University of Alberta

Protein Isolation from Mechanically Separated Turkey Meat (MSTM)

by

Yuliya Victorivna Hrynets

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

Department of Agricultural, Food and Nutritional Science

Fall 2010

Edmonton, Alberta

EXAMINING COMMITTEE

Dr. Mirko Betti, Agricultural, Food and Nutritional Science

Dr. Andreas Schieber, Agricultural, Food and Nutritional Science

Dr. Jonathan M. Curtis, Agricultural, Food and Nutritional Science

Dr. Ellen Goddard, Rural Economy

ABSTRACT

Mechanically separated turkey meat (MSTM) is one of the cheapest sources of protein; however its use for production of further-processed poultry products is limited due to undesirable composition. pH-shifting extraction was applied to overcome the problems associated with MSTM. In the first study the effect of acid pH-shifting extraction with the aid of citric acid and calcium ions on lipids and heme pigments removal from MSTM was investigated. The maximum removal of total, neutral and polar lipids was achieved with addition of 4, 6 and 2 mmol/L of citric acid, respectively. Addition of 6 or 8 mmol/L of citric acid was the most efficient for total heme pigments removal. In the second and third studies chemical, functional and rheological properties of proteins isolated from MSTM were investigated as influenced by different (2.5, 3.5, 10.5 and 11.5) extraction pH. Gel-forming ability was found the highest for pH 3.5 extracted protein.

ACKNOWLEDGMENTS

I am greatly indebted to my graduate advisor, Dr. M. Betti, for his valuable help, mentoring and encouragement throughout my study. Thank you for your infailing interest and guidance, which made the completion of this work possible.

I extend my gratitude to my advisory committee members Dr. Schieber and Dr. Curtis for their help and advice. My appreciation is also expressed to Dr. E. Goddard for agreeing to be an external examiner.

Special thanks go to Dr. D. A. Omana for all his practical advices he gave me in scientific work and writing.

I also wish to express my thanks and gratitude to Yan Xu and Takuo Nakano for their great help with laboratory analyses and methodologies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFW: Air-flotation washing

ANOVA: Analysis of variance

ANS: 1-anilino-8-naphthalenesulfonate

ATP: Adenosine triphosphate

BCAA: Branched-chain amino acids

CA: Citric acid

CAC: Codex Alimentarius Commission

CFC: Chicken Farmers of Canada

CFIA: Canadian Food Inspection Agency

CFR: Code of Federal Regulations

CIE: International Commision of Illumination

Cys: Cysteine

DVB: Dynamic viscoelastic behaviour

EAI: Emulsifying activity index

EDTA: Ethylenediaminetetraacetic acid

ESI: Emulsion stability index

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

FE: Foam expansion

FSIS: Food Safety and Inspection Service

FVS: Foam volume stability

GRAS: Generally Regarded as Safe

HACCP: Hazard Analysis Critical Control Point

Hb: Hemoglobin

His: Histidine

HMM: Heavy meromyosin

HSD: Honestly significant difference

ICMSF: International Commission on Microbiological Specifications for Foods

Iso: Isoleucine

LC: Light chain

Leu: Leucine

LMM: Light meromyosin

LVR: Linear viscoelastic region

Lys: Lysine

Mb: Myoglobin

MDA: Malondialdehyde

MDB: Mechanically deboned beef

MDTM: Mechanically deboned turkey meat

Met: Methionine

MHC: Myosin heavy chain

MLC: Myosin light chain

MSM: Mechanically separated meat

MSPM: Mechanically separated poultry meat

MSTM: Mechanically separated turkey meat

NCC: National Chicken Council

ND: Not detected

PER: Protein efficiency ratio

Phe: Phenylalanine

pI: Isoelectric point

PL: Phospholipids

PUFAs: Polyunsaturated fatty acids

RFI: Relative fluorescence intensity

ROS: Reactive oxygen species

R-SH: Reactive sulfhydryl groups

SAS: Statistical analysis system

SAW: Salt-alkaline washing

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SH: Sulfhydryl groups

STPP: Sodium tripolyphosphate

TAG: Triacylglycerols

TBA: Thiobarbituric acid

TBARs: Thiobarbituric acid reactive substances

TCA: Trichloroacetic acid

TEAA: Total essential amino acids

Thr: Threonine

TNEAA: Total nonessential amino acids

T-SH: Total sulfhydryl groups

USDA: United States Department of Agriculture

Val: Valine

WHC: Water-holding capacity

WOF: Warmed-over flavour

CHAPTER 1. LITERATURE REVIEW

1.1 Development of mechanical deboning process

A few decades ago the primary focus of the poultry industry was on production of whole poultry carcasses. Changes in people's lifestyle, which included health concerns and economic consciousness, forced the poultry industry to evolve in order to better meet consumer's expectations and fulfill their demands. Recently, consumer preferences shifted from buying whole birds to buying further-processed products. For instance, in 1962 whole birds accounted for 83% of the broiler processing market, while in 2008 only 11% of broilers were sold as whole birds (NCC, 2009). This change is accompanied by increased poultry consumption worldwide and it is expected that by 2020 poultry will be the overall meat of choice (Bilgili, 2002). Recognition of poultry meat as a healthy component of a diet, possibility to purchase convenient ready-to-eat type of products and higher product diversification, all contribute to the increased demand for poultry products. Increasing demand for cut-up and further-processed poultry is associated with the increased supply of necks, backs and frames, which require proper utilization. About 24% of these parts are edible (Trindade et al., 2004). In an attempt to recover the meat that is firmly attached to the poultry carcass and facilitate its conversion to more saleable foods, a mechanical separation (deboning) process was developed. The first mechanical deboners were designed for fish in Japan in early 1940's. The mechanical separation of poultry began in late 1950's in U.S. (Trindade et al., 2004). This process offers an excellent

opportunity to improve the edible yield from processing plants and increase the availability of animal protein in the food supply (Young et al., 1983). Around 700 million kg of mechanically separated poultry meat (MSPM) is produced each year in the U.S. alone (Stanley, 2008). The separation process involves grinding meat and bone together, and forcing the mixture through a fine screen of a mechanical deboner (e.g. Paoli, Beehive, Bibun) (Froning, 1981; Oliphant, 1997). The "soft" materials, including meat, fat and connective tissue are separated from the hard material (bone) by the application of high pressure. The composition of mechanically separated meat (MSM) is highly variable and depends on species, animal age, bone to meat ratio, skin content, the type of machine and its setting (Crosland et al., 1995; Henckel et al., 2004). Due to paste-like texture and relatively low price, MSPM is used in production of further-processed products, such as bologna, frankfurters, turkey rolls, nuggets, hamburgers, etc. The increased use of MSPM for further-processed products has highlighted the need to improve meat quality associated with organoleptic and functional characteristics (Sosnicki and Wilson, 1991).

1.1.1 Deboning equipment

Availability of mechanical deboners reduces labor costs drastically and allows to retrieve the meat which cannot be removed from bones by hand deboning. MSM is produced by machines which first grind or crush bones, followed by separation of bone and tendon by forcing the tissue through a sieve (Hedrick et al., 1994). Two general categories of deboning equipment are used in

the poultry industry. The first category includes equipment where the meat is forced from the outside to the inside through perforations in the drum and the bone residue goes to the outside of the drum. In the other equipment category the meat is forced from the inside to the outside through a perforated cylinder, with the bone part left inside (Froning, 1981; Froning and McKee, 2001). Three basic deboner types are currently available on the market (Barbut, 2002). In the first, belt-drum type, raw material is passed between a rubber belt and a micro-grooved steel drum. The meat is squeezed through the perforated steel drum, while the harder bones and connective tissue remain outside. In the second, rotating auger type, bones and frames go through a bone cutter to reduce their size. Then, the ground mixture is subjected to a screw-driven boning head. Under pressure, the material is squeezed out through the perforated steel cylinder encasing the auger. The remaining bones and connective tissue are pushed forward and exit at the end of the head. The third type of the deboner is a hydraulically pressed batch system. Similarly to the rotating auger machines, bones are pre-cut prior to be deboned in a batch-type chamber. Inside, the material is forced against a stationary slotted surface by a high pressure hydraulic-powered ram piston. The material is squeezed through the cylinder openings under high pressure (Barbut, 2002). Deboners can process from 230 to 9000 kg of product per hour depending on the size and capacity (Froning and McKee, 2001).

1.1.2 Regulations of mechanical deboning process

In most countries MSM is generally considered be of poor quality and therefore subjected to strict regulations regarding its use as a binding agent or as a meat source for the production of minced meat products (Henckel et al., 2004). The norms and guidelines are generally based on protein, fat and calcium content, the size and number of solid particles (bone particles) in the separated meat and on use and storage of MSM. A minimum protein content of MSM should be 10%, or 14% if destined for retail sale (CFIA, 2008). MSM shall have a fat content no more than 30% (CFR, 2005). The legislation regarding handling, processing, use, nutritional standards and microbiological quality are variable between different countries. For example, in Denmark, if MSM is used at levels less than 2%, it does not have to be declared on the label. In Australia, exported product is labelled as “edible mechanically deboned meat” and contains a statement declaring maximum calcium, moisture and minimum protein content. FSIS (1982) of US imposed some restrictions on the amount and type of products, where MSPM might be used. Most of them are based on some particular component presented in MSPM, like calcium.

CFIA (2008) regulations regarding labelling, stated that MSM may not be described simply as "meat" on food labels, but must be labelled as "mechanically separated" species (e.g. beef, pork, chicken). CFIA (2009) allows the use of MSM in standardized and non-standardized cooked meat products; fresh and preserved sausages, and uncooked burger products (sold only in a frozen state). The use of

MSM in comminuted meat, e.g. ground beef, ground pork or in meat patties is prohibited.

1.2 Composition and associated problems of mechanically separated poultry meat (MSPM)

1.2.1 Protein quality

Increased utilization of MSM in production of sausages and other further-processed products raise the question about the nutritional value of these products for human consumption. Protein quality specified in CFR (2005) requires that MSM have a minimum protein efficiency ratio (PER) of 2.5 or have an essential amino acids content of at least 33% of the total amino acids present. PER is a method to determine the quality of food protein by comparing weight gain to protein intake (Grodner, 2004). MacNeil et al. (1978) reported PER values of 2.65 for skinless mechanically separated broiler necks and 2.45 for a combination of skinless mechanically separated backs and necks. The reason might be due to a partial removal of connective tissue during processing, such as collagen, which has poor amino acids composition (Newman, 1981). The difference in PER values among different studies is based on variability in the bone source and the levels of connective tissue and calcium present (Happich et al., 1975).

MacNeil et al. (1978) observed the following essential amino acids composition of deboned skinless broiler necks (grams of amino acid residue per 100g of total amino acid residues): histidine (His) 2.61, lysine (Lys) 8.74,

threonine (Thr) 4.74, cysteine (Cys) 0.65, valine (Val) 4.10, methionine (Met) 2.82, isoleucine (Iso) 3.63, leucine (Leu) 8.25 and phenylalanine (Phe) 3.89. Table 1.1 represents the approximate composition of different poultry parts after deboning.

Table 1.1. Proximate composition of mechanically separated meat (MSM) produced from different parts of poultry carcass

Part of the carcass	Moisture (%)	Protein (%)	Fat (%)	Reference
Turkey frame	72.7	16.4	10.4	(Radomyski, 2000)
Turkey frame	73.7	12.8	12.7	(Essary, 1979)
Turkey frames with skin and drumsticks	69.8	17	12.8	(Radomyski, 2000)
Chicken backs and necks	66.6	14.5	17.6	(Grunden et al., 1972)
Chicken backs	62.4	13.2	21.2	(Froning, 1970)
Skinless necks	76.7	15.3	7.9	(MacNeil et al., 1978)
Chicken backs	63.9	13.98	21.16	(Henckel et al., 2004)
Hen thigh	69.9	19.5	8.8	(Kondaiah and Panda, 1992)

The variability in moisture, protein and fat content of MSPM is described by a wide range of factors, including the type of machine used, the history of the material subjected to deboning and the preparation procedures used (Goldstrand, 1975).

1.2.2 Lipids and heme pigments

The considerable shearing action caused by mechanical deboning results in cellular disruption, the extent of which depends on the screen sizes of the

machine. As a consequence, bone marrow released from the broken bones, accumulates in the resultant product causing increased heme pigments and lipids content. These pigments and lipids fractions dilute the amount of protein in the resulting product. Mechanical deboning causes a three-fold increase in heme protein content compared to hand-deboned meat (Froning and Johnson, 1973). Hemoglobin (Hb) and myoglobin (Mb) are the main pigments responsible for the color of MSPM. Other pigments are also present in meat, but in small amounts, therefore their effect on the meat color is minimal (Elkhalifa et al., 1988). Aeration during the deboning process facilitates oxidation of myoglobin to oxymyoglobin. Further oxidation, due to the interaction with the metal surfaces of the separator and the continuous availability of oxygen, results in metmyoglobin formation, giving an undesirable brown color to the product (Janky and Froning, 1975). Hb also contributes to the color instability of MSPM, since it can be easily oxidized and is susceptible to heat denaturation during processing. Abnormal brown, green and gray color defects were observed in further-processed products containing MSPM (Froning and McKee, 2001). Moreover, heme pigments are known to have a pro-oxidant effect on lipids. Bone marrow contains 0.09-0.23% of iron, which has been identified as an oxidation catalyst in muscle tissue (Skibsted et al., 1998). Under some conditions heme pigments leak iron to form more simple iron species, which may bind to negatively charged phospholipids and catalyze Fenton-type reactions in the membranes (Carlsen et al., 2005).

The lipid content of chicken bone marrow is 46.5%, wherein neutral lipids constituted 98.4% of the total lipids, which are predominantly triacylglycerols (TAG), and 1.7% of phospholipids (PL) fraction (Moerck and Ball, 1973). Inclusion of bone marrow leads to a higher variation in the fatty acids content and a higher percentage of cholesterol and PL in mechanically separated chicken meat (Al-Najdawi and Abdullah, 2002). The PL fraction of MSPM is highly unsaturated (Gomes et al., 2003). Even though unsaturated fatty acids are beneficial to human health, they are more prone to oxidation, causing problems with shelf life stability and sensorial quality. Besides the bone marrow lipids, subcutaneous fat, skin and abdominal fat also contribute to the high lipid content of MSM.

1.2.2.1 Problems associated with lipids and heme pigments content

One of the major problems with MSPM is the rapid onset of oxidative deterioration, which limits its acceptability for meat products production. Oxidation has been implicated in a number of deleterious effects on lipids and pigments, causing discoloration, drip-losses, off-flavor development, reduced shelf life, loss of nutritional value and functionality (Matsushita, 1975; Gray et al., 1996; Morrissey et al., 1998; Coronado et al., 2002). The problem of lipid oxidation has an economic impact on the meat industry as it leads to the development of potentially toxic reaction products (aldehydes, ketones, alkanes, etc.) and chemical spoilage in the food system (McCarthy et al., 2001; Reig and Toldra, 2010). Poultry meat is notably sensitive to lipid oxidation because of its

high content of polyunsaturated fatty acids (PUFAs) (Botsoglou et al., 2002). MSM is particularly susceptible to lipid oxidation because of its high fat content (Mielnik et al., 2003). Lipids in meat are commonly classified into two types: depot or intermuscular and intramuscular or tissue lipids (Watts, 1962; Love and Pearson, 1971). The neutral lipids (mostly TAG) are the principal components of intermuscular lipids, which are generally localized in specialized connective tissue in relatively large deposits. Intramuscular lipids are integrated into and are widely distributed throughout the muscle tissues and contain a high amount of PL (Watts, 1962). Incorporation of myoglobin and hemoglobin during the deboning process also accelerates the rate of oxidative changes (Love and Pearson, 1971). Lipid oxidation catalyzed by iron porphyrins may decompose PUFAs and destruct pigments (Kendrick and Watts, 1969). Mb has been shown to be the major catalyst for lipid oxidation; however its mode of action is controversial. It has been suggested that the interaction of Mb with hydrogen peroxide (H_2O_2) or lipid hydroperoxides results in the formation of ferrylmyoglobin, which initiates free radical chain reactions (Harel and Kanner, 1989; Rao et al., 1994). Mb is also a source of iron, which has a strong catalytic effect on lipid oxidation (Ahn and Kim, 1998). Lee et al. (1975) suggested that the highest pro-oxidant activity occurs at the linoleic acid to heme pigments ratio of 500:1. They also reported that the ratio for mechanically deboned chicken was 480:1, which shows the critical role of interaction between lipids and pigments on the oxidative stability of a meat

system. Kendrick and Watts (1969) observed the fast destruction of heme compounds as a result of contact with linoleic acid.

One of the most widely used methods to determine oxidative stability of meat is the measuring of malondialdehyde (MDA), considered the most abundant and highly reactive product of PUFAs peroxidation (Janero, 1990). The evaluation of MDA is based on the reaction with thiobarbituric acid reactive substances (TBARs), followed colorimetric assay. Johnson et al. (1974) indicated that mechanically separated turkey had minimal lipid oxidation when stored up to 10 weeks. In contrast, Smith (1987), who investigated the lipid oxidation rate with the same raw material, reported that lipid oxidation occurred rapidly during the first seven weeks of frozen storage, followed by a decrease in TBARs number during the latter part of storage. Froning et al. (1971) reported that mechanically separated turkey stored at -24 °C for 90 days showed high TBARs values. Those differences may be attributed to a wide range of factors, such as oxygen availability, light and temperature (Monahan, 2000). For instance, Pettersen et al. (2004) investigated the different packaging effects on lipid oxidation of MSTM and found that MSTM stored in air had higher TBARs values compared to meat stored in a vacuum or modified atmosphere packaging. The study of Schnell et al. (1973) showed that the particle size had an effect on the oxidative stability of MSPM as evaluated by TBARs numbers, with the level being inversely proportional to particle size.

One of the issues having arisen with MSM is the cholesterol content, which is affected by the amount of bone marrow, body fat and skin (Al-Najdawi and Abdullah, 2002). The major concern is that overconsumption of MSM may result in health problems for persons prone to hyperuricemia (abnormally elevated blood level of uric acid) or hypercholesterolemia (Young, 1985). However, MSPM is used in the production of further-processed products, where cholesterol content should be declared on the label. Therefore, it was suggested that those people who need to control the cholesterol level in their diet would be able to make an educated decision of whether to purchase the product, based on the information provided on the product label (Froning and McKee, 2001). Serdaroglu and Turp (2005) reported a cholesterol content of 63.6 mg in 100 g of MSTM. Ang and Hamm (1982) reported 81 mg of cholesterol in 100 g of mechanically separated broiler meat.

Along with lipid oxidation, color and flavour are also characteristics that need to be vastly improved upon for MSPM. The darker color of MSM is reported to be due to the release of heme pigments and the reduction in connective tissue content, which does not contain pigments (Field, 1975). Often the color evaluation is based on measurements of tristimulus colorimetry, including L*, a* and b* values. The L* value on a 0 to 100 scale denotes the color from black (0) to white (100). The a* value denotes redness (+) or greenness (-), and the b* value denotes yellowness (+) or blueness (-) (Chamul, 2007). The lightness “L”, redness “a” and yellowness “b” of mechanically separated chicken were 53.4; 16.4 and

7.0, respectively (Perlo et al., 2006). Froning et al. (1973) studied the effect of skin content on MSPM color and found that fat from skin increased the dilution of heme pigments, resulting in lighter, less red and more yellow final product. Dhillon and Maurer (1975) compared the color stability between MSPM and ground beef after storage during 6 months at -25 °C. They found that redness was more intense for ground beef; however after prolonged storage no difference was observed. MSPM also often has a rancid flavour and aroma if not chilled immediately following manufacture. It sometimes has a “burned” flavour and aroma because manufacturers were attempting to achieve maximum recovery by increasing the back-pressure in the deboner (Borchert, 1998).

Lipids play an important role in flavor perception as they are carriers of lipophilic flavor molecules including off-flavor. Hydroperoxides, the products of lipid oxidation, are essentially odourless, but will decompose to a variety of volatile and non-volatile secondary products (Mottram, 1994). It is recognized that a problem with warmed-over flavour (WOF) usually occurs in refrigerated cooked meats within 48 hours of storage; however the researchers, Gray and Pearson (1987), reported on development of WOF in MSM as well. In the study of Mielnik et al. (2002), it was found that the addition of MSTM to comminuted sausages facilitated the development of rancid flavour during storage at -25 °C, as determined by the concentration of volatile compounds. They also found that TBARs values and volatile compounds were highly correlated with rancid flavour. The flavor stability of MSM depends on material composition, deboner

type, quantity of heme compounds, contact with metal parts and the temperature of deboning (Ockerman and Hansen, 2000).

MSM is already being used in the formulation of emulsified products (sausages, balls, loaves, etc.). However, comminuted consistency and puree form sometimes limit its use in products (Kondaiah and Panda, 1992). MSM reduced the palatability of products in which it is incorporated (Ockerman and Hansen, 2000). Several authors reported on undesirable texture of MSM (Froning, 1981; Field, 1988). High lipid content was shown to have a negative effect on texture, making it softer (Raphaelides et al., 1998). The same authors also mentioned on the grainy or gritty structure, due to possible presence of bone particles.

1.2.3 Calcium and bones content

One of the important points of criticism raised by consumers for acceptance and usage of MSPM is high calcium content, which is commonly associated with the high amounts of microscopic bone particles (Henckel et al., 2004; Branscheid et al., 2009). Presence of organoleptically detectable bone markedly decreases the acceptability of any product utilizing MSM (Chant et al., 1977). According to the regulation CFR (1995) calcium content of MSM from turkey or mature fowl should not exceed 0.235% and no greater than 0.175% in products made from broilers processed at the age from 6 to 8 weeks. The reason for this difference in requirements is that mature fowls have more brittle bones and turkeys have larger bones; therefore slightly higher calcium content in the final product is expected (Froning and McKee, 2001). The CFR (2005) requires that the content of solid

bone fragments be less than 3% on the meat wet weight, at least 98% of the bone particles must have a maximum size no greater than 0.5 mm and no bone particles larger than 0.85 mm in their greatest dimension. Particle size is important since larger particles might cause a gritty structure and potential dental problems. The percentage of calcium or bone in MSM is variable and depends on the amount of meat attached to the bone, the size of grinder plate, the extent to which the bones were broken, the yield of processing and the part of the carcass subjected to deboning (Field et al., 1974; Goldstrand, 1975).

Nutritionists reported that many population groups, particularly adolescent and older females, consume significantly less calcium than recommended (Miller et al., 2001). MSM helps to maintain the balance in calcium to phosphorus ratio and prevent calcium deficiencies in the human diet (Lutwak, 1975). Posner (1969) reported that nonreversible hydrolysis of bone occurs in aqueous media at physiological pH values. Field (1988) confirmed that bone particles from MSM are totally solubilized in HCl solutions equal to the concentrations of HCl in a stomach and present no hazard to consumers. The bone source of calcium is especially useful for people who cannot tolerate milk as a calcium source due to deficiency of lactase enzyme (Ockerman and Hansen, 2000).

1.2.4 Microbiological quality

MSM is highly perishable, since it usually contains high microbial contamination. The level of contamination depends on the slaughtering and deboning conditions, times and temperatures to which the product is exposed

during processing. The main reasons for the microbial load of MSM are poor hygienic measures, including environment, handlers, equipment, and also improper holding temperatures during deboning and storage. The deboning process may increase the temperature of the recovered meat (Newman, 1981). The screw press types of machine (e.g. Beehive, Paoli) were reported to cause an increase in temperature between 10 ° to 13 °C (Mawson and Collinson, 1974; Meiburg et al., 1976). In some cases the use of a pre-grinder may further increase the temperature by as much as 17 °C (Paoli, 1976). These relatively high temperatures and paste-like structure create an excellent environment for bacterial growth. The release of intracellular fluids, which are rich in nutrients, availability of air, temperature and a higher pH all contribute to microbial multiplication (Froning, 1981; Field, 1988). Mulder and Dorresteijn (1975) observed that transmission of pathogenic bacteria to the final material during different stages of processing was frequent and not influenced by the method of separation. Frequently the source of contamination (*Salmonella, Campylobacter, Listeria* spp. and *Clostridium perfringens*) comes from animal carcasses (ICMSF, 2005). MSPM is often heavily contaminated, both with spoilage and pathogenic bacteria, including salmonellae (Ostovar et al., 1971). The results of the study by Malicki et al. (2006) showed that the average counts of psychrotrophic and mesophilic bacteria, proteolytic bacteria, lactic acid bacteria and *Pseudomonas* spp. were much higher in MSTM compared to raw turkey breast. The high surface-to-volume ratio and homogeneous structure of MSM facilitate the spread of bacteria

throughout. USDA/FSIS has established the HACCP procedure to control microbiological quality of mechanically separated poultry (USDA/FSIS, 1999).

1.3 Characteristics of skeletal muscle proteins

Muscle proteins are generally classified into three main groups: sarcoplasmic, myofibrillar and stromal or connective tissue proteins. This division is based on their function in a muscle and solubility in aqueous solvents. The sarcoplasmic proteins contribute to around 30% of the total protein and are water-soluble proteins. They include hemoglobin, myoglobin, cytochromes and glycolytic enzymes (Wang, 2006).

Myofibrillar proteins, which are the major proteins in muscle cells, comprise about 60% of the total muscle proteins and are considered to be soluble in relatively concentrated salt solutions (0.3-1.0 M) (Damodaran, 1997).

Based on the functional role in muscle, myofibrillar proteins are further divided into contractile, regulatory and cytoskeletal proteins. The most abundant contractile protein is myosin (Xiong, 2004). Myosin (Figure 1.1) has a molecular weight of approximately 480 kDa and consists of six subunits; two heavy and four light chains, arranged into a molecule with two pear-shaped globular heads attached to a long α-helical tail. Hydrolysis of a myosin heavy chain with trypsin yields light meromyosin (LMM) and heavy meromyosin (HMM). Treatment of myosin with papain generates two identical globular heads (subfragment 1) and a myosin rod (LMM and HMM S-2) (Smith, 1994). The two globular heads are

relatively hydrophobic and are able to bind to actin. The tail portion is relatively hydrophilic and responsible for the assembly of myosin into thick filaments (Xiong, 1997).

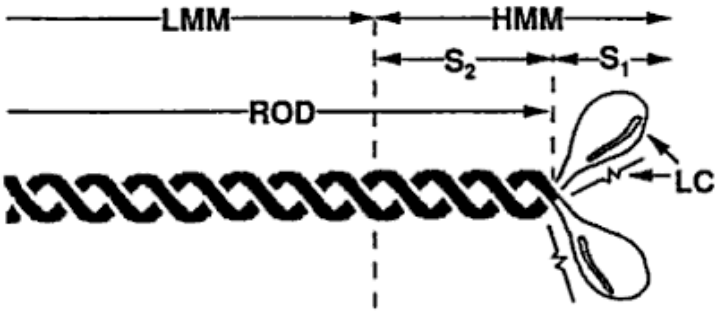

Figure 1.1. Schematic representation of the myosin molecule (LMM; light meromyosin; LC, light chain; HMM, heavy meromyosin; S1, subfragment 1; S2, subfragment 2). Reprinted from PROTEIN FUNCTIONALITY IN FOOD SYSTEMS. EBOOK by N. S. Hettiarachchy and G. R. Ziegler. Copyright 1994 by Marcel Dekker, Inc. Reproduced with permission of Marcel Dekker, Inc. in the format of Dissertation via Copyright Clearance Center.

Actin, the second most abundant contractile protein, forms the thin filament of the sarcomere. Actin molecule consists of two protein strands twisted upon one another (Hossner, 2005). During contraction, actin and myosin interact, resulting in the formation of the actomyosin complex. Troponin and tropomyosin are regulatory proteins, which are associated with thin filaments, while cytoskeletal titin and nebulin are the structural components of myofibrils (Aberle et al., 2001). Stromal proteins (connective tissue) constitute about 10% of the total protein and are considered to be insoluble in an aqueous medium. There are two types of

connective tissue: proper and supportive. Connective tissue that covers the muscle, muscle bundle and muscle fiber (epimysium, perimysium and endomysium, respectively) is known as connective tissue proper (Alvarado and Owens, 2006). Bones and cartilage refer to supportive connective tissue. There is also an extracellular matrix, which is fibrous in structure and made up of proteins called stromal. The major stromal protein is collagen (Ponce-Alquicira, 2004). Elastin and reticulin are minor constituents of the stromal fraction. Meat tenderness often decreases with animal age as a result of higher cross linkages that occur in the collagen.

1.4 Protein extraction techniques

One of the possibilities to overcome the problems associated with MSM is to extract muscle proteins in order to prepare functional protein isolates, which can be used for the production of further-processed meat products and other food applications. Two main extraction technologies are used in this regard, and both were initially developed for the extraction of proteins from fish. These technologies include surimi and pH-shifting (acid and alkaline extraction processes). Since the MSM is a material different from fish, the extraction processes need to be optimized in order to be suited to poultry muscle proteins. In the following paragraphs a detailed discussion about the steps involved in these two technologies will be provided.

1.4.1 Surimi processing

Surimi is one of the major fish meat transformations (Martin-Sanchez et al., 2009). It is a wet concentrate of myofibrillar proteins made from raw minced fish flesh. It is an intermediate product for the production of a variety of foodstuffs (FAO, 2005a; 2005b), such as the traditional Japanese kamaboko or shellfish imitation products, which include crabsticks, crab legs, crab meat and others (Carvajal et al., 2005; Blanco et al., 2006). Before 1960 surimi was produced and used within a few days as chilled raw material because the product was unstable during frozen storage due to the denaturation of actin and myosin. Discovery of cryoprotectants, such as sugars and polyphosphates helps maintain protein functionality during the frozen storage (Nishiya et al., 1960; Tamato et al., 1961; Park and Lin, 2005). About 95% of all surimi produced is in a frozen state and the term “frozen surimi” is more related to the addition of cryoprotectants than to freezing by itself (Sonu, 1986). The production of surimi includes the following steps: raw materials preparation, deboning, washing, refining, dewatering, addition of cryoprotectants and freezing. The details on each of the processing steps in surimi production are further discussed. There are three methods of material preparation before deboning. Method selection depends on the desired quality of the final product (Park and Morrissey, 2000). This step affects the quality and yield, because endogenous and microbial proteases from guts and skin affect the gel-forming ability of surimi if they are present in high amounts (Martin-Sanchez et al., 2009). The next step is deboning, performed by using a

perforated drum that minces the fish and removes any bones by forcing the tissue through 3-5 mm perforations. Once the raw fish flesh has been obtained, cyclic washings are applied to remove sarcoplasmic proteins (enzymes and heme proteins), fat and other impurities which might decrease the surimi value (Vilhelmsson, 1997; Hultin et al., 2005). This also increases the quality of myofibrillar proteins, which in turn positively affect the functional properties (Hall and Ahmad, 1997). Generally, three cycles of 10 minutes washing with water: mince ratios of 3:1 or 4:1 are used in industrial applications (Park and Morrissey, 2000). After each washing the dewatering step is applied.

The over usage of water is one of the major problems of surimi processing leading to an increase in utility costs and pollution problems (Park and Morrissey, 2000). However, the quality of the final surimi is highly dependent on the amount of water used in its production. Several studies have been investigating the improvement of the washing procedure. Chen (2002) used air-flotation washing (AFW) to achieve a higher removal of unwanted compounds by air infused into cold water. Hultin et al. (2005) and Balange and Benjakul (2009) used salt-alkaline washing (SAW) to aid in the removal of heme pigments. The yield from AFW was slightly higher compared to SAW, but the surimi obtained from SAW showed a slightly higher ability to form a gel.

After washing, the meat is passed through a refiner to remove the small parts of bones, skin and connective tissue (Venugopal, 2006). As a result of repeated washing cycles the moisture content increases from 82-85% to 90-92%.

It is important to remove the excess water before the addition of cryoprotectants. The removal of water, thereby increasing the concentration of proteins, is achieved by using a highly efficient screw press machine. To improve water removal, a mixture of NaCl and $CaCl_2$ (0.1 - 0.3%) could be added to the final wash (Park and Lin, 2005). The addition of salt also facilitates protein unfolding, resulting in better gel strength; however it also accelerates protein denaturation and consequently might decrease the shelf life (Park and Morrissey, 2000). The traditionally used cryoprotectants include: 5% sorbitol, 4% sucrose and 0.3% polyphosphates. Sorbitol and sucrose act as cryoprotectants and also stabilize the protein gel network during freezing. Sucrose also inhibits ice crystal formation and water migration from proteins. Phosphates have the ability to increase water retention and the ability of proteins to reabsorb liquid during thawing (Rasco and Bledsoe, 2006). After mixing with cryoprotectants, fish flesh is formed into 10 kg blocks, put in plastic bags and frozen for 2.5 hours or until the core temperature reaches -25 °C. Frozen surimi is further stored at -20 °C. The final surimi contains 15-16% of protein, 75% moisture and 8-9% of freezing stabilizers (Shaviklo, 2007). The yield of surimi is relatively low, since one-third of the fish flesh is lost during the washing steps. In general, less than 25% of the fish weight is recovered as surimi (Rasco and Bledsoe, 2006).

1.4.2 Acid and alkaline extraction processes (pH-shifting method)

Considering the disadvantages of the surimi processing, including inefficient removal of membrane lipids and excessive water usage, a new pH-

shifting method has been developed at the University of Massachusetts (Hultin and Kelleher, 1999). The pH-shifting process utilizes the principle of pH-dependent protein solubility. First, proteins are solubilized in either acidic or alkaline mediums, followed by precipitation at the isoelectric point (pI) (pH about 5.0-5.5), with the possibility of final neutralization of pI precipitated proteins. Figure 1.2 represents the acid/alkaline extraction process. During step 1, water is added to finely ground raw material at a ratio from 1:6 to 1:9 and homogenized.

During step 2 of the acid pH-shifting process, myofibrillar and sarcoplasmic proteins are solubilized by adjusting the mixture to pH 2.5-3.5, usually by the addition of 2 N HCl. For the alkaline pH-shifting process, the water/meat slurry is subjected to solubilization at a pH of 10.5-11.5, usually by the addition of 2 N NaOH (Kristinsson et al., 2005). During step 3 skin, bone particles and membrane lipids (under favourable conditions) are separated from the myofibrillar and sarcoplasmic proteins by centrifugation (Hultin, 2000). Usually three fractions are formed after first centrifugation: a bottom layer composed of skin, bone particles, connective tissue proteins and impurities; a middle layer composed of a soluble protein fraction and a neutral lipids fraction on the top.

During step 4, the pH of the soluble protein fraction is adjusted to the pI of about 5.0-5.5, to induce precipitation of both myofibrillar and sarcoplasmic protein fractions. The final protein isolate is recovered by centrifugation. Sarcoplasmic protein fraction, which is mostly washed off during surimi processing, is largely precipitated along with myofibrillar protein in the final

isolate of the pH-shifting extraction. The moisture of the final isolate may vary from 82 to 90% depending on the initial source subjected to the extraction. The final pH of the sample can also be readjusted. In the final step, the cryoprotective substances are added to the protein isolate. Acid- and alkali-produced protein isolates have a "Generally Regarded as Safe" (GRAS) status in the US (FDA, 2004).

