023-0.038 g/100 mL for KCl).

Mojet and others (2001) investigated the effect of gender and age on the threshold sensitivity of the basic tastes using 22 young (age 19-33 years old; 11 M and 11 F) and 21 elderly (age 60-75 years old; 10 M and 11 F) subjects. They reported that the age effect was significant but not the gender. In their study, the detection thresholds were 0.030 and 0.033 g/100 mL for young M and young F, respectively, for NaCl; 0.034 and 0.037 g/100 mL for young M and young F, respectively, for KCl.

Regarding the gender effect on saltiness recognition thresholds, both M and F followed a similar pattern for the individual threshold values (Figure 3.2). However, F had higher individual BET values for KCl in the solution and emulsion 1 than did M (F = 0.0951 vs. M = 0.0690 g/100 mL for solution, and F = 0.1038 vs. M = 0.0673 g/100 mL for emulsion 1). On the other hand, M had a slightly higher individual BET value for KCl in emulsion 2 than F (M = 0.1449 vs. F = 0.1189 g/100 mL). For the bitterness recognition thresholds, both M and F were comparable in

their individual threshold values (Figure 3.2). However, F had higher individual BET values for caffeine and KCl for emulsion 2 than did M (F = 0.1131-0.1379 vs. M = 0.0760-0.0761 g/100 mL, Figure 3.2). Due to the fewer numbers of panelists in each category (M and F), practical conclusions cannot be generalized from the obtained results. Future studies with an increased number of panelists in each category (M and F) are needed.

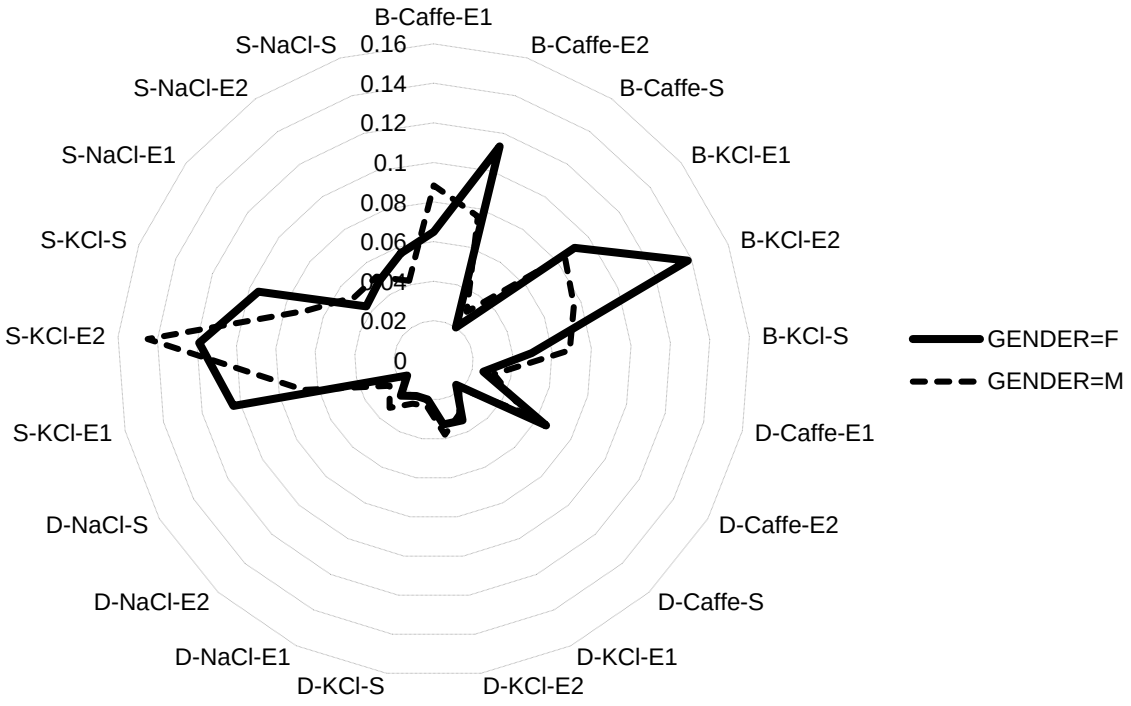

Figure 3.2 A plot of the individual Best Estimate Thresholds (BET's in g/100mL) by gender*
*Gender (F = 8 females and M = 7 males). 3 threshold types: detection (D), saltiness recognition (S) and bitterness recognition (B). 3 tested compounds (NaCl, Caffeine (Caffe) and KCl). 3 system [Solution (S), emulsion 1 (E1 = viscosity of 257 cP) and emulsion 2 (E2 = viscosity of 59 cP)]

3.4 Conclusions

Understanding factors affecting saltiness and bitterness perception becomes critical in order to choose an appropriate approach to sodium-reduction in foods. In this study, we evaluated sensory detection and recognition thresholds of NaCl, caffeine, and KCl in the solution and emulsion systems using the ASTM E-679-04. The major finding was that emulsions did not significantly affect the saltiness recognition threshold of NaCl and KCl; however, emulsions exhibited bitterness-suppressing effects toward caffeine and/or KCl. This finding would prompt more in-depth studies as to how other emulsion characteristics affect saltiness and bitterness perception in the reduced sodium food system. In future studies, effects of increasing viscosities on the bitterness perception of aqueous solutions and emulsion systems should be evaluated and compared. Understanding bitterness suppression may also be useful in the constant search for the perfect non-nutritive sweetener, which should also be further investigated.

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CHAPTER 4.
OIL AND TASTANT CONCENTRATIONS AFFECT SALTINESS AND BITTERNESS PERCEPTION OF OIL-IN-WATER EMULSIONS

4.1 Introduction

High sodium consumption is a major contributor to high blood pressure which is the leading cause of stroke, coronary heart diseases, and kidney failure in the US (CDC 2015). Reducing sodium has proven to be a difficult task as salt (NaCl), the major contributor of sodium, not only plays an important role in taste, but is also used for preservation, structuring and other functional purposes (Kilcast and Angus 2007). One approach to sodium reduction is the use of salt substitutes such as potassium chloride (KCl) (Liem and others 2011), but KCl has a drawback of imparting bitterness and metallic aftertaste (Sinopoli and Lawless 2012). Another sodium reduction approach includes the modification of food structure for improving sodium release and saltiness perception (Kuo and Lee 2014; Busch and others 2013; Stieger and van de Velde 2013; Thurgood and Martini 2010). In liquid and semi-solid foods, this approach includes the modification of physical properties such as microstructure, viscosity, overall salt distribution, and the use of inert fillers that concentrate salt in the aqueous phase (Busch and others 2013; Stieger and van de Velde 2013), which can also be applied to emulsion systems.

Several natural and processed foods consist of either partial or entire emulsions, or they have been in an emulsified state during their production (McClement 2005). To our knowledge, most emulsion researches have focused on texture and flavor/aroma releases rather than perception of the basic tastes. From this limited research, two contrasting postulates regarding the effects of oil on taste perception have been proposed. First, oil can form a physical barrier (mouth-coating) between the tastants and receptor cells, and hence decreasing the perceived intensity (Lynch and other 1993; Metcalf and Vickers 2002). Second, oil may increase the concentration of water-

soluble tastants, and hence creating a more intense taste perception (Metcalf and Vickers 2002). Moreover, oil may affect taste perception by increasing viscosity, and altering the diffusion coefficients and retention times of taste substances in the oral cavity (Mela and others 1994; Barylko-Pikielna and others 1994). Consequently, sensory perception of basic tastes can be affected by physical properties of emulsions (Suzuki and others 2014; Rietberg and others 2012; Dresselhuis and others 2008).

The effects of oil addition on saltiness perception in emulsions were described (Suzuki and others 2014). As NaCl and KCl are water-soluble, they are expected to be fully partitioned in the aqueous phase, resulting in increased perceived saltiness in emulsions with higher oil concentrations (Kuo and Lee 2014; Koriyama and others 2002). Malone and others (2003) reported that saltiness perception in emulsions was dependent on the concentration of salt in the aqueous phase, the total aqueous phase volume in the emulsion, and the formation of an oily mouth-coating that reduces the mass transfer of the tastant to the taste receptors.

In previous investigations, contrasting conclusions were found regarding the effects of oil on bitterness perception. Keast (2008) stated that increasing milk fat content increased the bitterness of caffeine. He attributed this effect to the interaction of caffeine molecules with milk proteins and carbohydrates. Pripp and others (2004) concluded that increasing oil viscosity was not effective in reducing bitterness of olive oil phenolics. They hypothesized that, at high oil viscosity, the mass transport of tastants decreased, and hence a lower tastant concentration at the interface between sample and taste receptors. Metcalf and Vickers (2002) reported that emulsions with added oil had less bitter taste and more intense sweet, salty, sour, and umami tastes than those with added water. The majority of bitter compounds are hydrophobic and they can reside in lipophilic environments. Therefore, oil may suppress bitterness through a dilution effect of the

bitter compounds in the water-phase of emulsions (Coupland and Hayes 2014; Metcalf and Vickers 2002). Moreover, the type and characteristics of the oil may affect the perceived bitterness (Kuo and Lee 2014). For instance, Koriyama and others (2002) concluded that bitterness suppression of quinine sulfate was higher for tuna oil compared to that of soybean and corn oils.

Suzuki and others (2014) studied the effect of lipid content on saltiness perception in oil-in-water emulsion systems. However, they only measured salty taste quality using NaCl, and no other research has attempted to investigate saltiness perception of NaCl and KCl, and bitterness perception of caffeine and KCl in emulsion systems. Therefore, the objective of this study was to evaluate saltiness of NaCl and KCl, and bitterness of KCl and caffeine in emulsions prepared with different concentrations of canola oil (20, 40 or 60%) and tastants [NaCl (0.50, 0.75 or 1.00%), KCl (0.50, 1.00, or 1.50%), or caffeine (0.05, 0.10, or 0.15%)] using a trained Spectrum™ descriptive panel.

4.2 Materials and Methods

4.2.1 Preparation of Sample Solutions and Emulsions

Sodium chloride (NaCl; Morton International, Inc., Chicago, IL, USA), caffeine (caffeine anhydrous 80 mesh, AnMar, Bridgeport, CT, USA), and potassium chloride (KCl; 99% FCC grade, Extracts & Ingredients, LTD., Union, NJ, USA) were thoroughly dissolved in spring water (Ozarka®, Nestlé Waters North America, Greenwich, CT, USA.), and solutions were used as reference samples (Table 4.1). Each aqueous solution was poured into 1 L glass bottle and kept at ambient temperature (25°C). Before serving, 25 mL of solution was poured into plastic cups with lids that were coded with three-digit random numbers. Reference samples were coded with the associated reference intensity numbers (Table 4.1) and kept at ambient temperature (25 °C) until used. For emulsion preparation, the Tic Pretested®Ticaloid®210 S powder (gum acacia and

xanthan gum; Tic Gums®, Inc., White Marsh, MD, USA) was used to increase the viscosity of the aqueous phase of the emulsion.

Table 4.1 Saltiness and bitterness references for the Spectrum™ descriptive analysis method

Attribute	Definition	Reference intensity	Preparation Method	% Solution
Saltiness	A fundamental taste of which the taste of sodium chloride in water is typical	7.5	2.25 g NaCl in 500 mL of water	0.45
		10.0	2.75 g NaCl in 500 mL of water	0.55
		12.5	3.10 g NaCl in 500 mL of water	0.63
		18.0*	5.00 g NaCl in 500 mL of water	1.00
		22.0*	7.00 g NaCl in 500 mL of water	1.40
Bitterness	A fundamental taste of which the taste of caffeine in water is typical	2.0	0.25 g caffeine in 500 mL of water	0.05
		5.0	0.40 g caffeine in 500 mL of water	0.08
		10.0	0.75 g caffeine in 500 mL of water	0.15

*Source: Kwan (2004).

The Tandem® 552K (a mixture of mono- and diglycerides, polysorbate, water and proply gallate), obtained from Caravan® ingredients (Lenexa, KS, USA), was used as an emulsifier. 1% of Tic Pretested®Ticaloid®210 S powder was mixed with the aqueous phase, and 1% of Tandem® 552K was mixed with the oil phase. NaCl, caffeine, or KCl was dissolved in the aqueous portion (water+Tic gum) of the emulsion, and then mixed with canola oil (CWP, Cal Western Packaging Corp., Memphis, TN, USA) and the emulsifier. Emulsions were mixed for 10 minutes at high-speed using a hand-held blender (Model # 59780R, Hamilton Beach® Brands Canada, Inc., Picton, Onratio, Canada). Each emulsion was poured into 1 L glass bottle and kept at ambient temperature

(25 °C) prior to testing. Before serving, 25 mL of emulsion was poured into plastic cups with lids that were coded with three-digit random numbers. NaCl, KCl, and caffeine concentrations in emulsions (Table 4.2) were chosen such that their saltiness and bitterness intensities would fit within the range of reference concentrations for solutions using the Spectrum^{TM} intensity line scale (Table 4.1). Preliminary studies demonstrated that saltiness intensity of NaCl was roughly 1.5 times higher than that of KCl at a given tastant concentration; therefore, selected KCl concentrations in emulsions were higher than those of NaCl (Table 4.2). Two independent batches for each emulsion were prepared.

4.2.2 Emulsions Physical Properties

Emulsion samples (100 mL) were placed in 200 mL beakers, and viscosity in centipoise (cP) units was measured at 20 ± 0.5 °C with a viscometer (model DV-II+, Brookfield Engineering Labs Inc., Middleboro, MA, USA) at 50 rpm using an RV-IV spindle, with data gathered by Wingather V2.1 software (Brookfield Engineering Labs Inc.). The pH of emulsions was measured using an Orion 520 pH meter (Orion Labs, Tucson, AZ). Two measurements were taken from each of the two independent batches of the emulsion systems.

4.2.3 Sensory Analysis

4.2.3.1 Panelist Recruitment

The research protocol for this study was approved (IRB# HE 15-9) by the Louisiana State University Agricultural Center Institutional Review Board. Panelists from a pool of faculty, staff, and students of the Louisiana State University were recruited and pre-screened using the following criteria: availability, health, general product attitudes, sensory awareness, and rating ability. Panelists were screened with acuity sensory tests in which they had to be able to detect, recognize, and describe sensory characteristics of salty and bitter compounds using NaCl, caffeine, and KCl.

Additionally, they were tested for ability to evaluate intensities using matching, ranking, and rating tests.

Table 4.2 Factorial arrangement for two treatment factors (tastant concentration and oil concentration) for each tastant

Tastant	Variable level	
	Tastant concentration % (X_1)*	Oil concentration % (X_2)*
NaCl	0.50	20
	0.75	20
	1.00	20
	0.50	40
	0.75	40
	1.00	40
	0.50	60
	0.75	60
	1.00	60
KCl	0.50	20
	1.00	20
	1.50	20
	0.50	40
	1.00	40
	1.50	40
	0.50	60
	1.00	60
	1.50	60
Caffeine	0.05	20
	0.10	20
	0.15	20
	0.05	40
	0.10	40
	0.15	40
	0.05	60
	0.10	60
	0.15	60

*In RSM, the independent variables were the tastant concentration (X_1) and the oil concentration (X_2), and the dependent variables (Y) were either saltiness or bitterness intensities.

A panel of sixteen people (N=16) with age ranging from 20 to 30 years was selected to participate in the Spectrum^{TM} descriptive analysis method (Sensory Spectrum, New Providence, NJ, USA) for measuring intensities of salty and bitter tastes in emulsion systems.

4.2.3.2 Training and Orientation of Panelists

The training program was required for all panelists. The main purposes of training were to ensure an accurate evaluation of the sensory characteristics, and to provide a similar frame of reference in terminology and scaling among all panelists. An initial general orientation session (1 h) was conducted to expose panelists to the underlying technical principles, methodology and terminology of salty and bitter tastes. Following this orientation, six practice sessions (1.5 h each session; 9 h total) were scheduled for reviews of samples, references, evaluation procedures and results. A 15- or 22-cm line scale anchored at the ends with the terms “none” and “extreme” was used, where panelists indicated the perceived intensities by marking a vertical line on the scale. For reference samples, sodium chloride solutions were used for salty references and caffeine solutions were used for bitter references. Reference intensity scores, preparation methods and concentrations of each reference are shown in Table 4.1. Once panelists had completed their training, practice samples were provided to them to evaluate. This practicing time lasted 10 to 15 h or until their scores were <10% standard deviation from the known reference intensity values.

4.2.3.3 Product Evaluation

All evaluations were performed in partitioned sensory booths illuminated with cool, natural, fluorescent lights between 10:00 am-11:15 am. A total of 10 sessions were performed to evaluate all emulsion samples over a period of 7 wks. Panelists were advised not to drink, eat, or smoke 1 h prior to the test.

To avoid biases, panelists did not receive any monetary incentive for participation; however, upon completion of the study, all panelists were invited to an appreciation dinner reception to acknowledge their contributions. Unsalted plain crackers and water were provided to cleanse the palate during the evaluation. A 22-cm anchored scale was used to measure the saltiness intensities where 0 = none and 22 = extreme (Kwan 2004). A 15-cm anchored scale was used to measure the bitterness intensities where 0 = none and 15 = extreme. Two replications of each sample were performed for both saltiness and bitterness perceptions. Individual scores were collected and analyzed statistically. The Compusense *five* (Compusense Inc., Guelph, Canada) computerized data collection system was used to develop the questionnaire, and to collect the data.

4.2.4 Design of the Experiment and Statistical Analysis

A randomized complete block design (RCBD with a full factorial treatment arrangement was used to systematically investigate the main effects and interactions of two factors [three levels of tastant concentration as X_1 * three levels of oil concentration as X_2, Table 4.2] on physical characteristics (viscosity and pH), and saltiness and bitterness intensities (considering the panelists as blocks). The experimental results were analyzed using a two-way Analysis of Variance (ANOVA) to determine differences in physical characteristics, and saltiness and bitterness intensities of emulsion systems. The Tukey's studentized range test was performed for *post-hoc* multiple comparisons. For response surface methodology (RSM), descriptive data were fitted with the second order polynomial equation [$\hat{Y} = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$], where $\hat{Y}$ was the predicted response (saltiness or bitterness intensities); b_0 was the value of the fitted response at the center point of the design; b_1 and b_2 were linear regression terms; b_{11} and b_{22} were quadratic regression terms; and b_{12} was the cross-product regression term. Multivariate analysis of variance (MANOVA) was conducted to determine the overall difference among the

emulsion samples considering all sensory and physical characteristics simultaneously. Subsequently, principal component analysis (PCA) was performed to demonstrate the relationship among physical characteristics, taste qualities, and emulsion samples. Statistical Analysis Software® (SAS 2012) at α=0.05 was used for all data analysis.

