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Physiochemical and Rheological Properties of Alkaline Isolated Poultry Proteins

by

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DEDICATION

To my father, Ali Moayedi Mamaghani, who always watches over me from the sky with his special smile.

To my mother, Sorour, who has always supported me in all ways…without you I would never be at this stage.

To Pouya, Lida, and Alireza, my motivators and supporters, without your great help, this would not have been possible.

ABSTRACT

Chicken dark meat has been considered as a major underutilized commodity due to the increasing demand for further processed breast meat products. Alkali aided protein extraction is an option to increase the utilization of chicken dark meat. First, the effect of pH (10.5-12.0) on alkaline extraction of chicken dark meat has been studied, and protein yield, composition, color, and **TBARs** of the extracted meat have been determined. Second, textural and rheological properties and water holding capacity **(WHC)** of alkali extracted chicken dark meat have been evaluated. The highest protein yield (94.2%) was obtained at pH 12.0. Lipid content of the extracted meat decreased by 50% compared to chicken dark meat. WHC, hardness and chewiness of extracted meat were greater at higher pH. The gel from recovered meat with added cryoprotectants showed more stability. This process may offer the possibility to use the underutilized poultry resources for preparation of functional foods.

 Keywords: Chicken dark meat, alkali aided protein extraction, composition, TBARs, color, texture, rheology, water holding capacity, cryoprotectant.

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LIST OF ABBREVIATIONS

AA: Arachidonic acid

DHA: Docosahexaenoic acid

DMA: Dynamic mechanical Analysis

DMCM: Dark minced chicken meat

EM: Extracted meat

EPA: Eicosapentaenoic acid

FA: Fatty acids

GC: Gas chromatography

LA: Linoleic acid

LC ω-3 PUFA: Long chain omega-3 polyunsaturated fatty acids

LNA: alpha-linolenic acid

MDA: Malonaldehydes

MT: Metric tons

MUFA: Monounsaturated fatty acids

PL: Phospholipids

PUFA: Polyunsaturated fatty acids

SFA: Saturated fatty acids

TBA: Thiobarbituric acid

TBARs: Thiobarbituric acid reactive substances

TG: Triacylglycerols

TPA: Texture profile analysis

WHC: Water holding capacity

1 LITERATURE REVIEW

1.1 'Low Value' Poultry Dark Meat

Poultry meat production worldwide approached 94.7 million metric tons (MT) in 2009 (FAO, 2009). Currently the US, China, Brazil and European Union (EU) with 19.4, 12.1, 11.3, and 8.5 million MT respectively, are the primary broiler producers (USDA, 2009a,b,c,d). In 2008 the amount of poultry production in Canada reached 1.2 million MT (Statistics Canada, 2009).

According to the US Livestock Marketing Information Centre, estimated per capita total red meat and poultry consumption in the US in 2007 was 110.7 kg (World Poultry, 2008). Chicken and turkey meat consumption in the US had a dramatic increase from 12 kg in 1950, to 52 kg per capita in 2007 (USDA/ERS, 2007). Chicken meat consumption in Canada was 21.5 kg per capita in 1989 while in 2008 it reached the level of 31.8 kg (CFC, 2008). The major factors which contributed to the increase in Canadian poultry consumption were population growth, demand for non red meat species, awareness of perceived health benefits of poultry meat, and continuing consumer preference for convenient and creative value-added chicken products with reduced meal preparation time (AAFC, 2006; Goddard *et al*., 2007).

Poultry meat can be categorized into high-value (mostly white meat) and low-value (mostly dark meat) (Peterson and Orden, 2005). Although, chicken meat mainly consists of broilers (high value), spent breeder hens and males, and spent table egg layers are also categorized in the group of low value poultry meat (Bilgili, 2000). Due to the low quality and yield of the meat from broiler breeders and spent layers, they are regarded as 'Low Value' meat, and there is little demand for them in food processing (AFAC, 2003). In the United States, white meat is preferred over dark meat (USDA, 2000). Therefore, with the increasing demand for further processed breast meat products (i.e. white meat), chicken dark meat has been regarded as a by-product (USDA, 2004). The major concerns with chicken dark meat which affect consumers' selection and satisfaction are color (due to heme pigments containing iron), high fat content and poor shelf stability (Froning,

1995; Fletcher, 1997; Betti and Fletcher, 2005). The differences in protein, lipid, moisture and iron content of chicken white and dark meat are presented in Table 1.1.

Low value dark poultry meat should not be considered only as muscle tissue which is processed into traditional forms of food, but rather as a source of functional ingredients for food product development. Agricultural commodities which are fractionated into components have been widely used as ingredients in other food products to improve functional properties. For example, isolated soybean proteins are used as binders, extenders, and fillers. In addition, isolated soy proteins can improve water-holding capacity, and lighten dark meat (Owens, 2001). Therefore, this idea can be also applicable to poultry dark meat.

Since meat quality is affected by the muscle structure in the living animal, it is important to overview chemical and physical composition of muscle proteins, which is provided in the following sections.

1.2 Structure and Protein Composition of Muscle

1.2.1 Skeletal Muscle

A schematic diagram of the muscle structure is shown in Figure 1.1. As can be seen in the diagram, a skeletal muscle is formed by many muscle bundles. Epimysium is the cover for muscle bundles. Perimysium is a connective tissue which separates muscle bundles from one another. Endomysium is a thin layer by which muscle fibers are separated from each other. Muscle fibers are the units which form a muscle bundle. Each muscle fiber consists of myofibrils and each myofibrill contains myofilaments. The Sarcomere is a small muscle unit which is involved in muscle contraction. There is a dark area in which a thick filament (myosin) and a thin filament (actin) overlap. This area is known as the A-band. There is a light area in the A-band which contains no thin filaments called the H-zone. The Z-line is the area which determines the borders of a sarcomere, and it is composed of narrow dark bands of proteins (Strasburg *et al.,* 2008). Muscle contraction occurs when thick filaments slide toward the Z-line (Barbut, 2002). There is a great variation in the size of the skeletal muscles from breast muscle to the muscle which control the movement of the eyes.

1.2.2 Muscle Proteins

Based on their solubility function, proteins in skeletal muscle have been categorized into sarcoplasmic, myofibrillar, and stromal proteins (Strasburg *et al.*, 2008). Sarcoplasmic proteins include proteins located in the sarcoplasm (cellular fluid) of the myofiber including myoglobin, hemoglobin, cytochromes, glycolytic enzymes and creatine kinase. These proteins are also called 'water soluble' proteins. This fraction constitutes about 30% of the total muscle protein content (Scopes, 1970).

Myofibrillar proteins include 50-60% of muscle proteins. These proteins are salt soluble and thus they are called 'salt soluble' proteins. Myosin and actin which are categorized in this group, are thick and thin filaments, respectively (Strasburg *et al.*, 2008). They play the main role in muscle contraction and consist of about 65% of the total myofibrillar proteins and around 40% of the total muscle protein content (Yates and Greaser, 1983). Other proteins included in this group are tropomyosin, troponin, C-Protein, α-Actinin, and β-Actinin (Barbut, 2002). According to the fact that actin and myosin are the most abundant proteins among the myofibrillar proteins, the solubility of all other proteins in this group will be affected by these two proteins, and greatly influence the functional properties of the processed meat products. Myosin which constitutes about 45% of the myofibrillar proteins is the main skeletal muscle protein involved in muscle contraction. Myosin forms the thick filaments and is the abundant protein present in the A-band area (Yates and Greaser, 1983). Myosin with molecular weight of approximately 540,000 daltons, is a long rod-shaped protein composed of six subunits including two heavy chains and four light chains. The heavy chains consist of the myosin head. Movement occurs when the myosin heads connect with actin (Barbut, 2002). Thin filaments are composed of three main myofibrillar proteins including actin, tropomyosin and troponin. Actin with a molecular weight of 42,000 daltons, makes up the thin filaments. The actin molecule is composed of two chains which are twisted together. Tropomyosin, another protein of the thin filaments, surrounds the actin molecule. It consists of 5% of the myofibrillar proteins and is a rod shaped protein. Troponin, another protein of the thin filaments, is composed of 5% of the myofibrillar proteins and is in globular shape. There are three types of troponin which include troponin-C, troponin-I, and troponin-T. They are all located along the actin filament.

When Troponin-C binds with calcium ions, the tropomyosin moves and as a result, the myosin binding site in actin molecule becomes exposed which leads to the connection of actin and myosin (Jackson *et al.*, 1975).

Stromal proteins, which comprise 10-20% of total muscle protein content, provide strength and protection for muscle tissue. The composition and abundance of these kinds of proteins greatly affect the quality of meat products. The major protein of this group is collagen (Strasburg *et al.,* 2008). Other proteins of this group are elastin and mitochondrial proteins (Barbut, 2002). Collagen contributes to meat toughness and increases with the age of the animal. Thus, the meat from older animals is of lower quality because it is more tough compared to the meat from the younger ones (McCormick, 1999; Purslow, 2005). During meat processing, cooking leads to breaking and partial solubilization of collagen which will increase meat tenderness (Resurreccion, 1994).

1.2.3 Muscle Fiber Types

According to muscle appearance, it is possible to classify the muscle fibers into red and white. The poultry dark meat (e.g. thigh) is mainly composed of red fibers, and due to this reason its color is dark compared to white meat (i.e. breast). The myoglobin content of red fibers is higher than those in white fibers leading to the color difference (Barbut, 2002). On the other hand, according to the type of metabolism, muscle fibers can be divided into two groups of oxidative and anaerobic (glycolytic). Thus, based on this classification, muscle fibers can be grouped into type I (slow oxidative), type IIa (fast oxidative glycolytic), and type IIb (fast glycolytic) (Strasburg *et al.,* 2008). Type I fibers, which are red fibers, (e.g. in thigh muscle) have high myoglobin content which supplies the oxygen for the oxidative metabolism, and fatigue occurs slowly. Mitochondria are the organelles which are responsible for aerobic respiration of the cell. The reactions during aerobic respiration require oxygen for ATP production, which is the energy source for all the cell activities. Since the metabolism of type I fibers is oxidative, they have higher number of mitochondria and the size of mitochondria is larger compared to the white fibers. Furthermore, the lipid content of the fibers is higher. These characteristics enable type I fibers to contract for longer time. The speed of contraction for these fibers is lower,

and their diameter is smaller than the white fibers. In contrast, type IIb fibers (e.g. in breast muscle) have low myoglobin content and their metabolism is glycolytic. Thus, fatigue occurs faster. Glycolytic metabolism can happen in the presence or absence of oxygen. Due to this fact, type IIb fibers have the lowest number of mitochondria, and also the size of their mitochondria is smaller compared to the red fibers. The lipid content of these fibers is also lower than the red fibers. All these characteristics lead fatigue to happen faster in these muscles compared to the red muscles. In addition, the contraction speed of type IIb fibers is higher than the red fibers and their diameter is larger compared to the red fibers (Barbut, 2002). Type IIa fibers are red, have medium amount of myoglobin and fatigue occurs on a medium speed compared to type I and type IIb fibers (Strasburg *et al*., 2008). According to the research by Smith *et al.* (1993) chicken *Pectoralis* muscle (i. e. breast) contained 100% Type IIb muscle fibers. According to Baeza and Brillard (1999), on average, chicken *Sartorius* muscle (i. e. thigh) was composed of type I (16%), type IIa (54%), and type IIb (30%) fibers.

1.3 Alternatives to Increase 'Low Value' Dark Meat Utilization

The 'low value' meat includes both dark meat fishes and poultry dark meat. The most important options to increase the utilization of 'low value' dark meat include traditional surimi technology and pH shifting processing. These technologies aim to concentrate and isolate muscle proteins from this type of raw materials.

1.3.1 Conventional (Traditional) Surimi Processing Technology

Surimi is a Japanese word which literally means "minced meat" and refers to a frozen concentrate of fish myofibrillar proteins which has been stabilized by antidenaturants (known as cryoprotectants). Surimi preparation was discovered 900 years ago by Japanese fishermen. The ancient surimi process involved mincing the fish with salt and spices, grounding it into a paste and cooking it to achieve a shelf stable gelled product. With the addition of cryoprotectants, which are sugars, surimi will be protected against freeze denaturation (Zamula, 1985; Vieira, 1996). As a result, surimi has a longer stability when stored in frozen conditions. For the first time cryoprotectants were used by Nishiya *et al*. (1960). In their study, after dewatering of fish myofibrillar proteins, they added carbohydrates (sucrose and sorbitol) and froze the product afterwards. The results

indicated that freeze denaturation of proteins was inhibited. The discovery revolutionized the surimi industry. Figure 1.2 shows an outline of the surimi manufacturing process. As the figure shows, surimi production includes several steps which involve preparing the fish for deboning, deboning process, mincing, washing and refining processes, dewatering, addition of cryoprotectants, and finally freezing the final surimi product. The details of each step are described in the following paragraph. Due to its reasonable price and good sensory characteristics, surimi seafood is a well-known product in the market (Shie and Park, 1999).

The aim of deboning process is to separate the flesh from the fish; usually carried out using mechanical meat separators. Before proceeding to deboning, fish should be prepared to get deboned. One method for fish preparation is removing head, gut and clean the belly walls (Pigott, 1986). The main concern at this step is the complete removal of viscera from the meat. Otherwise, the stability of the meat will be considerably decreased due to microbial infection (Park and Lin, 2005). Deboning and mincing processes are both using the same procedure, which is utilization of a roll-type meat separator. During these operations, the prepared fish is pressed through a perforated drum. While meat passes through the perforations and is conducted to the inner drum, bone, skin, etc. will be left in the outer drum. The optimum size of the perforations is suggested to be 3- 4 mm in diameter (Takeda, 1971; Lee, 1984).

The minced meat thus obtained is then water washed. During the washing process, the minced meat is washed with iced water with the ratio of 1:4 or 1:5, and this process is repeated twice or three times. Salt can be added to the minced meat at the level of 0.2 to 0.3% to help in removing the water from the meat (Southeast Asian Fisheries Development Center, 1988).

Sarcoplasmic proteins and lipids are removed by the washing process. Sarcoplasmic proteins include myoglobin, hemoglobin and other components, and lipids include neutral lipids (triacylglycerols) and polar lipids (mainly phospholipids). The presence of lipids (especially polar lipids) and sarcoplasmic proteins put the storage stability of myofibrillar proteins at risk (Scopes, 1970; Park and Lin, 2005; Betti *et al.*, 2009). On the other hand, the amount of water used for washing is of great importance

due to economic issues. Therefore, choosing a suitable washing procedure which uses reasonable amount of water and at the same time efficiently removes the dispersed fat and water-soluble proteins is highly important. According to Adu *et al.* (1983) some factors such as freshness of fish or the structure of washing unit affect the washing process.

The aim of the refining process is to remove stromal proteins (mainly connective tissues' components such as collagen) from the meat using a refiner. The quality of the refining process varies with the size of the refiner screen size and speed. For example, a larger screen size and fast speed leads to higher yields as well as greater amount of impurities in the isolated proteins. The composition of the fraction separated from the myofibrillar proteins by the refiner was reported as protein (15.4%), moisture (81.4%), lipid (1.9%) and ash (1.0%) (Kim and Park, 2003) in which the composition of the protein part was mainly stromal proteins. After the washing process, the water content of the meat is increased by approximately 10% (from 82% to 92%). The washed minced meat is further passed through a screw press. The goal of this process is to dewater the meat which means to remove the extra water gained from the washing process by applying mechanical force. According to Park and Lin (2005) by the use of salts, such as NaCl and $CaCl_2$ at the level of 0.1 to 0.3%, separation of the moisture from the screw press will be improved.

As mentioned before, cryoprotectants play an important role in surimi production, because they prevent muscle protein denaturation during frozen storage. Cryoprotectants such as sucrose and sorbitol (9% w/w) are used for this purpose. A number of studies performed to present new cryoprotectants to the industry. For example, trehalose (a disaccharide) and a short-chain glucose polymer have been introduced by Cargill Corporation and Roquette Corporation respectively (Hunt *et al.*, 2001, 2002). Although the mechanism of cryoprotectants are not fully understood yet, the addition of cryoprotectants leads to improved water holding capacity of the myofibrillar proteins, which further leads to a smoother paste in the final product. The last step in surimi production is freezing; usually done at -30 °C. The temperature for transportation of the frozen surimi is approximately -20 °C (Southeast Asian Fisheries Development Center,

1988). A quality control test for the surimi product is the detection of metals. In order to prevent lipid oxidation and increasing the surimi shelf life, it is of great importance to detect metals in surimi final product, which is a part of surimi HACCP program. The common metals detected are ferrous, copper, aluminum, and stainless steel, and according to the FDA's Health Hazard Evaluation Board metal fragments which have a length of 7-25 mm are considered hazardous (FDA, 2001).

1.3.2 pH Shifting Processing: A New Technology for the Recovery of Functional Proteins

Traditional surimi processing has been revolutionized by the introduction of a new technique. The new technology which is named pH shifting processing is based on shifting pH values, and is utilized in acid and alkaline solubilization processes (Hultin and Kelleher, 1999, 2000). This process consists of different phases. The first phase is homogenization of minced muscle with water. The ratio of meat to water varies between 1:5 to 1:9 (Nolsøe and Undeland, 2009). Homogenization is followed by the addition of acid or alkali solution to bring the pH above 10.5 or below 3.5 where proteins have the maximum solubility (see chapter 2). In order to remove lipids and stromal proteins from the muscle, centrifugation is performed. During centrifugation, materials are separated based on their density. Thus, fat (with least density) will be present as the top layer, while other impurities which have more density (skin, bone and cellular membranes) will be precipitated at the bottom of the centrifuge tube. Therefore, the middle fraction contains solubilized proteins which will be collected and then adjusted to the isoelectric point of the myofibrillar proteins, which is pH of 5.2 to 5.5. At this pH value, because myofibrillar proteins have no net charge, they will precipitate. Another centrifugation step is used to collect the sedimented proteins. After collection of the functional proteins the pH value can be re-adjusted to the natural pH of the muscle. The final step is adding cryoprotectants and freezing the final product. pH shifting processing is an ideal method for the utilization of the low-value raw meat, especially because this kind of raw material has high amount of fat which can be difficult to separate by conventional surimi process.

The advantages of pH shifting processing over traditional surimi production include higher protein yields (85% vs. 70%) due to more recovery of sarcoplasmic

proteins, and more efficient removal of lipids, connective tissue and other impurities thanks to the use of high-speed centrifugation (Kristinsson and Hultin, 2003; Hultin *et al.*, 2005; Sanmartin *et al.,* 2009). Also by utilizing pH shifting processing, there is no need for deboning the raw fish material (Nolsøe and Undeland, 2009). Using optimum amount of water, both neutral and polar fractions of lipids can be separated from the meat and thus, the shelf life of the final product will be increased due to reduction in lipid oxidation. Also, the waste water obtained from the pH shifting processing contains less solid parts due to the use of centrifugation (Park *et al.*, 2003).

1.3.2.1 Factors Influencing Protein Recovery using pH shifting Processing and their Comparison with Traditional Surimi Production

Due to the fact that traditional surimi production was not efficient enough, pH shifting processing was introduced as a new technology to improve and overcome problems in surimi processing. Most of the studies in this field are focused on fish as raw material. Therefore, in this section, comparisons between traditional surimi production and acid / alkaline solubilization process for fish have been reviewed, and relevant studies on poultry sources have also been included.

