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Effect of Bitterness Blockers in Partial and Complete Replacement of Sodium Chloride with Potassium Chloride on the Physicochemical and Sensory Characteristics of Marinated Chicken Breast Fillets

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EFFECT OF BITTERNESS BLOCKERS IN PARTIAL AND COMPLETE REPLACEMENT OF SODIUM CHLORIDE WITH POTASSIUM CHLORIDE ON THE PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN BREAST FILLETS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

High sodium intake has negative health implications on hypertension and cardiovascular diseases. Brines used in chicken marination are composed of salt (NaCl), phosphates and seasonings. Potassium chloride (KCl) is the most common sodium replacer but its use is typically limited to less than 50% substitution due to its undesirable bitterness and metallic aftertaste. The objective of this study was to determine the effect of 5' adenosine monophosphate (AMP) and glycine, as bitterness blockers, on physicochemical, sensory characteristics and purchase intent of sodium reduced chicken breast fillets. Chicken breast was injected with a 20% pick-up solution. Salt treatments (trts) were 50, 75 and/or 100% KCl substitutions with two levels of glycine (0.1 and 0.2%) and two levels of AMP (0.01 and 0.02%) based on the solution weight. Replacing NaCl by KCl at levels of 50-100% did not have a significant effect on sensory acceptability (aroma, flavor, juiciness, tenderness, bitterness, saltiness and overall liking), physicochemical traits (water holding capacity, and moisture) and positive emotions (good, happy, pleased and satisfied). Negative emotions (unsafe, worried and guilty) showed significant differences after sodium nutrient content claim per product had been presented, decreasing significantly in trts with 75 and 100% NaCl substitution. Overall liking and "satisfied" emotion were critical attributes influencing purchase intent (Overall liking odds ratio= 2.5 to 4.2; satisfied odds ratio 2.26 to 2.35). JAR results showed all treatments were considered "not salty enough" reflected by the low liking scores (neither like nor dislike and/or like slightly). A reduction of 75 to 100% NaCl significantly decreased tenderness when measured instrumentally. Initial pH values were significantly more acidic for breasts before injection ($P < 0.05$). A lower level of glycine and AMP caused significantly higher pH values but they were still in a normal range. In conclusion, it was feasible to reduce sodium in chicken breast marination, taking into account

sensory and physicochemical parameters, using KCl levels from 50 to 100% salt substitution with the use of bitterness blockers in concentrations of 0.01 and 0.02% of the injected solution.

CHAPTER 1. INTRODUCTION

Chicken breasts are widely consumed and the preferred white meat in the United States. Broiler meat production in the U.S. is always rising, since data for 2016 already indicate a rise in 3.1% (The Poultry site 2016). The U.S. poultry industry has developed products requiring less preparation time such as skinless, boneless chicken breasts, pre-marinated cuts, and micro-waveable chicken dishes. Demand for white meat in the US has expanded, raising the market price. Thus, productions of both white and dark meat are higher, suggesting that the price of dark meat has declined, as these are less desirable to American consumers due to health concerns (more fat than breasts) and versatile, convenient preparation. At the same time that chicken breast demand has expanded in the US, the demand of leg quarter export to Mexico, Asia, Canada, Cuba and Angola has increased (USDA 2016).

Chicken cut outs marination is one of the convenient ways the poultry industry enhances meat products (30% US production). Marination is achieved by adding a solution with solutes such as salt. Moreover, this leads to added sodium even before common home preparation. About 90% of the population of the United States consumes excess sodium, at a mean intake of about 3,400mg/day. Evidence shows that high sodium intake is strongly linked with high blood pressure, which contributes to nearly 1,000 deaths per day and is a leading risk factor for heart disease and stroke, the first and fourth leading causes of death, respectively (Gunn and others 2013). Salt from meat products accounts for 30% of daily sodium consumption because it’s the main ingredient in processed meats that contributes greatly to flavor, preservation and textural properties (Petracci and others 2013).

The objective of this study was to evaluate the effect of KCl and bitterness blockers, AMP and Glycine, on the quality of marinated chicken breasts.   One of the specific objectives of this thesis was to perform physicochemical analysis to evaluate texture, moisture, pH, color and water holding capacity affected by NaCl substitution with KCl and the addition of bitterness blockers. Sensory attributes were evaluated for acceptability, emotions and purchase intent as affected by different sodium reduced marinated chicken treatments.

This thesis is divided in four chapters, where the first is a brief introduction and justification of this study. The second and third chapters comprise a literature review and explained materials and methods, respectively. The fourth chapter describes the results and discussion. A summary and conclusions are contained in the fifth chapter. After the references section, the appendices include additional materials used in this study. The vita of the author is also provided.

CHAPTER 2. LITERATURE REVIEW

2.1 Poultry industry

The human body uses protein to create, maintain or repair existing cells. It also produces enzymes, which help catalyze processes such as digestion, metabolism and fat storage. Chicken meat consists of high-quality protein that contains 8 essential amino acids and is relatively low in fat. Furthermore, fat found in chicken is mostly unsaturated, which is beneficial against heart disease being recommended by physicians and nutrition counselors as an alternative to red meat (beef and pork) (Pearson and Dutson 1997).

As a result, consumption of poultry in the US has led to increasing production rates. According to the USDA (2015), the value of broilers produced during 2014 was $32.7 billion, 6 percent higher than 2013. The total number of broilers produced in 2014 was 8.54 billion, which was slightly higher than 2013. In comparison to other poultry sources, broiler meat production is always in a rise, since data for 2016 indicate a rise in 3.1 percent, while turkey production reports indicate a decrease in 4.1 percent (The poultry site 2016).

Poultry, meat and fish market share in the US is dominated by JBS that generates a 16.2% share of market value. JBS USA processes, packages and distributes fresh whole and chicken parts to the customers in the US, Mexico and Puerto Rico. Following JBS other companies such as Kraft and Tyson have 15.7% and 16.2% market share (Marketline 2014). On the other hand, there was an estimated per capita consumption of 90.1 lb of chicken products in 2015 and it has been projected a rise in consumption for 2016 of up to 91.8 lb (National chicken council 2016). This makes the US the largest consumer of broiler meat. As expected, it’s considered the largest producer in the world exporting 19% of its production. Forty percent of broilers are marketed as chicken in cut-up parts (leg quarters or breast).

Broiler leg quarters have a low domestic price but have a higher demand in foreign markets. The most consumed part of broilers in the U.S. market is breast meat. Increasing production to meet the domestic demand for white meat likewise increases the supplies of dark meat available for export. Lower priced leg quarters have expanded broiler meat exports to many countries looking for lower cost protein. Breasts account for US 51% broiler meat consumption (USDA 2013). Actually about 30 percent of poultry is enhanced by some marinating technique. From the enhancement methods commonly used, injection is used in around 53.4% of the cases, 28.1% vacuum tumbling and 13.4% marination by soaking or immersion (USDA 2012).

2.1.1 Poultry marination

Poultry marination is a processing technique that has been employed for decades with the objective to improve yields. Marinating solutions are composed of water and solutes such as salts, phosphates and seasonings. Water is added to meat products to increase yield and enhance meat characteristics such as tenderness. The marinade solution must be at an optimal temperature (-2-2 °C) allowing for optimal solubility of myosin and actin (0-3 °C) as well as reducing possible microbial contamination.

Marination can be done by 3 major methods: immersion, injection and tumbling. Immersion involves soaking the meat and allowing absorption under refrigerated conditions. However, this process is slow, therefore, not widely used, and only used when absorption is intended to take place during distribution. Injection involves multiple spring-loaded injections that penetrate the meat and force marinade into tissue under some pressure. Automated systems involve using high-speed belts where parts pass under the needles for injection. Among the benefits of this method, are its speed and the variety of products that can be processed. Tumbling allows massaging inside of a closed drum container usually with a partial vacuum. However, it is not suitable for skin on products because susceptible damage may occur (Mead 2004).

Salt is the most commonly used ingredient in food products. In meat products, salt is used majorly to enhance flavor, lower water activity, increase water binding capacity without pH changes, and act in conjunction with phosphates to solubilize protein and improve emulsification of fat. Phosphate salts added to marination solutions in meats add beneficial value in meat products. They act as protein solubilizers breaking actin and myosin (structural components of muscle myofibrils responsible for muscle contraction and relaxation) bridges formed post rigor (stiffening state that occurs post mortem), increase ionic strength thus improving water-binding capacity and have bacteriostatic effect. The most common phosphates used in meat industry are tripolyphosphates (Freiner 2006). However, the use of phosphates is limited to 0.5% in the final product due to possible adverse effects in health. Lowered mineral absorption in the intestinal tract, increased bone diseases and chronic kidney disease are some of the associated risks (Sherman and Metha 2009). In addition to salt and phosphates, other products seldom added to meat injections are gums or hydrocolloids. The most commonly used gum in chicken injections is carrageenan that originates from seaweed. It acts as a thickener to reduce cook loss and increase yield by not interacting with protein activation in meat. However, when used in marination it should be added after salt and phosphate solubilization to reduce surface tension for better carrageenan dispersion. Percentages of carrageenan in poultry brines are about 0.5% (FAO 2003).

2.2 Sodium in meat products

Salt (as sodium chloride) is added to meat products because of its effects on flavor, texture and microbial safety. The goal in applying salt substitutes to food products is to use a product that does not contain sodium but has the same sensory properties (Henney and others 2010). While sodium (Na^+) is responsible for producing salty taste, the chloride ion (Cl^-) modifies the taste and binds to specific proteins to achieve desired texture (Kilcast and others 2007). For this reason using chloride salts with other ions such as potassium, calcium and magnesium have become potential as salt substitutes. Flavor enhancers and non-chloride salts have also been explored as possible sodium replacers and will also be discussed in this thesis.

In order to maintain a healthy lifestyle all essential nutrients should be consumed in the right amounts, being sodium one of them. Hence, deficiency conditions are likely to develop if sodium intake is below the recommended levels for prolonged periods. Likewise, a high intake for long periods may develop toxicity and adverse effects. About 90% of the population of the United States consumes excess sodium at a mean intake of about 3,400mg/day. Evidence shows that high sodium intake is strongly linked to high blood pressure, which contributes to nearly 1,000 deaths per day and is a leading risk factor for heart disease and stroke, the first and fourth leading causes of death, respectively (Gunn and others 2013). In 2015 the Dietary Guidelines for Americans (DGA) recommended a consumption of less than 2,300 mg Na per day for general population and 1,500 mg/day for higher risk population groups such as: people 51 years and older and people with hypertension, diabetes or chronic kidney disease (CDC 2016).

Salt from meat products account for 30% of daily sodium consumption because it is a main ingredient in processed meats that contributes greatly to flavor, preservation and textural properties (Petracci and others 2013). Fresh meat is accountable for less than 100mg Na per 100g of product such as poultry meat which has 77g NaCl /100g (USDA 2015). Some meat products that are classified as emulsions (bologna, frankfurters or mortadella) require high concentrations of sodium chloride for water-holding capacity, emulsification, fat-binding properties and stability

(Totosaus and Perez-Chabela 2009). These properties affect the sensory quality of the meat product, therefore, reducing the sodium content can result in negative changes in sensory properties.

2.3 Sodium replacers in meat products

From a technological point of view, it has been demonstrated that sodium can be reduced in meat products. Moreover, sensorial traits have become a challenge to overcome when using non-sodium alternatives. Meat products have been recently studied in order to develop acceptable low sodium alternatives to consumers, as there are few new products that meet this condition. According to a survey performed by Hendriksen and others (2015) using evidence provided by the Dutch National Consumption Survey and Food Composition Table, if sodium from the most processed food groups was reduced by 50%, sodium intake could be reduced by 38% and low sodium alternatives can yield a 47% sodium reduction.

Substituting potassium chloride (KCl) for sodium chloride (NaCl) is one of the most widely used methods to reduce the sodium content of foods. The substitution is favorable because of similar ionic strength and chemical properties, although KCl has certain adverse effects on taste. As atomic weight of an ion increases, such as the increase from sodium to potassium, there is an increase in metallic and bitter tastes and because of this KCl results in both salty and bitter tastes and thus affects the sensory properties of the products containing KCl (Murphy and others 1981).

Using magnesium in replacement of sodium is of interest to health not only because of the relationship between sodium and hypertension, but also because magnesium is an essential element that has important roles in the body (Barat and others 2013). Like KCl, magnesium chloride ($MgCl_2$) may generate off-flavors but there are also other sensory effects to be considered. Magnesium is more electronegative than sodium or potassium (Mg^{2+} compared to Na^+ and K^+) so it will bind to polar protein groups during the salting process, thus increasing protein interactions and decreasing salt penetration. For this reason the salting process of meat products using magnesium salt substitutes would take longer than using NaCl. Alino and others (2010) studied this property on the effect of dry-cured hams containing magnesium chloride as a salt substitute. Because magnesium ions have difficulty in penetrating the muscle, there are lower salt contents in the inner parts of the ham, which could produce undesired textural properties and quality defects such as pastiness. However, the protein binding that occurs with $MgCl_2$ can be useful for meat emulsions (mortadella, frankfurters and bologna) because it contributes to the emulsion stability due to the strong protein-magnesium interactions (Horita and others 2011).

Similar to magnesium, calcium is an ideal sodium replacer because of its added health benefits. The National Institute of Health states that hypertension is not only related to high sodium intake but is also associated with poor dietary calcium intake due to its role in blood pressure regulation. Many have proposed that meat products are an excellent opportunity to increase calcium in the diet and more recently the possibility of calcium salts to replace NaCl in meats is of growing interest (Gimeno and others 2001). Like other salt replacers, calcium has certain negative effects on the sensory properties of meat products and should only be used as a partial NaCl replacer to minimize these effects. One major change in sensory quality is the textural property of meat products that use calcium salts tends to differ from those products with NaCl and be less desirable. Color is another important sensory attribute affected by the use of calcium salts, which could also affect consumer acceptance. In a sensory evaluation of color intensity of sausages with $CaCl_2$ the panelists were able to detect slightly yellow shades in the product which lowered acceptability score compared to the control (Gimeno and others 1999). Color development in meat products depends on the formation of heme pigments, and as the sodium content is decreased the color development will be lower resulting in the yellowish color as opposed to redness of sausages (Gimeno and others 2001).

Because magnesium and calcium are both divalent cations, it is expected that they exhibit similar effects on the sensory properties of food. $MgCl_2$ and $CaCl_2$ were characterized for their similar bitter and metallic taste, and the only significant difference in taste perception between the two is the slightly higher saltiness of $CaCl_2$ (Lawless and others 2003).

Non-chloride salts that contain calcium should also be used as part of a blend rather than on their own. Choi and others (2014) conducted a consumer acceptance test using frankfurter sausages with a blend of calcium ascorbate and potassium lactate (K-lactate). They explained that the use of salt replacers resulted in softer and less gummy products that were also confirmed in sensory evaluation. However, no difference was found in acceptability between the frankfurter sausages with the non-chloride salt blend and a control. Calcium salts give the best results for flavor but cannot achieve the appropriate texture of meat products due to their divalent nature, whereas potassium salts can achieve this texture but are more likely to result in off-flavors.

