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Characterization and Value-Added Utilization of the Proteins Extracted from the By Products of Catfish Fillet Processing Plant

Haoran Gao

Characterization and value-added utilization of the proteins extracted from the by-products of catfish fillet processing plant

By

Haoran Gao

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By

Haoran Gao

Approved:

_____

Sam K. C. Chang
(Major Professor)

_____

Peter J. Bechtel
(Committee Member)

_____

Barakat S. M. Mahmoud
(Committee Member)

_____

Ramakrishna Nannapaneni
(Committee Member)

_____

Marion W. Evans, Jr.
(Graduate Coordinator)

_____

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Haoran Gao

Date of Degree: December 9, 2016

Institution: Mississippi State University

Major Field: Food Science and Technology

Major Professor: Dr. Sam K. C. Chang

Title of Study: Characterization and value-added utilization of the proteins extracted from the by-products of catfish fillet processing plant

Pages in Study 75

Candidate for Degree of Master of Science

Proteins in catfish by-products were extracted by two methods: Alkaline extraction (AE) and salt extraction (SE). Properties of the fish protein isolate (FPI) were measured by protein yield and content, moisture content, SDS-PAGE protein patterns, color and texture profile, and compared with commercial surimi products. Our results showed that catfish frame had higher protein yield and color similarity with commercial products than the head; AE-FPI had higher yield and gel strength than SE-FPI; SDS-PAGE protein patterns of FPI from catfish frame by SE method was comparable with commercial surimi products. Based on the results, further optimization of the recovery yield of protein extracted by alkaline extraction method, and effect of microbial transglutaminase (MTGase) on gelation properties under various concentration and reaction time was investigated. Results indicate that the protein yield reached up to 60%, and the addition of MTGase in protein isolate effectively improved the gel forming ability.

DEDICATION

I wish to express my sincere appreciation to my major advisor Dr. Sam K. C. Chang for his invaluable advising, encouraging through my entire graduate study, and his rigorous, scientific attitude inspired me so much.

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CHAPTER I

INTRODUCTION

In Mississippi, channel catfish farming ranks No.1 with over half of U.S. total catfish sales in 2015 (USDA National Agricultural Statistics Service, 2015). Catfish farming is also important warm water aquaculture in Southeastern states include Alabama, Arkansas and Louisiana. However, the increasing feed cost (grain and soybean) and fierce competition of imported catfish products from Asian countries caused a dramatic decline of domestic catfish production. The U.S. catfish industry was on the highest mark in 2003 when 662 million pounds of round weight catfish were processed (2014 Catfish Industry Review and 2015 Outlook). In spite of the declination, catfish farming and processing industries remain as an important industry in Mississippi and other Southeastern states, since the catfish industry's existence is vital to the success of the local community economy by providing many employment opportunities to many low-income families in the region. Therefore, enhancing profitability of the catfish industry is of a high priority to the U.S. agriculture.

The catfish by-products, including heads, skin, frame and viscera (Figure 1.1), account for 55-65% of the whole fish mass. Though the fact that catfish by-products contain 35% of the protein content of whole fish, which currently, were regarded as waste and may cause environmental pollution if not utilized.

There are two extraction methods mainly used for protein recovery that has been assayed: Alkaline extraction (AE) and salt extraction (SE). Unlike mechanical meat recovery such as mechanical deboning, Alkaline extraction allows selective, pH-induced water solubility of meat proteins with concurrent separation of lipids and removal of materials not intended for human consumption, such as bones and skin (Gehring and others, 2011). According to Chen and Jackzynski (2007), protein isolated from rainbow trout by-products by alkaline solublization followed by isoelectric precipitation has a high protein content between 77.9% and 89.0% on a dried basis. Results from Kristinsson and others (2005) indicated that the recovery yield of protein extracted by AE method from catfish fillet (70%) is significantly higher than the conventional surimi process (40%). However, the yield data of catfish by-products by the AE method has not been reported. In addition, since the catfish head accounted for almost 30% of the whole fish mass compared to the frames (16-18%), their protein yield and functional properties need to be studied individually. In addition to the isoelectric solubilization, fish muscle protein can also be solubilized with salts such as NaCl, KCl and LiCl under neutral pH conditions (Regenstein and Rank, 1980; Ravesi and Anderson, 1969). With low salt, water-soluble or sarcoplasmic proteins are extracted, while at higher salt concentrations, the salt-soluble or myofibrillar proteins are extracted (Regenstein and Stamm, 1979). Kelleher and Hultin (1991) used LiCl and NaCl as protein extractants in red hake. Munasinghe and Sakai (2004) have investigated the highest protein extractabilities of NaCl, KCl and LiCl for pork. However, no investigation has been done to explore the extractability or yield of protein recovered from fish by-products. In this study, proteins were isolated from catfish by-products by two methods (Isoelectric

solubilization/precipitation and salt extraction) and two by-product sources (catfish heads and frames), in order to identify the optimum extraction method of protein in yield rate and its functional properties.

Based on the preliminary results, alkaline extraction has significantly higher recovery yield than that of salt extraction method. However, poorer gel forming ability was found on alkaline processed surimi than that by salt extraction. Hence, the optimal conditions for maximizing the protein recovery yield by alkaline extraction, and approaches for improving the gelling properties of alkaline processed surimi need to be further investigated. Protein extractability depends on extracting procedure that include: volume of extract solution, method, duration of homogenization, centrifugal force and time, and others. (Munasinghe and Saikai, 2004). Hence, a further study was performed to investigate the effect of extracting conditions including extraction time, solid/liquid ratio, solibilization pH on protein recovery yield. Among the approaches for improving the texture of surimi products, microbial transglutaminase (MTGase) has been widely used to induce the polymerization of proteins, thereby increasing the gel strength of surimi (Benjakul and others, 2008; Duangmal and Taluengphol, 2010; Jiang and others, 2000). Benjakul and others (2008) reported that the addition of MTGase from Streptoverticillium mobaraense to mince from lizardfish effectively increased breaking force and deformation of gel. In this study, protein isolate recovered by alkaline extraction (AE) was added with MTGase under various concentrations and reaction times to investigate its effectiveness for improving gel forming ability.

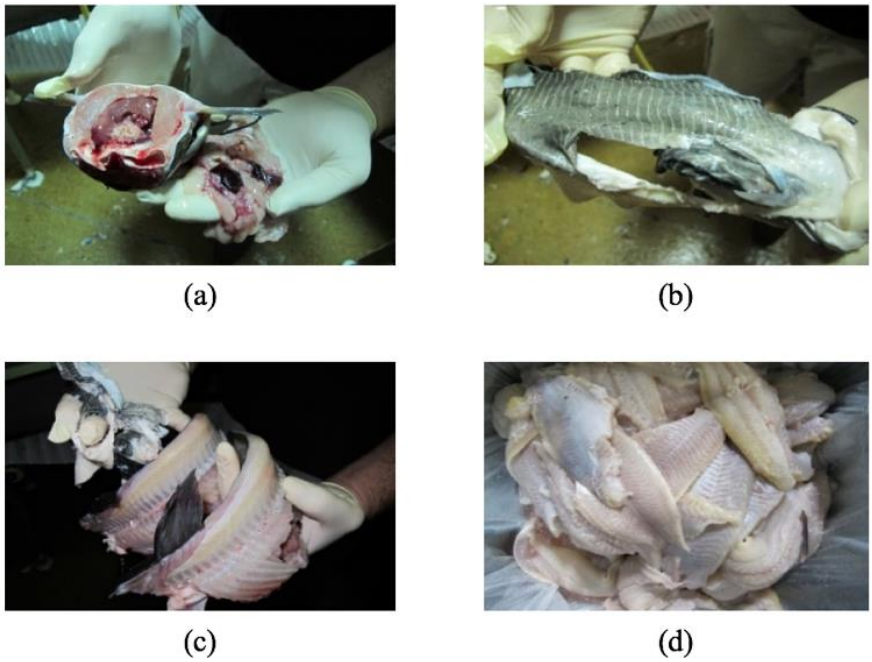

Figure 1.1 Catfish by-products and fillets

(a)-head; (b)-skin; (c)-frame; (d)-fillet.

CHAPTER II

LITERATURE REVIEW

2.1 An overview of seafood by-products

Marine and aquatic species represent a valuable source of protein and essential micronutrients for balanced nutrition and good health. In addition, the benefit from those marine species and seafood is in a great part, dependent on the utilization of the catch. The world's aquaculture and capture fisheries supplied about 148 million tons of fish in 2010, of which over 85% was used for human consumption. However, when fish are processed mechanically on a commercial scale, typically 60-70% of the live fish weight may be discarded as the processing by-products with only 30-40% of live fish weight being marketable as fillet (Gildberg 2002). The mass of flesh leftover on the by-products, typically accounts for 20-30% of the whole fish weight. A large quantity of seafood by-products, which include heads, frames, skins, and viscera currently remain unused or are used for animal feed and sold to oil rendering plants.

The terms "waste", "co-products" and "by-products" are used frequently to describe the secondary products generated as a result of muscle food (seafood and poultry) processing, of which include both edible and inedible materials leftover following processing of the primary product (Gehring and others, 2009). And among these terms, negative connotations may often conjoured from "waste", which imply that those materials are valueless. The terms "by-product" or "co-product" imply that the

secondary products could be valuable if they are recovered properly. Moreover, discarding those seafood by-products could cause environmental pollution. It has been estimated that the value addition of human food developed from the by-product will increase significantly in the future (Kristinsson and Rasco, 2000; Gildberg, 2002). Hence, technologies fostering more efficient use of these by-products to fulfill the needs of human nutrition are required.

Utilization of these processing by-products may be exercised in various ways leading to: 1. Animal and aquaculture feed production, which is similar to the use of whole fish for fish meal and fish oil production. 2. Food ingredients production, such as production of surimi from fish frames. 3. Production of value-added products for pharmaceutical, nutraceutical and chemical industries. Novel and specialty products with potential biological activity or functionality provide a means for value-added utilization of seafood by-products. Taking advantages of the specific flavor of a certain species may used as food ingredients, such as those from cook water of crab and lobster (Jayarajah and Lee, 1999; Yang and Lee, 2000), or for rendering a specific functional property such as health food ingredients may be used for nutritional purposes; these include proteins, lipids, minerals and vitamins. Finally, by-products may be applied for nutraceutical and specialty applications. In this category, protein hydrolysates, fish oils, hormones, glucosamine, chitin/chitosan, flavorants and enzymes as well as other physiologically active ingredients may be included.

2.2 Protein-rich seafood by-products

Seafood by-products are an excellent source of high quality proteins that may supply a major part of the essential amino acids that are required for a balanced nutrition.

Recovery of proteins from by-products may be carried out by different processes using mechanical separation from frames, solvent extraction or hydrolysis. While hydrolysis of fish proteins by endogenous enzymes prior to or during primary processing may lead to fish quality deterioration, such processes may be intentionally carried out to produce specialty products. Thus, production of fish sauce and silage from fish and processing discards can be carried out. In addition, enzymes that are commercially available may be used to produce protein hydrolysate that could be used in a variety of applications. Protein hydrolysates are nearly colorless and appear like milk powder; they may be used in applications where water solubility and water-holding capacity are important. Protein hydrolysates may possess biological activity in enhancing immune response and may also render antioxidant as well as angiotensin converting enzyme (ACE) inhibitory activity (Je and others, 2004) among others.

