SALT CONCENTRATION AND SPECIES AFFECTS PROTEIN EXTRACTABILITY AND PROCESSED MEAT CHARACTERISTICS

BY

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THESIS

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Abstract

Excessive sodium consumption is one contributor to high blood pressure and cardiovascular disease, and the Institute of Medicine has recommended that salt levels be reduced in food, including processed meats. As such, it is critical to understand the interaction of salt level and meat species with regards to characteristics of further processed meat. Salt-soluble protein extraction, important in forming meat emulsions, is dependent on several factors including species of protein source. As such, the purpose of our first study was to quantify the amount of salt-soluble proteins in various muscles of three species commonly used in meat products - beef, pork, and chicken. Pork and beef serratus ventralis (dark) and semitendinosus (light) muscles, and chicken pectoralis major muscles (light) and the entire thigh (dark) were used. Samples were analyzed for salt-soluble protein content at salt (NaCl) concentrations ranging from 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, to 3.5%. Overall, there was an increase in extractable salt-soluble protein content as the salt concentration increased. The interaction of species and salt showed all species were different (P < 0.05) from one another when compared at the same salt concentration when corrected for fat and moisture content with chicken showing the greatest extractability. In chicken, extractable salt-soluble protein was increased (P < 0.01) in light muscles compared with dark muscles. In industry, most processed products include 1.5-3.5% salt, and at these levels we have demonstrated that chicken meat had more salt-soluble proteins to aid in the processing of meat products. Given the advantage of chicken overall and in chicken light muscles compared with dark muscles, the purpose of the second study was to evaluate characteristics of products manufactured from pectoralis major muscle (light) and the entire thigh (dark) of chicken. The study was designed as a complete randomized design in a 2x4 factorial arrangement with factors of muscle type (light and dark) and salt concentration (0.5%, 1%, 1.5%, and 2%). Bologna was manufactured and analyzed for salt-soluble protein
content, proximate analysis, cooking yield, pH, Minolta L*, and binding strength. In thigh muscle bologna batches, raw and cooked moisture content, as well as cooked fat content, was increased over breast muscle batches (P < 0.01). Increased retention of moisture in thigh muscle bolognas contributed to improved cooked yields compared to breast muscle bolognas (P = 0.01). We were especially interested in whether characteristics of low-salt (≤ 1%) light muscle bolognas were similar or superior to higher salt (≥ 1.5%) dark muscle bolognas. Therefore, we used a single degree of freedom contrasts to compare these products. Low salt breast bolognas exhibited slightly higher (0.05 percentage units) salt-soluble protein extractability (as a percentage of crude protein) compared with higher salt thigh meat bolognas. The increase in salt-soluble protein content, however, was not reflected in a higher break strength value for low-salt breast bologna compared to high-salt thigh bologna (P < 0.01). Hardness increased in low-salt breast bologna compared to high-salt thigh bologna (P < 0.01). Low salt breast bologna showed acceptable processing characteristics and textural properties, therefore it can be used to manufacture low salt products.
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Chapter I
Review of Literature

Introduction

Today’s consumer devotes less time to preparing meals, so they look to ready-to-eat products to reduce time spent in the kitchen. However, these products traditionally tend to be higher in sodium (975mg sodium/100g), than unprocessed meat, such as steaks or roasts (50-90 mg sodium/100g) (Romans, Costello, Carlson, Greaser, and Jones, 1994). In 2010, the Dietary Guidelines for Americans listed reducing sodium as a major goal (USDA and USDHHS, 2010). In addition, the Institute of Medicine recommends that intake of sodium not exceed 2300 mg per day for people over the age of 14. At this level of intake, sodium is not likely to trigger adverse health effects, such as increased risk of developing hypertension (Dahl, 1972). However, average sodium intake for Americans over the age of two is approximately 3400 mg per day, nearly 1.5 times more than recommended levels (USDA-ARS and USDHHS-CDC, 2006). Thus, it is imperative that consumers lower sodium intake as one way to ensure a healthy lifestyle. In response to desires for lowered sodium, meat processors offer products with reduced sodium levels. The challenge for the industry, however, is to create high-quality products that remain acceptable to the consumer despite reductions in salt.

Salt, defined in this paper as sodium chloride (NaCl), was initially used by people to preserve meat. Currently, salt is the most widely used non-meat ingredient in processed foods and functions to enhance the flavor and texture of meat products. Terrell (1983) described the following to be advantages of salt addition to meat products: extraction of proteins to increase hydration and water binding capacity, increased binding properties of proteins to improve
texture, increased viscosity of meat batter facilitating the incorporation of fat to form stable batters, increased pH of meat systems, flavor enhancement and bacteriostatic effects.

**Protein Extraction**

Muscle protein is categorized into three classes of proteins, differing in function and their ability to be solubilized in salt solutions. The first class, sarcoplasmic proteins, account for approximately 30% of total muscle proteins and are soluble at low ionic strength (>0.3mM) (Scopes, 1964). Proteins from the sarcoplasm, including myoglobin and metabolic enzymes make up this class of proteins. The second class of proteins is the myofibrillar proteins, which comprise approximately 50-60% of muscle proteins and are soluble at salt concentrations greater than 0.3M. Myosin and actin contribute 45 and 20%, respectively, to the total myofibrillar protein content making these two proteins the most important when considering protein extraction in further processed products (Scopes, 1964). The third class of proteins is known as the stromal proteins, which are not soluble in salt solutions unless heat is added. These stromal proteins constitute approximately 10-20% of the total muscle proteins and consist of collagen and elastin.