The use of umami-containing substances is of growing interest because of their flavor enhancing properties and addition of these flavor enhancers to foods with salt replacers may be able to counter the decline in consumer acceptance (Keast and Hayes 2011).

Milk minerals have also been proposed for use as salt enhancers in meat products. A study by Paulsen and others (2014) compared the use of these flavor enhancers to KCl alone, along with non-chloride salts (K-lactate and sodium diacetate). A control with 100% NaCl was used to compare other sodium reduced formulated sausages and a trained sensory panel evaluated differences in flavor. Both the KCl substituted sausage and non-chloride salt sausage were rated significantly higher in meat taste than the control. The sensory panel detected textural differences in the non-chloride salt sausages, which were significantly harder and less juicy than the control. Up to 40% of sodium reduction in sausages with milk minerals was possible without significant differences in flavor as observed in a descriptive analysis.

2.3.1 Potassium chloride

In general KCl is recognized as the best salt substitute for meat products because of the technological advantages it has (Horita and others 2014). However, the metallic and bitter tastes accompanied by KCl substitutes raise great concern in sensory aspects. In meat products, KCl substitution is less effective in maintaining meat functionality, thus some adjustments must be made to the formulation of low sodium products (Petracci and others 2013).

Recommended daily intakes of potassium for adolescents and adults are 4700 mg/day, while 3000 mg/day for children 1 to 3 years of age; 3800 mg/day for 4 to 8 years of age, and 4500 mg/day for 9 to 13 years. Consequently, the actual average potassium intakes in the United States are considered low, barely reaching 43% of the recommended level (USDA 2005). Increasing potassium intake by approximately 1.8 to 1.9 g/day has proved to lower the blood pressure of hypertensive people. However, an increase in the diet is not sufficient to raise the potassium intake since other indicators such as excretion of sodium and lower sympathetic nervous activity seem to be needed to reduce blood pressure using potassium (Whelton and others 1997).

Results from Soglia and others (2014) indicated that when 0.5% salt is used in poultry marinating solutions, NaCl could be replaced with a maximum amount of up to 50% KCl without any negative effects on the texture or flavor. However, they suggested a large-scale consumer test to verify this results since only a taste profile was obtained from trained panelists. Moreover, higher levels of KCl have been reported to decrease hardness in meat products. Alves dos Santos (2014) stated that a 75% KCl replacement reduced hardness, chewiness and cohesiveness of cooked sausages, although not necessarily desirable in this type of products.

KCl substitution may only be appropriate in small amounts (under 30%) so that flavor of meat products is not affected, but with lower concentrations of KCl the sodium content in the products will remain high. Because of this, researchers have looked for bitterness blockers and flavor enhancers to diminish this effect.

2.4 Bitterness blockers

Taste receptors are a short range of sensors used to detect chemicals in nutritionally or toxicologically relevant concentrations. Today's bitter taste receptors are the consequence of poisonous bitter compounds to which our ancestors were exposed. Bitter compounds are related to toxicity reason why humans usually adapt or prefer low or moderate bitterness diets (Reed and Knaapila 2010).

The use of products that can improve sensory characteristics of functional bitter compounds has led to a series of studies in search of bitterness blockers and flavor enhancers. In sodium-reduced systems, the use of enhancers is advantageous because their presence increases the perception of saltiness without adding significant salt content (Kilkast and Angus 2007). NaCl is usually substituted by KCl to lower sodium in the diet, although KCl concentrations have been limited to 50% substitution due to undesirable effects in flavor such as bitterness (Desmond, 2006).

An alternative way to block bitterness is by the application of flavor enhancers. Such substances usually posses umami flavors and offer a unique approach to reduce sodium. Soy sauce, monosodium glutamate (MSG) and monoinosinate (IMP) act by enhancing saltiness and serve as functional ingredients in addition to flavor. Mixtures of NaCl, KCl and soy enhancers have shown to produce successful sensory and functional results in sodium-reduced frankfurters (McGough and others 2012). IMP produces beefy flavors as opposed to GMP that produces oak-mushroom flavor. Functional levels of 5'-ribonucleotides required to promote enhancement may be in the 0.02–0.04% range (Löliger 2000).

MSG's sensory function lies in its ability to enhance the presence of other active compounds. Usually described as producing brothy, mouth watering sensations, low levels of MSG are detectable as equally as saltiness and higher than bitterness. Additionally, MSG contains 1/3 less sodium compared to table salt. Synergism is a key factor when using glutamates in combination with nucleotides (Löliger 2000).

As NaCl enhancer, potassium lactate (K-lactate) has been applied on fermented sausages with positive effects of boosting flavor. However, it increases hardness and cohesiveness as well as pastiness being a disadvantage in meat products (Guardia and others 2008). Sodium lactate is also commonly used in meat and poultry products as antimicrobial agent and salty flavor enhancer being able to reduce sodium.

On the other hand, yeasts extracts are natural sources that have been used as flavorings and as precursors of the formation of tastes and aromas in meat products. According to findings of Bastianello and others (2011) yeast extracts were successful in blocking bitterness of 50% NaCl substituted fermented sausages with KCl. Yeast extracts are commonly composed of 55% amino acids that are responsible of enabling acceptable sensory qualities through the increase of volatile compounds from the fermentation of amino acids.

Other bitterness blockers that have been used in combination with KCl are the amino acids, L-lysine and L- arginine. When added to reduced sodium foods, their salty taste is enhanced and provide a significant amount of dietary protein (Kilkast and Angus 2007). Among other amino acids used as bitter blockers in meats are glycine and taurine.

2.4.1 Glycine

Glycine, the smallest amino acid, reduces water activity and can improve microbiological stability. While it has been demonstrated that amino acids by themselves don’t have strong taste individually, their taste is intensified in presence of nucleotides (Kuninaka 1960).

T2R sites are a subfamily of G (gustducin) protein taste receptors that are activated by bitter compounds. Pydi and others (2014) applied low molecular weight compounds such as glycine along with other amino acids and peptides (L-ornithyl-alanine (OA) and gamma-aminobutyric acid (GABA)) to block bitterness of quinine at these receptor sites. OA has been demonstrated to act as a bitterness blocker of potassium salts. To determine its efficacy, glycine was used first as a ligand binder providing positive results in combination with GABA which can be used in the elimination of bitter taste in dietary foods.

Tamura and others (2014) has demonstrated that glycine and its ester are better seasonings than metal chloride salts (KCl) or citric acids. When compared to glycine, KCl has an inferior quality of saltiness, hence having poor enhancing effect on NaCl, and bitterness is exhibited. Glycine has been used as NaCl substitute in 20% substitution levels of fermented sausages and substitutions of 30-40% in dry cured loins, respectively (Gou and others 1996).

Other amino acids like Lysine, which is considered essential, at a concentration of 0.313% of the final product in combination of other amino acids and nucleotides (GMP, IMP, and others) can improve sensory characteristics and maintain physical properties of 50% NaCl substituted fermented cooked sausages with potassium chloride (Bastianello and others 2011).

2.4.2 AMP

Non- protein substances in meat composed by purine or pyrimidine linked to ribose, adenine, guanine and others are called ribonucleotides. AMP is a structural component of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and can be found in all living organisms. In metabolism, cyclic AMP is used for glucose mobilization, energy and muscle concentration (William and others 2007). Crustaceans, mollusks and some vegetables are rich in AMP that aids the formation of IMP (Löloger 2000). AMP, IMP and GMP have been used lately to develop new products that impart flavor due to their umami properties, thus blocking the bitter taste of foods (Aramouni and Deschenes 2015). AMP is often used in meat and poultry soups as a flavor enhancer or as additives for specific nutritional purposes.

AMP works as a bitterness blocker specifically by inhibiting the activation of the transducin-like protein gustducin. Ming and others (1999) developed *in vitro* studies where AMP inhibited activation of transducin by taste membranes and blocked behavioral and gustatory nerve responses casued by denatonium benzoate and quinine hydrochloride bitter compounds. Results suggested that AMP acts as a cell-surface receptor. They also implied that certain artificial sweeteners might inhibit taste receptors, behavioral and gustatory nerve responses on gustducin, which is known as bittersweet mixture suppression.

Recent research using ribonucleotides to mask bitter flavors of KCl in sodium substituted meat formulations have been developed. Bastianello and others (2012) added lysine, GMP and IMP as bitterness blockers in low sodium sausages using KCl. Results indicated that adding a mixture of these nucleotides (in concentrations of 0.06% of the final product) along with lysine resulted in suppressing sensory defects caused by 50% NaCl substitution. However, individual inclusions didn't decrease KCl off flavors. A similar research performed in sausages using lysine, taurine, GMP and IMP with 50% sodium replacement with KCl (Batianello and others 2012). Researches found that a mixture of amino acids and 5'-ribonucleotides (added at 0.03% of the product) was efficient in suppressing bitterness flavors by providing increased saltiness and masking the metallic aftertaste of potassium.

In addition to flavor, AMP (10-40 mM in 50 mL solution) contributes to meat quality improvement as proposed by Wang and others (2016) when employing solutions with AMP to duck breast meat. Conversion of AMP to IMP, dissociation of actomyosin, and fiber shrinkage prevention are some of the results obtained from the nucleotide inclusion in this study

CHAPTER 3. MATERIALS AND METHODS

3.1 Chicken breast marination

Boneless skinless chicken breasts without marination were obtained from a local retail store (Winn Dixie, Baton Rouge, LA), packed and sealed in polystyrene trays (Figure 1).

Figure 1. Boneless skinless chicken breasts obtained from retail store (Winn Dixie, Baton Rouge, LA)

Multiple packs of samples purchased for each repetition were selected from the same "sale by date" period of time. Chicken breasts were marinated using commercial procedures at the Louisiana State University (Baton Rouge, LA) meat processing plant. Marination was performed by needle injection using a manual injector (Model 5318 Home 1 gallon sprayer, Project Source™ Cincinnati, OH) immediately after purchase. A system of injection heads (4-needle) was manually attached, which are commonly used for brine injections in poultry industry (Figure 2 and 3). Two individual repetitions were performed for physicochemical as well as for sensory analysis. Each batch was composed of 39 breasts for physicochemical analyses and 52 breasts for sensory analyses. Batches were evenly divided into the 13 treatments (13 marinade solutions, see Table 1 for reference) for both analyses, leaving 3 and 4 breasts per treatment for physicochemical and sensory analyses, respectively. Marinated chicken breasts were packed per treatment using a vacuum sealer (Model L10, Turbovac, Hertogenbosch, The Netherlands) in commercial plastic bags (3 mil/75 micron, nylon/polyethylene; UltraSource, Kansas City, MO). Packed treatments were stored for 24 h at 4 °C.

Figure 2. Manual injector and a 4-needle set attached used to inject chicken breasts (Model 5318 Home 1 gallon sprayer, Project Source™ Cincinnati, OH).

Figure 3. Method for chicken breast injection performed manually using a home sprayer.

3.1.1 Solution preparation

Chicken breasts were marinated by injection with 20% solution (based on meat weight, w/w) according to industry standards and percentages commonly applied in previous studies (Lee and others 2012; Broadway and others 2011). Total salt content in the brine solution injected was limited to 0.75% (w/w) of the solution (Table 1). A salt mixture of NaCl (Morton, Illinois, USA) and KCl (Spectrum® P1255, NJ, USA) was added based on this percentage. The salt mixture was managed independently from the bitter blocking mixture since previous studies have shown that mixtures of salts and amino acids were perceived less salty than NaCl by itself (Waimaleongora, 2002). Saha and others (2009) found that consumers rated the saltiness perception of fillets marinated with 0.5% and 0.75% of the final product as just about right (JAR).

Table 1. Salt and bitterness blocker mixture per treatment.

Treatment*	NaCl %	KCl%	Glycine%**	AMP%**
A	100	0	0	0
B	50	50	0.1	0.01
C	50	50	0.1	0.02
D	50	50	0.2	0.01
E	50	50	0.2	0.02
F	25	75	0.1	0.01
G	25	75	0.1	0.02
H	25	75	0.2	0.01
I	25	75	0.2	0.02
J	0	100	0.1	0.01
K	0	100	0.1	0.02
L	0	100	0.2	0.01
M	0	100	0.2	0.02

*A mixture of NaCl+KCl is 0.75% w/w based on the weight of the solution injected.
**A mixture of Glycine (0.1 or 0.2%) plus AMP (0.01 or 0.02%) based on the weight of the solution injected.

Sodium substitutions with KCl at levels of 50% or higher have been demonstrated to have a decreasing saltiness perception effect as reported by Soglia and others (2014). This is when the addition of bitterness blockers becomes beneficial. Bitterness blockers such as glycine (U.S.P. F.C.C., JT Baker, PA, USA) and AMP (Adenosine-5'–monophosphate free acid, Zhen-Ao GROUP Co., Ltd., China) were also added to reduce the sensory bitterness of KCl. Wang and others (2015) marinated duck fillets with solutions containing concentrations of 0.1, 0.2, 0.3 and 0.4% AMP. This demonstrated that at increasing AMP concentrations, tenderness increased. Conversion of AMP to IMP in meat has been known to contribute to meat flavor. Addition of these flavor enhancers allows the inclusion of higher levels of KCl in breast meat, which have been poorly studied due to the negative flavor effects. Percentages of AMP evaluated in the present study were 0.01 and 0.02% of the brine solution. Glycine has also been known to have an effect on enhancing saltiness perception and suppressing bitterness as shown by Gou and others (1996) who evaluated sodium substitution with potassium lactate and glycine in cured pork loins and fermented sausages. Glycine percentages in the present study were limited to 0.1 and 0.2% of the brine solution based on previous studies (Alves dos Santos 2014 and Bastianello and others 2011) that used Lysine and Taurine at 0.075% and 1%. Alves dos Santos (2014) evaluated these amino acids in combinations with IMP/AMP and monosodium glutamate (MSG) on the sensory qualities of fermented cooked sausages with sodium reduced up to 75%. Phosphate (Brifisol® 450Super, BK Giulini Corp., Simi Valley, CA) was limited to 0.45% (w/w) of the brine solution since it is limited to 0.5% of the final product (USDA, 2016) when used to reduce moisture loss, flavor protectant or accelerator (Tarté 2009). Proportions of total bitterness blockers (both AMP and glycine) were based on the total salt (mixture of KCl and NaCl) added and ranged from 1:3.4 to 1:6.8.

3.1.2 Cooking procedure

After 24 h of marination, breasts were placed in individual aluminum foil pans per treatment for baking. Cooking was undergone in a Comination oven (CTP6-10, Alto-Sham Combitherm, Wisconsin, USA) using steam and convection heat at 93 °C (200°F) until an internal temperature of 76°C (168 °F) was reached. A probe thermometer, included in the oven, was inserted into the middle of the thickest part (about 2.5 cm) of the breast to confirm temperature. According to USDA (Food safety.gov 2012), the minimum internal cooking temperatures for poultry breasts is 74 °C (165 °F), since color or appearance is not considered an indicator of doneness. Complete doneness took a total of 60 minutes. Low cooking temperatures (below 100 °C) result in slow heating, minimal Maillard reaction and appearance of boiled meat, which contribute to a more uniform appearance than browning (Bejerholm and others 2014). After internal temperature was reached, the samples were cooled under a hood with current airflow to 20 °C during a resting period of 30 min. Resting has been known for a better redistribution of juices in the meat before cutting. Further processing such as cutting and/or physicochemical analysis was performed after resting for 20 minutes.