4.3 Results and Discussion

4.3.1 Viscosity and pH of Emulsions

ANOVA results (Table 4.3) indicated that the tastant type (NaCl, KCl, and/or caffeine), as a main effect, was significant ($P < 0.05$) for viscosity and pH. Oil concentration was a significant main effect for viscosity, while tastant concentration for pH. A significant interaction ($P < 0.05$) was observed between tastant type and oil concentration for viscosity.

Regardless of the type and concentration of tastant (NaCl, KCl, or caffeine), increasing oil concentrations increased viscosities of emulsions (Table 4.4). The emulsion viscosities increased from 269.80-303.00 cP to 587.80-666.00 cP and to 1774.80-2130.40 cP when oil concentration increased from 20% to 40% and to 60%, respectively. Compared to oil concentration, the type and concentration of tastant had lesser effects on viscosity. Only at 60% oil, emulsions with caffeine showed higher viscosities compared to those with NaCl and KCl (2114.00-2130.40 vs. 1774.80-1874.70; Table 4.4). Variations in viscosity may affect taste perception of oil-in-water emulsions (Pripp and others 2004; Smith and others 1996). Moskowitz and Arabie (1970) found that increasing viscosity of solutions decreased the basic taste intensities.

There were significant ($P < 0.05$) variations in pH values depending of the type of tastant in the emulsions. Emulsions with KCl (pH 7.54-8.72) had higher pHs than those with caffeine (pH 5.82-5.98) and NaCl (pH 4.88-5.19) (Table 4.4).

Table 4.3 ANOVA table for saltiness and bitterness perception, and viscosity and pH of oil-in-water emulsions

Effects*	Saltiness		Bitterness		Viscosity (cP)		pH	
	F Value**	Pr > F**	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Tastant type (A)	192.25	<.0001[S]	18.92	<.0001[S]	86.99	<.0001[S]	4687.49	<.0001[S]
% tastant (B(A))***	155.82	<.0001[S]	73.9	<.0001[S]	0.17	0.9827	61.89	<.0001[S]
% oil (C)	53.72	<.0001[S]	3.81	0.0226[S]	14055.7	<.0001[S]	1.99	0.1428
A x C	0.79	0.4565	2.3	0.1016	59.85	<.0001[S]	2.36	0.0599
C x B (A)	1.10	0.3588	0.91	0.5110	0.66	0.7722	0.29	0.9888

*ANOVA, Analysis of variance [2 and/or 3 tastants (NaCl and KCl for saltiness; Caffeine and KCl for bitterness; NaCl, KCl, and caffeine for viscosity and pH); 3 tastant concentrations (0.5, 0.75 and 1.0% for NaCl; 0.5, 1.0 and 1.5% for KCl; 0.05, 0.10 and 0.15% for caffeine); 3 oil concentrations (20, 40 and 60%)].

**F value, Mean square/Mean square error.

***% tastant (B) effect was nested within the tastant type (A) = B(A).

[S] Effects were considered significant when Pr > F was < 0.05.

Table 4.4 Viscosity and pH of oil-in-water emulsions

Tastant	% Oil	% Tastant	Viscosity (cP)*	pH*
NaCl	20%	0.50%	289.50 ± 13.44^{g}	5.19 ± 0.24^{e}
		0.75%	284.00 ± 5.66^{g}	5.17 ± 0.15^{e}
		1.00%	279.90 ± 4.38^{g}	5.07 ± 0.17^{efg}
	40%	0.50%	600.40 ± 42.43^{f}	5.09 ± 0.14^{ef}
		0.75%	595.60 ± 45.82^{f}	5.07 ± 0.18^{efg}
		1.00%	587.80 ± 5.37^{f}	4.90 ± 0.15^{fg}
	60%	0.50%	1774.80 ± 14.71^{d}	5.04 ± 0.10^{efg}
		0.75%	1783.20 ± 28.85^{cd}	4.95 ± 0.20^{fg}
		1.00%	1806.50 ± 24.47^{cd}	4.88 ± 0.12^{g}
KCl	20%	0.50%	269.80 ± 20.08^{g}	7.54 ± 0.24^{c}
		1.00%	288.30 ± 2.12^{g}	8.19 ± 0.20^{b}
		1.50%	284.10 ± 4.38^{g}	8.72 ± 0.40^{a}
	40%	0.50%	632.30 ± 46.24^{ef}	7.63 ± 0.30^{c}
		1.00%	626.60 ± 1.13^{ef}	8.28 ± 0.34^{b}
		1.50%	666.00 ± 30.26^{e}	8.73 ± 0.36^{a}
	60%	0.50%	1845.60 ± 16.40^{bc}	7.64 ± 0.24^{c}
		1.00%	1874.70 ± 25.88^{b}	8.25 ± 0.33^{b}
		1.50%	1807.60 ± 21.21^{cd}	8.68 ± 0.25^{a}
Caffeine	20%	0.05%	294.00 ± 2.83^{g}	5.82 ± 0.06^{d}
		0.10%	295.00 ± 8.20^{g}	5.91 ± 0.09^{d}
		0.15%	303.00 ± 9.90^{g}	5.98 ± 0.01^{d}
	40%	0.05%	635.40 ± 13.58^{ef}	5.91 ± 0.02^{d}
		0.10%	641.30 ± 21.35^{ef}	5.92 ± 0.03^{d}
		0.15%	640.60 ± 14.42^{ef}	5.92 ± 0.02^{d}
	60%	0.05%	2118.50 ± 0.99^{a}	5.87 ± 0.04^{d}
		0.10%	2114.00 ± 97.86^{a}	5.85 ± 0.04^{d}
		0.15%	2130.40 ± 65.62^{a}	5.85 ± 0.01^{d}

*Mean and standard deviation values (N=2). $^{a\text{-}g}$Within each tastant, mean values with the same letter in each column are not significantly different ($P \geq 0.05$).

Tastant concentration had a significant effect on pH. For instance, as the tastant concentration increased, pH of emulsions with NaCl slightly (but not significant) decreased, those with KCl increased, but those with caffeine remained unchanged. For a given tastant, oil

concentration had minimal effects on pH of emulsions. Changes in pH mainly affect the acidity and sour taste of liquid products. Schiffman and others (2000) did not find a significant effect of pH on sweet taste. Fontoin and others (2008) demonstrated that increasing pH values from 2.5 to 4.0 did not have a significant effect on bitter taste in wine model solutions. Research investigating effects of pH on taste perception in emulsion systems is limited and should be further investigated.

4.3.2 Saltiness Intensity of Emulsions

ANOVA results (Table 4.3) indicated that the main effects including tastant type (NaCl and KCl), tastant concentration, and oil concentration were significant ($P < 0.05$) for saltiness intensity. However, none of the interactions (tastant type and oil concentration, and tastant concentration and oil concentration) were significant ($P \geq 0.05$).

The descriptive saltiness mean intensity scores of the emulsion systems are shown in Table 4.5. In general, emulsions with NaCl had higher saltiness intensities compared to those with KCl at a given oil and tastant concentration. For instance, at a fixed 40% oil and 0.5% tastant, saltiness of NaCl = 13.07 vs. KCl = 6.37, and at 60% oil and 1.0% tastant, saltiness of NaCl = 20.63 vs. KCl = 15.22. Moreover, oil concentration had a significant effect ($P < 0.05$) on saltiness intensity. Generally, increasing oil concentrations increased saltiness intensities for a given tastant concentration. For instance, at 0.5% NaCl, saltiness intensities increased from 9.22 to 13.07, and to 14.22 when oil concentration increased from 20%, 40% and to 60%, respectively. Likewise, at 0.5% KCl, saltiness intensities increased from 4.85 to 6.37 and to 7.74 when oil concentration increased from 20%, 40% and to 60%, respectively.

Table 4.6 shows parameter estimates of predictive regression models for saltiness intensities. For NaCl, the linear, quadratic, and cross product terms were significant ($P < 0.05$). However, for KCl, only the linear term was significant.

Table 4.5 Saltiness and bitterness intensities of oil-in-water emulsions

Tastant	% Oil	% Tastant	Saltiness*	Bitterness*
NaCl	20%	0.50%	9.22 ± 4.44^{fg}	-**
		0.75%	15.28 ± 3.17^{d}	-
		1.00%	18.58 ± 1.94^{bc}	-
	40%	0.50%	13.07 ± 4.05^{e}	-
		0.75%	17.95 ± 2.74^{c}	-
		1.00%	20.39 ± 1.54^{ab}	-
	60%	0.50%	14.22 ± 3.97^{de}	-
		0.75%	18.83 ± 2.41^{abc}	-
		1.00%	20.63 ± 1.66^{a}	-
KCl	20%	0.50%	4.85 ± 3.66^{i}	2.40 ± 2.03^{h}
		1.00%	10.02 ± 5.57^{f}	3.91 ± 2.81^{fg}
		1.50%	14.73 ± 4.67^{de}	5.11 ± 3.62^{ef}
	40%	0.50%	6.37 ± 4.76^{hi}	2.83 ± 2.77^{gh}
		1.00%	13.12 ± 4.85^{e}	4.64 ± 2.53^{ef}
		1.50%	17.77 ± 4.55^{c}	6.76 ± 3.28^{cd}
	60%	0.50%	7.74 ± 5.23^{gh}	2.58 ± 2.24^{gh}
		1.00%	15.22 ± 3.73^{d}	5.44 ± 3.00^{de}
		1.50%	19.33 ± 2.76^{abc}	7.51 ± 3.85^{bc}
Caffeine	20%	0.05%	-**	2.53 ± 1.81^{h}
		0.10%	-	5.40 ± 2.45^{de}
		0.15%	-	8.50 ± 2.76^{ab}
	40%	0.05%	-	3.19 ± 2.35^{gh}
		0.10%	-	5.49 ± 3.09^{de}
		0.15%	-	8.28 ± 2.92^{ab}
	60%	0.05%	-	2.70 ± 2.50^{gh}
		0.10%	-	5.27 ± 2.71^{ef}
		0.15%	-	8.94 ± 2.84^{a}

*Mean and standard deviation values (N=2). Values were based on a 22-cm scale for saltiness and 15-cm scale for bitterness. [a-i]Within each tastant, mean values with the same letter in each column are not significantly different ($P \geq 0.05$).
**Not determined.

Table 4.6 Parameter estimates of predictive regression models for saltiness and bitterness intensities in response surface methodology

Taste	Tastant	Regression effects*				Residual*	Parameters estimates**
		Linear	Quadratic	Cross product	Total model	Lack of fit	
		Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	
Saltiness	NaCl	**<.0001**	**<.0001**	**0.0065**	**<0.001**	0.9408	$-17.53+53.36X_1+0.40X_2-21.38X_1X_1-0.15X_1X_2-0.003X_2X_2$
	KCl	**<.0001**	0.1554	0.2818	**<0.001**	0.7129	$-6.13+17.18X_1+0.15X_2-4.0X_1X_1+0.04X_1X_2-0.001X_2X_2$
Bitterness	Caffeine	**<.0001**	0.6205	0.7697	**<0.001**	0.5870	$0.64+30.45X_1+0.02X_2+122.50X_1X_1+0.07X_1X_2-0.00X_2X_2$
	KCl	**<.0001**	0.7359	**0.0338**	**<0.001**	0.9436	$0.28+2.69X_1+0.03X_2-0.53X_1X_1+0.06X_1X_2-0.00X_2X_2$

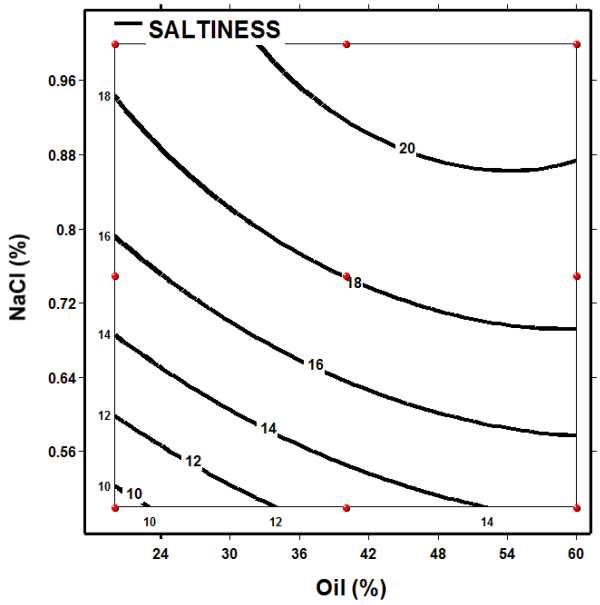
*Effects were considered significant when Pr > F was < 0.05 (Bolded probabilities).

**For saltiness, X_1 = % of NaCl or KCl and X_2 = % of oil; for bitterness, X_1 = % of caffeine or KCl and X_2 = % of oil.

The estimate b_1 for % tastant concentration of NaCl was around 3.1 times higher than that of KCl (53.36 vs. 17.18). This indicated that the magnitude of saltiness intensity in emulsions was about 3.1 times more susceptible to changes in % NaCl concentrations than changes in % KCl concentrations, disregarding the quadratic and cross product terms in the models. The estimate b_2 for % oil concentration ranged from 0.15 to 0.40, indicating that increasing oil concentration increased the saltiness intensities of emulsions. The estimate b_2 for % oil concentration was 2.7 times higher for emulsions with NaCl than with KCl (0.40 vs. 0.15), indicating that the oil concentration had a larger effect on saltiness intensities in emulsions with NaCl than with KCl. The quadratic and cross product terms for emulsions with NaCl were significant (Table 4.6). As shown in Figure 4.1, the effect of oil on saltiness intensities was not linear. In general, oil inserted a larger effect when incorporated at 20 to 40% compared to at 40 to 60% (Figure 4.1). Contrarily, the quadratic and cross product terms for emulsions with KCl were not significant ($P \geq 0.05$; Table 4.6), indicating the linear effects of tastant and oil concentrations. As mentioned above and shown in Figure 4.1, increasing oil concentrations increased the saltiness intensity of emulsions. For instance, to achieve a saltiness intensity of 14, it would require a NaCl concentration of 0.70%, 0.58% and 0.57%, respectively, at 20%, 40% and 60% oil in the emulsions. However, it would require a KCl concentration of 1.43%, 1.09% and 0.93% (Figure 4.1). This indicated that across all emulsion systems (20, 40 or 60% oil), saltiness intensities of emulsions with NaCl were about 1.5-2.0 times higher than those with KCl at any given tastant concentration (Table 4.5; Figure 4.1).

From MANOVA results (Appendix C.f), the variable that explained the majority of the variance among emulsions was viscosity (r = 0.882) in the first canonical discriminant function. In the second canonical discrimination function, pH explained most of the remaining variance among emulsions (r = 0.544).

NaCl: $\hat{Y} = -17.53 + 53.36X_1 + 0.40X_2 - 21.38X_1X_1 - 0.15X_1X_2 - 0.003X_2X_2$ ($R^2 = 0.58$)



KCl: $\hat{Y} = -6.13 + 17.18X_1 + 0.15X_2 - 4.0X_1X_1 + 0.04X_1X_2 - 0.001X_2X_2$ ($R^2 = 0.54$)

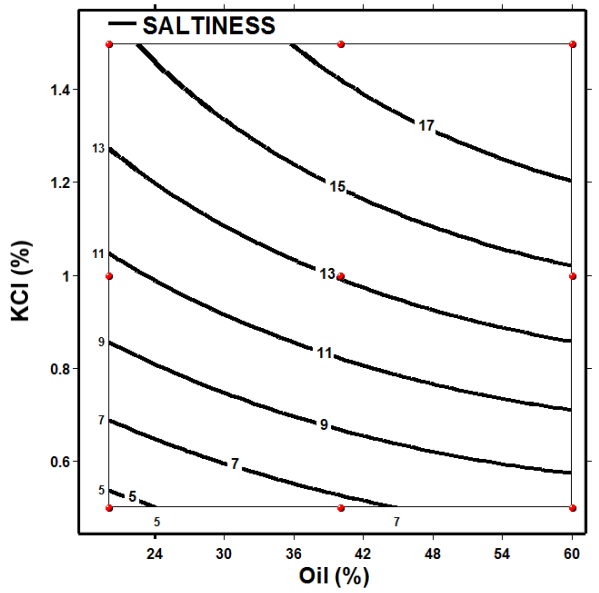


Figure 4.1 RSM contour plots for saltiness intensity* with design points and surface plot for emulsions with NaCl and KCl

*A panel of 16 panelists with two independent replications (N=2). See Table 4.2 for % oil, % NaCl and % KCl in oil-in-water emulsions. NaCl (%) or KCl (%) = X_1 and Oil (%) = X_2.

From PCA results (Figure 4.2), two emulsion groups were clearly distinguishable in PC1. Within PC1, all emulsions with NaCl had higher saltiness intensities compared to those with KCl. Besides, emulsions with higher oil concentrations (60%) showed higher saltiness intensities compared to emulsions with lower oil concentrations (20 to 40%). Additionally, saltiness intensity was positively correlated with viscosity and negatively correlated with pH in the first principal component (PC1; 38.41%). Limited research has been done to investigating the effects of pH on basic taste perception in emulsion systems. Seki and others (1990) found that when the pH of the sample solution was high, the saltiness perception of peptides was weak. In this study, emulsions with KCl (pH 7.54-8.72) had higher pHs than those with NaCl (pH 4.88-5.19) (Table 4.4); this may likely and partially explain the observed greater saltiness intensities of NaCl than KCl in emulsions (Table 4.5). Keast and Breslin (2003) stated that saltiness in solutions was enhanced with the introduction of sourness using a binary system at low and medium intensities. However, these limited researches were done in solution systems and hence further research is needed to investigate the effect of pH and binary interactions on taste perception in oil-in-water emulsions.

Other studies have contrasting conclusions on the effects of oil on saltiness perception. Metcalf and Vickers (2002) found that saltiness intensity was marginally affected by oil in emulsions. Hughes and others (1997) stated that fats and oils as hydrophobic compounds acted as barriers against sodium migration, hence disfavored its release. Moreover, oil can coat the tongue surface and prevent the taste buds from accessing sodium in the oral cavity (Lynch and others 1993). On the other hand, Malone and others (2003) demonstrated that at a given salt (NaCl) concentration, the perceived saltiness increased with increasing oil concentrations.