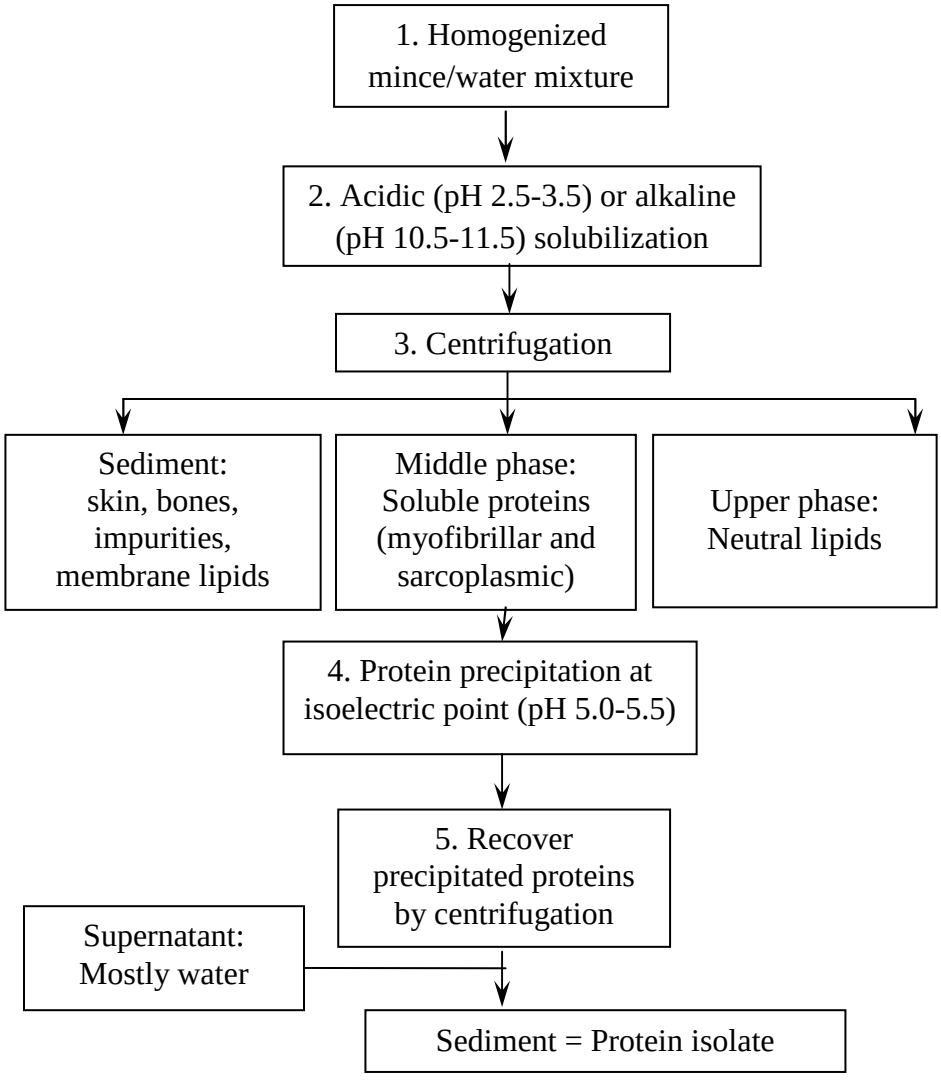

Figure 1.2. Schematic diagram the pH-shifting process (Adopted from Ingadottir, 2004).

1.4.2.1 Protein yield

The new pH-shifting protein extraction method has many advantages over surimi processing. Minced materials might be directly subjected to the acid or alkaline processing, since the undesirable compounds, such as bone parts, skin, fat and impurities are removed by centrifugation. pH-shifting provides a higher processing yield, because sarcoplasmic protein fraction is precipitated together with myofibrillar proteins (Nolsoe and Undeland, 2009). The process is faster since washing and refining procedures are excluded. It also decreases the amount of water used, in turn decreasing water waste (Shaviklo, 2007). Moreover, the water obtained from pH-shifting process has less solid parts as a result of centrifugation (Park et al., 2003). Under optimum conditions (meat to water ratio) neutral and polar lipids might be removed from the initial material, increasing its oxidative stability during storage.

Protein yield is one of the important factors, which have economic implications for the processor. Protein yield of the pH-shifting process depends on three main factors: solubility of proteins at extreme acid or alkaline pH, the size of the sediment after centrifugations and protein solubility at the pH of precipitation. Preferably, the initial solubilization should be high, while the other two factors should be low (Nolsoe and Undeland, 2009). Hultin and Kelleher (2000) demonstrated that 94.4% of mackerel light meat could be recovered using the acid-aided process. Undeland et al. (2002) used acid or alkaline solubilization to extract proteins from herring (*Clupea harengus*). The study showed that 92% and

89% of the initial muscle proteins were solubilized at pH 2.7 and 10.8, respectively and resulted in protein yields of 74% and 68%, respectively. The same results were reported in the study of Kristinsson and Liang (2006) on the pH-shifting processes of Atlantic croaker (*Micropogonias undulates*). The authors found that the acid-aided process led to higher protein recoveries (78.7%) compared to alkaline treatments (65%). Kristinsson et al. (2005) reported a 71.5% yield for acid-processed and 70.3% for alkali-recovered protein from Channel catfish (*Ictalurus punctatus*). Rawdkuen et al. (2009) obtained higher recovery yield from tilapia in the acid-aided process (85.4%), followed by the alkaline-aided process (71.5%) and surimi (67.9%). The same results on the higher protein yield from acid-aided extraction process were reported in the studies on the other fish species, such as mullet catfish, Spanish Mackerel, croaker (Kristinsson and Demir, 2003) and Pacific whiting (Choi and Park, 2002). The reason for a lower protein yield with alkaline extractions was suggested to be due to less protein precipitation on pH readjustment to 5.5 compared to the acid process (Kristinsson and Hultin, 2004). However, Kristinsson and Ingadottir (2006), who investigated protein yields from tilapia (*Orechromis niloticus*) light muslce, found no significant difference in protein yield between acid and alkaline treatments. They reported protein yields from 56% to 61% for the acid-aided process, and from 61% to 68% for the alkali-aided process. Batista et al. (2007) reported that protein yields from sardine (*Sardina pilchardus*) muscle achieved 77% and 73% for the alkaline and acidic processes, respectively.

Kim et al. (2003) studied the influence of solubilization pH on protein yield from acid- (pH 2 and 3) and alkaline-aided (pH 10.5, 11 and 12) extractions from Pacific whiting using a 1:10 fish meat to water ratio. The study revealed the highest protein yield at solubilization pH of 12 (around 70%) and the lowest at pH 10.5 (around 60%). No difference was found in protein yield between solubilization at pH 2 and 3 (62-63%). The authors suggested that the difference in protein yields was due to the effect of the meat to water mixing ratio, since the fish proteins were highly soluble at pH of 10.5 when a 50-fold dilution rate was implemented. Therefore, not only pH, but also the dilution factor has an influence on the final protein yield.

Liang and Hultin (2003) used alkaline extraction at pH 10.8 and precipitation pH 5.2 for protein recovery from mechanically deboned turkey. The ratio of meat to water was 1:6 (wt/vol), and centrifugation (10,000 × g). They found that protein yields were 62.2% for coarsely ground and 63.9% for finely ground mechanically deboned turkey. Apart from pH of solubilization, other factors, such as meat to water ratio and the particle size of initial material have influenced on the final protein yield.

Kelleher and Hultin (2000) using an acid solubilization with isoelectric precipitation achieved a yield of 83.5% for protein recovered from chicken breast muscle and a yield of 68.6% for protein recovered from chicken thigh and leg muscles. The difference in protein yield was suggested due to the varying amounts of connective tissue in the starting materials. Betti and Fletcher (2005)

conducted the study to determine the effects of extraction pH and precipitation pH on the protein yields from boneless and skinless broiler leg meat. The effect of 8 extraction pH (8.0 - 12.0) and 8 precipitation pH (3.8 - 5.2) was investigated. The highest yields, over 70%, were found at extraction pH above 10.5 and precipitation pH above 4.4. Omana et al. (2010) studied the effect of different alkaline solubilization pH (10.5; 11; 11.5; 12) and pI precipitation (pH 5.2) on the protein yield from dark chicken meat. The authors reported the highest yield at pH 12 (94.2%).

1.4.2.2 Lipids reduction and stability of isolated proteins to oxidative deterioration

Lipid oxidation is one of the problems associated with a high lipid content of the material subjected to the pH-shifting extraction. Therefore, decreasing the amount of lipids is important. During the pH-shifting process the amount of lipids that can be removed depends on lipid content of the starting material, viscosity of the homogenate after solubilization and the speed of the first centrifugation (Nolsoe and Undeland, 2009). Acid solubilization (pH 3) of catfish protein removed 74% of fat (Dewitt et al., 2007). Batista et al. (2007) reported that fat content reductions in sardine muscle were 65.3% and 51.0% for the protein recovered after alkaline and acid solubilization, respectively. Kristinsson and Demir (2003) investigated the lipid reduction of acid and alkaline recovered fish proteins. The lipid reduction for acid-recovered meat was 38.1% for croaker, 58% for mullet, 76.9% for mackerel and 85.4% for catfish. For alkaline-recovered meat

the values were 68.4%, 81.4, 79.1% and 88.6%, respectively to the species. Rawdkuen et al. (2009) found that 67.8%, 85.2 and 88.6% of lipids were reduced in the tilapia muscle after being processed with surimi, acid- and alkaline-aided treatments, respectively. Kristinsson et al. (2005) reported a higher lipid reduction of 88.6% for alkaline extraction (pH 11.0) compared to 85.4% acidic extraction (pH 2.5) of proteins from Channel catfish. When the same authors compared the pH-shifting and surimi proces, the latter provided less lipids removal (58.3%). The higher efficiency of the pH-shifting method for lipids removal is due to the use of centrifugation, which causes precipitation of the membrane phospholipids to the bottom layer of the centrifuge tube, and separation of neutral lipids to the top (Hultin and Kelleher, 2000). This separation is based on the difference in density and solubility (Kristinsson et al., 2005). Kristinsson and Liang (2006) also showed a higher lipids removal by the alkali-aided process (68.4%) compared to the acidic (38.1%) process. The higher lipid removal for alkali-treated samples is thought to be due to the greater emulsification ability of the proteins at alkali pH, since some of the proteins might be lost in a top neutral lipid phase (Kristinsson et al., 2005). In comparison to acid and alkaline extractions, surimi processing showed lower lipid reduction (16.7%).

Liang and Hultin (2005b) studied the effect of calcium chloride and citric acid addition on the improvement of PL removal from fish muscle homogenate solubilized at pH 10.5. They found that more than 85% of PL were removed with calcium chloride concentration of more than 20 mM in the presence of 1 mM of

citric acid. When the effect of these two compounds was measured during acidic extractions (pH 3) of proteins from cod (*Gadus morhua*) muscle, it was found that with 8 mM of calcium chloride and 5 mM citric acid addition, 90% of PL removal was achieved (Liang and Hultin, 2005a).

Using Blue Mussels (*Mytilus edulis*) with 13.5% (dry weight) of initial fat content, Vareltzis and Undeland (2008) studied the effects of acid (pH 2.8) and alkaline (pH 11.1) extractions on lipids removal. They reported that acid-aided extractions provided lower lipid content (11.0% on dry weight) compared to alkaline (18.8% on dry weight). The addition of 5 mM of citric acid and 10 mM of calcium chloride to the homogenate of blended mussels prior to acid or alkaline solubilization, greatly decreased lipid content in both acid and alkaline treated samples.

Froning and Johnson (1973) used centrifugation to improve the composition of mechanically deboned fowl meat. They found that using centrifugation conditions of 20,000 rpm for 15 min at 5 °C decreased total fat content by 62.8%. Dawson et al. (1988) used a water washing process with the addition of bicarbonate (pH 8.0), followed by precipitation at pH 6.8 to remove lipids and pigments from mechanically separated chicken meat. The study resulted in 88.3% reduction of lipid content compared to the raw material. Liang and Hultin (2003) found the decrease in lipids content from 10.8% in the original coarsely ground mechanically deboned turkey to 0.9% in the resultant alkali-extracted protein isolate, and from 19.3% in the original finely ground mechanically deboned

turkey to 1.0% in the resultant protein isolate. No difference in lipid reduction was found between the different alkaline pH of extraction (10.5, 11.0, 11.5 and 12.0) for dark chicken meat in the study of Moayedi et al. (2010), however around 50% reduction of lipids was achieved compared to that of raw material.

Kristinsson et al. (2005) in the study on the Channel catfish reported that none of the processing methods, including acid and alkaline extractions, led to significantly higher TBARs values compared to the starting raw material.

Kristinsson and Liang (2006) compared the levels of lipid oxidation of Atlantic croaker (*Micropogonias undulates*) processed with acid, alkaline extractions and surimi. The TBARs value of the acid-aided isolates was significantly higher than alkaline and surimi process, and remained higher throughout the storage at 4 °C during a period of 14 days. The higher lipid oxidation for acid-extracted isolates is believed to be due to the increased pro-oxidative potential of the heme pigments, which would have denatured at low pH and then partly coprecipitated with the muscle proteins at pH 5.5.

Froning and Johnson (1973) showed that centrifugation of mechanically deboned fowl resulted in higher oxidative stability compared to the raw material. Dawson et al. (1988) conducted a study on the extraction of myofibrillar proteins from mechanically separated chicken meat using a phosphate solution (pH 8.0) and precipitation at pH 6.8. They indicated that, even though lipid content decreased by 88.3%, extracted meat was more susceptible to lipid oxidation as compared to the raw material. The authors suggested that this susceptibility was

as a result of the unsuccessful removal of PL. The same results were found in the study of Moayedi et al. (2010) on alkaline extraction of proteins from dark chicken meat. Higher amounts of TBARs were observed in the alkali-extracted meat compared to the raw material, as a result of the poor removal of PL.

Undeland et al. (2005) tested the effect of the addition of antioxidants on reduction of lipid oxidation during acid-extraction (pH 2.7) process and during the storage of herring protein isolate. The following antioxidants were tested: erythorbate (0.2%, 9.3 mM), sodium tripolyphosphate (STPP; 0.2%, 5.4 mM), ethylenediaminetetraacetic acid (EDTA; 0.044%, 1.5 mM), and milk proteins (4%). The study revealed that the addition of erythorbate alone, or in combination with STPP/EDTA, decreased lipid oxidation during processing. During ice storage, the highest stability was obtained when EDTA was used instead of STPP.

Vareltzis et al. (2008) determined the effect of citric acid and calcium chloride ion on the oxidative stability of acid (pH 3.0) extracted proteins from ground cod muscle. The authors showed that treatments with 5 mM of citric acid and 10 mM of calcium chloride markedly inhibited lipid oxidation.

1.4.2.3 Color properties

The acceptable color of meat products has one of the strongest influences on consumer preferences (Mugler and Cunningham, 1972). Myoglobin is a protein responsible for the red colour of meat. Myoglobin does not circulate in the blood, but is fixed in the tissue cells and is purplish in color. The remaining colour comes from the hemoglobin which occurs mainly in the circulating blood and a

small amount can be found in the tissues after slaughter (Muchenje et al., 2009). The color of the protein isolate depends on the initial characteristics of the material subjected to the extraction process, including part of the carcass and the amount of pigments present. Undeland et al. (2002) found that protein isolate from the light herring muscle obtained from alkaline extraction had higher lightness, yellowness and whiteness compared to acidic extractions. Choi and Park (2002) compared the color characteristics between acid-extracted and surimi processed protein isolates from Pacific whiting. Protein isolates obtained from surimi isolates were lighter and whiter compared to acid-extracted. The same results, of lower lightness from acid-produced brisling sardine (*Opisthonema liberate*) protein isolates compared to the isolates made from surimi, were reported by Cortes-Ruiz et al. (2001). Perez-Mateos and Lanier (2007) found the highest whiteness for surimi process, followed by alkaline and then acid-extracted proteins from Atlantic menhaden (*Brevoortia tyrannus*). In comparison among the effect of surimi, acid and alkaline pH-shifting extractions, Yongsawatdigul and Park (2004), found that the lightest isolates were produced from surimi processing, followed by acid and alkali-processed protein isolates. They reported that surimi exhibited whiter appearance due to the removal of myoglobin during washing.

Dawson et al. (1988) found that bicarbonate washing treatments greatly lightened (L* = 64 vs 49) the color and decreased the redness (a* = 4.5 vs 9.0) of mechanically deboned chicken meat. Hernandez et al. (1986) used phosphate

buffers of pH 6.4, 6.8, 7.2 and 8.0 to extract pigments from mechanically deboned turkey meat (MDTM). They reported that lightness of MDTM increased by 51.1%, redness decreased by 64.0% and yellowness increased by 26.0% when washed with 0.04 M phosphate buffer with a pH of 8.0.

1.5 Functional properties of muscle proteins

Protein functionality is a complex of physicochemical properties that allows protein molecules to interact among themselves and their environment to generate the quality and stability of the final product (Xiong, 2004).

These properties greatly influence product texture, cooking yield, appearance, and hence, palatability and consumer acceptance. In meat processing the most important functional properties are classified into three categories, based on protein interactions: protein-water interaction (e.g. solubility); protein-fat interaction (e.g. emulsification); and protein-protein interaction (e.g. gelation). Depending on the product formulation and processing, muscle proteins undergo specific structural changes, producing various physical characteristics of the final product (Xiong, 2004). Therefore, for the successful use of fish and poultry protein isolates in food formulation it is necessary that these characteristics are preserved during the extraction process.

Functional properties are affected by the primary structure of proteins. The proportion of amino acids, as well as their sequence, will affect the hydrophilic-hydrophobic properties. Conformation of the protein chain will influence protein

solubility characteristics and intermolecular interactions during heat treatments. Hydrophobic-hydrophilic areas of the protein exposed to the medium, electrical charges and surface polarity will also be affected by the conformation of protein molecules (Sebranek, 2009). Protein functional properties are also influenced by the processing history of the material and interaction with a range of environmental factors. This is where methods of processing play a key role. Physical stresses, which include muscle fragmentation (grinding or chopping) or tumbling of the meat pieces cause a great force to the final proteins modifications, achieved through changing ionic strength, pH medium and moisture content (Sebranek, 2009). In meat processing, the protein properties of interest typically include: protein solubility, water-holding capacity, foaming characteristics, ability to form a heat-set gel and emulsifying capacity (Hsin–Sui, 1997).

1.5.1 Solubility

Solubility can be defined as the amount of protein that goes into a solution under specified conditions (e.g. pH, salt and protein concentration and temperature) (Singh and Flanagan, 2006). Solubility of protein is an indicator of protein denaturation, which in turn affects other functional properties such as foaming, emulsification and gelation (Stefansson, 1994). pH and ionic strength are the most important factors affecting protein solubility.

The degree of protein solubility is a result of electrostatic and hydrophobic interactions between protein molecules. Protein surface has a net charge, which depends on the pH of the environment. When a protein has an equal number of

positive and negative charges on its surface the solubility is minimal and that pH is referred to as the pI. At pH values above or below the pI, proteins carry negative or positive charges, respectively. In both cases the presence of a charge results in increased solubility. Therefore, most proteins show a U-shaped curve, when solubility is plotted against pH (Damodaran, 1996).

Addition of salt also has a significant effect on solubility. An increase in salt concentration (within particular limit) causes an effect called “salting-in” and is attributed to the ability of salt ions to enhance the surface charge of the protein. However, at high concentrations of salt (above 1 M), salt ions will compete with protein for the available water, resulting in precipitation of proteins. This effect is referred to “salting-out” (Culbertson, 2006).

1.5.2 Water-holding capacity

Meat as a complex biological system meat consist of 75% water, with the balance being made of 20% protein, about 2% fat, 0.5% of carbohydrates and about 2.5% of minor components (e. g. minerals, phosphorus compounds, and vitamins) (Pedersen et al., 2003). The majority of water in a muscle is held in space between thick (myosin) and thin (actin) filaments of the muscle cells post-mortem (Bond et al., 2004). Once muscle is harvested, the amount and location of water in the meat can change depending on the tissue itself and how the product is handled (Honikel, 2004). The water-holding capacity (WHC) of muscle is an important parameter because it influences both, qualitative and quantitative characteristics of meat and meat products (Kauffman et al., 1986). WHC

represents the ability of meat to retain its inherent water. It is usually expressed in terms of expressible moisture, drip loss or cook loss, whereby the latter two are the essential problems in meat processing. Since water is the large portion being added to the meat products, it is critical to provide as much water-holding ability as possible. Maintenance of a high water holding ability is a key factor when product appearance, eating quality and yields are considered.

The minimum WHC of proteins is at its pI. This is a result of decreased repulsion between protein molecules and reduction of space within the myofibril (Huff-Lonergan and Lonergan, 2005). When the pH is above or below the pI, increased electrostatic repulsion between proteins induces protein swelling and WHC increases (Gault, 1985; Miller, 1998). Sodium chloride plays an important role in water retention, providing solubilization of myofibrillar proteins. One explanation of the effect of sodium chloride on improving WHC is the swelling capability myofibrillar proteins, which can swell double, depending on the salt concentration. Chloride ions tend to increase electrostatic repulsive forces between proteins. With increasing repulsive forces, the protein structures unfold. Sodium ions surround the filament, resulting in local concentration differences and increase the pressure within myofibrils. This induces swelling and provides a higher number of protein side chains to bind water (Miller, 1998). Improving WHC can be achieved not only by changes in pH, but also by the addition of food salts, such as lactic, citric or ascorbic. An increase in water binding with the

addition of acid showed a strong effect of lower pH on proteins, which causes swelling of fibers (Medynski et al., 2000).

1.5.3 Emulsification

Meat emulsion is a two-phase system, consisting of fairly coarse dispersion of a solid (fat) in a liquid (water) in which the solid is not miscible. Proteins are good emulsifiers which assist in keeping the water and oil together in meat emulsions. In meat emulsion, myofibrillar proteins have the most important role, when compared to sarcoplasmic meat proteins (Zorba et al., 1993). Sarcoplasmic proteins are able to emulsify fat, but with certain limitation, while myosin, due to polar and non-polar amino acids structure and high length-to-diameter ratio, can increase flexibility and ordering of the molecules at the surface of the film (Xiong, 1997). Jones (1984) proposed that the hydrophobic heads of myosin orient toward a lipid phase, whereas the more polar tail region orients toward an aqueous phase.

The major factor for producing a stable emulsion is to create a protein film on the surface of lipid droplets to decrease interfacial tension (Damodaran, 1996). The stability and rheological properties of the emulsions are influenced by many factors, such as pH, viscosity of the aqueous phase, time and temperature of processing and meat-to-fat ratio. Bull (1971) reported that as the proteins approach their pI, the protein molecules generally lose their net charge and exhibit maximal attraction to each other. This is accompanied by a decrease in solubility, resulting in a decreased emulsification capacity.

1.5.4 Foaming characteristics

The foaming properties include the ability to produce a large interfacial area of foam per unit of protein and to stabilize film against internal and external forces. Foamability is associated with the film-forming ability at the air-water interface (Mita et al., 1977; Mita et al., 1978). Proteins that rapidly adsorb at the newly created air-liquid interface and readily undergo unfolding and molecular rearrangement at the interface, possess better foamability (Yu and Damodaran, 1991). The stability of the protein-stabilized foam is also dependent on the rheological properties of the protein film. This is because a viscous, gel-like, cohesive film with high elasticity produces more stable foams. The foaming properties of proteins are related to their hydrophobicity and their charge (Indrawati et al., 2008). For example, proteins that are highly charged and less hydrophobic may not be able to form a cohesive network due to a strong electrostatic repulsion at the interface (Damodaran, 1996). Apart from this, several additional factors such as protein concentration, ionic strength, pH, temperature and the presence of other food components affect foamability of proteins (Damodaran, 1990). Usually an increase in protein results in increasing rate of adsorption of protein at the air-water interface, leading to a reduction in surface tention, and thus increase in foamability (Cho et al., 1996; Cornec et al., 2001). The foaming properties might also be enhanced by partial heat denaturation (Mitchell, 1986; Damodaran, 2005). This improvement happens due to an increase in surface hydrophobicity, which decreases the energy barrier for

adsorption (Indrawati et al., 2008). In regard of pH influence on foamability, the reduced electrostatic repulsion between molecules at the pI allows a higher amount of protein adsorption at the interface (Yu and Damodaran, 1991). This increases the thickness of the film and improves its rheological characteristics (Indrawati et al., 2008).

1.5.5 Gelation

One of the very important functional properties of muscle proteins is their ability to form a gel. A gel is an intermediate stage between a solid and a liquid, where proteins form a three-dimensional network that is able to immobilize water, exhibiting a no steady-state flow (Fennema, 1996).

Gelation has been described as a multi-stage process involving: the initial denaturation of native protein structure and unfolding of the protein molecules, followed by aggregation and cross-linking between proteins. While heated, the non-covalent bonds (ionic, hydrogen, van der Waals forces and hydrophobic interactions) which provide secondary and tertiary structures become weakened, and break down with increasing temperature. This process is associated with protein denaturation. Unfolding of the protein structure usually occurs at the initial stages of denaturation. Finally protein-protein interaction will lead to the formation of a three-dimensional gel network. A balance of attractive forces and repulsive forces between proteins is necessary to provide good network formation (Arntfield et al., 1990). For example, if the network is too weak, the viscosity will increase, but the flow of the fluid still be possible and a gel will not be formed. In

contrast, if interactions between proteins are too strong, the network might break up expelling the water (Mangino, 1992). Properties of a gel are affected by the type and number of protein-protein interactions, aggregation and arrangement of unfolded proteins, which are determined by pH, ionic strength, heating temperature and rate, fiber type, salt and protein concentration (Zayas, 1997; Xiong, 2004). The pH has a great effect on protein structure, amount of water bound and protein-protein interactions. To attain gel formation it is also critical to maintain a proper pH to achieve the optimum balance between unfolding and denaturation extension, as well as attractive and repulsive forces between protein chains (Mangino, 1992; Feng and Hultin, 2001).

Lan et al. (1995) concluded that the heating rate is important for gelation in order to have a proper sequence (arranged unfolding, aggregation and gel formation). Temperature is an important aspect, influencing the quality characteristics of the final gel. Thus, determination of optimal heating and cooling procedures is important. For example, for some species gel strength may be improved by holding the gel at 50 °C before the final heating to 80-90 °C. However, for other species, that heating pattern results in a loss in quality, due to proteolytic degradation of muscles (Hultin, 2002).

Salt in general affects the structure of protein molecules and their interaction with water. Thus, it influences both the solubility of protein and the rate of thermal denaturation. In combination with temperature and pH, there is generally an optimal level of salt that favors gel formation.

The proteins that mainly contribute to gel formation and gel strength are myofibrillar. Among them, the most important is myosin (Sasaki et al., 2006). The gel formation of myosin gradually increases with thermal treatment (Liu and Foegeding, 1996). Usually gelation starts with the myosin molecule unfolding at 35-40 °C. Above 40 °C head-to-head interactions alter the formation of aggregates. Continuous heating to 55 °C and above will increase cross linking between aggregates to form a three-dimensional gel.

The two most widely used tests to measure the strength of a given gel are the large and small oscillatory strain tests. Large strain (fracture) test is used to analyze cooked and cooled gels. Small oscillatory strain test (no deformation) is usually used to control the sample during cooking.

Large strain testing is carried out by torsional, puncture, folding and uniaxial compression tests. Small oscillatory method is used to monitor the solid-to-gel transition during heat-induced gelation (Tabilo-Munizaga and Barbosa-Canovas, 2005). To discriminate the “real” gel from the “weak” gel or solid, the storage modulus (G’) and loss modulus (G’’) are evaluated (Matsumura and Mori, 1996). Storage modulus (G’) is associated with elasticity, while loss modulus (G’’) represents viscous characteristics of the gel.

1.6 Changes in protein conformation as a result of the pH-shifting extraction process

During acid and alkaline extraction processes the protein is exposed to extreme pH conditions which induce its partial unfolding. This partial unfolding accelerates changes in the protein structure and conformation, resulting in some modifications to the protein properties after refolding (Kristinsson and Hultin, 2003a). Study of Kristinsson (2001) showed that Hb underwent conformational changes when subjected to low and high pH, with final refolding at the pI. It has been revealed that the Hb molecule was fully dissociated at pH from 1.5 to 3.5; hence the heme group lost contact with the distal histidine. In contrast, when subjected to alkali treatment in the pH range from 10 to 12, almost no effect on Hb conformation was observed. This was reported to be due to strong coordination of heme to distal histidine in alkali-treated proteins. The author also found that helical structure of myosin rods was little affected at low and high pH, but the head group was substantially unfolded.

Kristinsson and Hultin (2003a) stated that cod myosin conformation and structure varied after treatments at an extreme pH. The authors showed that, unlike alkaline pH, at pH 2.5, the rod portion of the myosin completely separated due to an electrostatic repulsion. The arrangement of the globular head portion of myosin was altered at both acidic and alkaline pHs. After readjusting the pH to the neutrality (pH 7.5), a reassociation of the heavy chain occurred in the case of the low pH treatment, while light chains remained dissociated after both pH

treatments. The same authors also asserted that the loss of myosin ATPase activity (adenosine triphosphate) and the more reactive sulfhydryl groups were as a result of the irreversible changes in the globular head region. (Mohan et al., 2007) confirmed that acid and alkaline treatments had a more drastic effect on the head group of myosin from mullet (*Mugli cephalus*) than on its rod part.

The study of Kristinsson and Hultin (2003b) showed that the unique structure that proteins possess after a pH treatment is responsible for improved functional properties (e.g. gelation, emulsification). The partially unfolded/refolded protein structure is more flexible and thus able to form a better network during a heat induced gelation. A number of studies (Choi and Park, 2002; Undeland et al., 2002; Kristinsson and Demir, 2003) reported that the quality of gels obtained from pH-shifting extracted protein was the same or better compared to gels produced using the surimi processing.

The solubility as a function of salt concentration (0-600 mM KCl) did not show any difference for native or refolded protein; moreover emulsification properties of treated myosin were improved compared to myosin in its native state. When studied the pH-shifting effect on cod myofibrillar proteins, Kristinsson (2001) found that treated proteins had a higher solubility than the native proteins. In general, studies showed that both myosin and myofibrillar proteins functionality was improved after alkaline, rather than acid process. Raghavan and Kristinsson (2007) studied the changes in conformation of catfish (*Ictalurus punctatus*), as affected by the different anions (Cl^-, SO_4^{2-} and PO_4^{3}), pH

(1,5; 2.0 and 2.5) and salt addition. It was found that the G' (storage modulus) of different acid treatments decreased in the following order: Cl^- > SO_4^{2-} > PO_4^{3-}. Among the different pH treatments, the *G*' of myosin treated at pH 1.5 was significantly higher than myosin treated at pH 2.5. Results indicated that addition of NaCl during unfolding and refolding (pH 7.3) caused less denaturation than if NaCl was added after refolding.

In another study Raghavan and Kristinsson (2008) the effect of different pH (11.0; 11.5 and 12.0) and bases (KOH and NaOH) on the conformation of catfish myosin was determined. It was found that unfolding/refolding (pH 7.3) treatments increased the G' value for all treatments, especially for pH 11, indicating a greater level of protein denaturation. The presence of salt provided certain stability to myosin conformation during alkaline unfolding and denaturation. Moreover, KOH resulted in a higher denaturation and a higher gelling ability (G') compared to NaOH.

1.7 Possibilities for co-extraction of valuable compounds from mechanically separated turkey meat (MSTM)

A significant amount of time and effort has been put in to the development of techniques for myofibrillar protein extraction from low-value muscle foods. In contrast to the successful exploitation of proteins, there has been no progress in methods aimed for co-extraction of other valuable compounds during the extraction process, including lipids, pigments and connective tissue. Considering

the possible application of co-extraction technology, it may provide great opportunities to establish a new generation reprocessing system. Figure 1.3 represents the potential co-extraction of valuable compounds from MSTM.

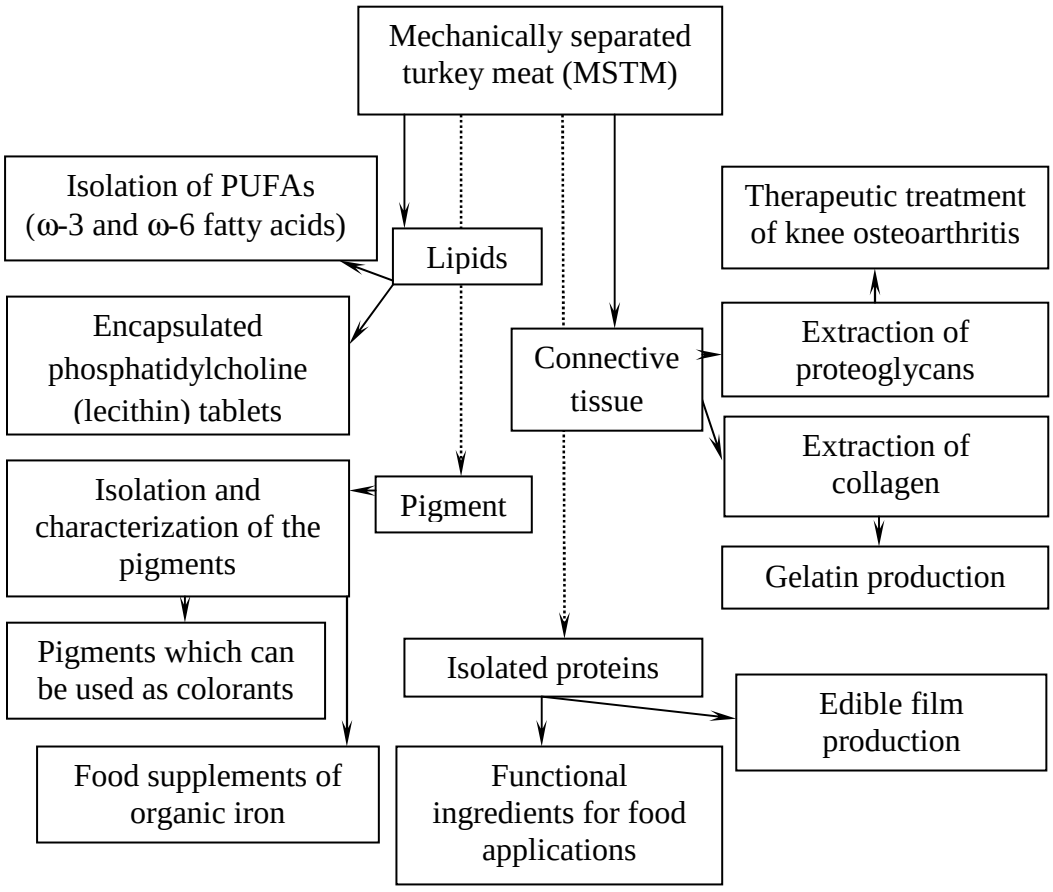

Figure 1.3. Opportunities for co-extraction of valuable compounds from mechanically separated turkey meat (MSTM).

The pH-shifting process results in significant removal of lipid fraction, including phospholipids, which are usually discarded. The study of Miller et al. (1982) showed that phosphatidylcholine was the major phospholipids in MSM.

Phosphatidylcholine (lecithin) is known as a supplement used for a variety of therapeutic reasons. The phospholipids fraction also consists of ω-3 and ω-6 fatty acids, which are recognized to have a beneficial influence on health. Pigments are also a part of the extraction process, which are no further utilized. However, it may be used as a good supplier of organic iron. Connective tissue may be used in several ways. Connective tissue, collagen, may be purified and added back to the protein isolates to improve its gel forming ability. Collagen preparations can be successfully used as a carrier of foodstuffs or cosmetic additives (Dubois, 1992). For instance, Briassoulis (2004) suggested the use of collagen in the form of a biodegradable encapsulating material. Collagen preparations are also used as carriers of antioxidants in the production of wiener-type and liver sausages. This limits lipid oxidation more than the direct addition of antioxidants to meat products (Waszkowiak and Dolata, 2007). For example, in meat products, collagen is used to make edible casings and as an ingredient in sausage products to increase the protein content of the product. It is also added to emulsified type of products to improve their emulsion stability. Heat pre-denatured collagen, when added in a quantity no more than 10%, improved binding strength and juiciness of restructured low-fat and low-salt beef (Kenney et al., 1992). To make the native collagen tissue from animal carcasses useful for food applications, it has to be converted to a gelatin by means of denaturation, followed by partial hydrolysis. Currently, gelatin derived from connective tissues, bones and skins of cows and pigs is being used.