Carotenoids and their oxygenated derivatives (xanthophylls) are another group of bioactives that are present in salmonid fish, crustaceans and their processing by-products (Shahidi and others, 1998). These are often present in combination with proteins, known as carotenoproteins. Extraction and isolation of carotenoproteins as ingredients for potential use in salmonid fish aquaculture has been reported (Cremades and others, 2003).

Digestive proteases from fish and shellfish discards may be used as industrial processing aids (Shahidi and Kamil, 2001). Suggested uses of digestive proteases from fish include acids for cheese making, herring fermentation, fish skinning, roe processing and production of specialty kits, as well as medical applications.

Since seafood is considered a worldwide prime source of proteins with high biological value, fish processing by-products is an ideal and cheap source for the the extraction of valuable ingredients (Harnedy and FitzGerald 2012). Raw materials from fish by-products have received considerable attention in recent years as an alternative for collagen and gelatins extraction. Several unique applications of collagen and gelatin derived from fish by-products have been reported, due to the enriched properties of fish collagen. The high hydroxyproline content of collagen has been reported to reduce pain in osteoarthritis patients supplemented with collagen/gelatin hydrolysate (Moskowitz 2000).

2.3 An overview of catfish

2.3.1 Catfish production, sales and consumption in the U.S.

Catfish, primarily the channel catfish (Ictalurus punctatus), is the largest aquaculture industry in terms of production and value in the United States. From the first commercial production in ponds in the 1960s, catfish production has grown rapidly to reach annual sales of $361 million in 2015. The combined production and sales in Southeastern states include as Mississippi ($201 million), Arkansas ($21 million), Alabama ($104 million) and Texas ($16 million), account for 94% of the U.S. catfish sales. However, water acres used for U.S. catfish production have decreased by 64% from 2002 to 2015 (Figure 2.1- USDA National Agricultural Statistics Service 2015). Fierce international competition is one of the important factor that led to the current situation. From 2005 to 2013, due to the lower price of fish products imported from Asia countries especially Vietnam and China, market share of domestic processed fillets has decreased from 80% to 20%. However, the emerging customers in the U.S. tend to buy

seafood products locally grown with known production methods and approved, safe practices. Dramatic increase of feed cost (corns and soybean prices) for catfish raising is another contributed impact factor for the catfish processing industry. The average annual feed cost of catfish with crude protein has doubled from 2005 ($220/ton) to 2014 ($470/ton). Many catfish raising farmers who used to plant row crops in the Delta regions have converted their pond back to corns and soybean production to reduce the feed cost.

American's preference for seafood species have been changed over the last decade (Figure 2.2). Shrimp is the most preferred seafood products since 2003. Tilapia is the number four preferred seafood since 2006. The farm-raised catfish dropped from fifth to eighth from 2000 to 2013 with 0.56 pounds per person per year.

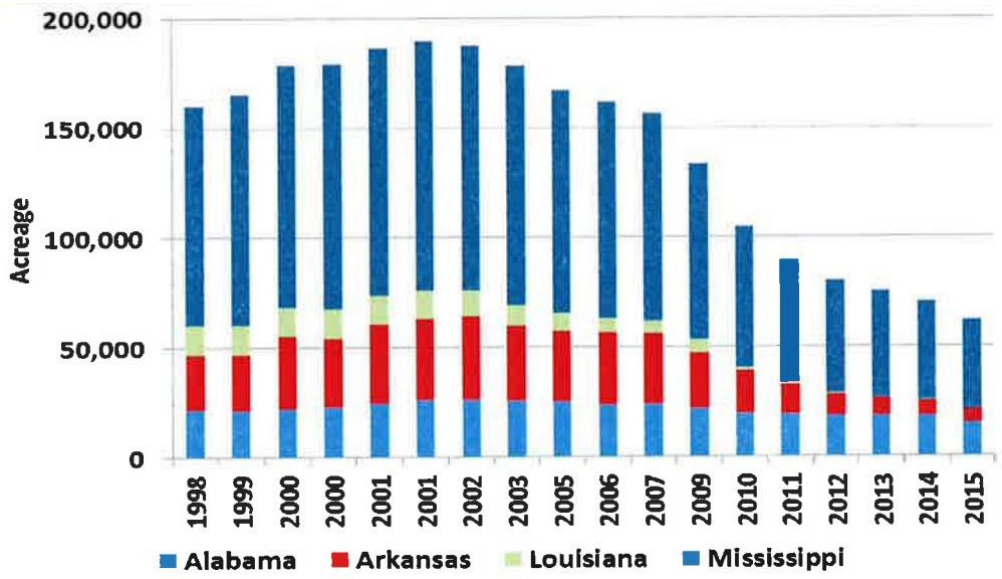

Figure 2.1 Water acreage used in U.S. catfish production, Jan 1998- Jan 2014. (Source: January 2015 NASS Catfish Production report).

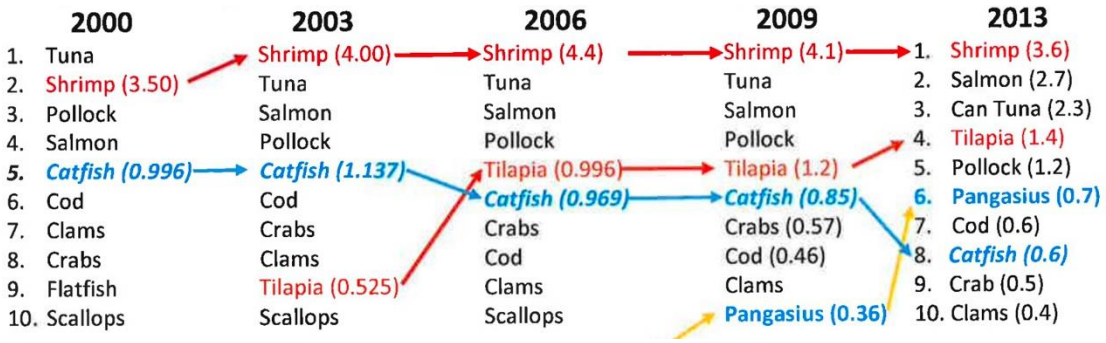

Figure 2.2 U.S. top ten seafood consumed, per capita consumption

(Source: Fisheries of the United States, 2013, NOAA Office of Science and Technology, 101).

2.3.2 Catfish composition

2.3.2.1 Protein

Myofibrillar proteins are the proteins organized into myofibrils, which are commonly viewed as salt-soluble proteins. Thick and thin myofilament are the two major components of sarcomere, the contractile units of myofibril. It contains myosin (55-60%), actin and other proteins. With both fibrous and globular properties (Figure 2.3), myosin is a large protein with molecular weight of 470 kDa. A myosin molecule is composed of two 220 kDa heavy chain and two different light chains with molecular weight range from 17 to 22 kDa. Myosin is largely responsible for functional properties of muscle tissues, including gelation and water-binding. Actin comprises about 15-30% of the myofibrils with a molecular weight of 43 kDa. Other small fractions of protein associated with myosin, such as tropomyosin and troponin, function as regulatory proteins to initiate and terminate the contraction process of muscle. In addition, those fractions serve to maintain the structural integrity of sarcomeres. During surimi processing procedures, disassembling the sarcomere is prerequisite for obtaining heat-induced gel structure with

evenly distributed protein. Z-disk protein and interfilamentous proteins are cytoskeletal proteins, which is critical to maintain the ultrastructure of myofibril.

In surimi processing, extensive washing is utilized to remove water soluble substances, mainly sarcoplasmic proteins (Lin and Park, 1996), which has a molecular weight ranging from 12 to 97 kDa (Figure 2.4). Sarcoplasmic proteins are composed of a group of protein with enzymes involved in muscle metabolism. In contrast to myosin or actomyosin with large fibrillar or rod-like conformations, it has a globular tertiary structure. This protein is usually removed during the washing step in order to concentrate myofibrillar proteins (Park and Lin, 2005). Moreover, sarcoplasmic protein could adversely affect surimi gelation by interfering with myofibrillar protein interactions (Rodger and Wilding, 1990). A positive effect to myofibrillar protein gelation could be occurred by the activity of endogenous muscle transglutaminase (TGase) and endogenous proteinase inhibitors. Heme proteins is one of the important component of sarcoplasmic proteins and it is responsible for the pigmentation of unleached mince. It contained hemeglobin and myoglobin, which are the main proteins that existed in blood and red muscles. Removal of those protein during the seafood processing is essential since that the existed ferric iron would play a role of facilitating lipid oxidation, which could cause the premature denaturation and aggregation of myofibrillar proteins, and finally, the declination of gelling properties.

Stroma protein or mainly the collagens are proteins existed in the connective tissue of seafood species. Due to its salt-soluble properties, this protein is usually retained with myofibrillar protein during surimi processing. It can be converted into gelatin when temperature increased, and thus interfere with the gelation properties of myofibrillar

proteins. The degree of this effect varies from land animals and fish species due to different content of stroma proteins. The meat of land animals presents high concentration of this protein, it can accumulate in unsightly pockets in a heat-induced gelled meat product. However, since there is only a small percent existed in fish species, the residual stroma proteins in meat of fish species have a negligible effect to the gelling property of surimi product. Collagen has a wide range of applications in the leather ad film industries, in cosmetic and biomedical materials, and as food (Kittiphattanabawon and others, 2005).

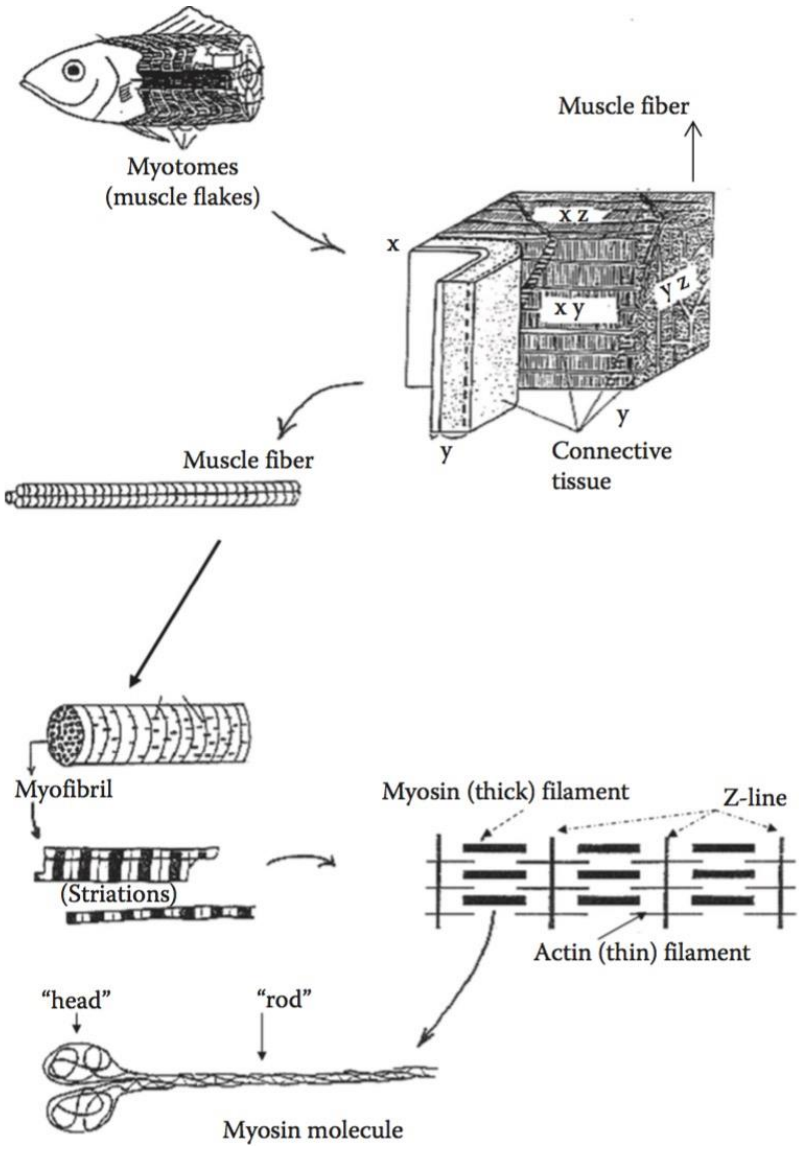

Figure 2.3 Diagram depicting successively greater detail in fish muscle microstructure.