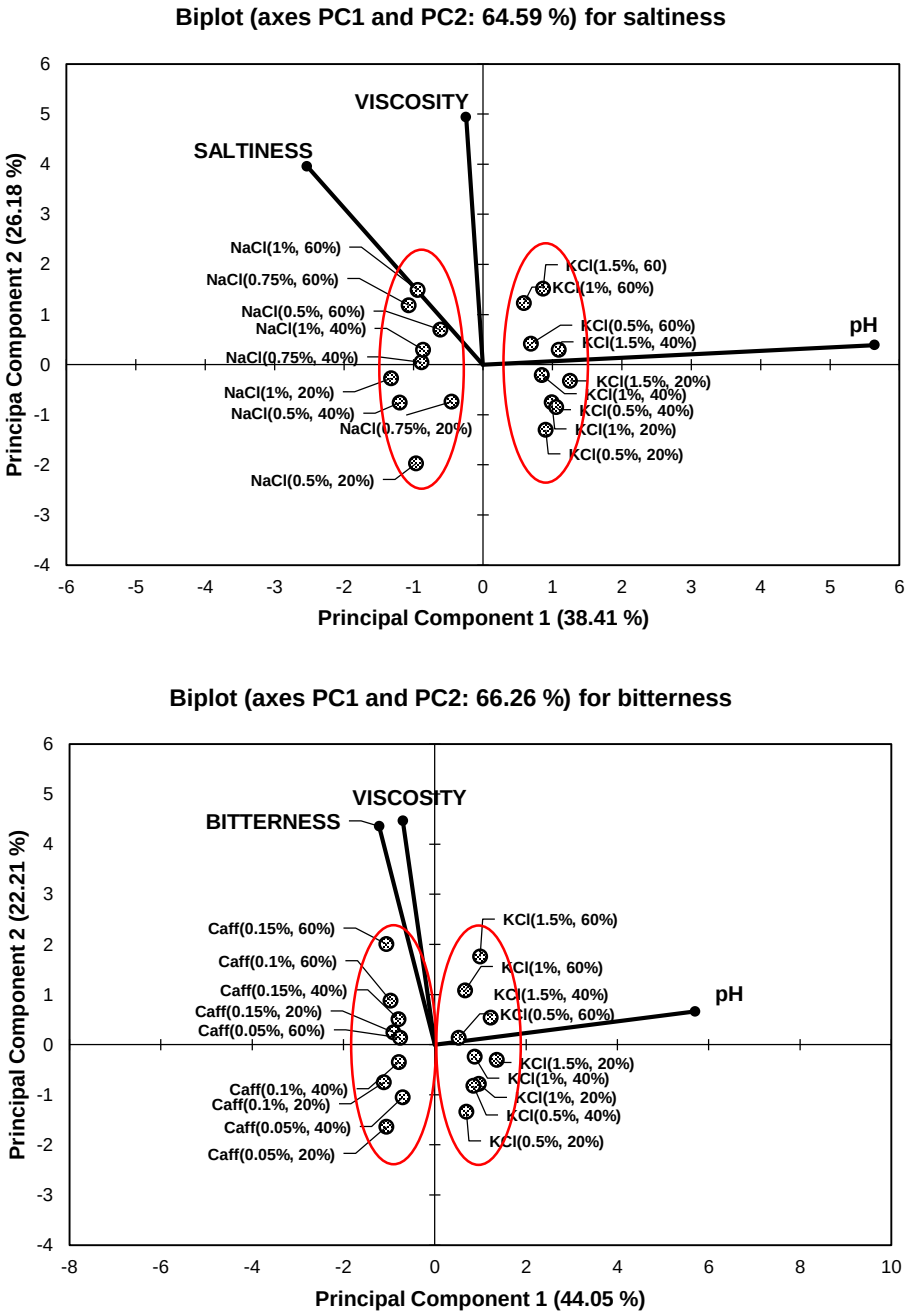


Figure 4.2 Principal component analysis (PCA) product-attribute bi-plot*
A score plot of the first and second principal component (PC1 and PC2) visualizing among emulsions, sensory taste perception (saltiness and/or bitterness) and physical characteristics (viscosity and pH). See Table 4.2 for % oil, % NaCl, % KCl, and % caffeine in oil-in-water emulsions.

Malone and others (2003) proposed that perception of saltiness was dependent on the salt concentration in the aqueous phase and the total aqueous phase volume in the emulsion. Some studies showed that oil/fat components may sensitize the sodium taste receptor cells, resulting in a higher response toward saltiness (Gilbertson and others 2005). Suzuki and others (2014) reported that NaCl saltiness intensity (expressed as a function of the amount of NaCl in the aqueous phase) decreased as an oil phase was introduced in the system due to an emulsion dilution effect. However, they stated that, when expressing saltiness intensity as a function of the amount of NaCl in the entire emulsion, saltiness intensities were significantly higher in emulsions compared to in solution systems; they further concluded that the presence of lipids may enhance saltiness perception, especially at 40% oil in emulsions. In the present study, oil showed a greater effect on saltiness intensities in emulsions with 20 to 39% oil compared to those of emulsions with 40 to 60% oil (Figure 4.1). Moreover, the present study showed drastic differences in the perceived saltiness intensities between NaCl and KCl in emulsion systems, and that oil had a larger effect on saltiness of NaCl than KCl in emulsions (Table 4.5 and Figure 4.1).

4.3.3 Bitterness Intensity of Emulsions

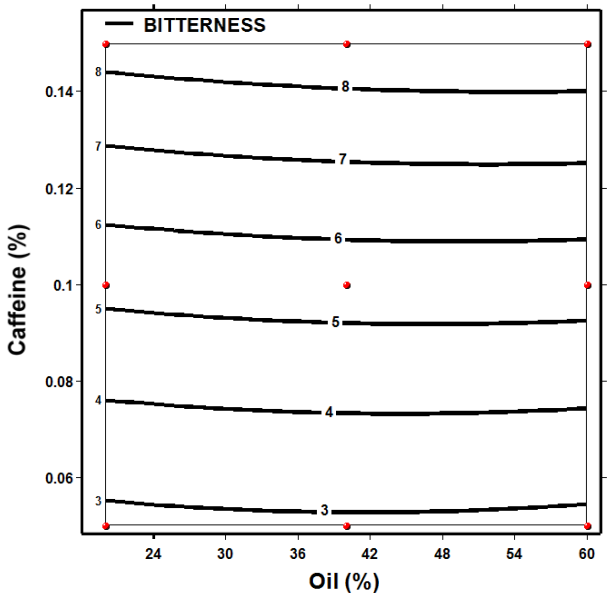
ANOVA results (Table 4.3) indicated that the main effects including tastant type (KCl and/or caffeine), tastant concentration, and oil concentration were significant ($P < 0.05$) for bitterness intensity. However, none of the interactions (tastant type and oil concentration, and tastant concentration and oil concentration) were significant ($P \geq 0.05$). Bitterness intensity mean scores varied from 2.48 to 7.51 in emulsions with KCl (0.5-1.5%), and from 2.53 to 8.94 in emulsions with caffeine (0.05-0.15%) (Table 4.5). Without exception, increasing tastant concentration increased bitterness intensity. Generally, increasing oil concentration slightly increased bitterness intensity at a given tastant concentration with some exceptions. For instance,

at 1.0% KCl, bitterness intensities increased from 3.91 to 4.64 and to 5.44 when oil was incorporated at 20%, 40% and 60%, respectively. However, this increase was not observed in emulsions with caffeine.

Table 4.6 shows parameter estimates of predictive regression models for bitterness intensities. For KCl, the linear and cross product terms were significant ($P < 0.05$). However, for caffeine, only the linear term was significant ($P < 0.05$). The estimate b_1 for tastant concentration of caffeine was about 11.3 times higher than that of KCl (30.45 vs. 2.69). This indicated that a smaller quantity of caffeine (<10 times) would generate a similar magnitude of bitterness intensities compared to a larger quantity of KCl. The positive estimate b_2 for % oil concentration ranged from 0.02 to 0.030, indicating that increasing oil concentration increased the bitterness intensities of emulsions. The estimate b_2 for % oil concentration was 1.5 times higher for emulsions with KCl than with caffeine (0.03 vs. 0.02), indicating that the oil concentration had a larger effect on bitterness intensities in emulsions with KCl than with caffeine. Comparing salty and bitter tastes in emulsions, the oil concentration term (b_2) was more pronounced for saltiness than for bitterness (0.15-0.40 vs. 0.02-0.03, Table 4.6), indicating the larger effect of oil on saltiness than bitterness. For caffeine, the quadratic and cross product regression terms were not significant, indicating linear effects of tastant and oil concentration on bitterness. Contrarily, the significant cross product term was observed for KCl, indicating that increasing KCl concentrations had different effects on bitterness intensities, depending on the oil concentration. These effects can be observed in the RSM contour plots for bitterness of caffeine and KCl in emulsions (Figure 4.3).

Figure 4.3 shows that increasing oil concentration increased the bitterness intensity of KCl in emulsions. For instance, to achieve a bitterness intensity score of 5, it would require a KCl concentration of 1.28%, 0.75% and 0.48% in emulsions with 20%, 40% and 60% oil, respectively.

Caffeine: $\hat{Y} = 0.64 + 30.45X_1 + 0.02X_2 + 122.50X_1X_1 + 0.07X_1X_2 - 0.00X_2X_2$ ($R^2 = 0.45$)



KCl: $\hat{Y} = 0.28 + 2.69X_1 + 0.03X_2 - 0.53X_1X_1 + 0.06X_1X_2 - 0.00X_2X_2$ ($R^2 = 0.26$)

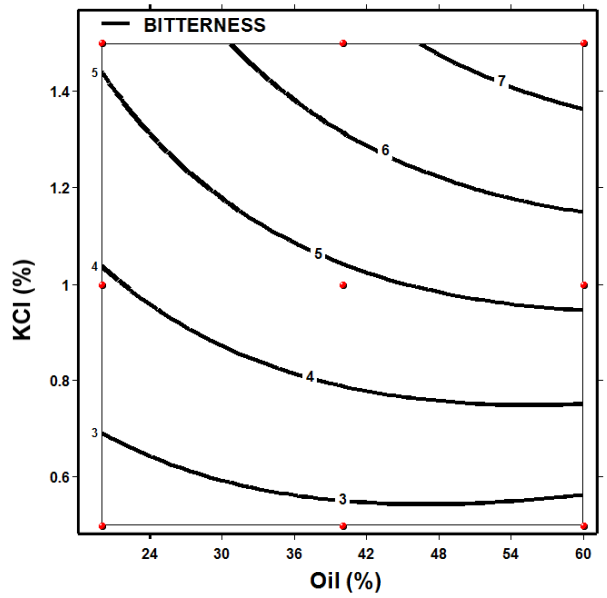


Figure 4.3 RSM contour plots for bitterness intensity* with design points and surface plot for emulsions with NaCl and KCl

*A panel of 16 panelists with two independent replications (N=2). See Table 4.2 for % oil, % caffeine and %KCl in oil-in-water emulsions. Caffeine (%) or KCl (%) = X_1 and Oil (%) = X_2.

This indicated that KCl bitterness intensity was 2.7 higher in emulsion with 60% oil compared to emulsion with 20% oil, at a given KCl concentration. On the other hand, bitterness of caffeine was marginally affected by oil concentrations in emulsions. For instance, to achieve a bitterness intensity score of 5, it would require a caffeine concentration of 0.092%, 0.082%, and 0.073% in emulsions with 20%, 40% and 60% oil, respectively.

From MANOVA results (Appendix C.f), the variable that explained the majority of the variance among emulsions was viscosity (r = 0.921) in the first canonical discriminant function. From PCA results (Figure 4.2), bitterness intensity was positively correlated with viscosity and negatively correlated with pH in the first principal component (PC1; 44.05%). Two emulsion groups were distinguishably separated; those with caffeine vs. those with KCl in PC1. In general, emulsions with higher oil concentrations (60% or 40%) had higher bitterness intensities compared to those of emulsions with lower oil concentrations (20%).

The effect of oil on bitterness perception in emulsions has not been fully elucidated. In general, oil may suppress bitterness in emulsions. Metcalf and Vickers (2002) reported that emulsions with added oil had less bitter taste than those with added water. Koriyama and others (2006) concluded that tuna oil in emulsions suppressed the bitterness perception of quinine sulfate due to a decreased tastant concentration in the aqueous phase. Thurgood and Martini (2010) reported that bitterness intensities were lower in emulsion systems compared to those in aqueous solutions. Contrarily, Keast (2008) stated that as the milk fat content increased from 0% to 4%, the level of caffeine bitterness significantly increased due to interactions of the caffeine molecules with milk proteins and carbohydrates.

Collectively, the suppression of bitterness by oil/fat is not universal and depends on the properties of the molecules responsible. For instance, caffeine is more hydrophilic than quinine,

so its partitioning into a lipid phase would be expected to be less substantial in emulsion systems (Coupland and Hayes 2014). In this current study, oil slightly increased the perceived bitterness in emulsion systems. Although caffeine can reside in the lipid phase and hence its concentration is diluted in the water-phase, its perceived bitterness did not significantly ($P \geq 0.05$) change with increasing oil concentrations (Table 4.5). The opposite effect was observed in emulsions with KCl (a water-soluble molecule), in which the presence of oil likely increased the KCl concentration in the aqueous phase of the emulsion, resulting in an increased perceived bitterness (Table 4.5). This study demonstrated that increasing oil concentrations increased the perceived bitterness intensity, and this effect was more pronounced for KCl than caffeine, under specific conditions evaluated in this study.

4.4 Conclusions

Limited research has been done to understand the taste perception of oil-in-water emulsions. In this study, saltiness of NaCl and KCl, and bitterness of KCl and caffeine were evaluated for oil-in-water emulsions prepared with different oil and tastant concentrations. Results demonstrated that oil had a saltiness-enhancing effect on NaCl and KCl in emulsions, and such effect was more pronounced for NaCl than KCl. Additionally, oil affected saltiness and bitterness in emulsions in a different manner, and such effect was more pronounced for saltiness than bitterness. Oil concentration had a marginal effect on the bitterness perception of caffeine, but the opposite effect was observed with emulsions with KCl in which increasing oil concentration likely increased the perceived bitterness. This study proved that the explanation of the dilution effect imparted by oil in emulsions is inadequate to describe the effect of oil on taste perception. Other mechanisms, including the oil mouth-coating, the sensitizing of taste receptor cells imparted by

oil, the effects of pH and binary or tertiary interactions, may be involved in taste perception, and these effects need to be further investigated.

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CHAPTER 5.
PSYCHOPHYSICAL EFFECTS OF INCREASING OIL CONCENTRATIONS IN SALTINESS AND BITTERNESS PERCEPTIONS OF OIL-IN-WATER EMULSIONS

5.1 Introduction

Salt (sodium chloride) is the most commonly used food additive in the food industry worldwide (Heshmati 2014). High intakes of dietary sodium are associated with cardiovascular diseases, the leading causes of deaths in the United States (CDC 2015). Hence, there are several approaches to reduce sodium in diets including stealth sodium reduction, saltiness potentiation, multisensory applications, physical modification of salt crystals, and utilization of sodium replacements (Kuo and Lee 2014). Potassium chloride (KCl) is a potential salt substitute but it has a drawback of introducing bitterness, metallic aftertaste, and off-taste (Hooge and Chambers 2010; Sinopoli and Lawless 2012). Another approach for reducing sodium is modifying the food matrix properties which have a significant role on the sodium release and saltiness perception (Kuo and Lee 2014; Busch and others 2013; Thurgood and Martini 2010). In liquid products, this approach includes the modification of physical properties such as viscosity, overall salt distribution, and the use of inert fillers that concentrate salt in the aqueous phase (Busch and others 2013), which can also be applied to emulsion systems.

An emulsion is a mixture of two immiscible liquids in which one liquid is dispersed as small spherical droplets (a discontinuous phase) in the other (a continuous phase) (McClement 2005). To our knowledge, most of the food emulsion research has focused on texture and flavor/aroma releases rather than the effect of emulsions on the perception of basic tastes. Oil affects the taste perception by increasing the viscosity of liquid foods and affecting the diffusion coefficients and retention times of taste substances in the oral cavity (Mela and others 1994; Barylko-Pikielna and others 1994). Therefore, sensory perception of basic tastes can be affected

by various physical properties of emulsions (Suzuki and others 2014; Rietberg and others 2012; Dresselhuis and others 2008).

In emulsion systems, the presence of lipids may enhance saltiness perception. Suzuki and others (2014) hypothesized that oil contributes to mouth-coating of the tongue that can delay the "washing out" of the water phase, causing an increased saltiness perception. In food models, Shamil and others (1991-1992) stated that fat increased saltiness perception in salad cream, and decreased bitterness perception in Cheddar cheese. Since NaCl and KCl are water-soluble molecules, they are expected to be fully partitioned in the aqueous phase resulting in increased perceived intensities in emulsions with higher oil concentrations (Kuo and Lee 2014; Koriyama and others 2002). Malone and others (2003) indicated that saltiness perception in emulsion systems was dependent on the concentration of salt in the aqueous phase, the total aqueous phase volume in the emulsion, and the formation of an oily mouth-coating that reduces the mass-transfer between tastants and taste receptors.

In case of bitterness perception, Metcalf and Vickers (2002) reported that emulsions with added fat had less bitter taste and more intense sweetness, saltiness, sourness, and umami taste than those with added water. Pripp and others (2004) concluded that oil had a limited effect on the bitterness reduction of olive oil phenolic compounds. Keast (2008) stated that as the milk fat content increased from 0% to 4%, the level of caffeine bitterness significantly increased, and he attributed this effect to the interaction of caffeine molecules with milk proteins and carbohydrates. Bennett and others (2012) did not find significant differences in the bitterness of ibuprofen among different levels of fat in milk but they reported that changes in the milk viscosity affected the bitterness perception. Therefore, the suppression of bitterness by oil is not universal and depends on the properties of the molecules responsible for the taste perception. For instance, caffeine is

more hydrophilic than quinine, so its partitioning into a lipid phase would be expected to be less substantial in emulsion systems (Coupland and Hayes 2014). Thus, bitterness perception depends on the availability of the bitter molecules to the taste receptor cells.

In measuring human perceived intensities, the most important variable is the concentration of the stimulus. Moskowitz and Arabie (1970) and Stevens (1969) reported that taste intensity follows a power function of the concentration. The Stevens' power law: $\Psi = k\,(\Phi)^n$ is a generally well-established psychophysical expression (Stevens 1969) where Ψ is the response (intensity) to concentration (Φ) of stimuli. In the last decade, several investigations have been dedicated to describe the use of electronic-tongues (E-tongue) in food applications. These devises are considered analytical instruments that reproduce taste sensations (Escuder-Gilabert and Peris 2010). Although there are a number of E-tongue applications related to taste and aroma of foods and beverages, there is limited research investigating basic tastes in emulsion systems.