Moreover, possible extraction of proteoglycans from connective tissue might be used in the pharmaceutical industry. For example, chondroitin sulphate (the major proteoglycan) is used in the treatment of joint ailments. Glucosamine (component of cartilage extracellular matrix), either alone or in conjunction with chondroitin sulphate, is the most commonly used supplement for preventing or relieving the symptoms of osteoarthritis. In conclusion, there is great potential to convert and utilize more of MSTM co-extraction by-products as valuable products.

1.8 Research objectives and outline of the thesis

The objective of this thesis project was to increase the utilization of mechanically separated turkey meat (MSTM) by pH-shifting extraction of protein isolates. In order to accomplish this goal different sub-objectives were defined.

(a) Determine the optimum extraction pH values to achieve the highest protein solubility

(b) Study the effect of addition of citric acid and calcium chloride on the composition and protein yield of proteins isolated from MSTM

(c) Assess the effect of different extraction pH on composition of protein isolates obtained from mechanically separated turkey meat (MSTM)

(d) Establish the effect of pH treatments on lipid composition and oxidative stability of MSTM protein isolates

(e) Characterize color characteristics from isolates obtained from different extraction pH

(f) Define the effect of pH of extraction of rheological and textural properties of recovered MSTM protein

(g) Investigate the protein functionality as affected by different extraction pH

The outline of the thesis is as follows. Chapter 1 is a review of literature pertaining to the factors affecting the composition of mechanically separated meat and different protein extraction techniques (surimi and pH-shifting).

The effect of addition of different volumes of citric acid and calcium chloride during acid pH-shifting protein extraction on improving the composition

of final protein isolates is presented in Chapter 2. Protein yield, lipid content (total, neutral and polar), oxidative stability (TBARs) and total heme pigments content are investigated. The results presented in this chapter have been accepted for publication in the Journal of Poultry Science.

Chapter 3 deals with the evaluation of the effect of different extraction pH on the composition and chemical properties of the extracted MSTM proteins. The influence of 4 extraction pH values on protein yield and extractability, surface hydrophobicity, sulfhydryl group content, SDS-PAGE profile, lipid and amino acid composition are elucidated. This chapter also provides information on additional co-extraction opportunities of collagen and glycosaminoglycan. The results of this study have been accepted with a minor revision in the Process Biochemistry Journal.

Chapter 4 attempts to identify the protein functionality in terms of the cooking and water losses, emulsifying and foaming abilities. Textural and rheological properties of protein isolates as influenced by extraction pH are also determined. The results presented in this study have been published in the Journal of Food Science.

Chapetrs 5 and 6 are concluding chapters, where the summary, future research and industrial relevance are described.

1.9 REFERENCES

Aberle, E. D., J. C. Forrest, E. D. Gerrard, E. W. Mills, H. B. Hedrick, M. D. Judge, and R. A. Merkel. 2001. Principles of Meat Science. 4th ed. Kendall Hunt Publishing Company, Dubuque, Iowa.

Ahn, D. U., and S. M. Kim. 1998. Prooxidant effects of ferrous iron, hemoglobin, and ferritin in oil emulsion and cooked meat homogenates are different from those in raw-meat homogenates. Poult. Sci. 77:348-355.

Al-Najdawi, R., and B. Abdullah. 2002. Proximate composition, selected minerals, cholesterol content and lipid oxidation of mechanically and hand-deboned chickens from the Jordanian market. Meat Sci. 61:243-247.

Alvarado, C. Z., and C. M. Owens. 2006. Poultry: Chemistry and Biochemistry. Pages 31:1-31:14 in Handbook of Food Science, Technology and Engineering. Y. H. Hui, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Ang, C. Y. W., and D. Hamm. 1982. Proximate analyses, selected vitamins and minerals and cholesterol content of mechanically deboned and hand-deboned broiler parts. J. Food Sci. 47:885-888.

Arntfield, S. D., E. D. Murray, and M. A. H. Ismond. 1990. Influence of protein charge on thermal properties as well as microstructure and rheology of heat induced networks for ovalbumin and vicilin. J. Texture Stud. 21:295-322.

Balange, A. K., and S. Benjakul. 2009. Effect of oxidised tannic acid on the gel properties of mackerel (*Rastrelliger kanagurta*) mince and surimi prepared by different washing processes. Food Hydrocolloids. 23:1693-1701.

Barbut, S. 2002. Poultry Products Processing. An Industry Guide. CRC Press, Boca Raton, FL.

Batista, I., C. Pires, and R. Nelhas. 2007. Extraction of sardine proteins by acidic and alkaline solubilization. Food Sci. Tech. Int. 13:189-194.

Betti, M., and D. L. Fletcher. 2005. The influence of extraction and precipitation pH on the dry matter yield of broiler dark meat. Poult. Sci. 84:1303-1307.

Bilgili, S. F. 2002. Poultry meat processing and marketing - what does the future hold. Poult. Int. 40:12-22.

Blanco, M., C. G. Sotelo, M. J. Chapela, and R. I. Perez-Martin. 2006. Towards sustainable and efficient use of fishery resources: present and future trends. Trends Food Sci. Technol. 18:29-36.

Bond, J. J., L. A. Can, and R. D. Warner. 2004. The effect of exercise stress, adrenaline injection and electrical stimulation on changes in quality attributes and proteins in Semimembranosus muscle of lamb. Meat Sci. 68:469-477.

Borchert, L. L. 1998. Poultry meat quality - its impact on processed meat products. Pages 89-91 in Proc. 47th Ann. National Breeders Roundtable. St. Louis, Missouri.

Botsoglou, N. A., E. Christaki, D. J. Fletouris, P. Florou-Paneri, and A. B. Spais. 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. Meat Sci. 62:259-265.

Branscheid, W., M. Judas, and R. Horeth. 2009. The morphological detection of bone and cartilage particles in mechanically separated meat. Meat Sci. 81:46-50.

Briassoulis, D. 2004. An overview on mechanical behaviour of biodegradable agricultural films. J. Polymer. Environ. 12:65-81.

Bull, H. B. 1971. An Introduction to Physical Biochemistry. 2nd ed. F.A. Davis Company, Philadelphia, PA.

CFIA (Canadian Food Inspection Agency). 2008. Mechanical separation of muscle tissue from bones - production of finely textured meat (FTM) and mechanically separated meat (MSM). 4:2:5. Accessed June, 2010. http://www.inspection.gc.ca/english/fssa/meavia/man/ch4/4-2-7-3e.shtml

CFIA (Canadian Food Inspection Agency). 2009. Use of mechanically separated meat (MSM). 7:22:5. Accessed June, 2010. http://www.inspection.gc.ca/english/fssa/meavia/man/ch7/7-13-22e.shtml

Carlsen, C. U., J. K. S. Moller, L. H. Skibsted. 2005. Heme-iron in lipid oxidation. Coord. Chem. Rev. 249:485-498.

Carvajal, P. A., T. C. Lanier, and G. A. MacDonald. 2005. Stabilization of proteins in surimi. Pages 163-225 in Surimi and Surimi Seafood. 2nd ed. J. W. Park, ed. Taylor & Francis Group, Boca Raton, FL.

Chamul, R. 2007. Quality measurements in beef. Pages 341-355 in Handbook of Meat, Poultry and Seafood Quality. M. L. N. Leo, and T. Boylston, eds. Blackwell Publishing, Victoria, Australia.

Chant, J. L., L. Day, R. A. Field, W. G. Kruggel, and Y. O. Chang. 1977. Composition and palatability of mechanically deboned meat and mechanically separated tissue. J. Food Sci. 42:306-309.

Chen, H. H. 2002. Decoloration and gel-forming ability of horse mackerel mince by air-flotation washing. J. Food Sci. 67:2970-2975.

Cho, D., G. Narsimhan, and E. I. Frances. 1996. Adsorption dynamics of native and alkylated derivatives of bovine serum albumin at air-water interface. J. Colloid Interface Sci. 178:348-357.

Choi, Y. J., and J. W. Park. 2002. Acid-aided protein recovery from enzyme-rich Pacific whiting. J. Food Sci. 67:2962-2967.

CFR (Code of Federal Regulations). 1995. Poultry Products Inspection Regulations. No: 381.173. Mechanically Separated (Kind of Poultry). http://ecfr.gpoaccess.gov/cgi/t/text/textidx?c=ecfr&sid=8ca5e8b03c15708cd8a925ec3d35e431&rgn=div6&view=text&node=9:2.0.2.1.35.16&idno=9 Accessed June, 2010.

CFR (Code of Federal Regulations). 2005. Animals and Animal products. No: 319.5. Mechanically Separated (Species). Accessed June, 2010. http://cfr.vlex.com/vid/319-mechanically-separated-species-19611341.

Cornec, M., D. A. Kim, and G. Narsimhan. 2001. Adsorption dynamics and interfacial properties of α-lactalbumin in native and molten globule state conformation at air-water interface. Food Hydrocolloids. 15:303-313.

Coronado, A. S., G. R. Trout, F. R. Dunshea, and P. N. Shah. 2002. Antioxidant effects of rosemary extract and whey powder on the oxidative stability of wiener sausages during 10 months frozen storage. Meat Sci. 62:217-224.

Cortes-Ruiz, J. A., R. Pacheco-Aguilar, G. Garcia-Sanches, and M. E. Lugo-Sanches. 2001. Functional characterization of protein concentrates from bristly sardine under acidic conditions. J. Aquat. Food Prod. Tech. 10:5-23.

Crosland, A. R., R. L. S. Patterson, R. C. Higman, C. A. Stewart, and K. D. Hargin. 1995. Investigation of methods to detect mechanically recovered meat in meat products I: Chemical composition. Meat Sci. 40:289-302.

Culbertson, J. D. 2006. Food protein functionality. Pages 7:1-7:12 in Handbook of Food Science, Technology and Engineering. Y. H. Hui, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Damodaran, S. 1990. Interfaces, protein films, and foams. Pages 1-72 in Advances in Food and Nutrition Research. J. E. Kinsella, ed. Academic Press, London, UK.

Damodaran, S. 1996. Functional properties. Pages 167-224 in Food Proteins: Properties and Characterization. S. Nakai, and H. W. Modler, eds. Wiley-VCH, Inc., New York, NY.

Damodaran, S. 1997. Food proteins: An overview. Pages 1-24 in Food Proteins and Their Applications. S. Damodaran, and A. Paraf, eds. Marcel Dekker, Inc., New York, NY.

Damodaran, S. 2005. Protein stabilization of emulsions and foams. J. Food Sci. 70:R54-R66.

Dawson, P. L., B. W. Sheldon, and H. R. Ball. 1988. Extraction of lipid and pigment components from mechanically deboned chicken meat. J. Food Sci. 53:1615-1617.

Dewitt, C. A. M., R. L. Nabors and C. W. Kleinholz. 2007. Pilot plant scale production of protein from catfish treated by acid solubilization/isoelectric precipitation. J. Food Sci. 72:351-355.

Dhillon, A. S., and A. J. Maurer. 1975. Stability study of comminuted poultry meats in frozen storage. Poult. Sci. 54:1407-1414.

Elkhalifa, E. A., P. P. Graham, N. G. Marriott, and S. K. Phelps. 1988. Color characteristics and functional properties of flaked turkey dark meat as influenced by washing treatments. J. Food Sci. 53:1068-1071.

Essary, E. O., and S. J. Ritchey. 1968. Amino acid composition of meat removed from boned turkey carcasses by use of commercial boning machine. Poult. Sci. 47:1953-1955.

Essary, E. O. 1979. Moisture, fat, protein and mineral composition of mechanically deboned poultry meat. J. Food Sci. 44:1070-1073.

Feng, Y. M., and H. O. Hultin. 2001. Effect of pH on the rheological and structural properties of gels of water-washed chicken breast muscle at physiological ionic strength. J. Agric. Food Chem. 49:3927-3935.

Fennema, O. R. 1996. Food Chemistry. 3rd ed. Marcel Dekker Inc., New York, NY.

Field, R. A. 1988. Mechanically separated meat, poultry and fish. Pages 83-128 in Edible Meat By-Products. A. M. Pearson, and T. R. Dutson, eds. Elsevier Applied Science, New York, NY.

Field, R. A. 1975. Recovery of protein from bones by mechanical deboners. Proc. 21st Eur. Meat Res. Wkrs. p. 100. Congress Secretariat, Convention Bureau, Main Station, Berne, Switzerland.

Field, R. A., M. L. Riley, and M. H. Corbridge. 1974. Influence of yield on calcium content of mechanically deboned lamb and mutton. J. Food Sci. 39:285-287.

FAO (Food and Agriculture Organization). 2005a. Code of practice for fish and fishery products. CAC/RCP 52-2003:134. Accessed June, 2010. ftp://ftp.fao.org/docrep/fao/011/a1553e/a1553e00.pdf

FAO (Food and Agriculture Organization). 2005b. Proposed draft code of practice for fish and fishery products-other sections. Appendix IX: Optional Final Product Requirements – Frozen Surimi. Accessed June, 2010. http://www.fao.org/docrep/meeting/005/w9253e/w9253e0o.htm#bm24.26

FDA (Food and Drug Administration). 2004. GRAS Notice No. GRN 000147. http://www.cfsan.fda.gov/~rdb/opag147. html Accessed May, 2010.

Froning, G. W. 1970. Poultry meat sources and their emulsifying characteristics as related to processing variables. Poult. Sci. 49:1625-1631.

Froning, G. W., R. G. Arnold, R. W. Mandigo, C. E. Neth, and T. E. Hartung. 1971. Quality and storage stability of frankfurters containing 15% mechanically deboned turkey meat. J. Food Sci. 36:974-978.

Froning, G. W., L. D. Satterlee, and F. Johnson. 1973. Effect of skin content prior to deboning on emulsifying and color characteristics of mechanically deboned chicken back meat. Poult. Sci. 52:923-926.

Froning, G. W., and F. Johnson. 1973. Improving the quality of mechanically deboned meat by centrifugation. J. Food Sci. 38:279-281.

Froning, G. W. 1981. Mechanical deboning of poultry and fish. Pages 109-147 in Advances in Food Research, 2nd ed. C. O. Chichester, E. M. Mrak, and G. F. Stewart, eds. Academic Press, New York, NY.

Froning, G. W., and S. R. McKee. 2001. Mechanical separation of poultry meat and its use in products. Pages 243-256 in Poultry Meat Processing. A. R. Sams, ed. CRC Press, Washington, DC.

Gault, N. F. S. 1985. The relationships between water-holding capacity and cooked meat tenderness in some beef muscles as influenced by acidic conditions below the ultimate pH. Meat Sci. 15:15-30.

Goldstrand, R. E. 1975. Mechanically deboned meats yields and product characteristics. Proc. Recip. Meat Conf. 28:116-126.

Gomes, H. A., E. N. Silva, M. R. L. Nascimento, and H. T. Fukuma. 2003. Evaluation of the 2-thiobarbituric acid method for the measurement of lipid

oxidation in mechanically deboned gamma irradiated chicken meat. Food Chem. 80:433-437.

Gray, J. I., and A. M. Pearson. 1987. Restructured meat and poultry products. Pages 221-269 in Advances in Meat Research. A. M. Pearson, and T. R. Dutson, eds. Van Nostrand Reinhold Comp., New York, NY.

Gray, J. I., E. A. Gomaa, and D. J. Buckley. 1996. Oxidative quality and shelf life of meats. Meat Sci. 43:111-123.

Grodner, M., S. Long, and S. Deyoung. 2004. Foundations and Clinical Applications of Nutrition: a Nursing Approach. 3 rd. ed. St. Louis, Mosby.

Grunden, L. P., J. H. MacNeil, and P. S. Dimick. 1972. Poultry products quality: chemical and physical characteristics of mechanically deboned poultry meat. J. Food Sci. 37:247-249.

Hall, G. M., and N. H. Ahmad. 1997. Surimi and fish-mince products. Pages 74-92 in Fish Processing Technology. 2nd ed. G. M. Hall, ed. Blackie Academic and Professional, London, UK.

Happich, M. L., C. E. Swift, and J. Naghski. 1975. Equations for predicting PER from amino acid analysis - a review and current scope of application. Pages 125-135 in Protein Nutritional Quality of Foods and Feeds. M. Friedman, ed. Marcel Dekker Inc., New York, NY.

Harel, S., and J. Kanner. 1989. Hemoglobin and myoglobin as inhibitors of hydroxyl radical generation in a model system of “iron redox” cycle. Free Radic. Res. Commun. 6:1-10.

Hedrick, H. B., E. D. Aberle, J. C. Forrest, M. D. Judge, and R. A. Merkel. 1994. Principles of meat processing. Pages 117-139 in Principles of Meat Science. 4th ed. E. D. Aberle, Forrest, J. C., Gerrard, D. E., and E. W. Mills, eds. Kendall Hunt Publishing Company, Dubuque, IA.

Henckel, P., M. Vyberg, S. Thode, and S. Hermansen. 2004. Assessing the quality of mechanically and manually recovered chicken meat. Food Sci. Technol. 37:593-601.

Hernandez, A., R. C. Baker, and J. H. Hotchkiss. 1986. Extraction of pigments from mechanically deboned turkey meat. J. Food Sci. 51:865-867.

Honikel, K. O. 2004. Water-holding capacity of meat. Pages 389-400 in Muscle Development of Livestock Animals: Physiology, Genetics and Meat Quality. M. F. te Pas, Everts, M. E., and H. P. Haagsman, eds. CABI Publishing, Cambridge, MA.

Hossner, K. L. 2005. Hormonal Regulation of Farm Animal Growth. CABI Publishing, Wallingford, Oxfordshire, UK; Cambridge, MA.

Hsin-Sui, C. 1997. Solubility and gelation of chicken breast muscle proteins as affected by salts. PhD Diss. Univ. Massachusetts, Amherst, MA.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanism of water-holding capacity of meat: the role of postmortem biochemical and structural changes. Meat Sci. 71:194-204.

Hultin, H. O., and S. D. Kelleher. 1999. Process for isolating a protein composition from a muscle source and protein composition. Advanced Protein Technologies Inc., assignee. US. Pat. No. 6,005,073.

Hultin, H. O., and S. D. Kelleher. 2000. Surimi processing from dark muscle fish. Pages 59-77 in Surimi and Surimi Seafood. J. W. Park, ed. Marcel Dekker Inc., New York, NY.

Hultin, H. O. 2002. Recent advances in surimi technology. Pages 241-251 in Recent Advances in Marine Biotechnology. M. Fingerman, and R. Nagabhushanam, eds. Science Publisher, Inc., Enfield, New Hampshire.

Hultin, H. O., H. G. Kristinsson, T. C. Lanier, and J. W. Park. 2005. Process for recovery of functional proteins by pH-shifts. Pages 107-139 in Surimi and Surimi Seafood. 2nd ed. J. W. Park, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Ingadottir, B. 2004. The use of acid and alkali-aided protein solubilization and precipitation methods to produce functional protein ingredients from tilapia. MS Thesis. Univ. Florida, US.

Indrawati, L., Z. Wang, G. Narshimhan, and J. Gonzalez. 2008. Effect of processing parameters on foam formation using a continuous system with a mechanical whipper. J. Food Eng. 88:65-74.

ICMSF (International Commission on Microbiological Specifications for Foods). 2005. Microorganisms in Foods: Microbial Ecology of Food Commodities. 2nd ed. Kluwer Academic and Plenum Publishers, New York, NY.

Janero, D. R. 1990. Malonaldehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9:515-540.

Janky, D. M., and G. W. Froning. 1975. Factors affecting chemical properties of heme and lipid components of mechanically deboned turkey meat. Poult. Sci. 54:1378-1387.

Johnson, P. G., F. E. Cunningham, and J. A. Bowers. 1974. Quality of mechanically deboned turkey meat: effect of storage time and temperature. Poult. Sci. 53:732-736.

Jones, K. W. 1984. Protein-lipid interaction in processed meats. Proc. Ann. Recip. Meat Conf. 37:52-57.

Kauffman, R. G., G. Eikelenboom, P. G. van der Wal, B. Engel, and M. Zaar. 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Sci. 18:307-322.

Kelleher, S. D., and H. O. Hultin. 2000. Functional chicken muscle protein isolates prepared using low ionic strength, acid solubilization and precipitation. 14:76-81.

Kendrick, J., and B. Watts. 1969. Acceleration and inhibition of lipid oxidation by heme compounds. Lipids. 4:454-456.

Kenney, P. B., C. L. Kastner, and D. H. Kropf. 1992. Raw and pre-heated epimysium and gelatin affect properties of low-salt, low-fat, restructured beef. J. Food Sci. 57:551-554.

Kim, Y. S., J. W. Park, and Y. J. Choi. 2003. New approaches for the effective recovery of fish proteins and their physicochemical characteristics. Fish Sci. 69:1231-1239.

Kondaiah, N., and B. Panda. 1992. Processing and utilization of spent hens. World Poult. Sci. 48:255-268.

Kristinsson, H. D. 2001. Conformational and functional changes of hemoglobin and myosin induced by pH: Functional role in fish quality. PhD Diss. Univ. Massachusetts, Amherst, MA.

Kristinsson, H. G., and H. O. Hultin. 2003a. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J. Agric. Food Chem. 51:7187-7196.

Kristinsson, H. G., and H. O. Hultin. 2003b. Effect of low and high pH treatment on the functional properties of cod muscle proteins. J. Agric. Food Chem. 51:5103-5110.

Kristinsson, H. G., A. E. Theodore, N. Demir, and B. Ingadottir. 2005. A comparative study between acid- and alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. J. Food Sci. 70:C298-C306.

Kristinsson, H. G., and B. Ingadottir. 2006. Recovery and properties of muscle proteins extracted from tilapia (*Oreochromis niloticus*) light muscle by pH shift processing. J. Food Sci. 71:132-141.

Kristinsson, H. G., and H. O. Hultin. 2004. Changes in trout hemoglobin conformations and solubility after exposure to acid and alkali pH. J. Agric. Food Chem. 52:3533-3643.

Kristinsson, H. G., and N. Demir. 2003. Functional fish protein ingredients from fish species of warm and temperate waters: comparison of acid- and alkali-aided processing vs. conventional surimi processing. Pages 277-298 in Advances in Seafood By-Products. P. J. Bechtel, ed. Univ. of Alaska Press: Fairbanks, AK.

Kristinsson, H. G., and Y. Liang. 2006. Effect of pH-shift processing and surimi processing on Atlantic croaker (*Micropogonias undulates*) muscle proteins. J. Food Sci. 71:C304-C312.

Lan, Y. H., J. Novakofski, R. H. McCusker, M. S. Brewer, T. R. Carr, and F. K. McKeith. 1995. Thermal gelation of pork, beef, fish, chicken and turkey muscles as affected by heating rate and pH. J. Food Sci. 60:936-940.

Lee, Y. B., G. L. Hargus, J. A. Kirkpatrick, D. L. Berner, and R. N. Forsythe. 1975. Mechanism of lipid oxidation in mechanically deboned chicken meat. J. Food Sci. 40:964-967.

Liang, Y., and H. O. Hultin. 2003. Functional protein isolates from mechanically deboned turkey by alkaline solubilization with isoelectric precipitation. J. Muscle Foods. 14:195-205.

Liang, Y., and H. O. Hultin. 2005a. Separation of membranes from acid-solubilized fish muscle proteins with the aid of calcium ions and organic acids. J. Agric. Food Chem. 53:3008-3016.

Liang, Y., and H. O. Hultin. 2005b. Separation of muscle membrane from alkali-solubilized fish muscle proteins. J. Agric. Food Chem. 53:10012-10017.

Liu, M. N., and A. E. Foegeding. 1996. Thermally induced gelation of chicken myosin isoforms. J. Agric. Food Chem. 44:1441-1446.

Love, J. D., and A. M. Pearson. 1971. Lipid oxidation in meat and meat products - a review. J. Am. Oil. Chem. Soc. 48:547-549.

Lutwak, L. 1975. The role of dietary calcium: phosphorus ratio in human nutrition. Page 63. Proc. Meat Ind. Res. Conf.

MacNeil, J. H., M. G. Mast, and R. Leach. 1978. Protein efficiency ratio and levels of selected nutrients in mechanically deboned poultry meat. J. Food Sci. 43:864-865.

Malicki, A., S. Bruzewicz, A. Zechalko-Czajkowska, K. Szybiga, W. Kopec, and T. Trziszka. 2006. Comparison of the microbiological qualities of turkey myofibrillar protein isolate, mechanically recovered turkey and turkey breast. EJPAU 9.

Mangino, M. E. 1992. Gelation of whey protein concentrates. Food Technol. 46:114-117.

Martin-Sanchez, A. M., C. Navarro, J. A. Perez-Alvarez, and V. Kuri. 2009. Alternatives for efficient and sustainable production of surimi: a review. Comp. Rev. Food Sci. Food Safety. 8:359-374.

Matsumura, Y., and T. Mori. 1996. Gelation. Pages 76-106 in Methods of Testing Protein Functionality. G. M. Hall, ed. Blackie Academic and Professional, London, UK.

Matsushita, S. 1975. Specific interactions of linoleic acid hydroperoxides and their secondary degraded products with enzyme proteins. J. Agric. Food Chem. 23:150-154.

Mawson, R. F., and B. R. Collinson. 1974. Evaluation of Beehive mechanical deboning process. Meat Indus. Res. New Zeal. 391:71.

McCarthy, T. L., J. P. Kerry, J. F. Kerry, P. B. Lynch, and D. J. Buckley. 2001. Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Meat Sci. 58:45-52.

Medynski, A., E. Pospiech, and R. Kniat. 2000. Effect of various concentrations of lactic acid and sodium chloride on selected physicochemical meat traits. Meat Sci. 55:285-290.

Meiburg, D. E., K. E. Berry, C. L. Brown, and S. Simon. 1976. Studies on the utilization of mechanically deboned and mechanically separated meat. Page 79. Proc. Meat Ind. Res. Conf.

Mielnik, M. B., K. Aaby, K. Rolfsen, M. R. Ellekjer, and A. Nilsson. 2002. Quality of comminuted sausages formulated from mechanically deboned poultry meat. Meat Sci. 61:73-84.

Mielnik, M. B., K. Aaby, and G. Skrede. 2003. Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. Meat Sci. 65:1147-1155.

Miller, G. J., J. Frey, J. E. Kunsman, and R. A. Field. 1982. Bovine marrow lipids. J. Food Sci. 47:657-665.

Miller, R. 1998. Functionality of non-meat ingredients used in enhanced pork. National Pork Board/Am. Meat Sci. Assoc. Fact Sheet. Pages 1-12. Des Moines, IA.

Miller, G. D., J. K. Jarivs, and L. D. McBean. 2001. The importance of meeting calcium needs with foods. J. Am. Coll. Nutr. 20:168-185.

Mita, T., E. Ishido, and H. Matsumoto. 1977. Physical studies on wheat protein foams. J. Colloid Interface Sci. 59:172-178.

Mita, T., E. Ishido, and H. Matsumoto. 1978. Physicochemical studies on wheat protein foams: II. Relationship between bubble size and stability of foams prepared with gluten and gluten components. J. Colloid Interface Sci. 64:143-153.

Mitchell, J. R. 1986. Foaming and emulsifying properties of proteins. Pages 291-338 in Developments in Food Proteins. B. J. F. Hudson, ed. Elsevier Applied Science Publishers, New York, NY.

Moayedi, V., D. A. Omana, J. Chan, Y. Xu, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Composition and stability to lipid oxidation of recovered proteins. Poult. Sci. 89:766-775.

Moerck, K. E., and H. R. Ball. 1973. Lipids and fatty acids of chicken bone marrow. J. Food Sci. 38:978-980.

Mohan, M., D. Ramachndran, T. V. Sankar, and R. Anandan. 2007. Influence of pH on the solubility and conformational characteristics of muscle proteins from mullet (*Mugli cephalus)*. Process Biochem. 42:1056-1062.

Monahan, F. J. 2000. Oxidation of lipids in muscle foods: fundamental and applied concerns. Pages 3-25 in Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality. E. Decker, Faustman, C., and C. J. Lopez-Bote, eds. John Wiley and Sons, Inc., New York, NY.

Morrissey, P. A., P. J. A. Sheehy, K. Galvin, J. P. Kerry, and D. J. Buckley. 1998. Lipid stability in meat and meat products. Meat Sci. 49:73-86.

Mottram, D. S. 1994. Some aspects of the chemistry of meat flavour. Pages 210-230 in The Flavour of Meat and Meat Products. F. Shahidi, ed. Chapman and Hall, London, UK.

Muchenje, V., K. Dzama, M. Chimonyo, P. E. Strydom, A. Hugo, and J. G. Raats. 2009. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. 112:279-289.

Mugler, D. J., and F. E. Cunningham. 1972. Factors affecting poultry meat color - A review. World Poult. Sci. 28:400-406.

Mulder, R. W. A. W., and L. W. J. Dorresteijn. 1975. Microbiological quality of mechanically deboned poultry meat. Proc. Eur. Symp. Poult. Meat Qual. 50:1-7.

NCC (National Chicken Council). 2009. Statistics and research. How broilers are marketed. Accessed June, 2010. http://www.nationalchickencouncil.com/statistics/stat_detail.cfm?id=7

Newman, P. B. 1981. The separation of meat from bone - A review of the mechanics and the problems. Meat Sci. 5:171-200.

Nishiya, K., F. Takeda, K. Tamato, O. Tanaka, T. Fukumi, T. Kubo, and N. Toriyabe. 1960. Study on freezing of Alaska Pollock surimi and its application – III. Influence of salt on quality of frozen fish meat. Hokkaido Fish. Expt. Sta. 17:373-384.

Nolsoe, H., and I. Undeland. 2009. The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. Food Bioproc. Technol. 2:1-27.

Ockerman, H. W., and C. L. Hansen. 2000. Animal By-product Processing and Utilization. Technomic Publishing Company, Inc., Lancaster, PA.

Oliphant, G. G. 1997. Meat and meat products. Pages 1-46 in Food Industries Manual. 24th ed. M. D. Ranken, Kill, R. C., and C. G. J. Baker, eds. Chapman and Hall, London, UK.

Omana, D. A., Y. Xu, V. Moayedi, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Chemical and functional properties of recovered proteins. Process Biochem. 45:375-381.

Ostovar, K., J. H. MacNeil, and K. O'Donnell. 1971. Poultry product quality: Microbiological evaluation of mechanically deboned poultry meat. J. Food Sci. 36:1005-1007.

Paoli, S. 1976. British Patent 1, 464,642.

Park, J. W., and M. T. Morrissey. 2000. Manufacturing of surimi from light muscle fish. Pages 23-58 in Surimi and Surimi Seafood. J. W. Park, ed. Marcel Dekker Inc., New York, NY.

Park, J. W., C. H. Jung, J. S. Kim, D. M. Cho, M. S. Cho, and Y. J. Choi. 2003. Surimi processing using acid and alkali solubilization of fish muscle protein. J. Korean Food Sci. Nutr. 32:400-405.

Park, J. W., and T. M. Lin. 2005. Surimi: Manufacturing and evaluation. Pages 33-106 in Surimi and Surimi Seafood. 2nd ed. J. W. Park, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Pedersen, D. K., S. Morel, H. J. Andersen, and S. B. Engelsen. 2003. Early prediction of water-holding capacity in meat by multivariate vibrational spectroscopy. Meat Sci. 65:581-592.

Perez-Mateos, M., and T. C. Lanier. 2007. Comparison of Atlantic menhaden gels from surimi processed by acid or alkaline solubilization. Food Chem. 101:1223-1229.

Perlo, F., P. Bonato, G. Teira, R. Fabre, and S. Kueider. 2006. Physicochemical and sensory properties of chicken nuggets with washed mechanically deboned chicken meat: Research note. Meat Sci. 72:785-788.

Pettersen, M. K., M. B. Mielnik, T. Eie, G. Skrede, and A. Nilsson. 2004. Lipid oxidation in frozen, mechanically deboned turkey meat as affected by packaging parameters and storage conditions. Poult. Sci. 83:1240-1248.

Ponce-Alquicira, E. 2004. Poultry: canned turkey ham. Pages 417-433 in Food Processing: Principles and Applications. 1st ed. J. S. Smith, and Y. H. Hui, eds. Blackwell Publishing, Oxford, UK.

Posner, A. S. 1969. Crystal chemistry of bone material. Physiol. Rev. 49:760-792.

Radomyski, T. 2000. Characteristic of main protein fractions in male turkey meat. EJPAU 3.

Raghavan, S., and H. G. Kristinsson. 2007. Conformational and rheological changes in catfish myosin as affected by different acids during acid-induced unfolding and refolding. J. Agric. Food Chem. 55:4144-4153.

Raghavan, S., and H. G. Kristinsson. 2008. Conformational and rheological changes in catfish myosin during alkali-induced unfolding and refolding. Food Chem. 107:385-398.

Rao, S. I., A. Wilks, M. Hamberg, and Ortiz de Montellano, P. R. 1994. The lipoxygenase activity of myoglobin. Oxidation of linoleic acid by the ferryl oxygen rather than protein radical. J. Biol. Chem. 269:7210-7216.

Raphaelides, S. N., S. Grigoropoulou, and S. Petridis. 1998. Quality attributes of pariza salami as influenced by the addition of mechanically deboned chicken meat. Food Qual. Pref. 9:237-242.

Rasco, B., and G. Bledsoe. 2006. Surimi and surimi analog products. Pages 160:1 -161:1 in Handbook of Food Science, Technology and Engineering. Y. H. Hui, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Rawdkuen, S., S. Sai-Ut, S. Khamsorn, M. Chaijan, and S. Benjakul. 2009. Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. Food Chem. 112:112-119.

Reig, M., and F. Toldra. 2010. Detection of chemical hazards. Pages 469-481 in Handbook of Meat Processing. F. Toldra, ed. Wiley-Blackwell, Iowa. US.

Sasaki, T., C. Yuan, and K. Konno. 2006. Role of cooling process in the thermal aggregate formation by carp myosin rod. J. Food Sci. 71:C75-C80.

Schnell, P. G., K. R. Nath, J. M. Darfler, D. V. Vadehra, and R. C. Baker. 1973. Physical and functional properties of mechanically deboned poultry meat as used in the manufacture of frankfurters. Poult. Sci. 52:1363-1369.

Sebranek, J. G. 2009. Functional properties of muscle proteins: implications for processed meat product characteristics. Proc. Recip. Meat Conf. Rogers, Arkansas. 62:1-7.

Serdaroglu, M., and G. Y. Turp. 2005. Effects of deboning on chemical composition and some properties of beef and turkey meat. Turk. J. Vet. Anim. Sci. 29:797-802.

Shaviklo, G. R. 2007. Quality assessment of fish protein isolates using surimi standard methods. UNU–Fisheries Training programme. Reykjavik, IC.

Singh, H., and J. Flanagan. 2006. Milk proteins. Pages 26:2-26:18 in Handbook of Food Science, Technology and Engineering. Y. H. Hui, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Skibsted, L. H., A. Mikkelsen, and G. Bertelsen. 1998. Lipid-derived off-flavours in meat. Pages 217-256 in Flavor of Meat, Meat Products and Seafoods. F. Shahidi, ed. Blackie Academic and Professional, New York, NY.

Smith, D. M. 1987. Functional and biochemical changes in deboned turkey meat due to frozen storage and lipid oxidation. J. Food Sci. 52:22-27.

Smith, D. M. 1994. Protein interactions in gels: protein-protein interactions. Pages 209-224 in Protein Functionality in Food Systems. N. S. Hettiarachchy, and G. R. Ziegler, eds. Marcel Dekker Inc., New York, NY.

Sonu, C. S. 1986. Surimi. National Marine Fisheries Service, NOAA. Page 122. Technical Memorandum, NMFS, Terminal Island, CA.

Sosnicki, A. A., and B. W. Wilson. 1991. Pathology of turkey skeletal muscle: Implications for the poultry industry. Food Struct. 10:317-326.

Stanley, D. W. 2008. Case studies of innovation in the Canadian food industry. Pages 1-149. Dept. Food Sci.