(T Nakagawa, S Watabe, K Hashimoto. Nippon Suisan Gakk 54: 993-98, 1998.)

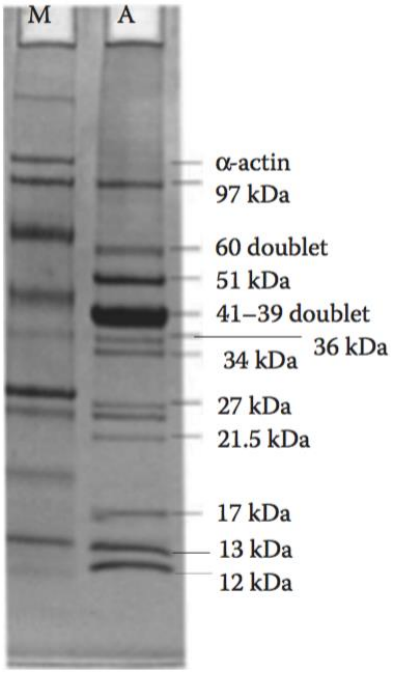

Figure 2.4 SDS-PAGE pattern of sarcoplasmic proteins extracted from sea bass. M: protein markers

(Ladrat and others, 2003.)

2.3.2.2 Lipid

Lipids are the fundamental components that serve essential physiological and biochemical functions of all aquatic organisms. However, the distribution of lipid varies in position of fish body and fish species. It concentrated primarily in the subcutaneous layer of the fatty fish, in the liver of the lean fish, in muscle tissue and in mature gonads (Sikorski and others, 1990). The lipid content of farm-raised catfish is low (7%) compared to other animal based foods such as meat (10%) and poultry (9%). It is known that fish species is a good source of polyunsaturated fatty acids (PUFA), especially the n-3 fatty acid for human nutrition. Sun and others (2002) reported that viscera from salmon was a good source of EPA and DHA and by using microbial lipases the concentration of these omega-3 PUFAs could be doubled.

2.3.2.3 Enzymes

Enzymatic technique has been commonly used for processing in food industry. The enzymes extracted from aquatic species have drawn the attention of many researchers since their unique specificity and characteristics. In addition, recovery of enzymes from seafood by-products could add values to the seafood industries. There is a large group of enzymes that can be extracted from seafood and seafood by-products, include digestive proteinases, cellular and extracellular proteinases, lipases, chitinases, phospholipases, polyphenoloxidase, transglutaminases and others. Studies of the purified enzymes from fish species and its by-products include carboxypeptidases from tilapia stomach (Taniguchi and others, 2001), transglutaminase from walleye pollock liver (Kumazawa and others, 1996), carbohydrates from butterfish, silver drummer, and marble fish (Skea and others, 2005).

Compared with enzymes extracted from terrestrial animals and plant sources, aquatic-based enzymes usually have special physical, chemical and catalytic properties (Shahidi and Kamil, 2001). Since some of the aquatic species accustomed to live in the habitat with cold temperatures, their enzymes present cold-adapted properties. Several enzymes have been shown to be salt tolerant as well, which can be an advantage in certain food applications (Cavicchioli and others, 2002). The solid and liquid by-product streams of seafood processing plant is an important source of enzyme extraction and utilization, since those by-products are often discarded or conventionally processed into fish feed or fertilizer. However, the economic feasibility for commercializing the enzyme extraction may depend on quality of raw materials, extraction techniques and potential markets.

2.3.2.4 Pigments

Pigments in aquatic species means all compounds that are naturally present in aquatic animals, plants and algae. Those pigments may include carotenoids, which account for the red, yellow color as present in salmon and trout fillet, crab and lobster. Colors ranging from brown to black that usually found in eyes and skins of fish species are contributed by the existence of melanins, which is formed by enzyme catalyzed oxidation of phenolic compounds. The green chlorophyll pigments that presents in all photosynthetic organisms enabled those species play a role of producers in food chain.

In addition to impart bright red, yellow and orange colors to these species, the carotenoid pigments also possess antioxidant properties by virtue of highly unsaturated nature, which enabled oxidation of themselves instead of other molecules.

Melanins are formed from amino acid, phenolic compounds and tyrosine by oxidation and followed by polymerization, thus it has a large molecular weight. Skins, eyes and peritoneal lining of fish species are typical body parts that melanins present to protect these tissues against ultraviolet radiation. However, in crustacean species such as prawn, shrimp, lobster and crab, melanosis or blakspot are the usual form of melanins presents. Benjakul and others (2005) has studied this phenomenon with the goal to impair the effect of polyphenoloxidases in crustacean species. In fact, these melanins have been recovered and applied for human use for protecting skin or minimizing the glare of eyes from radiation of sunlight.

2.4 Surimi

2.4.1 An overview of surimi

Surimi is minced fish meat subjected to washing to remove fat, water-soluble proteins (sarcoplasmic protein) and undesirable muscle components such as blood and pigments (Kaewudom and others, 2013) to make fish ball, fish cake, fish sausage and imitation crabsticks. Surimi technology originated in Japan approximately 900 years ago, and Japan has had a leading global role in developing surimi and surimi seafood. However, the modern surimi technology falls before and after 1960 when it was discovered that sugar was an effective cryoprotectant for fish protein. The development of crabstick in Japan in 1973-1975 facilitated the globalization of surimi, which were expanded to United States, Europe and Russia. In the United States, the production of surimi from Alaska pollock started with the legal justification of the Magnuson Act in 1976. The commercial surimi production had changed significantly in the past twenty years. For the raw materials of surimi manufacture, it has greatly diversified from Alaska pollock as a single sourch to pollock, and to tropical fish species. The production yield has increased from 12% to 15% in the 1990s to 30% or higher in 2010, which made fishery resources more sustainable.

2.4.2 Surimi gelation chemistry

In the creation of any sort of surimi based product, the gelation process is the most important step in achieving the desired texture (Duangmal and Taluengphol, 2010). Fukazawa and others (1961) and MacFarlane (1977) reported that it is myosin that mainly contributes to gel formation and water holding in cooked comminuted meat products. The mechanism of gelation is depicted in Figure 2.5 and Figure 2.6. Individual

myosin or filaments should be freed from the complex structure of muscle. Once the myofibrillar protein is unfolded or denatured during thermal processing, it will expose reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds. A three-dimensional network is formed as gel when bonding is sufficient. There are four types of chemical bonds contribute to the gel formation: hydrogen bonds, hydrophobic interactions, ionic linkages and covalent bonds.

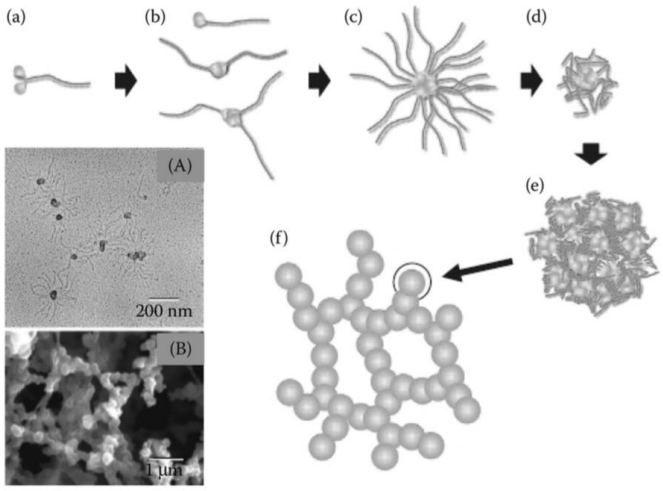

Figure 2.5 Heat-induced gel formation of myosin monomer.

(a) Unheated; (b) cluster formation in early heating; (c) Daisy wheel formation of myosin head-to-head; (d) denaturation of tail portions; (e) clusters bound through denatured tails; (f) gel network formation. (Photomicrographs (A) TEM (Transmission Electron Microscope) image of clusters (0.5 M KCI, pH 6, 40°C, 10 min); (B) FE-SEM (Field Emission Scanning Electron Microscope) image of gel.)

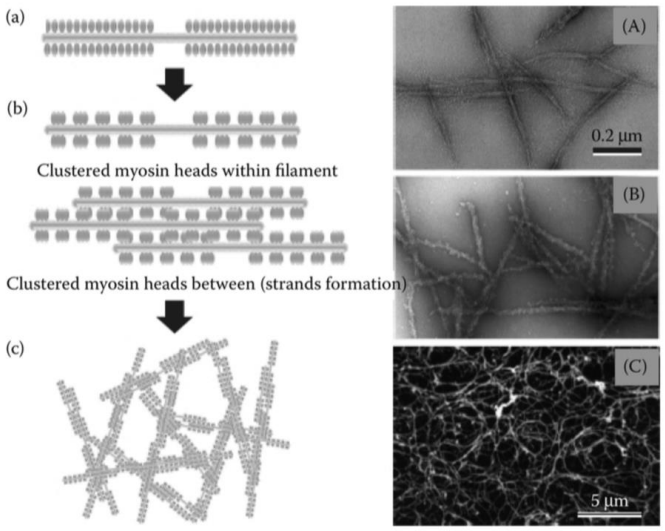

Figure 2.6 Heat-induced gel formation of myosin filaments

(a) Unheated; (b) cluster formation in early heating; (c) gel network formation; (A) TEM (Transmission Electron Microscope) image of unheated filaments; (B) TEM image of after heating at 40°C for 10 min (0.1 M KCI, pH 6) (Same magnification shown in (A); (C) SEM (Scanning electron microscope) image of filament gel. (K, Yamamoto, 2008).

2.4.3 Surimi gelation chemistry

For surimi products, there are mainly three functional properties: color, texture, flavor. Controlling color and flavor is relatively easy because of linear responses (Park, 2014). However, textural properties were influenced by the addition of ingredients in a non-linear fashion, and hence, texture is more difficult to control. The major ingredients used for texture and flavor fortification of surimi are protein additives, starch, and hydrocolloids.

Dried protein additives approximately have 60-95% protein content, thus are usually used as nutritional fortifier in surimi. More importantly, protein additives could provide functional advantages when added in surimi: it can improve the elasticity of gels and inhibit enzyme activity. The commercially available protein additives include frozen

egg white, dried egg white, bovine plasma protein, whey gluten, soy protein isolate, whey protein isolate. Among those isolated proteins used as ingredients, soy proteins are probably the most widely employed (Pietrasik and Li, 2002).