3.2 Analyses

3.2.1 Texture

Tenderness is considered a critical characteristic in meat products (Bourne, 1982). The American Meat Science Association (AMSA, 2015) recommended the Warner Bratzler as the standard method for tenderness measurement in meat products. This method shears or cuts the meat product into separate pieces reporting a maximum force, which is considered a measure of meat tenderness (Kilkast 2004).

Tenderness of 78 cooked chicken breasts was instrumentally measured using a Warner Bratzler shear force with a texture analyzer (model TA-XTPLUS Texture Technologies Corp., New York, USA). A 5 kg load transducer and a cross speed of 200 mm/min was applied. The weight calibration was performed before measurements with a 2 kg weight standard and the height calibration was set at 55 mm. The set up used included: pretest speed of 2-mm/ sec, test speed of 3.30 mm/sec and post-test speed of 10 mm/sec. A 5-kg load cell has been proven to draw results of maximum 50N or 5.1 kg, which is in accordance with broiler breast shear values found in research performed by Lopez and others (2012) and Saha and others (2009). The waiting time between each shear was 10 seconds.

The sampling method for texture evaluation was performed as described by Zhuang and Savage (2009) with slight modifications, which mainly involved the separation of 3 middle section 1.9 cm strips (Figure 4).

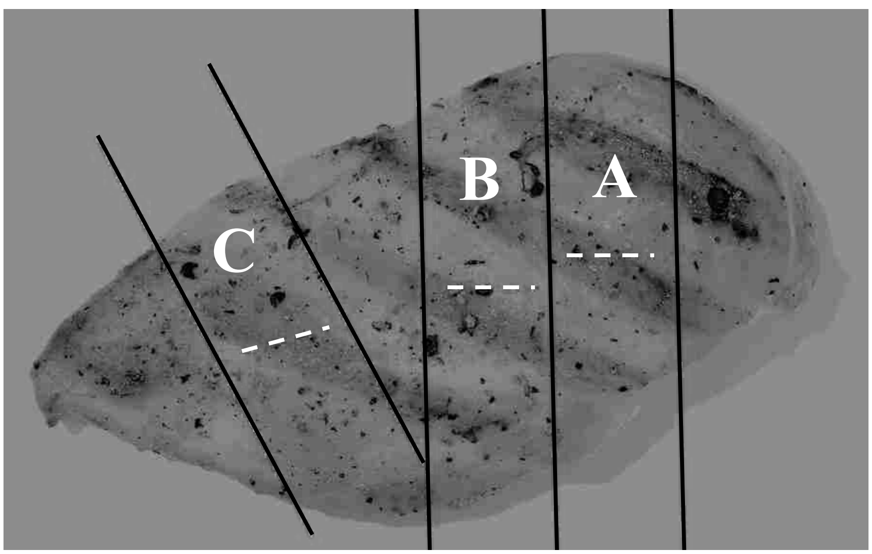

Figure 4. Diagram for sectioning the cooked chicken breast to obtain samples of 3 x 1.9 x 1.9 cm strips for Warner-Bratzler shear measurements

*White dash lines represent the middle section where the shear was performed.

Strips of 3 x 1.9 x 1.9 cm (length x width x height) wide were aligned parallel to the muscle fibers and adjacent to the cranial end when separated. Each strip was sheared in the center and heights at each shear point were standardized to 1.9 cm. Length of the strips were uniformly cut to measure 3 cm. Hardness and toughness were obtained and used as predictors for tenderness assessment. Three measurements were taken from each of the three chicken breasts for a total of 9 measurements per treatment. Averages were calculated and were used as a representative instrumental tenderness per treatment.

3.2.2 Color

Color is the first attribute observed by consumers and is usually an indicator of the product's quality. This study measured color instrumentally in 4 different times using a handheld Minolta colorimeter model CM-508d Series (Osaka, Japan) with a 10º standard observer and D65 illuminant. CIELAB is an approximately uniform color scale and is organized in a cube form space where L*axis runs from top to bottom and a* and b* axes run horizontally from left to right perpendicular to each other. The parameters measured were L *, a *, b* (HunterLab 2007). Prior to repetition of analysis the equipment was calibrated with a white standard (values for the white standard tile were L* = 95.3, a* = −0.4, b* = 4.7) and blank calibration. Fletcher and others (2000) determined a significant relation between raw pH and cooked color of poultry meat. While raw color doesn't directly affect cooked color, darker color ranges found in raw chicken may influence consumer acceptance. L* values represent whiteness (100) or blackness (0) measurements on the axis. The a* values correspond to red-green hues with positive corresponding to redness and negative values to greenness. The b* axis corresponds to yellow-blue hues, being positive values equal to yellowness and negative representing blueness (Sharma 2003).

Color measurements were made directly on the surface of the chicken breasts on two locations of the dorsal (upper) and ventral (bottom) sides. Two breasts per treatment were measured for a total of 4 dorsal and 4 ventral measurements per treatment. The times in which color was measured were: before injection (right after unpacking), after injection, 24 h after injection (under refrigeration at 4 °C) and on cooked samples.

3.2.3 pH

Quality characteristics such as color and water holding capacity (WHC) have been related to pH changes in meat. Although problems in poultry related to raw meat color are considered sporadic but sometimes related to raw meat pH (Fletcher 1999). Post mortem ultimate pH may have an influence in water holding capacity, consequently at higher ultimate pH less moisture will be lost (Lawrie, 1998).

A handheld portable pH meter (Model 160, Hach, Loveland, CO) with a probe and built-in thermometer was used. Calibration with 4 and 7 pH standards was performed before each repetition. Measurements were taken until readings were stable. Sampling was performed four different times as with color measurements; before injection (right after unpacking), after injection, 24 h after injection (under refrigeration at 4 °C) and on cooked samples. Two locations were punctured at both ends of the breast. Two breasts were evaluated per treatment for a total of four measurements per treatment per time measured.

3.2.4 Moisture

Moisture present in a product can determine its stability and quality and varies widely depending on the type and characteristics of the product. Upon cooking, moisture and some fat are lost, while protein, ash and cholesterol are concentrated (Owens and others 2010) For moisture content determination using oven drying methods, the sample is heated under specific conditions. Weight loss over a period of time, using a simple equation shown below (Equation 1) is used to calculate the moisture content of the sample (Nielsen 2010).

This study performed moisture content evaluation using a conventional oven, which is an official AOAC method No. 948.12 (AOAC 1990). A VWR forced air safety oven (Model 1330FMS, VWR Signature™, Oregon, USA) was set to 100 °C a day before the samples were analyzed to dry crucibles. Once the crucibles were dried the samples were prepared. Samples were homogenized by blending 5 g from three breasts per treatment using a commercial blender (Model: CB15V, Waring®, Conneticut, USA). Two 3 g samples were weighed into the crucibles using an XS precision balance (XP6002S, Mettler Toledo, Columbus OH). Crucibles were properly handled using tongs and weights were recorded. Samples were then placed in the conventional oven at 100 °C for 24 h. After this period of time, crucible weight plus dried sample weight was recorded and calculation for moisture percentage was made. Moisture sampling was performed in duplicates per treatment.

Equation 1. Moisture calculation.

$$\% \text{ Moisture} = \frac{(\text{wt of wet sample} + \text{crucible}) - (\text{wt of dried sample} + \text{crucible})}{(\text{wt of wet sample} + \text{crucible}) - (\text{wt of crucible})} \times 100$$

3.2.5 Water holding capacity (WHC)

This study measured different variables to determine WHC. Initial weights of three chicken breasts per treatment were taken before injection using a precision balance (SG32000 Balance, Mettler Toledo, Columbus OH). The method employed consisted of measuring gravimetrically right after injection as performed by Lopez and others (2012). Sampling units for marinade uptake were the same as used for the initial weights.

Equation 2. Marinate uptake.

$$\text{Uptake \%} = \frac{\text{Raw chicken breast immediately after injection} - \text{Raw chicken breast before injction}}{\text{Raw chicken breast before injection}} \times 100$$

After injection, samples were packed and refrigerated at 4 °C for 24 h. Purge loss was measured gravimetically using a precision balance according to the calculation methods of Soglia and others (2014). The same sampling units used for marinade uptake were subsequently used for purgeloss.

Equation 3. Purge loss.

$$\text{Purge loss \%} = \frac{\text{Raw chicken breast immediately after injection} - \text{Raw chicken breast 24 h post injection}}{\text{Raw chicken breast immediately after injection}} \times 100$$

Cook loss was measured gravimetrically with an analytical balance to evaluate WHC after cooking based on the methods of Soglia and others 2014. Cooking of the chicken breast took place in a combination oven at 93 °C (200 °F). An internal temperature of 76 °C (168 °F) was reached in 60 minutes for complete doneness of the chicken breasts after which the samples were cooled to 20 °C for a period of 30 min. After cooling the samples were weighed in an analytical balance. The samples used to perform cook loss were the same samples used throughout the study for all water holding capacity measurements. According to Lawrie (1998), moisture loss percentage of cooked meat to 80 °C should be around 14%.

Equation 4. Cook loss.

$$\text{Cook loss \%} = \frac{\text{Raw chicken breast 24h post injection} - \text{Cooked chicken breast breast}}{\text{Raw chicken breast 24h post injection}} \times 100$$

3.2.6 Preliminary Online Emotion survey

In addition to physicochemical analyses, this study measured the emotions elicited by the consumption of chicken breast injected with low sodium brines. Researches have recently been including emotions as a factor to determine food choices made by consumers since food affects the way we feel. In a preliminary study, 68 individuals completed an online survey (appendix A) administered through Qualtrics (Qualtrics, Utah, USA). Individuals chose the emotions that described their feelings about baked chicken breast consumption. A check-all-that-apply list (Table 2.) was provided with the aim that it would produce meaningful and condensed results.

Table 2. A list of 39 emotion terms for prescreening.

1	Active	21	Loving
2	Adventurous	22	Merry
3	Affectionate	23	Mild
4	Aggressive	24	Nostalgic
5	Bored	25	Peaceful
6	Calm	26	Pleased
7	Daring	27	Pleasant
8	Disgusted	28	Polite
9	Eager	29	Quiet
10	Energetic	30	Satisfied
11	Enthusiastic	31	Unsafe (regarding nutrition facts)
12	Free	32	Steady
13	Friendly	33	Tame
14	Glad	34	Tender
15	Good	35	Understanding
16	Good-natured	36	Warm
17	Guilty	37	Whole
18	Happy	38	Wild
19	Interested	39	Worried
20	Joyful		

*From EsSense® profile (King and Meiselman 2010).

The shortened list of terms was subsequently included in the consumer study. The list of emotion terms used was obtained or adapted from the EsSense® profile predetermined list validated by King and Meiselman (2010). Terms are presented in alphabetical order so consumers get acquainted easily. King and Meiselman (2010) reported that the order presented does not have significant effects on results. They also proved that a crucial factor in measuring consumer emotions linked to products is whether the person consumes the product. Therefore, an initial question of consumption of chicken breasts was included. If consumers denied consumption, the survey automatically ended.

3.2.7 Consumer acceptance test

The sensory characteristics of the 13 treatments of injected breast fillets were evaluated through a consumer acceptance test. Two hundred sixty (260) untrained consumers were randomly chosen from Louisiana State University (LSU), Baton Rouge Campus. All the participants met with the following criteria: 18 years of age or older, not allergic to chicken, NaCl, potassium chloride (KCl), phosphates, glycine or AMP; and willingness to participate for approximately 7-10 minutes to complete the survey. Consumers were required to read and sign a consent form (IRB # HE 15-9) approved by the Louisiana State University Institutional Review Board. The survey was completed electronically using the Compusense (Compusense® five, Release 5.6 with Compusense Inc., Guelph, Ontario) at the Sensory laboratory located in the Animal and Food Science laboratories building of LSU.

Demographic information was requested such as gender, race and age. Screening questions, using a binomial scale (yes/no), regarding usual consumption of salt and consideration of reducing salt in the diet were asked. Further, consumers rated color, aroma, flavor, tenderness, juiciness, saltiness, bitterness and overall liking of the product based on the 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). The attributes of tenderness, juiciness, saltiness and bitterness were rated as well using a Just about right scale (JAR) (for tenderness: 1=not tender enough, 2=JAR, 3=too tender; for juiciness: 1=not juicy enough, 2=JAR, 3=too juicy; for saltiness: 1= not salty enough, 2=JAR, 3=too salty; for bitterness: 1= not bitter, 2=moderately bitter, 3=too bitter) since they are the most important attributes related to chicken breast marination. Overall liking, a shortened list of emotions (good, happy, pleasant, satisfied, worried, unsafe and guilty) and purchase intent were evaluated before and after additional information about the reduction of sodium in marinades of the chicken breast were evaluated (Table 5 and 6). The content claims presented per treatment are detailed in Table 3. These nutrient content claims were calculated based on USDA natural sodium content in skinless boneless chicken breasts and adding up the concentrations of total salt added per treatment.

Table 3. List of nutrient content claims for sodium content per treatment and KCl level.

Trt*	KCl %	Content claim**
A	0% (Control)	This product was prepared with similar sodium per serving than some commercial products.
B-E	50%	This product was prepared with 22%-35% less sodium per serving than some commercial products.
F-I	75%	This product was prepared with 34%-45% less sodium per serving than some commercial products.
J-M	100%	This product was prepared with 66%-72% less sodium per serving than some commercial products.

*Refer to Table 1 for treatment descriptions.

**Content claims were calculated based on sodium added compared to comercial information available online for marinated chicken breasts.

Comparable data was obtained from commercial products enhanced with marinade solutions. According to the FDA (2013), to label a product as “low sodium” it must contain at least 25% less sodium per RACC (Recommended amount customarily consumed), which in this case were 114 g of ready to cook chicken breast. It can be inferred from the previous Table that all the levels of substitution (50-100%) can be labeled as “low sodium” taking into account that sodium was reduced from 35% up to 72% when compared to commercial products (180 mg Na/114 g chicken).

Purchase intent was evaluated with a binomial scale (yes/no). Emotions were rated on a 5-point scale (1 = not at all, 2 =slightly, 3 = moderately, 4 = very much, 5 = extremely). Using the Balanced Incomplete Block Design (BIB) Plan 11.21, t = 13, k = 3, r = 6, b = 26 $e_{2=}$ 0.72, λ^{I} = 1 (Cochran 1957), the consumers were presented with 3 out of the 13 chicken breast treatments. These formulations were randomly coded with a 3-digit number and a total of 60 observations per treatment were evaluated. The consumers were given two cubic samples of 2 x 2 x 2 cm^3, placed in lidded transparent 2 oz. cups (Pro Pack, Houston, TX). Room temperature drinking water (Nestle Waters, Greenwich, CT) and unsalted crackers (Nabisco, Northfield, IL) were provided for palate cleansing between sample testing.