A variety of processes were used for separation of myofibrillar proteins from fat and pigments, and to increase oxidative stability of the final poultry products. One of the methods was the use of centrifugation (Froning and Johnson, 1973; Dhillon and Maurer, 1975). Young (1975) performed an aqueous extraction using a solution having a pH of 7.0 followed by precipitation at a pH of 4.5, for the protein recovery from mechanically deboned chicken meat (MDCM). Hernandez *et al.* (1986) used phosphate buffer solution at a pH of 8.0 for protein extraction, and efficiently removed fat and pigments from mechanically deboned turkey meat (MDTM). An aqueous washing with added sodium bicarbonate (pH: 8.0) was used by Dawson *et al.* (1989) for myofibrillar proteins' isolation from mechanically deboned chicken meat. Some researchers such as Jelen *et al.* (1982), McCurdy *et al.* (1986), Liang and Hultin (2003), and Betti and Fletcher (2005) extracted myofibrillar proteins from poultry dark meat and poultry residues by the use of high extraction pH and low precipitation pH.

Protein recovery, which is an important issue in both surimi and pH shifting processes, is affected by some other factors. One of these factors is protein solubility at alkali and acid pH ranges; which should be high to obtain good protein yields. Another factor is the size of the sediments formed after the centrifugations which should be low to increase the recovered proteins. The last factor is the solubility of the proteins at the precipitation pH, which should be low in order to increase the proteins' precipitation after the centrifugation process. Kristinsson and Ingadottir (2006) who worked on tilapia fish using alkaline and acid solubilization processes indicated protein yields of 61-68% for the alkaline process, while the yields for the acid process was reported as 56-61%. Therefore, in their study higher yields were obtained from the alkaline solubilization process. On the other hand, a study performed in 2002 by Undeland *et al.* who worked on white muscle of herring fish indicated protein yields of 68% and 74% for alkaline and acid protein extraction, respectively. The reason for lower yield for alkaline solubilization was due to the fact that during the first centrifugation larger sediment was formed, which reduced the yield.

Batista (1999) tested alkali process on hake and monkfish and obtained the average protein yield of 71.8%. To study the relationship of protein yield with solubilization pH, Kim *et al.* (2003) used a 1:10 fish meat to water ratio, and acidic and alkaline pH treatments. The results showed that at a pH of 12.0, the yield of proteins was approximately 70% (maximum level), while the yield was around 60% (minimum level) when a pH of 10.5 was used. However, due to economical issues, the amount of water used in the process is of high importance. Thus, an efficient amount of water should be chosen in a way to meet both yield and economical matters.

Froning and Johnson (1973) studied the effect of centrifugation on the quality of mechanically deboned fowl meat. The centrifugation process led to the formation of three phases including fat, aqueous, and meat fractions. The results indicated that the protein content of the final product was significantly increased from 14.2% to 22.7 % using centrifugation.

In a study conducted by Young (1975), protein extraction from mechanically deboned poultry meat was carried out. Protein extraction was performed using solvents

with pH range of 5.5 to 7.5, and precipitation pH range of 4.0 to 7.5. Results suggested the use of extraction pH values of above 6.5, with a precipitation pH of 4.5 for obtaining maximum yield of 83.7%.

In a Study by Liang and Hultin (2003) alkaline solubilization (pH: 10.8) and acid precipitation (pH: 5.2) was used for protein isolation from mechanically deboned turkey meat. The ratio of meat to water was 1:6 (w:v), and dewatering was performed by centrifugation at 10,000 × g for 25 min at 0 °C to 10 °C. The moisture content of the isolated proteins was adjusted to approximately 80%, and cryoprotectants (sorbitol, sucrose, sodium tripolyphosphate, and sodium bicarbonate) were added before freezing the final product. The results showed that protein content of the final product was increased from 12.0% to 17.0% compared to the raw meat.

Betti and Fletcher (2005) studied the effect of extraction and precipitation pH on the yield of broiler dark meat. They studied extraction pH range of 8.0 to 12.0, followed by precipitation pH range of 3.8 to 5.2. The better dry yields (above 70%) were obtained at extraction pH values of above 10.5 and precipitation pH values of above 4.4.

Due to the problems associated with lipid oxidation, it is of great importance to obtain a low level of lipid content in the final product. Several studies have been performed on lipid reduction using acid or alkaline protein extractions. Lipid removal depends on some factors such as the speed which is chosen for the first step of centrifugation process, the lipid content of the raw meat and the water amount used in the process. Since water is a polar solvent, using high amounts of water helps to separate the polar fraction of lipids from the muscle proteins. More details are given on this topic in chapter 2. During the first step of centrifugation process, the membrane lipids precipitate, which reduce the risk of lipid oxidation in the final product. Richards and Hultin (2001) studied the effect of lipid content on lipid oxidation in minced washed cod fish containing 0.1% fat. The results indicated high level of lipid oxidation for the final product. This result could emphasize the role of hemoglobin as a strong prooxidant, which causes lipid oxidation even when the amount of fat in the minced washed cod fish was as low as 0.1%. Thus, in order to prevent lipid oxidation in the presence of strong prooxidants like hemoglobin, the lipid content of the final product should be very low. The following

studies were conducted on lipid reduction using acid, alkali and traditional surimi processing.

The aim of a research conducted on several fish species including catfish, Spanish mackerel, mullet, and croaker by Kristinsson and Demir in 2003, was to compare lipid removal using surimi processing with acid and alkali aided process. The results indicated that the highest amount of lipid loss was found using alkaline solubilization followed by acid aided and traditional surimi process. A lipid removal of 68.4% (croaker), 81.4% (mullet), 79.1% (mackerel), and 88.6% (catfish) was reported using alkaline process. The lipid reduction values for acid process for the mentioned fish species were 38.1%, 58.0%, 76.9%, and 85.4%, respectively. Finally, the values for traditional surimi process were 16.7%, 10.4%, 72.1%, and 58.3%, respectively. The main difference between the processing of surimi and the acid or alkaline process was the dewatering step. In acid and alkali processes, centrifugation is used to remove the excess water from the recovered proteins, while in surimi technology cheesecloth is used for this matter, which probably affected removal of lipids. A study conducted by Cortes-Ruiz *et al.* (2001) on lipid reduction of fish (Bristly sardine) led to a 67.4% lipid reduction in surimi process, and 88.3% for the protein recovered by acid solubilization process, while the total lipid content of the fish was 3.3%.

Another study on lipid reduction was conducted by Kristinsson and Liang (2006). In this study Atlantic croaker fillets containing 3.1% lipid, was used for acid, alkaline, and surimi processing. The dewatering step was performed using centrifugation (10,000 × g) for 20 min for acid and alkali process, and by cheesecloth for the surimi process. The results showed a fat reduction of 68% for the acid process, 38% for the alkali process, and 17% for the surimi process.

A study conducted by Kristinsson *et al.* (2005) on channel catfish meat compared lipid reduction using three processes of acid and alkali protein extraction with traditional surimi processing. The results showed that the highest lipid reduction (88.6%) for alkaline processing, while the value for acid and surimi processing were 85% and 58%, respectively. The catfish meat was skinless fillets with a lipid content of 4.7% to 9.8%. While the dewatering step for surimi process involved squeezing by cheesecloth, this step

for acid or alkali process was operated by centrifugation (10,000 $\times$ g) for 20 min in 2 steps. The reason for the noticeable difference in lipid reduction between the conventional surimi process and the acid and alkaline processes was due to the first centrifugation step in acid and alkali processes (Kristinsson and Demir, 2003). Lipid removal was greater in acid and the alkaline processing than the traditional surimi processing due to the use of centrifugation. In most of the comparative studies on the acid and alkaline solubilization processes in fish, lipid reduction was higher by alkaline solubilization.

A study by Froning and Johnson (1973) was performed to improve the quality of mechanically deboned fowl meat by centrifugation. The results showed that centrifugation process (20,000 rpm for 15 min at 5 °C) had a significant effect on fat reduction from mechanically deboned poultry meat. Total fat content of the recovered proteins using centrifugation was decreased by 62.8%. The final product of an alkaline extraction followed by acid precipitation from mechanically separated poultry residues showed a 51.3% decrease in total fat content (Jelen *et al.*, 1982).

In a study by Dawson *et al.* (1989) an aqueous washing process was used to remove fat and pigments from mechanically deboned chicken meat. MDCM was mixed with a solution of water with added sodium bicarbonate (pH: 8.0) to solubilize myofibrillar proteins which are salt soluble. The ratio of meat to water was 1:4 (w:w), a pH of 6.8 was used to precipitate myofibrillar proteins, and separate them from pigments, and dewatering was performed by centrifugation. The results of this study showed that fat content of the extracted MDCM was decreased by 88.3% compared to the raw MDCM. The initial fat content of the MDCM was 12.8%.

Total fat content of the final product was decreased by 93.3% in a study using alkaline solubilization (pH: 10.8) and acid precipitation (pH: 5.2) which was performed on mechanically deboned turkey meat. The ratio of meat to water was 1:6 (w:v), and centrifugation (10,000 $\times$ g for 25 min, at 0 - 10 °C) was used for dewatering (Liang and Hultin, 2003).

Another factor for the quality of meat products is lipid oxidative stability which is related to the product storage stability. Minced meat products as well as products containing greater amount of prooxidants are those which are at high risk of lipid oxidation. This could be explained by the fact that after mincing process with the increased lipid surface being exposed to oxygen and prooxidants, lipid oxidation increases. Because of the activation of heme proteins in acidic conditions leading them to act as a prooxidant, the acid solubilization process caused more lipid oxidation than the alkaline process. In addition, the exposure of minced washed cod fish to acidic conditions (pH: 2.5) for 20 minutes makes the muscle more susceptible to oxidation (caused by hemoglobin) compared to native washed minced fish meat (Kristinsson, 2001). Acidic conditions exist in both acid and alkali processes. However during the acid solubilization process there is a greater exposure to acidic conditions. As has been mentioned before, there is an exposure to low pH (isoelectric point i.e. pH: 5.2-5.5) for myofibrillar proteins' precipitation, which would lead to lipid oxidation. Kristinsson and Liang (2006) reported more lipid oxidation for the recovered proteins from Atlantic croaker fish by acid process compared to the alkaline process. In a study conducted by Kristinsson and Demir (2003) on some fish species, lipid oxidation of the protein isolates using acid, alkali, and surimi process was evaluated. The species of fish used in this study included catfish, croaker, mullet, and Spanish mackerel, and the temperature of the water used in the process was 4 °C. After the process, the protein isolates obtained from all three processing methods were put in freezer bags, and kept in the fridge (4 °C). Afterwards, every third day samples were chosen and were put in the freezer (-70 °C) to prevent lipid oxidation. After this treatment, thiobarbituric acid reactive substances (TBARs) were measured as a method for lipid oxidation evaluation. The results indicated that the lowest amounts of lipid oxidation were found with surimi process compared to acid and alkali processing. The highest level of lipid oxidation was found with the acid solubilization process followed by the alkali aided process. The reason for the more lipid oxidative stability of surimi process would be that during this process, the minced meat is not subjected to low pH, which prevents hemoglobin and myoglobin activation leading to reduction in lipid oxidation.

A potential solution to reduce lipid oxidation is the addition of compounds such as metal chelators which can act like antioxidants and improve lipid oxidative stability of the recovered proteins. A study by Undeland *et al.* (2005) indicated that chelating agents such as ethylenediaminetetraacetic acid (EDTA) (0.04%) and sodium tripolyphosphate (0.2%), and also reducing agents such as erythorbate (0.2%), could act as antioxidants and improve lipid oxidative stability of acid-processed fish and also during the storage of the protein isolate. The results of this work showed that while the addition of only erythorbate or in combination with EDTA and sodium tripolyphosphate had a significant effect in decreasing lipid oxidation in the final protein isolate, without using these antioxidants, severe lipid oxidation occurred in the protein isolate.

Lipid oxidative stability of mechanically deboned poultry meat which was centrifuged was higher than those without centrifugation (Froning and Johnson, 1973). This finding indicated that fat reduction caused by centrifugation led to higher stability to lipid oxidation for the final product.

In a study conducted by Dawson *et al.* (1990) myofibrillar proteins from mechanically deboned chicken meat were extracted by a phosphate solution (pH: 8.0), followed by a precipitation pH of 6.8, and dewatered by centrifugation. The results of the study indicated that even though total fat content decreased by 88.3%, extracted meat was more susceptible to lipid oxidation than the raw meat. The lower lipid oxidative stability of extracted meat compared to the raw meat was due to unsuccessful removal of phospholipids which are more susceptible to lipid oxidation.

1.3.3 Functional Properties of Muscle Proteins and their Link to pH Shifting Process and Traditional Surimi Production

1.3.3.1 Protein Solubility

The definition of solubility or extractability of proteins is the percent of the total proteins extracted by water or a suitable solvent in specific conditions (Sikorski, 2007). One of the major factors affecting protein solubility in aqueous solutions is the pH of the

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solution. In alkaline and acidic conditions, proteins carry a negative and positive charge, respectively. This leads to repulsion between the proteins which finally cause proteins to be solubilized. On the other hand, because of the fact that proteins carry no net charge at the isoelectric point, they precipitate at this pH. For the isolation of different kinds of proteins from food, solubility difference is considered as an important issue (Kristinsson, 2001). Another factor which affects solubility is denaturation of the proteins. Solubility may decrease when proteins are denatured. According to Sikorski (2007) during heating, denaturation which is followed by aggregation of the proteins leads to an increase in hydrophobicity, and as a result, solubility reduces. The other critical factor which affects protein solubility is the ionic strength of a solution. Ionic strength determines the concentration of ions in a solution, and is calculated by the following formula:

$$\mu = \frac{1}{2}\sum C_i Z_i^2$$

In this formula C_i is the concentration of an ion and Z_i is the net charge of the ion (Fox and Foster, 1957). When ionic strength of a salt solution is low (i. e. lower than 0.5) protein solubility can increase or decrease depending on the amino acid composition on the protein surface. For example, for soy proteins, because hydrophobic (non-polar) amino acids are located on the surface of the protein molecule, they are unable to bind to the ions of the salt solution, and therefore, solubility of the proteins is reduced. In contrast, β-lactoglobulin and myosin have hydrophilic (polar) amino acids located on the surface of the protein molecule, thus they can bind to the ions in the salt solution, and increase protein solubility. With the increase in ionic strength of a salt solution (i. e. μ >1), different ions affect solubility in different ways. For example, for salt solutions containing ions of SO_4^{2-} and F^-, solubility decreases with the increase in the salt concentration (Damodaran, 2008). It means that salt solutions containing these ions are able to precipitate proteins from the solution. This concept is named as 'salting out'. On the other hand, when salt solutions containing ions of Br^-, I^-, SCN^-, and ClO_4^- are added to the protein solution, solubility increases with the increase in the salt concentration. This phenomenon is named 'salting in' (Sikorski, 2007). When ionic strength of a solution is kept at a constant amount, protein solubility can either increase or decrease

based on the anions and cations which are present in the solution. In this condition, while anions which most increase protein solubility involve SCN^-, ClO_4^-, I^-, Br^-, Cl^-, F^-, and SO_4^{2-} respectively, cations which most decrease protein solubility include Ca^{2+}, Mg^{2+}, Li^+, Na^+, K^+, and NH_4^+, respectively. This ranking of anions and cations is named the Hofmeister series (Damodaran, 2008).

1.3.3.2 Protein Gelation

Proteins from different sources of animal or plant have the gel forming ability. In case of myofibrillar proteins, when they are extracted from muscle and exposed to heat, they form an irreversible semisolid, three-dimensional structure, which is called a gel. Since myofibrillar proteins form the major component of processed meat products, gelation is an important factor in meat products industry (Acton *et al.*, 1978; Barbut, 1995).

Myofibrillar protein gelation process involves several steps. During the first step, muscle fibers are extracted by the use of salt. Afterwards, with the application of heat, protein denaturation occurs. The next step is aggregation, and it happens when unfolded proteins connect with the surrounding proteins. The last step occurs when aggregates cross-link which leads to the formation of a three-dimensional gel network and water entrapment within the network (Tarte and Amundson, 2006).

1.3.3.3 Rheological Properties

1.3.3.3.1 Dynamic Mechanical Analysis (DMA)

Dynamic mechanical analysis (DMA) is a method which is used to study dynamic viscoelastic behavior (DVB) of a material. For this purpose, dynamic or oscillatory tests are performed. During DMA, dynamic stress at a given frequency is applied to a material. Viscoelastic materials show both elastic (solid) and viscous (liquid) behaviors. The rheological factors which show DVB of the material, includes the storage modulus (G'), the loss modulus (G''), and loss angle (Tan delta). The storage modulus (G') is related to the elastic (solid) character of the material which indicates the energy which is stored during deformation (Peleg *et al.*, 1989; Hamann *et al.*, 1990). On the other hand, the loss modulus (G'') is related to the viscous (liquid) behavior of the material which indicates

the energy which is dissipated as the material is deformed (Vodovotz *et al.*, 2001). For example if we assume a solid which is ideally elastic, all the energy is stored (i.e. G"= 0) but for a viscous material in which all the energy is dissipated, G' is equal to zero. Tan delta which is defined as the ratio of G"/G' (Dogan and Kokini, 2007), shows the degree of viscoelasticity of a sample. If tan delta is a low value for a material, it means that the material has a higher elasticity (higher G' value) and is a more solid-like material.

1.3.3.4 Textural Properties

Texture of foods is an attribute which is strongly related to consumer preference, selection, and satisfaction of food products. Food industry efforts are to develop the proper texture for food products (Rohm, 1990; Gunasekaran and Ak, 2003). According to the International Organization for Standardization, texture of a food is defined as the rheological and structural attributes of a food product which is perceived by human senses (ISO, 1992). Texture of meat is an attribute that is determined by several factors such as hardness, springiness, chewiness, and cohesiveness. Differences in meat texture are related to the composition and structure of the meat including different kinds of proteins as well as fat and connective tissue. Some other factors such as cooking also affect meat texture (Bailey, 1972; Solomon *et al.*, 2009).

1.3.3.4.1 Textural Profile Analysis (TPA)

Textural properties of a material are measured by a technique called textural profile analysis. According to Mallikarjunan (2006), cohesiveness, springiness, chewiness, resilience and hardness are the textural attributes which are measured by the utilization of TPA technique. Definition of these parameters is important because each factor deals with different aspects of a material's texture. While the amount of work needed to chew a solid sample until the swallowing state is determined by chewiness value, the ability of a material to recover its original shape after the removal of the force is known as springiness. Resilience, on the other hand, determines an extent to which the sample can fight to get back to its original position. Cohesiveness determines the amount of deformation for a material before it breaks, and finally, hardness is the maximum force needed to compress a material. By determining each of the above factors, some information could be obtained about the texture of a material.