Suzuki and others (2014) studied the effect of lipid content on human saltiness perception using the Steven's power law. However, they only measured one taste quality (i.e., saltiness) using NaCl. In addition, no research has attempted to investigate saltiness perception of NaCl and/or KCl, and the bitterness perception of caffeine and/or KCl in emulsion systems. Moreover, no research has been done to compare saltiness and/or bitterness perception obtained from human perception vs. E-tongue in emulsion systems. Thus, the objective of this research was to measure saltiness and bitterness intensities of emulsions prepared with different concentrations of oil (0%, 20%, and 40%) and different concentrations of tastants (NaCl, caffeine, and/or KCl) using the Spectrum™ descriptive method and the E-tongue.

5.2 Materials and Methods

5.2.1 Preparation of Solutions and Emulsions

Sodium chloride (NaCl, Morton International, Inc., Chicago, IL, USA), caffeine (caffeine anhydrous 80 mesh, AnMar, Bridgeport, CT, USA), and potassium chloride (KCl, 99% FCC grade, Extracts & Ingredients, Ltd., Union, NJ, USA) solutions were thoroughly dissolved in Ozarka® spring water (Nestlé Waters North America, Greenwich, CT, USA.). Each aqueous solution was poured into 1 L glass bottle and kept at ambient temperature (25 °C). Before serving, 25 mL of solution was poured into plastic cups with lids that were coded with three-digit random numbers. Reference samples were coded with the associated reference intensity values (Table 5.1) and kept at ambient temperature (25 °C) prior to testing.

Table 5.1 Saltiness and bitterness references for the Spectrum™ method

Attribute	Definition	Reference intensity	Preparation Method	% Solution
Saltiness	A fundamental taste of which the taste of sodium chloride in water is typical	7.5	2.25 g NaCl in 500 mL of water	0.45
		10.0	2.75 g NaCl in 500 mL of water	0.55
		12.5	3.10 g NaCl in 500 mL of water	0.63
		18*	5.00 g NaCl in 500 mL of water	1.00
		22*	7.00 g NaCl in 500 mL of water	1.40
Bitterness	A fundamental taste of which the taste of caffeine in water is typical	2.0	0.25 g caffeine in 500 mL of water	0.05
		5.0	0.40 g caffeine in 500 mL of water	0.08

*Source: Kwan (2004).

For preparing the emulsions, one texture modifier and one emulsifier were used. The texture modifier Tic Pretested®Ticaloid®210 S Powder (gum acacia and xanthan gum; Tic Gums®, Inc., White Marsh, MD, USA) was used to increase the viscosity of the aqueous phase of the emulsion. The emulsifier Tandem® 552K (a mixture of mono- and diglycerides, polysorbate, water and proply gallate) was obtained from Caravan® ingredients (Lenexa, KS, USA). A concentration of 1% of Tic gum was mixed with the aqueous phase, and 1% of Tandem emulsifier was mixed with the oil phase. NaCl, KCl, or caffeine were first dissolved in the aqueous portion (water+Tic gum) of the emulsion, and then mixed with canola oil (CWP, Cal Western Packaging Corp., Memphis, TN, USA) and the emulsifier. Final concentrations of each tastant and oil are shown in Table 5.2. Emulsions were mixed for 10 minutes at high-speed using a hand-held blender (Model # 59780R, Hamilton Beach® Brands Canada, Inc., Picton, Onratio, Canada). Each emulsion was poured into 1 L glass bottle and kept at ambient temperature (25 °C) prior to testing. Before serving, 25 mL of emulsion was poured into plastic cups with lids that were coded with three-digit random numbers. Viscosity of emulsions was measured in centipoise (cP) at 20±0.5 °C using a viscometer (model DV-II+, Brookfield Engineering Labs Inc., Middleboro, MA, USA) at 50 rpm using a RV-IV spindle, with data gathered in Wingather V2.1 software (Brookfield Engineering Labs Inc.).Two independent batches for each emulsion were prepared.

5.2.2 Sensory Analysis

5.2.2.1 Panelist Recruitment

The research protocol for this study was approved (IRB# HE 15-9) by the Louisiana State University Agricultural Center Institutional Review Board. Panelists from a pool of faculty, staff, and students at Louisiana State University were recruited and pre-screened using the following criteria: availability, health, general product attitudes, sensory awareness, and rating ability.

Table 5.2 Tastant and oil concentrations used for the Spectrum™ descriptive analysis and E-tongue evaluations

Tastant	Tastant concentration %				
NaCl	0.500	0.625	0.750	0.875	1.000
KCl	0.500	0.750	1.000	1.250	1.500
Caffeine	0.050	0.075	0.100	0.125	0.150
Test	Oil concentration %				
Descriptive test	0	20	40		
E-tongue	0	20			

Panelists were screened with acuity sensory tests in which they had to be able to detect, recognize, and describe sensory characteristics of salty and bitter compounds using NaCl, caffeine, and KCl. Besides, they were tested for ability to evaluate intensities using matching, ranking, and rating tests. Panelists who self-indicated sensory deficits (ageusia and/or anosmia) or kidneys/liver problems were excluded from this study. A panel of sixteen people (N=16) with age ranging from 20 to 30 years was selected to participate in the Spectrum™ method (Sensory Spectrum, New Providence, NJ, USA) for measuring intensities of salty and bitter tastes in solutions and/or emulsion systems.

5.2.2.2 Training and Orientation of Panelists

The training program was required for all panelists to be able to discriminate and quantify the sensory characteristics of products following the Spectrum™ method. The main purposes of training were to ensure an accurate evaluation of the characteristics, and to provide a similar frame of reference in terminology and scaling among all panelists. An initial general orientation session (1 h) was conducted to expose panelists to the underlying technical principles, methodology and terminology of salty and bitter tastes. Following this orientation, six practice sessions (1.5 h each session; 9 h total) were scheduled for reviews of sample references, evaluation procedures and

results. A 15- or 22-cm line scale anchored at the ends with the terms “none” and “extreme” was used, where panelists indicated the perceived intensities by marking a vertical line on the scale. For reference samples, sodium chloride solutions were used for salty references and caffeine solutions were used for bitter references. Reference intensity scores, preparation methods and concentrations of each reference are shown in Table 5.1. Once panelists had completed their training, practice samples were provided to them to evaluate. This practicing time lasted 10 to 15 h or until their scores were <10% standard deviation from the known intensity scale values.

5.2.2.3 Product Evaluation

A total of 12 sessions were performed to evaluate all solution and emulsion samples over a period of 8 wks. All sample evaluations were performed in partitioned sensory booths illuminated with cool, natural, fluorescent lights. Besides, evaluation sessions were conducted at 10:00 am (2 h before the regular lunch time of panelists), and panelists were advised not to drink, eat, or smoke 1 h prior to the test. To avoid biases, panelists did not receive any monetary incentive for participation; however, at the end of the study, all panelists were acknowledged for their contributions at an appreciation dinner reception. Unsalted, plain crackers and water were provided to cleanse the palate during the evaluation. A 15-cm anchored scale was used to measure the bitterness intensities where 0 = none and 15 = extreme. A 22-cm anchored scale was used to measure the saltiness intensities where 0 = none and 22 = extreme (Kwan 2004). Two replications of each sample were performed for both saltiness and bitterness perceptions. Individual scores were collected and analyzed statistically. The Compusense *five* (Compusense Inc., Guelph, Canada) computerized data collection system was used to develop the questionnaire, and to collect the data.

5.2.3 Taste Analysis Using the Electronic-Tongue (E-tongue)

To compare the descriptive panel and the E-tongue analysis, the same tastant (NaCl, caffeine, or KCl) concentrations of solutions and 20% oil emulsions tested for human evaluations were measured using an α-Astree II electronic tongue (Alpha M.O.S. Co., Toulouse, France). Data were collected from two liquid cross-selective sensors. Each sensor had a specific organic membrane that could produce a response to salty and bitter taste qualities. Any interactions at the membrane interface were detected by the sensor and converted into an electronic signal. Electrodes were dipped in a 75 mL sample for 120 sec, and data were recorded using the Alpha M.O.S. software. Three measurements were taken from each of the two independent replications of the solution and emulsion systems. Emulsions with 40% oil were not measured since their viscosities were above the recommended specifications for the sensors.

5.2.4 Design of the Experiment and Statistical Analysis

A randomized complete block design (RCBD), considering the panelists as blocks, with a full factorial treatment arrangement was used to systematically investigate the main effects and interactions of two emulsion factors [five levels of tastant concentration by three levels of oil concentration (0, 20 and 40%) for the descriptive panel evaluation and five levels of tastant concentration by two levels of oil concentration (0 and 20%) for the E-tongue evaluation] on the saltiness and bitterness intensities (Table 5.2). The independent variable was the concentration of tastant. The dependent variables were either saltiness or bitterness intensities. The experimental results of the RCBD with a full factorial treatment arrangement were analyzed using a two-way Analysis of variance (ANOVA) to determine differences in saltiness and bitterness intensities of solution and emulsion systems. Data from the descriptive panel and E-tongue evaluations of each oil concentration were fitted using linear regressions as in [*Intensity = Intercept + Slope*

(Concentration of tastant)], where *Intensity* referred to either saltiness or bitterness intensity values; *Concentration of tastant* referred to the concentration of NaCl, KCl, or caffeine in the solution or emulsion systems; *Intercept* was the *Intensity* value when the *Concentration of tastant* was 0; *Slope* was the rate of change of the *Intensity* as the *Concentration of tastant* changed by one unit.

Data from the descriptive panel were also fitted using the Stevens' power functions [$\Psi = k\,(\Phi)^n$], where Ψ was the response (intensity) to stimuli of concentration (Φ). The constant k was a scaling constant that reconciled the units used to measure Ψ and Φ, whereas the constant n was a measure of the growth rate of the perceived intensity as a function of the stimulus concentration (Moskowitz and Arabie 1970; Meilgaard and others 2006; Suzuki and others 2014). Coefficients of determination of the regression models (R^2) were also obtained. Analysis of Covariance (ANCOVA) was used to evaluate differences between solution and emulsion systems for the fitted linear and Steven's power models. Statistical Analysis Software® (SAS 2012) at α=0.05 was used for the regression analyses of the experimental data.

5.3 Results and Discussion

5.3.1 Saltiness Perception Using Descriptive Panel

The estimated parameters for saltiness intensities of NaCl and/or KCl solutions and oil-in-water emulsions (20 and/or 40% of oil) using linear regressions and the Stevens' power law are shown in Table 5.3. For saltiness linear regression, data were fitted with R^2 values of 0.88-0.94 for NaCl, and 0.91-0.94 for KCl solutions or emulsions. In general, estimated linear slopes for NaCl systems were higher compared to those of KCl (23.78 vs. 15.47, 18.19 vs. 14.19, and 15.68 vs. 13.40 for 0, 20, and 40% oil systems, respectively).

Table 5.3 Parameters for saltiness and bitterness intensities of oil-in-water emulsions using linear regression and the Stevens' power law

Type of perception	Type of tastant	Type of regression	Estimated parameters*	Oil concentration (%)		
				0**	20**	40**
Saltiness	NaCl	Linear Regression	Intercept	***-4.98***^{B}	1.80^{A}	***5.35***^{A}
			Slope	***23.78***^{A}	***18.19***^{AB}	***15.68***^{B}
			R^2	0.94	0.82	0.88
		Stevens' Power Law	Log k	***1.27***^{A}	***1.31***^{A}	***1.32***^{A}
			n	***1.40***^{A}	***0.96***^{B}	***0.68***^{B}
			R^2	0.92	0.84	0.86
	KCl	Linear Regression	Intercept	***-5.70***^{B}	-2.61^{AB}	-0.05^{A}
			Slope	***15.47***^{A}	***14.19***^{A}	***13.40***^{A}
			R^2	0.91	0.92	0.94
		Stevens' Power Law	Log k	***0.95***^{B}	***1.05***^{A}	***1.12***^{A}
			n	***1.51***^{A}	***1.28***^{A}	***1.14***^{A}
			R^2	0.87	0.94	0.93
Bitterness	Caffeine	Linear Regression	Intercept	0.89^{A}	***-1.71***^{B}	***-1.52***^{B}
			Slope	***62.78***^{A}	***72.22***^{A}	***65.94***^{A}
			R^2	0.92	0.94	0.94
		Stevens' Power Law	Log k	***1.72***^{B}	***2.08***^{A}	***1.94***^{AB}
			n	***0.87***^{B}	***1.36***^{A}	***1.25***^{A}
			R^2	0.92	0.95	0.92
	KCl	Linear Regression	Intercept	1.00^{A}	0.79^{A}	-0.09^{A}
			Slope	***3.38***^{A}	***3.32***^{A}	***4.12***^{A}
			R^2	0.83	0.79	0.90
		Stevens' Power Law	Log k	***0.64***^{A}	***0.61***^{A}	***0.60***^{A}
			n	***0.68***^{A}	***0.87***^{A}	***1.00***^{A}
			R^2	0.73	0.86	0.94

*Data points were fitted using a linear regression model: Intensity = Intercept + Slope (Concentration of tastant), and the Stevens' power function: $\log(\Psi) = \log k + n * \log(\Phi)$, where Ψ is the response (intensity) to stimuli of concentration (Φ). R^2 is the coefficient of determination of the regression models.

**Bold italicized values indicate that the parameter was significantly different from 0 ($P < 0.05$).

$^{A-B}$Parameter values with the same letter in each row are not significantly different ($P \geq 0.05$).

This indicated that for a given change in concentration of either NaCl or KCl, the change in saltiness intensity was higher for NaCl compared to that of KCl. Besides, the estimated NaCl linear slopes decreased significantly ($P < 0.05$) with increasing oil concentrations (from 23.78 at 0% oil to 15.68 at 40% oil). This indicates that the addition of oil in the systems reduced the rate of change in saltiness intensities imparted by NaCl. For instance, doubling NaCl concentration from 0.5 to 1.0% would produce a greater change (1.30-1.50 times higher) in saltiness perception in solutions compared to that in emulsion systems. On the other hand, the estimated KCl linear slopes decreased slightly but not significantly ($P \geq 0.05$) with increasing oil concentrations (15.47 at 0% oil to 13.40 at 40% oil).

Data fitted using the Stevens' power law showed R^2 values of 0.84-0.92 for NaCl and 0.87-0.94 for KCl solutions and/or emulsions (Table 5.3). In general, the estimated exponent *n* values were higher for KCl systems compared to those of NaCl systems (1.51 vs. 1.40, 1.28 vs. 0.96, 1.14 vs. 0.68 for 0, 20, and 40% oil systems, respectively). The exponent *n* parameter determines the type of response obtained for a specific stimulus; for instance, if *n* is greater than 1.0, the response toward a specific stimulus accelerates with concentration, whereas if *n* is lower than 1.0, the response decelerates with concentration (Shallenberger 1993). If the *n* exponent appears to be 1.0, the intensity of the taste is linearly related to the stimulus concentration (Moskowitz and Arabie 1970). The intercept *k* may change from experiment to experiment without affecting the exponent (Stevens 1969). For the present study, the estimated *n* indicated that oil had a decelerating effect (n = 0.68-0.96) on the saltiness perception of NaCl. However, oil imparted an accelerating effect (n = 1.14-1.28) on the saltiness perception of KCl (Table 5.3). These findings indicated profound differences between NaCl and KCl in terms of the perceived saltiness intensity in oil-in-water emulsions. Compared with NaCl, KCl demonstrated to be less susceptible to the taste decelerating

effect imparted by oil, i.e., the effects of oil on the saltiness perception of NaCl were larger compared to that of KCl, which substantiated the results of the linear regression mentioned above.

Suzuki and others (2014) concluded that the response toward NaCl saltiness intensity decreased as an oil phase was introduced in the system. They reported estimated *n* values of 0.87, 0.66, and 0.47 for 0, 20, and 40% oil systems, respectively. Differences in results reported by Suzuki and others (2014) and in the present study could be attributed to the different methods of scaling used (magnitude estimation rating vs. linear scale rating), and the different experimental conditions for preparing the emulsions. Moskowitz and Arabie (1970) stated that the estimated *n* for saltiness of solutions was approximately 1.40 (similar to the value found in the present study), suggesting that the perceived saltiness intensity increased as a positively accelerating function of concentration. Moreover, Moskowitz and Arabie (1970) found that the rate of saltiness intensity perception was diminished when the apparent solvent viscosity increased (from 1 to 1000 cP). Viscosities in the present study increased from 1.0 cP for 0% oil to 280-290 cP for 20% oil, and to 588-600 cP for 40% oil in NaCl systems. Thus, the decelerating effect in salty taste imparted by oil was partially due to the increased viscosity of the emulsion systems. Hughes and others (1997) stated that fats and/or oils as hydrophobic compounds acted as barriers against sodium migration, hence disfavor its release. Moreover, oil was found to coat the tongue surface, thus preventing the taste buds from accessing sodium in the oral cavity (Lynch and others 1993).

For the present study, linear model approximations (R^2 = 0.82-0.94) fitted the data as closely as power function models (R^2 = 0.84-0.94). Hence, both models may explain the behavior of taste intensities across the tested tastant concentrations. However, when using linear models, making conclusion outside the range of concentrations tested can be misleading since the relationship between perceived intensity and tastant concentration may not be linear outside this

range and extrapolation can lead to errors in prediction. Comparisons of different systems (0, 20, and/or 40% oil) on the saltiness perception of NaCl and KCl are shown in Figure 5.1. For both salts, increasing oil concentrations increased saltiness intensities at various salt concentrations. However, the effect of oil on saltiness perception decreased with simultaneously increasing oil and salt concentrations; this effect was more obvious for NaCl than KCl. Moreover, at a given salt concentration, saltiness intensities were higher for NaCl than for KCl regardless the systems used (0, 20, and/or 40% oil; Figure 5.1). For instance, 0.75% NaCl vs. 0.75% KCl had saltiness intensity values of 18.50 vs. 11.19, 15.72 vs. 8.25, and 12.29 vs. 4.20 for 0, 20, and 40% oil systems, respectively (Figure 5.1).