3.3 Statistical analysis

Data for the physicochemical characteristics of the chicken breast treatments were analyzed using the Glimmix procedure with one way ANOVA and the Post-Hoc Tukey's Studentized range test ($\alpha = 0.05$) to describe the changes of these characteristics over time for each treatment (for variables: color and pH, before injection (1), after injection (2), 24 h after storage (3) and cooked (4)) (See appendix B for SAS codes of statistical analysis). The null hypothesis (Ho) of the study is that no differences existed (for sensory acceptance or physicochemical characteristics) among the treatments. While the alternate hypothesis (Ha) is that differences exist (for the same parameters) among the salt substitute treatments.

For the consumer acceptability test, the analysis performed were one way ANOVA, and Post-Hoc Tukey's Studentized range test ($\alpha = 0.05$) to detect and group the sensory characteristics of the different treatments. The MANOVA and descriptive discriminative analysis (DDA) were done to determine overall differences among the 13 chicken samples. DDA helped determine which attributed contributed to overall product differences. A t test was performed to determine differences before and after additional information had been given. Attributes influencing purchase intent were assessed using logistic regression and the Mc Nemar tests to determine changes in purchase intent. JAR was analyzed using a penalty analysis. These analyses were performed using the Statistical Analysis System 9.3 (SAS NC, USA).

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Pre screening of emotion terms

Pre screening of emotion terms from the EsSense® profile list was performed during the preliminary online survey. A shortened list of emotion terms was obtained by selecting the positive and negative emotions with frequencies greater than 40% as shown on Table 4.

Table 4. Response frequency for determination of shortened list of emotion terms presented during sensory evaluation.

Emotion Term*	Response (%)	Emotion Term	Response (%)
Active	32%	Loving	3%
Adventurous	18%	Merry	3%
Affectionate	7%	Mild	11%
Aggressive	11%	Nostalgic	7%
Bored	18%	Peaceful	3%
Calm	39%	Pleased	70%
Daring	8%	Pleasant	46%
Disgusted	37%	Polite	0%
Eager	3%	Quiet	8%
Energetic	29%	Satisfied	68%
Enthusiastic	15%	Unsafe (regarding nutrition facts)	93%
Free	15%	Steady	0%
Friendly	18%	Tame	8%
Glad	10%	Tender	14%
Good**	97%	Understanding	7%
Good-natured	28%	Warm	14%
Guilty	40%	Whole	17%
Happy	53%	Wild	0%
Interested	32%	Worried	90%
Joyful	0%		

*From EsSense® profile.
**Emotions with > 40% frequencies are selected.

A total of 68 respondents determined the most significant emotions elicited by the consumption of baked chicken breast were good, happy, satisfied and pleased for positive emotions. For negative emotions worried, unsafe and guilty were the most important.

4.2 Sensory characteristics of marinated chicken breasts

4.2.1 Consumer study

Mean scores and standard errors are reported in Table 5 for color, aroma, flavor, tenderness, saltiness and bitterness. Generally all attributes had a mean score greater than "4" with most of them having mean scores of "5" and "6". This means mostly the products were "neither liked nor disliked" and "liked slightly" for most of the attributes.

Table 5. Mean Acceptability Scores for color, aroma, flavor, juiciness, tenderness, saltiness and bitterness.

Trt*	Color	Aroma	Flavor	Juiciness	Tenderness	Saltiness	Bitterness
A	6.03^{a**}	5.32^{NS}	5.35^{NS}	5.34^{NS}	6.05^{NS}	5.26^{NS}	5.37^{NS}
B	5.20^{cd}	5.37	5.33	5.84	6.19	5.03	5.46
C	5.63^{abc}	5.29	5.66	5.87	6.03	5.08	5.48
D	5.62^{abc}	5.30	5.82	5.85	6.21	5.19	5.36
E	5.38^{bcd}	4.85	5.17	5.59	5.94	4.88	5.49
F	5.11^{d}	5.13	5.47	5.48	5.93	5.12	5.50
G	5.44^{bcd}	4.86	5.63	5.64	5.75	4.94	5.24
H	5.40^{bcd}	5.25	5.36	5.31	6.03	4.85	5.34
I	5.06^{d}	5.10	5.14	5.63	5.94	4.60	5.13
J	5.71^{ab}	5.26	5.54	5.57	6.20	5.16	5.41
K	5.25^{bcd}	4.94	5.24	5.17	5.92	5.11	5.51
L	5.35^{bcd}	5.13	5.32	5.46	5.92	5.15	5.42
M	5.50^{bcd}	5.04	5.45	5.61	5.93	4.96	5.21
Std. Error	0.19	0.18	0.23	0.23	0.21	0.21	0.17

*Refer to Table 1 for treatment descriptions.
**Means and standard errors with the same letters are not significantly different (P >0.05). NS: Not significant.

The different formulations showed no significant differences for the attributes aroma, flavor, juiciness, tenderness, saltiness and bitterness. However, color presented differences between treatments with the control (Treatment A) (100% NaCl) having the highest score with a mean score of "6.03".

Overall, color was rated as "neither like nor dislike" for all treatments except the control which was slightly different, although significant. However, acceptability differences in color relate to those observed by Bastianello and others (2011). A treatment with 50% NaCl and 50% KCl plus a mixture of amino acids and nucleotides showed to have lower color acceptability than the control, and this difference was linked to lower instrumental values for a* (redness). In this study, instrumental color showed significantly lower values for L* a* and b* values for treatment F only (Table 14), meaning this treatment was instrumentally darker, less red and less yellow. Luminosity has demonstrated to be the most informative parameter responsible for color change and acceptability. We can conclude that differences in color liking can be linked to minor color differences found instrumentally. Likewise, variability among panelists can't be disregarded due to their different experience and expectation.

Results are in accordance with Lee and others (2012) where a trained panel found no significant differences in sensory attributes among breast fillets marinated with different sodium substituted marinades (25-100% substitution). Similar results were also found in 25 and 50% KCl substitution of frankfurter sausages with poultry meat where no differences were found in appearance and aroma (Horita and others 2014). The lack of differences in flavor, bitterness, aroma, saltiness and tenderness might be due to the low concentrations of salt added (0.75%) compared to products containing 2-3% total salt content such as sausages (Bastianello and others (2011); Alves dos Santos (2014). Marinated chicken is sold commercially with the purpose of further preparation such as seasoning or basting before cooking.

4.2.2 Nutrient content claim effect in overall liking and emotions

Overall liking was evaluated last on the list of attributes rated on the 9-point hedonic scale. Subsequent evaluation of emotions towards consumption of the baked chicken breast samples was performed on a 5-point emotion scale (Table 6).

Table 6. Mean overall liking and emotion scores and t tests performed to compare responses before and after sodium nutrient content claims.

Trt *	Overall liking		Good		Happy		Pleased		Satisfied		Unsafe		Worried		Guilty	
Time	Before**	After ϕ	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
A	5.52^{ns}	5.40^{NS}	2.53^{ns}	$2.41^{NS\rho}$	2.44^{ns}	2.33^{NS}	2.52^{ns}	2.46^{NS}	2.54^{ns}	2.51^{NS}	1.37^{ns}	1.62^{A}	1.40^{ns}	1.62^{A}	1.31^{ns}	1.65^{A}
B	5.61	5.54	2.72	2.80	2.60	2.57	2.80	2.72	2.55	2.66	1.48	1.41^{AB}	1.49	1.39^{B}	1.31	1.50^{AB}
C	5.80	5.80	2.78	2.89	5.50	2.80	2.54	2.87	2.66	2.78	1.48	1.37^{BC}	1.37	1.27^{B}	1.31	1.34^{BC}
D	5.77	5.81	2.81	2.94	2.58	2.72	2.69	2.75	2.69	2.80	1.40	1.35^{BC}	1.37	1.30^{B}	1.21	1.15^{C}
E	5.41	5.38	2.60	2.77	2.52	2.64	2.60	2.63	2.62	2.59	1.46	1.41^{ABC}	1.32	1.34^{B}	1.25	1.32^{BC}
F	5.43	5.48	2.48	2.74	2.28	2.52	2.51	2.62	2.51	2.62	1.39	1.35^{BC}	1.28	1.26^{B}	1.11	1.34^{BC}
G	5.51	5.39	2.60	2.65	2.48	2.55	2.57	2.55	2.68	2.70	1.34	1.31^{BC}	1.51	1.16^{B}	1.22	1.34^{BC}
H	5.29	5.44	2.41	2.64	2.32	2.41	2.30	2.54	2.41	2.54	1.70	1.40^{ABC}	1.42	1.37^{B}	1.36	1.29^{BC}
I	5.42	5.56	2.52	2.87	2.42	2.67	2.50	2.78	2.52	2.70	1.40	1.33^{BC}	1.46	1.33^{B}	1.25	1.25^{C}
J	5.72	5.86	2.66	2.96	2.51	2.70	2.68	2.93	2.63	2.83	1.41	1.20^{BC}	1.38	1.19^{B}	1.38	1.25^{C}
K	5.14	5.40	2.57	2.79	2.27	2.46	2.38	2.70	2.45	2.53	1.40	1.17^{C}	1.41	1.19^{B}	1.23	1.15^{C}
L	5.56	5.69	2.53	2.72	2.33	2.70	2.50	2.77	2.59	2.86	1.49	1.22^{BC}	1.40	1.20^{B}	1.24	1.29^{BC}
M	5.46	5.68	2.64	2.87	2.35	2.64	2.58	2.91	2.60	2.79	1.34	1.32^{BC}	1.35	1.34^{B}	1.23	1.26^{C}
Std. Error	0.21	0.22	0.11	0.12	0.12	0.12	0.12	0.13	0.12	0.13	0.09	0.08	0.08	0.08	0.07	0.08

*Refer to Table 1 for treatment description.

** Means (before) in each column are not significantly different (P >0.05) among treatments. ns= not significant.

ϕ Means (after) in each column with same capitalized letters are not significantly different among treatments (P > 0.05). (NS)= not significant.

ρ Bold means and letters are significantly different between before vs. after nutrient content claim.

In addition, all of these attributes were evaluated before and after presenting a sodium nutrient content claim related to the sample being presented. Treatments were not significantly different ($P \geq 0.05$) from one another regarding overall liking before or after additional information was provided.

All emotions (positive and negative) were not significantly different among treatments for rating performed before the nutrient content claim was specified. But, negative emotions (Unsafe, Worried and Guilty) showed significant differences among treatments after the nutrient content claims. This shows that consumers experienced slightly more unsafe, worried and guilty emotions after consuming the control than any of the other treatments with some lower sodium levels. Claims linked to treatments with 100% sodium substitution (J, K and M) had a higher decrease in guilty emotion.

T-tests were performed to compare emotion response evaluated before and after the nutrient content claim. Treatments G-M (75 and 100% NaCl substitution) presented a significant increase in rating of good, happy and pleased emotions. However, the increase wasn't large enough (<1 point in scale) to change from "slightly" to "moderately" (from 2 to 3 in the 5 point emotion scale). A significant increase for treatments G, J, L and M was observed for "satisfied" emotion, however an increase (from 2 "slightly" to 3 "moderately") in the emotion scale wasn't observed. A decrease in "good" emotion was observed when the content claim for Trt A (control, 100% NaCl) was presented.

Contrastingly, negative emotions (worried and guilty) increased significantly after the nutrient claim for Trt A (control) was presented. Consumers felt significantly less unsafe when presented with treatments G, J and L, with 75% and 100% NaCl substitution respectively. Worried emotion significantly decreased for treatments C, F, J and L (Trts under 50, 75 and

100% NaCl substitution). Similarly a numerical decrease was observed in other treatments except the control, while not being significant. Guilty emotion was not significantly (positively or negatively) affected by the sodium nutrient content claims.

These results (Table 6) demonstrate the effect of a sodium nutrient content claim on the emotions of consumers. Generally negative emotions will decrease and positive emotions will increased if a reduction in a risk factor is stated to consumers. Schouteten and others (2015) evaluated the impact of reduced sodium statement on emotions and overall liking elicited by cheese consumption. Similar to our findings, overall liking results showed no significant differences in acceptance between the control and reduced salt-labeled cheese. This can be explained by the fact that consumers believe that reduced salt products would not taste good (Verbeke 2006). If this hypothesis is true, our results of “neutral acceptability” and no significant increase in overall liking of the product may be related to the fact that low salt products aren’t perceived as tasty as saltier options. After tasting the product the nutrient content claim had little impact on consumer emotion, since few differences were observed by Schouteten and others (2015). They stated that only the emotion term “glad” was significantly increased for the control. Although, this can relate to our study where a significant increase in emotions after the sodium nutrient content claim was presented yet the difference wasn’t large enough to increase in emotion scale similar.In a similar study, Schouteten and others (2016) evaluated the change in liking of insect, plant and meat based burgers under blind, expected and informed conditions. Low liking was observed for insect based burgers before health information was presented. However, under informed conditions, insect based burgers were perceived more nutritious than meat based. Thus, this explains that consumers under informed conditions may be willing to compromise taste if a health statement is stated. However, this was not the case in this study because overall liking remained unchanged and barely increased for treatments with the highest KCl substitution. An interesting fact is that the low sodium treatments were equally liked compared to the control and remained in the "neither like nor dislike" acceptability score. Knowing that the taste of ready to cook chicken breasts is considered flat may be the reason for these results. Likewise, Verbeke (2006) determined that consumers associate nutrient content claims of healthier food options to lack of taste explaining why information about low sodium chicken breasts didn't affect overall liking.

4.2.3 Purchase intent using the McNemar test statistic

Purchase intent of sodium substituted marinated chicken breasts was measured in this study after acceptability of sensory attributes, JAR scales and emotions. As with emotions and overall liking, purchase intent was evaluated after the sodium nutrient content claim was presented. A McNemar's statistical procedure (Table 7) was used to evaluate significant effects of the different nutrient content claims on the purchase decision.

Asymptotic probability was used to determine significance of purchase intent, as it is more discriminative when evaluating large number of treatments (>10). According to McNemar's test, only Trt I (34-45% Na reduction) increased significantly in positive purchase intent. However, most of the treatments showed a numerical increase with the exception of Trt F (34-45% Na reduction), which purchase intent remained unchanged despite the given sodium nutrient content claim statement. Treatments B (22-35% Na reduction), H (34-45% Na reduction) and J (66-72% Na reduction), although not significant at $\alpha = 0.05$, almost reached a significant increase in purchase intent. If an $\alpha = 0.1$ were to be used, these treatments could have had significantly increased the purchase intent of marinated chicken breasts with lower sodium than commercial products. Treatment A (similar sodium as some commercial products), contrastingly, had lowered purchase intent after sodium nutrient content claim.

Table 7. Purchase intent of sodium reduced marinated chicken breasts before and after sodium nutrient content claim using the McNemar's test.