Starch is one of the most widely used additives in surimi based products because of its high capacity to swell, and retain water, which enhances gel strength, reduces the amount of surimi used, and ensures storage stability for frozen crabsticks (Campo and Tovar, 2008). Starch is composed of microscopic granules in stratified layers. The granules consist of two major polymers: amylose and amylopectin. Park (2014) reported that high-amylose starch, such as corn and wheat, form brittle gels, whilst high amylopectin starches, such as waxy maize and tapioca, form adhesive and coherent gels. Except for those native starches, modified starches is a series of starch derivatives synthesized through physical, chemical or enzymatic processing methods, which attempt to alter the molecular size and physico-chemical properties for specific applications (Sun and others, 2014).

2.4.4 Surimi production in the U.S.

Due to the fact that the Magnuson Act of 1976 legally justified, Alaska pollock surimi started to produce in the United States. Despite a variety of difficult obstacles, joint ventures grew rapidly. Eventually, it led to a bond between fishermen, and a foundation was formed upon which many of today's relationships was built.

Since 1984, with the first surimi production in the United States, a total of 17 land-based surimi plants were built and, as of 2012, only nine plants currently produce surimi from either pollock or pacific whiting in North America (USDT, American

fisheries, 2012). After about 30 years of surimi production in the Unites States, the surimi yield significantly increased from 12% to over 30%.

In 1973-1975, the Japanese invented the crabsticks, which became a corner stone for surimi to globalize. Consumption of surimi crabsticks reached over 5 million pounds in 1981. The consumption of crabstick increased from 30 million pounds to 75 million pounds in 1983-1984. Consumption continued to grow as 11 different plants started to make crabstick in 1984-1986 and reached 160 million pounds in 1990. After the rapid growth, during 1990-2007, the market matured and showed growth. Due to the surimi crisis in 1991-1992 and 2007-2008, production/consumption significantly reduced. The current U.S. market in 2012-2013 would be near 200 million pounds, including approximately 20 million pounds of imports (Park, 2014).

As for the product distribution in the market, more than 90% is flake type, 5% stick type, and the rest is either shreds or molded shrimp (Yuge and Nozaki, 2010). 85-90% of surimi are used for salad making. The remaining 0-15% is used in producing California rolls. These two product applications have controlled US production and market. The US surimi seafood industry needs to lead its consumers to try premium crabstick as a snack, seafood pasta with premium texture and fired surimi seafood. Seafood pasta should be promoted as having high protein and being gluten-free. Fired surimi seafood can be marketed as a healthy food as it gives just 2% total fat while providing the same good taste of typical food (10-20%) fat.

2.5 Methods for protein recovery from by-products

2.5.1 Mechanical technique for flesh recovery

In large fish species such as cod and haddock, the cheeks and tongues are usually removed manually. Regenstein (2004) reported that equipment to remove the cheek has not been widely adopted though it is commercially available.

According to the FAO Fisheries and Aquaculture Department's report, ninety percent of world fish production destined for nonfood purposes ends up as fish meal or oil, and the remaining 10% is largely utilized as a feed in aquaculture and for animals (FAO 2007).

Those mechanically recovered flesh from fish by-products is normally termed as fish mince. Fish-bone separators are developed from the first machines applied in fruit and meat industry and later specialized to perform in fish industry. The separation techniques of this machine is based on the screening of flesh from bones through a perforated filter. Despite the apparently simple operating principles, the relationships between pressure, perforation size, and perforation area with yield, contaminant levels, and shear damage, are complex (Grantham 1981). However, fish mince currently has lower acceptance in market due to the aesthetic deterioration that occurs during the flesh-bone separation process. Compare with the actual flesh in by-products, fish mince is darker in color and more heterogeneous, which is due to the mixing of components such as blood, pigments within skins that can also pass through the perforations of the drum.

2.5.2 Non-mechanical technique for flesh recovery

2.5.2.1 Enzymatic method for protein recovery

Enzymatic methods have become an important and indispensable part of the process used by the modern food and feed industry in order to produce a large and diversified range of products for human and animal consumption (Shahidi and Kamil 2001). Protein hydrolysates have been made from various fish species by bacteria fermentation (Rai and others, 2013; Ruthu and others, 2014) or by enzymatic hydrolysis (Cai and Wang, 2009; Liu and Guo, 2009, Hathwar and others, 2011; Kumar and Bhalla, 2003; Benhabiles and others, 2012). Numerous studies (Kristinsson and Rasco, 2000) shared a common objective that enzymatic proteolysis as applied to proteinaceous fish by-products. Fish protein hydrolysates have a wide range of potential utilizations, such as peptone ingredient in microbial growth media (Aspmo and others, 2005; Dufosse and others 2001); fertilizer or new source of bioactive peptides (Byun and Kim 2001, Guerard and others 2001); animal feed or food. Recently, hydrolysates of herring protein have been found to possess antioxidant activity (Halldorsdottir and others 2013) and Angiotensin-I converting enzymes (ACE) inhibition activity (Taheri and others 2014; Kristinsson and others, 2014).

Proteases is one of the most well applied enzymes in food industry. According to their specific peptide bond cleavage, proteases can be classified in four major classes: cysteine proteinases, metalloproteinases, serine proteinases and aspartic proteinases. By hydrolyzing mechanism, proteases can be further divided into endoproteinases and exoproteinases. The breakdown of proteins into peptides is brought about primarily by endoproteinases which cleave the peptide bond within protein molecules, usually at

specific residues to produce relatively large peptides (Kristinsson and Rasco 2000). Exopeptidases such as carboxypeptidases and aminopeptidases serves to reduce the peptides into amino acids.

Employing and autolytic process or by using added proteolytic enzymes are the normal biochemical process for converting fish by-products into fish protein hydrolysates. This process is often used in fish sauce and fish silage production, in which hydrolytic enzymes from the fish itself play a key role in the solubilization and degradation of the tissue proteins (Gildberg and others 2000).

2.5.2.2 Chemical methods for protein recovery

2.5.2.2.1 Fish protein concentrate

Fish protein concentrate (FPC) is a product that is produced by removing fat and water and gaining higher protein concentration (Ibrahim 2009). Experiments with FPC began in Sweden in 1936 and were important for fish silage production (Sanmartin and others, 2009). With the objective to exploring new ways to produce fish protein concentrates on large scale for seafood industry stimulation and confrontation of global malnutrition, the National Marine Fisheries Service (NMFS) in U.S. initiated a research program with respect to this area in the early 1960s (Snyder 1967). First, the minced by-products are extracted with isopropanol at 20-30 °C for 50 min and subjected to centrifugation. The supernatant was collected and extract with isopropanol for times, 75 °C for 90 min with isopropanol and 75 °C for 70 min with azeotropic isopropanol, individually. After another batch of centrifugation, the collected supernatant is then dried, milled and screened, which makes the final products with colorless, odorless and high biological values. Mackie (1974), Cheftel and others (1971) reported that fish protein

concentrate is poorly soluble or dispersible in foods, which greatly limits its applicability. Venugopal and others (1996) also reported the poor emulsification properties of FPC. However, some of the other researches indicate that FPC has good use if produced properly. For instance, water holding capacity and cooking yield of the burger patties was increased by adding FPC, which was extracted from sardine by-products (Vareltzis and others 1990). Moreover, FPC could be a good substrate for enzymatic hydrolysis for making fish protein hydrolysates since it provides a largely oil-free substrate and has partially denatured proteins which are highly susceptible to enzymatic hydrolysis (Kristinsson and Rasco 2002). However, general poor functionality, off-flavors and colors, high cost of production and possible traces of solvent in the final product have made solvent extracted FPC commercially unsuccessful regardless of intensive efforts (Mackie 1982).

2.5.2.2.2 Isoelectric solubilization/precipitation

Developed by Herbert O. Hultin and coworkers (Hultin and Kelleher 1999; Hultin and others 2004), acid and alkaline solubilization and isoelectric precipitation of muscles are specifically designed to recover functional proteins from fish by-products. The myofibrillar proteins obtained a large net charge at high or low pH values, which cause them to solubilize. As the proteins solubilize at extremes of pH, the cellular lipid membrane encasing the myofibrillar proteins is disrupted causing a dramatic drop in solution viscosity (Kristinsson 2002; Kelleher and others 2004). The alkaline extraction (AE) is a pH-shift process in essence, which induces the water solubility of myofibrillar proteins. Following separation between myofibril and insolubles, a subsequent pH-shift induced protein precipitation (Figure 2.7). Centrifugation is used for separating lipids

(top layer) and other residues (bones, skins, collagens) by sedimentation from dissolved proteins (middle layer). This process efficiently recovers protein isolates with high quality in terms of nutrition and functional properties from sources difficult to process such as krill, fish, chicken, and beef processing by-products (Chen and Jaczynski 2007; Gigliotti and others 2008; Jaczynski 2009; Nolsoe and Jaczynski 2009). There are several essential benefits of AE recovered protein compared with that by mechanical process. A major advantage of AE process is that those undesirable compounds such as skins, bones, cholesterol, lipids and other contaminating materials can be removed during the first centrifugation step (Hultin and Kelleher 2000). Moreover, the extreme pH-shift during AE result in mild, non-thermal pasteurization (Lansdowne and others 2009). Marmon and others also reported that dioxin and polychlorinated biphenyls (PCBs) are significantly reduced in the AE-recovered fish proteins.

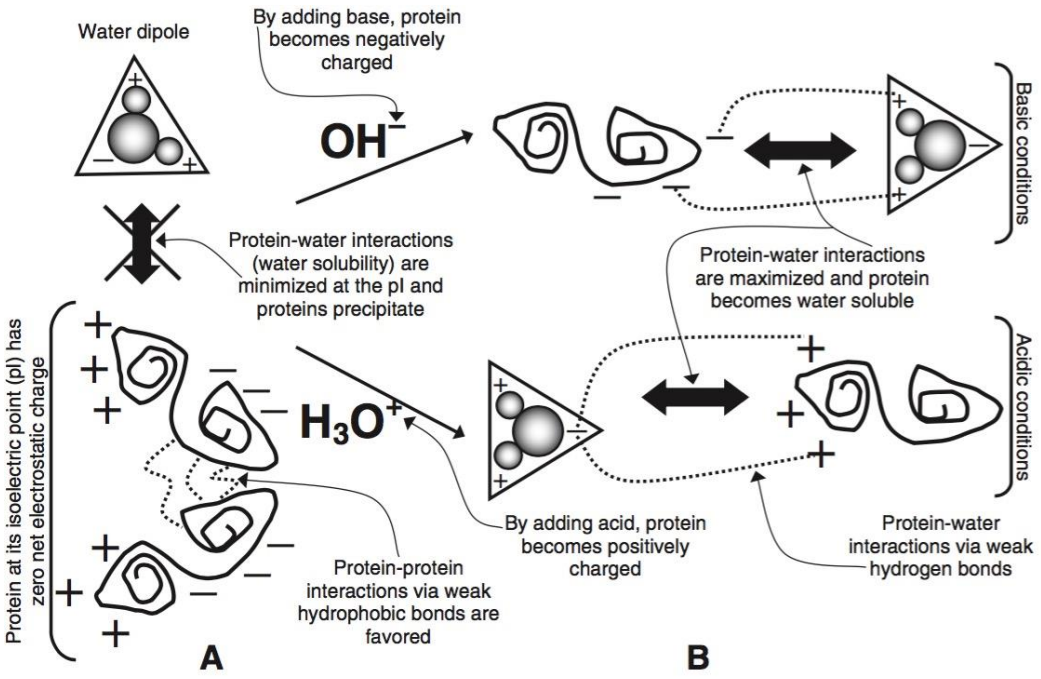

Figure 2.7 A protein at its isoelectric point (pI) has a zero net electrostatic charge

A. At its pI, protein–water interactions are at its minimum, while protein–protein interactions via weak hydrophobic bonds are at its maximum, causing protein precipitation. B. Protein–water interactions prevail under acidic or basic conditions far from the pI, resulting in protein solubility in water. (Gehring and others, 2011).