Stefansson, G. 1994. Solubility of Cod myofibrillar proteins at low ionic strength. PhD Diss. Univ. Massachusetts. Amherst, MA.

Tabilo-Munizaga, G., and G. V. Barbosa-Canovas. 2005. Rheology for the food industry. J. Food Eng. 67:147-156.

Tamato, K., O. Tanaka, F. Takeda, T. Fukumi, and K. Nishiya. 1961. Study on freezing of Alaska Pollock surimi and its application – IV. On the effect of sugar upon the keeping quality of frozen Alaska pollock meat. Hokkaido Fish. Expt. St. 32:50-60.

Trindade, M. A., P. E. Felicio, and C. J. C. Castillo. 2004. Mechanically separated meat of broiler breeder and white layer spent hens. Sci. Agric. 61:234-239.

Undeland, I., S. D. Kelleher, and H. O. Hultin. 2002. Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. J. Agric. Food Chem. 50:7371-7379.

Undeland, I., G. Hall, K. Wendin, I. Gangby, and A. Rutgersson. 2005. Preventing lipid oxidation during recovery of functional proteins from herring (*Clupea harengus*) fillets by an acid solubilization process. J. Agric. Food Chem. 53:5625-5634.

USDA/FSIS (United States Department of Agriculture/Food Safety and Inspection Service). 1999. Generic HACCP Model for Mechanically Separated Species/Mechanically Deboned Poultry. Accessed June, 2010. http://www.fsis.usda.gov/OPPDE/nis/outreach/models/HACCP-6.pdf

Vareltzis, P., H. O. Hultin, and W. R. Autio. 2008. Hemoglobin-mediated lipid oxidation of protein isolates obtained from cod white muscle as affected by citric acid, calcium chloride and pH. Food Chem. 108:64-74.

Vareltzis, P., and I. Undeland. 2008. Removal of lipids and diarrhetic shellfish poisoning toxins (DSP) from blue mussels (*Mytilus edulis*) by an acid or alkaline protein solubilization technique. J. Agric. Food Chem. 56:3675-3681.

Venugopal, V. 2006. Seafood Processing: Adding Value Through Quick Freezing, Retortable Packaging and Cook-Chilling. CRC Press, Taylor & Francis, Boca Raton, FL.

Vilhelmsson, O. 1997. The state of enzyme biotechnology in the fish processing industry. Trends Food Sci. Technol. 8:266-270.

Wang, B. 2006. Chemical composition of red meat. Pages 29:1-29:6 in Handbook of Food Science, Technology and Engineering. Y. H. Hui, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Waszkowiak, K., and W. Dolata. 2007. The application of collagen preparations as carriers of rosemary extract in the production of processed meat. Meat Sci. 75:178-183.

Watts, B. M. 1962. Meat Products. Pages 202-214 in Symposium on Foods: Lipids and Their Oxidation. H. W. Schulz, Day, E. A., and R. O. Sinnhuber, eds. AVI Publ. Co., Westport, CT.

Xiong, Y. L. 2004. Muscle proteins. Pages 100-122 in Proteins in Food Processing. R. Y. Yada, ed. CRC Press, Taylor & Francis, Boca Raton, FL.

Xiong, Y. L. 1997. Structure-function relationships of muscle proteins. Pages 341-392 in Food Proteins and Their Applications. S. Damodaran, and A. Paraf, eds. Marcel Dekker Inc., New York, NY.

Yongsawatdigul, J., and J. W. Park. 2004. Effects of alkali and acid solubilization on gelation characteristics of rockfish muscle proteins. J. Food Sci. 69:499-505.

Young, L. L., G. K. Searcy, L. C. Blankenship, J. Salinsky, and D. Hamm. 1983. Selected nutrients in ground and mechanically separated veal. J. Food Sci. 48:1576.

Young, L. L. 1985. Nucleic acid and purine content of the bone residue from a mechanical poultry deboning machine. Poult. Sci. 64:359-374.

Yu, M., and S. Damodaran. 1991. Kinetics of protein foam destabilization: evaluation of a method using bovine serum albumin. J. Agric. Food Chem. 39:1555-1562.

Zayas, J. F. 1997. Functionality of proteins in food. 1st ed. Springer-Verlag, Berlin, Germany.

Zorba, H. Y., H. Y. Gokalp, H. Yetim, and H. W. Ockerman. 1993. Model system evaluations of the effects of different levels of K_2HPO_4, NaCl and oil temperature on emulsion stability and viscosity of fresh and frozen turkish style meat emulsions. Meat Sci. 34:145-161.

CHAPTER 2. IMPACT OF CITRIC ACID AND CALCIUM IONS ON ACID SOLUBILIZATION OF MECHANICALLY SEPARATED TURKEY MEAT (MSTM): EFFECT ON LIPID AND PIGMENT REMOVAL[1]

2.1 INTRODUCTION

Consumption of poultry meat has greatly increased over the past decades. In particular, consumer tastes began to shift from a preference for whole carcasses toward cut-up parts (breast, thighs, wings, etc.) and processed poultry products. The increased demand for these types of products caused an increased availability of neck, back and frame supplies that can be processed into MSPM. The latter is used for production of frankfurters, fermented sausages and restructured meat products (Dhillon and Maurer, 1975). The mechanical deboning process includes grinding meat and bone together and forcing the mix through a fine screen to remove bone particles (Froning, 1981). During this process, in addition to the extreme mechanical stress, extraction of considerable amount of lipids and heme components (Hb and Mb) from the bone marrow and aerated conditions results in problems with lipid oxidation and color instability of the final product. Hb and Mb are known to be the main pro-oxidants in muscle foods (Richards et al., 1998); their oxidation is usually associated with color problems in muscle foods. Therefore, removing pigments from MSPM could have a beneficial effect on color intensity and fast lipid rancidity, which in turn extend the shelf life stability of raw and cooked MSPM-based products.

[1] *A version of this chapter has been accepted for publication in the Journal of Poultry Science. (Authors: Y. Hrynets, D. A. Omana, Y. Xu and M. Betti).*

Membrane polar lipids being rich in PUFAs are considered to be the primary substrates for lipid oxidation as compared to neutral lipids (triacylglycerols). Since the phospholipid fraction (major part of polar lipids) of MSPM is highly unsaturated (Gomes et al., 2003) it is desirable to remove as much phospholipid as possible, which in turn might greatly increase the stability of proteins to lipid oxidation. While the hydrophobic triacylglycerols are fairly easy to separate from minced muscles, the membrane lipids are relatively difficult to remove because of their amphiphilic nature (Gehring et al., 2009). To overcome the problems resulting from mechanical deboning, Japanese researchers developed a process involving water washing of fish muscle minces that result in a functional protein ingredient called “surimi”. However, low processing yield, inefficient removal of membrane lipids and excessively large volumes of water are the factors that limit the usage of this process (Hultin and Kelleher, 2000a).

A new approach to extract functional proteins from underutilized muscle protein sources has been introduced by Hultin and Kelleher (1999). The process is based on pH-dependent solubility of muscle proteins for their separation and recovery from the undesirable components, such as oxidatively unstable lipids in cellular membranes (Kristinsson and Hultin, 2003). This process involves protein solubilization at acid or alkaline conditions and recovering of the solubilized proteins by precipitation at their isoelectric point. Hultin and Kelleher (2000b) reported removal of 37% and 51% of phospholipids from chicken breast and thigh muscles respectively at pH 2.8. The study of Undeland et al. (2002) on recovery

of functional proteins from herring muscle achieved 20-30% phospholipids removal by an acid solubilization process. In order to improve the stability of extracted protein to lipid oxidation, Liang and Hultin (2005a) also examined the effect of acid solubilization of fish proteins. They reported that treatment with citric acid and calcium ions could aid in removal of membrane lipids from cod muscle homogenates at pH 3.0. The authors suggested that citric acid and calcium ions are able to disconnect the linkages between membranes and cytoskeletal proteins, which are further separated via high-speed centrifugation.

Earlier studies showed that calcium ions were able to facilitate aggregation or fusion of membrane/phospholipids vesicles (Fraley et al., 1980; Wilschut et al., 1980). The efficiency of calcium ions in precipitation of phospholipoproteins of cheese whey was also reported (Maubois et al., 1987). However, no work has been carried out on the effect of citric acid and calcium chloride during protein extraction from MSTM. Hence, the objective of the present study was to investigate the effects of citric acid and calcium chloride on improving the efficiency of recovered proteins from MSTM. An additional objective was to optimize the concentration of citric acid and calcium chloride to obtain high protein yields along with lipid and pigment removal. The effect of these compounds to improve the oxidative stability of the recovered proteins from MSPM was also assessed.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Mechanically separated turkey meat (MSTM) was obtained from Lilydale Inc. (Edmonton, AB, Canada). MSTM (250 g) was filled into polyethylene bags and kept at -20 °C until use. Before extraction, samples were thawed overnight at 4 °C. All chemicals used were reagent grade and obtained either from Fisher Scientific (Waltham, MA) or Sigma-Aldrich Co. (St. Louis, MO).

2.2.2 Extraction procedures

Acid-aided protein recovery from MSTM was done as per the method of Liang and Hultin (2003) with some modifications (Betti and Fletcher, 2005). Cold (1-3 °C) distilled water/ice mixture and 200 g of MSTM were mixed at 1:5 ratio (meat: water/ice, wt/vol) followed by addition of respective concentration of citric acid and/or calcium chloride. Extraction steps were performed at low temperature (4 °C). The mixture was homogenized using a 900-Watt Food Processor (Wolfgang Puck WPMFP15, W.P. Appliances Inc., Hollywood, FL, US) for 15 min. After homogenization, 1200 mL of the meat slurry was transferred to a beaker and placed at 4 °C for 30 min. Thereafter, the pH of the homogenate was adjusted to 2.5 by drop-wise addition of 2 N HCl to achieve the maximum protein solubility. This solubilization pH was chosen based on the solubility study conducted during our preliminary work, where the highest value for MSTM proteins at acidic conditions was obtained at pH 2.5 (reported in chapter 3). The pH-adjusted homogenate was centrifuged using an Avanti® J-E refrigerated

centrifuge (Beckman Coulter Inc., Palo Alto, CA, US) at 25,900 × *g* for 20 min at 4 °C. Centrifugation resulted in the formation of three phases: an upper phase of MSTM neutral lipids; a middle phase of soluble proteins and a bottom phase of insoluble proteins and membrane lipids. The soluble protein fraction (middle layer) was collected and pH was adjusted to 5.2 to isoelectrically precipitate proteins. The precipitated proteins were further centrifuged at 25,900 × *g* for 20 min at 4 °C. The precipitate was re-suspended in a water/ice mixture (water/ice, 350 mL/350 g) by homogenization for 10 min and the pH of the homogenate was then adjusted to 6.2. The proteins were finally collected via centrifugation at 25,900 × *g* for 20 min at 4 °C. The isolated proteins were stored at -20 °C until analysis.

2.2.3 Protein yield

Protein yield was estimated according to the method described by Omana et al. (2010). Protein yield was calculated based on a difference in total protein content between isolates and raw MSTM, and expressed in percentage. Protein content was estimated by the Biuret procedure as described by Gornall et al. (1949); Torten and Whitaker (1964). Bovine serum albumin (Hy-Clone, UT, US) was used as a standard.

2.2.4 Total lipid extraction

Total lipids were extracted from 10.0 g of processed meat and 5.0 grams of raw meat separately with 120 mL of chloroform: methanol solution (2: 1, vol/vol) by homogenization (Fisher Scientific, Power Gen 1000S1) at setting 1 for 10 min

accordingly to the method of Folch et al. (1957). After 30 min the homogenates were filtered through Whatman No. 1 filter paper. To allow clear phase separation 40 mL of 0.88% (vol/vol) sodium chloride solution was added and the mixture was carefully transferred to the separation funnel. After separation, the chloroform phase was filtered through anhydrous sodium sulphate (Fisher Scientific, NJ, US) placed on Whatman No. 1 filter paper. This filtration was done in order to remove the trace of water that possibly may present in chloroform phase. After the filtration chloroform phase was transferred into a pre-weighed round-bottom flask. The upper phase was discarded, since it was rich in non-lipid components. In order to remove solvent, lipid extracts were dried in a rotary evaporator (Rotavapor, RE 121, Buchi, Switzerland) under temperature not exceeding 40 °C. The flasks were then placed in a hot air oven at 60 °C for 30 min and weighed accurately after desiccation for 30 min. To allow further analysis of lipid classes the total lipid extract was dissolved in 10 mL of chloroform, transferred into pre-weighed vials and frozen at -20 °C. Lipid reduction was calculated from the difference in lipid content between raw and treated materials and expressed as percentage.

2.2.5 Fractionation of the main lipid classes

The total lipids extracted from MSTM were further separated into neutral and polar lipid fractions using the method described by Ramadan and Morsel (2003). The separation of these two lipid classes was carried out using a glass column (30 cm × 2 cm; height × diameter) (Chemiglass Life Sciences, NJ, US**)**

packed with slurry of silica gel (70-230 mesh; Whatman, NJ, US). This slurry was prepared by suspending silica gel in chloroform (1:5, wt/vol). Total lipid solution (9 mL) obtained from the lipids extraction was allowed to pass through the column. Chloroform (60 mL) was used as eluent initially to separate neutral lipids (triacylglycerols). After the removal of these triacylglycerols, 60 mL of methanol was applied to the column in order to elute polar lipids.

The obtained fractions were completely evaporated to dryness and kept in a hot air oven at 60 °C for 30 min. The final weight of the flasks was taken after desiccating for 30 min. Both neutral and polar lipid parts were determined gravimetrically and expressed as a percentage.

2.2.6 Thiobarbituric acid reactive substances (TBARs) determination

TBARs were determined in triplicate for each extraction group and also raw material using a modification of the procedure described by Kornbrust and Mavis (1980). Meat samples (3 g) were homogenized in 27 mL of 1.15% KCl with a Power Gen 1000 S1 homogenizer (Schwerte, Germany) for 1 min operated at setting 3. 1000 µl of 80 mM Tris-maleate buffer (pH 7.4), 400 µl of 2.5 mM ascorbic acid and 400 µl of 50 mM ferrous sulphate were added to 200 µl aliquot of the homogenate and incubated for 0, 30, 60, 100 and 150 min using a 37 °C water bath. After incubation 4 mL of TBA-TCA-HCl mixture (26 mM TBA (thiobarbituric acid), 0.92 M TCA (trichloroacetic acid) and 0.8 mM HCl) was added to the sample and thereafter the test tubes were kept in boiling water for 15 min. Then the samples were cooled to room temperature and the absorbance of

the solution was read at 532 nm against the blank containing all the reagents except homogenate. The readings were taken using a spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). TBARs concentration was calculated using the extinction coefficient of $E_{532} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$. The extent of lipid oxidation was expressed as nanomoles of malondialdehyde (MDA) per gram of meat.

2.2.7 Total pigment determination

The estimation of total pigment content was done by direct spectrophotometric measurement according to the method described by Fraqueza et al. (2006), with slight modifications. The pigments were extracted from 10 g of meat sample with the mixture of 40 mL of acetone, 1 mL of HCl, and 1 mL of water. The mixture was vortexed for 3 min and allowed to stand for 1 hour at room temperature. After the extract was filtered through Whatman No. 1 the absorbance was recorded at 640 nm against an acid-acetone blank using a UV/VIS spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The absorbance value was multiplied by a coefficient of 17.18 and the concentration of total heme pigments was expressed in milligrams of myoglobin per gram of meat.

2.2.8 Experimental design and statistical analysis

The experimental design is shown in Figure 2.1.

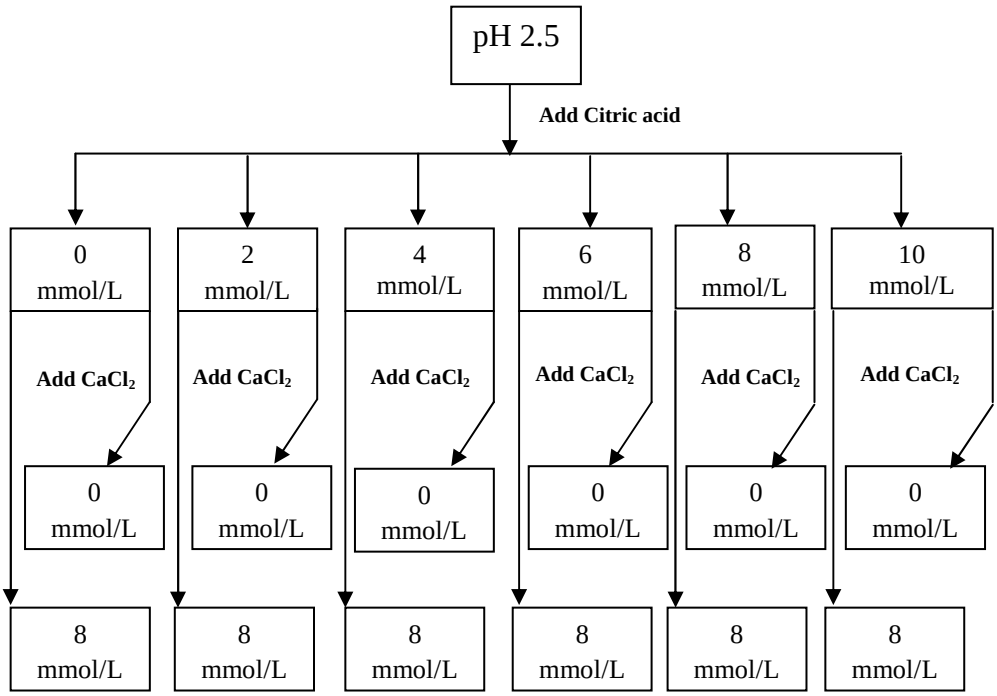

Figure 2.1. Experimental design of the protein extraction process.

The extraction range of variables and their respective levels were chosen based on literature data (Liang and Hultin, 2005a). The experiment was conducted as a 6 × 2 factorial arrangement with six levels of citric acid (0; 2; 4; 6; 8; 10 mmol/L) and two levels of calcium chloride (0 and 8 mmol/L) and was replicated three times resulting in a total of 36 extractions. Analysis of variance was performed by using SAS software (SAS Institute, 1988) in order to determine the significance of the effects of these variables. The model tested both the main effects for citric acid and calcium chloride. The interactions were determined using residual error:

$$Y = \mu + [CA] + [CaCl_2] + ([CA]*[CaCl_2]) + e$$

where Y - dependent variable, μ - treatments average, CA - citric acid, $CaCl_2$ - calcium chloride, e - residual error. Means were separated using Tukey's adjustment. Differences were considered to be significant based on 0.05 level of probability. The results were expressed as mean value ± standard deviation.

2.3 RESULTS AND DISCUSSION

2.3.1 Protein yield

A preliminary study by our group on solubility of MSTM proteins revealed the highest protein solubility at pH 2.5 among different acidic conditions investigated (reported in chapter 3); this pH was used for this study as a high recovery yield was expected. Moreover, the efficiency of acid extraction of protein might be a result of additional recovery of sarcoplasmic proteins along with myofibrillar protein fraction, as low pH facilitated protein solubilization, further resulting in higher protein yields (reported in chapter 3). This is in agreement with the basic principle of the pH-shifting process, which states an additional recovery of sarcoplasmic proteins during extraction process (Ingadottir, 2004). High protein yield is very important during industrial extraction of proteins for economical reasons. Results for protein yield are shown in Table 2.1.

Table 2.1. Effect of citric acid and calcium chloride on protein yield, lipid and total heme pigment content of proteins recovered from MSTM by the acid pH-shifting process[1]

Parameters	Treatments								P-values		
	Citric acid (CA)						Calcium chloride ($CaCl_2$)		Source of variation		
	0 mmol/L	2 mmol/L	4 mmol/L	6 mmol/L	8 mmol/L	10 mmol/L	0 mmol/L	8 mmol/L	Citric acid (CA)	Calcium chloride ($CaCl_2$)	Interaction (CA)* ($CaCl_2$)
n	6	6	6	6	6	6	18	18			
Protein yield (%)	$71.2^{a,b}$ (9.3)	$72.5^{a,b}$ (6.8)	$71.6^{a,b}$ (8.1)	85.6^{a} (5.2)	47.8^{c} (9.6)	$59.5^{b,c}$ (9.0)	66.7 (13.7)	69.4 (14.9)	<0.0001	0.3444	0.6545
Total lipid content (%)	2.2^{a} (0.5)	2.3^{a} (0.3)	1.4^{c} (0.3)	$1.9^{a,b}$ (0.3)	$1.7^{b,c}$ (0.2)	2.4^{a} (0.7)	2.0 (0.6)	2.0 (0.4)	0.0001	0.5032	<0.0001
Neutral lipid content (%)	1.03^{a} (0.30)	$0.68^{a,b}$ (0.14)	$0.65^{a,b}$ (0.35)	0.47^{b} (0.24)	$0.79^{a,b}$ (0.26)	$0.90^{a,b}$ (0.24)	0.76 (0.33)	0.75 (0.28)	0.017	0.8584	0.2937
Polar lipid content (%)	0.68^{a} (0.11)	0.29^{c} (0.12)	$0.53^{a,b}$ (0.16)	0.70^{a} (0.22)	$0.47^{b,c}$ (0.10)	$0.67^{a,b}$ (0.15)	0.55 (0.24)	0.56 (0.15)	<0.0001	0.8643	0.0045
Total heme pigment (mg/g of meat)	0.61^{c} (0.15)	1.01^{a} (0.13)	0.96^{a} (0.35)	0.55^{c} (0.35)	0.80^{b} (0.61)	0.93^{a} (0.14)	0.56^{b} (0.28)	1.06^{a} (0.23)	<0.0001	<0.0001	<0.0001

[1]Different letters in the same raw represent significant ($P < 0.05$) difference between means. Value in parenthesis represent standard deviation (n=3).

No significant interaction was found between CA and $CaCl_2$ effects (P = 0.6545). At the main effect levels, only CA significantly affected protein yield (P < 0.0001). Maximum protein yield (85.6%) was achieved when 6 mmol/L of CA was added to the MSTM homogenate. Further increase of CA concentration to 8 and 10 mmol/L caused reduction in recovery yield. In general, protein yield tends to be slightly higher when extractions were carried out at lower CA concentrations rather than higher. Even though the protein yield was slightly improved by addition of CA, the values were not significantly different from the control, indicating a small influence of CA on protein yield during acid extraction process. The protein yield obtained from acid extraction process depends on three main factors: the solubility of the protein during exposure to low or high pH, the size of the sediments after centrifugation and the solubility at precipitation pH (Nolsoe and Undeland, 2009).

2.3.2 Total lipid content

Lipids in meat are classified as neutral lipids (triacylglycerols) and polar lipids (PL) (Kono and Colowick, 1961). Neutral lipids are stored in connective tissue in relatively large deposits, whereas polar lipids are integrated into and widely distributed throughout the muscle tissues. In order to increase the utilization of the extracted proteins, total lipid from MSTM must be reduced.

The total lipid contents of protein isolates prepared as a function of CA and $CaCl_2$ concentration are shown in Table 2.1. A significant interaction between CA and $CaCl_2$ was found (P < 0.0001). Within these treatments the maximum

removal of total lipids from MSTM (94.7%) was achieved with addition of 4 mmol/L of CA (Figure 2.2).

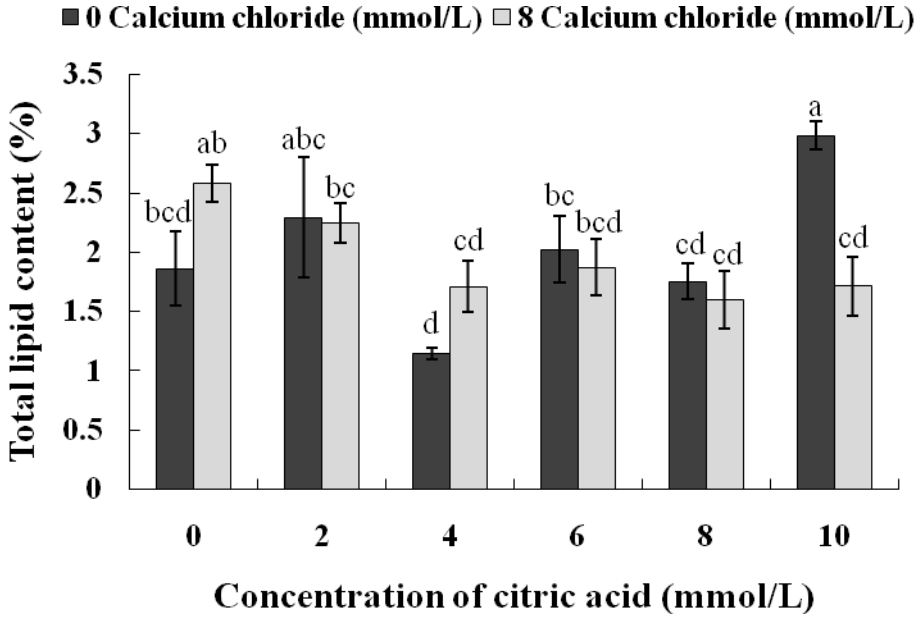

Figure 2.2. The effect of interaction between citric acid and calcium chloride on total lipid content of proteins recovered from MSTM by acid pH-shifting process. Results are presented as mean (n=3) ± standard deviation. Different letters within a figure represent significant ($P < 0.05$) difference between means.

The most evident effect from combination of CA and $CaCl_2$ was observed when 8 mmol/L of $CaCl_2$ was used in combination with 10 mmol/L of CA, which in turn decreased the total lipids to only 42.6% compared to that of CA without $CaCl_2$ addition. This suggests that addition of 8 mmol/L of $CaCl_2$ may diminish the effect of CA at higher concentrations. In general, all the combinations removed on average 90.8% of total lipid from MSTM. Statistical analyses also

showed the significant ($P < 0.0001$) main effect of CA on total lipid content of the protein isolates; as in the case of interaction the highest removal of total lipids from MSTM was achieved with the addition of 4 mmol/L of CA. However, no significant ($P = 0.5032$) difference was attained for total lipid content among the treatments when the main effect of $CaCl_2$ was analyzed.

For the comparison between initial material and extracted proteins the composition of raw MSTM is presented in Table 2.2.

Table 2.2. Properties of raw mechanically separated turkey meat (MSTM)[1]

Parameters	Raw MSTM
Total lipid content (%)	21.7 (1.2)
Neutral lipid content (%)	13.3 (1.6)
Polar lipid content (%)	6.3 (0.4)
Total heme pigment (mg/g of meat)	4.9 (0.4)

[1]Value in parenthesis represent standard deviation (n=3).

2.3.3 Neutral lipid content

Neutral lipids (triacylglycerols) are mainly rich in saturated and monounsaturated fatty acids and related to the sustainable energy source required for the broiler chicken (Betti et al., 2009). The neutral lipid content of the isolated proteins is presented in Table 2.1. Neutral lipid removal from MSTM was not affected by the interaction between CA and $CaCl_2$ ($P = 0.2937$). Furthermore, the main effect of $CaCl_2$ on neutral lipid content showed no significant ($P = 0.8584$)

influence. However, when the main effect of CA on neutral lipid content was evaluated, a significant (P = 0.017) effect was found. Addition of 6 mmol/L of CA resulted in maximum (96.5%) removal of neutral lipid fraction from MSTM. The lipid content in this treatment was found to be 2.2 times lower compared to control sample. Increasing the CA concentration to 8 and 10 mmol/L or decreasing to 4 and 2 mmol/L resulted in increasing of neutral lipid content. The results showed that the addition of different levels of CA improved the removal (93.3-96.5%) of neutral lipids from MSTM.

2.3.4 Polar lipid content

Although the polar lipids are less predominant in muscle tissues, they are considered to be more susceptible to oxidative changes compared to neutral lipids. This is attributed to high content of unsaturated fatty acids, close contact with catalysts of lipid oxidation such as reactive oxygen species (ROS), and the large surface area exposed to the aqueous phase (Gandemer, 1999). Thus, the removal of phospholipids is highly desirable in terms of improving stability of extracted proteins to lipid oxidation. Results for polar lipid fraction content are reported in Table 2.1. The interaction between CA and $CaCl_2$ was found to have a significant (P = 0.0045) effect on polar lipid content (Figure 2.3).

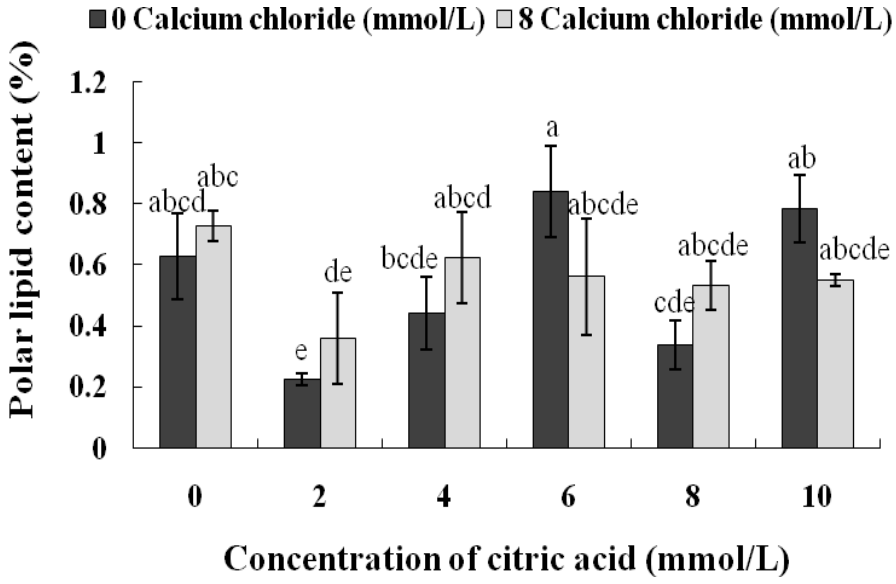

Figure 2.3. The effect of interaction between citric acid and calcium chloride on polar lipid content of proteins recovered from MSTM by acid pH-shifting process. Results are presented as mean (n=3) ± standard deviation. Different letters within a figure represent significant ($P < 0.05$) difference between means.

Maximum polar lipid removal was attained for treatment with addition of 2 mmol/L of CA; at these conditions 96.4% polar lipids were removed from MSTM to reach a final content of 0.22%. Polar lipid content with the addition of 2 mmol/L of CA was 3.1 times lower compared to the control sample, indicating an influential effect of CA for polar lipid removal from MSTM. Further increase in the CA concentration to 4, 6 and 10 mmol/L resulted in less efficient removal of polar lipids. With the addition of 8 mmol/L of $CaCl_2$ to 6 and 10 mmol/L of CA significant removal of polar lipids was observed; however addition of $CaCl_2$ to treatments with 2, 4 and 8 mmol/L of CA decreased the efficiency of polar lipids removal. Removal of polar lipids was considerably high with a range from 86.6 to

96.4% when different concentrations of CA were incorporated in the MSTM protein homogenate. The high efficiency of CA for polar lipid removal may be due to its ability to disconnect the linkages between cytoskeletal proteins and membrane lipids, linked together via electrostatic interaction. CA might play a role as a binding agent for the basic amino acid residues of cytoskeletal proteins competing with the acidic phospholipids of membranes (Liepina et al., 2003). As a result, membranes released from the cytoskeletal proteins might aggregate due to low pH achieved by addition of CA and then be removed by centrifugation (Liang and Hultin, 2005a). Removal of up to 90% of the phospholipids was achieved with addition of 5 mmol/L of CA along with 8 mmol/L of $CaCl_2$ in a study on acid solubilization of fish muscle proteins (Liang and Hultin, 2005a). In previous studies on removal of membrane lipids from fish muscles the addition of CA along with calcium chloride has been successfully used (Liang and Hultin, 2005a; 2005b). However, the present study revealed that addition of $CaCl_2$ did not have a significant (P = 0.8643) effect on polar lipid removal. This might be the result of differences in the composition of raw materials used for the extraction process. MSTM contains large quantities of bone particles and hence the calcium content in the raw material is expected to be high. For this reason, it is possible that the addition of 8 mmol/L of $CaCl_2$ to a starting material already rich in calcium ions showed no effect on polar lipid reduction.

2.3.5 Extent of lipid oxidation (TBARs)

Lipid oxidation is a complex process by which unsaturated fatty acids reacts with molecular oxygen via free radicals, and form peroxides or other products of oxidation (Gray, 1978). Secondary oxidation products, such as aldehydes, ketones and esters, are responsible for the increased deterioration and rancid flavour (Ladikos and Lougovois, 1990). Spectrophotometric detection of these compounds by TBARs test has been widely used for estimating oxidative stress effects on lipids (Gray, 1978).

The changes in TBARs values for raw MSTM and protein isolated at different extraction conditions are presented in Figure 2.4.

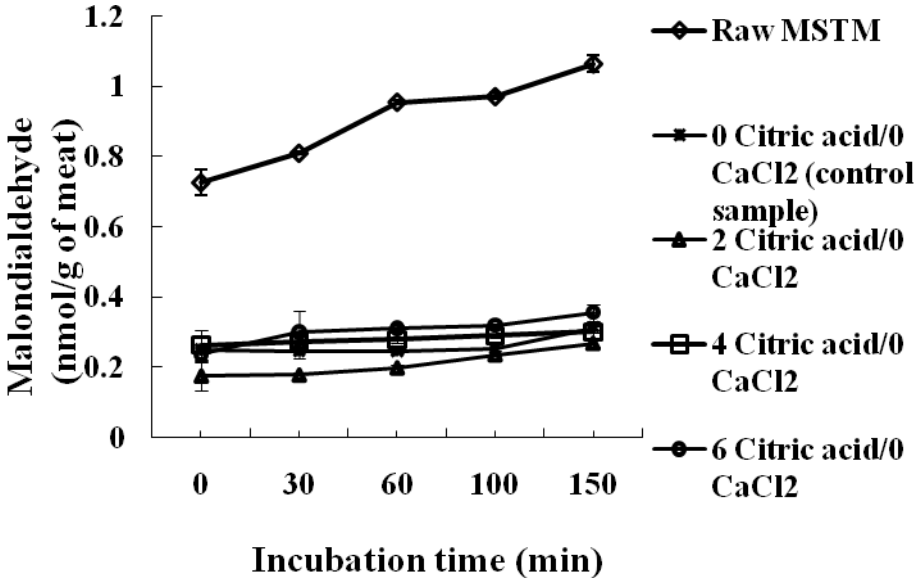

Figure 2.4. Effect of citric acid on oxidative stability of protein recovered from MSTM as determined by induced thiobarbituric acid reactive substances (TBARs) method. Results are presented as mean (n=3) ± standard deviation.

Since the addition of $CaCl_2$ did not show significant effect on removal of polar lipids from MSTM, the rate of lipid oxidation was tested only for samples treated with 0, 2, 4 and 6 mmol/L of CA. Increasing concentration of CA to 8 and 10 mmol/L also did not show significant improvement in polar lipid removal, therefore the lipid oxidation tests were not determined for these treatments. TBARs values at 0 min of incubation time were significantly ($P < 0.05$) lower for samples extracted with addition of 2 mmol/L of CA. When the incubation time reached 150 min the same samples tend to be significantly ($P = 0.0559$) lower from control and the other treatments. The lowest level of lipid oxidation in this treatment is probably due to the efficient removal of the majority of phospholipids as revealed by phospholipids analysis (Figure 2.3). Pikul et al. (1984) reported that 90% of thiobarbituric acid reactive substances (TBARs) formation was contributed by polar lipids of chicken meat. The highest MDA value, regardless of incubation time, was found for raw MSTM and the values were significantly ($P < 0.05$) higher compared to extracted samples. This study revealed the addition of 2 mmol/L of CA might act as a protection against lipid oxidation by removal of polar lipids. This effect might be also attributed to its ability to chelate pro-oxidants like iron and heme proteins, via bonds formed between the metal and carbonyl or hydroxyl groups of citric acid molecule (Ke et al., 2009). CA is also often used as an antioxidant to stabilize fish muscle during frozen storage (Pokorny, 1990) and is included among the antioxidants which are generally permitted in foods (E 330) (Mikova, 2001). Our results are in agreement with the

study of Vareltzis et al. (2008) who reported that low pH treatments improved the oxidative stability of protein isolates from cod muscle, while calcium chloride alone did not.