For a practical use, a table of saltiness equivalence was created to demonstrate concentrations of KCl and NaCl (only emulsions) systems needed to achieve similar saltiness intensities of NaCl in water solutions (Table 5.4). This table was created using the linear regression models established in Table 5.3 for solution and emulsion systems. For instance, to achieve a saltiness intensity similar to 0.50% NaCl in solutions, it would require a concentration of 0.82% KCl in solutions. In the same manner, to achieve a saltiness intensity similar to 1.00% NaCl in solutions, it would require a concentration of 1.41% KCl in 40% oil emulsion systems. However, considerations that KCl imparts bitterness and metallic aftertaste must be taken into account when formulating a sodium-reduced product.

5.3.2 Bitterness Perception Using Descriptive Panel

The estimated parameters for bitterness intensity of caffeine or KCl in solutions and oil-in-water emulsions (20 and/or 40% of oil) using a linear regression and the Stevens' power law are shown in Table 5.3. Data fitted using a linear regression showed R^2 values of 0.92-0.94 for caffeine, and 0.83-0.90 for KCl solutions and/or emulsions.

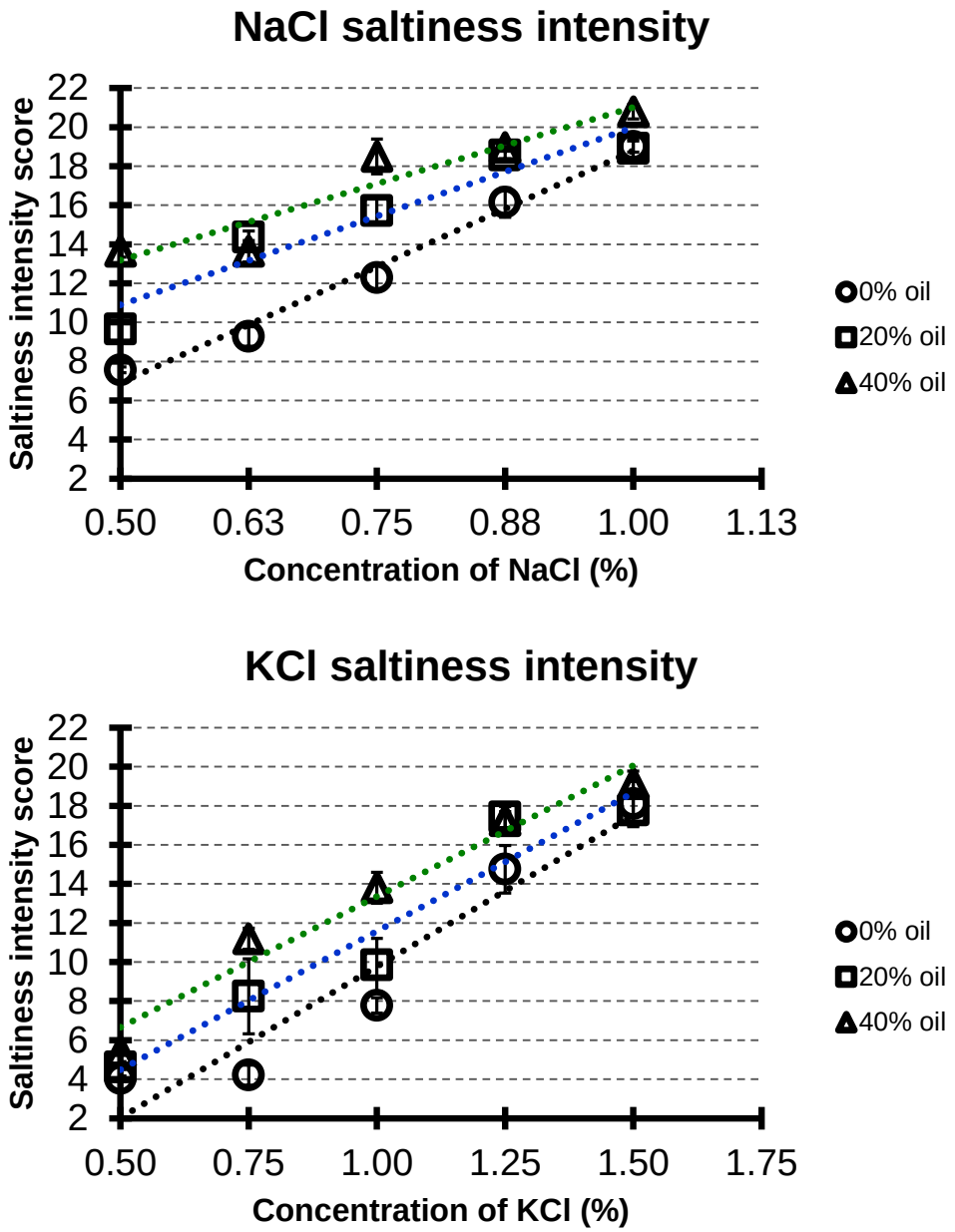

Figure 5.1 Effects of oil concentration on saltiness intensity* imparted by NaCl and KCl in oil-in-water emulsions

*Values represent the means and standard deviations of two replicates. A total of (N=16) trained panelists were used. Data points were fitted using a linear regression model: Intensity = Intercept + Slope (Concentration of tastant).

Table 5.4 Saltiness intensity equivalence between NaCl and KCl concentrations based on the Spectrum^{TM} descriptive panel

Type of system		NaCl solutions (%)								
		0.50	0.56	0.63	0.69	0.75	0.81	0.88	0.94	1.00
KCl (%)*	Solutions (0% oil)	0.82	0.91	1.01	1.1	1.2	1.3	1.39	1.49	1.58
	Emulsions (20% oil)	0.67	0.78	0.88	0.98	1.09	1.19	1.30	1.40	1.51
	Emulsions (40% oil)	0.52	0.63	0.74	0.85	0.96	1.07	1.18	1.30	1.41
NaCl (%)*	Emulsions (20% oil)	0.28	0.36	0.44	0.53	0.61	0.69	0.77	0.85	0.93
	Emulsions (40% oil)	0.10	0.19	0.29	0.38	0.48	0.57	0.67	0.76	0.86

*Values represent concentrations (%) of KCl or NaCl equivalent to concentrations of NaCl in solutions in terms of saltiness intensity for solutions and oil-in-water emulsion systems. Data points were fitted using a linear regression model: Intensity = Intercept + Slope (Concentration of tastant) from Table 5.3.

As expected, estimated linear slopes for caffeine systems were much higher compared to those of KCl (62.78 vs. 3.38, 72.22 vs. 3.32, and 65.94 vs. 4.12 for 0, 20, and 40% oil systems, respectively). This indicated that a smaller concentration of caffeine (in the range of 0.05 to 0.15%) had a much higher effect on bitterness intensity compared to a greater concentration of KCl (in the range of 0.50 to 1.50%). For caffeine, estimated linear slopes for emulsion systems were higher but not significant ($P \geq 0.05$) compared to that of the solution (65.94-72.22 vs. 62.78; Table 5.3). Similarly for KCl, emulsion slopes were not significantly ($P \geq 0.05$) different compared to that of the solution (3.32-4.12; Table 5.3). For caffeine, higher linear slopes indicated that the addition of oil in the systems increased the rate of change in bitterness intensities.

Data fitted using the Stevens' power law showed R^2 values of 0.92-0.95 for caffeine and 0.73-0.94 for KCl solutions or emulsions (Table 5.3). In general, the estimated *n* values were higher for caffeine systems compared to those of KCl systems (0.87 vs. 0.68, 1.36 vs. 0.87, 1.25 vs. 1.00 for 0, 20, and 40% oil systems, respectively). The estimated *n* values showed that oil had an accelerating effect (n = 1.25-1.36) on the bitterness perception of caffeine (n of solution = 0.87). For the KCl, the estimated *n* values of solution (0.68) and emulsion systems (0.87-1.00) were not significantly different ($P \geq 0.05$).

Comparisons of different emulsion systems (0, 20, and/or 40% oil) on bitterness perception of caffeine and KCl are shown in Figure 5.2. Generally, as concentration of tastant (caffeine and/or KCl) increased, the bitterness intensity increased. For caffeine, 20% and 40% oil emulsions had lower bitterness intensities compared to those of the solution regardless of caffeine concentrations (Figure 5.2); however, bitterness intensities were not significant different between 20% and 40% oil emulsions ($P \geq 0.05$). For instance, the solution had a bitterness intensity of 7.30 compared to 4.78-4.92 for emulsions at 0.10% caffeine.

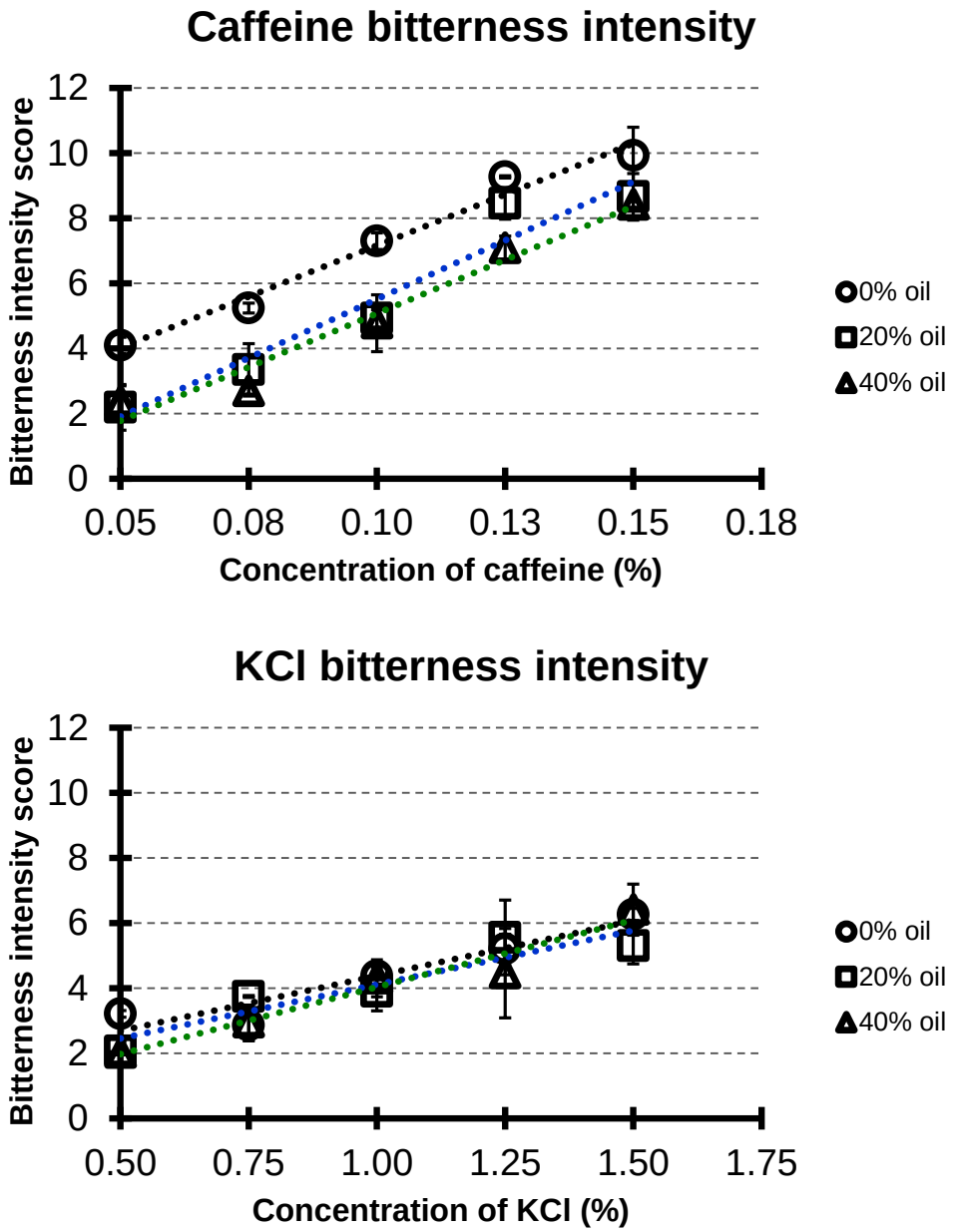

Figure 5.2 Effects of oil concentration on bitterness intensity* imparted by caffeine and KCl in oil-in-water emulsions

*Values represent the means and standard deviations of two replicates. A total of (N=16) trained panelists were used. Data points were fitted using a linear regression model: Intensity = Intercept + Slope (Concentration of tastant).

Metcalf and Vickers (2002) reported that samples with added oil had less bitter taste and more intense saltiness than those with added water. They concluded that bitter compounds are hydrophobic and can reside in lipophilic environments; therefore, oil in oil-in-water emulsions may suppress bitterness via a dilution effect of bitter compounds in the water-phase of the emulsions. Thurgood and Martini (2010) reported that intensities of sour and bitter tastes were lower in emulsion systems compared to aqueous solutions. Pripp and others (2004) concluded that oil had a limited effect on bitterness-reduction of olive oil phenolic compounds. Moskowitz and Arabie (1970) reported an estimated *n* bitterness parameter of 0.49, 0.58, and 0.64 for 1, 100, and 1000 cP quinine solutions, respectively. Moreover, they stated that bitterness intensities decreased with increased viscosities (1-1000 cP). Viscosity values of emulsions in the present study increased from 1.0 cP for 0% oil to 294-303 cP for 20% oil, and to 635-641 cP for 40% oil in caffeine systems. Thus, increasing the viscosity of the emulsion systems could have contributed to changing the growth rate (*n*) of bitterness intensities for caffeine.

For KCl, no significant differences were found ($P \geq 0.05$) between the solution and two emulsion systems (20% and 40% oil) at a given tastant concentration. The bitterness intensity, however, increased from 2.02-3.21 at 0.50% KCl to 5.29-6.41 at 1.50% KCl for all systems (Figure 5.2). These results indicated that oil had a bitterness-suppressing effect for caffeine but not for KCl at the concentrations (0.5-1.5%) and conditions evaluated in this study. Moreover, these results demonstrated that bitterness in emulsion systems was dependent on the type of molecule used. Since partial oil-soluble compounds such as caffeine can be diluted in emulsion systems, the bitterness intensities were lower compared to that of solutions (Goldstein 2001). Because KCl is 100% water-soluble, it did not show that dilution effect in our present study.

Collectively based on Table 5.3 and Figures 5.1 and 5.2, opposite effects for saltiness and bitterness perception imparted by oil were found based on the power functions. In general, oil decelerated saltiness intensity for NaCl but accelerated saltiness intensity for KCl while it accelerated bitterness intensity for caffeine in emulsion systems. Moreover, saltiness intensities in emulsions were higher compared to those in solutions, demonstrating a saltiness-enhancing effect of oil. Bitterness intensities in emulsions were lower compared to those in solutions for caffeine but they were similar for KCl. This demonstrated a bitterness-suppressing effect for caffeine but not for KCl imparted by oil.

5.3.3 Saltiness and Bitterness Measured by the E-tongue

Saltiness and bitterness intensities of NaCl, KCl, and caffeine for solutions and 20% oil emulsions measured by the E-tongue are shown in Figures 5.3 and 5.4. Similar to the descriptive panel results, 20% oil emulsions had higher saltiness intensities compared to those of the solutions for both NaCl and KCl (Figure 5.3). For instance, the 0.75% NaCl - 20% oil emulsion had an intensity value of 1396.4 compared to 1187.0 for the 0.75% NaCl solution. On the other hand, the 0.75% KCl - 20% oil emulsion had an intensity value of 1246.4 compared to 1190.5 for the 0.75% KCl solution. Figure 5.3 also shows that when the concentration of either tastant (NaCl and/or KCl) increased, the differences in saltiness intensity between solutions and 20% oil emulsions generally increased. This effect was opposite of that observed in the descriptive panel results in which the differences in saltiness intensity between solutions and emulsions decreased as the concentration of tastant increased.

Similar to the descriptive panel results, 20% oil emulsions had lower bitterness intensities compared to those of the solutions for caffeine (Figure 5.4). For instance, at 0.10% caffeine, the 20% oil emulsion had an intensity value of 1486.3 compared to 1923.3 for the solution.

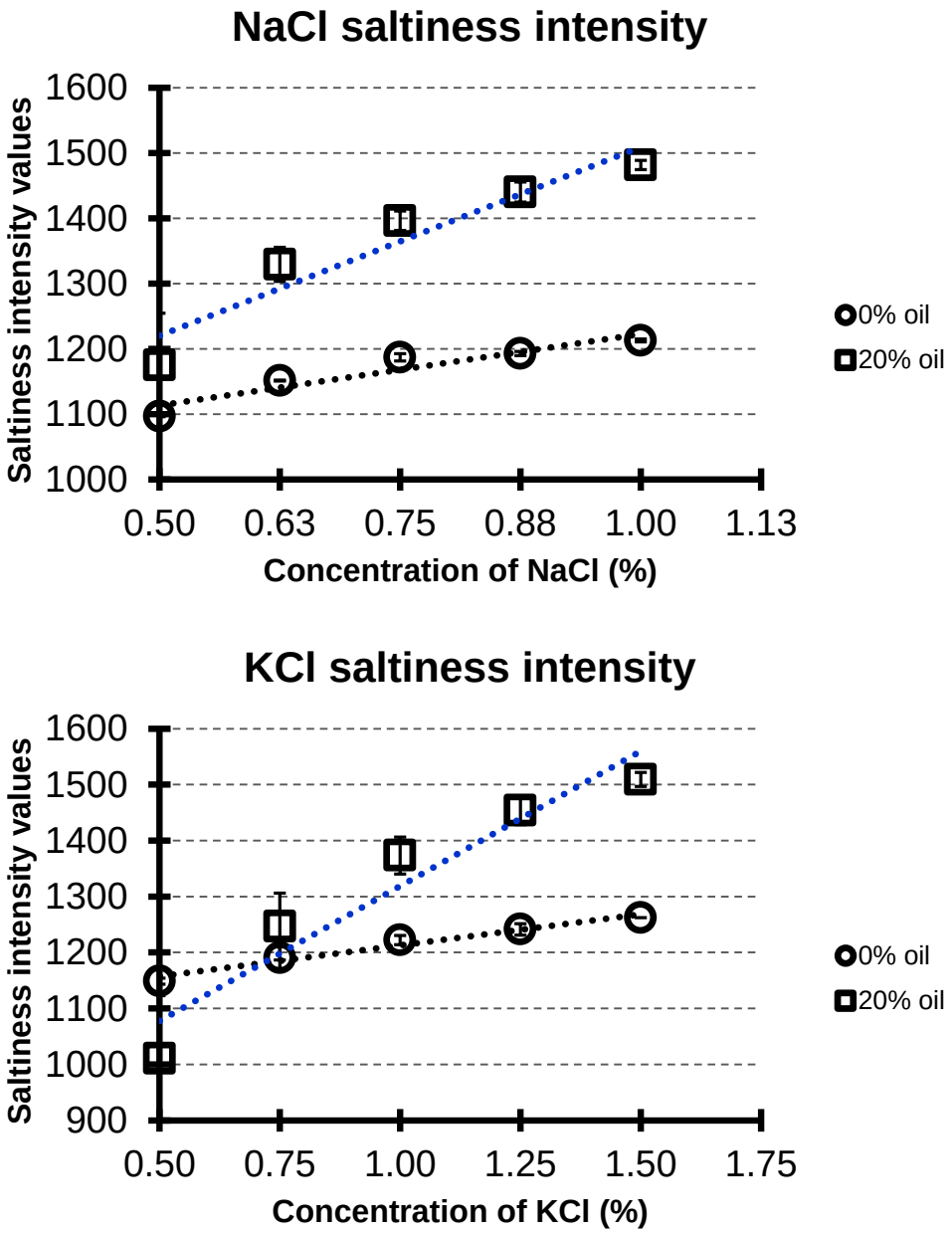

Figure 5.3 Effects of oil concentration on saltiness intensity* imparted by NaCl and KCl in oil-in-water emulsions using the E-tongue

*Values represent the means and standard deviations of two replicates. Intensities were obtained using the E-tongue (ASTREE). Data points were fitted using a linear regression model: Intensity = Intercept + Slope (Concentration of tastant).