2.5.2.2.3 Chemical hydrolysis

At high temperatures, proteins in fish by-products could be hydrolyzed with acid or base. Due to the cost-efficiency and simplicity, several processes have been proposed for the acid or alkaline hydrolysis of fish species (Hale, 1972). However, Blenford and Skanderby's (1994) research indicates that the final products with variable composition and functionality may result from this process, which mainly due to that it is difficult to control. Moreover, the extreme pH conditions and high temperatures may result in reduced nutrition functionality of the products, which have lower potential for using as seafood flavorings (Webster and others 1982; Loffler 1986).

Acid hydrolysis is more commonly used than the alkaline hydrolysis as a method to hydrolyze fish proteins. The process usually involves mixing of strong hydrochloric acid or sulfuric acid with raw materials, which was then subjected to high temperature or high pressure. Total hydrolysis can be achieved in 18 hours at 118 °C in 6 N hydrochloric acid (Thomas and Loffler, 1994). Resulting fraction containing the hydrolyzed proteins is then neutralized to pH 6.0-7.0 and dried or concentrated. (Thakar and others 1991).

Poor functionality and adverse nutritive values can occur in final product made by alkaline hydrolysis. However, several alkali treatment is still used in food industry for recovering and solubilizing a broad range of proteins (Kristinsson and Rasco 2002). One of the advantages of alkaline hydrolysis is that it modified and improved functional properties of otherwise highly insoluble FPC (Sikorski and Naczk 1981). Tannenbaum and others (1970) developed a process that with the use of high pH (12.5) and 95 °C for 20 min, the final product demonstrated an overall improvement in functional properties compared with the original FPC.

2.6 Utilization of seafood by-products

2.6.1 Food using of seafood products

2.6.1.1 Food ingredients

The relevance of the actual concept of by-products as all raw material, edible or inedible, leftover during the preparation of the main product (Gildberg, 2002) has become very important.

Fish heads are usually regarded as waste for disposal or processed to fish meal. However, markets in some region, especially in Asian coutries where the fresh or frozen fish heads are commercialized for human consumption. For instance, salmon heads in

Taiwan market has the same price per kilogram as the fillets. In Iceland, where fish is an important item in the diet, dried fish heads softened in dairy whey are used to prepare various dishes. The heads from tuna and some large size tropical fish are consumed grilled over an open fire or in an oven and are known as kabutoyaki in Japan (Tonsberg and others 1996).

The fish frames generated after filleting in processing plant is an essential source of food using. Frame mince can be used to make a variety of fish products either for human consumption or pet food manufacture (Arason, 2002).

This study's objective is to extract proteins from catfish by-product and to characterize their molecular and fundamental properties for potential utilization as surimi products.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Whole catfish, catfish fillets, catfish frames, catfish heads

Whole live catfish, catfish fillet and by-products (heads and frames) were collected from Country Select Catfish and Prime Line Catfish, two U.S. certified catfish processors located in Isola, MS 38754 and Scooba, MS 39358, respectively. All samples were washed with tap water to remove the blood on surface and packed with ice in a cooler box and transported to the Pilot Processing Plant in Department of Food Science, Nutrition and Health Promotion, Mississippi State University, MS 39759. Whole live catfish was used for preliminary study and was processed in a walk-in cold room at 4°C immediately after arrival. Catfish fillet, head and frames were stored in a walk-in freezer at -20°C for further use.

3.1.2 Chemicals

Sodium chloride, sodium hydroxide, sodium azide (NaN_3), hydrochloric acid, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), β-mercaptoethanol (β-ME), glycerol, tetramethylethylenediaminecc (TEMED), ammonium persulfate, acylamide, methanol, acetic acid, comassie brilliant blue G-250, bovine serum albumin (BSA), phosphoric acid, boric acid, bromocresol green, ethanol 95%, methyl red, sulfuric acid, potassium sulfate, cupric sulfate, titanium dioxide, tris-aminomethane

(THAM) were purchased from Sigma (St. Louis, MO, USA). Microbial transglutaminase (MTGase) from Streptoverticillium mobaraense (TG-K) containing 1% pure enzyme was supplied by Ajinomoto (Thailand) Co., Ltd. (Bangkok, Thailand).

3.2 Materials

3.2.1 Analysis of mass balance

The mass balance of catfish was measured based on the following process: 100 hundred whole live catfish (3-5 lbs) pressed through the assembly line of deheading, gutting, filleting and flesh-skin separating to obtain the weighed percentage parts of catfish head, frame, fillet, skin and viscera.

3.2.2 Moisture content determination

Four to five grams of extracted protein and minced fillet, individually, were placed on a weighed aluminum pan, and incubated overnight at 105°C. Dried products were placed in a desiccator for 10 min to cool before weighing for calculation of moisture content.

3.2.3 Protein content determination

3.2.3.1 Kjeldahl analysis (AOAC Itl., 2015, Method No. 991.22)

3.2.3.1.1 Digestion

The recovered protein isolates were freeze-dried into powder and subjected to Kjeldahl analysis for determining protein content. Approximately 0.2 g fish protein isolate (FPI) were weighed, recorded and placed in a digestion tube. One catalyst tablet and 7mL of concentrated sulfuric acid were added to each tube with FPI. The rack of

digestion tubes was placed on digestion block, which covered with exhaust system turned on. The digest was diluted with deionized water after the digestion is complete.

3.2.3.1.2 Distillation

The distillation system was started up and a receiving flask with appropriate volume of boric acid solution was placed so that the tube coming from the distillation of the sample was submerged in the boric acid solution. A set volume of NaOH solution was delivered to the tube, and a steam generator was used to distill the sample for a set period of time.

3.2.3.1.3 Titration and Calculation

The normality of the standardized HCl solution was recorded. The flask was placed on a stir plate and a stir bar were placed in flask to keep the solution stirring briskly while titrating. Each sample and blank were titrated with HCl standardized solution to the first faint grey color, and the volume of HCl titrant used was recorded. The following equation was used to calculate the Nitrogen content:

$$\% \text{ N} = \text{Normality HCl} * \text{corrected acid volume (mL)}/ \text{weight of sample (g)} * 14\text{g N / mol} * 100 \quad (3.1)$$

(the nitrogen to protein conversion factor used were 6.25)

3.2.3.2 Bradford protein assay

To prepare the protein sample, 2 g of protein isolate were solubilized in 10 mL 2% SDS and 1% β-mercaptoethanol solutions, and ground by mortar and pestle for 10 min to homogenize. The homogenate centrifuged at 3500 xg for 20 min to remove the undissolved debris. Bradford protein assay were used to determine the protein concentration of the supernatant. Samples were diluted 20 times with deionized water,

and 5 uL were mixed with 250 uL Bradford standard solution. A portion of 200 uL mixture were transferred to a 96-well plate and measured for absorbance at 595 nm. The final concentration of samples was adjusted to 2 mg/ml for SDS-PAGE electrophoresis.

3.2.4 Ash content determination (AOAC Itl., 2015, Method No. 942.05)

Before the analysis, crucibles and the lid were placed in the oven overnight at 550°C to burn off the impurities on the surface, and cooled in the desiccator for 30 min. Five grams of samples were weighed and placed into the crucible to heat over low Bunsen flame with lid half covered. The crucible and lid were then placed in oven and heat overnight at 550°C when fumes no longer produced. The lid was placed after complete heating to prevent loss of fluffy ash. The desiccator was cooled down, and crucible and lid were weighed when samples turned to gray. Ash content of the samples were calculated using the following equations:

Ash (%) = Weight of ash/ Weight of sample * 100

3.2.5 Lipid content determination (AOAC Itl., 2015, Method No. 983.23)

The bottle and lid were incubated at 105°C overnight to ensure that weight of the bottle was stable. Three to five grams of samples were weighed and wrapped with Whatman No.40 filter paper. Samples were placed into extraction thimble and transferred into soxhlet. 250 ml petroleum ether was poured into the bottle and took it on the heating mantle. Soxhlet apparatus were connected and cycled with water to cool before switched on the heating mantle. Sampled were heated for 14 hours at rate of 150 drop/min. A vacuum condenser was used to evaporate the solvent. The bottle was incubated at 80-90°c until solvent was completely evaporated. After drying, the bottle and lid were

cooled and weighed for its dried content. The lipid content of the samples was calculated with the following equation:

$$\text{Lipid (\%)} = \text{Weight of lipid/ Weight of sample} * 100 \quad (3.2)$$

3.2.6 Color property determination

Hunter color values ("L", "a", "b") of final precipitate (protein paste) were measured using a hand-held Tristimulus Minolta Chroma Meter (Minolta Ramsey, NJ, USA) by the method described in our study (Kong and others, 2008). The value L indicates the lightness ranged from 0 to 100; "a" was chromaticity where positive indicating redness and negative value indicating greenness; while positive "b" values indicate yellowness and negative "b" value indicating blueness. Three random spots on each sample were measured and the averages were recorded.

3.2.7 Alkaline extraction

The entire extraction procedures were carried out in a cool room at 4 °C. Heads and frames, respectively, were minced by a meat grinder (LEM Products #32, West Chester, OH) and washed two times with tap water (containing 0.5% EDTA) at ratio of 1:4 (mince: water, w/v) to remove the residual blood, sarcoplasmic protein and fat. The flow diagram (Figure 3.1) shows the process of catfish by-products protein extraction by AE method. 2N NaOH was used to adjust the protein slurry to pH 11 and extract for 30 min at 4 °C, and followed by centrifugation at 10000x g for 20 min to obtain the supernatant. The oil layer floating on the top was removed by decanting the protein solution through a hand sieve with a mesh size of 0.25 mm. The supernatant was then

adjusted to pH 5.5 with 2N HCl and centrifuged at 10000x g for 20 min. The precipitate was collected and used as the raw material for surimi making.