2.3.6 Total heme pigments

Color is an important factor which affects consumer's perception of product quality and influences purchasing decisions. It is also one of the key parameters when comparing different processing treatments. Overall, the market is most interested in protein isolates as white as possible (Tabilo-Munizaga and Barbosa-Canovas, 2004). The two major pigments responsible for the color of MSTM are myoglobin and hemoglobin (Hernandez et al., 1986); which are also known as catalysers of lipid oxidation in meat (Richards et al., 2005). Therefore, the effective removal of these pigments might not only improve the color characteristics of protein isolates, but also increase their stability to oxidative deterioration. The effect of CA and $CaCl_2$ in removing total pigments from MSTM during acid solubilization is shown in Table 2.1. The interaction between CA and $CaCl_2$ was found to have a significant ($P < 0.0001$) influence on the total pigment content of protein isolated from MSTM (Figure 2.5).

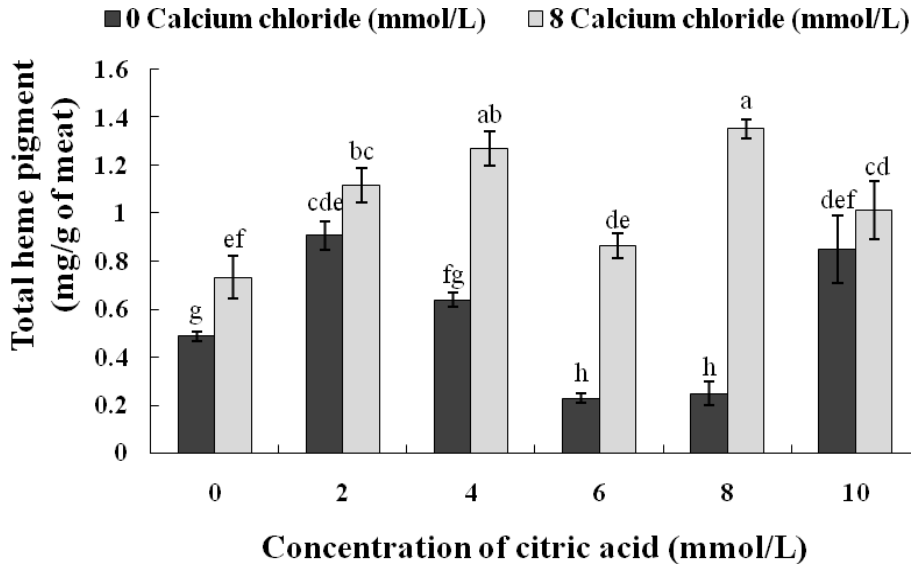

Figure 2.5. The effect of interaction between citric acid and calcium chloride on total heme pigment content of proteins recovered from MSTM by the acid pH-shifting process. Results are presented as mean (n=3) ± standard deviation. Different letters within a figure represent significant ($P < 0.05$) difference between means.

The lowest total pigment content was observed for treatments with the addition of 6 or 8 mmol/L of CA during extraction (0.23 and 0.25 mg/g of meat, respectively). The values were around half that of the control sample. In general, treatments with the combinations of CA and $CaCl_2$ removed from 72.2 to 95.3% of total heme pigments from MSTM. The present study also revealed that addition of $CaCl_2$ to protein homogenates decreased the effectiveness of pigment removal from MSTM. It has been reported that $CaCl_2$ increases the size of the aggregates caused by increased protein-protein and decreased protein-water interactions, by occupying negative charges on polypeptide chains (Maltais et al., 2005). Hence, it

may lead to precipitation of heme pigments to the bottom sediment during centrifugation after isoelectric precipitation.

2.4 CONCLUSION

In conclusion, CA significantly influenced protein yield, lipid and pigment removal during extraction of proteins from MSTM. The optimum concentration of CA for maximum protein yield was found at 6 mmol/L. However, 2 mmol/L of CA was the most efficient for the removal of phospholipids. This resulted in greater stability of isolated proteins to lipid oxidation compared to raw MSTM. CA also significantly affected the total pigment content of the protein isolates, which has a direct relation to the color of extracted meat. Increase in protein yield with efficient lipid removal during extraction will benefit industry in utilizing protein isolates from MSTM for the production of further-processed products in order to improve their functionality. Therefore, the addition of CA to the acid solubilization technique is a highly appealing alternative for extraction of proteins from MSTM to help overcome its compositional problems.

2.5 REFERENCES

Betti, M., and D. L. Fletcher. 2005. The influence of extraction and precipitation pH on the dry matter yield of broiler dark meat. Poult. Sci. 84:1303-1307.

Betti, M., Perez, T. I., M. J. Zuidhof, and R. A. Renema. 2009. Omega-3 enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipid classes. Poult. Sci. 88:1740-1754.

Dhillon, A. S., and A. J. Maurer. 1975. Stability study of comminuted poultry meats in frozen storage. Poult. Sci. 54:1407-1414.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:467-509.

Fraley, R., J. Wilschut, N. Duzgunes, C. Smith, and D. Papahadjopoulos. 1980. Studies on the mechanism of membrane fusion: Role of phosphate in promoting calcium ion induced fusion of phospholipid vesicles. Biochem. 19:6021-6029.

Fraqueza, M. J., A. S. Cardoso, M. C. Ferreira, and A. S. Barreto. 2006. Incidence of pectoralis major turkey muscles with light and dark color in a portuguese slaughterhouse. Poult. Sci. 85:1992-2000.

Froning, G. W. 1981. Mechanical deboning of poultry and fish. Pages 109-147 in Advances in Food Research, 2nd ed. C. O. Chichester, E. M. Mrak, and G. F. Stewart, eds. Academic Press, New York, NY.

Gandemer, G. 1999. Lipids and meat quality: lipolysis, oxidation, maillard reaction and flavour. Sci. Aliment. 19:439-458.

Gehring, C. K., M. P. Davenport, and J. Jaczynski. 2009. Functional and nutritional quality of protein and lipid recovered from fish processing by-products and underutilized aquatic species using isoelectric solubilization/precipitation. Curr. Nutr. Food Sci. 5:17-39.

Gomes, H. A., E. N. Silva, M. R. L. Nascimento, and H. T. Fukuma. 2003. Evaluation of the 2-thiobarbituric acid method for the measurement of lipid oxidation in mechanically deboned gamma irradiated chicken meat. Food Chem. 80:433-437.

Gornall, A. C., C. L. Bradwill, and M. M. David. 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem. 177:755-766.

Gray, J. I. 1978. Measurement of lipid oxidation. A review. J. Am. Oil. Chem. Soc. 55:539-546.

Hernandez, A., R. C. Baker, and J. H. Hotchkiss. 1986. Extraction of pigments from mechanically deboned turkey meat. J. Food Sci. 51:865-867.

Hultin, H. O., and S. D. Kelleher. 1999. Process for isolating a protein composition from a muscle source and protein composition. Advanced Protein Technologies, Inc, assignee. US. Pat. No. 6,005,073.

Hultin, H. O., and S. D. Kelleher. 2000a. Surimi processing from dark muscle fish. Pages 59-77 in Surimi and Surimi Seafood. J. W. Park, ed. Marcel Dekker Inc., New York, NY.

Hultin, H. O. and S. D. Kelleher. 2000b. High efficiency alkaline protein extraction. Univ. of Massachusetts, assignee. US. Pat. No. 6,136,959.

Ingadottir, B. 2004. The use of acid and alkali-aided protein solubilization and precipitation methods to produce functional protein ingredients from tilapia. MS Thesis. Univ. Florida, US.

Ke, S., Y. Huang, E. A. Decker, and H. O. Hultin. 2009. Impact of citric acid on tenderness, microstructure and oxidative stability of beef muscle. Meat Sci. 82:113-118.

Kono, T., and S. P. Colowick. 1961. Isolation of skeletal muscle cell membrane and some of its properties. Arch. Biochem. Biophys. 93:520-533.

Kornbrust, D. J., and R. D. Mavis. 1980. Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correction with vitamin E content. Lipids. 15:315-322.

Kristinsson, H. G., and H. O. Hultin. 2003. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J. Agric. Food Chem. 51:7187-7196.

Ladikos, D., and V. Lougovois. 1990. Lipid oxidation in muscle foods: A review. Food Chem. 35:295-314.

Liang, Y., and H. O. Hultin. 2003. Functional protein isolates from mechanically deboned turkey by alkaline solubilization with isoelectric precipitation. J. Muscle Foods. 14:195-205.

Liang, Y., and H. O. Hultin. 2005a. Separation of membranes from acid-solubilized fish muscle proteins with the aid of calcium ions and organic acids. J. Agric. Food Chem. 53:3008-3016.

Liang, Y., and H. O. Hultin. 2005b. Separation of muscle membranes from alkali-solubilized fish muscle proteins. J. Agric. Food Chem. 53:10012-10017.

Liepina, I., C. Czaplewski, P. Janmey, and A. Liwo. 2003. Molecular dynamics study of gelsolin-derived peptide binding to a lipid bilayer containing phosphatidylinositol 4,5-biphosphate. Biopolymers. 71:49-70.

Maltais, A., G. E. Remondetto, R. Gonzalez, and M. Subirade. 2005. Formation of soy protein isolate cold-set gels: protein and salt effects. J. Food Sci. 70:67-73.

Maubois, J. L., A. Pierre, J. Fauquant, and M. Piot. 1987. Industrial fractionation of main whey proteins. Int. Dairy Fed. Bull. 212:154-159.

Mikova, K. 2001. The regulation of antioxidants in food. Pages 267-284 in Antioxidants in Food: Practical Applications. N. Yanishlieva, J. Pokorny, and M. Gordon, eds. Woodhead Publishing Ltd, Cambridge, UK.

Nolsoe, H., and I. Undeland. 2009. The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. Food Bioproc. Technol. 2:1-27.

Omana, D., Y. Xu, V. Moayedi, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Chemical and functional properties of recovered proteins. Process. Biochem. 45:375-381.

Pikul, J., D. E. Leszczynski, and F. A. Kummerow. 1984. Relative role of phospholipids, triacylglycerols and cholesterol esters on malonaldehyde formation fat extracted from chicken meat. J. Food Sci. 49:704-708.

Pokorny, J. 1990. Stabilization of food. Pages 347-367 in Oxidation Inhibition in Organic Materials. J. Pospisil, and P. P. Klemchuk, eds. CRC Press, Taylor & Francis, Boca Raton, FL.

Ramadan, M. F., and J. T. Morsel. 2003. Determination of the lipid classes and fatty acid profile of Niger (*Guizotia abyssinica* Cass.) seed oil. Phytochem. Anal. 14:366-370.

Richards, M. P., S. D. Kelleher, and H. O. Hultin. 1998. Effect of washing with or without antioxidants on quality retention of mackerel fillets during refrigerated and frozen storage. J. Agric. Food Chem. 46:4363-4371.

Richards, M. P., M. A. Dettman, and E. W. Grunwald. 2005. Pro-oxidative characteristics of trout hemoglobin and myoglobin: a role for released heme in oxidation of lipids. J. Agric. Food Chem. 53:10231-10238.

Tabilo-Munizaga, G., and G. V. Barbosa-Canovas. 2004. Color and textural parameters of pressurized and heat-treated surimi gels as affected by potato starch and egg white. Food Res. Intern. 37:767-775.

Torten, J., and J. R. Whitaker. 1964. Evaluation of the Biuret and dye-binding methods for protein determination in meats. J. Food Sci. 29:168-174.

Undeland, I. A., S. D. Kelleher, and H. O. Hultin. 2002. Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. J. Agric. Food Chem. 50:7371-7379.

Vareltzis, P., H. O. Hultin, and W. R. Autio. 2008. Hemoglobin-mediated lipid oxidation of protein isolates obtained from cod white muscle as affected by citric acid, calcium chloride and pH. Food Chem. 108:64-74.

Wilschut, J., N. Duzgunes, R. Fraley, and D. Papahadjopoulos. 1980. Studies on the mechanism of membrane fusion: Kinetics of calcium ion induced fusion of phosphatidylserine vesicles followed by a new assay for mixing of aqueous vesicle contents. Biochem. 19:6011-6021.

CHAPTER 3. COMPARATIVE STUDY ON THE EFFECT OF ACID- AND ALKALINE-AIDED EXTRACTIONS ON MECHANICALLY SEPARATED TURKEY MEAT (MSTM): CHEMICAL CHARACTERISTICS OF RECOVERED PROTEINS[2]

3.1 INTRODUCTION

In recent years, consumption of poultry meat and further processed poultry products has greatly increased worldwide. One of the reasons for the increased consumer preference for poultry products is the greater availability of choice for poultry cuts, such as wings, thighs and breast. Consequently, in USA, the consumption of chicken and turkey from 1950 to 2007 increased from 12 to 52 kg per capita (USDA/ERS, 2007). In Canada, per capita consumption of chicken was 21.5 kg in 1989 and reached 31.8 kg in 2008 (CFC, 2008). As a result, considerable quantity of poultry carcass parts, such as necks, backs, and drumsticks, became available. Utilization of these less desirable parts can be achieved through mechanical deboning to produce mechanically separated poultry meat (MSPM) for the manufacture of variety meats, canned meats and emulsified-type products.

The main problem encountered with MSPM is due to its method of production, which includes grinding meat and bones together and forcing the mixture through a perforated drum with consequent separation into two fractions,

[2] *A version of this chapter was accepted for publication in the Process Biochemistry Journal (doi: 10.1016/j.procbio.2010.09.006). (Authors: Y. Hrynets, D. A. Omana, Y. Xu and M. Betti).*

such as mechanically separated meat and bone residue. This causes the release of a considerable amount of fat and heme components from the bone marrow which becomes incorporated into the meat product. Hence, the fundamental problems with proper utilization of MSPM are the high content of lipids, pigments and connective tissue (Yang and Froning, 1992), which lead to dark meat color, susceptibility to lipid oxidation, undesired textural properties and sometimes unpleasant odor due to the rancidity of fat. These properties may result in problems with further processing and consumer acceptance.

One of the alternatives to overcome these problems and make MSPM more suitable for further processing is the extraction of functional proteins from these raw materials. The most well known extraction method is surimi processing, which was initially developed for extraction of fish proteins (Pepe et al., 1997). However, low processing yield, inefficient removal of membrane lipids and excessively large volumes of water usage are limiting factors of this approach (Hultin and Kelleher, 2000). To address the problems of utilization of low-value meat, a new pH-shifting extraction method was developed at the University of Massachusetts (Hultin and Kelleher, 2000). The method also known as acid or alkaline extraction is based on the pH-dependent solubility principle. The technology involves initial solubilization of muscle proteins at low or high pH, followed by precipitation at the isoelectric point and further pH adjustment to the original meat pH. When exposed to acid and alkaline conditions, proteins carry a net positive and net negative charge, respectively, which is a key factor for

obtaining high solubility, along with low viscosity of the initial homogenate. Low viscosity provides separation of insoluble parts, especially membrane lipids, which are known to be the primary substrates for deteriorative changes in lipid oxidation (Maestre et al., 2009) and their removal is expected to increase the stability of the final isolate greatly.

Several studies on the utilization of recovered fish proteins by pH-shifting processes have been conducted. These studies showed high recovery yields and improved functionalities of the recovered proteins compared to proteins obtained using conventional surimi processing (Kristinsson and Demir, 2003; Kristinsson et al., 2005). Moreover, acid and alkaline treatments minimize the risk of lipid oxidation due to efficient removal of both neutral and membrane lipids under favorable circumstances (Nolsoe and Undeland, 2009). Other valuable components from MSPM are also of interest. One such component is glycosaminoglycan (which are the important constituents of proteoglycans), which can be obtained in different fractions during pH extraction. One of the applications of glycosaminoglycans, chondroitin sulfate, in particular, is the therapeutic treatment of knee osteoarthritis, which is the most frequently reported reason for long term disability in UK and Canada (Black et al., 2009; Badley, 1995). To the best of our knowledge, no publication has reported the analysis of proteoglycans during the protein isolation process.

Development of novel and economical protein sources is one of the major challenges for the present world food market. Thus, the application of the pH-

shifting process for efficient recovery of proteins from MSPM appears promising. In addition, it helps to improve the utilization of this low value meat source. Therefore, the objectives of this study were to determine the feasibility of pH-shifting process to recover proteins from MSPM and to investigate the effect of different pH treatments on chemical properties of these proteins.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Mechanically separated turkey meat (MSTM) was obtained from Lilydale Inc. (Edmonton, AB, Canada). The blocks of meat were cut in a frozen state into pieces (250 g) and filled into polyethylene bags and kept at -20 °C until use. Before extraction, samples were thawed overnight at 4 °C. All the reagents and chemicals used in the study were of analytical grade.

3.2.2 Methods

3.2.2.1 Protein solubility

In order to find the effect of different pH on the solubility of proteins in raw MSTM, a solubility curve was created, as described by Kim et al. (2003). Six grams of raw MSTM was mixed with 300 mL of refrigerated, deionized water in a homogenizer (Fisher Scientific, Power Gen 1000 S1, Schwerte, Germany) at a setting of 3 for 1 minute. The homogenate (30 mL) was adjusted from pH 1.5 to 12.0 in 0.5 intervals, using 0.2 M and 1 M HCl or NaOH, with the aid of a pH meter (Denver Instrument, Ultra Basic, UP-10, Colorado, US). The homogenate

was centrifuged at 25,900 × *g* at 4 °C for 20 min. The protein concentration of the supernatant was determined by Biuret method (Gornall et al., 1949). The protein solubility of the middle layer was expressed as milligram per gram of meat. Four replications were carried out for each measurement.

3.2.3 Extraction procedures

3.2.3.1 Preparation of protein isolate by acid-aided process

The acid-aided protein recovery from MSTM was done as per the methods of Liang and Hultin (2003) and Betti and Fletcher (2005) with some modifications. Protein extractions were carried out under low temperature conditions (4 °C) in order to maintain the functionality of the final product. For each test, 200 g of MSTM were homogenized with cold (1-3 °C) distilled water/ice mixture at 1:5 ratio (meat: water/ice, wt/vol) using a 900-Watt Food Processor (Wolfgang Puck WPMFP15, W.P. Appliances Inc., Hollywood, FL, US) for 15 min. After homogenization, 1200 mL of the meat slurry was transferred to a beaker and placed at 4 °C for 30 min. Further, the proteins in the homogenate were solubilized by drop-wise addition of 2 M HCl to reach the maximum solubility points at acid conditions with pH values of 2.5 and 3.5, as determined from the solubility profile. The protein suspension was centrifuged using an Avanti® J-E refrigerated centrifuge (Beckman Coulter Inc., Palo Alto, CA, US) at 25,900 × *g* for 20 min at 4 °C. After centrifugation, three layers were formed: an upper layer of MSTM neutral lipids; a middle layer of soluble proteins and a bottom layer of insoluble proteins and membrane lipids (this layer is termed

as the sediment fraction in this chapter). The middle layer of soluble proteins was collected and pH was adjusted to 5.2 by 2 M NaOH in order to isoelectrically precipitate proteins. The precipitated proteins were thereafter centrifuged at 25,900 × g for 20 min at 4 °C. The precipitate was re-suspended in water/ice mixture (water/ice, 350mL/350g) by homogenization for 10 min and pH of the homogenate was then adjusted to 6.2. The proteins were finally collected via centrifugation at 25,900 × *g* for 20 min at 4 °C. After complete extraction the moisture content of the resulting protein isolates was adjusted to 80%. Cryoprotectants (5% sorbitol, 4% sucrose, 0.3% tripolyphosphate, 0.4% sodium bicarbonate and 0.03% sodium nitrite) were mixed with protein isolates in a pre-chilled Wolfgang Puck WPMFP15 900-Watt Food Processor (W.P. Appliances Inc., Hollywood, FL, US). The isolated proteins were stored in the freezer at -20 °C until analysis.

3.2.3.2 Preparation of protein isolate by alkaline-aided process

The extraction process was carried out in the same sequence as acid-aided extractions, and differs just in the first solubilization step. Here, the MSTM proteins were initially solubilized at pH values of 10.5 or 11.5.

3.2.4 Total protein content and recovery yield

The total protein content of both the raw material and final protein isolates from different solubilization methods was estimated by the Biuret procedure (Gornall et al., 1949; Torten and Whitaker, 1964). Meat sample (1 g) was

dispersed in 20 mL of 0.5 M NaOH, heated in the boiling water for 10 min and cooled in an ice-water bath. After cooling the solution was filtered through Whatman No. 1 filter paper. Then, 15 mL of the filtrate was centrifuged with 15 mL of anhydrous ether (J-6B/P Beckman, Beckman® Instruments, Inc, CA, US) at 2278 × *g* for 10 min. After centrifugation, 1 mL of the lower phase was taken and mixed with 4 mL of Biuret reagent and the absorbance was measured at 540 nm (V-530, Jasco Corporation, Tokyo, Japan). Bovine serum albumin (Sigma-Aldrich, St Louis, MO, US) was used as a standard.

Protein recovery of acid- and alkaline-aided treatments was determined according to the method described by Omana et al. (2010). The recovery yield was expressed as a difference in total protein content of isolates (after isoelectric precipitation or final) and raw MSTM.

3.2.5 Protein extractability

Frozen MSTM protein isolates were thawed overnight at 4 °C. Sarcoplasmic and total protein extractability was determined by homogenizing (Fisher Scientific, Power Gen 1000S1, Schwerte, Germany) 2 g of sample at speed setting of 1 for 45 sec in 20 mL of 30 mM phosphate buffer (pH 7.4) and 50 mM phosphate buffer containing 0.55 M potassium iodide (pH 7.4), respectively. The homogenate was centrifuged in Avanti® J-E refrigerated centrifuge (Beckman Coulter Inc., Palo Alto, CA, USA) at 15,300 × *g* for 15 min at 4 °C. The supernatant was filtered through Whatman No. 1 filter paper and the protein

content of the clear filtrate was determined by Biuret method (Gornall et al., 1949).

3.2.6 Protein surface hydrophobicity

Sarcoplasmic and myofibrillar protein surface hydrophobicity were determined using 1-anilino-8-naphthalenesulfonate fluorescent probes (ANS; 8 mM in 0.1 M phosphate buffer, pH 7.0) according to the method described by Kim et al. (2003). Sarcoplasmic proteins were extracted by homogenizing 2 grams of meat sample in 20 mL of 30 mM phosphate buffer (pH 7.4) for 45 sec, followed by centrifugation at 15,300 × g for 15 min at 4 °C. The supernatant was used as sarcoplasmic protein solution. The pellet obtained after centrifugation was re-suspended in 50 mM phosphate buffer containing 0.55 M potassium iodide (pH 7.4), homogenized and centrifuged as described above. The supernatant was filtered through Whatman No. 1 filter paper and protein concentration was determined using Biuret method (Gornall et al., 1949). The protein solutions were serially diluted with the same buffer to the final volume of 4 mL to obtain concentrations ranging from 0.008% to 0.03%. After mixing with 20 µl of ANS solution, fluorescence was measured using a fluorescence plate reader (Fluoroscan Ascent FL; Thermo Electron Corp., Vantaa, Finland) at an excitation wavelength of 355 nm and emission wavelength of 460 nm. The net relative fluorescence intensity (RFI) was obtained by subtracting the RFI of each sample measured without ANS from that with ANS. The initial slope of the RFI versus

protein concentration (expressed in percents) was calculated by linear regression analysis and used as an index of the protein surface hydrophobicity.

3.2.7 Total and reactive sulfhydryl groups content

The estimation of total (T-SH) and reactive (R-SH) sulfhydryl groups were performed using protocols of Choi and Park (2002) and Kim et al. (2003), respectively. Protein extracts were prepared by homogenizing (setting 3 for 1 min) 2.5 gram of recovered protein in 25 mL of tris-glycine buffer (pH 8.0) containing 5 mM of EDTA. The homogenate was filtered before use. For T-SH estimation, to 1 mL of the filtrate, 4 mL of 10 M Urea and 50 µl of Ellman's reagent (10 mM 5,5'-dithiobis (2-nitrobenzoic acid)) were added and mixed well by vortex mixer (Fisher, Scientific, ON, CA). In case of R-SH, 1 mL of filtrate was mixed with 4 mL of tris-glycine buffer (pH 8.0) and 50 µl of Ellman's reagent. The mixture was kept for 1 h at 4 °C with intermittent stirring. The absorbance of the solutions was measured at 412 nm against a blank of Ellman's reagent at the same concentration without proteins using a spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The SH content was calculated by using molar extinction coefficient of 13, 600 M^{-1} cm^{-1} and expressed as µmol/g of protein. The protein content of the filtrate was determined by Biuret method (Gornall et al., 1949).

3.2.8 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins were separated according to the procedure described by Laemmli (1970). Precast 10-20% ready gels (Bio-Rad Laboratories Inc., Hercules, CA) were used to separate proteins in a Mini-PROTEAN tetra cell attached to a PowerPack Basic electrophoresis apparatus (Bio-Rad Laboratories Inc., 1000 Alfred Nobel Drive, Hercules, CA, US). For each sample, 20 μg of protein was loaded and ran at constant voltage of 200 V. Standard protein marker from Bio-Rad (Bio-Rad Laboratories Inc., Hercules, CA, US) was loaded into a separate well. After staining and destaining, gels were scanned using an Alpha Innotech gel scanner (Alpha Innotech Corp., San Leandro, CA) with FluorChem SP software.

3.2.9 Amino acid analysis

Amino acid analysis was carried out on a Beckman System 6300 High Performance Analyzer by post-column ninhydrin methodology after hydrolysis of proteins in 6 N HCl and 0.1% phenol for 1 h at 160 °C. Pickering Laboratories 15 cm sodium column and Pickering's sodium eluent buffers were used in the study. Data was collected and analyzed using Beckman System Gold software.

3.2.10 Total lipid extraction

Total lipid content was determined using the method of Folch et al. (1957). Accordingly, 10.0 g of processed meat and 5.0 grams of raw meat were separately extracted with 120 mL of Folch solution (chloroform: methanol solution, 2: 1,

vol/vol) by homogenization for 10 min. After 30 min, the homogenates were filtered through Whatman No. 1 filter paper. To allow clear phase separation, 40 mL of 0.88% (vol/vol) sodium chloride solution was added and the mixture was carefully transferred to a separating funnel. After separation, the chloroform phase was filtered through anhydrous sodium sulphate (Fisher Scientific, NJ, US) and transferred into a pre-weighed round-bottom flask, while the upper phase was discarded as it was rich in non-lipid components. Thereafter, the chloroform was evaporated at 40 °C using a rotary evaporator (Rotavapor, RE 121, Buchi, Switzerland). The flasks were then placed in a hot air oven for drying at 60 °C for 30 min and weighed accurately after desiccation for 30 min. For further analysis of lipid classes, the total lipid extract was washed with 10 mL of chloroform and dissolved lipids were transferred into pre-weighed vials and frozen at -20 °C. Lipid reduction was calculated from the difference in lipid content between raw and treated materials and expressed as percentage.

3.2.11 Fractionation of the main lipid classes

The method of Ramadan and Morsel (2003) was used to separate the triacylglycerols (neutral lipids) and phospholipid (polar lipids) fractions in total lipid extracts. The separation of two lipid classes was accomplished using a glass column (30 cm × 2 cm; height × diameter) (Chemiglass Life Sciences, NJ, US) packed with silica gel (70-230 mesh; Whatman, NJ, US) by applying the slurry of the adsorbent in chloroform (1:5, wt/vol). Lipid solution (9 mL) obtained from the total lipids extraction was applied to the column. Neutral lipids were eluted first

using 60 mL of chloroform. After the triacylglycerols were removed, 60 mL of methanol was applied to the column, which resulted in elution of polar lipids. The obtained fractions were completely evaporated to dryness and kept in a hot air oven at 60 °C for 30 min. The final weight of the flasks was taken after desiccating for 30 min. Both neutral and polar lipid parts were determined gravimetrically and expressed as percentage.

3.2.12 TBARs measurement

Lipid susceptibility to oxidation was measured by the induced thiobarbituric acid reactive substances (TBARs) method as a modification of the procedure of Kornbrust and Mavis (1980). Briefly, 3 g of sample was homogenized in 27 mL of 1.15% KCl with a Power Gen 1000 S1 homogenizer (Schwerte, Germany) for 1 min at setting 3. A 200 µl aliquot of the homogenate was mixed with 1000 µl of 80 mM Tris-maleate buffer (pH 7.4), 400 µl of 2.5 mM ascorbic acid and 400 µl of 50 mM ferrous sulphate and incubated for 0, 30, 60, 100 and 150 min in a 37 °C water bath. After incubation, 4 mL of TBA-TCA-HCl mixture (26 mM TBA (thiobarbituric acid), 0.92 M TCA (trichloroacetic acid) and 0.8 mM HCl) was added to the sample and further the test tubes were placed in boiling water for 15 min. After cooling to room temperature, the absorbance was recorded at 532 nm against the blank containing all the reagents except homogenate. TBARs concentration was calculated using the extinction coefficient of $E_{532} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$. The extent of lipid oxidation was expressed as nanomoles of malondialdehyde (MDA) per gram of meat.

3.2.13 Analysis of connective tissue components

Collagen and glycosaminoglycan concentration in raw meat, sediment after first centrifugation, and final isolates were estimated by analyzing hydroxyproline and uronic acid contents, respectively. Samples were dehydrated and defatted with several changes of acetone and then with chloroform: methanol (2:1, vol/vol) solution. For hydroxyproline analysis, dry-defatted samples (50-100 mg) were hydrolyzed in 6 N HCl in the presence of nitrogen at 110 °C for 20 h. Then, hydrochloric acid was removed by evaporation in a hot water bath (80 °C) with nitrogen flushing. The dried preparation was cooled to room temperature, dissolved in water and filtered (Whatman No. 1). The clear filtrate was subjected to the colorimetric method of hydroxyproline analysis as reported by Stegemann and Stalder (1967).

For uronic acid determination, dry-defatted samples (50-200 mg) were digested with twice crystallized papain (4 μg/mg of tissue) in 20 volumes of 0.1 M sodium acetate buffer (pH 5.5) containing 0.005 M EDTA and 0.005 M cysteine hydrochloride at 65 °C overnight. After proteolysis, trichloroacetic acid was added to each digest to a final concentration of 7% (wt/vol) and the mixture was held at 4 °C overnight. After the removal of precipitated proteins by filtration (Whatman No. 1), the filtrate was dialyzed in running tap water for 24 h and then for another 24 h in double deionized water at 4 °C. The uronic acid content in glycosaminoglycan containing fraction retained in the dialysis tube was determined by the carbazole reaction (Bitter and Muir, 1962; Kosakai and

Yosizawa, 1979) with glucuronolactone as a standard. The reaction mixture consisted of 0.5 mL of solution containing glycosaminoglycan or glucuronolactone standard, 3.0 mL of sulfuric acid reagent (0.2 M sodium tetraborate decahydrate in sulfuric acid) and 0.1 mL of 0.5% (wt/vol) carbazole in methanol.

3.2.14 Statistical analysis

The entire experiment, from MSTM through final protein isolate was replicated at least three times. The results were expressed as mean value ± standard deviation. Data were subjected to one-way-analysis of variance (ANOVA) using the General Linear Model procedure of the Statistical System Software of SAS institute (2006). To identify significant differences among mean values within the evaluated parameters at various pH treatments, HSD Tukey`s adjustment with a 95% confidence level ($P < 0.05$) was performed.

3. 3 RESULTS AND DISCUSSION

3.3.1 Protein solubility

The basis for using pH-shifting processing on MSTM utilization is the fact that solubilization of muscle proteins is maximum at low and high pH values. Solubility is not only significant for the determination of the optimum conditions for protein extraction, but also of great importance in food industry applications. The high solubility at certain pH values is required for efficient separation of the soluble proteins from undesirable meat constituents (lipids, connective tissue,

impurities, etc.). However, low solubility is needed to precipitate the solubilized proteins at pI (Kristinsson et al., 2005) for better recovery. In order to investigate the effect of different pHs on MSTM proteins, a solubility curve was constructed with pH range from 1.5 to 12.0 in 0.5 increments (Figure 3.1).

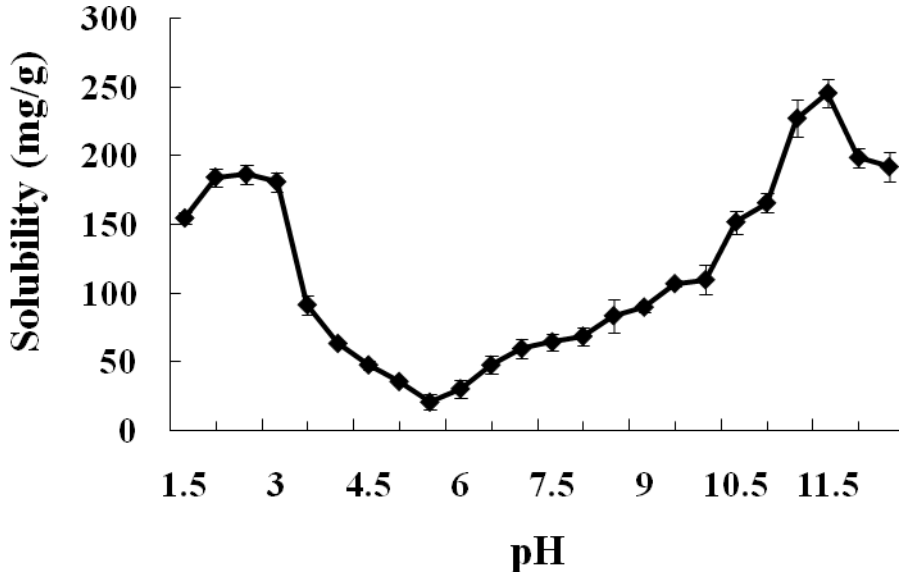

Figure 3.1. The solubility (mg/g) profile of mechanically separated turkey meat (MSTM) proteins at pH values from 1.5 to 12.0. Muscle tissue was homogenized in 50 volumes of deionized water and pH was adjusted by using 0.2 M and 1 M HCl or NaOH. Results are presented as mean (n=4) ± standard deviation.

The lowest solubility (or highest precipitation) in deionized water occurred at pH 5.5, which is in the range of isoelectric points for the majority of muscle proteins (Xiong, 1997). At the pI, the negative and positive charges are equal, thus association among protein molecules is strong due to the ionic linkages (Kinsella, 1984). As a consequence, protein-water interactions are replaced by protein-protein interactions and precipitation occurs. An increase in solubility was

observed with either acidification or alkalization, when the proteins become positively or negatively charged, respectively. These net charges provide more binding sites for water, resulting in electrostatic repulsion among molecules, hydration of charged residues and increased protein-solvent interactions contributing to the increased solubility (Hamm, 1994). The highest protein solubility in acidic conditions, (186.2 mg/g) was attained at pH 2.5, while for alkaline conditions a maximum value of 245.3 mg/g was found with pH 11.5. The rapid increase in solubility on the acidic side compared to the alkaline might be attributed to more ionizable groups with pKa values between 2.5 and 7.0 than between 7.0 and 11.0 (Undeland et al., 2002). The protein solubility profile showed a U-shaped pattern; however, unlike the typical solubility curve for fish muscle protein homogenates, the solubility was found to be the maximum at pH 11.5 and decreased at pH 12.0. Therefore, additional pH points 11.25 and 11.75 were added to the MSTM protein solubility analysis. The results confirmed the decreasing solubility with increasing pH from 11.5 to 12.0. This is in agreement with Omana et al. (2010), who reported the same trend for reduction in solubility from pH 10.5 to 12.0 for the chicken dark meat. This finding further indicated that poultry meat proteins are likely to behave differently when exposed to the extreme alkaline conditions compared to fish muscle proteins.