Further processed meat products typically begin with a primary comminution (reduction of particle size) phase. Comminution is essential in disrupting membranes and sarcolemma of muscle in order to free myofibrillar proteins and allow them to swell. After comminution, salt is added, increasing the water-binding capacity of myofibrillar proteins by creating a downward shift in the isoelectric point of these proteins, resulting in a larger net negative charge. Hamm (1961) theorized that salt caused a repulsion of peptide chains on myofibrillar protein, allowing water to enter this space. As the concentration of salt incorporated in products increases, the
amount of myofibrillar protein extracted increases until a threshold is reached at 1.2 M NaCl (Munasinghe and Sakai, 2004). Salt extraction of proteins is affected by pH, extraction volume, and homogenization time (Lan et al., 1993). The ideal extraction for beef and pork occur at a pH of 6.0 with an extraction volume of 15 X sample weight and a homogenization time of 90 to 120 seconds (Lan et al., 1993). Extraction also differs between species and muscles within species. Under ideal extraction conditions, pork longissimus dorsi muscle yielded greater values for salt soluble proteins as a percent of the sample weight when compared to that of beef longissimus dorsi muscle and semimembranosus muscle (Lan et al., 1993). The content of extractable salt-soluble proteins was highly variable due to differences in pH and collagen content among red meats commonly used in the manufacturing of further processed meats. Cow meat had the highest extractability (8.19%) and meat classified as “rework”, the lowest (1.23%) (Saffle and Galbreath, 1964). However, many studies have established that greater amounts of salt-soluble proteins could be extracted from breast muscles of poultry (turkeys, chickens, and ducks) when compared to the leg muscles of the same animals (Khan, 1962; Hudspeth and May, 1967; Maurer et al., 1969; McCready and Cunningham, 1971; Prusa and Bowers, 1984; Richardson and Jones, 1987). Constrained by the type of product being manufactured, the extractability of salt-soluble proteins is highly variable and dependent on several processing factors, such as pH and time. Therefore, it is important to characterize the extractability of different species and muscles to identify where they can best be used in further processed products.

**Formation of Meat Emulsions**

The ability of salt to solubilize myofibrillar proteins is one of the most important factors in the processing of emulsified meat products like bologna and frankfurters. These products are
formed by blending a mixture of lean meat, fat, water, salt and other ingredients into an emulsion forming a nearly homogenous product. Meat emulsions form a two-phase system with the dispersed phase containing fat particles and the continuous phase consisting of suspended proteins and water containing dissolved salts (Forrest et al., 2001). In this system, solubilized proteins add stability to the emulsion, acting as emulsifying agents and reducing surface tension formed when fat comes into contact with water (Schut, 1976). Solubilized protein molecules coat fat particles through protein’s affinity for both hydrophilic and hydrophobic particles. Protein molecules, once unfolded, are oriented around fat particles with hydrophobic side chains in contact with fat and hydrophilic side chains in contact with water (Forrest et al., 2001). Increasing the amount of solubilized protein will lead to an increase in the emulsion stability of the product, which is a measure of the ability of the emulsion to retain fat and water (Zorba et al., 1993). Also, increasing the solubilized protein concentration will lead to an increase in the emulsion capacity of the product, which is a measure of the ability of the emulsion to encapsulate fat (Swift and Sulzbacher, 1963). Although, bologna and frankfurters are classified as emulsions, they are not true emulsions. When making frankfurters not all of the fat particles in the system were uniformly surrounded by solubilized proteins leading to a more heterogeneous mixture than a true emulsion (Swasdee et al., 1982).

**Gelation**

After the emulsified product is placed into a casing, it is thermally processed allowing for muscle protein gelation to occur. The gelation of proteins in comminuted products is responsible for adhesion of meat to meat as well as binding fat particles within the meat. The process of forming this gel matrix takes place in three steps: the initial denaturing of proteins; the
aggregation of these individual proteins, and the crosslinking of the protein aggregates to form a continuous network after heating (Xiong, 2004). The ability of these myofibrillar proteins to form a stable gel matrix is influenced by structure, size, concentration and pH of the protein. It has been demonstrated that intact myosin contributes to the binding properties of gels and that actin alone cannot influence these properties (Samejima et al., 1969). However, the presence of actin at a myosin to actin ration (w/w) of 24 increased rigidity of gels when compared to gels prepared in the absence of actin (Morita and Ogata, 1991). The balance of proteins within a muscle also influences gel stability. Muscles containing large amounts of connective tissue are detrimental to the formation of a stable gel matrix due to their poor emulsifying properties and the propensity to shrink during the heating process. Therefore, the amount of myofibrillar proteins extracted in relation to the amount of collagen will influence the quality of the finished product.

Muscle type and pH influence gel stability and these factors interact with each other. Myofibrillar proteins from white muscles are able to form firmer gels than those from red muscles due to the differences in myosin isoforms (Xiong, 1994). Gels formed from chicken and turkey pectoralis muscles (predominantly white) were more rigid and less influenced by pH than gels formed from chicken leg muscles (predominantly red) (Foegeding, 1987; Xiong and Blanchard, 1994). Chicken breast muscles formed the strongest gels at a pH of 6.30, while the thigh muscles were strongest at 6.00 (Xiong and Brekke, 1991; Lesiow and Xiong, 2003). Pork longissimus dorsi muscles (predominantly white) were also able to form stronger gels when compared to pork serratus ventralis muscles (predominantly red) (Robe and Xiong, 1993). In contrast, beef semimembranosus muscles (predominantly white) and vastus intermedius muscles
(predominantly red) gels showed no differences when compared at similar pH (Vega-Warner et al., 1999). The ability to target the use of muscles at their optimum gelation pH and salt extraction level in further processed products would enhance the emulsion stability.