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1.3.3.5 Water Holding Capacity

Water holding capacity (WHC) which is one of the important attributes of both fresh meat or processed meat products indicates the extent to which meat can keep its moisture inside when some conditions apply (Swatland, 2002). These conditions can be the application of temperature (e.g. cooking), or compression. A number of factors affect WHC of meat such as pH, animal species, and age. The most important factor is the pH of the meat. In order to study the effect of pH on WHC of the meat, a study has been performed on beef (Grau *et al.,* 1953). The beef samples were immersed in buffer solutions with different pH values and their weight gain were measured afterwards. The results indicated the lowest weight gain for the samples which were immersed in the buffer solution with the pH value of 5.2. The reason could be explained due to the fact that pH of 5.2 is the isoelectric point for meat proteins. At this pH the net charge of the proteins is equal to zero, hence they are attracted to each other, and as a result they cannot hold water inside them. Thus, their WHC was at its minimum level (Honikel, 2009). The reason for WHC to be such an important factor for the meat is that it is associated with the appearance of the meat when exposed to heat treatment (i.e. cooking), and contributes to the juiciness of the meat. When it comes to comminuted meat products (e.g. sausages), WHC is particularly an important quality attribute. During the production of comminuted meat products, due to mincing process of the meat, structure of the meat proteins which hold the water is destroyed, and therefore, the ability of the meat to hold the water will be decreased (Lawrie and Ledward, 2006). Therefore, WHC greatly affects the quality of the comminuted meat products.

1.3.3.6 Cooking Loss

One of the important factors which determine the quality of the meat product is cooking loss. Cooking loss indicates the amount of water which is lost during cooking, and therefore, it is associated with water holding capacity of the meat. When meat is heated, if the meat has lower cooking loss, it will be understood that the meat had higher ability to hold water, and therefore, it has greater WHC. If the water holding capacity of a meat product is low, the meat will lose more amount of water during cooking leading to production of a drier product, which can be unacceptable to consumers (Barbut, 2002).

Cooking loss occurs with the denaturation of meat proteins. During cooking, the structure of meat proteins change by shrinkage of muscle fibers and connective tissues, and aggregation of proteins occurs leading to water release from the cells (i.e. decrease of WHC). The temperature at which the meat proteins denature is at the range of 37 °C to 75 °C, and the recommended internal temperature for cooking is 75 °C (Honikel, 2009).

1.3.3.7 Emulsification Capacity

Emulsification capacity, which is an important quality factor especially in comminuted meat products, is defined as the ability of the proteins to emulsify oil. In order to measure emulsification capacity, liquid oil is gradually added to meat protein solution, while using a homogenizer to mix the oil. The aim is to determine the point at which proteins cannot emulsify more oil. This point is known as the breakdown point, and is the state when oil and protein phases separate (Barbut, 2002). The breakdown point can be measured by a sudden decrease in viscosity or conductivity of the solution, since the fat phase has lower conductivity. The method is used to measure the emulsification capacity for proteins from chicken meat (Maurer *et al.*, 1969).

1.3.3.8 Protein Denaturation

In food processing, denaturation is defined as an irreversible change which occurs when proteins unfold and hydrophobic amino acids, which are originally located in the interior of the protein molecule, are exposed. A number of factors cause protein denaturation such as change in pH (by the addition of alkali or acid) and temperature (i.e. cooking), or addition of ions (e.g. NaCl). Denaturation affects a number of factors such as solubility and hydrophobicity of the proteins (Sikorski, 2007). There will be a decrease in solubility as well as an increase in hydrophobicity of the denatured proteins compared to the native state. Denaturation of meat proteins occurs at the temperature range of 30-55 °C. For example, the denaturation temperature for myosin is 50-55 °C (Lesiow and Xiong, 2001; Tarte and Amundson, 2006). According to Wang and Smith (1994) and Smyth *et al.* (1999) the denaturation temperature of myosin varies with pH and ionic conditions. A pH of 5.5-6.1 and ionic strength of 0.6 M NaCl are the typical conditions used in processed meat industry.

1.3.3.9 The Effect of Surimi and pH Shifting Process on Functional, Textural, and Rheological Properties of the Recovered Proteins

The gelation properties of the recovered proteins play an important role in the preparation of processed muscle food products. Two important factors which influence the quality of the gel formed by the recovered proteins are temperature and the quality of raw material used for processing. In an investigation conducted by Kristinsson and Demir (2003), surimi and acid / alkali process were tested on four different fish species, and the gel quality measurements were performed by oscillatory tests. The best gel was obtained for the protein isolate prepared using alkali process, and the gel obtained from the acid processed mince was of the lowest quality. The quality of the surimi gel was between the alkali and acid processed ones. These results could be explained by the lower protease activity during alkali process, which caused less protein breakdown. Thus, when the protein isolate obtained from the alkali process was exposed to gelation, due to the higher amount of protein-protein interaction and aggregation, the resultant gel had higher quality. According to Nolsøe and Undeland (2009) the results of several comparative studies on fish species about the quality of gel for the recovered proteins during acid and alkali process, indicated better gel formation for protein isolates obtained by alkaline aided process. Regarding the quality of raw material as well as comparison of acid and surimi process, a study was conducted on fresh sardine fish to compare different gels obtained from surimi and acid process based on textural profile analysis (hardness and cohesiveness, and elasticity) (Cortes-Ruiz *et al.,* 2001). The raw material for acid process included fresh sardines and sardines which were stored on ice for 5 days. The highest hardness value was reported for the proteins recovered by the acid process which used the 5-day stored sardines as the starting material. This could be explained by the more protein content (+ 15%) in the final product prepared using acid process. Higher hardness values were probably because of more protein-protein interaction and aggregation which might have led to harder but less elastic gels. Regarding textural properties, maximum values were reported for cohesiveness and elasticity of the gels obtained from the surimi process, and minimum values were associated with the proteins recovered by the acid process using the stored fish as the raw material.

In a study conducted by Li (2006) isolated myofibrillar proteins from spent hens were added to chicken breast and pork ham to evaluate textural properties. A solution containing 6% myofibrillar proteins was injected into chicken breast and pork ham at a ratio of 20% of the meat weight. The results showed higher hardness for chicken breast as well as higher hardness and chewiness for pork ham with added myofibrillar proteins compared to those without the added proteins. Therefore, the use of isolated proteins from low value poultry meat sources for functional properties' improvement of meat products was indicated in the study.

According to Froning and Johnson (1973) cooking loss of the recovered proteins from mechanically deboned poultry meat after centrifugation treatment was lower compared to those with no centrifugation treatment. The lower cooking loss for the recovered meat might be explained by the higher protein content of it, which led to higher water holding capacity for the recovered proteins.

An alkaline solubilization with an acid precipitation was performed on mechanically deboned turkey meat (Liang and Hultin, 2003). The authors found that cooking loss of the cooked protein isolate decreased significantly compared to that of cooked raw meat. This finding indicated that higher water holding capacity for the protein isolate was due to its higher protein content. In addition, a good gel forming ability was reported for the protein isolate.

1.3.4 Color and its Link to the Recovered Proteins from Surimi and pH Shifting Process

Color is an important meat quality which greatly affects consumers' preference (Froning, 1995). Total heme pigments including myoglobin and hemoglobin are responsible for the color of meat. The meat pigment is mainly myoglobin because hemoglobin, which is the blood pigment, will be mostly removed after the slaughter. Therefore, myoglobin is the determining factor for the meat color, and variations in meat color indicate the differences in myoglobin content. For example, poultry breast and thigh which are known as white and dark meat respectively, easily can be differentiated from each other (Schwartz *et al.*, 2009). Since myoglobin and hemoglobin contain iron, the

difference in iron content of white and dark chicken meat is the result of variations in total heme pigments (see Table 1.1).

The CIE Lab color scale which is based on the values of redness (a*), yellowness (b*), and lightness (L*) is a well-known method for measuring the color of a sample. In this regard, the moisture content of the sample is an important factor influencing the color measurements. If the sample has more water content, because of the greater light reflection, it will have more lightness value. The CIELAB color attributes for chicken dark and white meat are presented in Table 1.2.

The factors influencing the color of the recovered proteins are those associated with the raw starting material such as the amount of pigments, blood, and dark muscle. Therefore, different processes should be applied to different fish species for obtaining the lightest (in color) possible protein isolates, because consumers preferred the whitest color for the protein isolates (Tabilo-Munizaga and Barbosa-Canovas, 2004). This shows that color is an important factor which affects consumers' selection for the products made from isolated proteins. The results from comparative studies performed on surimi, acid and alkali process indicated the maximum whiteness value for the protein isolates obtained from the surimi process, followed by alkali and acid processes. According to Nolsøe and Undeland (2009) the greater whiteness value in surimi production could be explained by the washing process which might have helped to remove higher amounts of heme pigments from the protein isolate. The higher whiteness value for the proteins recovered by alkaline solubilization processes compared to the ones obtained from the acid solubilization process indicated that alkali aided process was more efficient in the heme pigments' separation from the fish meat.

A study by Hernandez *et al.* (1986) on mechanically deboned turkey meat indicated that protein extraction using phosphate buffer with a pH of 8.0 could efficiently remove heme pigments, increased lightness of the final product by 51%, and reduced its redness by 64%.

There was a significant increase in lightness value of the extracted meat compared to the raw meat from mechanically deboned chicken meat using an aqueous washing with

added sodium bicarbonate (pH: 8.0). Redness value of the final product was also significantly decreased (Dawson *et al.,* 1989).

1.3.5 The Effect of Surimi and pH Shifting Process on the Recovered Proteins' Stability during Storage

One of the important parameters which determines the quality of the protein isolate is the stability of the isolated proteins during frozen storage which is considered as an advantage for surimi production. As has been mentioned previously, in surimi technology cryoprotectants are compounds such as sugars (sucrose and sorbitol) which are able to reduce the protein denaturation during freezing and thawing process as well as prevent aggregation (Matsumoto, 1980). The reason is that cryoprotectants avoid proteins becoming unfolded (Carpenter and Crowe, 1988). Thus, in this way they increase the stability of protein products (e.g. surimi) when stored frozen. Chemicals which have been used as cryoprotectants in muscle proteins included sucrose, sorbitol, phosphates, and sodium tripolyphosphate (Nishiya *et al.,* 1961; Mahon, 1964; Matsumoto, 1978, 1979; Park and Lanier, 1987; Park *et al.*, 1988). One action which has been reported for phosphates (e.g. sodium tripolyphosphate) as a cryoprotectant is that they decrease the negative effect of frozen storage on myofibrillar protein solubility (Jittinandana *et al.*, 2003).

A study was conducted on the recovered proteins (Thawornchinsombut and Park, 2006) from Pacific whiting fish regarding the effect of frozen storage on textural properties. In order to determine the effect of cryoprotectants on the stability of proteins during storage, they divided each sample into two parts, and used cryoprotectants in one part but not in the other. Afterwards, the samples were exposed to freezing and thawing steps which were repeated three times. The temperatures for freezing and thawing were -18 °C and 4 °C, respectively. The results indicated that the best quality gels were obtained from the recovered proteins using an alkali process containing cryoprotectants. In contrast, the lowest gel quality was associated with the gels obtained from the protein samples without cryoprotectants. From this study, it can be concluded that using cryoprotectants for the protein isolates obtained from alkali process is of high importance to avoid freeze denaturation.

On the other hand, the addition of cryoprotectants is not the only way of increasing the stability of a protein product during frozen storage. Jaczynski *et al.* (2006) reported that by separation of membrane lipids from the protein isolate, even without using cryoprotectants, the stability of freeze-dried fish protein could be improved.

1.4 Potential Applications of the Recovered Proteins by pH Shifting Methods

The goal of pH shifting technology is the utilization of low value meat and development of new healthy value-added products. By the advantages which were mentioned for pH shifting technology, and by the comparison of different chemical and functional properties of the proteins recovered by this method, it can be concluded that pH shifting technology could better utilize low value dark meat than the traditional surimi processing. The US. Food and Drug Administration (2004) has confirmed the isolated proteins from seafood and poultry sources using pH shifting processing as Generally Regarded As Safe (GRAS).

The final product of the recovered proteins using pH shifting processing can be in the form of liquid or powder. Proteus Industries introduced isolated proteins as coating for battered and breaded products (www.proteusindustries.com). Other applications include using recovered proteins as ingredients to improve functional properties of food products. For example, isolated proteins can be used as emulsifiers (Petursson *et al.*, 2004) and to improve water holding capacity of whole-muscle meat products (Li, 2006). The coating product "Nutrilean™" which is in the form of powder has been produced by Proteus Industries using acid process on both chicken and fish sources. Nutrilean™ is a pure protein isolate, and it can be used as a batter ingredient which can be sprayed on to any kind of meat before frying. Nutrilean forms a thin barrier outside the coated product which prevents moisture from escaping. Thus, during frying, less oil can be absorbed into the product. This way, the fat content of the final fried product was reported to decrease by 25% to 75%. Water holding capacity of the final cooked product was also improved. As a consequence, moisture loss from drying out the product under the heat lamps in restaurants or fast foods was reduced leading to more juicy products. Even the color of the final product has been reported to improve to a richer bronze color, which is a more

natural color and is usually found in home-made products. According to the Proteus Industries the technology can be applied to meat from all sources. The food industries which are currently using Nutrilean technology involve Comfort Creek, Good Harbor, and Sunrise Foods.

1.5 Research Objectives

1.5.1 Main Objective

The objective of this thesis project was to examine the use of high pH extractions and low precipitation pH on poultry dark meat in order to produce functional proteins extracts suitable for the preparation of further processed meat products.

1.5.2 Specific Objectives

- Determine the effect of pH, in the range of 10.5 to 12.0, on alkaline solubilization process of chicken dark meat

- Evaluate the effect of pH on solubility and extractability of the proteins from chicken dark meat

- Investigate the effect of alkali aided process on protein yield and composition of chicken dark meat

- Establish the effect of alkali aided extraction on the distribution of fatty acids in between neutral and polar lipid fractions of chicken dark meat

- Study the effect of alkali aided extraction on color characteristics of the chicken dark meat.

- Evaluate the effect of alkali aided process on lipid oxidative stability of chicken dark meat

- Determine the effect of alkaline solubilization process on the textural and rheological characteristics of chicken dark meat

- Evaluate the influence of cooking on the water holding capacity of alkali extracted chicken dark meat

1.6 Approach

The objectives of this thesis were assessed in two phases followed by laboratory analysis.

Phase 1. Four extraction pH values (10.5, 11.0, 11.5, and 12.0) were chosen for alkali protein extraction from chicken dark meat. Each experiment and each assay was done at least in triplicate. Protein yield, protein and lipid composition, fatty acid profile, color characteristics and TBARS were studied in chicken dark and extracted meat.

Phase 2. Four extraction pH values (10.5, 11.0, 11.5, and 12.0) were chosen for alkali protein extraction from chicken dark meat. Each experiment and each assay was done at least in triplicate. Textural and rheological characteristics, as well as cooking loss and expressible moisture have been studied.

1.7 TABLES

Table 1.1. Protein (%), lipid (%), moisture (%), and iron (mg/100 g of meat) content of skinless chicken dark and white meat

Chicken Meat	Protein content	Lipid content	Moisture content	Iron content (mg/100g meat)
Dark Meat	20.1	4.3	75.9	1.0
White Meat	23.2	1.6	74.9	0.7

Modified from: USDA, 1999.

Table 1.2. Color attributes (L*, a*, and b*) of chicken dark and white meat

Chicken Meat	Lightness (L*)	Redness (a*)	Yellowness (b*)
Dark Meat	59.95	1.11	3.98
White Meat	62.11	0.99	3.55

Modified from: Perez-Alvarez, and Fernandez-Lopez, 2009.

1.8 FIGURES
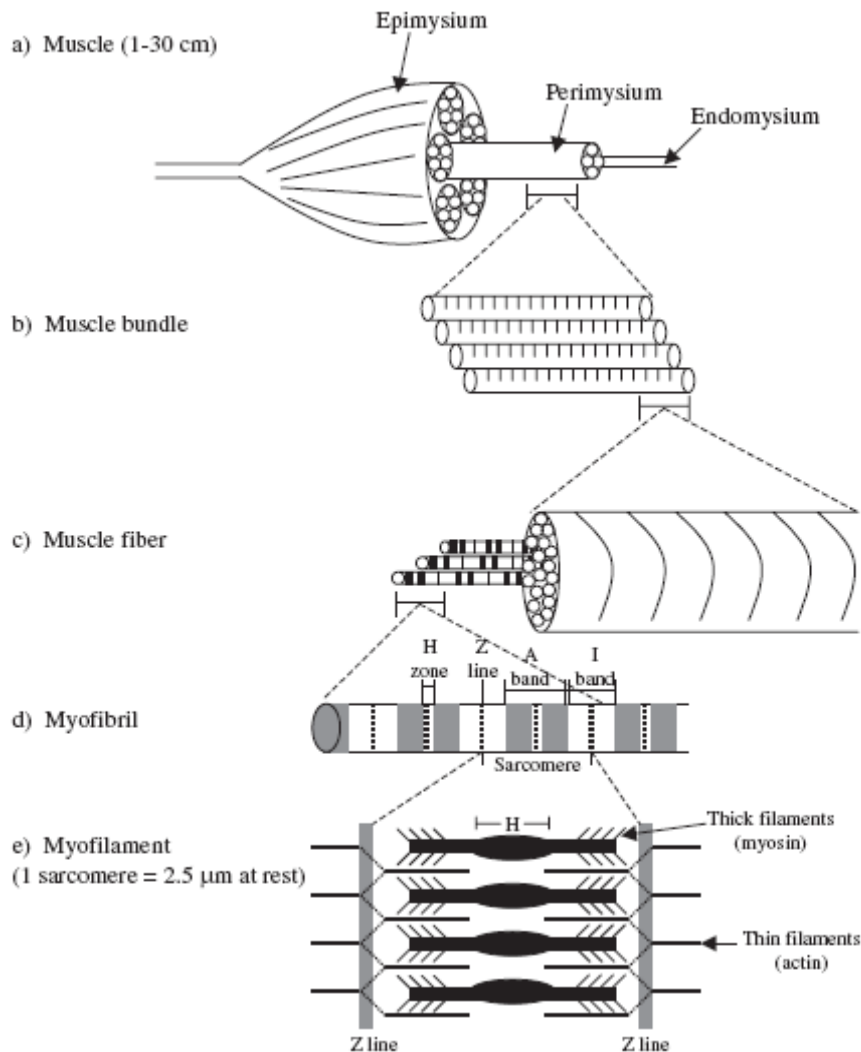
Figure 1.1. Schematic diagram of muscle structure, starting from a cross section of a whole muscle (a), including the layers of connective tissue, the muscle bundle (b), fibre (c), myofibril (d) and myofilaments (e). Reprinted from POULTRY PRODUCTS PROCESSING: AN INDUSTRY GUIDE. EBOOK by Shai Barbut. Copyright 2001 by Taylor & Francis Group LLC - BOOKS. Reproduced with permission of Taylor & Francis Group LLC – BOOKS in the format Dissertation via Copyright Clearance Center.
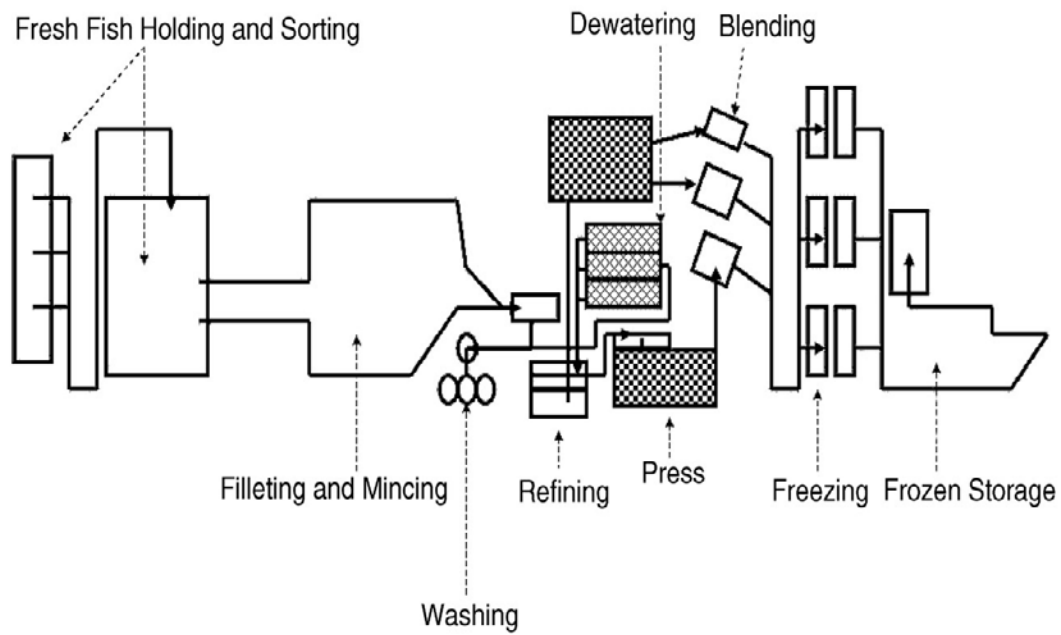
Figure 1.2. Flow chart of surimi manufacturing. Reprinted from SURIMI AND SURIMI SEAFOOD, SECOND EDITION by J. W. Park and T.M. JOHN LIN. Copyright 2005 by Taylor & Francis Group LLC - BOOKS. Reproduced with permission of Taylor & Francis Group LLC - BOOKS in the format Dissertation via Copyright Clearance Center.