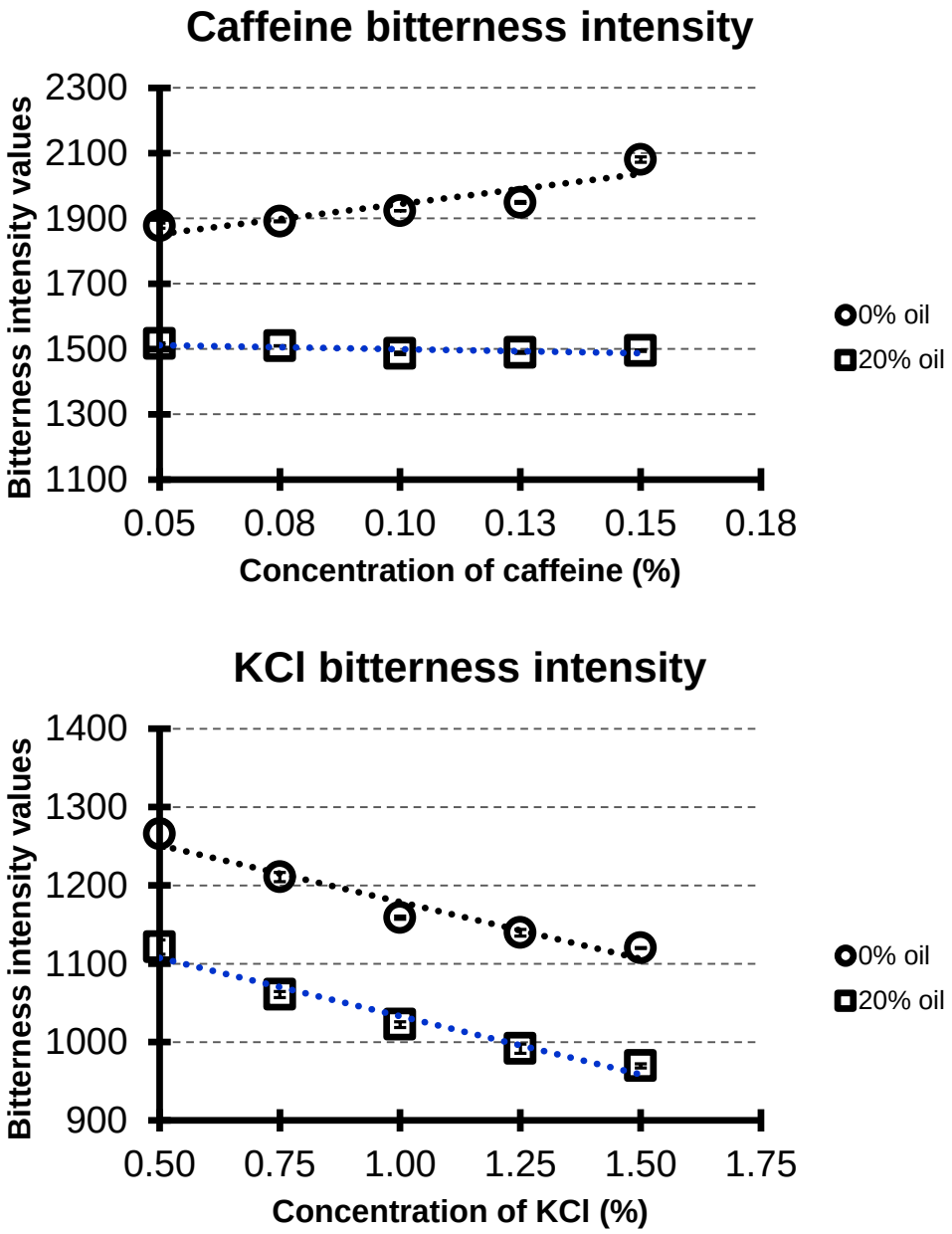

Figure 5.4 Effects of oil concentration on bitterness intensity* imparted by caffeine and KCl in oil-in-water emulsions using the E-tongue

*Values represent the means and standard deviations of two replicates. Intensities were obtained using the E-tongue (ASTREE). Data points were fitted using a linear regression model: Intensity = Intercept + Slope (Concentration of tastant).

Interestingly, while the bitterness intensity of the solution increased with increasing caffeine concentrations, the bitterness intensity of the emulsion remained somewhat constant (Figure 5.4). For KCl bitterness, results from the E-tongue were somewhat different compared to those of the descriptive panel. Although solutions had higher bitterness intensities compared to 20% oil emulsions, increasing concentrations of the tastant yielded lower bitterness intensities for both systems. Bitterness intensity decreased from 1265.7 at 0.50% KCl to 1120.0 at 1.50% KCl for solutions, and from 1121.1 at 0.50% KCl to 969.5 at 1.50% KCl for 20% oil emulsions.

Table 5.5 shows the Pearson's correlation coefficients (*r*'s) between the trained descriptive panel (Humans) and instrumental (E-tongue) in terms of the measured saltiness and bitterness intensities. For the saltiness perception, significant positive correlations (0.88-0.90; $P < 0.05$) were found between the descriptive panel and the E-tongue regardless of tastant and oil concentration. That was not the case for bitterness perception. For caffeine, a significant positive correlation (0.82; $P < 0.05$) was found for the solution. However, for the 20% oil emulsion, a significant negative correlation (-0.72; $P < 0.05$) was found between the human perception and the E-tongue. For KCl, significant negative correlations (-0.86 for 0% and -0.92 for 20% oil systems) were found.

Electronic-tongues use taste sensors that detect changes in the electrical potential of lipid/polymer membranes caused by the physicochemical interaction between the membranes and chemical substances (Tahara and Toko 2013). Potentiometric devices consist of ion-selective electrodes that are largely dependent on the aqueous medium in which they are immersed. This can explain the lower intensity readings observed in emulsion systems compared to solutions in the present study. However, human taste involves a more complex procedure that not only includes signals coming from the taste receptor cells but other senses that contribute as well to the taste sensation such as smell, touch, texture, sight and temperature (Cosio and others 2012).

Table 5.5 Pearson's correlation coefficients (*r*'s) between the trained descriptive panel (Humans) vs. instrumental measurement (E-tongue) in terms of the measured saltiness and bitterness intensities

Perception	Tastant	Oil Concentration (%)	Human vs. E-tongue correlation (*r*'s)*
Saltiness	NaCl	0	0.88
		20	0.90
	KCl	0	0.89
		20	0.89
Bitterness	Caffeine	0	0.82
		20	-0.72
	KCl	0	-0.86
		20	-0.92

*All correlation coefficients are significant ($P < 0.05$) for the null hypothesis (Ho): r = zero.

Moreover, binary and tertiary interactions could be involved in the taste perception of solution and oil-in-water emulsion systems. More studies are needed to confirm this finding regarding the relationship between the E-tongue and descriptive panels, particularly related to saltiness and bitterness perceptions.

5.4 Conclusions

This study demonstrated the psychophysical effects of oil on saltiness and bitterness perceptions in oil-in-water emulsions. Although the rate of growth (n) for saltiness in oil-in-water emulsions was lower compared to that of solutions, saltiness intensity values in emulsions were higher than in solutions, demonstrating a saltiness enhancing effect imparted by oil. For bitterness, intensities in emulsions were lower compared to those in solutions for caffeine but they were similar for KCl. This demonstrated that oil exhibited a bitterness-suppressing effect for caffeine but not for KCl. Saltiness intensities measured by the Electronic-tongue were congruent with the descriptive panel results. On the other hand, bitterness intensities of KCl showed an opposite pattern between the two methods.

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CHAPTER 6.
SUMMARY AND CONCLUSIONS

Salt (NaCl) is the most commonly used food additive in the food industry worldwide. Although sodium is vital in cells osmotic balance of the human body, diets in the US have overpassed the recommended daily amounts of sodium. High sodium consumption is a major contributor to high blood pressure which is a leading cause of stroke, coronary heart diseases, heart attack, and kidney failure. However, reducing sodium has proven to be a difficult task since salt (NaCl), the major contributor of sodium, not only plays an important role in taste, but also is used for preservation, structuring and other food functional purposes. Existing approaches to reduce sodium include stealth sodium reduction, saltiness potentiation, multisensory applications, physical modification of salt crystals, and utilization of sodium replacements. Potassium chloride (KCl) is a potential salt substitute but it has a drawback of imparting bitterness, metallic aftertaste, and off-taste. Another approach for reducing sodium is modifying the food matrix properties which have a significant role on the sodium release and saltiness perception. In liquid products, this approach includes the modification of physical properties such as viscosity, overall salt distribution, and the use of inert fillers that concentrate salt in the aqueous phase, which can also be applied to emulsion systems. To our knowledge, most of the food emulsion research has focused on texture and flavor/aroma releases rather than the effect of emulsions on the perception of basic tastes.

The aim of the present research was to study the saltiness and bitterness perceptions of oil-in-water emulsion systems. Three experiments were conducted that consisted of (I) determining the detection and recognition thresholds of salty and bitter tastes in aqueous solutions and oil-in-water emulsion systems, (II) characterizing the effects of concentrations of tastant and oil on the

saltiness and bitterness intensities in emulsions, and (III) studying the psychophysical effects of oil on saltiness and bitterness perception in emulsion systems, and to compare results from a descriptive panel to that from an electronic-tongue.

For experiment I, there were no significant differences in NaCl and KCl detection thresholds for solution and emulsion systems. Moreover, emulsions did not significantly affect the saltiness recognition threshold of NaCl and KCl; however, emulsions exhibited bitterness-suppressing effects toward caffeine and/or KCl. This finding would prompt more in-depth studies as to how other emulsion characteristics affect saltiness and bitterness perception in the reduced sodium food system at threshold levels.

For experiment II, the study demonstrated that oil had a saltiness enhancing effect on NaCl and KCl in emulsions. Moreover, oil had a larger effect on the perceived saltiness of NaCl than KCl in emulsions. On the other hand, oil had a marginal effect on the bitterness perception of caffeine. Regarding the physical parameters, viscosity largely contributed to overall differences among emulsions. Further research is needed to investigate the effect of pH and binary interactions on taste perception in emulsion systems.

For experiment III, the study demonstrated the psychophysical effects of oil on saltiness and bitterness perceptions in oil-in-water emulsions. Although the growth rate (n) for saltiness in oil-in-water emulsions was lower compared to that of solutions, saltiness intensity values in emulsions were higher than in solutions, demonstrating a saltiness enhancing effect imparted by oil. For bitterness, intensities in emulsions were lower compared to those in solutions for caffeine but they were similar for KCl. This demonstrated that oil exhibited a bitterness-suppressing effect for caffeine but not for KCl. Saltiness intensities measured by the Electronic-tongue were

congruent with the descriptive panel results. On the other hand, bitterness intensities of KCl showed an opposite pattern between the two methods.

In conclusion, this research demonstrated that oil can suppress bitterness and enhance saltiness in emulsion systems. Moreover, this research proved that perceptions of saltiness and bitterness differed between solution and emulsion systems. Thus, conclusions from previous research on taste perception of solutions cannot be extrapolated to emulsion systems. Further research has to be done to test the binary and tertiary interactions among five basic tastes (saltiness, bitterness, sourness, sweetness and umami) in emulsions systems. Besides, more research about the effects of emulsion viscosity, pH, and oil type/concentration on the saltiness and bitterness perception in food models has to be done.

APPENDIX A: IRB APPROVALS

a. Application for Exemption from Institutional Oversight (2011)

LSU AgCenter Institutional Review Board (IRB)
Dr. Michael J. Keenan, Chair
School of Human Ecology
209 Knapp Hall
225-578-1708
mkeenan@agctr.lsu.edu

Application for Exemption from Institutional Oversight

All research projects using living humans as subjects, or samples or data obtained from humans must be approved or exempted in advance by the LSU AgCenter IRB. This form helps the principal investigator determine if a project may be exempted, and is used to request an exemption.

- Applicant, please fill out the application in its entirety and include the completed application as well as parts A-E, listed below, when submitting to the LSU AgCenter IRB. Once the application is completed, please submit the original and one copy to the chair, Dr. Michael J. Keenan, in 209 Knapp Hall.

- A Complete Application Includes All of the Following:
  - **(A)** The original and a copy of this completed form and a copy of parts B through E.
  - **(B)** A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1 & 2)
  - **(C)** Copies of all instruments and all recruitment material to be used.
    - If this proposal is part of a grant proposal, include a copy of the proposal.
  - **(D)** The consent form you will use in the study (see part 3 for more information)
  - **(E)** Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing and handling data, unless already on file with the LSU AgCenter IRB.
    Training link: (http://grants.nih.gov/grants/policy/hs/training.htm)

1) Principal Investigator: Witoon Prinyawiwatkul **Rank:** Professor **Student? Y(N)**
Dept: Food Science **Ph:** 8-5188 **E-mail:** wprinya@lsu.edu
2) Co-Investigator(s): please include department, rank, phone and e-mail for each

- If student as principal or co-investigator(s), please identify and name supervising professor in this space

None

3) Project Title: Consumer Acceptance of New Food Products
4) Grant Proposal?(yes or (no) **If Yes, Proposal Number and funding Agency**
Also, if Yes, either: **this application completely matches the scope of work in the grant Y/N**
OR
more IRB applications will be filed later Y/N
5) Subject pool (e.g. Nutrition Students) LSU Faculty, staff, students, and off-campus consumers

- Circle any **"vulnerable populations" to be used:** (children<18, the mentally impaired, pregnant women, the aged, other). Projects with incarcerated persons cannot be exempted.

6) PI signature **Date 12/9/2011 (no per signatures)
I certify that my responses are accurate and complete. If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I leave the LSU AgCenter before that time the consent forms should be preserved in the Departmental Office.

Committee Action: Exempted √ Not Exempted **IRB#** HE11-29

Reviewer Michael Keenan **Signature** Michael Keenan **Date** 12-14-2011

APPROVED BY
LSU AG CENTER
IRB AS HE11-29
ON 12-14-2011

Research Consent Form

I, _____, agree to participate in the research entitled "Consumer Acceptance of New Food Products" which is being conducted by Dr. Witoon Prinyawiwatkul, Professor of the Department of Food Science at Louisiana State University, Agricultural Center, 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. Up to 150 consumers will participate in this research. For this particular research, about 15-20 minutes 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 investigator any food allergies I may have.

2. The reason for the research is to gather information on sensory acceptability of new food products. The benefit that I may expect from it is a satisfaction that I have contributed to quality improvement of these products.

3. The procedures are as follows: 3-5 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 that of an allergic reaction common food ingredients [red beans, bell pepper, onion, garlic, celery, thyme, cayenne pepper, bay leaf, pork products, rice and rice products, milk and dairy products, wheat flour, tapioca flour, eggs, table sugar, vanilla, sweet potato, salt (sodium chloride) and salt substitute (potassium chloride), and plain unsalted crackers]. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.

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 investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael Keenan, Chair of LSU AgCenter IRB, (225) 578-1708. 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. Application for Exemption from Institutional Oversight (2015)

LSU AgCenter Institutional Review Board (IRB)
Dr. Michael J. Keenan, Chair
School of Human Ecology
209 Knapp Hall
225-578-1708
mkeenan@agctr.lsu.edu

Application for Exemption from Institutional Oversight

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  - **(A)** The original and a copy of this completed form and a copy of parts B through E.
  - **(B)** A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1 & 2)
  - **(C)** Copies of all instruments and all recruitment material to be used.
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  - **(D)** The consent form you will use in the study (see part 3 for more information)
  - **(E)** Beginning January 1, 2009: Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing and handling data, unless already on file with the LSU AgCenter IRB.
    Training link: (http://grants.nih.gov/grants/policy/hs/training.htm)

1) Principal Investigator: Witoon Prinyawiwatkul **Rank:** Professor **Student?** No
School of Nutrition and Food Sciences **Ph:** 8-5188
E-mail: wprinyawiwatkul@agcenter.lsu.edu **and** wprinya@lsu.edu

2) Co-Investigator(s): please include department, rank, phone and e-mail for each NONE
- If student as principal or co-investigator(s), please identify and name supervising professor in this space

3) Project Title: Consumer Acceptance and Perception of New and Healthier Food Products
4) Grant Proposal?(yes or no) NO **If Yes, Proposal Number and funding Agency**
Also, if Yes, either: **this application completely matches the scope of work in the grant Y/N**
OR
more IRB applications will be filed later Y/N
5) Subject pool (e.g. Nutrition Students) LSU Faculty, Staff, Students and off-campus consumers
- Circle any **"vulnerable populations" to be used:** (children<18, the mentally impaired, pregnant women, the aged, other). Projects with incarcerated persons cannot be exempted. **NONE**

6) PI signature ****Date** 3-12-2015 (no per signatures)
****I certify that my responses are accurate and complete.** If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU AgCenter institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at the LSU AgCenter for three years after completion of the study. If I leave the LSU AgCenter before that time the consent forms should be preserved in the Departmental Office.