Sensory

One of the challenges to reducing the inclusion level of salt in further processed products is consumer acceptability of lower sodium products. Consumers may not find the new flavor or textural properties to be similar enough to the products they are accustomed to eating. This is further complicated by the dual pressure to reduce the fat content of processed products as the combination of fat and salt in further processed products contributes many of the sensory characteristics found within these products (Ruusenen et al., 2003). Salt reduction, however, is possible while maintaining sensory properties. Panelists found the same degree of saltiness in hams containing 1.7, 2.0, and 2.3% salt (Ruusenen et al., 2001). Similarly, frankfurters could be manufactured with 1.3% salt and still maintain sensory hardness scores deemed previously to be acceptable to consumers (Matulis et al., 1995) and with no adverse effects to the physical properties of the product (Puolanne and Terrell, 1983a, 1983b). However, salt inclusions of less than 1.0% in frankfurters yielded poorly due to a lack of water retention (Puolanne and Terrell, 1983a, 1983b). The ability to reduce salt and maintain textural properties has been shown to a degree, however, to continue the reduction of salt and maintain acceptability gradual reductions may need to be made over a period of time.

Objectives

The purpose of this study was to identify species or muscles that would allow for salt reduction while maintaining acceptable protein extraction and product characteristics. The
species of interest are beef, pork and chicken because these species are commonly used by the meat industry for further processed products. It is expected that chicken will yield the highest amount of total extractable salt soluble proteins based on previous literature, however it is unclear what differences will be observed between beef and pork. Within a species, differences between muscle types and their suitability for further processing will also be determined. By identifying differences in extractability of salt soluble proteins that may exist between common livestock species such as beef, pork and chicken or between muscles, meat processors could choose protein sources that allow maximum myofibrillar protein extraction at reduced salt inclusion levels.

The objectives of this study were:

1) Quantify the amount of salt-soluble proteins in beef, pork, and chicken.

2) Determine further processed characteristics of products from white muscle and dark muscles over a range of salt inclusion levels.
References


Chapter II

Extraction of Salt-soluble Proteins in Meat is Affected by Species and Muscle Type

Abstract

Salt-soluble protein extraction, important in forming meat emulsions, is dependent on several factors including species of the protein source. The purpose of this experiment was to quantify the amount of salt-soluble proteins in various muscles of three species commonly used in meat products - beef, pork, and chicken. Pork and beef serratus ventralis muscles (dark) and the semitendinosus muscles (light), and chicken pectoralis major muscles (light) and the entire thigh (dark) muscles were used. Samples were analyzed for proximate composition and for salt-soluble protein content at salt (NaCl) concentrations of 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, and 3.5%. Overall, there was an increase in extractable salt-soluble protein content as the salt concentration increased. Chicken had the highest amount of extractable salt-soluble protein compared with beef or pork at all salt concentrations, when samples were corrected for fat and moisture content (P < 0.05). Chicken light muscles were able to extract more salt-soluble protein at most salt concentrations, except 0 and 2.5%, compared with chicken dark muscles (P < 0.01). This study demonstrates that chicken meat has increased amounts of extractable salt-soluble protein content compared to beef or pork.

Introduction

Extraction of myofibrillar proteins, commonly called salt-soluble proteins, is crucial in manufacturing of further processed meat products such as frankfurters and bologna. Once extracted, these proteins serve as emulsifying agents in meat batter and contribute to emulsion stability. Extraction is achieved through the addition of sodium chloride to meat. Recently, however, concerns have emerged in the medical community about excessive sodium intake and
its relation to cardiovascular disease (Page, 1976; Porter, 1983; Gleibermann, 1973; Tobian, 1997). Therefore, it is in the interest of meat processors to acceptably reduce sodium content in meat products.

One way to reduce the amount of sodium chloride included in further processed products is to target meat sources that are enriched in salt-soluble proteins. Greater amounts of salt-soluble proteins can be extracted from breast muscles of poultry (turkeys, chickens, and ducks) when compared with leg muscles of the same animals (Khan, 1962; Hudspeth and May, 1967; Maurer et al., 1969; McCready and Cunningham, 1971; Prusa and Bowers, 1984; Richardson and Jones, 1987). Under ideal extraction conditions, pork longissimus dorsi muscle yielded greater values for salt soluble proteins as a percent of the sample weight when compared with beef longissimus dorsi and semimembranosus muscles (Lan et al., 1993). There were no differences, however, in salt-soluble protein extraction between beef semimembranosus or longissimus dorsi muscles (Lan et al., 1993). Our objective was to determine differences in salt-soluble protein extraction between three species commonly used for further processing – beef, pork, and chicken and to determine effect of muscle type on protein extraction. For each species, a predominantly red fiber type muscle (serratus ventralis and entire thigh) and a predominantly white fiber type muscle (semitendinosus and pectoralis major) were analyzed for differences within species.
Material and Methods

Sample Collection

Five muscle samples (approximately 500 grams) were obtained from each of the following treatment combinations: serratus ventralis (dark) and semitendinosus (light) muscles from beef and pork, and from pectoralis major muscle (light) and the entire thigh (dark) from chicken two days post-harvest resulting in 30 total samples. Meat was trimmed of external fat, homogenized, vacuumed packaged and stored (-29°C) until analyses were conducted.

Proximate Analysis

The moisture and lipid content was determined for each sample using the method described by Novakofski et al. (1989). In preparation, all samples were trimmed of external fat and connective tissue, and homogenized using a Cusinart Food Processor. Moisture content was determined after samples had been placed in a drying oven at 110°C for 48h. Lipid content was then determined after extraction by washing samples in a mixture of chloroform and methanol.