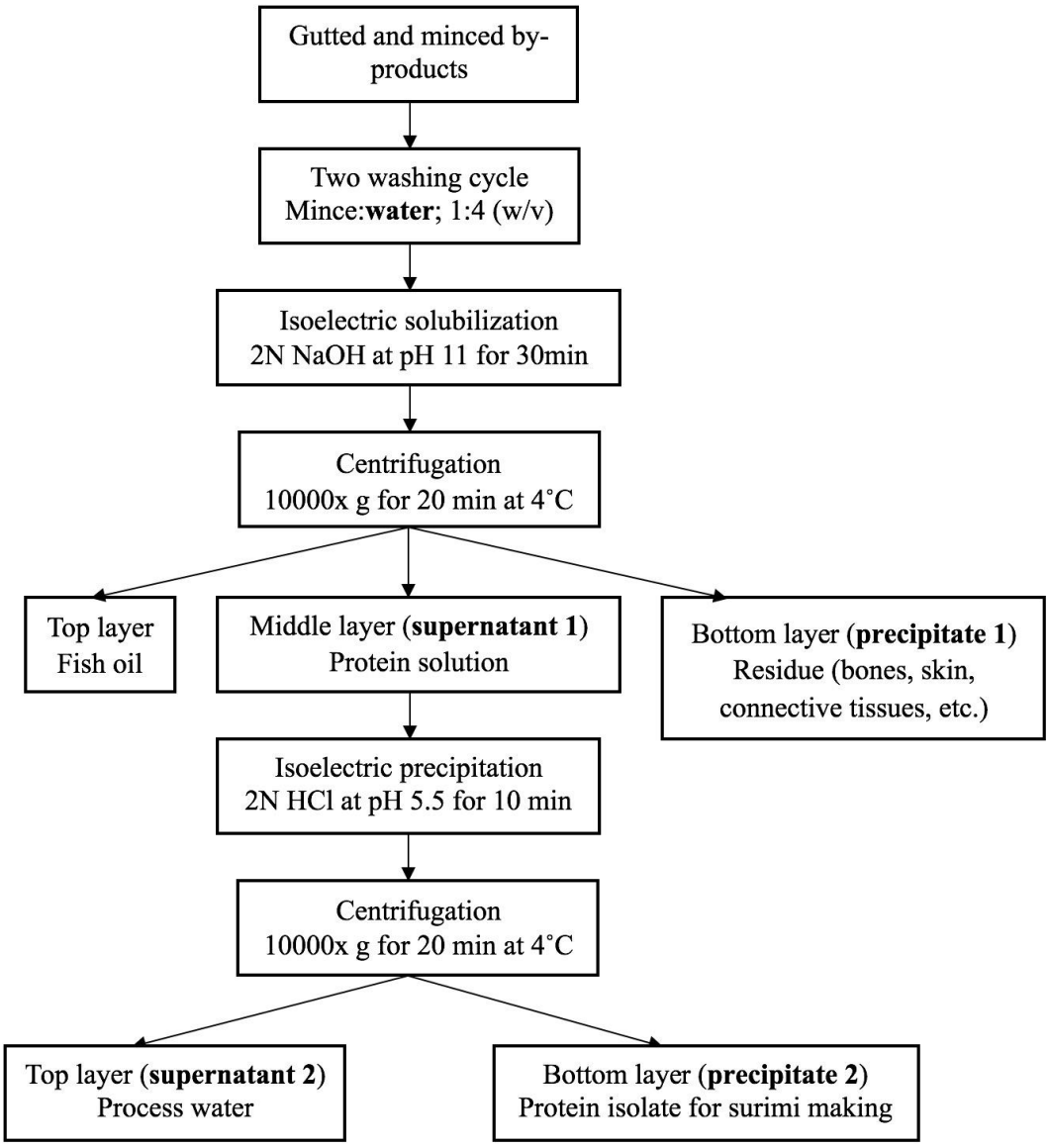

Figure 3.1 Flowchart of catfish by-products protein extraction by alkaline extraction method.

3.2.8 Salt extraction

The salt extraction was followed by the method described by Regenstein and Stamm (1979) with a slight modification as depicted in Figure 3.2. Minced catfish by-products were mixed with 1M NaCl solution at ratio of 2:5 (mince: salt solution, w/v). The mixture was homogenized and subjected to centrifugation at 10000x g for 20 min. The supernatant was collected and diluted in distilled water at ratio of 1:5 (mince: water) overnight at 4°C to remove the excessive salt. The solution was then centrifuged to get the precipitate, which was used as the raw material for surimi making. The supernatant was collected and stored in -80 °C freezer for further use.

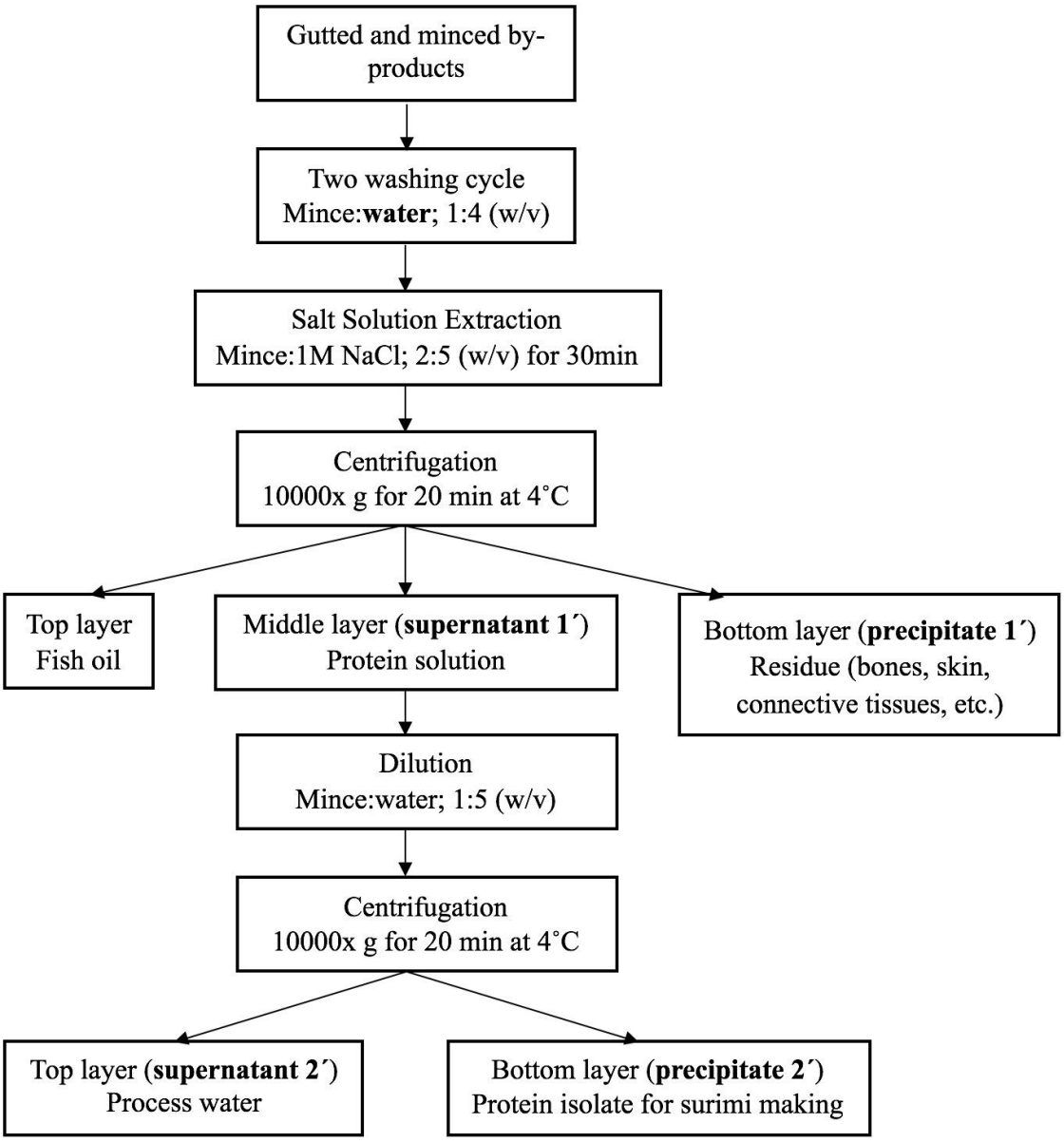

Figure 3.2 Flowchart of catfish by-products protein extraction by salt extraction method.

3.2.9 Puncture analysis by Instron

Cylinders and rack were removed from the refrigerator to a laboratory bench and stand at room temperature for 30 min. Surimi gels were removed from the cylinder and cut into specimens with 3 cm diameter and 1 cm height by a sharp knife. Fishballs were

cut into specimens with 3 cm diameter and 1 cm height and crabsticks were cut into surimi cubes with 1 x 1 x 1 cm length/height/wide, respectively, by a sharp knife immediately after products were cooled to room temperature. A food-grade aluminum tray was used to contain surimi specimens. Instrumental texture profile analysis of firmness was analyzed according to Bourne (1978) by using an Instron Universal Testing Machine (Model 1011, Instron Cooperation) as described in reports of Kong and others (2008) and Yuan and Chang (2008). A plunger probe with 8 mm diameter were used to test samples at a speed of 1.0 mm/sec and penetration depth was 95% of the surimi samples. Firmness (kg/cm^2) of surimi samples were recorded.

3.2.10 Water holding capacity determination

Water holding capacity was determined by the method of Himonides and others (1999), with slight modifications. For each treatment, three samples of 5 g each were separated. Samples were wrapped in individual Whatman filter papers (No. 41) and centrifuged at 1700x g for 30 min at 8°C. The amount of water drained from the surimi was estimated from the weight difference of the filter paper before and after centrifugation. The WHC of the surimi was calculated by the following equation: WHC g/kg= [(1-Mw/Ms)1000]. Mw was the mass (g) of expelled water and Ms was the initial mass (g) of the sample.

3.2.11 SDS-PAGE electrophoresis

SDS-PAGE (sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis) was used to analyze the patterns of fish proteins (Laemmli, 1970) in the original fish materials and extracted protein products. Before the SDS-PAGE electrophoresis, peotein

concentration of the samples were adjusted to 2mg/ml. However, all supernatants collected from the extraction has a lower protein concentration as 1mg/ml. After adjustment, 25 ul of the samples were loaded onto the polyacrylamide gel made of 10-20% gradient gel and subjected to electrophoresis. The 10-250 kDa molecular mass makers (BioRad, Hercules, CA) were used to estimate the molecular weight of proteins. After separation, the gels were stained for protein with Coomassie Brilliant Blue R-250 and destained. The protein patterns and concentrations, including different protein bands were determined using molecular mass standards after imaging using the BioRad imaging system (ChemiDoc XRS+, Hercules, CA).

3.2.12 Extraction kinetic study of the yield of alkaline processed protein isolate

3.2.12.1 Extraction time and solid/liquid ratio

Minced catfish by-products were used for analysis of extraction kinetics. The first step is to determine the FPI yield under different solid-liquid ratio and extraction time. 50 gram of minced by-products were mixed with 200 mL, 250 mL and 300 mL of distilled water to obtain different solid-liquid ratio of 1:4, 1:5, and 1:6, respectively. The slurry was extracted by alkaline extraction (Figure 1) with extraction time ranged from 30 min, 60 min, 90 min and 120 min. NaOH (2N) was used to adjust the protein slurry to pH 11 and extract at 4 °C. Followed by centrifugation at 10000x g for 20 min to obtain the supernatant, on which the oil layer floating on the top was removed by decanting the protein solution through a hand sieve with a mesh size of 0.25 mm. The supernatant was then adjusted to pH 5.5 with 2N HCl and centrifuged at 10000x g for 20 min. The precipitate was collected and used as the raw material for surimi making. The highest extraction yield of AE-FPI from catfish head was obtained at 60 min with solid-liquid

ratio of 1:4 (mince: water). Hence, the conditions above were used for analysis of FPI yield under different pH range (10, 10.5, 11, 11.5, 12) for protein solubilization in the AE procedure.