The pH-shifting process, which is widely used for extraction of proteins from fish sources, was found to be possible to apply for the recovery of poultry meat proteins, MSTM in particular. Based on the solubility study, four pH values

(2.5; 3.5; 10.5 and 11.5) were selected as solubilization pHs for the protein extraction from MSTM.

3.3.2 Protein content and recovery yield

A high recovery yield is important for economic reasons. The yield of protein achieved by acid and alkaline treatments is predominantly driven by three major factors: the solubility of the protein during exposure to low or high pH, the size of the sediments after centrifugation and the solubility at precipitation pH (Nolsoe and Undeland, 2009). The results obtained for different extraction stages are shown in Table 3.1.

Table 3.1. Protein content (%) and recovery yield (%) during different stages of protein extraction from MSTM[1]

Extraction pH	Protein yield after pI, %	Final protein content, %	Final recovery yield, %
pH 2.5	70.6 ± 1.7	18.5^{b} ± 0.6	66.4^{a} ± 5.4
pH 3.5	69.1 ± 2.2	18.2^{b} ± 1.3	57.1^{b} ± 4.7
pH 10.5	67.3 ± 6.9	19.6^{a} ± 0.2	63.6^{ab} ± 6.3
pH 11.5	68.7 ± 1.4	19.0^{ab} ± 0.2	64.8^{a} ± 2.5

[1]Results are presented as mean (n = 4) ± standard deviation.
Different letters within a column indicate significant difference; $P<0.05$.
Protein content and moisture of raw MSTM were 10.3% and 64.8%, respectively.
* pI refers to the isoelectric precipitation.

The yield of the proteins recovered by isoelectric precipitation indicated no significant difference (P = 0.7972) due to the extraction pH. The final protein

content was found to be different between acid and alkaline treatments, with a tendency to increase from low to high pH values. Final protein content was found to be maximum (19.6%) when MSTM was solubilized at pH 10.5, and minimum when solubilized at pHs 2.5 and 3.5. Protein yield did not show any statistical difference between the extractions carried out at pH 2.5, 10.5 and 11.5, while yield from extraction at pH 3.5 was considerably lower ($P = 0.0097$). The increase in recovery yield for pHs of 2.5 and 11.5 is highly associated with the solubility profile (Figure 3.1), which showed the highest solubility at these pH values. Slight decrease of recovery yield at pH 10.5 resulted mainly from decreased amount of solubilized proteins as indicated by the MSTM solubility profile. In general, the percentage loss in recovery yield between precipitation (pH 5.2) and re-adjusting to pH 6.2 was found to be around 6%. The results indicated that optimizing pH during solubilization is a prerequisite step to achieve the maximum protein recovery from MSTM.

3.3.3 Extractability of recovered proteins

Extractability is an important property since the amount of protein available in the solution affects the functional properties expected from proteins. The conformation of proteins, which is related to the environment, plays a significant role in determination of protein functionality. Also protein extractability relates to the surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interactions (Damodaran, 1997).

The highest total protein extractability was observed at pH 10.5, with a value of 73.7 mg/g (Figure 3.2).

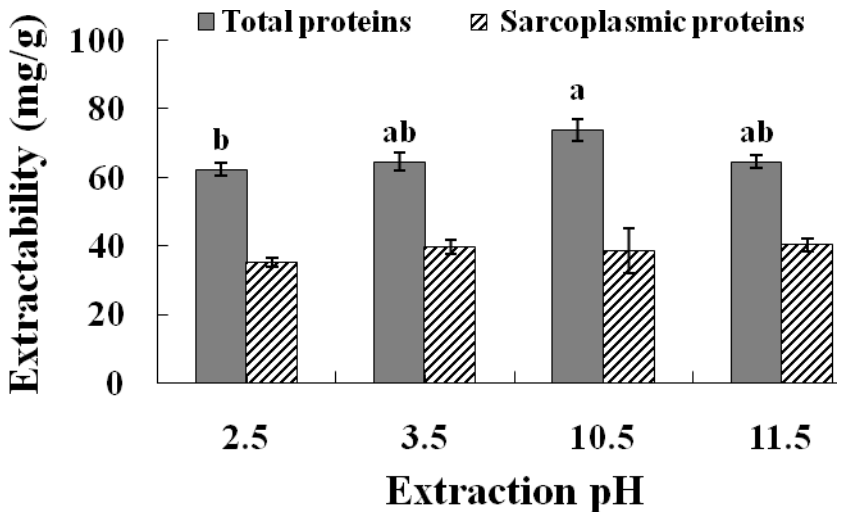

Figure 3.2. Extractability of proteins recovered from MSTM by acid- and alkaline-aided extractions. Sarcoplasmic proteins were solubilized in phosphate buffer, while total proteins were solubilized in phosphate buffer (pH 7.4) containing potassium iodide. Results are presented as mean (n=4) ± standard deviation. Different letters for respective parameters in the figure represent significant ($P < 0.05$) difference.

The difference in extractability between solubilization pHs can be explained by the different degrees of denaturation and the consequence of different degree of protein refolding after pH readjustment to 6.2. Our results indicated that protein isolates at pH 10.5 were less denatured compared to those prepared at pH 2.5, 3.5 and 11.5. The lowest amount of solubilized total proteins (62.3 mg/g) was found at extraction pH of 2.5. Kristinsson and Hultin (2004) showed that lower solubility was a result of improper protein unfolding. Sarcoplasmic protein extractability from recovered proteins as a function of pH was not significantly (*P*

= 0.0563) different among treatments (Figure 3.2). The sarcoplasmic protein fraction comprised around 58% of total soluble proteins, which confirms the fundamental theory of the pH-shifting method, that a sizeable amount of sarcoplasmic proteins are recovered during acid- and alkali-aided processes (Ingadottir, 2004).

3.3.4 Protein surface hydrophobicity

Hydrophobic interactions play a major role in defining the conformation and interactions of protein molecules in solution, thereby affecting the stability of native protein structures. Surface hydrophobicity of proteins helps to determine the rate of protein unfolding due to different processing methods (Mohan et al., 2006).

Myofibrillar protein hydrophobicity (Figure 3.3) was shown to be significantly different ($P < 0.0001$) between treatments and the trend was similar to that observed in protein extractability (Figure 3.2).

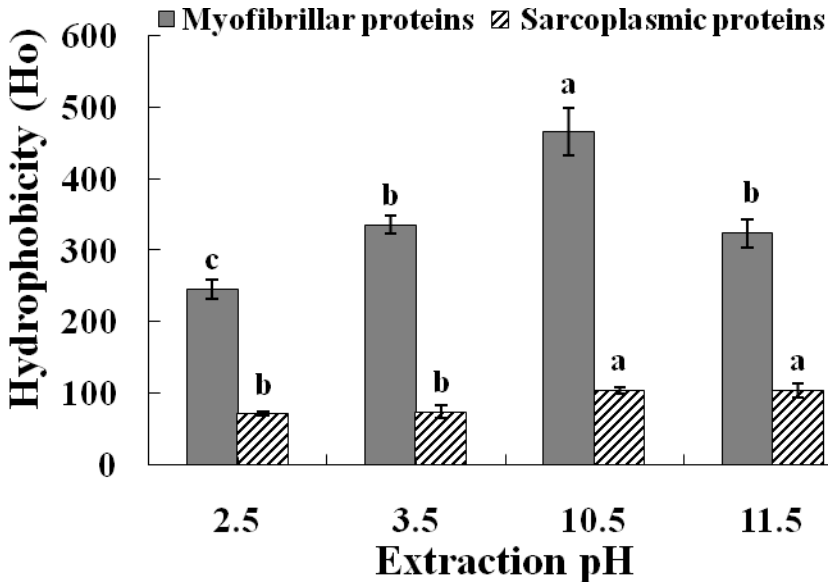

Figure 3.3. Surface hydrophobicity of myofibrillar and sarcoplasmic proteins at different extraction pH values. Hydrophobicity is expressed as initial slopes of relative fluorescence intensity versus protein concentration in the presence of 1-anilino-8-napthalenesulfonate. Results are presented as mean (n=4) ± standard deviation. Different letters for respective parameters in the figure represent significant ($P < 0.05$) difference.

Extraction at pH 10.5 resulted in the highest myofibrillar hydrophobicity (Ho = 465). Similar values were observed for extractions conducted at pH values of 3.5 and 11.5 while at pH 2.5 extracted samples represented the lowest value. The myofibrillar hydrophobicity was found to increase with an increase in total protein extractability (Figures 3.2 and 3.3). Even though the observed results appear to be in contradiction, it is important to point out that protein extractability depends not only on the amount of hydrophobic groups exposed to the protein surface, but also on the intrinsic factors such as protein conformation and surface polarity/hydrophobicity ratio (Kinsella, 1982). In these circumstances, although proteins isolated at pH 10.5 showed the highest surface hydrophobicity and

protein extractability, it might be possible that after the readjustment to pH 6.2, the amount of polar and ionic groups were still predominant over the non-polar groups even if these latter were exposed to the surface. Therefore, exploring the surface polarity/hydrophobicity ratio could be a better indicator of protein denaturation than surface hydrophobicity by itself.

Sarcoplasmic protein hydrophobicity of the extracted proteins was significantly higher ($P < 0.0001$) for the alkali processed samples compared to acidic treatments (Figure 3.3). The cause of increased hydrophobicity might be due to the change in protein conformation, particularly due to partial protein unfolding. As a result, the intramolecular bonds which stabilize protein structure are ruptured, thus facilitating the exposure of hydrophobic groups to the surface (Lin and Park, 1998).

3.3.5 Sulfhydryl groups content

Sulfhydryl group is considered to be the most reactive functional group in proteins. The total and reactive sulfhydryl content (Figure 3.4) of proteins extracted at different pH values indicated no significant difference between treatments ($P = 0.5825$ and $P = 0.9841$, respectively), even though hydrophobicity was higher at pH 10.5.

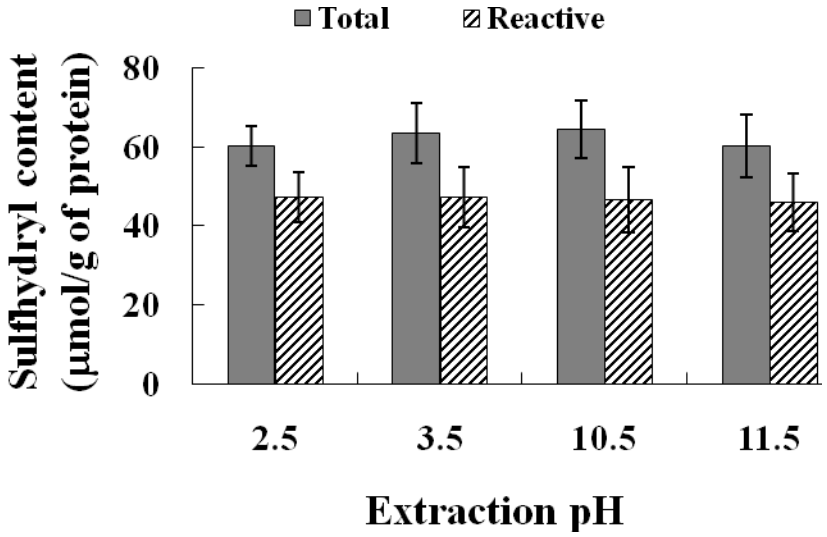

Figure 3.4. Total and reactive (free) sulfhydryl group content of proteins recovered from MSTM at different extraction pH values. Analyses were performed by using Ellman's reagent. Results are presented as mean (n=4) ± standard deviation.

Monahan et al. (1995) also observed an increase in hydrophobicity with no change in sulfhydryl group content, probably due to the SH-SS exchange reactions. However, an increase in total and reactive sulfhydryl group content was found for pH treated samples compared to raw MSTM (data not shown), which is probably related to the protein unfolding, resulting in exposure of sulfhydryl groups to the protein surface. The ratio T-SH/R-SH for raw and processed meat was also characterized. For raw MSTM, the ratio was equal to 1.42. A slight decrease of T-SH/R-SH ratio for protein isolates (1.32 and 1.36 for acid and alkaline extractions, respectively) was observed, which may be the result of increasing the amount of disulfide bond formation (Pires et al., 2008).

3.3.6 SDS-PAGE profile

Protein bands corresponding to myosin heavy chains (MHC) and actin were most abundant after isoelectric precipitation fractions and in the final protein isolate (Figure 3.5).

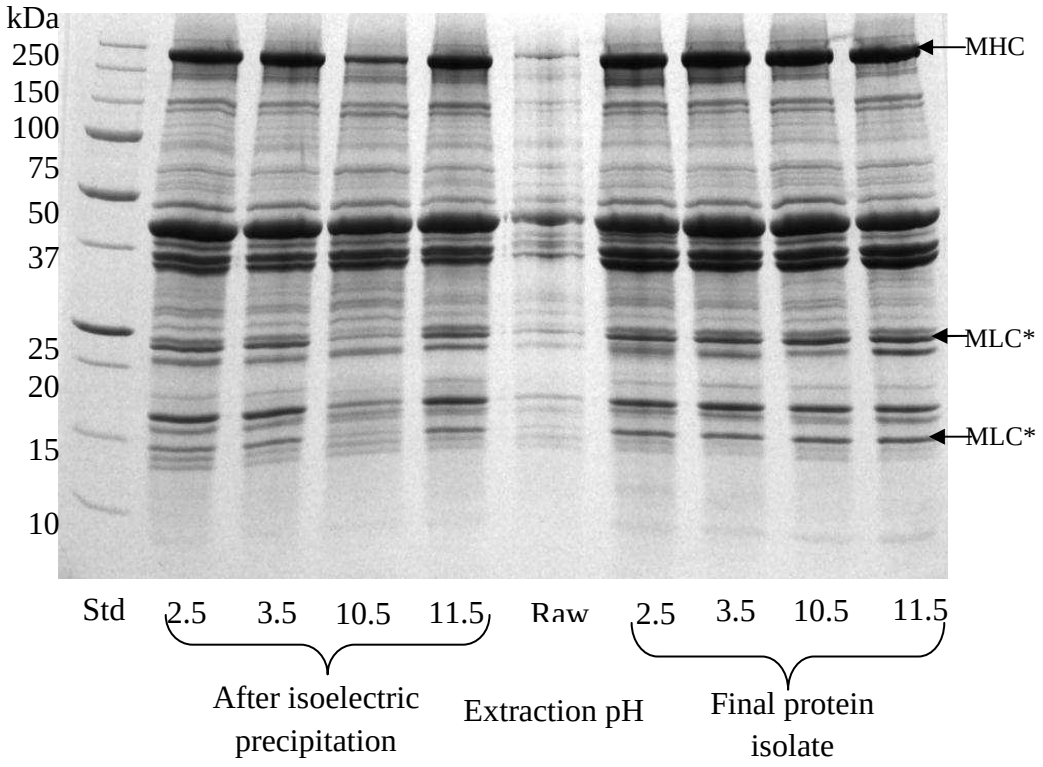

Figure 3.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of different samples from acid and alkaline extraction processes. Std - the molecular weight standard.
MHC – myosin heavy chains.
*MLC – myosin light chains.

No difference in protein profile was observed among different pH treatments for the final protein isolates. The presence of myosin light chains of low molecular weight showed the degradation of myosin into its subunits. The

intensity of bands corresponding to myosin heavy chain and actin increased in the extracted samples suggesting that the concentration of these proteins increased in the final protein isolates. Hence, this may have effects on the improved functionality of proteins in the final isolates compared to that of raw material. Our recent study (chapter 4) showed appreciable gelation, emulsion and foaming characteristics of the MSTM protein isolates. With improved functionalities of protein isolates, MSTM can be better utilized for value-added processing.

3.3.7 Amino acid composition

The amino acids composition of raw MSTM and protein isolates obtained by extractions at different pH is shown in Table 3.2.

Table 3.2. Amino acid composition of raw mechanically separated turkey meat (MSTM) and protein recovered from MSTM at different extraction pH values

Amino acid	Raw MSTM	Extracted with pH 2.5	Extracted with pH 3.5	Extracted with pH 10.5	Extracted with pH 11.5
Alanine	9.3 ± 0.7	10.0 ± 0.5	10.0 ± 0.3	8.6 ± 1.2	10.0 ± 0.4
Arginine[1]	6.2^{a} ± 0.4	5.0^{b} ± 0.2	5.4^{b} ± 0.4	5.1^{b} ± 0.1	5.2^{b} ± 0.2
Aspartic acid	9.6^{c} ± 0.2	11.5^{ab} ± 0.5	11.3^{abc} ± 0.6	10.2^{bc} ± 1.2	12.0^{a} ± 0.1
Glutamic acid	15.8^{b} ± 0.6	21.6^{a} ± 1.3	20.8^{a} ± 1.3	18.1^{ab} ± 3.2	20.5^{ab} ± 1.6
Glycine	7.6 ± 0.4	5.9 ± 0.1	6.1 ± 0.3	6.6 ± 1.1	7.0 ± 0.9
Histidine[1]	3.3^{a} ± 0.01	0.7^{b} ± 1.2	0.5^{b} ± 0.9	0.6^{b} ± 0.6	ND^{b}
Isoleucine[*, 2]	5.3 ± 0.3	4.5 ± 0.2	4.8 ± 0.2	5.1 ± 0.7	4.9 ± 0.2
Leucine[*,2]	8.5 ± 1.3	8.1 ± 0.5	9.2 ± 1.0	9.1 ± 2.7	8.5 ± 0.6
Lysine[*]	6.5^{b} ± 0.3	10.2^{a} ± 0.8	9.7^{a} ± 1.3	7.8^{ab} ± 0.2	8.8^{ab} ± 0.06
Methionine[*]	2.2^{a} ± 0.2	ND^{b}	0.3^{b} ± 0.3	ND^{b}	ND^{b}
Phenylalanine[*]	3.6 ± 0.01	3.3 ± 0.6	3.2 ± 0.2	3.7 ± 0.7	3.6 ± 0.3
Proline	4.1 ± 0.08	3.4 ± 0.2	3.4 ± 0.3	3.5 ± 0.5	3.4 ± 0.1
Serine	4.3^{a} ± 0.2	3.3^{b} ± 0.2	3.6^{ab} ± 0.3	3.9^{ab} ± 0.4	3.8^{ab} ± 0.1
Threonine[*]	4.8 ± 0.5	4.0 ± 0.2	3.9 ± 0.6	4.1 ± 0.6	4.1 ± 0.5
Tyrosine	2.9 ± 0.2	2.9 ± 0.7	2.0 ± 0.8	1.3 ± 0.1	2.2 ± 0.9
Valine[*, 2]	5.90 ± 0.26	5.7 ± 0.2	5.7 ± 0.1	5.8 ± 0.6	5.4 ± 0.1
TEAA:TNEAA, %	36.8 ± 0.6	35.8 ± 0.4	36.9 ± 1.0	42.3 ± 7.1	36.0 ± 0.2

Values are expressed as mole / 100 moles (relative %)
Results are presented as mean (n = 3) ± standard deviation. Different letters within a row indicate significant difference; $P < 0.05$.
TEAA – Total essential amino acids; TNEAA – total nonessential amino acids.
ND - The amount of amino acid is below the detectable level.
*- Essential amino acids. Body cannot synthesize them, therefore it should derive from food or amino acids supplements.

1 - Essential for infants, since their bodies cannot produce them yet.
2 - Branched-chain amino acids (BCAA). Important to maintain muscle tissue and also during times of physical stress and intense exercise.

Glutamic acid was found to be the predominant amino acid and was significantly ($P = 0.0143$) higher in acid treated samples compared to raw meat. A possible explanation for such an increase may be partially due to the oxidation of proline to glutamic acid, as reported by Stadtman (1993). Despite the extractions did not statistically affect proline concentration, values were lower in acid treated samples compared to the starting material. Lysine, an essential dietary amino acid, was found to be significantly ($P = 0.0023$) increased for the acid treated samples compared to the raw meat. No significant difference ($P > 0.05$) was found between raw and processed meat for alanine, glycine, isoleucine, leucine, phenylalanine, proline, threonine, tyrosine and valine. A significant ($P < 0.0001$) loss of methionine for the pH treated samples was observed. The reason for the methionine loss might be due to its oxidation during the extraction process, where the proteins are exposed to acidic or alkali environment. It was reported that methionine can be oxidized to methionine sulfoxide and methionine sulfone during processing (Rutherfurd and Moughan, 2008). The amino acid histidine was found to decrease 82% on average for all extraction pH values, excluding pH 11.5 where it was not detected. It was reported that histidine is an essential amino acid for infants (Sathe et al., 2002); however histidine is identified as a precursor for production of histamine. Histamine is known to be a cause of allergic reactions (Jutel et al., 2005). The reduction in histidine content might be due to its oxidation

during the extraction process. Cheng and Kawakishi (1994) reported that histidine is sensitive to oxidation and aspartic acid was identified as the major oxidation product of histidine (Dean et al., 1989; Ueda et al., 1994). Oxidation of histidine in the present study is further confirmed by the significant (P = 0.0074) increase in aspartic acid content in the protein isolates compared to raw MSTM. Our results are in agreement with the study of Shahidi and Synowiecki (1996) who reported a decrease in methionine and histidine content along with increasing of glutamic acid content during alkaline extraction of mechanically separated seal meat. The ratio of total essential amino acids to total amino acids showed no statistically significant (P = 0.1575) differences.

3.3.8 Lipid reduction and TBARs

Lipid reduction is the principal factor for producing functional protein isolates from MSTM since the raw material is highly rich in triacylglycerols and membrane phospholipids. The latter contribute greatly to the oxidative reactions due to the high content of unsaturated fatty acids (Hultin, 1995). The amount of lipids that can be removed is linked to the fat content of the starting material (Nolsoe and Undeland, 2009). The total, neutral and polar lipids content of raw MSTM were 23.5, 14.3 and 7.5%, respectively. Acid and alkaline extractions of MSTM resulted in protein isolates with significantly (P < 0.0001) reduced lipid content compared to the initial material (Table 3.3).

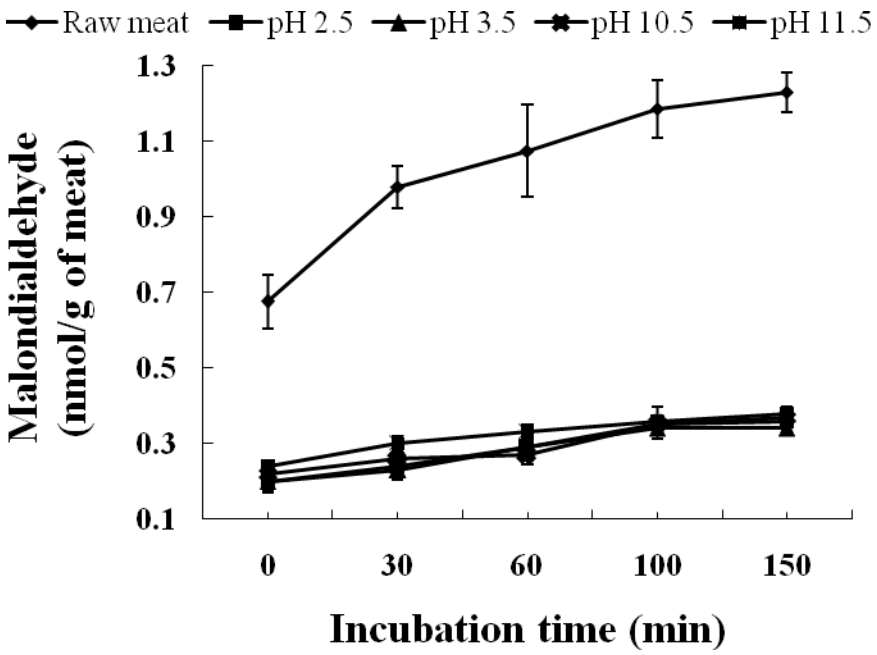

Figure 3.6. Effect of time and extraction pH on oxidative stability of raw material and proteins recovered from MSTM as determined by the induced thiobarbituric acid reactive substances (TBARs) method. Results are presented as mean (n=4) ± standard deviation.

However, no significant (P > 0.05) difference was found among pH treatments for removal of total, neutral and polar lipids, which on average were equal to 92.3, 93.0 and 90.7%, respectively. A large reduction of lipids from MSTM by the pH-shifting technique was expected, as at extreme pH values, the proteins are solubilized, favoring the release of the storage and membrane lipids (Rawdkuen et al., 2009). During the centrifugation step the lipids are released to the aqueous environment due to the differences in solubility and particle density (Kristinsson et al., 2005). The meat: water ratio (1:5, wt/vol) used in the study also contributed to the high removal of lipids from MSTM (Nolsoe and Undeland,

2010). Several studies have showed that a pH-shifting process is effective for lipids removal. Kristinsson and colleagues (2005) reported an 85.4% and 88.6% lipid reduction from skinless catfish fillets as affected by acid and alkaline extractions. Kristinsson and Demir (2003) found a lipid reduction of 76.9% for acid and 79.1% for alkaline extractions from mackerel fish.

The effect of different extraction pHs on TBARs development in the MSTM protein isolates is shown in Figure 3.6. Analysis on lipid oxidation showed no significant difference among different pH treatments ($P > 0.05$). However, there was a significant ($P < 0.001$) decrease in the amount of MDA for recovered meat compared to the raw meat. The reason for decreased lipid oxidation is probably due to the higher removal of membrane lipids. No difference in the amount of MDA in the samples processed at different pH is consistent with the analysis of polar lipids content (Table 3.3), wherein no differences were found among the various extraction pH conditions.

Table 3.3. Lipid composition of protein isolates recovered from MSTM at different extraction pH values[1]

Treatment pH	Total lipids		Neutral lipids		Polar lipids	
	Content, %	Reduction, %	Content, %	Reduction, %	Content, %	Reduction, %
			Acid extraction process			
pH 2.5	1.77 ± 0.14	92.5 ± 0.6	0.96 ± 0.06	93.3 ± 0.5	0.66 ± 0.08	91.2 ± 1.0
pH 3.5	1.82 ± 0.09	92.3 ± 0.4	0.98 ± 0.14	93.1 ± 1.0	0.72 ± 0.09	90.4 ± 1.3
			Alkaline extraction process			
pH 10.5	1.83 ± 0.26	92.2 ± 1.1	1.04 ± 0.2	92.7 ± 1.4	0.73 ± 0.15	90.3 ± 2.0
pH 11.5	1.80 ± 0.20	92.3 ± 0.8	1.05 ± 0.09	92.7 ± 0.7	0.70 ± 0.08	90.7 ± 1.0

[1]No statistical differences were observed. Data were statistically analyzed by one-way ANOVA. Results are presented as mean (n=4) ± standard deviation.
The total, neutral and polar lipids content of raw MSTM were 23.5, 14.3 and 7.5%, respectively.

3.3.9 Analysis of connective tissue fractions

The extracellular matrix of connective tissue is composed of collagen fibers embedded in an amorphous ground substance containing glycosaminoglycans (Nakano et al., 2004). Glycosaminoglycans are linear unbranched polymers of repeating disaccharide units of hexosamine and uronic acid. Thus, the amount of uronic acid residues is important for quantitative analysis of glycosaminoglycans. Collagen is the major protein of connective tissue, with relatively small amounts of glycosaminoglycans. Determination of the amino acid hydroxyproline is an accurate way of measurement of collagen, since there are no other known animal proteins containing any appreciable amounts of this amino acid (Gross, 1958). The collagen and glycosaminoglycans concentrations were estimated by determining hydroxyproline and uronic acid, respectively, in MSTM sediment obtained after the first centrifugation and in the final protein isolate (Table 3.4).

Table 3.4. Hydroxyproline and uronic acid contents (μg/mg of dry-defatted weight) in mechanically separated turkey meat (MSTM) and its protein fractions obtained during extraction process[1]

Treatment	Hydroxyproline content in the sediment after 1st centrifugation	Hydroxyproline content in the final protein isolate	Uronic acid content in the sediment after 1st centrifugation	Uronic acid content in the final protein isolate
Raw MSTM	$11.6^{c} \pm 0.8$	–	$1.7^{c} \pm 0.1$	–
pH 2.5	$50.6^{a} \pm 4.0$	0.5 ± 0.1	$7.6^{a} \pm 0.1$	$0.5^{b} \pm 0.1$
pH 3.5	$31.7^{b} \pm 5.2$	0.6 ± 0.2	$4.3^{b} \pm 0.0$	$0.4^{b} \pm 0.0$
pH 10.5	$23.3^{bc} \pm 4.5$	0.6 ± 0.3	$1.6^{c} \pm 0.2$	$0.9^{a} \pm 0.0$
pH 11.5	$59.1^{a} \pm 5.5$	0.3 ± 0.1	$3.5^{b} \pm 0.5$	$0.8^{a} \pm 0.1$

[1]Results are presented as mean (n=2) ± standard deviation. Different letters within a column indicate significant difference; $P < 0.05$.

The hydroxyproline concentration in the final isolates (< 1 μg/mg) was on average 23 and 82 times lower compared to MSTM (≈ 12 μg/mg) and the sediment fraction (23.3-59.1 μg/mg), respectively, with no significant difference among pH treatments (P = 0.5026). This indicates that the myofibrillar and sarcoplasmic proteins were the major part of the extracted proteins, while most of the connective tissue (collagen) were present in the sediment. At acidic extraction conditions, the concentration of hydroxyproline in the sediment was 1.6 times higher at pH 2.5 compared to pH 3.5.

A similar trend between these pH values was observed for the uronic acid concentration. The ratio of uronic acid to hydroxyproline, which represents the estimation of amorphous ground substance to collagen fiber, was similar between pH 2.5 and 3.5 and was almost identical to the value (0.14) corresponding to MSTM. When the proteins were extracted in alkaline conditions, it was found that hydroxyproline and uronic acid concentrations in the sediment fractions were more than two times higher (P = 0.0005 and P < 0.0001, respectively) at pH 11.5 than pH 10.5. However, the uronic acid values were lower compared to the corresponding values observed at acidic pH, which resulted in the lower (> 3 times) uronic acid to hydroxyproline ratio. Further studies are needed to explain pH dependent variations in the ratio of uronic acid to hydroxyproline.

The results give an insight into the possibility of extracting proteoglycans from the sediment fraction during the co-extraction of valuable components from MSTM.

3.4 CONCLUSION

The study demonstrated that at pH 2.5, 10.5 and 11.5, the proteins from MSTM were most soluble, leading to higher protein yields at these pH values. Among the different extraction pH values, the highest total extractability was achieved at pH 10.5. Acid- and alkaline-aided extractions were equally effective in removing total, neutral and polar lipids from MSTM. Consequently, TBARs analysis showed no difference between acid and alkaline treatments; however the values were significantly lower compared to raw MSTM ($P < 0.0001$). SDS-PAGE profiles for both acid and alkaline extractions indicated higher concentration of myosin heavy chains and actin compared to MSTM indicating concentration of myofibrillar proteins. No statistical difference was found in the ratio of total essential amino acids to total amino acids between MSTM and extracted proteins. Analysis of uronic acid content revealed that most of the proteoglycans accumulated in the sediment fractions, hence paving the way for a co-extraction technology of the valuable components from MSTM.

3.5 REFERENCES

Badley, E. M. 1995. The effect of osteoarthritis on disability and health care in Canada. J. Rheumatol. Suppl. 43:19-22.

Betti, M., and D. L. Fletcher. 2005. The influence of extraction and precipitation pH on the dry matter yield of broiler dark meat. Poult. Sci. 84:1303-1307.

Bitte, T., and H. M. Muir. 1962. A modified uronic acid carbazole reaction. Anal. Biochem. 4:330-334.

Black, C., C. Clar, R. Henderson, C. MacEachern, P. McNamee, Z. Quayyum, P. Royle, and S. Thomas. 2009. The clinical effectiveness of glucosamine and chondroitin supplements in slowing or arresting progression of osteoarthritis of the knee: a systematic review and economic evaluation. Health Technol. Assess. 13:1-148.

CFC (Chicken Farmers of Canada). 2008. Annual report 2008. Chicken Farmers of Canada. Accessed Jan. 2010. http://www.chicken.ca/app/wa/mediaEntrymediaEntryId=5230.

Cheng, R. Z., and S. Kawakishi. 1994. Site-specific oxidation of histidine residues in glycates insulin mediated by Cu^{2+}. Eur. J. Biochem. 223:759-764.

Choi, Y. J., and J. W. Park. 2002. Acid-aided protein recovery from enzyme-rich Pacific whiting. J. Food Sci. 67:2962-2967.

Damodaran, S. 1997. Food proteins: an overview. Pages 1-24 in Food Proteins and Their Applications. S. Damodaran, and A. Paraf, eds. Marcel Dekker Inc., New York, NY.

Dean, R. T., S. P. Wolff, and M. A. McElligott. 1989. Histidine and proline are important sites of free radical damage to proteins. Free Rad. Res. Comm. 7:97-103.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:467-509.

Gornall, A. C., C. J. Bradwill, and M. M. David. 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem. 177:755-766.

Gross, J. 1958. Studies on the formation of collagen: I. Properties and fractionation of neutral salt extracts of normal guinea pig connective tissue. J. Exp. Med. 107:247-263.

Hamm, R. 1994. The influence of pH on the protein net charge in the myofibrillar system. Rec. Meat Conf. Proc. 47:5-9.

Hultin, H. O. Role of membranes in fish quality. 1995. Pages 13-36 in Proceedings from the conference: Fish quality-role of biological membranes. F. Jessen, ed. Nordic Council of Ministers. Copenhagen, DN.

Hultin, H. O., and S. D. Kelleher. 1999. Process for isolating a protein composition from a muscle source and protein composition. Advanced Protein Technologies, Inc, assignee. US. Pat. No. 6,005,073.

Hultin, H. O., and S. D. Kelleher. 2000. Surimi processing from dark muscle fish. Pages 59-77 in Surimi and Surimi Seafood. J. W. Park, ed. Marcel Dekker, New York Inc., NY.

Ingadottir, B. 2004. The use of acid and alkali-aided protein solubilization and precipitation methods to produce functional protein ingredients from tilapia. MS Thesis. Univ. Florida, US.

Jutel, M., K. Blaser, and C. A. Akdis. 2005. Histamine in chronic allergic responses. J. Invest. Allergol. Clin. Immunol. 15:1-8.

Kim, Y. S., J. W. Park, and Y. J. Choi. 2003. New approaches for the effective recovery of fish proteins and their physicochemical characteristics. Fish Sci. 69:1231–1239.

Kinsella, J. E. 1982. Relationships between structure and functional properties of food proteins. Pages 51-103 in Food Proteins. Fox P. F. and J. J. Condon, eds. Applied Science Publishers, London, UK.

Kinsella, J. E. 1984. Milk proteins: physicochemical and functional properties. CRC Crit. Rev. Food Sci. Nutr. 21:197-262.

Kornbrust, D. J., and R. D. Mavis. 1980. Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correction with vitamin E content. Lipids. 15:315-322.

Kosakai, M., and Z. Yosizawa. 1979. A partial modification of the carbazole method of Bitter and Muir for quantitation of hexuronic acids. Anal. Biochem. 93:295-298.

Kristinsson, H. G., and H. O. Hultin. 2004. Changes in trout hemoglobin conformations and solubility after exposure to acid and alkali pH. J. Agric. Food Chem. 52:3633-3643.

Kristinsson, H. G., and N. Demir. 2003. Functional fish protein ingredients from fish species of warm and temperate waters: comparison of acid- and alkali-aided processing vs. conventional surimi processing. Pages 277-298 in Advances in Seafood By-Products. P. J. Bechtel, ed. Univ. of Alaska Press: Fairbanks, AK.

Kristinsson, H. G., A. E. Theodore, N. Demir, and B. Ingadottir. 2005. A comparative study between acid- and alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. J. Food Sci. 70:298-306.

Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature. 277:680-685.

Liang, Y., and H. O. Hultin. 2003. Functional protein isolates from mechanically deboned turkey by alkaline solubilization with isoelectric precipitation. J. Muscle Foods. 14:195-205.