TRT*	mg Na/114g chicken breast^	PIb (%)**	PIa (%)	McNemar's Tests Statistic	Asymptotic PR>S	95% Lower CL	95% Upper CL
A	150.21	50.00	46.67	1.00	0.32	0.74	0.99
B	116.61	41.67	46.67	3.00	0.08***	0.79	1.00
C	116.61	55.00	58.33	1.00	0.32	0.74	0.99
D	116.61	58.33	60.00	0.14	0.70	0.59	0.93
E	116.61	40.00	43.33	0.67	0.41	0.64	0.95
F	99.81	51.67	51.67	0.00	1.00	0.65	0.95
G	99.81	51.67	53.33	0.33	0.56	0.79	1.00
H	99.81	45.00	50.00	3.00	0.08***	0.79	1.00
I	99.81	45.00	51.67	4.00	0.05***	0.74	0.99
J	51.00	50.00	58.33	3.57	0.06****	0.61	0.93
K	51.00	46.67	48.33	1.00	0.32	0.90	1.00
L	51.00	56.67	61.67	1.29	0.26	0.59	0.92
M	51.00	46.67	50.00	2.00	0.16	0.84	1.00

*Refer to Table 1 for treatment description.
**PIb: purchase intent before; PIa: purchase intent after
***Not statistically significant at α=0.05, but potentially significan in case of increasing significant level to α=0.1.
^By calculation.
Statistical significant p-values in bold (P<0.05) based on McNemar Asymptotic probability.

A previous study (Bower and others 2003) performed in fat spreads evaluated purchase intent of reduced fat products. Health statements presented appeared to have slight effect on purchase intent and willingness to pay more. Liking of the taste of the low fat product was a more important factor. Tuorila and Cardello (2002) evaluated consumer liking of juices with functional ingredients that caused bitter off flavors. Their results indicate that the first impression in liking of the product was a very important predictor of future consumption, hence purchase intent. This is an interesting fact to note in relation to the present study since liking of most of the sensory attributes were not significantly affected by any of the low sodium formulations neither positively nor negatively. Lack of flavor impression of the chicken breasts can be accounted for the lack of significance in purchase intent of low sodium marinated chicken breasts. Likewise, further preparation to increase flavor is expected such as seasoning, marinating or basting before cooking and consumption of chicken breasts.

A similar study performed by Carraro and others (2012) evaluated the quality and acceptance of sodium reduced bologna sausages using herbs and spices. Sausages that replaced 50% NaCl with KCl solely without the addition of other ingredients (spices and herbs) had a negative effect purchase intent. Nevertheless, when the other ingredients (seasonings) were added the purchase intent was increased. On the other hand, sensory attribute acceptability of the sausages wasn’t significantly affected by KCl substitution.

4.2.4 Predicting purchase intent using logistic regression analysis (LRA)

LRA predictive model was performed to predict the probability of a significant purchase intent of the different treatments based on the sensory attributes measured (Table 8). Hedonic acceptability of the products was evaluated only before the nutrient content claim was presented. However, emotions and overall liking as well as some preliminary demographic information was also included in the analysis after the nutrient content claim was stated as seen in Table 5. The attributes flavor and saltiness were significant predictors of purchase intent even before presenting the nutrient content claim. The odds ratio value for flavor and saltiness were 1.4 and 1.24, respectively. This means that for every 1-unit increase in flavor acceptability score (based on a 9-point hedonic scale) the probability of the product being purchased would be 1.4 and 1.24 times higher than not being purchased.

Table 8. Odds ratio for predicting purchase intent before and after sodium nutrient content claim by logistic regression procedure.

	Before*		After	
Variables	Pr > ChiSq**	Odds ratio	Pr > ChiSq	Odds ratio
Gender	0.9035	1.0280	0.2247	1.332
Lower sodium	0.2450	1.4400	0.477	0.798
Salt user	0.0332	0.5080	0.0028	0.361
Color	0.5275	1.0630	-	-
Aroma	0.3548	0.9110	-	-
Flavor	0.0047	1.4000	-	-
Tenderness	0.1308	0.8580	-	-
Juiciness	0.5991	1.0510	-	-
Saltiness	0.0273	1.2440	-	-
Bitterness	0.4390	1.0820	-	-
Overall liking	<.0001	2.5050	<.0001	4.204
Good	0.9035	0.9730	0.2504	0.759
Happy	0.2513	1.2650	0.059	1.559
Pleased	0.8204	0.9500	0.9619	1.012
Satisfied	<.0001	2.2680	<.0001	2.355
Unsafe	0.7808	0.9270	0.6708	1.185
Worried	0.1620	0.6350	0.5403	0.786
Guilty	0.3976	1.2550	0.5361	0.86

*Purchase intent asked before and after content claim was presented to consumers
**Statistically significant p-values in bold print (P<0.05)

The fact that consumers considered themselves as frequent salt users was a predictor of purchase intent before and after the nutrient content claim was presented. Contrastingly, this means that with an increase in not being a frequent salt user purchase intent is reduced. This implies that people who don't frequently consume salt are less likely to purchase the product and intention was even lower after the nutrient content claim was explained. Overall liking and the emotion "satisfied" of the samples were significant predictors before additional information about the content claims was presented. However, the additional information increased the odds ratios from 2.505 to 4.204 and from 2.268 to 2.355, respectively. This indicates that every 1- unit increase in overall liking (9-point hedonic scale) and 1- unit increase in satisfied emotion (5-

point scale) will likely increase the purchase intent of the product up to 420.4% and 235.5%, respectively. This demonstrated a clear positive effect of the sodium nutrient content claims on purchase intent of sodium reduced marinated chicken breasts

Kim and others (2012) evaluated consumer awareness of salt consumption, sodium reduction and sodium labeling. Interestingly, they found that consumers don't link sodium with heart disease problems or bone health as opposed to kidney disease. They also concluded that reduced and low sodium products would be more likely bought by consumers than sodium free-labeled products. Purchase intent decreased when the adjective "free" was added to unhealthy ingredient claims such as sodium. This fact refutes with the results in the present study where non-salt consumers wouldn't potentially buy marinated chicken breasts prepared with "less" sodium than commercial products. Previous knowledge of the potential risks of high sodium consumption and the given sodium nutrient content claims didn't influence purchase intent of non-salt consumers. Mitchell and others (2013) evaluated the purchase intent of sodium reduced vegetable soup, finding positive correlations between acceptability and purchase intent. This is in accordance with our results where flavor and saltiness were the most important attributes contributing to a positive purchase intent, although not significantly different in acceptability among treatments (Table 5). Wong and others (2013) indicated that when sodium nutrient content claims were specified higher purchasing intent in soups was observed. They also determined that consumer reaction to sodium content claims depend more on attitude toward the food and their trust in the health claims on food labels and to a less extent on demographics.

These results highlight the importance of acceptable taste and saltiness characteristics for positive purchase intent of a food item. Taste has a highly important influence on the purchase intent process and is non negotiable to compromise on. Overall liking of the product is of great importance, remaining a decisive issue even after knowing the nutrient claims. This means that sodium reduction strategies must be acceptable regarding sensory attributes and consumers must feel satisfied to have positive purchase intent.

4.2.5 Overall product differences and discriminating sensory and emotion attributes

MANOVA and DDA were performed to determine which attributes were responsible for overall product differences (Table 9). From the MANOVA Wilk's lambda *P*-value, there were overall significant differences among sodium reduced marinated chicken treatments based on all combined attributes ($P<0.05$).

Among acceptability attributes, the attribute that contributed the most to overall product differences is color (canonical correlation, cc= 0.408) in the first canonical dimension (Can 1) with 22.7% variables explained. This relates to the results from Table 5 where no significant differences were found in liking among treatments for all attributes except for color. Emotions evaluated before the sodium nutrient content claim didn't contribute to overall product differences (canonical correlation < 0.2). However, emotions (especially negative) evaluated after the sodium nutrient content claim showed to have an effect of overall product differences. The DDA performed identified guilty, unsafe and worried as the 3 most important emotions that impart significant differences in the first canonical dimension (Can 1) with 22.7% variance explained. The second canonical added up to 39% of variance explained in comparison to Can 1, although no attribute was considered of significance contribution to overall differences. The third canonical (Can 3) likewise reported color (cc=0.338) as the major factor contributing to overall product differences with 52.7% variance explained. Canonical correlations weren't overall significantly high because the weighed relevance of all the attributes tested might have diluted their significance.

Table 9. Canonical structure describing group differences among low sodium marinated chicken breasts.

	Canonical Structure		
Variable	Can1	Can2	Can3
Color	0.408	0.140	0.338
Aroma	0.202	0.284	0.141
Flavor	0.062	-0.066	0.156
Tenderness	0.098	0.195	0.030
Juiciness	-0.016	0.081	-0.162
Saltinnes	0.218	-0.014	0.228
Bitterness	0.092	-0.009	0.224
Good[B]	-0.009	0.061	-0.001
Happy	0.045	0.037	-0.128
Pleased	0.012	0.103	-0.178
Satisfied	-0.001	-0.044	0.051
Unsafe	0.052	-0.008	0.046
Worried	0.058	0.133	0.032
Guilty	0.195	0.245	-0.051
Overall liking	0.106	0.048	0.133
Good[A]	-0.293	0.267	0.091
Happy	-0.158	0.066	0.095
Pleased	-0.161	0.239	0.198
Satisfied	-0.085	0.088	0.218
Guilty	0.488	0.033	-0.303
Unsafe	0.394	-0.002	-0.262
Worried	0.413	0.144	-0.251
Overall liking	0.001	0.180	0.232
Cumulative variance	0.227	0.39	0.527
Wilk's Lambda *P* value		0.0107	

Based on pooled within group variances.
[B] Before nutrient content claim was presented.
[A] After nutrient content claim was presented.

The canonical results for sensory attributes correlate to previous findings in this study where unnoticeable differences in sensory liking were observed, except for color. However, no significant differences at “neither like nor dislike” and “like slightly” scores suggest that treatments with high KCl substitution are similarly accepted as the Control, which can be attributed to the lack of flavor perception of chicken breasts.

The addition bitterness blockers (glycine and AMP) to 100% sodium substituted chicken marinades with KCl maintains similar sensory perceptions compared to the control (blocking bitter and metallic aftertastes) although is not related to increase or decrease product acceptance. Sodium nutrient content claims presented in response to sodium substitution increased consumer awareness against sodium's health effects. In response, negative emotions showed to be more sensitive to determine overall product differences rather than positive emotions and sensory attributes. After the sodium nutrient content claims were presented, negative emotions increased significantly for the control and decreased for treatments with 75 and 100% sodium reduction according to t tests performed (Table 6).

4.2.6 Penalty analysis for JAR scale

Penalty analysis was performed for 4 attributes in this study including saltiness, juiciness, bitterness and tenderness as these were the most important attributes related to meat product acceptability. Penalty analysis determines if liking was affected by the intensity of the attribute presented in each treatment.

Penalty analysis for saltiness is presented in Figure 5. "Not enough" saltiness presented the highest mean drops based on more than 30% of the responses for most treatments. This supports the results obtained in Table 5, where consumer's acceptability for saltiness was rated as "dislike slightly" and "neither like nor dislike".

This may be due to the fact that low salt levels were added (0.75% of the brine solution) and no other seasoning was used. More than 50% of the consumers considered treatments A, E, F and H as “not salty enough” with concerning mean drops of -2.31, -2.19, -2.13 and -2.48, respectively when saltiness is rated “not enough” on the JAR scale.

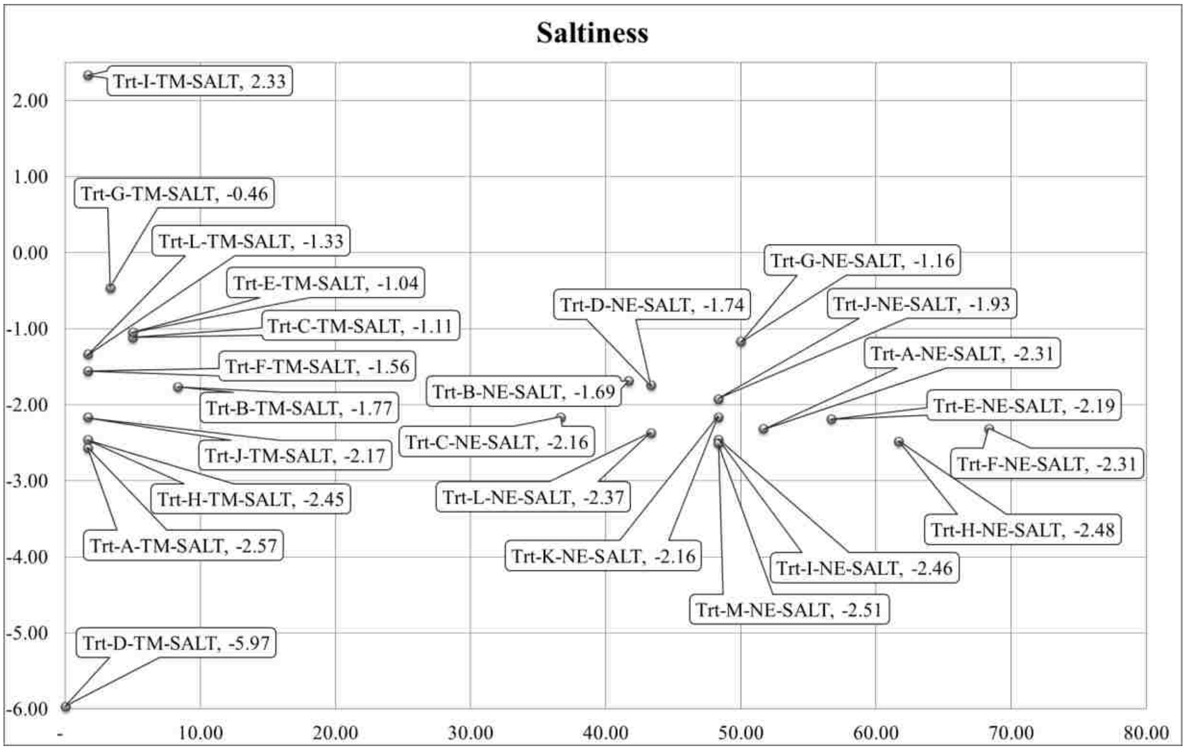

Figure 5. Mean drops and frequency responses for saltiness on a JAR scale.

For juiciness “not enough” juiciness showed the highest mean drops for more than 20% of the respondents (Figure 6). Overall concerning mean drops belong to “not juicy enough” classified treatments. Treatment K (100% KCl, 0.1% glycine and 0.01% AMP) was classified as not juicy enough by more than 45% of the consumers, which had a very concerning mean drop of -2.45. These results from the JAR scale can help understand the juiciness acceptability results that were also rated as “neither like nor dislike” to “like slightly”, which can be explained by the JAR ratings of “not juicy enough”. On the other hand, “too juicy” was elucidated by less than 15% of the frequency responses, meaning the reduced sodium chicken breasts were mostly “not juicy enough”.