Salt-Soluble Protein Content

The extractability of both sarcoplasmic and myofibrillar proteins were determined using the method described by Boler et al. (2011). A master mix extraction buffer of 0.01M 2-[N-Morpholino]ethanesulfonic acid was used to make the following eight NaCl extraction buffers 0% (0M), 0.5% (0.09M), 1% (0.17M), 1.5% (0.26M), 2% (0.34M), 2.5% (0.43M), 3% (0.51M) and 3.5% (0.6M) by volume. All extraction solutions were buffered to a pH of 6.0 according to Lan et al. (1993). Ground muscle samples (0.1 g) were placed in 2.0 mL microcentrifuge tubes with 4mm stainless steel beads and 1.5 mL extraction buffer. Samples were homogenized (TissueLyser II, Qiagen Inc., Valencia, CA) for 1 minute. Homogenates were centrifuged
(Microfuge® 18 Microcentrifuge, Beckman Coulter Inc., Germany) at 15000 x g for 20 min at 4°C. Supernatants were collected for each sample, diluted 10 fold, and protein content quantified using a BCA protein assay kit (Thermo Scientific Pierce Protein Research Products, Rockford, IL). The amount of solubilized protein was determined using a second order polynomial equation. Data were expressed as a percentage of wet weight and as the percentage of solids weight (weight minus fat and moisture).

$pH$

Meat (5g) was placed in a 50 mL conical tube with 20 mL of deionized water and homogenized (Model PT 10/35, Brinkmann Instruments Co., Switzerland) for 30 seconds. pH was measured with an automatic temperature compensation epoxy body probe attached to a bench meter (Model 917001, Model 501, Orion Research Inc., Jacksonville, Florida) that had been calibrated with pH 4.00 and 7.00 buffers.

Statistical Analysis

Data were analyzed as a randomized complete design in a 3x2 factorial arrangement with salt as a continuous variable. The statistical model included the main effects of species, salt, muscle type (light or dark) and their interactions. Differences between moisture, fat, and pH were determined using MIXED procedure of SAS (SAS Inst. Inc., Cary, N.C.) and means separated using the PDIFF option with a Bonferroni adjustment. Salt-soluble protein content were analyzed as repeated measures using an unstructured covariance structure with sample serving as the subject. Means of salt-soluble protein content were separated with the PDIFF option with a Bonferroni adjustment. Differences were deemed significant at P < 0.05.
Results and Discussion

Proximate Analysis and pH

For moisture content, the main effects of species and muscle type were significant (P < 0.01), however, there was an interaction of species and muscle type (P < 0.01). In beef and chicken, moisture content was increased in light muscles compared with dark muscles, but in pork, dark muscle had more moisture compared to light muscles (Table 1). Similarly, for fat content, the main effects of species and muscle type were significant (P < 0.01), however, there was an interaction of species and muscle type (P < 0.01). In beef and chicken, fat content was increased in dark muscles compared with light muscles, but in pork, light muscles had more fat compared to dark muscles. There is an inverse relationship seen between moisture and fat content, but this relationship is different for the muscles of pork when compared to beef and chicken muscles. Generally, it has been accepted that glycolytic (light) muscles contain less fat when compared to oxidative (dark) muscles since the main energy source of light muscles are carbohydrate glycogen (Leseigneur-Meynier and Gandemer, 1991). The observed values of chicken light and dark muscles and beef light and dark muscles were consistent with other findings (Xiong et al., 1993; Seggern et al., 2005). However, pork muscles did not follow this trend, perhaps due to the fact that metabolic pathways were more intermediate in the two muscles examined; still, the observed trend was substantiated by other work (Beecher et al., 1965).

For pH, the main effects of species and muscle type were significant (P < 0.01), however, there was an interaction of species and muscle type (P < 0.01). For chicken and beef, pH was lower in light muscles compared to dark muscles, which was expected due to the method of metabolism, associated with each muscle type; however, there was no difference between pork
muscles. Ultimate pH is dependent on lactate, the final product of glycolysis, which builds up in muscles after harvest due to stopped blood circulation. Muscles, whose metabolic pathway is primarily glycolytic like that of light muscles, will produce more lactate, which will drive ultimate pH downward (Lee et al., 2010). The values observed for pH of chicken, beef, and pork were consistent with the literature, in that light muscles reported lower values compared to dark muscles (Xiong et al., 1993; Seggern et al., 2005; Gil, et al., 2008). However, differences in pH were controlled for when running the salt-soluble protein assay by buffering the solution to pH of 6.0 for all samples.

Salt Soluble Protein Content

For salt-soluble protein content as a percentage of wet weight, the main effects of species, salt, and muscle type were significant (P < 0.01), however, there was an interaction of species and muscle type (P < 0.01), species and salt (P < 0.01), and species, muscle, and salt (P < 0.01). Overall, increasing amounts of protein were extracted as salt concentration increased (Figure 1). The increase in extractable salt-soluble protein content as the salt concentration increases is known as a salting-in effect, and for sodium chloride this effect is seen when salt is added at concentrations of 0.3 to 1.0 M (Hultin et al., 1995). In chicken light muscles, extractable salt-soluble protein content increased (P < 0.01) at a more rapid rate with increasing salt levels compared with all other species and muscle combinations resulting in the interaction of species, muscle, and salt (Figure 1). Thus, the effect of increasing salt concentrations on the solubility of muscle proteins is not only dependent on species, but also on muscle type being extracted. The greater extractability of chicken light muscle compared to chicken dark muscle was in agreement with other researchers (Khan, 1962; Hudspeth and May, 1967; Maurer et al., 1969; McCready and Cunningham, 1971; Prusa and Bowers, 1984; Richardson and Jones, 1987). The lack of
differences between pork and beef muscles may be due to the increase in the degree of similarity between muscle fiber types within the muscles, as opposed to the chicken muscles, which were predominantly type I or type IIb fibers. The interaction of species and salt showed similar trends, in that, all species were significantly different (P < 0.05) from one another when compared at the same salt concentration, except for beef and chicken at 0% (Figure 2A). Also of importance, chicken muscles were able to extract the same amount of salt-soluble proteins at 1% salt as beef muscles extracted at 2.5% salt, and that pork muscles never equaled this amount even at 3.5% salt (Figure 2A).