Lin, T. M., and J. W. Park. 1998. Solubility of salmon myosin as affected by conformational changes at various ionic strengths and pH. J. Food Sci. 63:215-218.

Maestre R., M. Pazos, J. Iglesias, and I. Medina. 2009. Capacity of reductants and chelators to prevent lipid oxidation catalyzed by fish hemoglobin. J. Agric. Food Chem. 57:9190-9196.

Mohan, M., D. Ramachandran, and T. V. Sankar. 2006. Functional properties of Rohu (*Labeo rohita*) proteins during iced storage. Food Resr. Inter. 39:847-854.

Monahan, F. J., J. B. German, and J. E. Kinsella. 1995. Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. J. Agric. Food Chem. 43:46-52.

Nakano, T., Y. W. Wang, L. Ozimek, and J. S. Sim. 2004. Chemical composition of the infrapatellar fat pad of swine. J. Anat. 204:301-306.

Nolsoe, H., and I. Undeland. 2009. The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. Food Bioproc. Technol. 2:1-27.

Omana, D., Y. Xu, V. Moayedi, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Chemical and functional properties of recovered proteins. Process Biochem. 45:375-381.

Pepe, T., M. Trotta, I. Marco, A. Anastasio, J. M. Bautista and M. L. Cotesi. 2007. Fish species identification in surimi-based products. J. Agric. Food Chem. 55:3681-3685.

Pires, C., I. Batista, V. Godinho, and M. L. Nunes. 2008. Functional and biochemical characterization of proteins remaining in solution after isoelectric precipitation. J. Aquatic Food. Prod. Technol. 17:60-72.

Ramadan, M. F., and J. T. Morsel. 2003. Determination of the lipid classes and fatty acid profile of Niger (*Guizotia abyssinica* Cass.) seed oil. Phytochem. Anal. 14:366-370.

Rawdkuen, S., S. Sai-Ut, S. Khamsorn, M. Chaijan, and S. Benjakul. 2009. Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. Food Chem. 112:112-119.

Rutherfurd, S. M., and P. J. Moughan. 2008. Determination of sulphur amino acids in foods as related to biovalidity. J. AOAC. Int. 91:907-913.

SAS user's guide. Statistics, Version 9.0, SAS Institute. Inc., Cary, NC, US. 2006.

Sathe, S. K., B. R. Hamaker, K. W. C. Sze-Tao, and M. Venkatachalam. 2002. Isolation, purification, and biochemical characterization of a novel water soluble protein from Inca Peanut (*Plukenetia volubilis* L.). J. Agric. Food Chem. 50:4906-4908.

Shahidi, F., and J. Synowiecki. 1996. Alkali-assisted extraction of proteins from meat and bone residue of harp seal (*Phoca groenlandica*). Food Chem. 57:317-321.

Stadtman, E. R. 1993. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu. Rev. Biochem. 62:797-821.

Stegemann, H., and K. Stalder. 1967. Determination of hydroxyproline. Clin. Chim. Acta. 18:267-273.

Torten, J., and J. R. Whitaker. 1964. Evaluation of the Biuret and dye-binding methods for protein determination in meats. J. Food Sci. 29:168-174.

Ueda, J. I., T. Ozawa, M. Miyazaki, and Y. Fujiwara. 1994. Activation of hydrogen peroxide copper (II) complexes with some histidine-containing peptides and their SOD-like activities. J. Inorganic Biochem. 55:123-130.

Undeland, I. A., S. D. Kelleher, and H. O. Hultin. 2002. Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. J. Agric. Food Chem. 50:7371-7379.

USDA/ERS. 2007. Farm animal statistics: meat consumption. Factory farming. Accessed January, 2010. http://www.hsus.org/farm/resources/pubs/stats_meat_consumption.html.

Xiong, Y. L. 1997. Structure-function relationship of muscle proteins. Pages 341-392 in Food proteins and their applications. S. Damodaran and A. Paraf, eds. Marcel Dekker Inc., New York, NY.

Yang, T. S., and G. W. Froning. 1992. Changes in myofibrillar protein and collagen content of mechanically deboned chicken meat due to washing and screening. Poult. Sci. 71:1221-1227.

CHAPTER 4. EFFECT OF ACID- AND ALKALINE-AIDED EXTRACTIONS ON FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF PROTEINS RECOVERED FROM MECHANICALLY SEPARATED TURKEY MEAT (MSTM)[3]

4.1 INTRODUCTION

Development of novel techniques for the recovery and utilization of poultry by-products has been a very active area of research in recent years. These techniques are aimed at isolating the protein fraction from these underutilized meat sources since protein isolates are an important ingredient in the human diet (Young, 1975). Mechanically separated meat is one of the cheapest sources of protein which can be recovered efficiently using different extraction techniques. In the year 2000, more than 182 million kg of mechanically separated poultry meat (MSPM) were produced in the United States (Ockerman and Hansen, 2000). MSPM contains 13-15% of protein (Froning and Johnson, 1973); however, the mechanical deboning process results in the release of a considerable amount of fat and heme components from the bone marrow and through disruption of muscle cells. Therefore, the utilization of MSPM is limited due to its dark color and undesirable textural properties (Froning, 1976). Protein recovery using a pH-shifting process can be used to overcome these problems. The major advantage of this process is that poultry muscle proteins can be recovered economically at a

[3] *A version of this chapter is published in the Journal of Food Science (2010; 75:E477-E486). (Authors: Y. Hrynets, D. A. Omana, Y. Xu and M. Betti).*

relative high recovery rate and the isolated muscle proteins may have improved functionality (Undeland et al., 2002; Kristinsson and Demir, 2003).

Gelation is one of the most important functional properties of muscle proteins during thermal processing. A protein gel is a continuous network structure of macroscopic dimensions immersed in a liquid medium and exhibiting no steady-state flow (Ziegler and Foegeding, 1990). Proteins in gels are important because they contribute to water entrapment and fat particles immobilization in processed meat and poultry products through adhesive forces (Ziegler and Acton, 1984). These properties are critical for the creation of unique textural properties of cooked products, such as frankfurters, bologna, loaves and rolls. Myofibrillar proteins are responsible for the formation of viscoelastic gel matrices and strong cohesive membranes on lipid globules in emulsions (Xiong, 1997). Kristinsson and Hultin (2003) showed that fish proteins possess a unique structure after pH-shifting extraction, which leads to improvement of functional characteristics, such as gelation, emulsification and solubility.

After acid- and alkaline-aided extractions, partially unfolded/refolded protein structure is more flexible and is able to form better protein networks during heating (gelation) and is able to adsorb more readily to interfaces and yield lower interfacial tension (emulsification) (Ingadottir and Kristinsson, 2010). Protein extractions obtained using acid and alkaline treatments of several fish species showed equal or better gel-forming ability compared to those obtained from surimi processing (Kristinsson and Demir, 2003; Undeland et al., 2002).

Darker color is another problem with MSPM, due to the release of a substantial quantity of hemoglobin from bone marrow during mechanical deboning (Froning, 1976). Hemoglobin oxidizes easily and is highly susceptible to heat denaturation during processing and storage. Hemoglobin content was found to be tripled after mechanical deboning of whole fowl carcass (Froning and Johnson, 1973). The study of Moayedi et al. (2010) on the alkaline extraction of proteins from chicken dark meat resulted in increased lightness of protein isolates, which was attributed to the removal of heme pigments (myoglobin and hemoglobin).

Since the utilization of MSPM is also limited due to its poor texture, the assessment of textural properties of proteins isolated from MSPM is of much importance. Texture is an important sensory evaluation parameter that is used as an indicator of food quality (Ross, 2009). Human perception of meat palatability is obtained by an interaction of sensory and physical properties during chewing. Evaluation of texture involves measuring the response of a food when it is subjected to forces, such as cutting, shearing, chewing and compressing. Texture profile analysis (TPA) is a widely used method to determine the textural properties of food. Data on the functional and rheological characteristics of acid- and alkali-recovered protein isolates from mechanically separated turkey meat is not available. Therefore, the objective of this study was to determine the effect of the pH-shifting technique on improving color and textural properties of MSTM. The effect of both, acid and alkaline extractions was investigated with the

emphasis on functional, textural and rheological characteristics of the recovered proteins.

4.2 MATERIALS AND METHODS

4.2.1 Raw material and chemicals

Mechanically separated turkey meat (MSTM) was obtained from Lilydale Inc. (Edmonton, AB, CA). MSTM (250 g) was filled into polyethylene bags and kept at -20 °C until use. Before extraction, samples were thawed overnight at 4 °C. All the reagents and chemicals used in the study were of analytical grade.

4.2.2 Preparation of protein isolate by acid-aided process

The acid-aided protein recovery from MSTM was accomplished by the method described by Liang and Hultin (2003) and Betti and Fletcher (2005) with slight modifications. Protein extractions were conducted under low temperature conditions (4 °C) in order to maintain the functional properties of the final product. To prepare the protein isolate, 200 g of MSTM was homogenized with cold (1-3 °C) distilled water/ice mixture at 1:5 ratio (meat: water/ice, wt/vol) using a 900-Watt Food Processor (Wolfgang Puck WPMFP15, W.P. Appliances Inc., Hollywood, FL, US) for 15 min. Homogenate (1200 mL) was transferred to a beaker and allowed to stand for 30 min at 4 °C. The proteins in the homogenate were then solubilized by drop wise addition of 2 M HCl to reach the maximum solubility at pH values of 2.5 and 3.5, as determined from the solubility profile, as reported in chapter 3. Acidic homogenates were centrifuged in Avanti® J-E refrigerated centrifuge (Beckman Coulter Inc., Palo Alto, CA, US) at 25,900 × *g*

for 20 min at 4 °C. Three phases were formed after centrifugation: a top phase of MSTM neutral lipids; a middle phase of soluble proteins and a sediment phase, containing a water-insoluble protein fraction and membrane lipids. The middle layer of soluble proteins was collected and pH was adjusted to isoelectric point (pH 5.2) with 2 M NaOH. The precipitated proteins were recovered by centrifugation at 25,900 × g for 20 min at 4 °C. The precipitate was re-suspended in water/ice mixture (water/ice, 350mL/350g) by homogenization for 10 min. The pH of the resultant homogenate was adjusted to 6.2 by drop wise addition of 2 M NaOH. Proteins were finally collected by centrifugation at 25,900 × g for 20 min at 4 °C. The moisture content of the recovered protein isolates was adjusted to 80%. Cryoprotectants (5% sorbitol, 4% sucrose, 0.3% tripolyphosphate, 0.4% sodium bicarbonate and 0.03% sodium nitrite) were mixed with protein isolates in a pre-chilled Wolfgang Puck WPMFP15 900-Watt Food Processor (W.P. Appliances Inc., Hollywood, FL, US). The isolated proteins were stored in the freezer at -20 °C until analysis.

4.2.3 Preparation of protein isolate by alkaline-aided process

For the alkali extraction process the procedures described above were followed except for the first solubilization step. Here the MSTM proteins were initially solubilized at pH values of 10.5 or 11.5.

4.2.4 Cooking loss

Raw samples were prepared by manually grinding protein isolate samples (12 g) with 2.5% NaCl in a pestle and mortar for 10 min. The paste was packed in

polypropylene capped tubes (1.7 cm × 10 cm, Simport, QC, CA) without air pockets. The stuffed tubes were then heated in the water bath at 95 °C until the internal temperature reached 75 °C. The internal temperature was measured using thermocouples, inserted in the centre of the sample. After cooking, the gel was removed from the tubes and accurately weighed individually. The samples were then stored in polyethylene bags at 4 °C overnight prior to texture profile analysis. The cooking loss was calculated as follows:

$$\text{Cooking loss (\%)} = \frac{\text{(Original sample weight – weight after cooking)}}{\text{Original weight}} \times 100$$

4.2.5 Determination of expressible moisture (EM)

The expressible moisture of the protein isolates, as an estimation of water loss, was evaluated by using a texture profile analyzer (TA-XT Express, Stable micro systems, Ltd., Surrey, England), which was set to the adhesive test mode prior to the measurements. Three grams of meat sample were placed on the pre-weighed filter paper (Whatman No. 1), and pressed between two glass plates, with a target force of 1000 g for 2 min. After squeezing, the filter paper along with the absorbed water was immediately weighed. Expressible moisture was expressed as percentage. Six press tests were performed for each treatment. The following formula was used to calculate the expressible moisture:

$$\text{Expressible moisture (\%)} = \frac{\text{(Wet paper – Dry paper)}}{\text{Meat weight}} \times 100$$

4.2.6 Emulsifying activity index (EAI) and emulsion stability index (ESI)

The measurements of emulsifying activity index and emulsion stability index were conducted according to the method described by Moure et al. (2002) with slight modifications. Oil-in-water emulsion was prepared by mixing corn oil with protein solution (myofibrillar or sarcoplasmic at the concentration of 0.4 mg/ml) at 1:3 ratio (vol/vol) in an homogenizer (Fisher Scientific, Power Gen 1000 S1, Schwerte, Germany) operated for 1 min at setting 3. Immediately after homogenization, 0.05 mL of emulsion was diluted to 5 mL with 0.1% sodium dodecyl sulphate (SDS) solution and the absorbance was measured at 500 nm in a 1-cm path cuvette using a spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The EAI was calculated from the following equation:

$$EAI = 2.33 \times A_0$$

where A_0 is the absorbance estimated just after emulsion preparation. The emulsion stability index was determined by measuring the absorbance of these emulsions after 10 min of standing. The ESI was deduced as follows:

$$ESI = 10 \times \frac{A_0}{A_0 - A_{10}}$$

where A_{10} is the absorbance determined after 10 min.

4.2.7 Foam expansion (FE) and foam volume stability (FVS)

The measurement of foamability was performed as per the method described by Wilde and Clark (1996). Known volumes of proteins (myofibrillar and sarcoplasmic) were whipped using a vortex mixer (Fisher Scientific, On, CA) at speed 10 for 2 min.

Foamability or foam expansion was expressed as percentage volume increase after mixing using the following equation:

$$\text{Foam expansion (\%)} = \frac{\text{Foam volume (mL)}}{\text{Initial liquid volume}} \times 100$$

The stability of the foam volume was calculated as percentage of foam remaining after 30 min at 25 °C.

$$\text{Foam volume stability (\%)} = \frac{\text{Volume of foam (mL) retained after 30 min}}{\text{Volume of foam after whipping}} \times 100$$

4.2.8 Total pigment determination

The total pigment content was evaluated by direct spectrophotometric measurement according to the method of Fraqueza et al. (2006), with slight modifications. For each run, 10 g of the sample was weighed into 50 mL capped glass tubes and 40 mL of acetone, 1 mL of HCl, and 1 mL of water, were added. The mixture was vortexed for 3 min and allowed to stand for 1 hour at room temperature. The extract was filtered through Whatman No. 1 filter paper, and the absorbance was read at 640 nm against an acid-acetone blank using a UV/VIS spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The absorbance value was multiplied by a coefficient of 17.18 and the concentration of total heme pigments was expressed in milligrams of myoglobin per gram of meat.

4.2.9 Color characteristics

The color characteristics of samples were measured on the surface of raw MSTM and freshly prepared protein isolates using a Minolta CR-400 (Konica Minolta Sensing Americas, Inc, Ramsey, NJ 07446). A white standard plate was

used to calibrate the colorimeter. Tristimulus color coordinates L*, a* and b* were recorded. The L* value on a 0 to 100 scale denotes the color from black (0) to white (100). The a* value denotes redness (+) or greenness (-), and the b* value denotes yellowness (+) or blueness (-). Three readings per treatment sample were taken and the average reading was recorded. The intensity of the red, saturation, Hue and whiteness were calculated as follows:

Intensity of the red = a* / b*

Saturation = $(a^2 + b^2)^{1/2}$

Hue = arctan b*/a*

Whiteness = $100 - [(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

4.2.10 Texture profile analysis

Texture profile analysis was carried out on cooked samples by employing a texture profile analyzer (TA-XT Express, Stable micro systems, Ltd., Surrey, England). The samples were cut into cylinders (17 mm diameter, 10 mm height) and subjected to the TPA mode analysis. Three samples per treatment were compressed to 50% of their original height for 2 cycles with the aluminium cylinder probe (d = 5 cm). The time between two compressions was set as 1 s. Determination of texture attributes was performed at the trigger force of 5 g with the speed of 5 mm/s. Attributes were calculated as follows. Hardness: the maximum force required for the first compression. Chewiness: the work needed to chew a solid sample to a steady state of swallowing. Springiness: the ability of the sample to recover to its original shape after the first compression. Cohesiveness: represents how well the product withstands a second deformation relative to how

it behaved under the first deformation. Measurements of samples were carried out at room temperature. Data were recorded and analyzed automatically by software provided with the instrument.

4.2.11 Dynamic viscoelastic behaviour of isolated proteins

The dynamic viscoelastic behaviour (DVB) of isolated proteins during heating and cooling was monitored using a Physica MCR Rheometer (Anton Paar GmbH, Virginia, US) under oscillatory mode, employing a 2.5 cm parallel plate measuring geometry. Four grams of protein isolate were mixed thoroughly with 2.5% of sodium chloride (w/w) in a pestle and mortar to obtain a fine ground paste. The paste was subjected to DVB measurements. The gap between measuring geometry and peltier plates was adjusted to 1.0 mm. Approximately 2 g of paste was placed on the peltier plate at 4 °C. Once the sample was pressed by lowering the measuring geometry plate, excess sample was removed with a stainless steel spatula. The samples were heated from 4 ° to 80 °C at a rate of 2 °C/min and cooled from 80 ° to 4 °C at the same rate. To determine the linear viscoelastic region (LVR) an amplitude sweep was carried out in a range of deformation from 0.1 to 10%. After determining LVR, measurements of the samples were conducted by applying a controlled strain (0.5%) with a constant frequency set at 1 Hz. The two sine waves had a phase difference tan δ, which gave elastic (storage modulus G') and viscous (loss modulus G'') elements of gel. These two values along with tan δ were recorded simultaneously throughout the heating and cooling processes by the instrument. Four replications were

performed, each using a fresh paste preparation and the average values were plotted.

4.2.12 Statistical analysis

All data were analyzed by one-way-analysis of variance (ANOVA) using General Linear Model procedure of the Statistical System Software of SAS institute (Version 9.0, SAS Inst., Cary, NC, US, 2006) and reported as means and standard deviation among means. The entire experiment, from MSTM through final protein isolate was replicated at least three times. Comparison of means within the evaluated parameters at various pH treatments was carried out by HSD Tukey`s adjustment with a 95% confidence level. Significance of difference was established at $P < 0.05$.

4.3 RESULTS AND DISCUSSION

4.3.1 Cooking loss and expressible moisture

The ability of meat proteins to retain water is one of the most important quality attributes influencing product yield and it also has an impact on eating quality of the product (Cheng and Sun, 2008). Cooking loss provides an insight into the tenderness of a meat product, which is related to the ability of proteins to bind water and fat. Expressible moisture is a measure of the water-holding capacity (WHC) of meat proteins and changes in WHC indicate the changes in the charge and structure of myofibrillar proteins (Hamm, 1975). In the present study the effect of different pH of extraction on WHC was assessed by estimation of

cooking and water losses. No significant (P = 0.5699) difference was found for cooking loss (Figure 4.1) among different treatments.

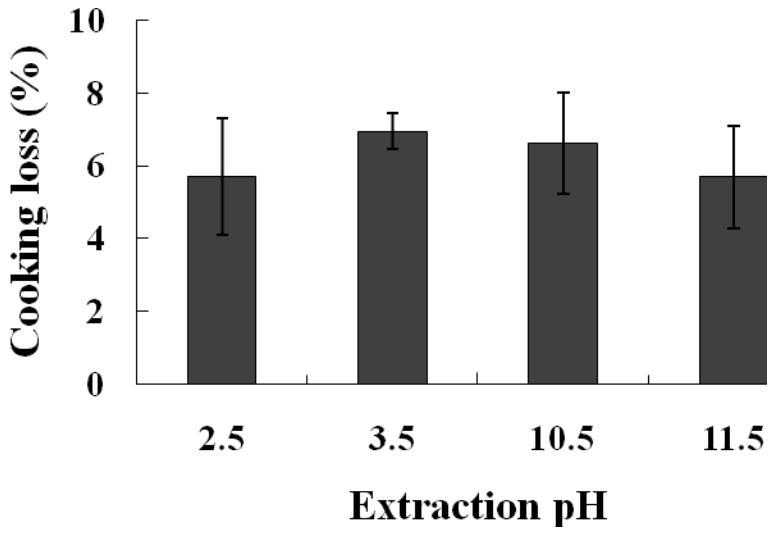

Figure 4.1. Cooking loss of proteins recovered from MSTM at different extraction pH. No statistical differences were observed. Data were statistically analyzed by one-way ANOVA. Results are presented as mean (n=4) ± standard deviation.

However, cooking loss of protein isolates was significantly lower (6.23% on average; P < 0.0001) compared to raw MSTM (29.27%; Table 4.1).

Table 4.1. Characteristics of raw mechanically separated turkey meat (MSTM)[1]

Parameter	Value
Cooking loss (%)	29.27 ± 5.1
Expressible moisture (%)	46.70 ± 0.61
EAI (myofibrillar proteins)	2.37 ± 0.18
EAI (sarcoplasmic proteins)	1.01 ± 0.07
ESI (myofibrillar proteins)	56.67 ± 3.19
ESI (sarcoplasmic proteins)	7.80 ± 0.53
FE (myofibrillar proteins) (%)	93.30 ± 20.82
FE (sarcoplasmic proteins) (%)	10.0 ± 0
FVS (myofibrillar proteins) (%)	61.69 ± 10.85
FVS (sarcoplasmic proteins) (%)	ND
Total heme pigments (mg/g of meat)	3.77 ± 0.69
Hardness (gram force)	142.82 ± 20.33
Springiness	0.66 ± 0.03
Chewiness	43.63 ± 11.27
Cohesiveness	0.46 ± 0.02

[1]Results are presented as mean (n=3) ± standard deviation.
ND denotes – not detectable.

Such a significant difference in cooking loss between raw and processed meat is probably due to the difference in composition of those two materials. Total lipid content of raw meat and isolated proteins was 23.50% and 1.81%, respectively (reported in chapter 3). Therefore, while subjected to heat treatment raw meat will be loosing more fat in addition to the water loss resulting in higher cooking loss.

The results obtained from the analysis of expressible moisture, as an evaluation of water loss, are presented in Figure 4.2.

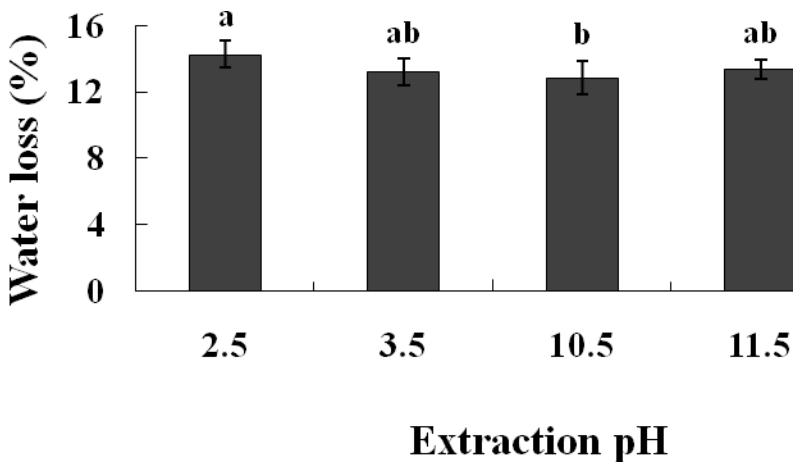

Figure 4.2. Expressible moisture (expressed as a water loss) of proteins recovered from MSTM at different extraction pH. Results are presented as mean (n=6) ± standard deviation. Different alphabetical letters in the figure represent significant ($P < 0.05$) difference between means.

Expressible moisture varied among treatments and the highest (14.26%; $P = 0.0249$) was obtained for samples processed with pH 2.5. Proteins extracted with pH 10.5 represented the lowest water loss of 12.86%. This decrease in water loss, which refers to the higher ability to retain water, is probably the result of higher protein content of samples extracted with pH 10.5, as reported in chapter 3. Extraction of proteins at this pH also resulted in the highest surface hydrophobicity of myofibrillar proteins (reported in chapter 3). The exposure of hydrophobic amino acids to the protein surface may increase the number of hydrophobic interactions, leading to the formation of a gel network with higher ability to entrap water (Niwa, 1992). Water loss was found to be significantly ($P < 0.0001$) higher for raw MSTM (46.70%; Table 4.1) compared to the processed meat. These results suggest that WHC of MSTM could be greatly improved by the extraction treatments.

4.3.2 Emulsifying activity index (EAI) and emulsion stability index (ESI)

Emulsion is a heterogeneous system consisting of at least two immiscible liquid phases, one of which is dispersed in the other in the form of droplets. Emulsion is stabilized through physical entrapment of fat globules within protein matrix followed by formation of an interfacial protein film around the small fat globules (Barbut, 1995). The ability of protein to adsorb at the water-oil interface during the formation of emulsion avoiding flocculation and coalescence is indicated by EAI. On the other hand, ESI estimates the rate of decrease of the emulsion turbidity due to droplet coalescence and creaming, leading to emulsion destabilization. Therefore, EAI and ESI increase when proteins favor emulsion formation and stabilization, respectively (Selmane et al., 2008). The emulsification properties of acid and alkaline extracted proteins were evaluated by their ability to form and stabilize emulsion with oil and the results are presented in Table 4.2.

Table 4.2. Emulsifying activity index (EAI), emulsion stability index (ESI), foam expansion (FE) and foam volume stability (FVS) of myofibrillar and sarcoplasmic proteins extracted from MSTM at different extraction pH[1]

Extraction pH	EAI myofibrillar proteins	EAI sarcoplasmic proteins	ESI myofibrillar proteins	ESI sarcoplasmic proteins	FE myofibrillar proteins (%)	FE sarcoplasmic proteins (%)	FVS myofibrillar proteins (%)	FVS sarcoplasmic proteins (%)
pH 2.5	2.41 ± 0.90^{ab}	1.98 ± 0.06	39.62 ± 1.63	5.07 ± 0.57^{b}	190 ± 17	72 ± 13^{b}	56 ± 17	53 ± 9
pH 3.5	2.40 ± 0.09^{b}	1.94 ± 0.09	38.18 ± 0.98	5.86 ± 0.23^{ab}	163 ± 12	110 ± 10^{a}	63 ± 3	68 ± 9
pH 10.5	2.63 ± 0.09^{ab}	1.89 ± 0.10	44.52 ± 4.01	6.60 ± 0.38^{a}	170 ± 27	118 ± 8^{a}	70 ± 4	69 ± 13
pH 11.5	2.66 ± 0.11^{a}	1.75 ± 0.10	45.66 ± 4.77	6.42 ± 0.17^{a}	172 ± 3	103 ± 6^{a}	72 ± 7	76 ± 5

[1]Results are presented as mean (n=3) ± standard deviation. Different alphabetical letters within a column represent significant ($P < 0.05$) difference between mean values.

EAI of myofibrillar proteins was significantly different (P = 0.0184) among treatments, with the highest value obtained at pH 11.5. In general, alkali extracted protein showed slightly higher EAI compared to acid extracted protein. Among the myofibrillar and sarcoplasmic proteins, the latter showed significantly lower emulsification ability (P = 0.0010). This was expected since the capability of sarcoplasmic proteins to emulsify fat is limited by their globular nature (Tantikarnjathep et al., 1983).

Among treatments, there was a tendency to be significantly higher for (P = 0.0592) ESI of myofibrillar proteins for alkali extracted samples. The latter values were also around 7 times higher compared to the ESI of sarcoplasmic protein fraction. This effectiveness of myofibrillar proteins is probably due to the ability of myosin to display both hydrophobic affinity for fat and hydrophilic affinity for water. Myosin provides a distribution of polar and non-polar amino acids, thus enhancing the orientation between two unlike phases (Sebranek, 2009). High length to diameter ratio of the myosin molecule also contributes to the molecular flexibility and rearrangement at the protein film interface (Xiong, 1997). ESI of sarcoplasmic proteins showed significant (P = 0.0039) difference in stability indexes with alkali extracted samples representing the highest values (6.42 and 6.60%). This is in agreement with our previous findings (reported in chapter 3) on surface hydrophobicity of sarcoplasmic proteins, where significantly higher values were reported for alkali extracted samples compared to acid extractions. The exposure of a higher number of hydrophobic areas to the protein surface

probably enhances the ability of alkali treated samples for emulsion stabilization (Damodaran, 2005).

4.3.3 Foam expansion (FE) and foam volume stability (FVS)

Food foams are dispersions of gas bubbles in a continuous liquid or semisolid phase. Foaming is responsible for the desirable rheological properties of many foods. The behaviour of the proteins at the liquid/air interface is important since the formation of protein film around air bubbles is essential for foam capacity and stability (Xiong, 1997). The foam expansion and foam volume stability of protein isolates from MSTM prepared at different extraction pH are presented in Table 4.2. The foaming properties between the pH treatments were found to have no significant differences ($P > 0.05$), except foam expansion of sarcoplasmic proteins. The latter was significantly ($P = 0.0014$) lower when proteins were extracted at pH 2.5. It was reported that foamability is related to the protein surface hydrophobicity (Damodaran, 1994). High surface hydrophobicity improves foam characteristics as the unfolding of protein molecules expose hydrophobic groups resulting in increased interaction at the air/water interface (Were et al., 1997). In our previous study (chapter 3) we found that surface sarcoplasmic protein hydrophobicity was lower for acid treated samples compared to alkaline extractions. Therefore, reduced foam expansion of sarcoplasmic proteins at pH 2.5 might be associated with the low hydrophobicity at this pH. FE of sarcoplasmic proteins were found to be significantly ($P = 0.0019$) lower compared to that of myofibrillar proteins. However there was no significant ($P =$

0.8550) difference among the FVS of these two protein fractions. This suggests that even though myofibrillar proteins have higher ability to form the foam, the stability might be maintained at the same level by both myofibrillar and sarcoplasmic protein fractions. FE (myofibrillar and sarcoplasmic) and FVS of sarcoplasmic proteins were found to be significantly ($P < 0.0001$) lower for raw MSTM (Table 4.1.) compared to processed samples. This denotes that conformational changes of proteins during acid and alkaline treatments lead to improvement of foaming properties.

4.3.4. Total pigments and color characteristics

Color is an important factor for determining consumers' perception of product quality and significantly influences purchasing decisions. Color is also a principal characteristic when different processing treatments are compared, especially considering increased interest of current markets in isolates as white as possible (Tabilo-Munizaga and Barbosa-Canovas, 2004). The two pigments which are mainly responsible for the color of MSTM are myoglobin and hemoglobin (Hernandez et al., 1986), thus their effective removal could greatly improve color characteristics of recovered meat. The total pigment analysis showed significantly ($P < 0.0079$) higher content in alkali extracted samples (0.56 mg/g of meat) compared to that of acid treatments (0.44 mg/g of meat) (Figure 4.3.).

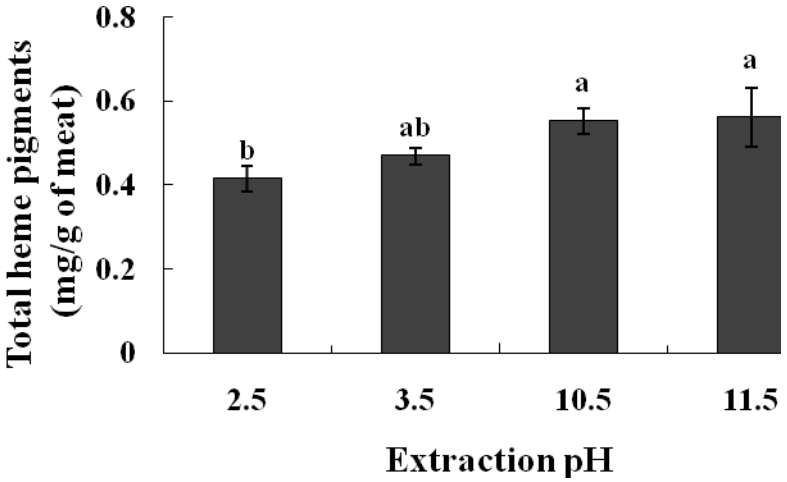

Figure 4.3. Total heme pigments content of proteins recovered from MSTM at different extraction pH. The original material (raw MSTM) contained 3.77 mg of total heme pigments per 1g of meat. Results are presented as mean (n=3) ± standard deviation. Different alphabetical letters in the figure represent significant ($P < 0.05$) difference between means.

The highest total pigment removal was found when MSTM proteins were extracted at pH 2.5 (88.96%), while the lowest was for extractions at pH 10.5 and 11.5. Chaijan et al. (2006) reported that sarcoplasmic proteins (including myoglobin and hemoglobin) and other proteinaceous materials were not removed in the alkaline-aided process, since myoglobin might be tightly bound with muscle proteins and co-precipitated during the extraction process. Our previous study on surface hydrophobicity of sarcoplasmic proteins (reported in chapter 3) showed higher values for alkali-extracted proteins compared to acid extractions.

Stronger protein-protein interactions at alkaline pH probably result in higher aggregation of sarcoplasmic proteins leading to precipitation into sediments after isoelectric precipitation. In general, extractions showed 86.72% removal of total pigments, resulting in around 0.5 mg of heme pigments per 1.0 g of meat. Comparable values were reported by Pikul et al. (1986) for turkey breast meat.

Color characteristics (L*, a*, b*, a*/b*, saturation, Hue and whiteness) of the recovered proteins by the pH-shifting process are presented in Table 4.3.

Table 4.3. Color characteristics of proteins recovered from MSTM at different extraction pH[1]

Parameter	Raw MSTM	2.5	3.5	10.5	11.5
a*	7.45 ± 0.24^{a}	3.66 ± 1.12^{b}	2.84 ± 0.76^{b}	4.51 ± 0.21^{b}	3.58 ± 1.19^{b}
b*	16.49 ± 0.36	14.43 ± 2.25	14.44 ± 0.98	16.05 ± 0.25	15.82 ± 0.39
L*	58.94 ± 1.55^{b}	69.84 ± 1.75^{a}	61.59 ± 4.74^{ab}	59.90 ± 2.75^{b}	57.40 ± 4.08^{b}
a*/b*	0.45 ± 0.02^{a}	0.27 ± 0.13^{b}	0.20 ± 0.07^{b}	0.28 ± 0.02^{ab}	0.22 ± 0.07^{b}
Saturation	18.10 ± 0.31^{a}	14.95 ± 1.88^{b}	14.74 ± 0.81^{b}	16.67 ± 0.20^{ab}	16.24 ± 0.64^{ab}
Hue	65.67 ± 0.95^{b}	75.23 ± 6.67^{a}	78.75 ± 3.68^{a}	74.30 ± 0.89^{ab}	77.36 ± 3.81^{a}
Whiteness	52.46 ± 1.27^{b}	64.82 ± 1.99^{a}	57.85 ± 4.38^{ab}	54.93 ± 2.47^{b}	53.19 ± 4.36^{b}

[1]Results are presented as mean (n=3) ± standard deviation. Different alphabetical letters within a row represent significant ($P < 0.05$) difference between mean values.