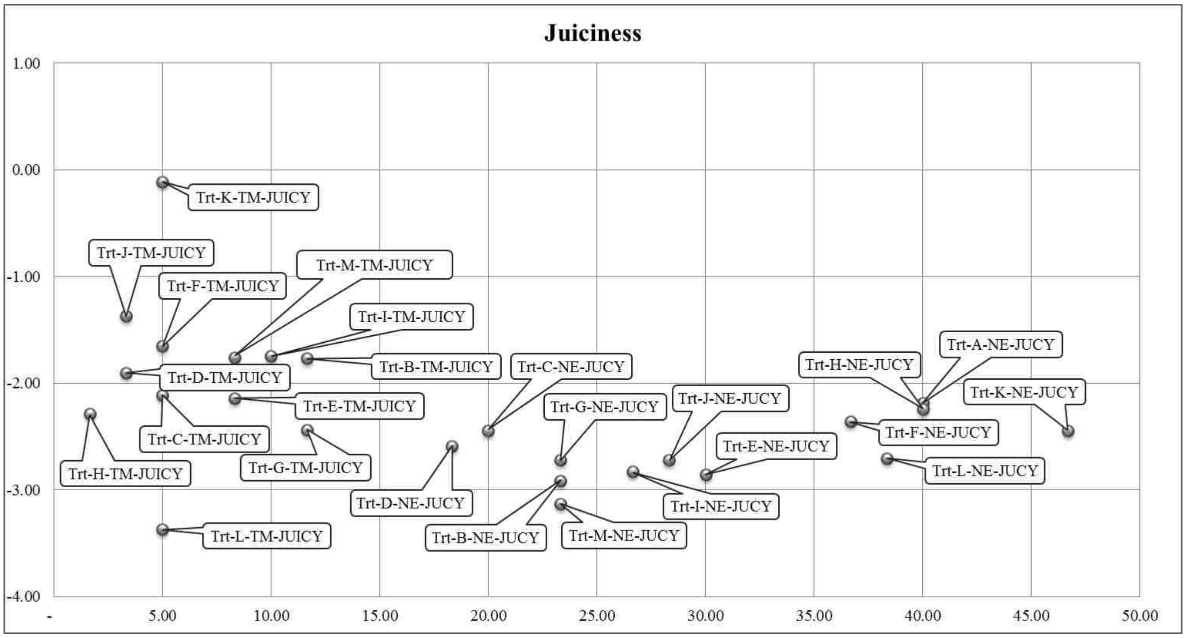

Figure 6. Mean drops and frequency responses for Juiciness on JAR a scale.

On the other hand, bitterness showed no concerning effects regarding perceptions of "too bitter" or "not bitter" as shown in Figure 7. Frequencies for "too bitter" are accountable for less than 10% of the frequency responses. Frequencies for "not bitter" are considered not concerning by more than 60% of the consumers. These results leave the concerning and very concerning zones with no treatments located in this zone for mean drops greater than -2 and for more than 20% of the consumer frequency responses.

This relates to ANOVA scores for bitterness liking that were rated as "neither like nor dislike" since the product was actually not considered bitter by the JAR scale. Results imply that salt substitution with KCl including bitterness blockers in chicken breast marinades produces chicken breasts with not concerning bitterness perception for consumers based on the penalty analysis.

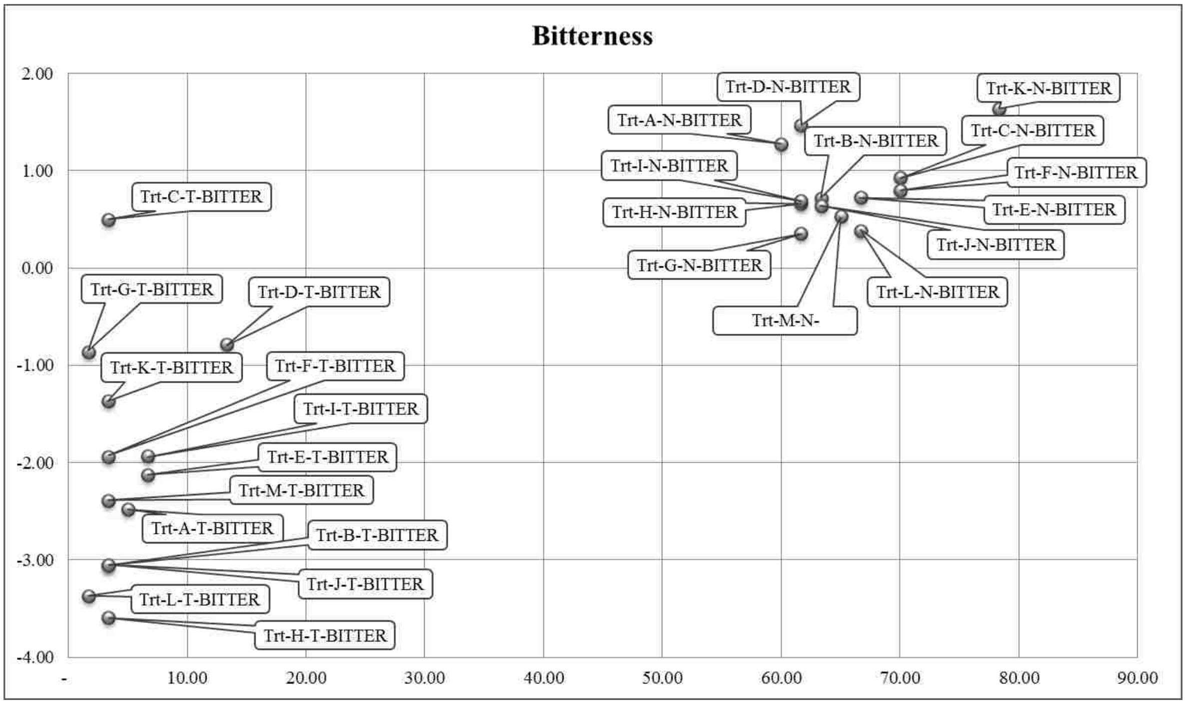

Figure 7. Mean drops and frequency responses for Bitterness on a JAR scale.

Degree of tenderness was also important in this study regarding the fact that it's one of the most important attributes in meat. More than 20% of the respondents stated that treatments A, F, H, I, K and L were "not tender enough" with concerning mean drops of -3.14, -2.67, -2.38, -2.78, -2.69 and -2.96 respectively (Figure 8). "Too tender" was elicited by treatments C and E with concerning mean drops of -2.81 and -2.91 for more than 20% of the participants. Moreover, when liking was rated, no significant differences were found among treatments and the liking score was "neither like nor dislike" or "like slightly". Nevertheless, these results can be compared to physicochemical results where increasing KCl substitution levels increased the hardness of chicken breast texture. This result links to treatments F, H, I, K and L with 75 and 100% of KCl inclusion. Treatments C and E belong to treatments with 50% KCl that were significantly more tender when evaluated instrumentally (Table 19).

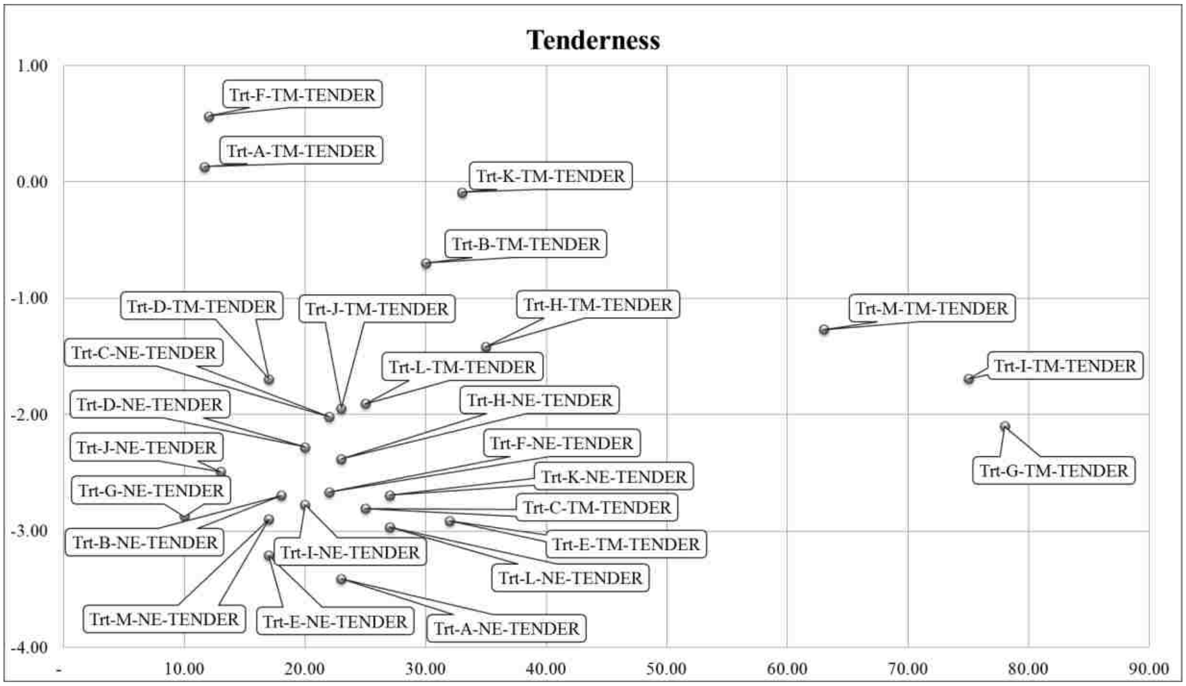

Figure 8. Mean drops and frequency responses for Tenderness on a JAR scale.

4.3 Physicochemical results

4.3.1 pH.

Table 10 shows the mean pH values for each treatment. Sodium level showed to have a significant effect on pH. The lowest pH values were observed in treatments A (control, 100% NaCl) and H (25% NaCl, 75% KCl, 0.2% gly and 0.01% AMP). Treatments B, E and J with the salt mixture containing low levels of glycine and AMP (0.1% Gly and 0.01% AMP) had significantly higher pH, or were more basic.

Marinated chicken breasts with 100% NaCl had the lowest pH. Both sodium and potassium salts have a low ability to lower pH even though these salts have been known to be effective against microbial inhibition (Toldrá 2015). Poulanne and others (2001) observed a weak tendency that low salt contents (0.5-1%) may produce a small decrease in pH values. Likewise, the pH value readings in this study might be influenced by salt concentration at the location where the pH was measured rather than by salt dilution. As expressed earlier, NaCl content caused a shift to lower pH values. Another explanation may be because NaCl has more Cl ions that usually produce a higher shift on pH than KCl. Cl^- ions are responsible for protein activation that consequently increases WHC and texture effects.

Table 10. Effects of NaCl replacement on the pH of chicken breasts.

Effect=Trt Method=LSD (P<.05)	
Trt*	Mean**
A	5.85^{d}
B	6.02^{ab}
C	5.90^{bcd}
D	5.88^{cd}
E	6.03^{a}
F	5.97^{abc}
G	5.88^{cd}
H	5.85^{d}
I	5.76^{abc}
J	6.04^{a}
K	5.90^{bcd}
L	5.90^{bcd}
M	5.88^{cd}
Std. Error	0.05

*Refer to Table 1 for treatment description.
**Means with the same letters are not significantly different (P>0.05).

A factorial experimental performed to observe interaction of main effects showed a significant effect in the processing times in which measurements were taken (Table 11).

Table 11. Mean pH values per processing time of low sodium marinated chicken breasts.

Method=LSD (P<0.05)	
TIME	Mean
Before injection	5.79^{d}
After injection	5.87^{c}
24 h at 4C	5.94^{b}
Cooked	6.16^{a}
Std. Error	0.03

*Means with the same letters are not significantly different (P >0.05).
Control not included in factorial 3 x 2 x 2 used to evaluate individual factors and interactions.

Not to be left unnoticed, the control was not considered in the factorial since the factorial 3x2x2 includes the three levels of NaCl/KCl (50,75 and 100%), 2 levels of glycine (0.1 and 0.2%) and 2 levels of AMP (0.01 and 0.02%). Time, being inherent to the process, is defined as the one of the four measurements taken: before injection, after injection, after storage (24 h at 4°C) and on cooked samples. The pH values significantly increased or became more basic through each stage of the process.

Lowest pH values were reported for the initial measurements taken before injection. Cooked chicken breasts had significantly the highest pH (Table 11). Results are in accordance with the values by Quiao and others (2001) for raw breast meat classified as lighter than normal (referring to color L*a*b* values) with pH of 5.81. Fletcher and others (2000) also reported a range of pH values from 5.76 to 5.93 for light to dark meat color and 6.09-6.21 for cooked breast meat. Lopez and others (2012) showed similar findings where pH values ranged from 5.79-5.89 for raw meat. In their study, Lopez and others (2012) also explained that large differences were not expected since sodium tripolyphosphate was added to the brine, which is considered as an alkaline phosphate and usually buffers the solution.

A significant effect was also observed in the interaction of glycine and AMP ($P<0.05$) comparing the four levels used in this study as seen in Table 12. The highest pH mean value was observed for the first level of bitterness blockers (0.1 Gly and 0.01 AMP), which was similar to the fourth level (0.2 Gly and 0.02 AMP). Intermediate concentration levels of AMP and glycine (0.1 Gly and 0.02 AMP, 0.2 Gly and 0.01 AMP) were similar and more acidic than extreme mixtures (lowest and highest concentration ratios).

Table 12. Bitter blocker effect on chicken breast pH.

Effect=Gly*AMP Method=LSD (P<0.05)		
Gly	AMP	Mean
0.1	0.01	6.01[a]
0.1	0.02	5.89[bc]
0.2	0.01	5.88[c]
0.2	0.02	5.96[ab]
Std. Error	0.03	

*Means with the same letters are not significantly different (P >0.05).
Control not included in factorial.

Despite differences in pH values, they are considered normal according to literature presented earlier (Quiao and others (2001); Fletcher and others (2000)). Contrastingly, being both extremes of bitterness blockers the highest pH, these results are partially similar to Wang and others (2016) for the fourth level of bitterness blockers with higher AMP (0.2 Gly and 0.02 AMP). Increasing concentrations of AMP applied to duck meat increased pH with every 10 mM increase. Bastianello and others (2012) also reported a slight but not significant increase in pH of fermented sausages containing a mixture of amino acids (Lysine) and nucleotides (IMP/GMP) compared to the control. Glycine on the other hand has not been reported to have an effect in pH as stated by Gelabert and others (2003) where no significant differences were encountered when substituting 10-20% NaCl on fermented sausages.

4.3.2 Water holding capacity

WHC followed similar sampling methods as color and pH, which were linked to specific processes: before injection, after injection, 24 h at 4C and cooked. Initial weights of chicken breast samples were recorded before injection and are a variable needed for further WHC parameter calculations. Furthermore, after injection samples were re weighed and marinade uptake was calculated using the formula indicated in the materials and methods section 3.2.5. After 24 h of storage under refrigerated conditions, samples were weighed for purge loss determination. Same weighing method was performed after cooking and cook loss detailed calculation can also be found in section 3.2.5 of this thesis.

Salt and bitterness blocker mixtures (Treatment) didn’t affect wáter holding capacity properties individually as seen in Table 13. No significant differences were observed from the KCl substituted treatments compared to the control (100% NaCl and no bitterness blockers).