Comparing salt-soluble protein content as a percentage of solids weight (weight minus fat and moisture), the main effects of species and salt were significant (P < 0.01), however, there was a significant interaction of species and muscle type (P < 0.01) and species and salt (P < 0.01). The interaction of species and muscle type was due to the increased salt-soluble protein content of chicken light muscle compared to chicken dark muscle and the lack of differences within beef and pork muscles (Figures 3A, B, C). The interaction of salt and species was due to the differences (P < 0.05) of all species, when compared at the same salt concentration (Figure 2B). When comparing the same salt concentration, chicken muscles expressed higher contents of salt-soluble proteins, followed by beef and finally pork (Figure 2B). Also of importance, chicken muscles were able to extract the same content of salt-soluble proteins at 1% salt as beef muscles at 3% salt; an amount that was not able to be extracted from pork even at 3.5% salt (Figure 2B). Within species, chicken light muscles demonstrated increased (P < 0.02) values in salt-soluble protein content compared to dark muscles at all salt concentrations, except 0 and 2.5% (Figure 3B). However, pork muscles were only different at 3.5% (P < 0.01)(Figure 3A), and beef muscles were different at 1% (P < 0.02), 2.5% (P < 0.05), and 3% (P < 0.05) (Figure
3C). The values for salt-soluble protein content as a percentage of solids weight may be more accurate in determining differences in the amount of protein available in these meat sources since it is corrected for the moisture and fat content.

Identifying salt-soluble protein content of meat sources is important because these proteins act as emulsifiers in further processed products (Hudspeth and May, 1967). In industry, most processes included of 1.5-3.5% salt, and at these levels we demonstrate chicken as a species will have more salt-soluble proteins than pork or beef to aid in the processing of meat products. Also, chicken light muscles would be even more beneficial due to the advantages in extractability over chicken dark muscles.

**Conclusions**

Overall, the largest quantity of salt-soluble proteins was extracted from chicken followed by that of beef and finally pork. Chicken light muscles exhibited higher extractability at low salt concentrations (0.5-2%) with light muscles containing more extractable salt-soluble proteins compared to dark muscles as a percentage of solids weight. There were no differences in pork muscles at these low salt concentrations (0.5%-2%). Beef muscles did exhibit a difference at 1%, when comparing within this low salt range (0.5%-2%), with dark muscles being greater than light muscles. This study suggests that chicken light muscles could be used at lower salt concentrations compared to chicken dark, or beef and pork muscles due to the increase in amount of salt-soluble protein extracted that is needed to manufacture further processed products.
References


Tables and Figures

Table 1. Effects of species and muscle on proximate composition and pH of raw materials

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Figure 1. Salt-soluble protein content of light and dark muscles of pork, beef and chicken (as a percentage of wet tissue weight). Within a level of salt, asterisks indicate a difference at $P < 0.05$ between chicken light and all other species and muscle combinations.

Species * Muscle * Salt $P = 0.01$
Figure 2. Salt-soluble protein content of chicken, beef, and pork (A) (as a percentage of wet tissue weight) (B) (as a percentage of solids weight basis (weight minus fat and moisture)). Within a level of salt, means with different letters are different at $P < 0.05$. 

Species * Salt $P < 0.01$

$\text{a, b, c}$
Figure 3. Salt-soluble protein content of light and dark pork muscles (A), chicken muscles (B), and beef muscles (C) (as a percentage on solids weight basis (weight minus fat and moisture)). Means lacking a common superscript are different at $P < 0.05$. 

Species * Muscle $P < 0.01$
Chapter III

The Effects of Varying Salt Concentrations on Protein Solubility and Product Characteristics of Bolognas Manufactured from Chicken Muscles

Abstract

This experiment evaluated product characteristics of bolognas manufactured from pectoralis major (light) and the entire thigh (dark) muscles of chicken at various salt levels. The study was designed as a complete randomized design in a 2x4 factorial arrangement with factors of muscle type (light and dark) and salt concentration (0.5%, 1%, 1.5%, and 2%). Bologna was manufactured and analyzed for salt-soluble protein content, proximate analysis, cooking yield, pH, Minolta L*, and binding strength. Increased retention of moisture in thigh muscle bologna contributed to improved cooked yields compared with breast muscle bolognas (P = 0.01). The comparison of low-salt breast bologna to high-salt thigh bologna indicated a difference (P < 0.01) 0.05 percentage units extractable salt-soluble proteins as a percentage of total crude protein, with low-salt breast exhibiting higher values. The increase in salt-soluble protein content was reflected in higher hardness scores in low-salt breast bologna compared to high-salt thigh bologna (P < 0.01). This study suggests that incorporating chicken light muscles into processed products would reduce the amount of salt needed to produce products with acceptable processing characteristics.

Introduction

Today, there is growing concern in the medical community that excess salt consumption is leading to the development of disease. Nearly 16.7 million deaths worldwide and 685,000 U.S. deaths annually are attributed to cardiovascular disease (Mackay and Mensah, 2004; Hoyert et al., 2006). Hypertension, a chronic, abnormally high systolic and diastolic arterial blood
pressure (Lopez et al., 2006) has been identified as a contributing risk factor to the development of cardiovascular disease (JNC VI, Working Group). In 1972, Dahl described the link between dietary sodium intake and the development of hypertension, which other researchers have further demonstrated (Page, 1976; Porter, 1983; Gleibermann, 1973; Tobian, 1997). Therefore, reducing sodium intake, including that in processed meats is crucial to improving human health.