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2 ALKALI AIDED PROTEIN EXTRACTION OF CHICKEN DARK MEAT: COMPOSITION AND STABILITY TO LIPID OXIDATION OF THE RECOVERED PROTEINS[1]

2.1 INTRODUCTION

Currently, with the increasing demand for further processed breast meat products, chicken dark meat has been considered a major underutilized commodity (USDA, 2004). The major concerns with chicken dark meat which affect consumers' selection and satisfaction are color, high fat content and poor shelf life stability (Froning, 1995; Fletcher, 1997). One of the alternatives to increase the utilization of chicken dark meat is to extract myofibrillar proteins and separate them from fat and pigments to enhance their application, which is required for the preparation of further processed meat products (Betti and Fletcher, 2005). The use of alkaline extraction followed by acid precipitation has been investigated for protein recovery from vegetable sources (Fletcher and Ahmed, 1977). Various researchers have examined potential extraction techniques for mechanically separated poultry meat (MSPM), meat offal, beef bones, and fish meat (Meinke *et al.*, 1972; Young and Lawrie, 1974; Jelen *et al.*, 1979; Lawrence *et al.*, 1982).

Japanese researchers developed a process for the recovery of proteins from fish resulted in 'Surimi', which is a functional protein ingredient. Surimi preparation involves washing the minced raw fish material in 5-10 °C water or slightly alkaline solution, with the aim of removing undesirable flavor components, pigments, blood and fat, leading to concentrated functional myofibrillar proteins (Lanier, 1986; Kristinsson and Ingadottir, 2006). An evolution of the conventional surimi preparation which is more suitable to extract proteins from pelagic fatty fishes is the alkaline solubilization technique developed by Hultin *et al.* (2000). In this new process, solubilized fish myofibrillar proteins at pH above 10.0 can be separated from fat and pigments by high speed

centrifugation and recovered by precipitation at the isoelectric point (Hultin and Kelleher, 1999; Hultin *et al.*, 2003; Kristinsson and Ingadottir, 2006). The extraction mechanism increases the charge on myofibrillar proteins by a pH-shifting processing technique.

The recent over-production of leg quarters and dark meat has resulted in a dramatically low market for these two raw materials. Thus, the production of a light-colored, low-fat protein isolate from the dark meat products, which can be used for the preparation of many processed food products, has received increased attention. It could be used, for example, as an alternative to phosphates for meat enhancement (Vann and DeWitt, 2007) and especially as a functional food ingredient for further processed meat products. The current study was performed to develop an efficient technology for extraction of myofibrillar proteins from chicken dark meat.

Hence, the objective of the present investigation was to determine the effect of pH, in the range of 10.5 to 12.0, on the alkaline solubilization of chicken dark meat. Aspects studied were the effect of alkali aided process on protein and lipid composition, lipid oxidation and color characteristics of the extracted meat.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Skinless frozen chicken thigh meat was obtained from Lilydale (Edmonton, AB, Canada). The chicken thigh meat was thawed (4 °C) overnight. The thawed chicken thigh meat was then ground using a meat grinder (Waring Pro, MG100NC, Taiwan), vacuum packed in 400 g plastic bags and was stored at -30 °C prior to analysis. The resulting dark minced chicken meat (DMCM) was used for this study.

2.2.2 Protein Solubility Curve

The protein solubility curve was prepared using a method by Kim *et al.* (2003). 6.0 g dark minced raw meat and 300 ml refrigerated distilled water were mixed in a homogenizer (Fisher Scientific, Power Gen 1000 S1, Schwerte, Germany) at setting 3 for 1 minute. Aliquots of homogenate (30 ml) were adjusted to a range of pH values (1.5 to

12.0) using 0.2 M and 1 M HCl or NaOH. The sample solutions were centrifuged at 25,900 g at 4 °C for 20 minutes. The middle liquid layer was used for protein analysis. Protein solubility was measured by the Biuret method (Gornall *et al.*, 1949) and expressed as mg/g of protein. Bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA) was used as the standard.

2.2.3 Protein Isolation

Protein isolation was performed as a modification of the methods of Liang and Hultin (2003), and Betti and Fletcher (2005). To prepare protein isolate, 400 g of frozen minced chicken thigh meat was partially thawed (4 °C) overnight it was then mixed with ice-distilled water (1:2.5, w:v) using a 900 Watt Food Processor (Wolfgang Puck, WPMFP15, W.P. Appliances, Inc., Hollywood, FL, USA) for 15 min. The homogenate was adjusted to pH 10.5 to 12.0 in 0.5 increments with a pH meter (UB10, Denver Instrument, Denver, CO, USA) using 2N NaOH over a 5 min period. After pH adjustment, the homogenate was allowed to stand still (4 °C for 30 min) and centrifuged (Beckman Coulter, Avanti® J-E centrifuge, CA, USA) at 25,900 × g for 20 min (4 °C). Three layers were formed after centrifugation: an upper fat layer, a middle aqueous layer of soluble proteins and a bottom sediment layer. The middle protein supernatant layer was carefully removed and the other two layers were discarded. The resultant protein supernatant was adjusted to pH 5.2 using 2N HCl over a 5 min period. The precipitated proteins were then centrifuged at 25,900 × g for 20 min (4 °C). The sedimented proteins were mixed with 700 g of ice-distilled water using the food processor for 7 min and adjusted to pH of 6.2, followed by centrifugation at 25,900 × g for 20 min (4 °C). The protein isolate were then weighed and protein yield was determined using the formula below. Moisture content of the resulting protein isolate was adjusted to 80% and stored at -30 °C. The resulting protein isolate was called extracted meat (EM).

$$\text{Protein yield (\%)} = \frac{\text{Protein content of EM (g) x 100}}{\text{Protein content of DMCM (g)}}$$

2.2.4 Determination of Total Protein Content

Total protein content was determined using the biuret procedure (Gornall *et al.*, 1949; Torten and Whitaker, 1964). The biuret reagent was prepared as described by

Layne (1957). The partially dispersed meat sample (1.0 g) in 20 mL of 0.5M NaOH was heated in a boiling water bath for 10 min and cooled in an ice-water bath. The solution was filtered to remove the elastin particles and some of the fat. Anhydrous ether (15 mL) was added to 15 mL of the filtrate, which was then centrifuged (J-6B/P Beckman, Beckman® Instruments, Inc, CA, USA) at $2,278 \times g$ for 10 min after shaking. After centrifugation, 1 mL of the lower aqueous layer was taken and mixed with 4 mL of biuret reagent. Optical density was measured at 540 nm after 30 min using a UV/VIS spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). Bovine serum albumin (HyClone, UT, USA) was used for preparation of the standard curve. Total protein content was measured on the DMCM as well as the EM.

2.2.5 Extractability of Recovered Proteins

The extractability of sarcoplasmic proteins in the protein isolate was measured by vortexing 2 g of sample with 20 ml of 30 mM phosphate buffer (pH 7.4) to create a homogenate solution. This solution was allowed to stand still overnight at 4 ^{o}C, and was then filtered using Whatman No. 1 filter paper. The protein content of the filtrate was estimated using the Biuret method (Gornall *et al.*, 1949). For measuring total protein extractability, 50 mM phosphate buffer containing 0.55 M potassium iodide (pH 7.4) was added to dissolve the proteins. Analysis was carried out in triplicate.

2.2.6 Determination of Total Fat Content

Total fat content was determined using a method described by Folch *et al.* (1957). 10.0 g of EM and 5.0 g of DMCM were separately mixed with 2:1 chloroform-methanol (v/v) solution (120 mL) using a vortex mixer for 10 min and stood still for 30 min. The slurry was filtered and the filtrate was transferred to a separation funnel, followed by addition of 40 mL of 0.88% NaCl solution. The funnel was then left for 30 min for phase separation. The bottom chloroform phase was filtered on an anhydrous sodium sulphate bed (Fisher Scientific, NJ, USA). 60 mL of the clear chloroform phase was taken out into a pre-weighed flask followed by rotary evaporation (Rotavapor, RE 121, Buchi, Switzerland) for 15 min at 40 °C. The flask was then put in a 60 °C oven for 30 min, followed by placing in a desiccator for 30 min before weighing it again to obtain the total

fat content. The residue was dissolved in 10 mL of chloroform and stored in the freezer (-30 °C) until required for analysis.

2.2.7 Separation of the Main Lipid Classes

The main lipid classes were separated using a method described by Ramadan and Mörsel (2003). A glass column (60 cm x 1.5 cm) was packed with activated silica gel (70–230 mesh; Whatman, NJ, USA) by applying a slurry of the silica gel in chloroform (1:5, w/v). Total lipids were dissolved in 9.5 mL of chloroform and applied to the column, which was then eluted sequentially with 50 mL chloroform (for neutral lipids), and with 50 mL methanol (for polar lipids) thereafter. The solvent from each fraction was evaporated by rotary evaporation and the percentage of each fraction was determined gravimetrically. The residues were dissolved separately in 2 mL of chloroform and stored in the freezer (-30 °C) until required for analysis.

2.2.8 Fatty Acid Analysis

Total fatty acid profile and composition of the neutral and polar lipid classes for raw thigh meat and extracted meat were analyzed by gas chromatography (GC) on 4 replicate samples. Derivatization was conducted using a solution of hydrochloric acid in methanol (methanolic HCl, 3N; Supelco, PA, USA) and according to manufacture instructions provided by Supelco. Extracted fat was diluted with chloroform. 2mL of methanolic HCl were added to 50µl of the extracted fat – chloroform mixture. In order to stimulate the reaction, samples were put into a 60 °C water bath for 120 min.

Fatty acid profile of the neutral and polar lipid classes was determined with a GC Varian (Varian Walnut Creek, California, USA) 3400 gas chromatograph equipped with a flame ionization detector and a SGE-BPX70 capillary column (60m x 0.25 mm x 0.25 µm film thickness; SGE Analytical Science Pty Ltd, Victoria, Australia). Operating conditions for the GC for fatty acid composition of neutral and polar lipid classes were as follows: initial temperature of 50 °C was held for 0.2 min; increased at 20 °C/min to 170 °C, which was maintained for 5 min. At a rate of 10 °C/min, a temperature of 230 °C was reached, which was maintained for 13 min. A Cool-on-Column injection method was used, with an initial and final injector temperature of 60 °C and 230 °C (30 min)

respectively, increasing at a rate of 150 °C/min. The temperature of the detector remained at 240 °C and the column head pressure of the carrier gas (helium) was 25 PSI. The fatty acid peak integration was performed using the Galaxie Chromatography Data System (Varian). Fatty acids were quantified using methyl-heptadecanoic acid as an internal standard (Sigma-Aldrich Co., MO, USA) and were identified by comparison of authentic standards (GLC-463 NU-CHEK PREP, INC. Elysian, MN, USA). Saturated fatty acids (SFA) levels were calculated as 14:0 + 15:0 + 16:0 (palmitic acid) + 18:0 (strearic acid) + 19:0 + 20:0 + 22:0. Monounsaturated fatty acids (MUFA) levels were calculated as 14:1 + 16:1 ω-7 cis +17:1 + 18:1 ω-7 trans + 18:1 ω-7 +18:1 ω-9 (oleic acid) + 20:1. Polyunsaturated fatty acids (PUFA) levels were calculated as 18:2 ω-6 (linoleic acid; LA) + 18:3 ω-3 (α-linolenic acid; LNA) + 18:3 ω-6 + 20:2 ω-6 + 20:3 ω-3 + 20:3 ω-6 + 20:4 ω-6 (arachidonic acid; AA) + 20:5 ω-3 (eicosapentaenoic acid; EPA) + 22:4 ω-6 + 22:5 ω-3 (docosapentaenoic acid; DPA) + 22:6 ω-3 (docosahexaenoic acid; DHA). Total ω-6 fatty acid levels were calculated as LA + 18:3 ω-6 + 20:2 ω-6 + 20:3 ω-6 + AA + 22:4 ω-6. Total ω-3 fatty acid levels were calculated as LNA + 20:3 ω-3 + EPA + DPA + DHA. Long chain ω-3 fatty acid (LC ω-3) levels were calculated as 20:3 ω-3 + EPA + DPA + DHA. Long chain ω-6 fatty acid (LC ω-6) levels were calculated as 20:2 ω-6 + 20:3 ω-6 + AA + 22:4 ω-6.

2.2.9 Susceptibility to Oxidation

Lipid susceptibility to oxidation was measured by using the induced thiobarbituric acid reactive substances (TBARs) test as a modification of the method of Kornbrust and Mavis (1980) on raw thigh meat as well as extracted leg meat. Four replicate measurements were conducted. To prepare homogenates, 3 g of the meat sample was homogenized with 25 mL of 1.15% KCl solution for 45 s. A 200 µl aliquot of the homogenate was incubated at 37 °C in 80 mM Tris-maleate buffer (pH 7.4) with 2.5 mM ascorbic acid and 50 µM ferrous sulphate in a total volume of 2 mL. At fixed intervals (0, 30, 60, 100 and 150 min), aliquots were removed for measurement of TBARS. TBARs were expressed as nanomoles of malondialdehyde (MDA) per milligram of meat.

2.2.10 Color Measurements

The CIE (1978) system color profile of lightness (L*), redness (a*) and yellowness (b*) was measured by a HunterLab colorimeter (LSXE/UNI, Virginia, USA) on EM samples immediately after extraction prior to frozen storage at -30 °C. A white standard plate was used to calibrate the colorimeter. Three readings of L*, a*, and b* values were taken from each batch of the raw dark and extracted meat samples, and the values were averaged. Whiteness, intensity of the red, saturation and HUE values were calculated according to the following formula.

$$Whiteness = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

$$Saturation = \sqrt{a^2 + b^2}$$

$$HUE = \arctan b / a$$

$$Intensity\ of\ the\ red = a^* / b^*$$

Hue angle measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006). As hue angle increases, visually perceived redness decreases (Little, 1975; Brewer *et al.*, 2006). Chroma refers to the brightness/colorfulness of an object (Fairchild, 2004).

2.2.10.1 Total Heme Pigments Content

Total heme pigments were extracted from 10.0 g of a homogenized meat sample within a solution of 40 mL acetone, 1 mL HCl and 2 mL water according to Hornsey (1956). The solution was filtered after 1-hour pause and the absorbance was measured using a UV/VIS spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan) at 640 nm wavelength. The absorbance value obtained was multiplied by the factor value 17.18, and the concentration of total hemic pigments was expressed in milligrams of myoglobin per gram of meat.

2.2.11 Statistical Analysis

Each experiment and each assay was done at least in triplicate. Reported results represent an average of each experimental assay. All data were subjected to Analysis of Variance using the general linear model procedure of SAS (SAS System, 2002). Differences between least squares means were determined using HSD Tukey differences, and were reported as significant at the $P < 0.05$ level. Linear regression analysis and Karl Pearson's coefficient of correlation has been determined to find the correlation between various parameters as a function of pH treatments.

2.3 RESULTS AND DISCUSSION

2.3.1 Protein Solubility

The solubility of proteins from chicken thigh meat has been measured in pH range of 1.5 - 12.0 (Figure 2.1). The highest value for protein solubility was determined at the extremes of pH in both acidic and alkaline range. The increased solubility at extreme pH values has been related to an increased positive and negative charge of the muscle proteins at low and high pH, respectively, leading to electrostatic repulsion between the proteins (Hamm, 1994). The protein solubility showed a U-shaped curve; however, unlike the typical solubility curve for fish muscle protein homogenates, the solubility was found to be a maximum at pH 10.5 and showed a marginal reduction up to pH 12.0. The reason for this marginal decrease in protein solubility may be due to the exposure of more hydrophobic groups at the higher pH values.

pH-shift solubilizing processing is a method which has been broadly used for fish muscle; however in this study we have shown that this process can also be applicable to underutilized chicken meat sources for protein extraction. Investigations by Kristinsson *et al.* (2005) on catfish muscle indicated that there is less protein denaturation in alkali aided process than in acid aided process. As a consequence, based on the solubility curve, higher alkaline pH, ranging from 10.5 to 12.0 was considered for the present study.

2.3.2 Protein Recovery and Protein Content

Alkaline pH treatments ranging from 10.5 to 12.0 were chosen for protein extraction according to the results obtained from the protein solubility curve. The yield of the protein extraction was significantly increased as the pH of extraction increased. The highest recovery of proteins which is equivalent to 94.2% was obtained at a pH of 12.0 (Figure 2.2). This high level of protein yield could be explained by the denaturation of myofibrillar proteins and the exposure of hydrophobic groups (Omana *et al.*, 2010). Denaturation of proteins at high pH values results in unfolding of proteins (Sikorski, 2007). As a consequence, hydrophobic amino acids will be exposed to the surface of the protein structure, which may lead to more aggregation and precipitation of myofibrillar proteins at the isoelectric point (Ingadottir, 2004; Gehring *et al.*, 2009). The reason for the higher protein yields in this study compared to Kristinsson and Ingadottir (2006), who recovered 68% of proteins from Tilapia fish muscle by alkali aided process, could be explained by the differences between chicken and fish proteins as starting materials. As expected, protein yield was negatively correlated with total and neutral lipid content of EM (R = -0.88 and R = -0.81; *P* < 0.00001), respectively (Figures 2.3 and 2.4).