Table 13. Effect of different levels of NaCl with KCl replacement on WHC of marinated chicken breasts.

	Marinade uptake	Purge loss	Cook loss
TRT*	Means		
A	13.50ns	8.00ns	30.00S
B	13.00	6.00	30.50
C	12.50	7.50	29.50
D	13.50	7.00	30.00
E	15.50	10.00	33.00
F	14.00	9.50	30.00
G	13.50	6.50	30.50
H	15.50	9.50	31.00
I	13.00	7.00	32.00
J	16.00	11.00	32.00
K	15.00	8.00	30.00
L	14.50	7.50	32.00
M	13.00	8.00	29.50
Std. Error	3.21	1.92	2.21

*Refer to Table 1 for treatment description.
ns: not significant

Table 14 indicates means stated in percentages and standard errors of water lost (e.g. purge loss or cook loss) or gained (e.g. marinade uptake) during each process rather than comparing the parameters over time. Since as mentioned before, time relates to a distinct process in this study.

Table 14. Time effect in water holding capacity of chicken breasts.

Effect=Time Method=LSD (P<0.05)	
	WHC
Time	Mean (%)
Marinade uptake	14.04
Purge loss	8.12
Cook loss	30.76
Std. Error	1.43

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

The mean actual marinade uptake for the 13 treatments was 14.04%, rather than the 20% aimed. However, since injection was performed manually, the pressure, volume and number of injections per breast could have affected the inclusion of brine as opposed to automated systems. Conversely vacuum tumbling pressure also used in other studies possibly expands muscle slightly, allowing better marination penetration as reported by Soglia and others (2014) and Smith and Young (2007). Likewise, polysaccharides such as pectin and mostly carrageenan are commonly added by the industry to help retain brine percentages ≥ 20% as presented by (Zheng and others 1998). Although, pectin used in their study didn't increase marinade uptake, it helped decrease purge and cook loss. Yet, this study's aim was to evaluate the direct effect of marinades with KCl substitutions and a mixture of bitterness blockers. The addition of chloride salts such as NaCl (2-3.5%) has been proven to increase ionic strength of water to protein, thus increasing hydration (Lawrie, 1998). Phosphates in addition to salt can shift the isoelectric point of proteins, to a positive pH. This allows protein extraction and solubilization thus increasing WHC. However, this study used lower concentrations of salt (0.75% w/w of brine), which is commonly used in the food industry to marinate chicken that may not be as effective as larger salt concentrations to increase hydration. Although a shift in pH was observed, this clearly didn't have an effect in increasing WHC parameters.

Meanwhile, purge loss had a mean of 8.12% (Table 15), with no significant differences found among treatments determining that Cl^- ion in KCl is as efficient as NaCl when maintaining moisture. However, Soglia and others (2014) reported a purge loss of 2.25-2.50% in vacuum tumbled marinated rabbit with a 20% pick up solution substituting NaCl up to 50% with KCl. Manual injection performed in this study causes surface rupture that can explain higher purge loss percentages since water can leak easily out of the meat matrix. Cook loss percentage (30.70%, Table 14), also measured in this study, is higher than the studies previously described that reported values ranging from 20- 24%. These results for cook loss follow the same principle that the marination procedure employed may have contributed to higher cook loss.

In general water holding capacity parameters were not optimal as expected, yet there were no significant differences neither among treatments with different levels of KCl replacement nor in bitterness blocker concentration. These results suggest that KCl maintained consistent levels of WHC at consistent NaCl concentration of 0.75% w/w of brine solution.

KCl concentrations have been shown to have no significant effect in marinade uptakes or purge loss in previous studies performed on rabbit and poultry meat by Soglia and others (2014) and Lee and others (2012), respectively. However, cook loss was significantly affected by 100% KCl substitution in marinated broiler breast fillets of the latter study. Lower amounts of sodium are accounted for lower WHC parameters. In this study, however, the addition of bitterness blockers, glycine and AMP might have enhanced WHC characteristics (treatments B-M containing 50, 25 and 0% NaCl compared to the control) counteracting the effect experienced by Lee and others (2012). Wang and others (2016) observed better water retention in duck breast meat at increasing concentrations of AMP. AMP increases ionic strength, altering solubility of myofibrillar proteins that bind water. Increased solubility is attributed to myofibril fragmentation index (MFI), which increases as AMP concentrations increase. Internally, "I" and "Z" bands were disrupted with AMP addition in duck meat, which are structural components of muscle myofibrils. These myofibrils take part in muscle contraction and basically contain the thin filaments (actin) (Du and McCormick 2009).

4.3.3 Color

Table 15 shows the mean and standard errors for the color L*a*b* values of marinated chicken breasts. Sodium replacement with KCL showed to have no significant effect on color.

Table 15. Effect of sodium replacement on the color of marinated chicken breast.

	Means		
Trt*	L*	a*	b*
A	64.76NS**	1.78NS*	8.54NS*
B	64.58	1.70	7.78
C	64.85	1.75	7.63
D	63.83	1.75	8.64
E	62.57	1.79	8.5
F	60.62	1.22	7.44
G	63.74	1.67	8.34
H	64.46	1.68	7.96
I	63.34	1.71	8.14
J	63.26	1.85	7.52
K	64.22	1.78	8.11
L	63.76	1.91	7.50
M	63.75	1.89	8.02
Std. Error	0.94	0.33	0.60

*Refer to Table 1 for treatment description.
**Means and standard errors with the same letters are not significantly different (P >0.05). NS: Not significant.

Color was measured at four different times during processing as with pH measurement. Mean values for each process time are observed in Table 16. No significant differences were found among treatments (P > 0.05) during raw conditions each time measured.

Table 16. Mean L*a*b* color values of low sodium marinated chicken breasts during each process time.

Effect=Time Method=LSD (P<0.05)			
	L*	a*	b*
	Mean		
TIME			
Before injection	57.89^{b}	1.04^{b}	5.93^{b}
After injection	59.26^{b}	0.98^{b}	5.13^{c}
24 h at 4C	59.62^{b}	0.91^{b}	5.79^{bc}
Cooked	77.62^{a}	4.04^{a}	15.2^{a}
Std. Error	0.65	0.23	0.42

*Means with the same letters are not significantly different (P >0.05).
NS: not significant

However, cooked color values were statistically different to all other raw values as expected. Raw color values (Table 16) (57.89-59.62 for L*; 0.9-1.04 for a*; 5.13-5.93 for b*) are in the normal range according to a study performed by Zhuang and Savage (2009) where the range of color values for chicken breast fillets was 47.4 to 61.5 for L*, -1.0 to 3.6 for a* and 5.7 to 6.4 for b*. Fletcher (1999) found that there is a noticeable variation of chicken breast color observed in retail packaging that may be due to live production, handling or processing. Qiao and others (2001) determined that raw chicken breast may be differentiated by L* values as values > 53 being lighter than normal, values between 48 and 51 are considered normal and values < 46 being darker than normal. This positions the L* color value for raw chicken breast reported in this study as lighter than normal. Qiao and others (2001) also observed a numerical increase in L*a*b* values of broiler breast meat over time from 0 to 24 h of measurements thus, stating that there is a highly significant correlation of color values at 0 and 24 h. Similar to our study, L* values numerically increased between before injection and 24 h post injection that could possibly be attributed to phosphate inclusion and pH increase. However a* and b* values decreased being significant only for b* values.

Raw chicken breasts were significantly different in b* values only when comparing before and after injection values. This significant decrease in yellowness can be related to a significant increase in pH observed after injection potentially caused by added phosphates and AMP. Cooked chicken breast color values, as expected were higher in all parameters measured since there is an evident shift from pink raw color to white cooked color in chicken breast. Still, these results are in accordance with the results of Fletcher and others (2000) where raw meat color demonstrated to have an effect on cooked meat color of broiler breasts. In their study, cooked breast L*a*b* color values for darker samples were 78.8, 3.2 and 12.1, respectively that relate to findings in this study. They also stated that cooking effect was more pronounced on originally darker than normal meat than in lighter than normal meat color, which relates to results in this study since all cooked colors are significantly similar (Table 17).

Table 17. Mean L* a* b* values per treatment for low sodium cooked marinated chicken breasts.

Cooked L* a* b* values			
Trt*	L* Mean	a* Mean	b* Mean
A	78.13^{ns}	4.18^{ns}	16.53^{ns}
B	77.13	3.99	15.64
C	78.94	4.19	15.07
D	77.89	4.27	16.44
E	78.31	4.15	15.95
F	76.49	2.28	16.28
G	79.41	4.04	15.66
H	80.24	4.17	15.23
I	78.06	4.63	16.01
J	79.05	4.03	14.16
K	76.11	3.98	15.05
L	76.03	4.14	14.76
M	78.85	4.21	15.25
Std. Error	1.35	0.42	1.3

*Refer to Table 1 for treatment description.
Ns: not significant

Water binding and pH are factors that affect meat color. Final muscle pH affects light reflectance properties and myoglobin chemical reactions. Marinade uptake and drip loss, which were WHC parameters measured, are affected by extent water retention and protein solubility. Sarcoplasmic protein denaturation may affect WHC that increases with higher pH values. Hence, this affects changes in color from the precipitation of sacrcoplasmic soluble proteins (Joo and others 1999). Color and WHC can also be explained in terms of myofibrillar volume since at lower WHC light is more scattered and meat appears pale. Since myofibrils and sarcoplasm between them are accounted for light scattering in meat, the color depends on their refractive index. With less WHC, the myofibrils are shrunk, producing more exudate that will be lighter scattering than normal (Offer and Trinick 1983). In conclusion, color changes may be due to WHC and level of sarcoplasmic denaturation. However, WHC variables remained unchanged for these treatments throughout the study. Differences in b* values throughout time may be due to disparities in sampling locations of the chicken breast surface as well as possible fat oxidation. Lipid oxidation leads to a yellow hue in meat. Oxymyoglobin auto oxidation and metmyoglobin pro oxidant activity can result in color deterioration of poultry (Kerry JP 2012).

Salt substitution demonstrated to have no significant effect on color values of cooked chicken breasts where substitutions were significantly similar to the control. Hence cooking faded the small color changes found in raw chicken breast as shown in Table 17, where no significant differences where found among cooked treatments.

4.3.4 Moisture

Moisture percentage evaluated in cooked chicken breast samples presented values from 65.15 to 68.84% (Table 18), which were not significantly different among treatments ($p > 0.05$).

Table 18. Mean moisture percentages per salt replacement of chicken breasts.

Effect=Treatment Method=LSD (P<0.05)	
	Moisture
Trt*	Mean
A	67.64^{ns}
B	67.48
C	66.52
D	66.48
E	65.57
F	66.62
G	65.11
H	67.29
I	65.11
J	67.29
K	67.52
L	68.16
M	68.84
Std. Error	2.19

*Refer to Table 1 for treatment description.
ns: Not significant

These results are comparable to WHC parameters since no differences were found among treatments, especially for cook loss determination as moisture was performed in cooked product. Moisture retention in meats varies with cooking method employed as well as cooking time and preparation method. Similarly, a study by Liao and others (2010) determined that roasted chicken breast without any type of marination or basting had mean moisture of 65.81% in accordance with the range obtained in this study. Higher moisture values, yet not significant, found may be due to the enhancing effect of phosphates and salt in water retention. Quiao and others (2002) also reported moisture comparable values of 70.61% for marinated cooked chicken breasts with a brine containing water, salt and phosphate. This increase in retention compared to Liao and others (2010) may be due to effects of marination. Addition of phosphates causes an increase in electrostatic repulsive forces expanding spaces between actin and myosin.

Synergistically salt enhances protein solubility, which leads to a higher ability to immobilize water (Petracci and others 2013).

4.3.5 Texture

Texture is considere done of the most important sensory and quality attributes to consumers. Table 19 shows texture results per treatment, which were performed on 1.9 cm strips of cooked chicken breasts at room temperature (20 °C) with sampling details as dicussed in section 3.2.1. As it can be observed, more tender chicken breasts belong to treatments with 50% NaCl substitution (Treatments B, C, D and E) with values of 19.51 to 21.67 N that were also significantly similar to the control. Least tender treatments (Treatments G, H, K and M) belong to those treatments with 75-100% NaCl substitutions with the exception of treatments F, J and L being numerically more tender and similar to the control (Trt A).

Table 19. Treatment effects on the firmness of marinated chicken breasts containing salt substitutes

	Firmness
Trt*	Mean (N)**
A	24.08[abc]
B	21.67[bc]
C	20.76[bc]
D	19.83[c]
E	19.51[c]
F	26.19[abc]
G	29.06[a]
H	29.69[a]
I	28.09[ab]
J	25.35[abc]
K	29.45[a]
L	24.59[abc]
M	29.16[a]
Std. Error	10.73

*Refer to Table 1 for treatment description.
*Means with the same letters are not significantly different (P >0.05).

Higher firmness (hardness) elicited by treatments with 75 and 100% KCl in NaCl substituted marinades resemble the results found by Lee and others (2012). This trend is believed to be due to a decreasing of sodium ions, which are considered to have greater protein extractability and solubility factor than potassium ions. This means that sodium has a higher tenderizing effect than potassium. As expected, glycine didn't have an effect in texture as previously stated by Gelabert and others (2003) where 10-20% substitution of NaCl with glycine showed no significant decrease in tenderness of fermented sausages. This demonstrated that glycine has less protein solubilization ability than NaCl (Gou and others 1996). Similarly, Bastianello and others (2011) found only a numerical decrease in hardness values of sodium-reduced sausages with KCl and a mixture of IMP and taurine as well as disidium guanylate and disodium inosinate demonstrating the mixture of amino acids and nucleotides didn't provide an enhancing effect on texture.

Moreover, an effect of AMP in texture was expected as previously described by Wang and others (2015). Increasing AMP concentrations (10-40mM) (2.75%-11%) decreased shear values of duck meat. AMP's tenderizing effect can be explained by the weakening actomyosin in meat structure. This was attributed to the fact that actin and myosin binding depends on the nucleotide bound or nucleotide analogue bound to myosin. Thus with AMP bound, the interaction of myosin with actin becomes weak (Spudich 2001).

To achieve an effective replacement of NaCl with KCl, around 15% more KCl should be added to achieve the same protein extractability and solubilization. So a 15% should have been added to the total salt content of 0.75% w/w of the brine (0.86% KCl w/w of brine in 100% NaCl substitution) to allow objective Cl^- ion comparisons with the control (100% NaCl). As the chloride ion is responsible for protein solubilization, differences in proportions of sodium and potassium must be compensated. KCl is composed of 48% potassium meaning 52% is chloride compared to NaCl that is 39% sodium and 61% chloride (Feiner 2006).

In Table 20 the effect of KCl concentration in reduced tenderness remains clear with levels higher than 50% KCl yielding harder meat texture instrumentally.

Table 20. Salt effect in tenderness values of chicken breast.