The results are shown in comparison to the initial material (raw MSTM). In general, acidic and alkaline isolates greatly ($P < 0.0001$) decreased in the redness (a*), with no difference being found within pH treatments. The decrease in redness is due to the removal of pigments during extraction (Figure 4.3). Yellowness (b*) values remained constant ($P = 0.0984$) for raw MSTM and different extraction treatments. Lightness (L*) was significantly increased ($P = 0.0035$) for the samples processed with extraction pH of 2.5. The concentration of total pigments is the influential factor for the L* values (Gasperlin et al., 2000). Thus, the extension of total pigment removal, which was observed to be higher for acid treated samples contributed to the increased lightness. Whiteness increased significantly ($P = 0.021$) compared to the raw meat, with the highest value (64.82) observed at pH 2.5. This is expected, because the whiteness values are mainly influenced by the lightness, which was the highest for samples extracted with pH 2.5. Both lightness and whiteness values are in agreement with the results obtained from the analysis of total pigment content, which indicated the highest removal at extraction pH of 2.5. A significant decrease ($P = 0.0036$) was observed for a*/b*, which indicates a decrease in intensity of the redness value. The ratio decreased from the original value of 0.45 found for raw MSTM to 0.24 in general for processed meat. High a*/b* obtained for the raw meat is primarily because of the high total pigment content. Saturation values determine how different the color is from gray and expressed as depth, vividness and purity (Elkhalifa et al., 1988). There was a significant decrease in saturation ($P = 0.0036$) observed between raw MSTM and acid extracted meat. As reported by Hernandez et al.

(1986), the samples having a dominant red color would give a higher saturation value than the samples with a more homogenous structure. In this study the lower purity of alkali extracted meat compared to acid treatments might be the result of higher total pigment content. Hue angle shows the degree of departure from the true red axis to the CIE (International Commission of Illumination) color space (Brewer et al., 2006). The Hue values were found to be significantly (P = 0.0046) higher for proteins recovered at pH 2.5; 3.5 and 11.5 compared to raw MSTM. This is expected, since increased Hue angle indicates a decrease in perceived redness (Brewer et al., 2006). As the result of extraction procedures, the red color was decreased due to the removal of heme pigments. Consequently samples decreased in darkness with the dominance of Hue, which is an indication that the color shifted slightly to the yellowish spectrum (Hoffman and Mellet, 2003).

4.3.5 Texture profile analysis and dynamic viscoelastic behaviour of isolated proteins

Complimentary information on textural properties of protein isolates was obtained using small and large deformation tests. A small deformation test was applied to investigate elastic and viscoelastic properties of gels, which is related to gel quality and strength. Uniaxial compression of a gel sample between two flat parallel plates (large deformation test) was used to determine textural properties, such as hardness, chewiness, springiness and cohesiveness.

Texture profile analysis (TPA) of the MSTM protein gels is summarized in Figure 4.4.

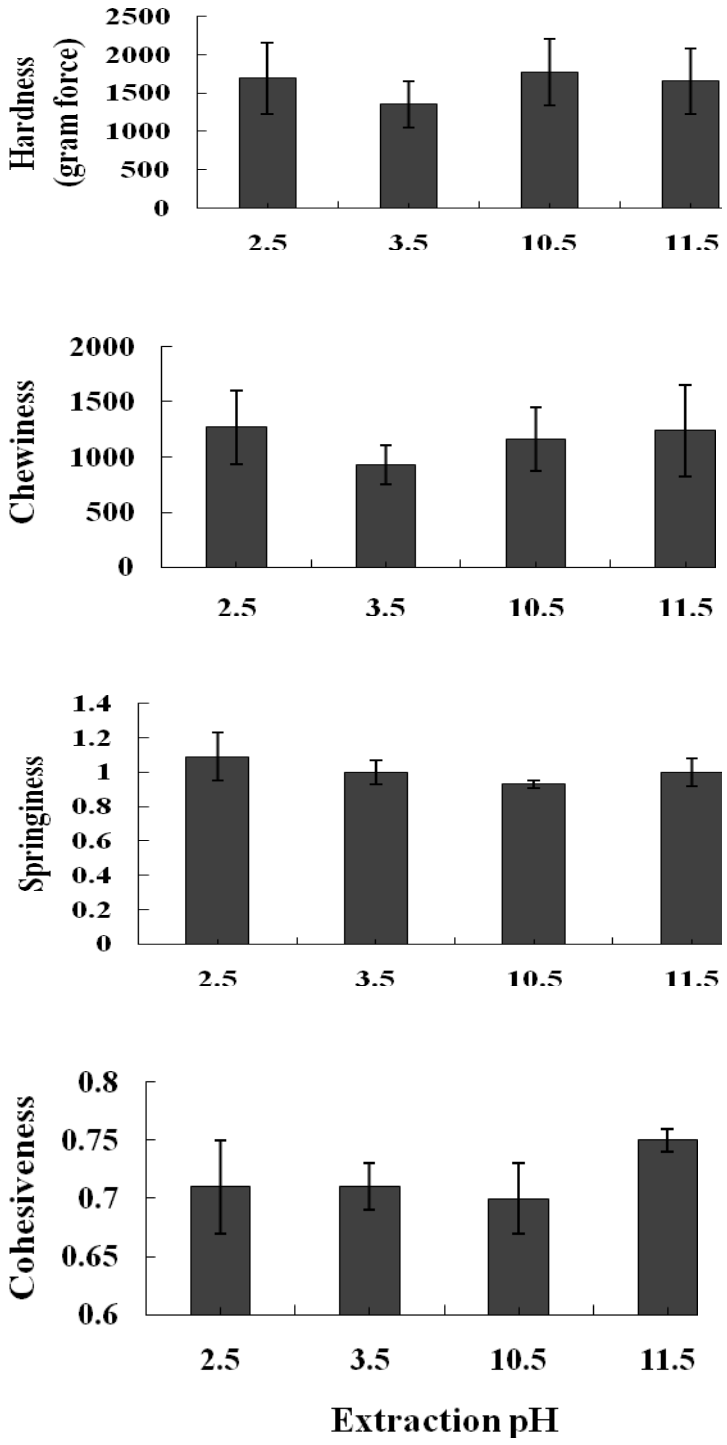

Figure 4.4. Hardness, chewiness, springiness and cohesiveness of proteins recovered from MSTM at different extraction pH. No statistical differences were observed. Data were statistically analyzed by one-way ANOVA. Results are presented as mean (n=3) ± standard deviation.

No significant differences ($P > 0.05$) were found for any of the parameters. Generally, the higher hardness of the gels developed from MSTM protein was observed at pH 10.5, with the value of 1773 gram force. The lowest value for chewiness (934) was observed at pH 3.5. The lower value for this parameter is associated with the higher ability to form a viscoelastic network (Figure 4.5 A, B), as chewiness represent the ability of the sample to regain its shape after compression. Chewiness is also one of the important characteristics, which associates with meat tenderness (Gullett et al., 2006). No significant difference found for springiness value is probably due to the same water content between samples, as the extraction process was followed by adjustment of water content to 80%. While no difference among treatments was found for cohesiveness, the samples extracted at pH 11.5 appeared to be higher.

Gelation of muscle protein is a multi-step thermodynamic process which involves protein unfolding and aggregation prior to the formation of three-dimensional network structures (Xiong and Blanchard, 1994). Hamann (1988) indicated that rheological parameters could be used to predict sensory characteristics, texture and functionality of comminuted meat products. The dynamic rheological technique is widely used for the evaluation of gelation of myofibrillar proteins. Viscoelastic properties of storage (G'), loss (G'') modulus and tan delta (δ) between acid and alkaline extractions were determined upon heating and cooling. Changes in storage modulus, loss modulus and tan δ during heating is given in Figure 4.5 A, B and C.

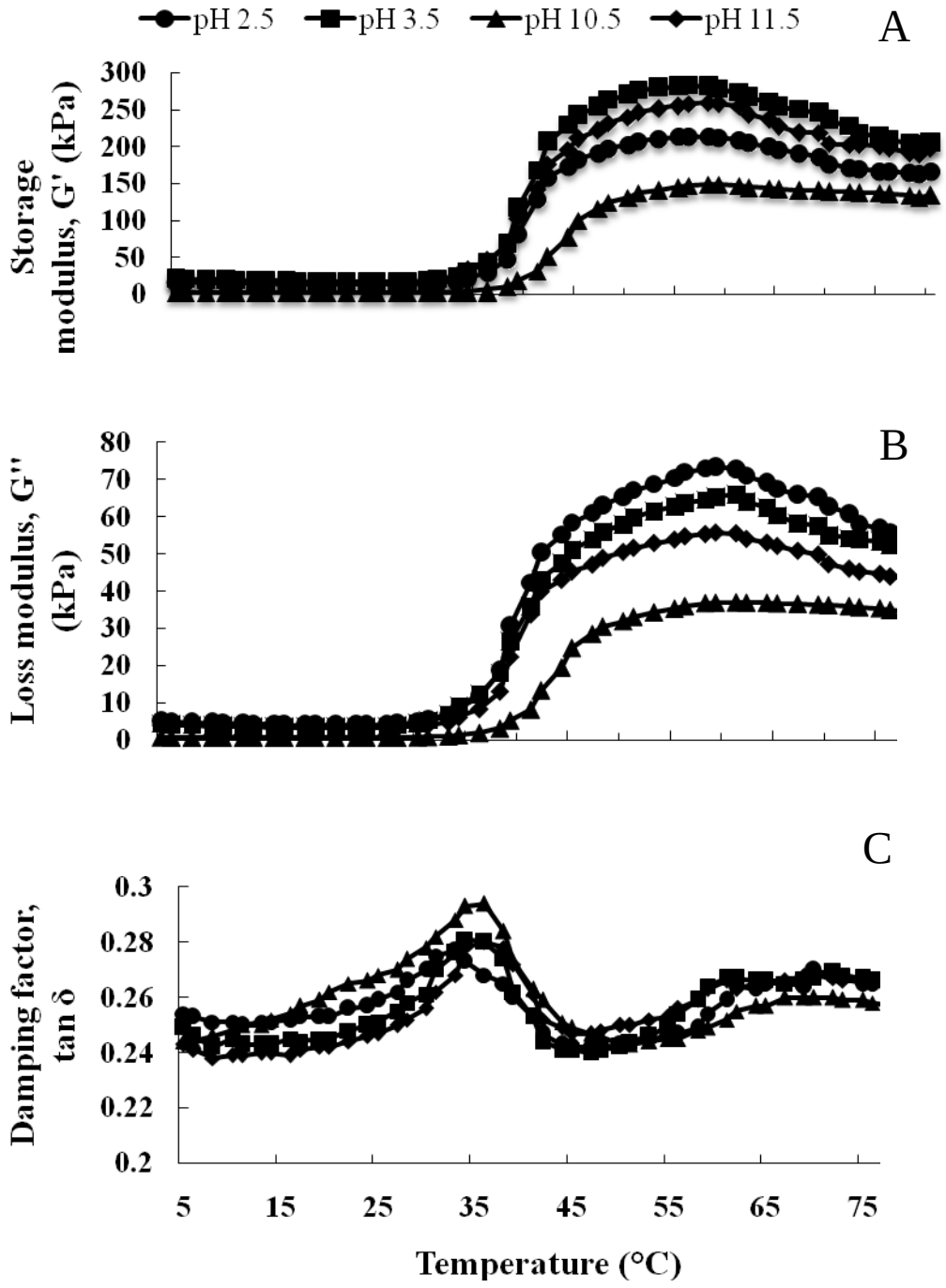

Figure 4.5. Changes in dynamic viscoelastic behaviour (DVB) of proteins recovered from MSTM at different extraction pH. The samples were prepared with 2.5% of NACl additon. The rheograms show storage modulus (G'), loss modulus (G'') and tan delta (δ) development during heating from 4 ° to 80 °C at 2 °C/min.

Proteins isolated at different pH values showed a similar trend for both G' and G'' values. However, the G' values were considerably higher in magnitude than the G'' values indicating the formation of more elastic gels. The G'' value is an estimation of energy dissipated as heat per sinusoidal cycle and is used to evaluate the gel viscosity.

During the heating phase, the G' showed only marginal change, until the temperature reached 36 °C, where onset of gelation occurred (Figure 4.5A). Storage modulus (G') is a measure of the energy stored in material and recovered from it per cycle of sinusoidal shear deformation and indicates solid or elastic characteristics. The increase in G' has been attributed to the ordered protein aggregation and formation of three-dimensional network with water entrapment in the matrix (Dileep et al., 2005). The gelation starts with unfolding of myosin molecules at 35 °- 40 °C (Sebranek, 2009). The same increasing pattern was observed in loss modulus for heat induced gelation of dark chicken meat protein isolates indicating the formation of a viscoelastic network (Omana et al., 2010). G' values increased until temperature reached 56 °- 58 °C; further increase in temperature caused weakening of the gels as shown by decrease in G' values. This decrease might be due to the result of denaturation of light meromyosin, leading to increased fluidity (Egelansdal et al., 1995). The maximum increase in G’ value was in the temperature range of 40 ° to 56.6 °C. The forces which are responsible for the formation of the gel network include hydrophobic interactions, disulphide cross bridges and hydrogen bonds (Hamann and MacDonald, 1992). Overall, the

patterns of slopes for acid and alkaline extracted MSTM proteins were similar, excluding pH 10.5.

Tan δ values indicated a major transition point at temperature of 47.3 °C for proteins extracted at pH 2.5, 3.5, 11.5 and 51.9 °C for proteins extracted with pH 10.5 (Figure 4.5 C). This transition point refers to the denaturation of the myosin molecule. This is consistent with rheological analysis of alkali-extracted proteins from dark chicken meat (Omana et al., 2010). The authors attributed the transition temperature at 50.1 °C to the denaturation of myosin. One minor transition point was observed for acid extracted samples at around 65 °C, which corresponds to the denaturation point of collagen (Martens et al., 1982). Above 35 °C tan δ values were found to be decreasing until the temperature reached 47 °C for pH 2.5, 3.5, 11.5 and 52 °C for the pH 10.5. In general, a decrease in tan δ indicates the formation of an ordered gel network. The use of tan δ to estimate the gel characteristics has the advantage of incorporating the contributions of both G' and G'' into a single parameter to evaluate the final network (Egelandsdal et al., 1986).

Storage modulus values of different protein isolates at various temperatures (5 °, 56.6 ° and 80 °C) are given in Figure 4.6.

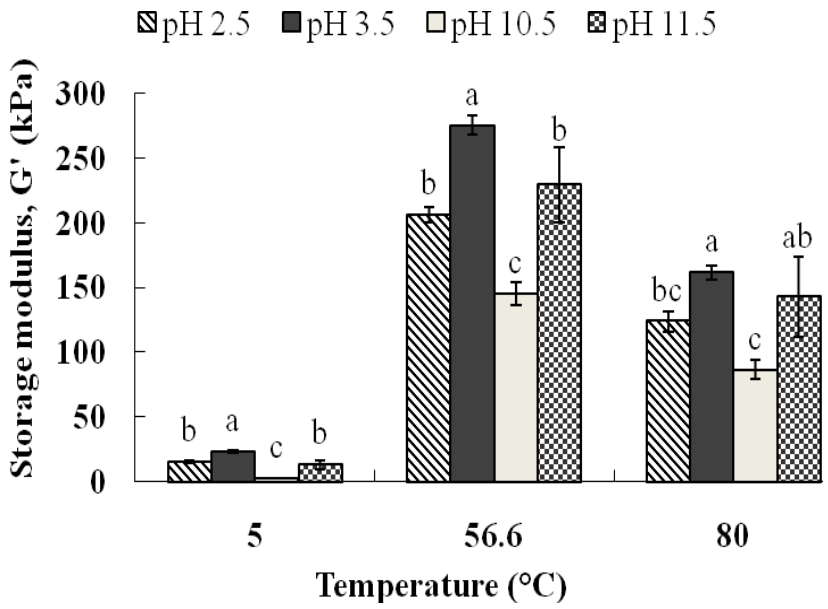

Figure 4.6. Average storage modulus (G', kPa) at 5 °, 56.6 ° and 80 °C for proteins recovered from MSTM at different extraction pH. Results are presented as mean (n=4) ± standard deviation. Different alphabetical letters in the figure represent significant ($P < 0.05$) difference between means.

The highest ($P < 0.0001$) G' value (at 5 °, 56.6 ° and 80 °C) was obtained for the sample extracted with pH 3.5. The lowest was observed with pH 10.5 extracted samples, while G' for proteins extracted at more extreme pH of 2.5 and 11.5 was not significantly different from each other. The same trend was observed with increasing temperature to 56.6 °C (the peak value for storage modulus). At 80 °C protein extracted with pH 3.5 possessed significantly ($P = 0.0005$) higher G’ compared to pH 2.5 and 11.5.

On cooling from 80 ° to 4 °C, all samples showed an increase in G' and G" as interactions between the proteins become stronger with the decrease in temperature (Figure 4.7.).

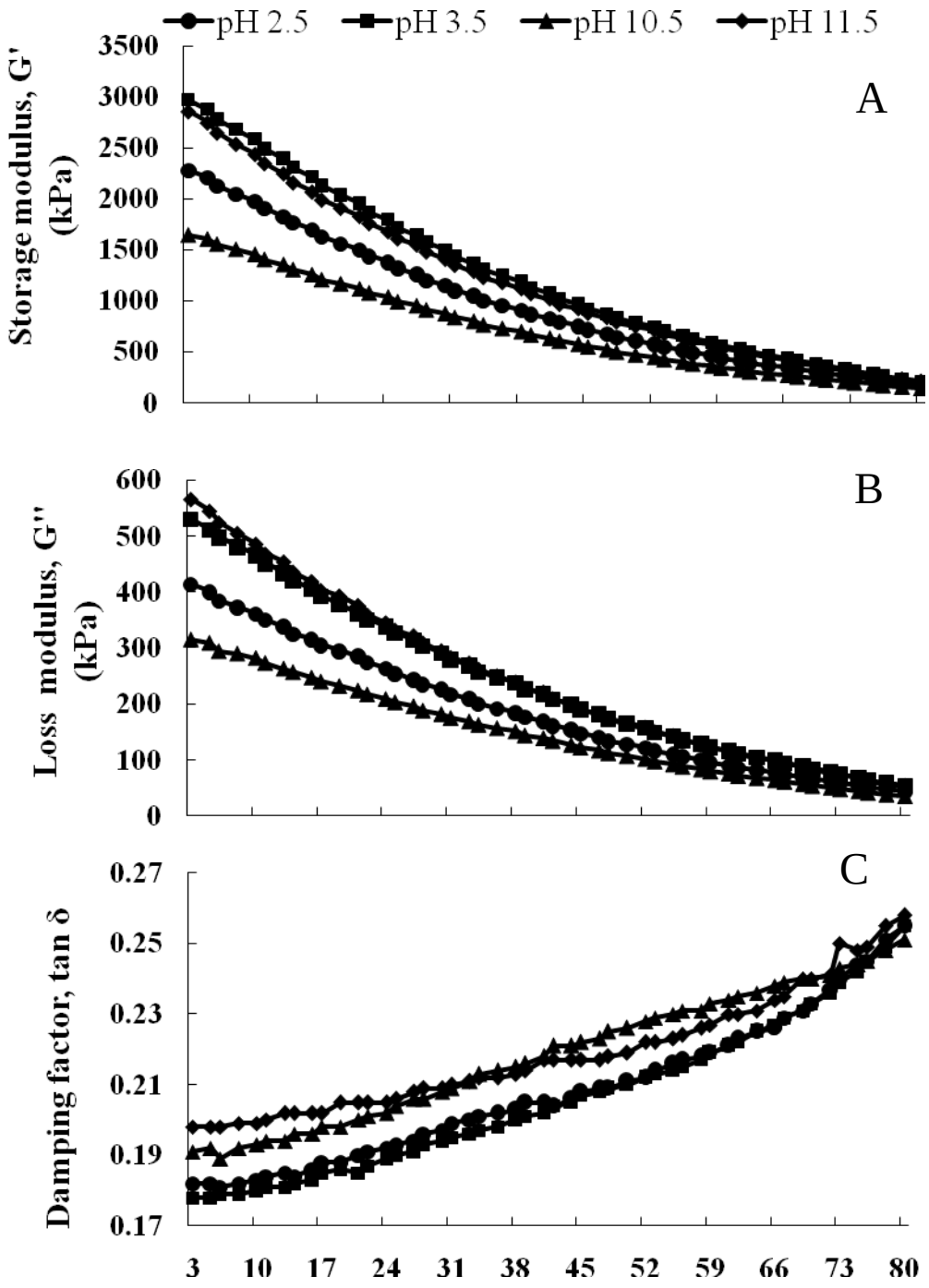

Figure 4.7. Changes in dynamic viscoelastic behaviour (DVB) of proteins recovered from MSTM at different extraction pH. The samples were prepared with 2.5% of NACl addition. The rheograms show storage modulus (G'), loss modulus (G'') and tan delta (δ) development during cooling from 80 ° to 4 °C at 2 °C/min.

However, a notable difference was observed for pH 10.5 extracted proteins, where G' and G'' showed the lowest values. During cooling the highest value was reached at the end of the gelation process. The increase in storage and loss modulus is attributed to the formation of hydrogen bonds during cooling (Hamann, 1988). High G' value during cooling is also an indication of the formation of a firm gel structure (Ingadottir and Kristinsson, 2010).

4.4 CONCLUSION

The present study indicated that functional properties and rheological characteristics of MSTM could be greatly improved by extraction procedures. Emulsion activity index of myofibrillar proteins was better at extraction pH of 11.5. Proteins extracted at pH 3.5 showed higher ability to form a viscoelastic gel network. Acid extractions were more efficient in heme pigment removal, which resulted in better color characteristics than alkali treated samples. Further research is needed to improve color properties of alkali extracted protein isolates. The study revealed that acid and alkaline processing can be the alternatives for recovering functional proteins from MSTM. In conclusion, proteins extracted at pH 3.5 were found to be the most suitable considering the rheological characteristics as well as pigment removal.

4.5 REFERENCES

Barbut, S. 1995. Importance of fat emulsification and protein matrix characteristics in meat batter stability. J. Muscle Foods. 6:161-177.

Betti, M., and D. L. Fletcher. 2005. The influence of extraction and precipitation pH on the dry matter yield of broiler dark meat. Poult. Sci. 84:1303-1307.

Brewer, M. S., Novakofski, J., and K. Freise. 2006. Instrumental evaluation of pH effects on ability of pork chops to bloom. Meat Sci. 72:596-602.

Chaijan, M., Benjakul, S., Visessanguan, W., and C. Faustman. 2006. Physicochemical properties, gel-forming ability and myoglobin content of sardine (*Sardinalla gibbosa*) and mackerel (*Rastrelliger kanagurta*) surimi produced by conventional method and alkaline solubilisation process. Eur. Food Res. Technol. 222:58-63.

Cheng, Q., and D. Sun. 2008. Factors affecting the water holding capacity of red meat products: A review of recent research advances. Crit. Rev. Food Sci. Nutr. 48:137-159.

Damodaran, S. 1994. Structure-function relationship of food proteins. Pages 190-225 in Protein Functionality in Food Systems. Hettiarachchy, N. S., and G. R. Zieger, eds. Marcel Dekker Inc., New York, NY.

Damodaran, S. 2005. Protein stabilization of emulsions and foams. J. Food Sci. 70:R54-R66.

Dileep, A. O., Shamasundar, B. A., Binsi, P. K., Badii, F., and N. K. Howell. 2005. Effect of ice storage on the physicochemical and dynamic viscoelastic properties of ribbonfish (*Trichiurus spp*) meat. J. Food Sci. 70:E537-E545.

Egelandsdal, B., Fretheim, K., and K. Samejima. 1986. Dynamic rheological measurements on heat-induced myosin gels: effect of ionic strength, protein concentration and addition of adenosine triphosphate or pyrophosphate. J. Sci. Food Agric. 37:915-926.

Egelandsdal, B., Martinsen, B., and K. Autio. 1995. Rheological parameters as predictor of protein functionality: A model study using myofibrils of different fiber-type composition. Meat Sci. 39:97-111.

Elkhalifa, E. A., Graham, P. P., Marriott, N. G., and S. K. Phelps. 1988. Color characteristics and functional properties of flaked turkey dark meat as influenced by washing treatments. J. Food Sci. 53:1068-1071.

Fraqueza, M. J., Cardoso, A. S., Ferreira, M. C., and A. S. Barreto. 2006. Incidence of pectoralis major turkey muscles with light and dark color in a portuguese slaughterhouse. Poult. Sci. 85:1992-2000.

Froning, G. W. 1976. Mechanically deboned poultry meat. Food Technol. 11:50-63.

Froning, G. W., Johnson, F. 1973. Improving the quality of mechanically deboned fowl meat by centrifugation. J. Food Sci. 38:279-281.

Gasperlin, L., Zlender, X., and V. Abram. 2000. Colour of normal and high pH beef heated to different temperatures as related to oxygenation. Meat Sci. 54:391-398.

Gullett, E. A., Buttenham, S., and T. Hore. 2006. Effect of age and cut on consistency of tenderness and leanness of beef. Food Qual. Pref. 7:37-45.

Hamm, R. 1975. Water holding capacity of meat. Pages 321-337 in Meat. Cole D. J. A., and R. A. Lawrie, eds. Butterworth, London, UK.

Hamann, D. D. 1988. Rheology as a means of evaluating muscle functionality of processed foods. Food Technol. 42:66-71.

Hamann, D. D., and G. A. MacDonald. 1992. Rheology and texture properties of surimi and surimi based foods. Pages 429-500 in Surimi Technology. Lanier, T. C., and C. M. Lee, eds. Marcel Dekker Inc., New York, NY.

Hernandez, A., R. C. Baker, and J. H. Hotchkiss. 1986. Extraction of pigments from mechanically deboned turkey meat. J. Food Sci. 51:865-867.

Hoffman, L. C., and F. D. Mellet. 2003. Quality characteristics of low fat ostrich meat patties formulated with either pork lard or modified corn starch, soya isolate and water. Meat Sci. 65:869-875.

Ingadottir, B., and H. Kristinsson. 2010. Gelation of protein isolates extracted from tilapia light muscle by pH shift processing. Food Chem. 118:789-798.

Kristinsson, H. G., and N. Demir. 2003. Functional fish protein ingredients from fish species of warm and temperate waters: comparison of acid- and alkali-aided processing vs. conventional surimi processing. Pages 277-298 in

Advances in Seafood By-Products. P. J. Bechtel, ed. Univ. of Alaska Press: Fairbanks, AK.

Kristinsson, H. G., and H. O. Hultin. 2003. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J. Agric. Food Chem. 51:7187-7196.

Liang, Y., and H. O. Hultin. 2003. Functional protein isolates from mechanically deboned turkey by alkaline solubilization with isoelectric precipitation. J. Muscle Food. 14:195-205.

Martens, H., Stabursvik, E., and M. Martens. 1982. Cooking related to thermal denaturation of muscle proteins. J. Texture Stud. 13:291-309.

Moayedi, V., D. A. Omana, J. Chan, J., Y. Xu, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Composition and stability to lipid oxidation of recovered proteins. Poult. Sci. 89:766-775.

Moure, A., H. Dominquez., M. E. Zuniga, C. Soto, and R. Chamy. 2002. Characterization of protein concentrates from pressed cakes of Guevina Avellana (Chilean hazelnut). Food Chem. 78:179-186.

Niwa, E. 1992. Chemistry of surimi gelation. Pages 389-427 in Surimi Technology. Lanier, T. C., and C. M. Lee, eds. Marcel Dekker Inc., New York, NY.

Ockerman, H. W., and C. L. Hansen. 2000. Animal by-product processing and utilization. p. 523. Lancaster: Technomic Publishing Company, Inc.

Omana, D. A., V. Moayedi, V., Y. Xu, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Textural properties and color characteristics of recovered proteins. Poult. Sci. 89:1056-1064.

Pikul, J., A. Niewlarowicz, and H. Kupijaj. 1986. The cytochrome c content of various poultry meats. J. Sci. Food Agric. 37:1236-1240.

Ross, C. F. 2009. Sensory science at the human-machine interface. Trends Food Sci. Technol. 20:63-72.

Sebranek, J. G. 2009. Functional properties of muscle proteins: implications for processed meat product characteristics. Proc. Recip. Meat Conf. Rogers, Arkansas. 62:1-7.

Selmane, D., C. Vial, and G. Djelveh. 2008. Extraction of proteins from slaughterhouse by-products: Influence of operating conditions on functional properties. Meat Sci. 79:640-647.

Tabilo-Munizaga, G., and G. V. Barbosa-Canovas. 2004. Color and textural parameters of pressurized and heat-treated surimi gels as affected by potato starch and egg white. Food Res. Intern. 37:767-775.

Tantikarnjathep, K., J. G. Sebranek, D. J. Topel, and R. E. Rust. 1983. Use of vacuum during formation of meat emulsions. J. Food Sci. 48:1039-1041.

Undeland, I. A., S. D. Kelleher, and H. O. Hultin. 2002. Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. J. Agric. Food Chem. 50:7371–7379.

Were, L., N. S. Hettiarachcht, and U. Kalapathy. 1997. Modified soy proteins with improved foaming and water hydration properties. J. Food Sci. 62:821-824.

Wilde, P. J, and D. C. Clark. 1996. Foam formation and stability. Pages 110-152 in Methods of Testing Protein Functionality. Hall, G. M., ed. Blackie Academic and Professional, London, UK.

Xiong, Y. L. 1997. Structure-function relationships of muscle proteins. Pages 341-392 in Food Proteins and Their Applications. Damodaran, S., and A. Paraf, eds. Marcel Dekker Inc., New York, NY.

Xiong, Y. L., and S. P. Blanchard. 1994. Dynamic gelling properties of myofibrillar protein from skeletal muscles of different chicken parts. J. Agric. Food Chem. 42:670-674.

Young, L. L. 1975. Aqueous extraction of protein isolate from mechanically deboned poultry meat. J. Food Sci. 40:1115-1118.

Ziegler, G. R., and J. C. Acton. 1984. Mechanism of the gel formation by proteins of muscle tissue. Food Technol. 38:77-82.

Ziegler, G. R., and E. A. Foegeding. 1990. The gelation of proteins. Adv. Food Nutr. Res. 34:231-237.

CHAPTER 5. PROJECT SUMMARY AND IMPLICATIONS

The desire of the food industry to convert low value meat products into value-added and high-priced commodities resulted in development of new processes, which helps in increased utilization of these raw materials. Mechanically separated meat is one of the cheapest sources of protein obtained by grinding meat and bones together and forcing the mixture through a perforated drum. Currently MSTM is considered as a low value by-product and available for the use in poultry products processing mainly for production of further-processed products of lower value. The use of MSTM for the production is limited due to its high lipid content, undesirable color and textural properties. Production of protein isolates by pH-shifting process is a new concept for protein recovery from low value meat. The benefits from this extraction process are due to possible improvement of protein functionality, which depends on the composition and chemical characteristics of protein isolates.

The aim of the first study was to improve the composition of MSTM by pH-shifting extraction with addition of citric acid and calcium chloride. In order to accomplish the goal, six levels of citric acid (0, 2, 4, 6, 8 and 10 mmol/L) and two levels of calcium chloride (0 and 8 mmol/L) addition were examined. The highest protein yield was found for treatment with 6 mmol/L of citric acid. In general, all the combinations removed an average of 90.8% of the total lipid from MSTM. The lowest amount of total lipid was obtained for samples treated with 4 mmol/L of citric acid. Maximum removal of neutral lipid and polar lipid was

attained with the addition of 6 and 2 mmol/L of citric acid, respectively. The isolated proteins were less susceptible to lipid oxidation compared to raw MSTM. The most efficient removal of total heme pigments was obtained with addition of 6 or 8 mmol/L of citric acid, while addition of calcium chloride had a negative effect on total pigment content. The study revealed that acid extractions with addition of citric acid resulted in significant removal of lipids and pigments from MSTM, improved stability of the recovered proteins to lipid oxidation and provided appreciable protein recovery yields.

Second and third parts of the project focused on the selection of the most suitable pH for protein extraction, therefore the effect of 4 pHs (2 acidic and 2 alkaline) was estimated. The evaluation and further comparison was based on the determination of chemical, functional and textural properties of protein isolates along with their composition.

The results did not show any statistical difference in protein yield between the extractions carried out at pH 2.5, 10.5 and 11.5. However, yield was considerably lower when pH of extraction was 3.5. No significant differences were found between the various pH of extractions on total, neutral and polar lipids, which on average were equal to 92.3, 93.0 and 90.7%, respectively. Analysis of lipid oxidation (TBARs) showed no difference between acid and alkaline treatments. SDS-PAGE analysis showed a greater concentration of myosin heavy chain and actin in protein isolates compared to the raw MSTM. Amino acid analysis of isolates showed higher concentration of glutamic acid and

reduced levels of histidine and methionine compared to raw MSTM. Uronic acid and hydroxyproline analysis revealed that most of the proteoglycans and collagen were deposited in the sediment fraction obtained during the centrifugation step following protein solubilization. Proteins extracted at pH 10.5 showed the lowest water loss. Emulsion and foaming properties were found to be slightly higher in alkali-extracted proteins compared to those for acid extractions. Textural characteristics (hardness, chewiness, springiness and cohesiveness) of recovered proteins were found to be unaffected by different extraction pH. The protein extracted at pH 3.5 formed a highly viscoelastic gel network, whereas the gel formed from proteins extracted at pH 10.5 was found to be weakest. Acid treatments were more effective for removal of total heme pigments from MSTM. Color characteristics of protein isolates were markedly improved compared to the initial material and tended to be better when subjected to acid extractions.

In conclusion, the determination of the optimal extraction pH depends on the final application of MSTM protein isolate. Considering the current trend of increasing demand for further-processed poultry products the following discussion is based on the opportunities to use MSTM protein isolate in these types of products. In order to find better extraction pH values the comparison was performed based on the difference in protein yield, color, lipid composition, nutritional value and functional properties.

Protein yield is very important due to economic considerations. A present study revealed that the lowest protein yield was achieved at extraction pH of 3.5.

Therefore, extraction pH values of 2.5; 10.5 and 11.5 were further considered in order to determine the optimum extraction pH. Another important property, which affects the acceptance of products, is color, especially lightness and whiteness. Higher values for lightness and whiteness were observed for isolates prepared at extraction pH of 2.5. No differences were found among pH treatments based on the lipid composition and nutritional value. However, considering that acidic pH could be more “natural” (due to HCl secretion in stomach during food digestion) the preference may be given to the acidic pH.

Among the functional properties studied, emphasis was mainly put on evaluation of WHC, determined by water and cooking losses. WHC is a very important property for the preparation of restructured poultry prouducts (i.e. battered and breaded products), which affects the yield and the quality of the end product. The highest water loss was obtained for the proteins extracted at pH 2.5; however processors are interested in cooking loss since the products will undergo cooking before consumption. There has been no difference found in cooking loss among different pH treatments. As a result we propose that pH 2.5 is most preferable for the extraction of proteins from MSTM. Moreover, from the first study it was found that the addition of 6 or 8 mmol/L of citric acid may aid in removal of total heme pigments. With this consideration we conclude that extraction pH 2.5 with the addition of 6 mmol/L of citric acid would be the best option for protein recovery from MSTM.

Future work can be focussed to study the effect of addition of protein isolates on sensorial characteristic of further processed poultry products. Another research aspect might include the potential possibility of co-extraction of other valuable components (i.e. connective tissue, phospholipids) from MSTM during the preparation of protein isolates.

CHAPTER 6. INDUSTRIAL RELEVANCE

Mechanically separated meat is a product obtained during the last stage in poultry processing. Currently it is taken from the processing plant by the pet-food industry or used for further-processed products of lower quality. The higher utilization of this MSTM by producers is limited due to the composition of the product. The limitations are based on high fat content, susceptibility to oxidative changes, dark color and the small particle sizes leading to undesirable textural properties. One of the approaches to overcome these problems is to extract and purify the functional proteins, which can be used as a food ingredient in the preparation of value-added meat products (i.e. burgers, nuggets, rolls). Protein isolate, in general, might be used in the food industry for nutritional reasons to increase the protein content and also to enhance the protein functionality, such as emulsification, foaming, water and fat absorption. In conclusion, the recovery of proteins from MSTM using the pH-shifting process could help the poultry processors to be more profitable and could also create an opportunity to produce functional ingredients to be used in a food industry. One of the most important advantages for the poultry industry could be the possibility of production a 100% poultry product, without any additions of soybean, eggs and milk proteins in the preparation of processed meat products.