The protein content was significantly increased when a pH of 12.0 was reached compared with the one of 10.5 (*P* < .0001) (Table 2.1). The highest level of protein content on wet basis, 19.4%, was reached at a pH of 12.0, however; it was not significantly different as compared to the values of chicken dark meat. It may indicate that the maximum protein could be extracted from chicken dark meat using alkali-aided processing at a pH of 12.0.

2.3.3 Extractability of Recovered Proteins

Extractability of recovered proteins is of great importance for the manufacture of processed muscle foods, including restructured meats. This is because most of the functional properties of muscle protein, such as emulsification and gelation, are related to this parameter.

Sarcoplasmic protein extractability from recovered proteins as a function of various pH was significantly decreased (*P* < 0.0001) compared to the extractability of

sarcoplasmic proteins in raw meat (Figure 2.5). The reduction was found to be most significant at pH 11.5 and pH 12.0. This reduction may be explained by the instability of proteins due to aggregation behavior (Mohan *et al.*, 2006) during the process of extraction. Total protein extractability showed a similar trend. Approximately a 50% reduction was found at pH treatments of 10.5 and 11.0 compared to the initial raw materials. The reduction was even more evident for the proteins recovered at alkali at pH values of 11.5 and 12.0 (Figure 2.5). The strong reduction of total protein extractability for samples extracted at higher pH may be explained by unfolding of myofibrillar proteins, leading to exposure of more hydrophobic groups to the exterior (Sarma *et al.*, 1999). However, the decrease in protein extractability at higher pH values might be a consequence of altered protein conformations induced by chemical denaturation, which could not be fully restored after pH readjustment. Kristinsson and Hultin (2003) showed that the conformation of myosin head could not revert to its native conformation during alkali treatment (pH 11.0) followed by pH (7.5) readjustment.

2.3.4 Lipid Profile

2.3.4.1 Total Lipid Content

The presence of high lipid content has important implications in the storage, processing, stability, and nutritional values of muscle food. It is also a reason for the rapid sensory quality losses observed. In order to increase the utilization of extracted proteins, total fat content from dark meat must be reduced.

Lipid content of the thigh meat was 6.3% (on wet basis). Fat content of the extracted meat by alkali treatments showed an average of 50% reduction compared to chicken dark meat with no significant difference among the pH treatments (Table 2.1). This reduction is possible because during the alkali solubilization process, when high pH values (10.5 - 12.0) are used, an increase in negative charge of muscle proteins occur, leading to electrostatic repulsion between the proteins (Hamm, 1994). As a result, proteins solubilize in alkaline solution. When this slurry is centrifuged, lipids, due to their low density, are separated from the protein phase (Hultin and Kelleher, 2000).

However, the low amount (~50%) of fat reduction found in the current investigation compared to other studies (65-70%) conducted in fish (Hultin and Kelleher, 2000; Nolsøe and Undeland, 2009) could be explained by the lower ratio of meat to water used for alkali extraction. In this study, the ratio of meat to water was 1:2.5 (w: v) while other investigations on mechanically deboned turkey used a ratio of 1:6 (w: v) (Liang and Hultin, 2003). In addition, research on fish alkali extraction indicated that a ratio of 1:5 is used in order to increase fat removal, especially polar lipids (Nolsøe and Undeland, 2009). The low amount of water used in this study was chosen because of the growing concern of water usage during food processing.

2.3.4.2 Neutral and Polar Lipids Content

In general, triacylglycerol (neutral lipid) content of the dark muscles, mainly rich in saturated fatty acids, is related to the sustainable energy source required by the animals, while the highly unsaturated nature of the polar lipids is necessary for the metabolic functional requirements of the membrane (Betti *et al.*, 2009). However, fatty acids composition is not the only difference between the neutral oils and the polar membranes lipids. Because the polar lipids (mainly phospholipids) are in the cell membranes, which exist primarily as a bi-layer, they have a very large surface area exposed to the aqueous phase of the cell compared to triacylglycerol (Hultin, 1995). As a consequence, even though the neutral fat predominates in dark meat, polar lipids would have ten times more exposure to pro-oxidants than the triacylglycerols at the surface of the oil droplets. In addition to their greater surface area, membrane lipids are found in association with components that can accelerate their oxidation, such as reactive oxygen species (ROS) (Hultin and Kelleher, 2000). For all these reasons, polar lipids must be strongly reduced during the extraction process.

A significant decrease ($P < 0.0001$) in neutral lipids in extracted meat was observed compared to chicken dark meat. The content of neutral lipids in chicken dark meat (% on wet basis) was 5.4 % (Table 2.1). On average, neutral lipid removal from chicken dark meat using the alkali aided process was 61.5%. In this study, in contrast to the neutral lipids, polar lipids were not affected by the alkali extraction (pH values 10.5 - 12.0). The unsuccessful removal of polar lipids may result from the lower amount of

water used for alkali extraction (2.5 vs. 5 (w: v)) (Nolsøe and Undeland, 2009). As previously discussed, polar lipids are difficult to remove because they are strongly bound to membrane proteins and hence, their extraction was difficult (Nylander, 2004). These results also concur with Dawson *et al.* (1990) who did not successfully remove polar lipids from MSPM by using slightly alkaline washing treatments. By contrast, a recent study conducted by Hrynets *et al.* (2009) using acid extraction in the presence of citric acid showed considerable reduction in the level of polar lipids of MSPM; it has been suggested that citric acid might play a role as a binding agent to the basic amino acid residues of cytoskeletal proteins competing with the acidic phospholipids of membranes (Liang and Hultin, 2005) In light of this, low pH of extraction and addition of citric acid may be exploited to improved removal of polar lipids from chicken dark meat.

2.3.5 Fatty Acid Profile

The fatty acid profile of neutral and polar lipids for chicken dark meat and extracted meat are presented in Tables 2.2 and 2.3.

2.3.5.1 Neutral Lipids Fraction

The predominant SFA was palmitic acid (C16:0) and among MUFA, oleic acid (C18:1 ω-9) was the most abundant fatty acid (Table 2.2). According to Wood *et al.* (2008), the content of oleic acid is higher in neutral lipids than in polar lipids fraction. The levels of SFA, MUFA and PUFA significantly decreased by 68%, 66% and 60%, respectively in the neutral lipids fraction of chicken dark meat due to alkali pH treatments ($P < 0.0001$). The lowest amounts of fatty acids were found at a pH of 11.5 (448, 978 and 339 mg per 100 g of chicken dark meat for SFA, MUFA and PUFA, respectively) (Table 2.3). The reduction in SFA after pH treatment was mainly due to a 68% decrease in palmitic acid. The reduction of MUFA was explained by the decrease of oleic acid. In fact, there was a 72% reduction in this fatty acid compared to chicken dark meat at a pH of 11.5. The decrease of LA was responsible for the significant reduction in PUFA levels in alkali pH treatments compared to chicken dark meat ($P < 0.0001$). The highest removal (72%) of LA was found at a pH of 11.5. In neutral lipids, LNA was the only ω-3 fatty acid found in chicken dark meat. The highest reduction of LNA in alkali treatments was also 72% at a pH of 11.5.

Total ω-6 fatty acids were significantly reduced by alkali extraction, with the maximum reduction at a pH of 11.5 (297 mg/100 g of chicken dark meat). The ω-6: ω-3 ratio for chicken dark meat was 7.20 and due to the similar reduction in total ω-3 and ω-6 fatty acids in the alkali treatments, the ratio of ω -6: ω -3 did not change significantly compared to chicken dark meat (Table 2.2). However, there was a significant improvement of the ratio (8.26) when a pH of 12.0 was used compared to the one at 10.5 (Table 2.2).

2.3.5.2 Polar Lipids Fraction

In chicken dark meat, palmitic acid, stearic acid, oleic acid and LA were the predominant fatty acids in polar lipids (87.8, 103, 155 and 122 mg per 100 g of meat for the fatty acids respectively) (Table 2.3). SFA levels did not decrease due to the alkali treatments. There was a significant increase in SFA of 47% at a pH of 11.5 compared to chicken dark meat (198 vs. 292 mg SFA/100 g of meat; $P = 0.0311$). The MUFA and PUFA levels in chicken dark meat did not significantly change due to the alkali treatments ($P = 0.2856$ and $P = 0.0730$ for MUFA and PUFA respectively).

Total ω -3 and LC ω -3 fatty acids tended to increase by alkali treatments while the increase was only significant at a pH of 10.5 ($P = 0.0093$ and $P = 0.0131$ for Total ω -3 and LC ω -3 fatty acids respectively). Long chain polyunsaturated fatty acids (LC PUFAs) including 22:4 ω -6, 20:3 ω -3, EPA, DPA and DHA were not detected in the neutral lipids fraction, and they were present only in polar lipids. These results are in agreement with Betti *et al.* (2009), who studied the distribution of fatty acids of chicken thigh (dark) meat in triacylglycerols and phospholipids, and found that LC PUFAs were absent (or present in a very low amount) in triacylglycerols compared to their level in phospholipids.

LC ω-6 and total ω-6 fatty acids on the other hand, were not significantly affected by the treatments ($P = 0.1315$ and $P = 0.0815$ for LC ω -6 and total ω-6 fatty acids respectively). Similarly, no significant change in ω-6: ω-3 ratio was observed due to alkali extraction in relation to chicken dark meat (Table 2.3). The ratio for chicken dark

meat was 7.47 and it varied from 5.54 to 8.23 in the different pH treatments, reaching its maximum at a pH of 12.0 (8.23).

As discussed previously, the unsuccessful removal of polar lipids did not decrease the concentration of LC PUFAs, which in some cases, (pH 10.5) they even increased. If this is potentially desirable from a nutritional point of view it does produce serious technological problems due to lipid oxidation during frozen storage (see below).

2.3.6 Susceptibility to Oxidation

Interactions between incubation time and alkali pH treatments used for extraction were found in the induced TBARs measured to test the oxidative stability of chicken dark meat and extracted meat (Figure 2.6). The MDA formation increased as the time of incubation increased, as expected. The highest MDA value was found after an incubation time of 150 min in chicken dark meat, as well as, in extracted meat. There was a significant increase ($P < 0.05$) in MDA formation in extracted meat compared to chicken dark meat, indicating that extracted meat was more susceptible to oxidation. This denotes that although total fat of chicken dark meat decreased by approximately 50% due to alkali extraction, polar lipids were not removed. These polar lipids contain more LC PUFAs, and hence extracted meat is more susceptible to oxidation than chicken dark meat. It confirms that LC PUFAs deposited in polar lipids are responsible for lipid oxidation (Erickson, 2002). This is further evidenced by the fatty acid data. In fact, the unsuccessful removal and concentration of LC PUFAs, including EPA, DPA, DHA, C20:3 ω-3 and C22:4 ω-6 in the polar fraction, which were not present in the neutral lipids, may be the reason for the increased lipid oxidation susceptibility in extracted meat compared to chicken dark meat.

2.3.7 Color Measurements and Total Heme Pigments

Color is an important attribute which influences consumers' selection of and satisfaction with meat products (Froning, 1995; Fletcher, 1997). The color measurements (L*, a*, b*, a*/b*, saturation, HUE and whiteness) and total heme pigments for chicken dark and extracted meat are described in Table 2.4. The individual color measurements of a* and b*, were significantly lower in extracted meat compared to chicken dark meat.

The application of alkali aided extraction to chicken dark meat lightened the color of the dark meat. This could be explained by the fact that during the alkali aided process, the pH is adjusted to the isoelectric point of myofibrillar proteins (pH: 5.2), which led to precipitation. Thus, by further centrifugation, sarcoplasmic proteins (total heme pigments included) which are water-soluble components (Scopes, 1970) will be easily separated from the myofibrillar proteins. The color values of redness (a*) and yellowness (b*) in extracted meat significantly decreased ($P < 0.0001$ and $P = 0.0016$) by 83% and 11%, respectively compared to chicken dark meat. The decrease in redness value could be explained by the fact that the raw meat presents red color because of the myoglobin (Terra *et al.*, 2009), and as a result, by removing myoglobin, the redness value decreased. The reduction in yellowness for extracted meat could be because of lipid removal. According to Irie (2001), carotene concentration is the main factor affecting yellowness of the fat in meat but carotene levels were not measured here.

The value of lightness (L*) significantly increased with alkali extraction compared to chicken dark meat ($P < 0.0001$). Moreover, studies on turkey muscle color indicated that total pigments concentration is one of the factors influencing meat L* values (Boulianne and King, 1995, 1998; Gasperlin *et al.*, 2000). Thus, the reason for increase in lightness values in extracted meat could be attributed to the removal of total heme pigments from chicken dark meat by alkali aided process. Whiteness values of the extracted meat significantly increased ($P < 0.0001$) compared to chicken dark meat, mainly because of the considerable increase in L* value which is the main factor affecting whiteness (Table 2.4). The intensity of the red (a*/b*) significantly decreased compared to chicken dark meat, due to the reduction of redness. The saturation value of extracted meat significantly decreased compared to chicken dark meat ($P < 0.0001$) indicating that extracted meat has less color intensity compared to chicken dark meat. This could be explained by the removal of total heme pigments by alkali extraction which may affect color intensity (Irie, 2001). The HUE value considerably increased in extracted meat ($P < 0.0001$) compared to chicken dark meat. This indicates that the visually perceived redness was reduced by the extraction process.

The values of a*, b* and L* for chicken breast was reported as 0.99, 3.55 and 62.11, respectively (Perez-Alvarez and Fernandez-Lopez, 2009) while the values obtained in our study for extracted meat were 1.54, 16.98 and 63.30 respectively. The comparison between these values indicates that L* and a* values for extracted meat are comparable with those in breast meat. Yellowness value (b*) in extracted meat is higher compared to chicken breast. The reason for this higher yellowness could be explained by the unsuccessful removal of polar lipids which contain higher amounts of LC PUFAs and oxidation of the remaining pigments to some extent in the EM (Irie, 2001).

Alkaline solubilization considerably decreased ($P < 0.0001$) total heme pigment content compared to chicken dark meat. The values reached the average of 0.60 mg/g which showed a 53% reduction compared to chicken dark meat (Table 2.4). According to Pikul *et al.* (1982) and Niewiarowicz *et al.* (1986) the values obtained in this study are similar to those found in chicken breast meat.

Despite all the color characteristics that were improved by alkali treatments, Omana *et al.* (2010) found different results when color was measured on EM samples stored for 30 d at -30 °C; L* and whiteness were lower in EM samples than minced chicken dark meat. The inefficient removal of polar lipids, which increased the unsaturation level in EM, and the pronounced oxidation of the remaining pigments probably resulted in a slightly darker meat as compared to the starting material although the index of red decreased. These results suggest that immediately after extraction, EM samples are distinguished by an overall bright white-yellow color and after 30 d of frozen storage, the formation of oxidized pigments, mainly met-myoglobin isolated proteins resulted in a more dark-yellow color.

2.4 CONCLUSIONS

This first experiment demonstrated that it is possible to recover protein yields of more than 80% in the pH range of 10.5 – 12.0. However, the isolated proteins were susceptible to lipid oxidation as revealed by TBARs values, even though the total fat content decreased to approximately 50%. The recovery process failed to remove polar

lipid and in some cases (pH 10.5), they even increased. However, neutral lipids were removed by 61.51%. Alkali aided extraction helped to lighten the color of the dark meat and reduction of heme pigments by 53%. In spite of the susceptibility to lipid oxidation of the recovered proteins, our earlier studies (Omana *et al.*, 2010) revealed that the proteins in the extracted meat obtained after treatment at pH 10.5 and 11.0 could regain the original conformation after pH readjustment to 6.2. Hence, it can be concluded that the meat extraction at these pH values can be utilized for product preparations with desired functional properties. The oxidation issue may be overcome by modifying the process to remove more polar lipids (i.e. addition of citric acid) or by adding a stabilizing agent such as vitamin E.

2.5 TABLES

Table 2.1. Protein (%) and lipid (%) composition of chicken dark and extracted meat (wet basis)

Parameters	Chicken Dark Meat	Extraction pH[a]				SEM	*Pr > F*
		10.5	11.0	11.5	12.0		
Protein content	20.6[a]	16.7[c]	17.1[c]	18.2[bc]	19.4[ab]	0.3	<.0001
Total fat content	6.26[a]	3.41[b]	3.15[b]	2.94[b]	2.97[b]	0.19	<.0001
Neutral lipids	5.43[a]	2.55[b]	2.06[b]	1.86[b]	1.90[b]	0.19	<.0001
Polar lipids	0.828	0.846	1.09	1.08	1.07	0.068	0.0261

[a-c]Least squares means in a row corresponding to a certain factor with different letters are significantly different ($P < 0.05$). The values presented are means of 4 replicate determinations.

Table 2.2. Level (mg fatty acid / 100 g of meat) of fatty acids in the neutral lipids of chicken dark and extracted meat

Fatty acids	Chicken Dark Meat	Extraction pH[a] 10.5	11.0	11.5	12.0	SEM	*Pr > F*
14:0	45.9[a]	17.0[b]	13.3[b]	11.7[b]	14.0[b]	1.5	<.0001
14:1	12.6[a]	5.03[b]	3.50[b]	2.52[b]	3.92[b]	0.64	<.0001
15:0	3.55[a]	2.61[a]	ND	0.547[a]	ND	1.05	0.0913
16:0	1430[a]	582[b]	456[bc]	345[c]	417[bc]	41	<.0001
16:1 ω-7	372[a]	149[b]	114[b]	93.8[b]	115[b]	13.9	<.0001
18:0	354[a]	154[b]	123[bc]	90.5[c]	108[c]	9.8	<.0001
18:1 ω-7	187[a]	80.0[b]	64.8[bc]	50.7[c]	63.0[bc]	6.2	<.0001
18:1 ω-9	3038[a]	1338[b]	1064[bc]	823[c]	990[bc]	94	<.0001
18:2 ω-6	1017[a]	533[b]	426[bc]	284[c]	325[c]	40	<.0001
18:3 ω-3	154[a]	103[ab]	82.2[bc]	42.1[c]	43.0[c]	12.5	<.0001
18:3 ω-6	11.5[a]	5.56[b]	4.21[bc]	2.40[c]	3.67[bc]	0.48	<.0001
20:1	27.5[a]	15.7[b]	11.9[bc]	7.78[c]	8.95[c]	1.11	<.0001
20:2 ω-6	4.96[a]	4.29[a]	3.47[a]	1.31[a]	2.41[a]	0.91	0.0798
20:3 ω-6	8.91[a]	5.53[b]	4.72[b]	2.57[c]	3.98[bc]	0.48	<.0001
20:4 ω-6	15.5[a]	14.9[a]	13.3[ab]	7.25[b]	9.65[ab]	1.42	0.0037
SFA*	1833[a]	756[b]	592[bc]	448[c]	539[bc]	52	<.0001
MUFA*	3636[a]	1588[b]	1258[bc]	978[c]	1181[bc]	112	<.0001
PUFA*	1212[a]	666[b]	534[bc]	339[c]	388[c]	53	<.0001
Total ω-3*	154[a]	103[ab]	82.2[bc]	42.1[c]	43.0[c]	12.5	<.0001
Total ω-6*	1058[a]	563[b]	452[bc]	297[c]	345[c]	42	<.0001
ω-6:ω-3	7.20[ab]	5.40[b]	5.43[b]	7.25[ab]	8.26[a]	0.54	0.0073

ND = not detected. SEM= standard error of the mean. [a-c] Values within a raw with no common superscript are significantly different ($P < 0.05$). The values presented are means of 4 replicate determinations. * SFA levels were calculated as 14:0 + 15:0 + 16:0 + 18:0. MUFA levels were calculated as 14:1 + 16:1 ω-7 + 18:1ω-7 +18:1 ω-9 + 20:1. PUFA levels were calculated as 18:2 ω-6 + 18:3 ω-3 + 18:3 ω-6 + 20:2 ω-6 + 20:3 ω-6 + 20:4 ω-6. Total ω-6 was calculated as 18:2 ω-6 + 18:3 ω-6 + 20:2 ω-6 + 20:4 ω-6. Total ω-3 was calculated as 18:3 ω-3.