Effect=KCL Method=LSD (P<0.05)	
	Firmness
KCL level	Mean (N)
50	20.44[b]
75	28.25[a]
100	27.13[a]
Std. Error	10.449054

*Means with the same letters are not significantly different (P >0.05).
Control not included in factorial.

Even though AMP previously demonstrated to aid in the actin and myosin dissociation it didn’t have an effect on decreasing tenderness this study. Okitani and others (2008) performed a study to evaluate the mechanism of action of AMP in myofibrillar proteins. They added 2 and 8mM (0.55-2.20%) concentrations of AMP to the actomyosin complex. After incubation, dissociation occurred almost completely in a 2h period. Thus, to observe a significant effect in texture AMP concentrations should be higher than the levels used in the present study.

CHAPTER 5. SUMMARY AND CONCLUSIONS

This thesis composed of several analyses with the objective of substituting salt (NaCl) in chicken breast marinades with KCl. AMP and glycine were added as enhancers or bitterness blockers of KCl.

A sensory analysis was performed to determine consumer acceptability of cooked chicken breasts containing different sodium substitutions and bitterness blocker levels. Each panelist (n=260) evaluated three of the 13 different formulations based on a BIB design. The attributes evaluated were color, odor, flavor, bitterness, saltiness, juiciness, tenderness and overall liking. Emotions and purchase intent were also evaluated before and after a sodium nutrient content claim was presented. These two components are considered novelty when compared to previous studies performed in reduced sodium chicken marinade systems. Emotions have become a new approach when measuring acceptability and purchase intent since they have shown to have a correlation with these factors afecting liking and purchase intent of a food product. A JAR scale was applied for the attributes bitterness, saltiness, juiciness, and tenderness. Overall, the acceptability of the treatments was situated between neither like nor dislike and like slightly for all attributes with no significant differences, except for color. Emotions were significantly affected by the sodium nutrient content claim presented since positive emotions good, happy, pleased and satisfied significantly increased for treatments with greater sodium reduction nutrient content claims, while negative emotions simultaneously decreased for these same treatments. This result was also in accordance with the Wilk's Lambda probability ($p < 0.0107$) indicating that overall differences existed among treatments when all sensory attributes for acceptability and emotions were simultaneously compared. Major differences were accounted for color and emotions guilty, unsafe and worried.

Purchase intent using the McNemar's statistical procedure showed a numerical increase in the percentage of consumers who would purchase the product after the nutrient content claim was presented. However, the increase was only significant for treatment I (25%NaCl, 75% KCl, 0.2% glycine, 0.02% AMP). Logistic regression was also applied to determine the degree in which demographic information, sensory attributes and emotions influence positive purchase intent. Flavor and saltiness predict a positive purchase intent before nutrient content claim was presented but not after. Overall liking and consumer emotion "satisfied" were significant predictors of purchase intent and tended to increase after the sodium nutrient content claim was presented. This determines that consumers would give less importance to flavor and saltiness if a sodium nutrient content claim was presented. However, emotion satisfied and overall liking would be more important predictors of purchase intent after a nutrient content claim is provided. The JAR scale showed interesting results that link to the overall low sensory acceptability scores. Consumers considered most of the treatments as "not salty enough", "not juicy enough" and "not tender enoguh" that might have affected the taste perception of the product. Moreover, bitterness wasn't concerned for all treatments that possibly demonstrate the effect of the bitterness blockers AMP and glycine in blocking KCl bitterness.

Physicochemical characteristics were generally not affected by different KCl replacements except for texture results. Values for pH and color were considered in a normal range according to the current literature with only expected differences found between process (raw to cooked). The different sodium replacements didn't affect the water holding capacity. Yet, lower WHC parameters were observed, possibly caused by the manual injection method performed in this study. Texture was moreover affected by KCl concentrations since cooked chicken breasts with 75% and 100% replacements were harder than the control and 50%

replacements. This is explained by the fact that KCl is not as effective as NaCl for solubilizing proteins because of the ratio of Cl^- ions as well as Na has a more tenderizing effect than K^+.

These results suggest that 100% replacement of NaCl with KCl is feasible if bitterness blockers, AMP and Glycine, are added to the formulations. Although high KCl levels instrumentally affect texture, it has no significant effect on consumer acceptance. Sodium nutrient claim information demonstrated to also have a significant effect on increasing purchase intent based on improving consumer emotion response.

Although the low sensory impressions elicited by the low sodium marinated chicken breast fillets, these results are applicable to products such as steamed chicken breasts usually used in salads. Consumer food trends are now related to changing lifestyle trends such as preparing more “natural” foods (foods with less ingredients) and consuming “healthier” foods (foods with less of a risky ingredient). In this case a slightly seasoned and reduced sodium product meets these concepts.

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APPENDIX A. PRELIMINARY ONLINE SURVEY.

Online Survey for Emotions
Marinated chicken breasts

1. Gender.
Male
Female

2. Do you consume baked chicken breasts?
Yes
No

3. Excessive sodium (salt) intake is one of the major causes of high blood pressure and other chronic diseases. Please, think about how the consumption of baked chicken breasts, which may contain high sodium makes you feel, and from the list below select the emotion descriptors that describe how you feel. Check all that apply.

Active
Adventurous
Affectionate
Aggressive
Bored
Calm
Daring
Disgusted
Eager
Energetic
Enthusiastic
Free
Friendly
Glad
Good
Good-natured
Guilty
Happy
Interested
Joyful
Loving
Merry
Mild
Nostalgic
Peaceful
Pleased
Pleasant
Polite
Quiet
Satisfied
Unsafe (regarding nutrition facts)
Steady
Tame
Tender
Understanding
Warm
Whole
Wild
Worried

APPENDIX B. SAS CODES

B.1 ANOVA for physicochemical analyses and interaction evaluation.

Note: Variable “firmness” only however, other variables were evaluated based on these codes. Similar ANOVA was performed for sensory hedonics and emotions.

```
dm 'log;clear';
Title 'anova for chicken breast';
data salt;
input Nacl      Kcl      glycine AMP  trtname $ Rep Firmness;
datalines;
;
proc means data=salt N Mean StdDev Min Max;
class  trtname;
var Firmness;
run;
proc glimmix data=salt;
title2 'anova saltiness lmr scale';
class trtname rep ;
model Firmness = trtname;
random rep;
lsmeans trtname/ lines;
run;
proc glimmix data=salt;
Title3 'interaction'
class Nacl KCl glycine amp rep;
model Firmness = KCl  glycine amp  kcl*glycine kcl*amp glycine*amp kcl*glycine*amp ;
random rep;
lsmeans kcl glycine amp kcl*glycine*amp/ lines;
run;
```

B.2 MANOVA codes

```
dm 'log;clear;output;clear';
option nonumber nodate;
Title1 'chicken breast';
data sensory;
input panelist  blindingcode   Color   Aroma Flavor Tenderness     TendJAR       Juiciness
        JucJAR          Saltinnes        SaltJAR         Bitterness       BittJAR
        Overalllikingbefore    Goodbefore    Happybefore   Pleasedbefore Satisfiedbefore
        Unsafebefore  WorriedbeforeGuiltybefore   Goodafter      Happyafter     Pleasedafter
        Satisfiedafter  Guiltyafter     Unsafeafter     Worriedafter   overalllikingafter
;
Datalines;
;
```

```
proc sort; by SAMPLE;
Proc candisc out=all mah;
Title2 'MANOVA - OVERALL';
class blindingcode;
var Color	Aroma	Flavor	Tenderness	Juiciness	Saltinnes
	Bitterness	Overalllikingbefore	Goodbefore	Happybefore	Pleasedbefore
	Satisfiedbefore	Unsafebefore	WorriedbeforeGuiltybefore	Goodafter
	Happyafter	Pleasedafter	Satisfiedafter	Guiltyafter	Unsafeafter	Worriedafter
	overalllikingafter;
run;
quit;
```

B.3 Logistic regression codes and McNemar.

```
dm "log;clear";
ods html close;
ods html;
data chickenbreast;
input trtname	gender	lowersodium	saltuser	color	aroma	flavor	tenderness	juiciness
	saltinnes	bitterness	overalllikingbefore	goodbefore	happybefore
	pleasedbefore	satisfiedbeforeunsafebefore	worriedbefore guiltybefore
	purchaseintbefore goodafter	happyafter	pleasedafter	satisfiedafter	guiltyafter
	unsafeafter	worriedafter	overalllikingafter	purchaseintafter;
datalines;
;
proc freq;
tables pib*pia;
proc sort;
by TRT;
proc freq;
by TRT;
tables Gender overalllikingbefore overalllikinglafter  purchaseintentafter  purchaseintentbefore;
tables  purchaseintentbefore * purchaseintentafter;

proc logistic data = chickenbreast;
model purchaseintbefore = gender	lowersodium	saltuser	color	aroma	flavor
	tenderness	juiciness	saltinnes	bitterness	overalllikingbefore
	goodbefore	happybefore	pleasedbefore	satisfiedbeforeunsafebefore	worriedbefore
	guiltybefore;
run;

proc logistic data = chickenbreast;
model purchaseintbefore = color	aroma	flavor	tenderness	juiciness	saltinnes
	bitterness	overalllikingbefore	goodbefore	happybefore	pleasedbefore
	satisfiedbeforeunsafebefore	worriedbefore guiltybefore;
run;
```

```
proc logistic data = chickenbreast;
model purchaseintafter = gender     lowersodium  saltuser goodafter     happyafter
        pleasedafter   satisfiedafter  guiltyafter     unsafeafter     worriedafter
        overalllikingafter;
run;

proc logistic data = chickenbreast;
model purchaseintafter = goodafter  happyafter     pleasedafter   satisfiedafter  guiltyafter
        unsafeafter     worriedafter  overalllikingafter;
run;

proc sort; by trtname;
/*the mcnemar test below to get the chi-sq and prob values*/;
proc freq; by trtname;
exact agree;
tables purchaseintbefore*purchaseintafter;
run;
```

B.4 T-tests

Note: Only “treatment 13” is shown however, other treatments were evaluated based on this codes. Variable overall liking done as an example but a procedure was done for each emotion tested before and after the nutrient content claim was presented.

```
dm 'log;clear';
ods listing;
title 'emotions t test for treatment 13 via anova with 2 treatments';
data salt;
input panelist  nacl     kcl     gly     amp     trt information $      overallliking   good    happy
        pleased          satisfied        unsafe  worried          guilty  ;
datalines;
;
proc means data=salt n mean stddev min max;
class information;
var overallliking        good    happy   pleased          satisfied        unsafe  worried          guilty;
run;
proc glimmix data=salt;
title2 't13';
class panelist   information;
model overallliking = information ;
random panelist;
lsmeans infornation/ lines;
```

APPENDIX C. CONSENT FORM.

Research Consent Form

I agree to participate in the research entitled "Effect of partial and complete replacement of salt on the physicochemical and sensory characteristics of marinated chicken breasts" which is being conducted by Witoon Prinyawiwatkul of the School of Nutrition and Food Science at Louisiana State University Agricultural Center, (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. Two hundred sixty consumers will participate in this research. For this particular research, about 5-10 minute participation will be required for each consumer.

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

2. The reason for the research is to evaluate how consumer liking of reduced sodium chicken marinades varies with different concentrations of sodium chloride, potassium chloride, glycine and AMP (Adenosine monophosphate). The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems related to such examination.

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

4. Participation entails minimal risk: The only risk may be an allergic reaction to chicken, sodium chloride (NaCl), potassium chloride (KCl), glycine (Gly), AMP (adenosine monophosphate), phosphates and unsalted crackers. However, because it is known to me beforehand that all those foods and ingredients are to be tested, the situation can normally be avoided.

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

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

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board.

Questions or problems regarding these activities should be addressed to Dr. Michael Keenan of LSU AgCenter at 578-1708. I agree with the terms above.

Please write your name down if you agree with the terms of this consent form:

____

APPENDIX D. SENSORY QUESTIONNAIRE.

Note: Example for control sample only.

Gender

- Female
- Male

Age (years):

- 18-25
- 26-35
- 36-45
- 46-55
- 56-65
- >65

Race

- Caucasian
- African American
- Hispanic/Latino
- Asian
- Other

Do you consider yourself a regular user of salt for cooking?

- Yes
- No

High sodium intake is associated with heart diseases. Would you consider lowering your sodium intake?

- Yes
- No

Please taste the following chicken breast samples in the order presented. Between the samples, drink water and eat unsalted crackers to clean your palate.

Sample %01
Color

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Aroma

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Flavor

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How would you rate the tenderness of this product?
Tenderness

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

JAR

Not tender enough	Just about right	Too tender

How would you rate the juiciness of this product?
Juiciness

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

JAR

Not juicy enough	Just about right	Too juicy

How would you rate the saltiness of this product?
Saltiness liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

JAR

Not salty enough	Just about right	Too salty

How would you rate the bitterness of this sample
Bitterness

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

JAR

Not bitter	Just about right	Too bitter

How would you rate the overall liking of this product?
Overall liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How do you emotionally feel when consuming this product?

Good

Not at all	Slighty	Moderately	Very much	Extremely

Happy

Not at all	Slighty	Moderately	Very much	Extremely

Pleased

Not at all	Slighty	Moderately	Very much	Extremely

Satisfied

Not at all	Slighty	Moderately	Very much	Extremely

Unsafe (health related)

Not at all	Slighty	Moderately	Very much	Extremely

Worried (health related)

Not at all	Slighty	Moderately	Very much	Extremely

Guilty (health related)

Not at all	Slighty	Moderately	Very much	Extremely

How likely will you purchase this product?

- Yes
- No

A diet high in sodium is linked to cardiovascular diseases. **This product was prepared with similar sodium percentage per serving to some commercial products.**

Please answer the following questions:

How do you emotionally feel when consuming this product?

Good

Not at all	Slighty	Moderately	Very much	Extremely

Happy

Not at all	Slighty	Moderately	Very much	Extremely

Pleased

Not at all	Slighty	Moderately	Very much	Extremely

Satisfied

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

Guilty (health related)

Not at all	Slighty	Moderately	Very much	Extremely

Unsafe (health related)

Not at all	Slighty	Moderately	Very much	Extremely

Worried (health related)

Not at all	Slighty	Moderately	Very much	Extremely

How would you rate the overall liking of this product?
Overall liking

Dislike Extrem ely	Dislike Very Much	Dislike Modera tely	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Modera tely	Like Very Much	Like Extrem ely

How likely will you purchase this product?

- Yes
- No

Please taste the following chicken breast samples in the order presented. Between the samples, drink water and eat unsalted crackers to clean your palate.

APPENDIX E. IRB APPROVAL.

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?** 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

VITA

Ana Ocampo was born and raised in San Pedro Sula, Honduras in 1989. She attended her elementary and high school in the present city. In 2008 she enrolled as an undergraduate at Zamorano University period under which she performed a senior internship at Purdue University in Indiana, USA. After graduating she worked for 1.5 years at Lacthosa dairy company in the Research and development department. Then she had the opportunity to work with CADECA poultry and meat processing company in the research and development department for a year. During the summer 2014, she was granted the opportunity of attending a summer internship at LSU. After completion she enrolled at LSU to pursue her Master's degree in the department of Food Science.