The need to reduce sodium levels in food products is evident; however, the use of sodium chloride in the further processing of meat products is essential. Salt disrupts the myofibrillar structure within muscle and allows myofilaments to swell and solubilize (Hamm, 1961). Increasing sodium chloride concentration increases salt-soluble protein extraction, resulting in increased amounts of fat and water that can be bound in products (Swift and Sulzbacher, 1963). As the amount of salt-soluble proteins acting as binding agents increases in a product, the binding strength of that product also increases (Acton, 1972). Break strength measures the texture profile of the cooked product and indicates ability to be handled for packaging and slicing (Herrero et al., 2008). In chicken loaves prepared entirely from either chicken breast or chicken thigh muscle, breast meat was superior in binding qualities when comparing break strength (Maesso et al., 1970). The purpose of this study was to determine the processing yield and textural properties of emulsified products manufactured from chicken breast and thigh muscles at various low salt (0.5-2%) concentrations.

**Materials and Methods**

*Raw Materials*

Approximately 120 kg of fresh, boneless and skinless chicken breast and thigh meat was obtained from a commercial poultry plant two days postharvest. Breast muscles were trimmed
to isolate pectoralis major muscles. Approximately 50 kg each of pectoralis major muscles and entire thighs were ground separately through a half-inch plate and mixed in a Hobart mixer to form a homogenate sample. For each muscle type, eleven (30 g) samples were collected to determine the proximate composition. While proximate composition analysis was being conducted, ground meat was stored in vacuumed packaged bags (4°C).

Proximate Composition

The moisture and lipid content was determined for each sample using the method described by Novakofski et al. (1989). In preparation, all samples were trimmed of external fat and connective tissue and homogenized using a Cuisinart Food Processor. Moisture content was determined after samples had been placed in a drying oven at 110°C for 48h. Lipid content was then determined after washing samples in a mixture of chloroform and methanol. Results of proximate composition analysis reported in Table 1.

Formulation

Two muscle combinations (pectoralis major muscle and thigh composite) and four sodium chloride concentrations (0.5%, 1%, 1.5%, and 2%) with four replicates per treatment were formulated for a total of 32 batches of bologna. Results of the proximate composition analyses were used to determine the appropriate fractions of raw materials for an 80:20 lean to fat ratio (Table 3). Pork back fat trim, free of visible lean, was added to reach the defined fat inclusion levels. Chicken was placed in a bowl chopper (Talsa, Windsor Food Machinery LTD, Kent, England) with seasonings and 1.5 pounds of ice and was allowed to mix for two revolutions. Pork fat trim was added and the mixture was chopped on a low blade speed for 1 minute and then on high for 1 minute. Batter was removed from the bowl chopper, and four samples (30g) were collected for proximate analysis, pH, and salt-soluble protein analysis.
Bologna batter was vacuumed stuffed into 5 3/8 inch collagen casings using a Handtmann stuffer (Model# VF 80, Biberach, Germany). After stuffing, chubs were weighed and individually identified. Product was allowed to rest at 2°C overnight before cooking. Bologna was cooked in an Alkar smokehouse (Lodi, Wisconsin) to an internal temperature of 66.1°C and chilled at 2°C for 24 hr. After chilling, the bolognas were weighed to determine cook loss and then peeled from casings. Bolognas were cut into six 2.54 cm pieces for texture analysis, proximate composition, pH, color.

**Salt-Soluble Protein Content**

The extractability of both sarcoplasmic and myofibrillar proteins were determined using the method described by Boler et al. (2011). A master mix extraction buffer of 0.01M 2-[N-Morpholino]ethanesulfonic acid was used to make the following eight NaCl extraction buffers 0% (0M), 0.5% (0.09M), 1% (0.17M), 1.5% (0.26M), 2% (0.34M), 2.5% (0.43M), 3% (0.51M) and 3.5% (0.6M) by volume. All extraction solutions were buffered to a pH of 6.0 according to Lan et al. (1993). Ground muscle samples (0.1 g) were placed in 2.0 mL microcentrifuge tubes with 4mm stainless steel beads and 1.5 mL extraction buffer. Samples were homogenized (TissueLyser II, Qiagen Inc., Valencia, CA) for 1 minute. Homogenates were centrifuged (Microfuge® 18 Microcentrifuge, Beckman Coulter Inc., Germany) at 15000 x g for 20 min at 4°C. Supernatants were collected for each sample, diluted 10 fold, and protein content quantified using a BCA protein assay kit (Thermo Scientific Pierce Protein Research Products, Rockford, IL). The amount of solubilized protein was determined using a second order polynomial equation. Data were expressed both as a percentage of wet weight and as a percentage of total crude protein.
pH

Meat (5g) was placed in a 50 mL conical tube with 20 mL of deionized water and homogenized (Model PT 10/35, Brinkmann Instruments Co., Switzerland) for 30 seconds. pH was measured with an automatic temperature compensation epoxy body probe attached to a bench meter (Model 917001, Model 501, Orion Research Inc., Jacksonville, Florida) that had been calibrated with pH 4.00 and 7.00 buffers.

Binding Strength and Compression Analysis

Binding strengths of bologna slices were analyzed using a cross bar and platform attached to a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) (Souza et al., 2011). The crossbar speed was 10 mm/sec and the load cell capacity was 100 kg. The crossbar descended until the sample was fractured and the peak force necessary to fracture the sample was recorded. Two samples were fractured and the average peak force was reported for each bologna.

The Bourne analysis (Bourne, 1978) was used to evaluate hardness of bologna. Four 2.54 cm cores were collected from each sample and compressed on Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). A 5.08 cm diameter plate compressed each core in two consecutive cycles to 75 percent of the samples original height with 2 s intervals between cycles. The cross-head moved at a constant speed of 5 mm/s. A force-time curve was plotted and the peak force of the first compression was used to determine hardness. The values for the 4 cores were averaged and reported as hardness of each bologna.
**Color**

Objective color scores (L*) were obtained using a Minolta CR-300 at a D65 light source and a 0° observer immediately from the cut surface of each bologna. For each sample, one measurement was taken from each half of the slice and the average of the two values was reported for each parameter.