Committee Action: Exempted ___ Not Exempted ✓ **IRB#** HE15-9

Reviewer Michael Keenan **Signature** Michael Keenan **Date** 3-16-2015

Research Consent Form

I, _____, agree to participate in the research entitled "Consumer Acceptance and Perception of New and Healthier Food Products" which is being conducted by Dr. Witoon Prinyawiwatkul, Professor of the School of Nutrition and Food Sciences at Louisiana State University, Agricultural Center, 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. Up to 300 consumers will participate in this research. For this particular research, about 15-20 minutes participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.
2. The reason for the research is to gather information on sensory acceptability, emotion and purchase intent of new and healthier food products. The benefit that I may expect from it is a satisfaction that I have contributed to quality improvement of these products.
3. The procedures are as follows: 3-5 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 that of an allergic reaction toward common food ingredients [red beans, bell pepper, onion, garlic, celery, thyme, cayenne pepper, bay leaf, pork products, rice and rice products, milk and dairy products, yogurt or fermented milk products, peanuts, mayonnaise products, wheat flour, tapioca flour, eggs, table sugar, vanilla, sweet potato, salt (sodium chloride) and salt substitute (potassium chloride and common amino acids such as glycine and lysine), and plain unsalted crackers]. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.
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 investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael Keenan, Chair of LSU AgCenter IRB, (225) 578-1708. 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: _____

APPENDIX B: PRELIMINARY WORK ON EMULSION CHARACTERISTICS

a. Emulsion Capacity

In preliminary studies performed in our lab, we measured the emulsion capacity (EC) of three commercial emulsifiers (TANDEM, DURFAX, and ULTRALEC), one commercial texture modifier (MODIFIED STARCH), and yolk (Figure A.1). The three commercial emulsifiers were TANDEM = Emulsifier Tandem® 552K (a mixture of mono- and diglycerides, polysorbate, water and proply gallate) which was obtained from Caravan® ingredients (Lenexa, KS, USA), DURFAX = Emulsifier Durfax 60 (nonionic and water dispersible Polysorbate 60 with tocopherols) which was obtained from IOI Loders Croklaan (Channahon, IL, USA), and ULTRALEC = Emulsifier Ultralec® (de-oiled soy lecithin) which was obtained from ADM® (Decatur, IL, USA). EC was determined using red-colored oil for enhancing the view of emulsion collapse, and was prepared by adding 0.3 g of biological stain (Oil Red O, 19819-6; SigmaeAldrich, St. Louis, MO, USA) to a liter of soybean oil (Great Value®; WalMart, AR, USA). Fifteen grams of the emulsifier, texture modifier, and/or yolk was mixed with 20 mL of soybean oil and 10 mL of vinegar, and emulsified at high speed using a hand blender (Hamilton Beach, Model 59780; Southern Pines, NC, USA) for 2 min. Then, 2 g of the resulting emulsion was taken and emulsified with 9 mL of 0.1 mol/L NaCl solution and 30 mL of red-colored oil at low speed for 2 min. Additional red-colored oil was dispensed from a burette at a speed of 0.1 mL s^{-1} while stirring at low speed until the emulsion broke. The breakpoint at which phase inversion occurred was considered as the EC. EC was expressed as mL of soybean oil added per g of emulsifier, texture modifier, and/or yolk. Three measurements were made for each treatment. Analysis of Variance and the *post-hoc* multiple comparison (Tukey studentized range test) at $\alpha = 0.05$ were performed on data collected.

As it is shown in Figure A.1, three commercial emulsifiers (TANDEM, DURFAX, and ULTRALEC) possessed significantly ($P < 0.05$) higher EC compared to the commercial texture modifier (MODIFIED STARCH) (143.08-159.06 vs. 42.40 mL of oil / g of emulsifier). Lecithin (ULTRALEC, 159.06 mL of oil / g of emulsifier) was significantly ($P < 0.05$) higher than TANDEM (143.08 mL of oil / g of emulsifier) but was not significant ($P \geq 0.05$) different from DURFAX. EC of egg yolk was comparable to the texture modifier (59.70 vs. 42.40 mL of oil / g of emulsifier) but was lower compared to the three emulsifiers. This shows that emulsifiers such as polysorbate and lecithin can form emulsion with greater amounts of oil compared to texture modifiers such as resistant starches.

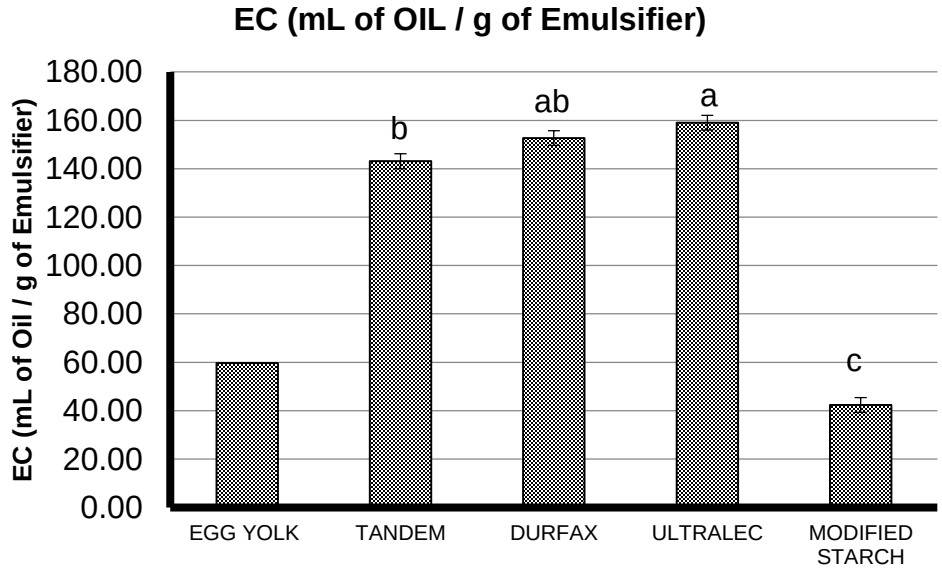


Figure A.1 Emulsion capacities (EC) of different emulsifiers*
*EGG YOLK (Wardy et al., 2011). TANDEM = Emulsifier Tandem® 552K (a mixture of mono- and diglycerides, polysorbate, water and proply gallate), DURFAX = Emulsifier Durfax 60 (nonionic and water dispersible Polysorbate 60 with tocopherols), ULTRALEC = Emulsifier Ultralec® (de-oiled soy lecithin), and MODIFIED STARCH = Food modified starch. $^{a\text{-}c}$ Bars with different superscripts on the top indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test. Standard error of this data set was 3.05 mL of oil / g of emulsifier.

b. Emulsion Viscosity

In an attempt to better understand the viscosity of emulsions, we performed some preliminary studies on the viscosity of several emulsions and solutions (Table A.1). A solution of distilled water and 1.0% of KCl (potassium chloride, 99% FCC grade) obtained from Extract & Ingredients, Ltd. (Road Union, NJ, USA) was prepared. Emulsions were prepared using distilled water, soybean oil (Great Value®; WalMart, AR, USA), and 0.5% of Durfax 60 (nonionic and water dispersible Polysorbate 60 with tocopherols) obtained from IOI Loders Croklaan (Channahon, IL, USA). Three emulsions were prepared at 56%, 35% and 14% of oil which represented 80%, 50%, and 20% of the amount of oil needed to reach the breakpoint in EC of Durfax 60 obtained in Figure A.1. Emulsions were prepared by adding the emulsifier to soybean oil and mixing using a hand blender (Model # 59780R, Hamilton Beach® Brands Canada, Inc., Picton, Onratio, Canada) at a low speed for 2 min at 25 °C. The mixture stood for 30 min at room temperature, and, subsequently, 1.0% KCl solution was added and mixed by using the hand blender at a high speed for 6 min at 25 °C. As a comparison between the viscosities of homogenized and nonhomogenized emulsions, an emulsion containing 35 % of oil was further homogenized by using a lab scale 2-stage homogenizer (APV Americas, Lake Mills, WI) at 9,400 psi. Thick solutions were prepared using 0.8%, 0.5% or 0.2% of Tic Pretested®Ticaloid®210 S Powder (gum acacia and xanthan gum; Tic Gums®, Inc., White Marsh, Md., USA). Thick solutions were prepared by adding the Tic® gum to the 1.0% KCl solution at a low speed using the hand blender for 4 min at 25 °C. Viscosity was measured using a viscometer (model DV-II +, Brookfield Engineering Labs Inc., Middleboro, MA., USA) at 30 rpm using a T-C spindle from the Helipath Spindle Set, with data gathered in Wingather V2.1 software (Brookfield Engineering Labs Inc.). Three measurements were made for each treatment. Analysis of Variance and the *post-hoc*

multiple comparison (Tukey studentised range test) at $\alpha = 0.05$ were performed on data collected. Table A.1 shows that distilled water and a solution of 1.0% KCl were not significantly different ($P \geq 0.05$) in terms of viscosity (6.80 vs. 7.37 cP). As the concentration of oil increased, the viscosity of emulsions increased exponentially from 9.13 cP with 14% of oil to 58.15 cP with 56% of oil. The viscosity of pure oil was 107 cP which was significantly higher ($P < 0.05$) than that of emulsions with 14% and 35% of oil (9.13-14.33 cP).

Increasing the concentration of Tic® gum to the thick solution had a similar effects of increasing the concentration of oil in the emulsions. The viscosity of thick solution raise exponentially from 17.74 cP with 0.2% gum to 773.50 cP with 0.8% gum. Viscosity of pure soybean oil was similar ($P \geq 0.05$) to the viscosity of the thick solution with 0.5% of gum, and the viscosity of the emulsion with 35% of oil was not significant different ($P \geq 0.05$) compared to the thick solution with 0.2% of gum. Comparing nonhomogenized and homogenized emulsions (14.27 vs. 14.06 cP), their viscosity values were not significant different ($P \geq 0.05$) using an emulsion with 35% of oil. The temperature for these viscosity values ranges between 20.40 and 23.84 °C.

Table A.1 Viscosities (cP) of different substances

Treatment	Viscosity (cP)*	Temperature (°C)*
Distilled water	6.80±0.60[d]	23.84±0.07[a]
KCl (1.0% Solution)	7.37±0.37[d]	20.40±0.30[b]
Soybean oil (100 % Oil)	107.56±4.55[bc]	23.80±0.10[a]
Emulsion (56% Oil = 80% EC, 1.0% KCl)**	58.15±3.41[cd]	20.55±0.66[b]
Emulsion (35% Oil = 50% EC, 1.0% KCl)**	14.33±0.37[d]	20.31±0.60[b]
Emulsion (14% Oil = 20% EC, 1.0 % KCl)**	9.13±0.44[d]	20.90±0.51[b]
Thick solution (0.8% Gum, 1.0 % KCl)	773.50±27.69[a]	23.35±0.05[a]
Thick solution (0.5% Gum, 1.0 % KCl)	146.92±41.25[b]	22.90±0.10[a]
Thick solution (0.2% Gum, 1.0 % KCl)	17.74±0.11[d]	22.95±0.05[a]
Emulsion (35% Oil = 50% EC, 1.0% KCl)** - Nonhomogenized	14.27±0.61[d]	20.65±0.25[b]
Emulsion (35% Oil = 50% EC, 1.0% KCl)** – Homogenized***	14.06±0.74[d]	20.40±0.27[b]

*Means ± standard deviations of 3 measurements.
[a-d] Means with different superscripts in a column indicate significant differences ($P < 0.05$) by Tukey's Studentized Range (HSD) test.
**Emulsions were prepared by using 0.5% of Durfax 60 emulsifier. EC = Emulsion capacity
***Homogenized at 9,400 psi. The other emulsions were nonhomogenized unless specified otherwise.

APPENDIX C: OIL-IN-WATER EMULSION EXHIBITS BITTERNESS-SUPPRESSING EFFECTS IN A SENSORY THRESHOLD STUDY IN CHAPTER 3

a. Research Consent Form

I, _____, agree to participate in the research entitled "**Sensory Threshold analysis on Solution and Emulsion systems**", which is being conducted by Witoon Prinyawiwatkul, Professor of the Department of Food Science at Louisiana State University Agricultural Center, 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. 15 panelists will participate in this research. For this particular research, about 7-12 min. participation per session for a total of 19 sessions 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 sensory thresholds of sodium chloride (NaCl), caffeine, and/or potassium chloride (KCl). 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.
4. Participation entails minimal risk: The only risk which can be envisioned is the allergic reaction toward **NaCl (regular salt), caffeine, KCl, Canola oil, and/or emulsifier products**. 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 AgCenter that 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. Michael Keenan of LSU AgCenter at (225) 578-1708.

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. Warm-up Session Questionnaire

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 **O**	–	no salty or bitter taste
Samples **B** and **C**	–	salty taste (C is more saltier than B)
Samples **D** and **E**	–	bitter taste (E is more bitter than D)
Samples **F** and **G**		salty and bitter tastes (G is more salty and bitter than F)

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

458	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
835	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
223	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
573	Sweet	Salty	Sour	Bitter	Unidentified	No Taste

Part III. Identify tastes

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.

Circle the taste(s) that you perceived

352	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
725	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
443	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
587	Sweet	Salty	Sour	Bitter	Unidentified	No Taste

Part III. Identify tastes

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.

Circle the taste(s) that you perceived

352	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
725	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
443	Sweet	Salty	Sour	Bitter	Unidentified	No Taste
587	Sweet	Salty	Sour	Bitter	Unidentified	No Taste

c. Threshold Evaluation Form

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

SESSION 1

INSTRUCTION:

1) Taste the samples from left to right. Two samples are identical; one is different.
2) Circle 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	Circle the odd sample			Circle the taste(s) which exhibits the difference	Remarks
1	835	689	767	Sweet – Salty – Sour – Bitter - Unidentified	
2	489	343	228	Sweet – Salty – Sour – Bitter - Unidentified	
3	925	674	391	Sweet – Salty – Sour – Bitter - Unidentified	
PLEASE TAKE A BREAK OF 4 MINUTES AND EAT SOME CRACKERS					
4	455	193	350	Sweet – Salty – Sour – Bitter - Unidentified	
5	635	916	549	Sweet – Salty – Sour – Bitter - Unidentified	
6	997	575	115	Sweet – Salty – Sour – Bitter - Unidentified	
7	383	705	249	Sweet – Salty – Sour – Bitter - Unidentified	

PLEASE STOP HERE

SESSION 2 **Date:** .....

Set	Circle the odd sample			Circle the taste(s) which exhibits the difference	Remarks
1	581	645	395	Sweet – Salty – Sour – Bitter - Unidentified	
2	957	738	161	Sweet – Salty – Sour – Bitter - Unidentified	
3	237	123	471	Sweet – Salty – Sour – Bitter - Unidentified	
PLEASE TAKE A BREAK OF 4 MINUTES AND EAT SOME CRACKERS					
4	499	343	710	Sweet – Salty – Sour – Bitter - Unidentified	
5	789	562	287	Sweet – Salty – Sour – Bitter - Unidentified	
6	445	119	358	Sweet – Salty – Sour – Bitter - Unidentified	
7	644	809	582	Sweet – Salty – Sour – Bitter - Unidentified	

PLEASE STOP HERE

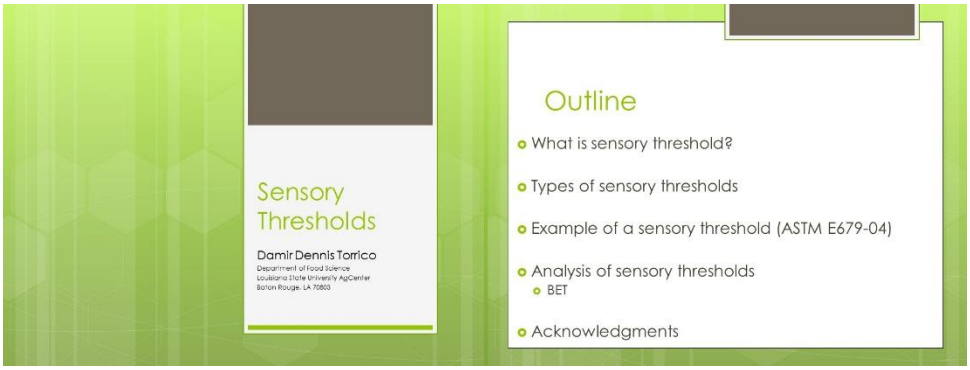
d. Sample Calculation of the Detection Group Best-Estimate Threshold of NaCl for the Method of Limits

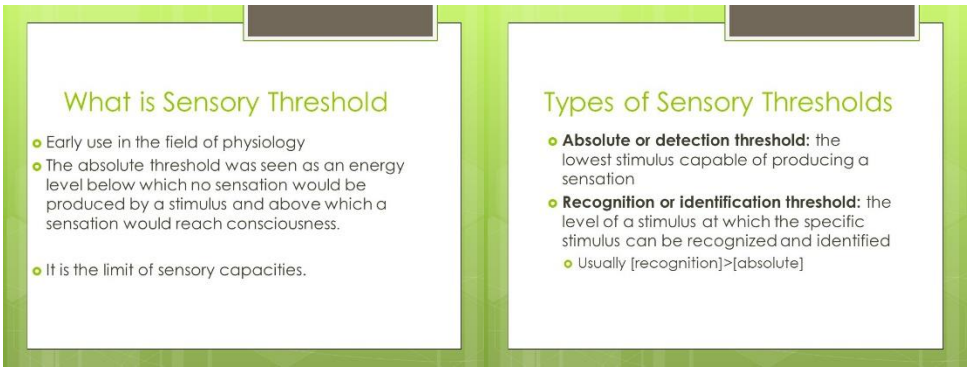
Panelists	Judgments (concentration increase -->)							Best-Estimate Threshold (BET)	
	0.005	0.01	0.02	0.04	0.08	0.16	0.32	BET	log10 BET
1	+	+	0	+	+	+	+	0.0283	-1.5485
2	0	+	+	+	+	+	+	0.0071	-2.1505
3	0	+	0	0	+	+	+	0.0566	-1.2474
4	0	0	+	+	+	0	+	0.0141	-1.8495
5	+	0	0	0	+	+	+	0.0566	-1.2474
6	+	+	+	0	+	+	0	0.0035	-2.4515
7	+	+	+	0	+	+	+	0.0035	-2.4515
8	0	+	0	0	0	+	+	0.1131	-0.9464
9	0	0	+	+	+	+	+	0.0141	-1.8495
10	0	0	+	+	+	+	+	0.0141	-1.8495
11	0	+	0	0	+	+	+	0.0566	-1.2474
12	+	+	+	+	+	+	+	0.0035	-2.4515
13	0	0	+	0	0	+	+	0.1131	-0.9464
14	+	+	0	0	+	+	+	0.0566	-1.2474
15	0	0	0	0	+	+	+	0.0566	-1.2474
								$\sum \log_{10}$ →	-24.7320
Group BET geometric mean								**0.0224** ←	-1.6488
Standard deviation									**0.5417**

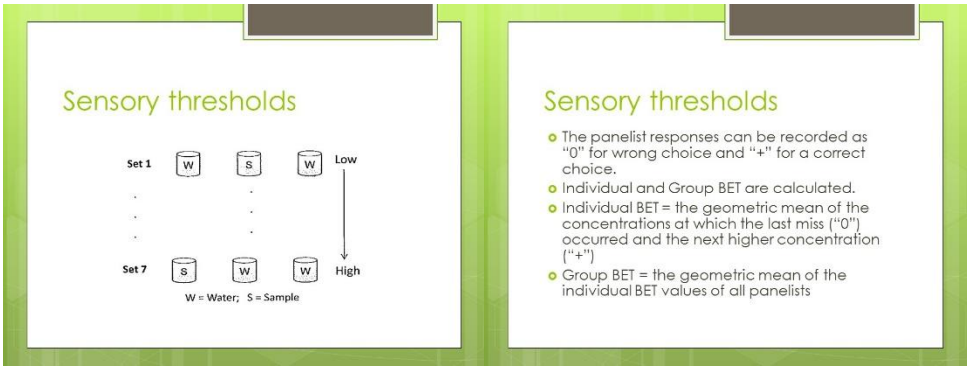
”0” indicates that the panelist selected the wrong sample of the set of three.