3.2.12.2 Effect of solubilization pH on protein recovery

The best conditions (reaction time and pH) for extraction above were used for the analysis of protein yield under various solubilization pH (10, 10.5, 11, 11.5, 12) of AE method.

3.2.13 Effect of MTGase on gel forming abilisty of alkaline processed protein isolate

3.2.13.1 Preparation of surimi gel

The procedure of surimi gel preparation was performed following the method described by Huang and others (1995). The moisture content of the surimi (minced fillet, AE-PI and SE-PI) was adjusted to 80% by combining with pH adjusted to 7, and mixed with 3% sorbitol, 3% NaCl, 3% potato starch by grinding using a mortar and pestle for 10 min to homogenize the mixture. In order to investigate the effect of MTGase on gel forming ability of AE-PI, various MTGase concentration from 0 (control), 0.2, 0.5, 1 u/g sample was sprinkled on the AE-PI, and homogenized by grinding using a mortar and pestle. Cylinders (60 mL capacity, 10 cm long and 3 cm diameter) were used to make gels by stuffing surimi into. The homogenate was then stored in a refrigerator at 4°C from 0 (control), 0.5 h, 1 h and 2 h. The cylinders were placed into a stainless steel rack and covered by a glass ball on the top to prevent evaporations. The rack holding the cylinders were then submerged vertically in a 40 °C water bath for 15 min and then in 90 °C water bath for 20 min. After cooking, the cylinders were cooled in an ice water (2 °C)

bath immediately for 10 min, and then stored in a refrigerator at 4 °C overnight before texture analysis. Commercial surimi products (fishball and crabstick, Wei-Chuan U.S.A. INC, Bell Gardens, CA 90201) were purchased from Asian Market (Starkville, MS 39759) and were then subjected to a 90 °C water bath for 10 min. After cooking, fishballs and crabsticks were cooled at room temperature and stored at 4 °C before texture analysis.

3.2.13.2 Cooking loss determination

The cylindrical surimi was weighed (X) before thermal treatment and after (Y) cooking to measure the differences. Cook loss (%) were expressed with following equation: Cooking loss (%) = 100* (X-Y)/X

3.2.13.3 Puncture analysis by texture analyzer

Puncture analysis of MTGase processed surimi samples was performed using a Model TA-XT2 texture analyzer (Stable Micro System, Surry, UK). Cylinders and rack were removed from the refrigerator to a laboratory bench and hold at room temperature for 30 min. Surimi gels were removed from the cylinder and cut into specimens with 3 cm diameter and 1cm height and tested. Breaking force (g) and deformation (mm) were measured by the texture analyzer equipped with a plunger with 5 mm diameter and the depression speed is 60 mm/min.

3.2.14 Data analysis

Data were subjected to analysis of variances using statistical analysis system (SAS, 2010). Differences among mean values were determined at $\alpha = 0.05$ using Fisher's

least significant difference (LSD) test. All data are reported as mean values ± standard deviation (SD).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preliminary study of catfish

The mass balance of catfish parts on wet weight basis was shown in Table 4.1. The percentage of fillet account for a whole catfish is about 30-40%, which is similar with that of Alaska Pollock (Kim and Park, 2007). In addition, their results pointed out that the Alaska Pollock head account for lower than 20% of the whole fish. Since the catfish head has a large portion (30%), its potential utilization as a source of protein recovery was assumed to be feasible and practicable.

Table 4.2 shows the moisture, protein content and Hunter color values of homogenized whole fish, catfish by-products and catfish fillet as is and in the dried mass based on 100 g of whole catfish. The yield of fillet by manual processing in our laboratory was only about 29% and the by-product is 71% of the original weight. Industrial processing gave higher fillet yields (35- 45%) depending on fish size, machine settings and product types. There is a significant difference ($P < 0.05$) with respect to the protein content between fillet (75.01% ± 3.75%), whole fish (41.59% ± 4.78%) and by-products (35.97% ± 0.49%). This was mainly due to fillets not containing bones and had less lipid content. The color properties of catfish and its by-products were shown in Table 2 and Figure 3. “L” value of fillet was significantly higher ($P < 0.05$) than that of whole catfish and catfish by-products, this is logical since melanin contribute to dark color of

skin and thus the lower lightness of whole catfish and by-products. Significantly higher ($P < 0.05$) "a" values of whole catfish and by-products than that of fillet might be due to the existence of higher quantities of myoglobin and hemoglobin within viscera that contributed to the red hue of samples. To utilize more than the fish fillet for consumable products, data on composition of different by-products are needed (Froese and Pauly, 2000). The moisture, protein, fat and ash content of the catfish heads and frames on a dry weight basis are listed in Table 4.3 (Data obtained from lab members Yuqing Tan with permission). catfish head and frames exhibited similar ($P > 0.05$) moisture contents (60.37 and 61.04%). The lipid content of catfish heads (7.71%) is significantly lower ($P < 0.05$) than that of catfish frames (11.03%), which has the similar lipid content with the processing frames of red rockfish and yellowtail as reported by Crawford and McNeil (1990). The protein content of catfish heads and frames were 26.84% and 38.47%, respectively. These high values probably reflect the conversion factor of 6.25 used in calculation of percent nitrogen to protein (Bechtel, 2003). In addition, the catfish frames had more amount of attached flesh than that of the heads. Low ash content was observed in both catfish heads (0.89%) and frames (0.67%).

Table 4.1 Mass balance of catfish

Processing Step	Processing Product/By-product	Percent of Whole Fish
Deheading	Head	25-28%
Gutting	Viscera, Stomach and Intestine	5-7%
Filleting	Frame	16-18%
	Fillet	37-42%
Mincing	Skin	3-6%
Washing	Blood	7-11%

Table 4.2 Weight percentage of parts, moisture content, protein content and hunter color value of catfish.

Samples	Weight Percentage of Parts (%)	Moisture Content (%)	Protein Content (%)	Hunter Color Value		
				L	a	b
Whole Catfish	100	71.46 ± 2.28[a]	41.59 ± 4.78[b]	49.62 ± 0.94[b]	10.21 ± 0.90[a]	11.07 ± 6.80[a]
Catfish Fillet	28.60 ± 2.03	70.70 ± 2.02[a]	75.01 ± 3.75[a]	73.32 ± 2.19[a]	6.99 ± 1.52[b]	10.07 ± 1.04[a]
By-products	71.32 ± 2.11	61.71 ± 1.30[b]	35.97 ± 0.49[b]	49.60 ± 1.66[b]	11.3 ± 0.77[a]	7.39 ± 0.93[b]

n=3
Moisture content was based on fresh weight
Protein content was based on dry weight
Hunter color value was measured after freeze-drying
Data in the same column shows different superscript letters are significantly different ($P < 0.05$)

Table 4.3 Proximate analysis of catfish by-products.

Samples	Protein Content (%)	Lipid Content (%)	Ash Content (%)	Moisture Content (%)
Head	26.84 ± 3.22^{b}	7.71 ± 0.05^{b}	0.89 ± 0.06^{a}	60.37 ± 1.48^{a}
Frame	38.47 ± 1.41^{a}	11.03 ± 0.11^{b}	0.67 ± 0.05^{b}	61.04 ± 2.11^{a}

Values are means ± SD (n=3). Data in the same column shows different superscript letters are significantly different ($P < 0.05$).

4.2 Preliminary study of catfish

4.2.1 Protein patterns of alkaline extraction and salt extraction

The SDS-PAGE analysis of the fractions collected in catfish frame protein extracted by both methods (AE and SE) are presented in Figure 4.1. Lanes 1-6 were various fractions by AE and lanes 7-10 are samples by SE method. The two washing cycles served as the leaching process of surimi manufacturing in that sarcoplasmic proteins were largely removed (Park and others, 2014), which was reflected by the decreased density of myosin light chain and hemoglobin shown in Lanes 1 and 2. After adjusting the catfish frame homogenate to pH 11 (Lane 3), myofibrillar protein was solubilized and that was evidenced by the myosin heavy chain band appeared in supernatant 2 (Lane 6). Declined quantity of myosin heavy chain in the precipitate 1 (Lane 4) as compared with supernatant 1 (Lane 3) showed the incomplete degree of extraction. A thorough extraction process is dependent upon the particle size of the mince, extraction time and others, which are the parameters that need to be further researched to obtain the highest yield. Lower quantity of myosin heavy chain band would have appeared in precipitate 1 (Lane 4) as the conditions above are optimized. For protein

patterns of the fractions by the SE method, supernatant 1′ (Lane 7) was collected after centrifugation of the mixture of minced frame in 6% NaCl solution. The myosin heavy chain band in the precipitate indicated an incomplete extraction (Lane 8). Precipitate 1′ was the residue after extraction and should include bone, skin, connective tissues, and the proteins were showed in lane 8. Only sarcoplasmic protein and actin band appeared on supernatant 2′ (Lane 9), which indicated the sedimentation of myofibrillar protein (final product) and that shown in precipitate 2′ (Lane 10).

However, significant amount of actin band in the first and second washed water, and myosin heavy chain in the second washed water were observed, indicating potential degradation of myofibrils by endogenous enzymes, which were released after the death of the catfish.

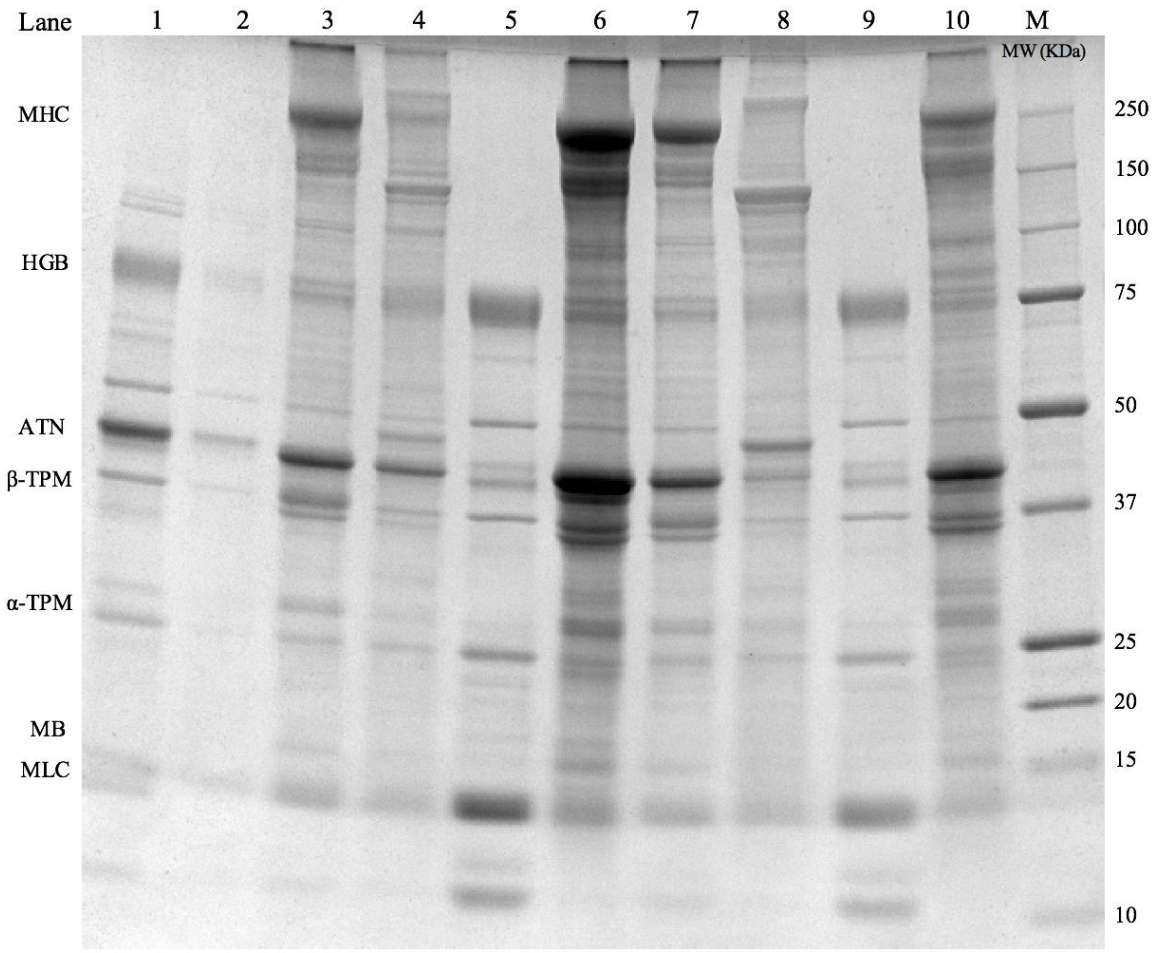

Figure 4.1 SDS-PAGE pattern of samples from catfish frame protein extracted by AE and SE method.