Table 2.3. Level (mg fatty acid / 100 g of meat) of fatty acids in the polar lipids of chicken dark and   extracted meat

Fatty acids	Chicken Dark Meat	Extraction pH[a]				SEM	*Pr > F*
		10.5	11.0	11.5	12.0		
14:0	5.88[b]	9.05[a]	7.52[ab]	7.54[ab]	7.44[ab]	0.62	0.0421
16:0	87.8[b]	105[ab]	143[a]	149[a]	137[ab]	11.3	0.0061
16:1 ω-7	12.6	11.8	11.0	11.3	10.1	1.1	0.7861
18:0	103	122	121	133	124	8	0.1681
18:1 ω-7	21.9[b]	24.4[ab]	28.5[ab]	31.7[a]	29.5[ab]	2.0	0.0216
18:1 ω-9	155	166	180	189	178	12	0.3055
18:2 ω-6	122[b]	153[ab]	163[ab]	171[a]	156[ab]	11	0.0459
18:3 ω-3	6.25[ab]	10.1[a]	7.65[ab]	5.83[ab]	4.45[b]	1.03	0.0165
20:0	1.19[ab]	1.08[ab]	1.03[ab]	1.53[a]	0.325[b]	0.276	0.0818
20:1	3.17	3.35	3.22	3.40	3.14	0.26	0.9356
20:2 ω-6	2.66	3.55	3.63	3.60	3.22	0.25	0.0724
20:3 ω-3	0.217	0.873	0.861	0.268	ND	0.245	0.0734
20:3 ω-6	6.77[b]	9.24[ab]	9.21[ab]	9.88[a]	9.32[ab]	0.65	0.0324
20:4 ω-6	54.3	70.0	59.8	68.0	66.0	4.7	0.1639
20:5 ω-3	3.77[b]	6.14[a]	5.46[ab]	5.12[ab]	4.56[ab]	0.46	0.0250
22:0	0.318	1.23	1.03	0.855	ND	0.365	0.1521
22:4 ω-6	9.30	12.4	11.1	12.5	12.0	0.82	0.0743
22:5 ω-3	10.6[b]	17.3[a]	16.0[a]	15.2[ab]	13.5[ab]	1.19	0.0116
22:6 ω-3	6.19[b]	10.3[a]	8.99[ab]	9.02[ab]	7.75[ab]	0.88	0.0472
SFA*	198[b]	239[ab]	274[ab]	292[a]	268[ab]	20	0.0311
MUFA*	192	206	222	236	221	14	0.2856
PUFA*	222	293	286	300	277	19	0.0730
LC ω-3*	20.8[b]	34.6[a]	31.3[ab]	29.6[ab]	25.8[ab]	2.5	0.0131
LC ω-6*	73.0	95.2	83.7	94.0	90.6	6.3	0.1315
Total ω-3*	27.0[b]	44.7[a]	39.0[ab]	35.4[ab]	30.3[b]	3.1	0.0093
Total ω-6*	195	248	247	265	246	17	0.0815
ω -6:ω-3	7.47[ab]	5.54[b]	6.31[ab]	7.60[a]	8.23[a]	0.45	0.0050

ND = not detected. SEM= standard error of the mean. [a-b] Values within a raw with no common superscript are significantly different (P < 0.05). The values presented are means of 4 replicate determinations. * SFA levels were calculated as 14:0 + 16:0 + 18:0 + 20:0 + 22:0. MUFA levels were calculated as 16:1 ω-7 + 18:1ω-7 +18:1 ω-9 + 20:1. PUFA levels were calculated as 18:2 ω-6 + 18:3 ω-3 + 20:2 ω-6 +20:3 ω-3 + 20:3 ω-6 + 20:4 ω-6 + 20:5 ω-3 + 22:4 ω-6 + 22:5 ω-3 + 22:6 ω-3. LC ω-3 levels were calculated as 20:3 ω-3 + 20:5 ω-3 + 22:5 ω-3 + 22:6 ω-3. LC ω-6 levels were calculated as 20:2 ω-6 + 20:3 ω-6 + 20:4 ω-6 + 22:4 ω-6. Total ω-3 was calculated as 18:3 ω-3 + 20:3 ω-3 + 20:5 ω-3 + 22:5 ω-3 + 22:6 ω-3. Total ω-6 was calculated as 18:2 ω-6 + 20:2 ω-6 + 20:3 ω-6 + 20:4 ω-6 + 22:4 ω-6.

Table 2.4. Color measurements and total heme pigments of chicken dark and extracted meat

Parameters	Chicken Dark Meat	Extraction pH[a]				SEM	*Pr > F*
		10.5	11.0	11.5	12.0		
a*	9.30[a]	1.74[b]	1.84[b]	1.44[b]	1.12[b]	0.22	< .0001
b*	19.1[a]	17.3[b]	17.5[b]	17.0[b]	16.1[b]	0.3	0.0016
L*	51.4[b]	63.2[a]	63.7[a]	63.6[a]	62.7[a]	0.8	< .0001
a*/ b*	0.486[a]	0.101[b]	0.105[b]	0.084[b]	0.065[b]	0.011	< .0001
Saturation	21.3[a]	17.3[b]	17.6[b]	17.1[b]	17.0[b]	0.3	< .0001
HUE	64.1[b]	84.2[a]	84.0[a]	85.2[a]	86.3[a]	0.6	< .0001
Whiteness	43.7[b]	58.6[a]	58.9[a]	59.2[a]	58.5[a]	0.8	< .0001
Total heme pigments (mg/g)	1.13[a]	0.580[b]	0.564[b]	0.695[b]	0.589[b]	0.030	<.0001

[a-b]Least squares means in a row corresponding to a certain factor with different letters are significantly different ($P < 0.05$). The values presented are means of 4 replicate determinations.

2.6 FIGURES
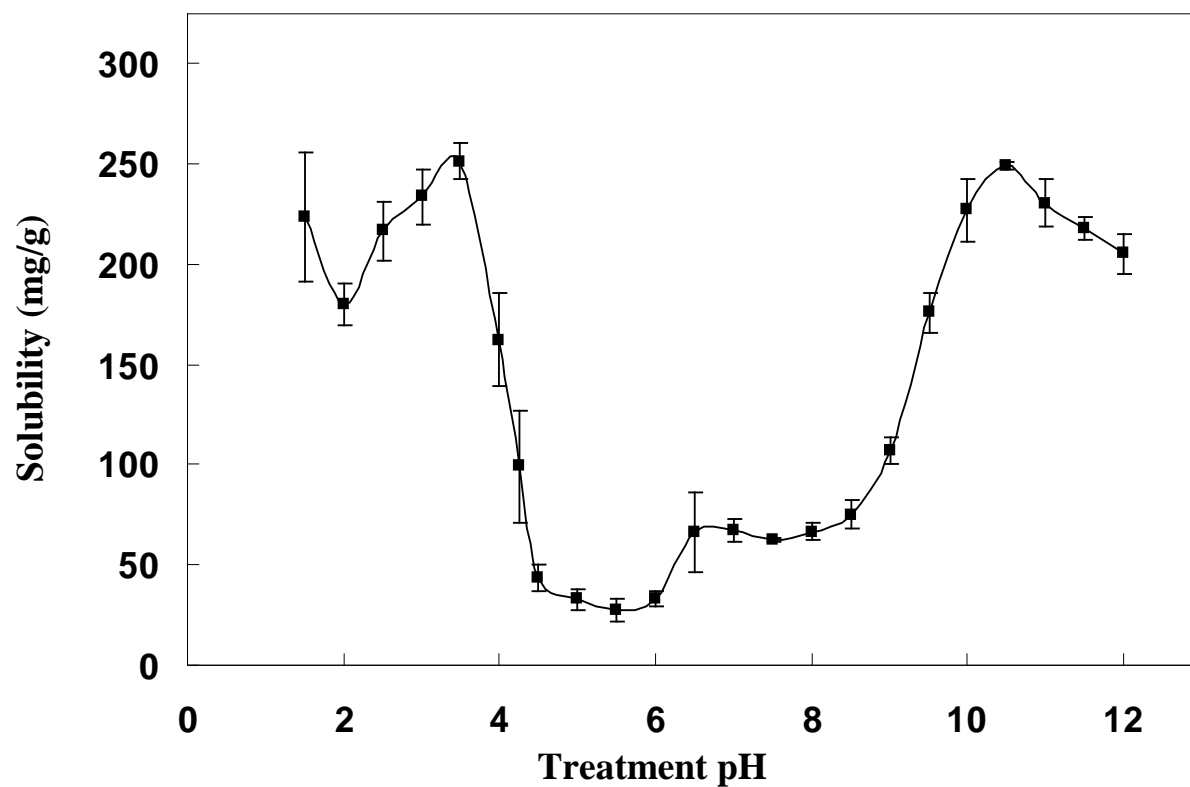
Figure 2.1. Solubility profile of chicken dark meat proteins as a function of pH. Protein homogenates were adjusted to a pH range of 1.5 to 12.0 using HCl or NaOH. The values presented are means of 4 replicate determinations.
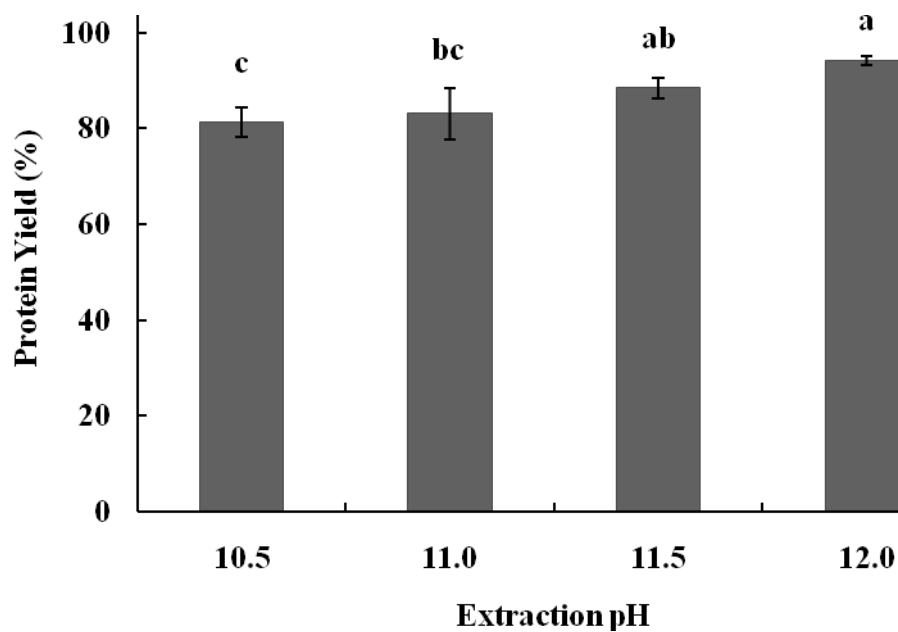
Figure 2.2. Effect of extraction pH on protein yield of extracted chicken dark meat. Dissimilar letters in the graph represent significant ($P < 0.05$) difference. The values presented are means of 4 replicate determinations.
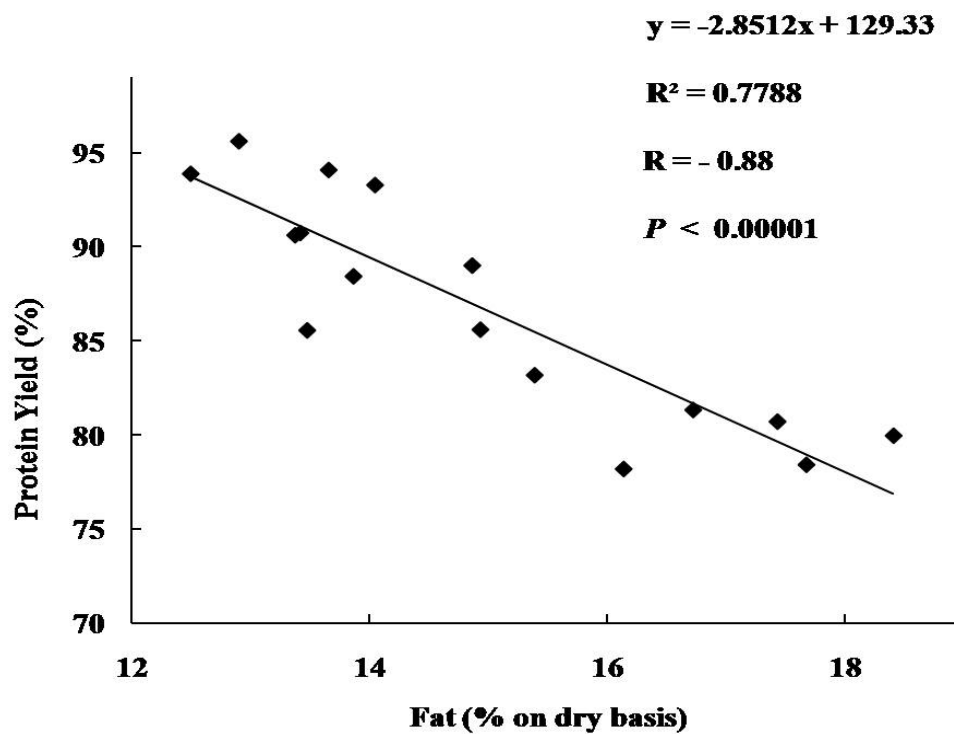
Figure 2.3. Regression analysis showing the relation between protein yield (%) and fat (% on dry basis) of extracted chicken dark meat as a function of extraction pH.
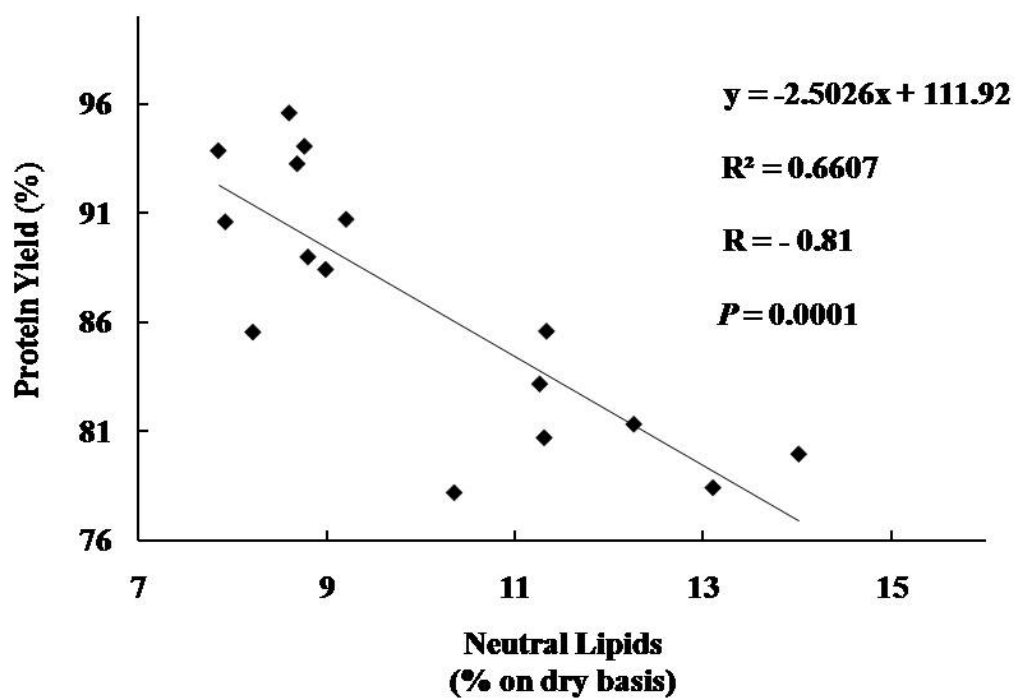
Figure 2.4. Regression analysis showing the relation between protein yield (%) and neutral lipids (% on dry basis) of extracted chicken dark meat as a function of extraction pH.
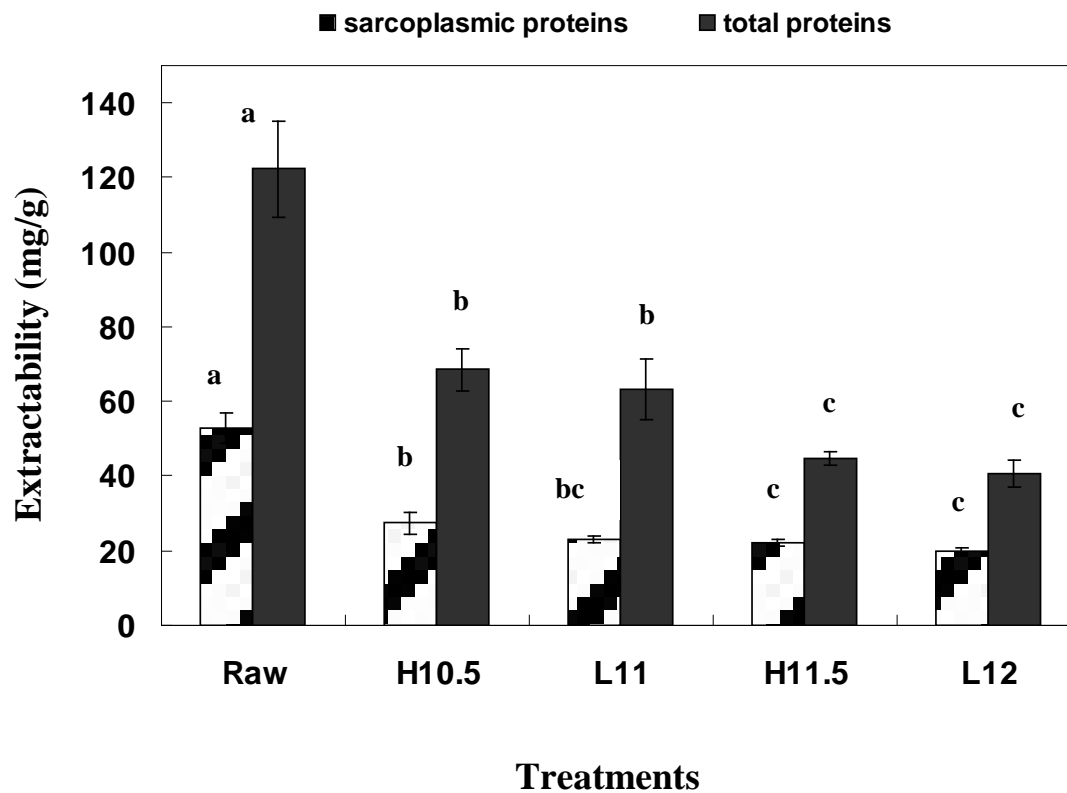
Figure 2.5. Extractability of recovered proteins after protein extraction by pH-shift method. Sarcoplasmic proteins were solubilized in phosphate buffer, while total proteins were solubilized in phosphate buffer (pH 7.4) containing potassium iodide. Dissimilar letters for respective parameters in the graph represent significant ($P < 0.05$) difference. The values presented are means of 4 replicate determinations.
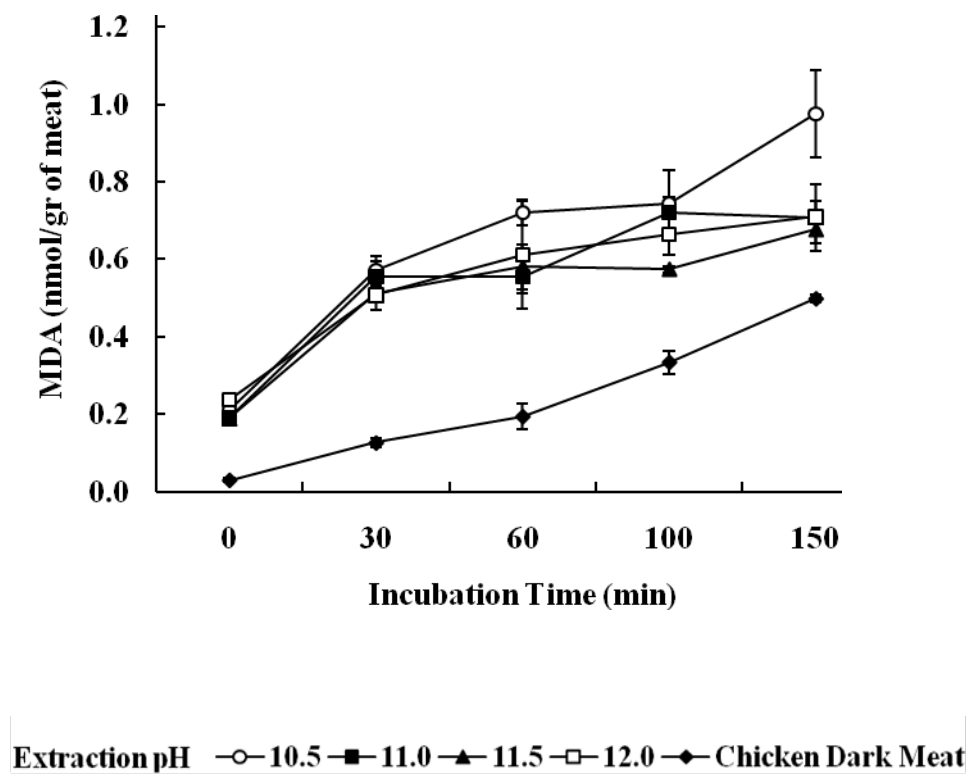
Figure 2.6. Effect of time and extraction pH on oxidative stability of chicken dark and extracted meat measured as induced TBA reactive substances (TBARS). The values presented are means of 4 replicate determinations.