**Statistical Analysis**

Data were analyzed as a randomized complete design in a 2x4 factorial arrangement. The statistical model included the main effects of muscle type (light or dark) and salt (0.5%, 1%, 1.5%, and 2%) and their interaction. Differences between moisture, fat, pH, textural properties and color were determined using MIXED procedure of SAS (SAS Inst. Inc., Cary, N.C.). Means for moisture, fat, pH, and color were separated using the PDIFF option and adjusted using Tukey’s W Procedure to control for experimentwise error. Salt-soluble protein content was analyzed as repeated measures using a first order auto regressive covariate structure with sample serving as the subject. Single degree of freedom contrast statements were used to compare low-salt (0.5-1%) breast bologna and higher-salt (1.5-2%) thigh bologna for salt-soluble protein content, hardness, and break strength. Differences were deemed significant at P < 0.05.

**Results and Discussion**

**Proximate Analysis and pH**

In thigh muscle bologna batches, raw moisture content was increased over breast muscle batches (Table 3). Also, the raw fat content of thigh muscle bologna was increased (P = 0.07) over breast muscle bologna (Table 3). The interaction of muscle and salt can be attributed to thigh bologna at 0.5% having a higher raw fat content (P < 0.05), compared to thigh bologna at
1.5% (Figure 4A). However, the raw fat content of breast bologna showed no differences at any of the salt concentrations. Differences in raw fat content between thigh and breast bolognas may be attributed to formulation error during the addition of fat trim to chicken trim and may have affected further characteristics.

To account for the variation in raw fat content, this parameter was used as a covariate for cooked fat content, cooked moisture content and cooked yield.

For cooked moisture content, thigh muscle bologna was increased compared to breast muscle bologna (P < 0.01); however, there was an interaction of salt and muscle type (P < 0.01). This interaction was due to thigh bologna having increased (P < 0.05) cooked moisture content at 0.5% salt compared to 1.5% salt (Figure 4B). However, breast bologna showed no differences in cooked moisture content (Figure 4B). The main effect of muscle (P < 0.01) was significant for cooked fat content, and there was an interaction of muscle and salt (P= 0.02) (Table 4). The interaction was due to breast bologna tending to decrease in cooked fat content with increased salt levels while thigh bologna exhibited the opposite trend (Figure 4C). Overall, thigh bologna had more cooked fat content compared to breast bologna at all salt concentrations (P < 0.01) (Table 4). The increase in moisture content and fat content of thigh muscles contributed to the improved cooked yields of thigh muscle bologna compared to breast muscle bologna. The main effects of species and muscle were significant (P < 0.01) for cooked yield as a percentage of product weight (Table 4). Increasing the salt concentration resulted in increased cooked yields for both bologna muscle types, however, thigh bologna had improved (P < 0.01) cooked yields compared to breast bologna. The findings of improved cooked yields of chicken products with the addition of increasing salt concentrations have been observed by other researchers (Acton,
1972; Lan et al., 1995; Somboonpanyakul et al., 2005). However, none of these papers compared the cooked yields of chicken breast and chicken thighs.

The main effect of muscle type was significant (P < 0.01) for raw pH (Table 3). Breast muscle bologna had lower pH values compared to thigh muscle bologna, which was expected due to the method of metabolism associated with each muscle type. The increase in lactate within the breast muscles drove the pH down compared to thigh muscle and values observed were similar to other findings (Lee et al., 2010; Xiong et al., 1993). Increased values for thigh pH may have led to an increase in water retention in the final product. However, pH was buffered for salt-soluble protein content analysis to a pH of 6.0.

Salt-Soluble Protein Content

A single degree of freedom contrast statement was used to evaluate salt-soluble protein content of low-salt (0.5%-1%) breast bologna and high-salt (1.5-2%) thigh bologna. Comparing the low-salt breast bologna group to the high-salt thigh bologna group a difference (P = 0.01) was detected, in that breast bologna extracted 1.05 percentage units more salt-soluble proteins as a percentage of wet weight compared to thigh bologna (Figure 5A). These differences persisted when salt-soluble protein content was corrected for total protein content of bolognas. The comparison of low-salt breast bologna to high-salt thigh bologna indicated a difference (P < 0.01) of only 0.05 percentage units extractable salt-soluble proteins as a percentage of total crude protein, with low-salt breast exhibiting higher values (Figure 5B). The increased extractability of breast muscle compare to thigh muscle was consistent with the findings of several researchers (Khan, 1962; Hudspeth and May, 1967; Maurer et al., 1969; McCready and Cunningham, 1971; Prusa and Bowers, 1984; Richardson and Jones, 1987). Overall, the increased amount of salt-
soluble proteins in low-salt breast muscle bologna should impact the products overall textural properties resulting in a firmer product when compared to high-salt thigh bologna.

**Binding Strength and Compression**

Break strength scores were expected to increase as the amount of extractable salt-soluble proteins increased, due to their role as a binding agent in further processed products. As the level of salt increased, break strength scores also increased, for both breast and thigh bologna (Figure 6A). When comparing low-salt breast bologna to high-salt thigh bologna, it took 3.30 kg more force to break the thigh bologna ($P < 0.01$) (Figure 6A). Even though it low-salt breast bologna contained a larger quantity of salt-soluble proteins compared to high-salt thigh bologna, that did not correlate to higher values for break strength.