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

e. Presentation for Panelists at the End of the Experiment







Analysis of Sensory Thresholds

Table A: Sample Calculation of the Group Best-Estimate Threshold of KCl for the Method of Limits

Panelists	Judgments: KCl concentrations, g/100 ml							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
Log Standard deviation								**0.201**	

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

"+" indicates that the panelist selected the correct sample.

GT = 0.0187 g/100 mL

References

- Prinyawiwatkul, W., Waimaleongora-Ek, P. A 3-Days Workshop: Sensory Characterization of Salt Substitutes and Its Food Applicatio. April 20-22, 2010.
- Lawless, H., Heymann, H. Sensory Evaluation of Food (Book). Springer Science+Business Media, LLC 2010.

Acknowledgments

- Dr. Witoon Prinyawiwatkul
- All panelists:
  - Srikanth
  - Luis
  - Wisdom
  - Mustafa
  - Behannis
  - Amelia
  - Chloe
  - Ronald
  - Pink
  - Adriana
  - Kennet
  - Busarawan
  - Tatiana
  - Parisut
  - John
  - June

f. Pooled within Canonical Structure (*r*'s) Describing Variables that Underlie Group Differences

	Saltiness		Bitterness	
	Wilks' Lambda	P-value	Wilks' Lambda	P-value
	0.000	<.0001	0.000	<.0001
Variables	Can 1*	Can 2*	Can 1*	Can 2*
Saltiness intensity	0.060	-0.359	-	-
Bitterness intensity	-	-	0.028	0.011
Viscosity	***0.882***	-0.271	***0.921***	0.286
pH	0.020	***0.544***	-0.047	0.318
Cumulative variance explained (%)	88.06%	96.81%	69.92%	93.52%

* Based on the pooled within group variances with $P < 0.05$ of Wilks' Lambda from MANOVA. Bolded and italicized values indicate attributes largely contributing to the overall differences among all treatments (NaCl, KCl and caffeine emulsions at different concentrations). Can 1 and Can 2 refer to the pooled within canonical structure in the 1st and 2nd canonical discriminant functions, respectively.

APPENDIX D: EFFECT OF OIL AND TASTANT CONCENTRATIONS ON PERCEPTIONS OF SALTINESS AND BITTERNESS IN OIL-IN-WATER EMULSIONS IN CHAPTER 4

a. Research Consent Form

I, _____, agree to participate in the research entitled "**Sensory Evaluation of Solution and Emulsion Systems**", which is being conducted by Witoon Prinyawiwatkul, Professor of the Department of Food Science at Louisiana State University Agricultural Center, 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. 15 panelists will participate in this research. For this particular research, about 15-20 min. participation per session will be required for each subject.

The following points have been explained to me:

7. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.
8. The reason for the research is to gather information on sensory thresholds of sodium chloride (NaCl), caffeine, and/or potassium chloride (KCl). 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.
9. 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.
10. Participation entails minimal risk: The only risk which can be envisioned is the allergic reaction toward **NaCl (regular salt), caffeine, KCl, Canola/Olive oil, and/or emulsifier products**. Individuals who have kidney problem should not participate in this study.
11. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
12. 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 AgCenter that 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. Michael Keenan of LSU AgCenter at (225) 578-1708.
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. Preliminary Evaluation Form for Screening

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

PART 1

INSTRUCTION:

4) You will be presented with 6 labeled samples (samples 1 to 6).
5) Please taste the sample starting with sample 1.
6) Identify the taste(s) of the sample, **only if you perceived,** and circle the taste that the sample exhibits (you can check more than one taste).
   Otherwise, circle "unidentified".

NOTE:

5) Take the whole sample into the mouth.
6) **Swirl** it for 2-3 seconds.
7) Expectorate and answer the question.
8) Rinse your mouth with water between samples.

Sample	Circle the taste(s) that the sample	Remarks
1	Sweet – Salty – Sour – Bitter - Unidentified	
2	Sweet – Salty – Sour – Bitter - Unidentified	
3	Sweet – Salty – Sour – Bitter - Unidentified	
4	Sweet – Salty – Sour – Bitter - Unidentified	
5	Sweet – Salty – Sour – Bitter - Unidentified	
6	Sweet – Salty – Sour – Bitter - Unidentified	

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

PART 2

INSTRUCTION:

1) You will be presented with 3 sets of 2 labeled samples in a random order.
2) Please taste the sample in the order presented, from left to right.
3) Rank samples for intensity. **No ties allowed!**

Set 1

- Rank the solutions in a descending order of **saltiness**

_____ **>** _____

Saltier Less salty

Set 2

- Rank the solutions in a descending order of **bitterness**

_____ **>** _____

More bitter Less bitter

Set 3

- Rank the solutions in a descending order of **saltiness**

_____ **>** _____

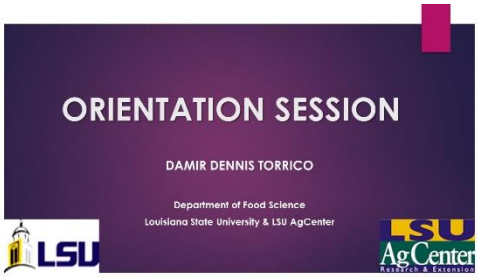
Saltier Less salty

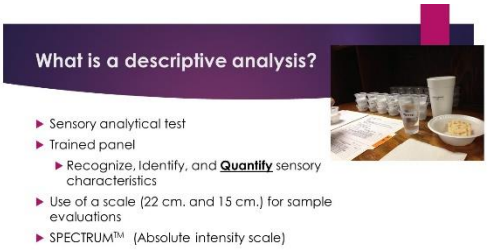
- Rank the solutions in a descending order of **bitterness**

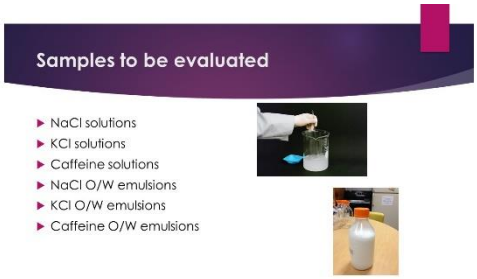
_____ **>** _____

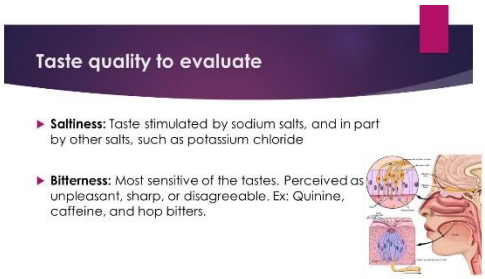
More bitter Less bitter

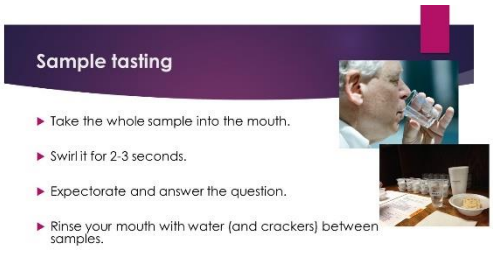
c. Orientation Session Presentation

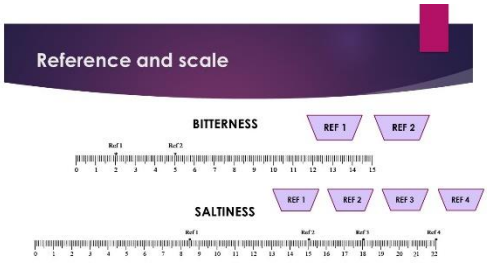


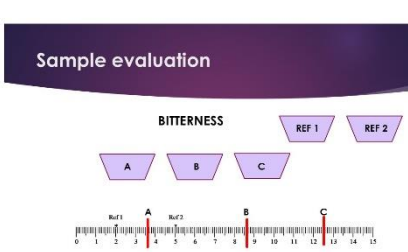












d. Training Sessions Forms

TRAINING SESSION 1

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

Saltiness intensity evaluation

INSTRUCTIONS:

1. Taste each reference sample: From Ref 1 to Ref 4 (Do not reverse the sequence)
2. Each reference represents an intensity value on the 22-cm scale. Associate this value with your perceived intensity
3. Taste the unknown sample
4. Rate the intensity of unknown sample on the 22-cm scale

Sample 857

Sample 458

TRAINING SESSION 1

Bitterness intensity evaluation

INSTRUCTIONS:

1. Taste each reference sample: From Ref 1 to Ref 3 (Do not reverse the sequence)
2. Each reference represents an intensity value on the 15-cm scale. Associate this value with your perceived intensity
3. Taste the unknown sample
4. Rate the intensity of unknown sample on the 15-cm scale

Sample 357

Sample 564

e. Samples Evaluation Forms

EVALUATION SESSION 1

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

Saltiness intensity evaluation

INSTRUCTIONS:

5. Taste each reference sample: From Ref 1 to Ref 4 (Do not reverse the sequence)
6. Each reference represents an intensity value on the 22-cm scale. Associate this value with your perceived intensity
7. Taste the unknown sample
8. Rate the intensity of unknown sample on the 22-cm scale

Sample 427

Sample 794

Sample 497

Sample 460

EVALUATION SESSION 1

Bitterness intensity evaluation

INSTRUCTIONS:

5. Taste each reference sample: From Ref 1 to Ref 3 (Do not reverse the sequence)
6. Each reference represents an intensity value on the 15-cm scale. Associate this value with your perceived intensity
7. Taste the unknown sample
8. Rate the intensity of unknown sample on the 15-cm scale

Sample 847

Sample 879

Sample 508

Sample 889

f. SAS Code: ANOVA for Saltiness and Bitterness Intensities

```
dm 'log;clear';
Title 'ANOVA for SALTINESS AND BITTERNESS';
data salt;
input PANEL SESSION TASTE $ PCTASTE PCOIL SALTINESS BITTERNESS;
datalines;
…
proc means data=salt N Mean StdDev Min Max; by TASTE;
class PCTASTE PCOIL;
var SALTINESS BITTERNESS;
run;

proc glimmix data=salt;
Title2 'NESTED MODEL';
class TASTE PCTASTE PCOIL;
model SALTINESS = TASTE PCTASTE PCOIL TASTE*PCOIL PCTASTE*PCOIL(TASTE);
random PANEL;
lsmeans PCTASTE*PCOIL(TASTE) / lines;
run;

proc glimmix data=salt;
Title2 'NESTED MODEL';
class TASTE PCTASTE PCOIL;
model BITTERNESS = TASTE PCTASTE PCOIL TASTE*PCOIL PCTASTE*PCOIL(TASTE);
random PANEL;
lsmeans PCTASTE*PCOIL(TASTE) / lines;
run;

quit;
```

g. SAS Code: RSM Example for NaCl Saltiness

```
data NaClsalt;
input PANEL SESSION TASTE $ PcNaCl PcOIL SALT;
label
  PcNaCl = "NaCl(%)"
  PcOIL = "Oil(%)"
  SALT = "Saltiness Intensity";
datalines;
…
proc rsreg data=NaClsalt;
model SALT = PcOIL PcNaCl/lackfit;
run;

ods graphics on;
proc rsreg data=NaClsalt plots= (surface) noprint;
model SALT = PcOIL PcNaCl/lackfit;
run;
ods graphics off;

ods graphics on;
proc rsreg data=NaClsalt plot=surface (3D) noprint;
model SALT = PcOIL PcNaCl/lackfit;
run;
ods graphics off;

data grid;
do SALT=.;
do PcOIL = 20 to 60 by 0.0008;
do PcNaCl = 0.5 to 1.0 by 0.0008;
output;
end;
end;
end;
run;

data new;
set NaClsalt grid;
run;

proc rsreg data=new out=predict noprint;
model SALT=PcNaCl PcOIL/lackfit predict;
```

```
run;

goptions /*reset=global*/ gunit=pct border cback =white
colors=();
proc gcontour data=predict;
axis1 label= ("Percentage of NaCl (%)");
axis2 label= (a=90 j=c "Percentage of oil (%)");
legend1 label = ('Saltiness Intensity');
plot PcOIL*PcNaCl=SALT/grid xticknum=9 yticknum=9 levels=8 to 20 by 2 pattern join
haxis=axis1 vaxis=axis2 legend=legend1;
run;

data grid1;
do SALT=.;
do PcOIL = 20 to 60 by 0.5;
do PcNaCl = 0.5 to 1.0 by 0.002;
output;
end;
end;
end;
run;

data new1;
set NaClsalt grid1;
run;

proc rsreg data=new1 out=predict noprint;
model SALT=PcNaCl PcOIL/lackfit predict;
run;


goptions /*reset=global*/ gunit=pct border cback =white
colors=();

proc g3d data=predict;
plot PcOIL*PcNaCl=SALT/grid caxis=black xticknum=7 yticknum=7 zticknum=7;
run;

quit;
```

h. SAS Code: MANOVA Example for Saltiness

```
dm 'log;clear';
Title 'MANOVA FOR SALTINESS';
%Include "C:\Users\dtorri1\Documents\biplot.sas";
%Include "C:\Users\dtorri1\Documents\equate.sas";
data mano;
input REP TASTE$ PCOIL PCTASTE TRT$ SALTINESS VISCOSITY PH;
datalines;
…
proc sort; by TRT;
run;

proc means data=mano N Mean StdDev Min Max; by TRT;
class TRT;
var SALTINESS VISCOSITY PH;
run;

proc candisc data=mano out=outcan mah;
Title2 'MANOVA - OVERALL';
class TRT;
var SALTINESS VISCOSITY PH;
run;

proc princomp data=mano cov out=comp1;
var SALTINESS VISCOSITY PH;
run;

proc gplot data=comp1;
plot Prin1*Prin2 = 1 / HRef =0 VRef = 0 VAxis=Axis1 HAxis=Axis2;
Axis1 Label = (A=90 j=c "Principal Component 1");
                ***Order = (-2 To 2 by 0.5) Length=1 in;

Axis2 Label = ("Principal Component 2");
                ***Order = (-2 To 2 by 0.5) Length=1 in;

Symbol1 C=Black V=Dot H=0.7 I=None PointLabel = (C=Black "#TRT");
run;

Title2 "Symmetric Biplot";
%Biplot (data=mano, var=SALTINESS VISCOSITY PH, Id=TRT, factype=SYM);
```

```
proc prinqual data=mano out=Results n=2 replace mdpref;
     title2 'Multidimensional Preference (MDPREF) Analysis';
     title3 'Optimal Monotonic Transformation of Preference Data';
     id TRT;
     transform monotone(SALTINESS VISCOSITY PH);
  run;


quit;
```

APPENDIX E: PSYCHOPHYSICAL EFFECTS OF INCREASING OIL CONCENTRATIONS IN SALTINESS AND BITTERNESS PERCEPTIONS OF OIL-IN-WATER EMULSIONS IN CHAPTER 5

a. SAS Code: Analysis of Covariance for the Linear and Steven's Power Models

```
dm 'clear;log;clear;output';
data REGPERCEP;
input PERCEPTION $ PANELIST SESSION TASTE $ TASTECON SOLUTION EMULTW
EMULFO INT LOGTASTECON LOGINT SOL_TC EMULTW_TC EMULFO_TC SOL_LTC
EMULTW_LTC EMULFO_LTC;

***SOLUTION, EMULTW, and EMULFO are dummy variables created for the three emulsion
***tested. SOL_TC EMULTW_TC EMULFO_TC are the multiplications of the taste
***concentration variable by each of the other dummy variables created.

datalines;
insert data;
proc sort; by PERCEPTION TASTE;
run;

proc reg; by PERCEPTION TASTE;
Title1 'LINEAR REGRESSION AND ANCOVA';
model INT = SOLUTION EMULTW TASTECON SOL_TC EMULTW_TC;
run;

proc reg; by PERCEPTION TASTE;
Title1 'LINEAR REGRESSION AND ANCOVA';
model INT = EMULTW EMULFO TASTECON EMULTW_TC EMULFO_TC;
run;

proc reg; by PERCEPTION TASTE;
Title1 'STEVENS POWER LAW AND ANCOVA';
model LOGINT = SOLUTION EMULTW LOGTASTECON SOL_LTC EMULTW_LTC;
run;

proc reg; by PERCEPTION TASTE;
Title1 'STEVENS POWER LAW AND ANCOVA';
model LOGINT = EMULTW EMULFO LOGTASTECON EMULTW_LTC EMULFO_LTC;
run;

quit;
```

VITA

Damir Dennis Torrico was born in January, 1985 in Cochabamba, Bolivia. In December 2006 he graduated from the Escuela Agricola Panamericana, Zamorano University, with a Bachelor of Science in Agro-Industry. After receiving his bachelor's degree, he worked for the Biological Control Laboratory at Zamorano University, as a researcher and production supervisor for one year before joining the master's program in the Food Science Department at Louisiana State University in 2009. He received a master's degree in food science in December 2010. He continued to work on his doctorate in the School of Nutrition and Food Sciences at Louisiana State University with a minor in experimental statistics, which he expects to complete in August 2015.