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3 ALKALI AIDED PROTEIN EXTRACTION FROM CHICKEN DARK MEAT: TEXTURAL AND RHEOLOGICAL CHARACTERISTICS OF RECOVERED PROTEINS[2]

3.1 INTRODUCTION

Due to the fact that poultry dark meat has been considered as a major underutilized commodity (USDA, 2004), finding new technologies to transform it into valuable foods has become a major challenge for the poultry industry in North America.

One of the alternatives to utilize the "low value" poultry dark meat is to recover the proteins without compromising the protein functionality. pH shifting processing has been widely used for the recovery of proteins from fish dark meat (Kristinsson and Hultin, 2003). This technology has several advantages over the conventional surimi processing. These advantages include economical feasibility, high recovery yield and improved functionalities of the recovered proteins (Undeland *et al*., 2002; Kristinsson *et al.*, 2005). Furthermore, the protein recovery from fatty fishes (more than 5% fat content) using pH shifting technology showed improvement in gel properties (Undeland *et al.*, 2002; Kristinsson and Hultin, 2003). Since the fat content of poultry dark meat is similar to that of fatty fishes, the pH shifting method is expected to be appropriate for poultry dark meat.

Problems with research on surimi and other materials rich in dark meat are color, poor gel properties, and lipid oxidation (Okada, 1980; Hultin and Kelleher, 2000). Due to the relationship of texture with gel forming ability of myofibrilar proteins, the study of gelation has been of interest. Research by Ashgar *et al.* (1985) indicated that myosin and actomyosin are the proteins which play the main role in gelation. According to Oakenfull *et al.* (1997), dynamic measurements which involve small deformations under oscillating stress may give more reliable information on the viscoelastic nature of the gel compared to large strain test. Meat texture is responsible for quality and affects consumer acceptance (Ordonez *et al.*, 2001; Stanley, 1976). The complex interaction between sensory properties and physical processes which occurs during chewing and determines human perception of meat quality. Instruments which measure food texture stimulate such interactions. Hence, texture evaluation can be performed by subjecting the

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food to shearing, chewing, compressing or stretching, and measuring the response of food to those conditions. Texture Profile Analysis (TPA) is broadly used to evaluate food texture (Tabilo *et al.*, 1999).

Alkaline solubilization followed by acid precipitation has been used on poultry meat in order to extract the proteins (Jelen *et al.*, 1982; McCurdy *et al.*, 1986). Since there is not much research performed on textural and rheological properties of alkali extracted proteins from chicken meat, this study was conducted to assess textural properties and rheological characteristics of alkali extracted chicken dark meat.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Frozen chicken thigh meat was obtained from Lilydale Inc. (Edmonton, AB, Canada) and used for this study. The chicken thigh meat was thawed at 4 ^{o}C overnight. It was then minced using a meat grinder (Waring Pro, Woodbridge, ON, Canada); vacuum packed in 400 g plastic bags and stored at -30 ^{o}C until analysis.

3.2.2 Methods

3.2.2.1 Extraction Method

The extraction was carried out as per the methods described earlier (Liang and Hultin, 2003; Betti and Fletcher, 2005) with modifications. For the extraction of protein isolate, 400 g of frozen chicken thigh meat was thawed at 4 ^{o}C overnight. The meat was further mixed with ice-distilled water mixture (1:2.5; w:v) using an homogenizer (Wolfgang Puck Appliances) for 15 minutes. Further, the pH of the resulting meat slurry was adjusted using 2 M NaOH with constant mixing. Individual lots were adjusted to pH 10.5, 11.0, 11.5, and 12.0 for alkaline hydrolysis/protein solubilization and allowed to stand still at 4 ^{o}C for 30 minutes. The slurry was centrifuged using an Avanti J-E refrigerated centrifuge (Beckman Coulter, Inc., Palo Alto, CA, USA) at 25,900 g for 20 minutes at 4 ^{o}C. Three layers were formed after centrifugation: an upper fat layer, a middle aqueous layer of soluble proteins and a bottom sediment layer. The middle protein layer was carefully removed and the pH was adjusted to 5.2 using 2 M HCl. The

precipitated proteins were separated by centrifugation at 25,900 g for 20 minutes at 4 ^{o}C. These separated proteins were further mixed with iced water and the pH was adjusted to pH 6.2. Hence, this method of recovery of proteins is also called pH-shift method. The proteins were finally separated by centrifugation at 25,900 g for 20 minutes at 4 ^{o}C. After determining the moisture content of the protein extract, the final moisture content was adjusted to 80%, then mixed with cryoprotectants (5% sorbitol, 4% sucrose, 0.3% sodium tripolyphosphate, 0.4% sodium bicarbonate and 0.03% sodium nitrite) using a Kitchen-Aid meat processor. The processor was pre-chilled and operated in a cold room (6-10 °C) to prevent the temperature of the protein isolate from exceeding 15 °C throughout the mixing process. The recovered proteins were then stored at -30 ^{o}C for one month, after which samples were drawn for analysis.

3.2.2.2 Cooking Loss

Samples weighing 25-30 g (W1) were packed in plastic tubes. The tubes were then heated at 95 ^{o}C, until the internal temperature of the samples reaches 75 ^{o}C. The temperature was checked using thermocouples inserted in the center of the sample. The samples were considered cooked when the internal temperature reached 75 ^{o}C. After cooking, the meat was weighed again (W2) to determine the loss in weight during cooking.

Cooking loss (%) = (W1- W2/ W1) x 100

3.2.2.3 Expressible Moisture

The expressible water content was determined using a texture profile analyzer (TA XT Express, Stable micro systems, Ltd., Surrey, England). A known quantity of cooked sample (approximately 300 mg) was placed on a pre-weighed filter paper (Whatman No.1), and sandwiched between two glass plates. Using the texture profile analyzer under adhesive test mode, the sample was tested with a target force of 1000 g. The hold time for this test was 2 minutes, which was sufficient to express the water content of the sample. After the test, the filter paper along with the absorbed water was immediately weighed. Expressible water is measured as the quantity of water released per gram meat and was expressed as a percentage.

Expressible water (%) = (wet paper - dry paper / meat weight) x 100

3.2.2.4 Textural Profile Analysis

The texture profile analysis was carried out using a texture profile analyzer (TA-XT Express, Stable micro systems, Ltd., Surrey, England) with the method developed by Zhang and Barbut (2005). Meat samples in cylindrical shape (1.0 cm-height and 4.0 cm-diameter) were prepared from the samples. The analysis was carried out in TPA mode, using the TA-XT Express software included with the equipment. A double compression cycle test was performed up to 50% compression of the original portion height with an aluminium cylinder probe of 5 cm diameter. A time of 1 s was allowed to elapse between the two compression cycles. The trigger force used for the test was 5 g, with a test speed of 5 mm/s. When the test was finished, the software calculated the values for hardness (maximum force in grams required to compress the sample), springiness (ability of the sample to recover its original form after deforming force was removed), chewiness (the work needed to chew a solid sample to a steady state of swallowing), cohesiveness (extent to which the sample could be deformed prior to rupture) and resilience (the ability of the sample for recovery of shape). The analysis was carried out for 6 replicates.

3.2.2.5 Dynamic Viscoelastic Behaviour of Recovered Proteins

The dynamic viscoelastic behavior (DVB) of raw chicken meat and treated samples in the temperature range of 7 °C to 100 °C was measured using a Physica MCR Rheometer (Anton Paar GmbH, Virginia, USA) under oscillatory mode, using a 2.5 cm parallel plate measuring geometry. Approximately 4 g of macerated meat was ground thoroughly to obtain a fine paste. The paste was used for DVB measurement. The gap between measuring geometry and the peltier plate was adjusted to 1000 µm. The measurements were made by applying a small amplitude oscillation (0.5%) with a frequency of 1.0 Hz. A heating rate of 2 °C/min was achieved through the peltier plate of the rheometer. The applied stress was compared with the resultant strain. The results of the measurements were expressed as the storage modulus (G′) and loss modulus (G″). An average of 3 replicates was used for plotting the results.

3.2.3 Statistical Analysis

Each experiment and each assay was done at least in triplicate. Reported results represent an average of each experimental assay. All data were subjected to Analysis of Variance using the general linear model procedure of SAS (SAS, 2006). Differences between least squares means

were determined using HSD Tukey differences, and were reported as significant at the $P < 0.05$ level.

3.3 RESULTS AND DISCUSSION

3.3.1 Cooking Loss

One of the factors which determine meat quality is water holding capacity. Expressible moisture and cooking loss are the factors which are responsible for tenderness of meat (El Rammouz *et al.*, 2004) and hence related to the water holding capacity of the proteins. Cooking loss measurement helps to assess the effect of extraction pH on the water holding capacity (WHC) of the recovered proteins. There was a decrease in cooking loss for proteins extracted at pH values above 11.0. The maximum reduction in cooking loss was for proteins recovered at pH values of 11.5 and 12.0 (Figure 3.1). The lower cooking loss indicates higher WHC of the proteins. The greater WHC for proteins extracted with pH values above 11.0 could be explained by their higher protein content (see Chapter 2). In fact during heating, proteins form a gel by entrapping water. Higher protein content leads to better gel formation due to greater WHC of the proteins.

For the raw meat on the other hand, cooking loss was found to be maximum compared to extracted meat, which means that WHC was at its minimum level. This could be explained by the absence of cryoprotectants in the raw dark meat which were only added to extracted meat. In fact cryoprotectants improve WHC by absorbing more water (Henry *et al.*, 1995). It has been reported by Ensoy *et al.* (2004) that the WHC of the surimi prepared from spent layers has been improved by adding cryoprotectants.

3.3.2 Expressible Moisture

Expressible moisture (water loss) is a factor which indicates meat texture, tenderness and juiciness. Cooking loss and water loss are the parameters which both indicate WHC of meat (Mallikarjunan and Hung, 1997). In the present study water loss decreased as the pH of extraction increased (Figure 3.2). Water loss was less than 10% which was significantly lower ($P < 0.05$) for extracted meat at pH values of 11.0, 11.5, and 12.0 compared to pH 10.5 and raw

dark meat. Kristinsson and Liang (2006) reported similar results for alkali-extracted fish proteins. In present investigation the lower expressible moisture obtained for protein isolates at the pH 11.0 and above, indicates higher WHC for the protein gel network at this pH values (Rawdkuen *et al.*, 2009).

An inverse relationship was found between water loss and hardness of the recovered meat (Table 3.2). Similar results were found by Rawdkuen *et al.* (2009) who conducted a study on tilapia fish. Higher pH values leads to unfolding the proteins and results in exposure of hydrophobic groups, which leads to an increase in hydrophobicity. As a result, during heating of such protein there will be better gel network formation due to higher hydrophobic interaction. Water loss of the proteins recovered at pH 10.5 was similar to the values for raw dark meat due to the low alkaline conditions which might lead to less exposure of hydrophobic groups compared to pH 11.0 and above.

3.3.3 Textural Profile Analysis/Dynamic Viscoelastic Behaviour

Alkali-aided protein isolates underwent less denaturation compared to acid-aided protein isolates as reported by Kristinsson *et al.* (2005). Texture profile analysis of alkali extracted proteins is showed in Table 3.1. Hardness was greater for protein isolates at higher extraction pH values. The maximum hardness (4956 gram force) was found to be for proteins extracted at pH 11.5. This could be explained by the fact that higher pH leads to more protein denaturation and exposure of more hydrophobic groups (Omana *et al.*, 2010), leading to more protein-protein and protein-cryoprotectant interactions. These interactions will lead to form a gel network with higher hardness values. It is hypothesized that such a gel network would differ from a typical gel network of myofibrillar proteins (Tadpitchayangkoon and Yongsawatdigul, 2009). Elastic gels are derived from numerous cross-links between the myofibrillar proteins. When this gel network is unevenly distributed due to local aggregation of myofibrillar proteins, it will lead to poor gel formation as indicated by the lower storage modulus (G′). According to Feng and Hultin (2001) due to the presence of local aggregates of myofibrillar proteins even though the gel appears harder, it can easily breaks under stress.

Another reason for an increase in hardness for extracted meat could be concentration of myofibrillar proteins. According to Chen *et al.* (1997) hardness increased for myofibrillar

proteins concentrated by washing procedures. Previous studies conducted on alkali extraction of chicken dark meat showed that samples at pH 11.5 and 12.0 had higher protein content (see Chapter 2). Thus, this higher protein content might be the reason for the greater hardness of these samples.

There was no significant difference in springiness values between extracted meats treated at different pH values. This could be explained by the fact that all the samples were adjusted to equal moisture content. There was a significant ($P < 0.05$) increase in chewiness values for extracted meat at pH values of 11.5 and 12.0. Chewiness denotes the resistance to compression force (Caine *et al.*, 2003). Greater chewiness values obtained for protein samples treated at higher pH, might be explained by the increase in WHC (Figure 3.2), which results in more chewiness. Cohesiveness and resilience values significantly ($P < 0.05$) increased for extracted meat compared to raw dark meat with no significant difference between the pH treatments. According to Caine *et al.* (2003) change in resilience value was mainly due to the fat content. The alkali-aided extraction led to a significant lipid loss. On average fat removal was approximately 50% between pH values of (10.5-12.0) (see Chapter 2). The difference of TPA factors between extracted meat and raw dark meat might be due to the variations in moisture, lipid, and collagen content.

The dynamic rheological measurement has been used to study the heat-induced gelation of myofibrillar proteins (Hamann, 1987). Increase in storage modulus (G') indicates elastic gel network formation. Thus the change in G' values is used to monitor gelation of proteins (Venugopal *et al.*, 2002). Loss modulus (G') indicates the viscous behavior of the sample. The ratio of G''/ G' during the oscillatory test indicates gel transition temperature and is expressed as tan delta.

Dynamic viscoelastic behaviour (DVB) of extracted proteins (without cryoprotectants) at various pH values is presented in Figure 3.3. The DVB for chicken dark meat indicated considerable gelation properties as it showed by G' values. Maximum storage modulus value (439 kPa) was found at 66.7 ^{o}C. The increase in value indicated that proteins had ordered aggregation and formed a 3-dimensional gel network which entraps water (Dileep *et al.*, 2005). The increase in storage (G') and loss modulus (G'') during heating indicate formation of a viscoelastic gel network. G' values were at their maximum in temperature range of 40 -60 ^{o}C.

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There was a dramatic decrease in G′ values for protein isolates at alkaline pH values. The highest G′ values for all the extracted meat samples were less than 200 kPa. This means that the proteins in extracted meat lost their ability to form an ordered gel network. This could be explained by the denaturation of proteins at alkaline pH values, which leads to the situation in which the proteins fail to form cross-links between each other, and thus not to form an ordered gel. Another reason for the decrease in G′ values might be the removal of collagen during alkali extraction. Collagen changes from quasi-crystaline to a random-like structure at temperatures around 60 $^{\circ}$C, leading to an increase in meat elasticity. Thus, collagen removal could lead to a reduction in elasticity as indicated by G′ values for extracted meat.

Even though the storage modulus of the recovered proteins was low, cryoprotectants helped to regain the elastic properties in recovered proteins (Figure 3.4). The maximum G values of the protein isolates decreased with higher pH values. This indicates that extracted proteins at higher pH values had lower ability to form an elastic gel network. The minimum G′ value (194 kPa) was observed for extraction carried out at pH of 12.0. As shown in Table 3.2, hardness increased for extractions at higher pH values, but G′ values were considerably lower compared to raw dark meat. This could be explained by the aggregation of denatured proteins during heating leading to formation of a rigid gel network (Tadpitchayangkoon and Yongsawatdigul, 2009). G′ values slightly decreased until the onset of gelation at the temperature of 30.3 $^{\circ}$C. Further increase in temperature led to an increase in G′ values for all the samples. The G values for raw dark meat increased until 66.7 $^{\circ}$C which reduced with further increase in temperature. However, there was an increase in G′ values in extracted meat even at temperatures above 80 $^{\circ}$C, indicating that the gel network is stable even at elevated temperatures. Tan delta values for raw dark meat showed two obvious transitions at 50.1 $^{\circ}$C and 96.5 $^{\circ}$C respectively. According to Wright *et al.* (1977), the transition at 55 $^{\circ}$C was due to myosin denaturation. They found another transition at 63 $^{\circ}$C which was because of denaturation of collagen and sarcoplasmic proteins. In our study, in case of extracted meat, only one major transition was found at 50.1 $^{\circ}$C. This could be explained by the removal of collagen in the extracted meat. The difference in gelation behavior between extracted meat and raw dark meat would be due to denaturation of proteins at high alkali pH values of extraction (Kristinsson and Hultin, 2003; Davenport and Kristinsson, 2004) and removal of collagen.