Bourne (1978) defined the parameter of hardness to be a measure of the first bite of a product. The first bite of low-salt breast bologna was increased ($P < .01$) by 2.19 kg of force compared to high-salt thigh bologna (Figure 6B). The increase in hardness scores agrees with previously published results which found that salt-soluble protein content increases corresponded to rigidity of the product (Somboonpanyakul et al., 2005; Barbut and Mittal, 1989). The acceptability of this product by the use of a sensory panel was not tested; however, Matulis et al. (1994) observed the acceptable hardness scores of frankfurters to be approximate six with a standard deviation of two, which would put low-salt breast bologna within this range.

**Color**

For $L^*$ color scores, the main effect of salt and muscle type were significant ($P < 0.01$), however, there was a significant interaction of salt and muscle type ($P = 0.05$). Increasing salt concentrations resulted in a decrease in $L^*$ color scores for both breast and thigh muscle bologna; however, at all salt concentrations breast muscle bologna was lighter compared to thigh
muscle bologna (Figure 7). This result was similar to other studies, which found that as the pH of the product measured increased there was a decrease in the recorded L* value due to an increase in the retention of surface water (Fletcher et al., 2000).

Conclusions

In thigh muscle bologna batches, raw and cooked moisture content as well as cooked fat content was increased over breast muscle batches (P < 0.01). The increased values for thigh pH may have led to an increase in water retention in the final product. The increase in retention of moisture of thigh muscles contributed to the improved cooked yields of thigh muscle bologna compared to breast muscle bologna (P = 0.01). The comparison of low-salt breast bologna to high-salt thigh bologna indicated a difference (P < 0.01) 0.05 percentage units extractable salt-soluble proteins as a percentage of total crude protein, with low-salt breast exhibiting higher values. The increase in salt-soluble protein content, however, was not reflected in a higher break strength value for low-salt breast bologna compared to high-salt thigh bologna (P < 0.01). Although the scores recorded for hardness did show an increase in low-salt breast bologna compared to high-salt thigh bologna (P < 0.01). Overall, textural properties did not show the same trend for each group compared and no human analysis was used to determine if these products would be acceptable. Furthermore, although thigh muscle bologna was improved in cooked yields and moisture content, it was not tested if breast muscle bologna would still be deemed acceptable by consumers. The ability of chicken to extract high amounts of salt-soluble proteins at low salt concentrations could be taken advantage of in the meat industry by manufacturing products that incorporate both chicken and beef or pork. This would allow for a variety of low sodium meat products to be manufactured.
References


### Tables and Figures

#### Table 2. Proximate Composition of breast and thigh muscles

<table>
<thead>
<tr>
<th></th>
<th>Moisture, %</th>
<th>Fat, %</th>
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</thead>
<tbody>
<tr>
<td>Breast</td>
<td>74.44</td>
<td>3.93</td>
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<tr>
<td>Thigh</td>
<td>71.36</td>
<td>11.90</td>
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#### Table 3. Formulation of chicken bologna batches

<table>
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<tr>
<th>Bologna</th>
<th>Breast</th>
<th>Thigh</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Pork Fat Trim, kg</td>
<td>1.13</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken Trim, kg</td>
<td>5.67</td>
<td>6.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw Moisture, %</td>
<td>70.43</td>
<td>71.60</td>
<td>0.11</td>
<td>&lt; 0.01</td>
<td>0.30</td>
<td>0.63</td>
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<tr>
<td>Raw Fat, %</td>
<td>12.81</td>
<td>13.17</td>
<td>0.14</td>
<td>0.07</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
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<tr>
<td>Raw pH</td>
<td>5.72</td>
<td>6.27</td>
<td>0.01</td>
<td>&lt; 0.01</td>
<td>0.23</td>
<td>0.58</td>
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</tr>
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Table 4. Effect of muscle and salt on processing characteristics of bologna

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Salt Concentration</th>
<th>P-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>Thigh</td>
<td>0.05%</td>
<td>1%</td>
</tr>
<tr>
<td>Cooked Moisture, %</td>
<td></td>
<td></td>
<td>67.94(a)</td>
<td>67.98(a)</td>
</tr>
<tr>
<td>Cooked Fat, %</td>
<td></td>
<td></td>
<td>15.19(a)</td>
<td>15.22(a)</td>
</tr>
<tr>
<td>Cook Yield, %</td>
<td></td>
<td></td>
<td>89.78(c)</td>
<td>91.66(b)</td>
</tr>
</tbody>
</table>

\(a\), \(b\), \(c\) Means lacking a common superscript are different \(P < 0.05\)

d pH of bologna batch after emulsification

e Moisture content of bologna after cooking

f Fat content of bologna after cooking

g \((\text{cooked weight}|\text{raw weight}) * 100\)

All means were calculated using raw fat content as a covariate
Figure 4. Effects of muscle and salt on processing characteristics of bologna. Raw fat content (A), cooked moisture content (B), and fat content as a percentage (C). Means lacking a common superscript are different at P < 0.05.
Figure 5. Salt-soluble protein content of breast and thigh as a percentage of wet weight (A) and as a percentage of total crude protein (B). Asterisks indicate differences between low-salt (0.5-1%) breast bologna and high-salt (1.5-2%) thigh bologna at $P < 0.05$. 

A

![Graph A: Salt-soluble protein content of breast and thigh as a percentage of wet weight.]

B

![Graph B: Salt-soluble protein content of breast and thigh as a percentage of total crude protein.]

Muscle $P < 0.01$
Figure 6. Force required to break slices of bologna (A) and force required to compress slices of bologna made from chicken breast and thigh muscles (B). Asterisks indicate differences between low-salt (0.5-1%) breast bologna and high-salt (1.5-2%) thigh bologna at $P < 0.05$. 

**A**

![Graph A](image1)

**B**

![Graph B](image2)
Figure 7. Minolta L* values of bologna made from chicken breast and thigh meat. Asterisks indicate differences between muscle types at $P < 0.05$. 

![Graph](image-url)