3.4 CONCLUSIONS

Cooking loss and water loss significantly reduced for recovered proteins at higher pH values. In comparison with raw dark meat, there was an increase in hardness and chewiness of extracted meat at higher pH values. Even though the hardness of protein extracts was higher, their lower G values showed less elasticity. The lower G values of the extracted meat as compared to the raw dark meat may be due to extensive denaturation of proteins as well as collagen removal during the process. Storage modulus of extracted meat was found to be increased with the addition of cryoprotectants. The gels obtained from extracted proteins with added cryoprotectants were more stable and were not disrupted even at temperatures above 80 ^{o}C. Unlike the raw dark meat, extracted meat showed a single transition due to the denaturation of myosin as indicated by tan delta values. In conclusion, this study revealed that proteins extracted at pH values of 10.5 and 11.0 posses good water holding capacity and appreciable gelling properties which make them a suitable functional ingredient for the preparation of further processed meat products.

3.5 TABLES

Table 3.1. Textural profile analysis of recovered proteins after protein extraction by pH-shift method[1]

Parameters	Raw	pH 10.5	pH 11.0	pH 11.5	pH 12.0
Hardness (g)	2836.83[b] (767.17)	3151.88[a,b] (1318.43)	3782.27[a,b] (1292.69)	4956.30[a] (1366.58)	4334.15[a,b] (424.38)
Springiness	0.98[a] (0.21)	1.17[a] (0.41)	0.97[a] (0.05)	1.24[a] (0.46)	1.36[a] (0.42)
Chewiness	1521.69[b] (641.08)	2840.10[a,b] (1941.44)	2753.09[a,b] (777.67)	4492.63[a] (1289.96)	4584.52[a] (1573.40)
Cohesiveness	0.54[b] (0.06)	0.73[a] (0.03)	0.77[a] (0.05)	0.77[a] (0.03)	0.77[a] (0.02)
Resilience	0.30[b] (0.04)	0.43[a] (0.03)	0.52[a] (0.07)	0.51[a] (0.06)	0.52[a] (0.03)

[1]Results are presented as means values (n = 6). Values in parenthesis denote standard deviations. Dissimilar superscripts in the same row denote significant difference ($P < 0.05$).
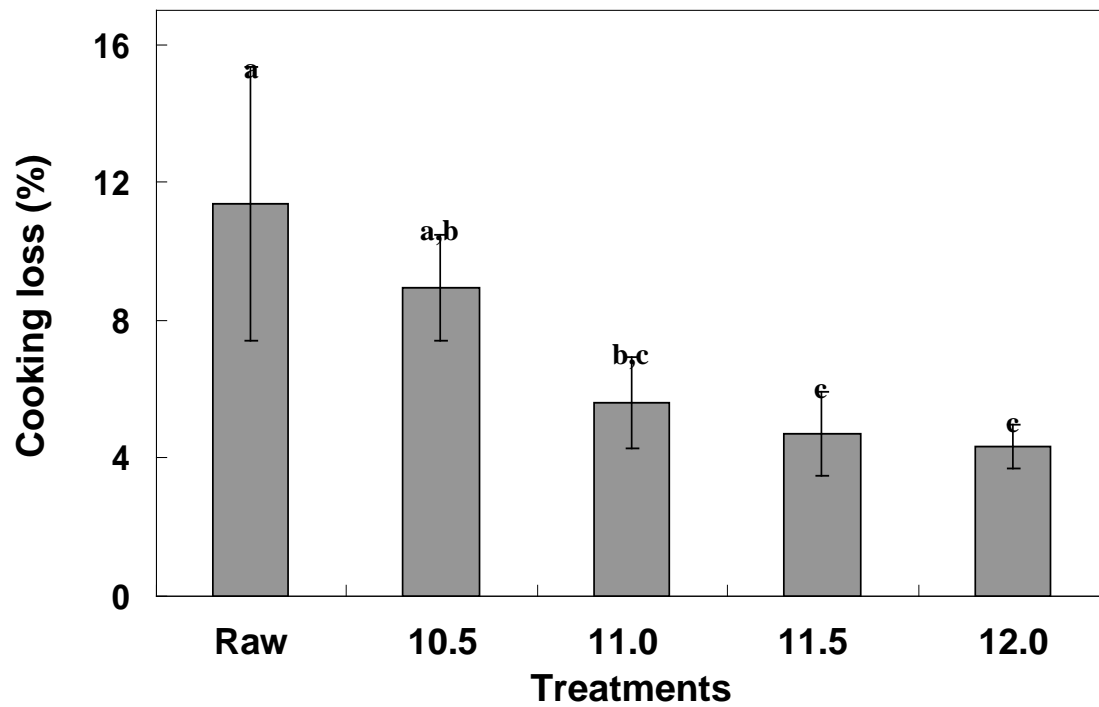
3.6 FIGURES

Figure 3.1. Cooking loss of different treatments during alkali extraction of Chicken dark meat. Dissimilar letters (a, b, c) in the figure denotes significant difference ($P < 0.05$). The values presented are means of 6 replicate determinations.
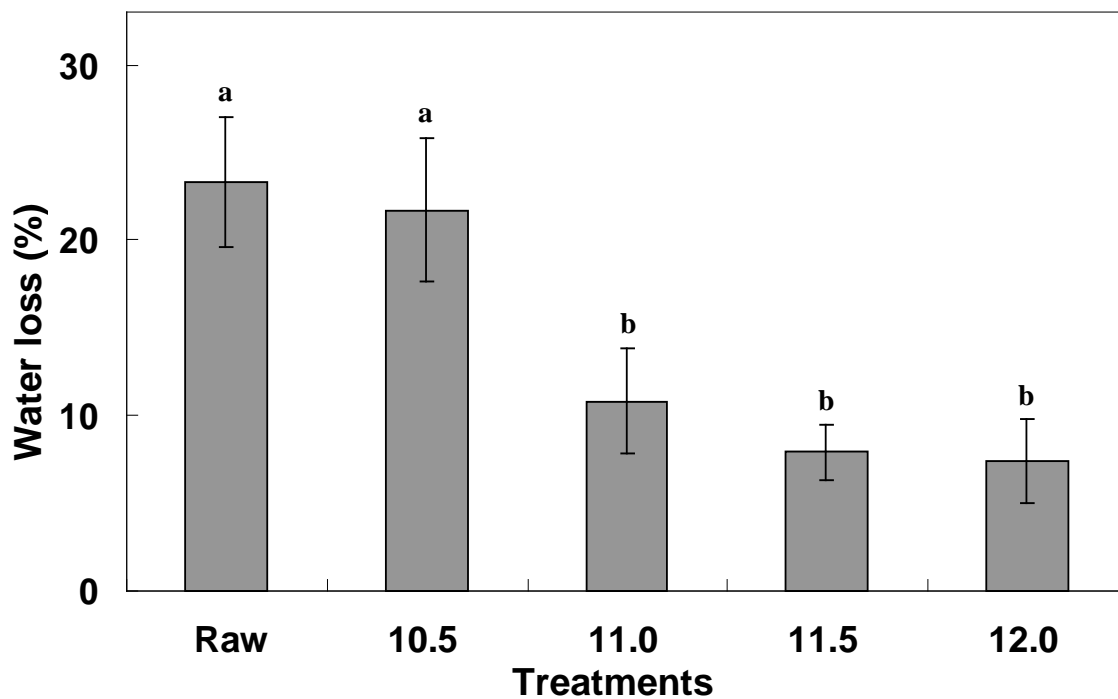
Figure 3.2. Expressible moisture of different treatments during alkali extraction of chicken dark meat. Dissimilar letters (a, b) in the figure denotes significant difference ($P < 0.05$). The values presented are means of 6 replicate determinations.
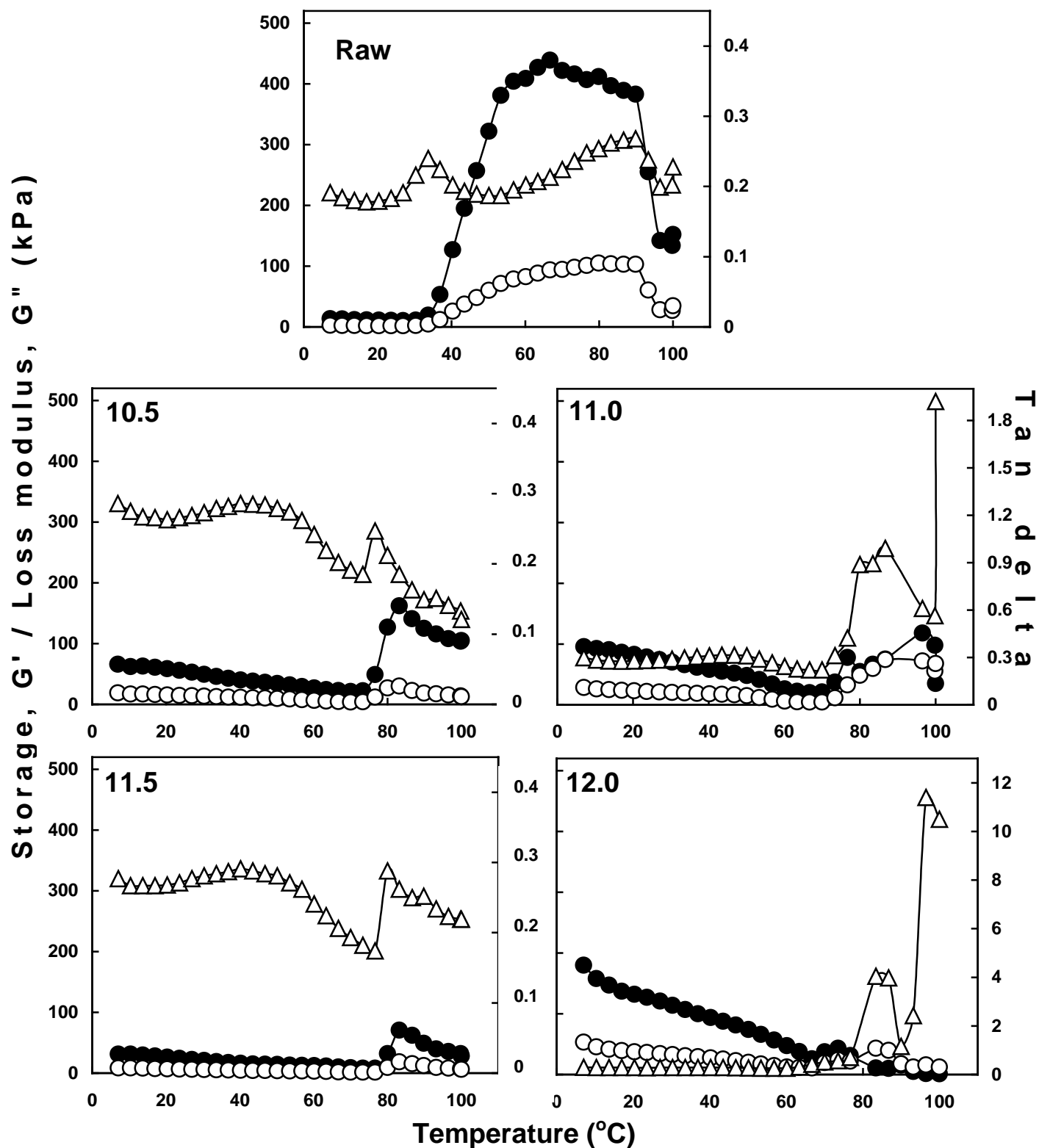
Figure 3.3. Dynamic viscoelastic behaviour (DVB) of recovered proteins from chicken dark meat after alkali extraction (without cryoprotectants). DVB of fresh chicken dark meat is given for comparison (G'(-●-) , G"(-○-) and Tan delta(-Δ-)). Storage/Loss moduli are drawn on the same scale for all the graphs.
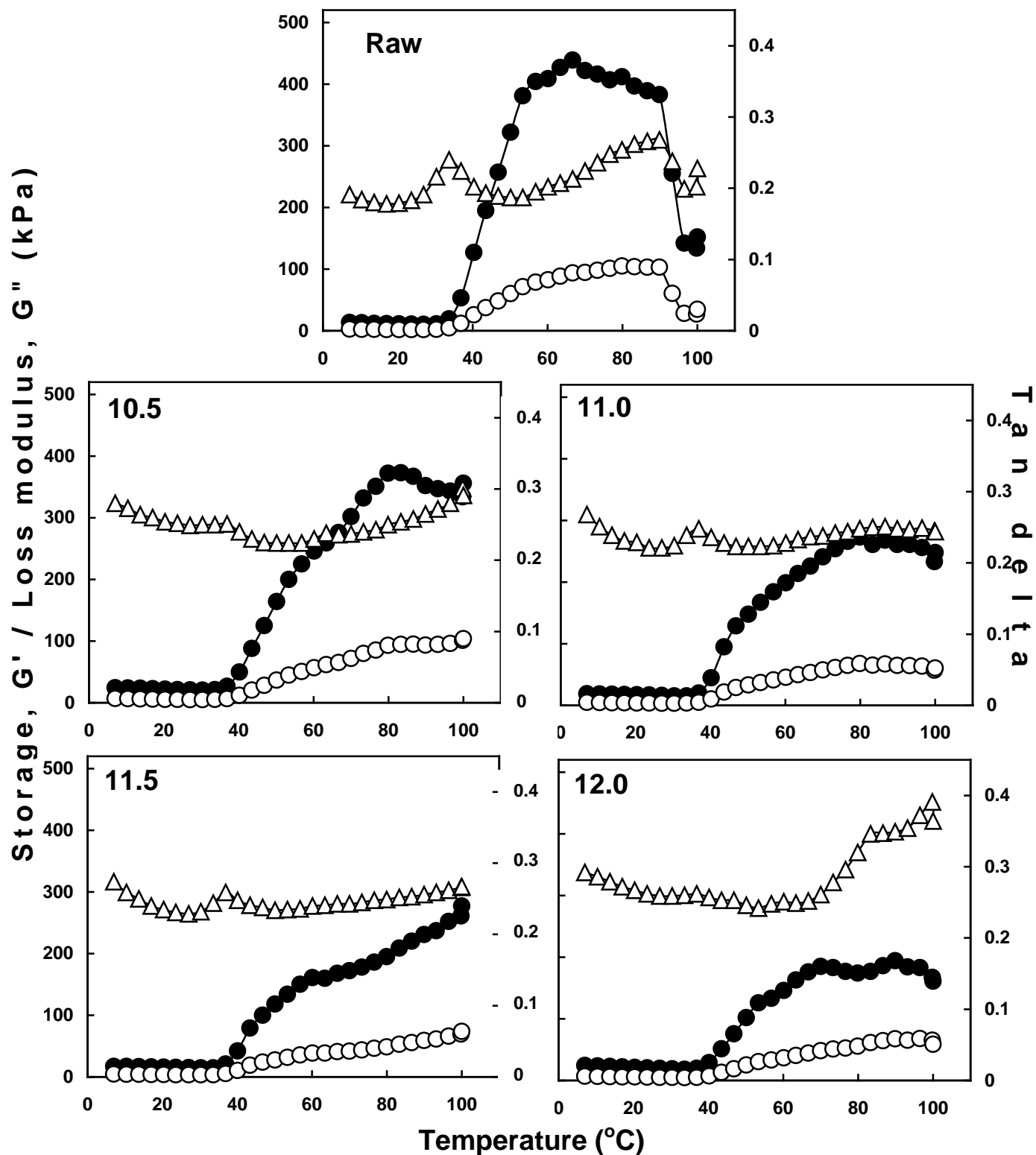
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Figure 3.4. Dynamic viscoelastic behaviour (DVB) of recovered proteins from chicken dark meat after alkali extraction, stored at -30°C for one month with added cryoprotectants. DVB of fresh chicken dark meat is given for comparison. (G'(-●-), G" (-○-) and Tan delta (-Δ-)). Storage/Loss moduli are drawn on the same scale for all the graphs.

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4 PROJECT SUMMARY AND IMPLICATIONS

Muscle protein extraction from "low value" poultry meat has been receiving increased attention. Color, high fat content and poor shelf life are the main concerns with "low value" poultry meat, and all affect consumers' selection and satisfaction. One of the attempts to increase the utilization of poultry dark meat is using alkaline solubilization processing to recover myofibrillar proteins and separate them from pigments and fat to increase their utilization for the preparation of further processed meat products.

This thesis has described alkaline extraction of chicken dark meat. The process resulted in a yield of more than 80% in the pH range (10.5 – 12.0) studied. Even though the total fat content decreased by approximately 50%, the current study demonstrated that the isolated proteins are susceptible to lipid oxidation as revealed by TBARs values. The alkaline solubilisation process failed to remove polar lipids and in some cases they even increased. However, neutral lipids were removed by 61.51%. Alkali aided extraction helped to lighten the color of the dark meat and reduction of heme pigments by 53%.

There was a significant decrease in cooking loss and water loss for the recovered proteins at higher pH values. Hardness and chewiness of extracted meat was found to be greater for extractions at higher pH values. Even though the hardness of protein isolates was higher the gels obtained from them were less elastic as indicated by G′ values. Cryoprotectants improved the gel forming ability of the extracted meat as indicated by rheological analysis. The gels thus obtained with added cryoprotectants were more stable and were not disrupted even at temperatures above 80 $^{\circ}$C. Raw dark meat showed two transitions corresponding to the denaturation of myosin as well as collagen and other sarcoplasmic proteins. However, the second transition was absent in the case of extracted meat, probably due to the removal of collagen. In conclusion, this study revealed that alkali-extracted proteins from chicken dark meat at pH values of 10.5 and 11.0 may have the possibility to be used as a functional ingredient for the development of new meat products.

Possible applications of the recovered protein include use as meat fillers or in the manufacture of casings and biodegradable packaging. Another advantage of using these proteins is that people seem to have fewer allergies to poultry proteins than those from milk, eggs, fish

and soybean sources. However, before any possible commercialization of this protein isolate in Canada, determination of potential unsafe compounds (i.e. lysino-alanine) needs to be evaluated. Remarkably, the U.S Food Drug Administration (2004) gave the status as safe (GRAS) to alkali- and acid-produced protein isolate from fish.

Further considerations include modifying the process to remove more polar lipids by adding more water and citric acid to the starting material, or adding stabilizing agents such as vitamin E to overcome the oxidation issue. A complete sensory profile analysis will be also important for the successful application of such extracts for food product development.