Lane l: first washed water; lane 2: second washed water; lane 3: supernatant 1; lane 4: precipitate 1; lane 5: supernatant 2; lane 6: precipitate 2; lane 7: supernatant 1′; lane 8: precipitate 1′; lane 9: supernatant 2′; lane 10: precipitate 2′; M: marker (from 10 to 250 KDa); MHC: myosin heavy chain; HGB: hemoglobin; ATN: actin; β-TPM: β-tropomyosin; α-TPM: α-tropomyosin; MB: myoglobin; MLC: myosin light chain.

4.2.2 Protein patterns of recovered isolate and commercial products

Protein patterns of the surimi proteins made from catfish by-products by both extraction method and commercial surimi products are shown in Figure 4.2. The higher quantity of actin band and myosin heavy chain in the surimi made from catfish frame by SE method (Lane 2) than that in surimi made from catfish head (Lane 1) was probably

because the frame had more residual fish muscle than the head. The protein pattern of surimi made by AE (Lanes 1 and 2) had more α-tropomyosin and β-tropomyosin band than that by SE method (Lanes 3 and 4), this may due to that the dilution step decreased the quantity of tropomyosin since it is water soluble. The protein patterns of commercial products (Lanes 5 and 6) were similar to that shown in surimi made by the SE method (Lanes 3 and 4).

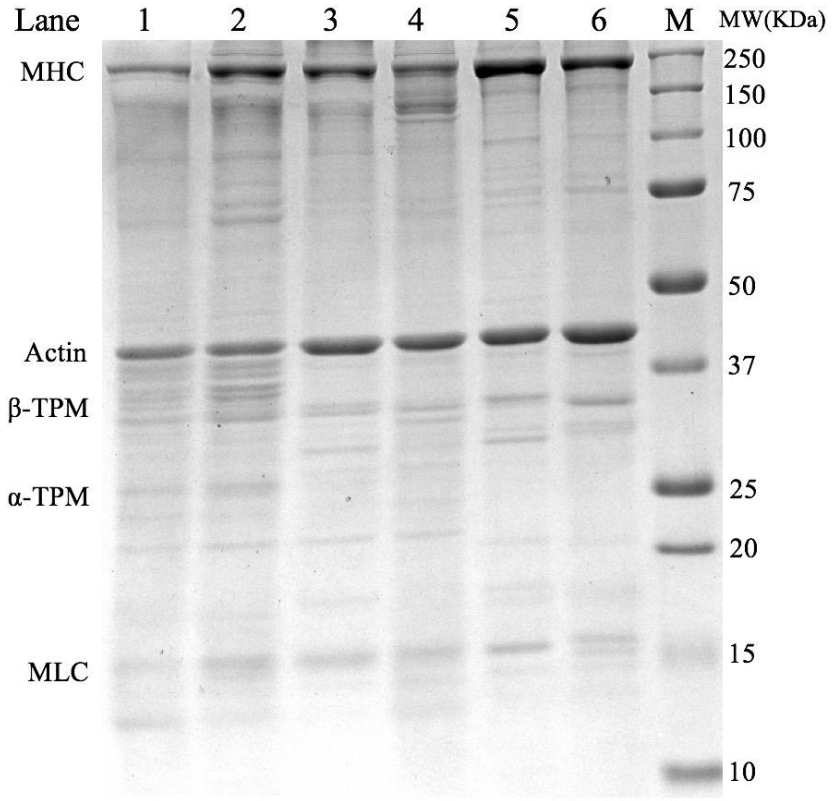

Figure 4.2 SDS-PAGE pattern of surimi made from catfish by-products and commercial products

Lane 1: surimi made from catfish head by AE method; lane 2: surimi made from catfish frame by AE method; lane 3: surimi made from catfish frame by SSE method; lane 4: surimi made from catfish head by SSE method; lane 5: fishball; lane 6: crabstick; MHC: myosin heavy chain; ATN: actin; β-TPM: β-tropomyosin; MLC: myosin light chain.

4.3 Surimi yield

Table 4.4 indicates that under the same method of extraction, surimi made from catfish head (AE: 36.64%, SE: 9.27%) had significantly lower ($P < 0.05$) yield than that of catfish frame (AE: 55.75%, SE: 16.13%). Catfish heads are comprised of meat, bone, connective tissues and viscera, whilst the frame had more quantity of remained meat after filleting. Under different extraction method of the same by-products (catfish heads or frames), surimi made by SE method had significantly lower ($P < 0.05$) yield than that by AE method, which might due to that extensive washing caused high loss of myofibrillar proteins and high moisture content in the washed mince (Lin and Park 1996). Hence, the dilution step in SE method may be regarded as another washing cycle that caused partial loss of myofibrillar protein. It is important to note, however, that the protein extractability of salt solutions mainly depended on the ionic strength, pH, and type of the salt (Franks, 1993). Therefore, we cannot preclude the possibility that higher recovery yield of protein by SE than AE method. Moreover, extracting procedure that include: volume of extraction solution, duration of homogenization, centrifugal force and time (Munasinghe and Saikai, 2004) need to be further explored for establishing the optimal extraction process. The moisture content of surimi made by SE method was significantly higher than that by AE method, and this was consistent with Lin and Park's research in 1996. Okada (1981) reported that the removal of water-soluble salt during washing causes the hydrophilicity to increase.

Table 4.4 Yield, Moisture content and hunter color value of surimi samples.

Samples	Yield (%)	Moisture Content (%)	Hunter Color Values		
			L	a	b
AE-Head	36.64 ± 1.31^{b}	63.21 ± 2.40^{c}	61.96 ± 0.28^{c}	5.44 ± 0.27^{b}	-2.38 ± 0.24^{d}
AE-Frame	55.75 ± 1.47^{a}	65.57 ± 1.37^{c}	67.13 ± 0.37^{b}	5.19 ± 0.31^{b}	2.23 ± 0.22^{b}
SE-Head	9.27 ± 0.88^{d}	83.11 ± 3.44^{a}	57.94 ± 0.17^{c}	3.12 ± 0.09^{d}	-2.98 ± 0.11^{d}
SE-Frame	16.13 ± 1.94^{c}	79.78 ± 3.27^{a}	67.75 ± 1.04^{b}	4.50 ± 0.04^{c}	2.17 ± 0.07^{b}
Fillet	_	70.70 ± 2.02^{b}	70.53 ± 0.20^{b}	6.34 ± 0.14^{a}	3.82 ± 0.13^{a}
Fishball	_	78.63 ± 1.01^{a}	74.51 ± 0.62^{a}	3.85 ± 0.06^{c}	1.59 ± 0.23^{c}
Crabstick	_	81.06 ± 2.30^{a}	72.01 ± 0.24^{a}	2.14 ± 0.03^{e}	1.77 ± 0.12^{c}

Values are means ± SD (n=3). Data in the same column shows different superscript letters are significantly different ($P < 0.05$).

4.4 Color property analysis

The "Lab" values of surimi gel made from catfish by-products by both extraction methods are presented in Table 4.4. In general, a washing process has been used widely in surimi production to remove sarcoplasmic proteins such as enzymes and haem proteins, pigments, odorous compounds, and other impurities that would reduce surimi quality (Hultin, Kristinsson, Lanier and Park, 2005; Martin-Sanchez and other, 2009). Pigment such as melanin presents in eyes, skin and black lining around the fish belly account for lower "L" values ($P < 0.05$) of surimi made from heads than that from frames. The color of cooked surimi gel slices was presented in Figure 4.3. Surimi made from catfish head by SE presents the lowest "L" value, this might due to that salt solution solubilize part of skin or connective tissues, and thus resulted in darker color than that from the frame and alkaline extracted head surimi. "L" value of surimi made from fillet had no significant differences ($P > 0.05$) as compared with commercial products. The "a" value of surimi made from catfish heads by AE was significantly ($P < 0.05$) higher than by SE. It could indicate that hame proteins that responsibly for the red hue of fish meat (Park, 1995) were decreased during dilution. A significant difference in "a" values

existed ($P < 0.05$) between surimi made from both methods and commercial products. As for "b" values, there were no significant differences ($P > 0.05$) between the two extraction methods but surimi made from frame showed higher value of yellowness than that from the heads.

4.5 Puncture analysis by Instron

For surimi gels, the force resistance to puncture, is an indicator for gel strength that affected by the moisture content and heating temperature (Huang and others, 1995). The surimi gels made from catfish fillet made in our laboratory had the same characteristics with commercial surimi products in texture profile and color, which were shown in Figure 4.3. The gel strength of surimi made from heads and frames were no significantly different ($P > 0.05$) within the same method of extraction. Figure 4.4 showed the surimi gels made from catfish heads by both extraction methods. As depicted in Figure 4.5, surimi made from catfish heads by SE showed significantly lower ($P < 0.05$) gel strength than that by the AE method. According to Chaijian and others (2010), the increase in breaking force and deformation of gel from protein isolate were possibly due to the partial denaturation of protein after alkaline treatment, leading to the exposure of reactive groups that subsequently underwent interaction during heat treatment. In addition, the reduction of lipid content was resulted in higher ability of proteins to interact with each other, thereby increasing breaking force of gels (Chanarat and Benjakul, 2013). To our surprise we discovered that the alkaline processed surimi gel has 20-25% cooking loss, suggesting the reduction in water holding capacity of AE processed gel (Davenport and Kristinsson, 2011). Whilst the SE processed surimi gel was elastic and had smoother surface, surimi gel made by AE method is brittle and has a coarse

appearance, showing less network flexibility. Chanarat and Benjakul's (2013) results suggested that protein isolate recovered by alkaline extraction had the decreased Ca^{2+}-ATPase and protein solubility, indicating protein denaturation.

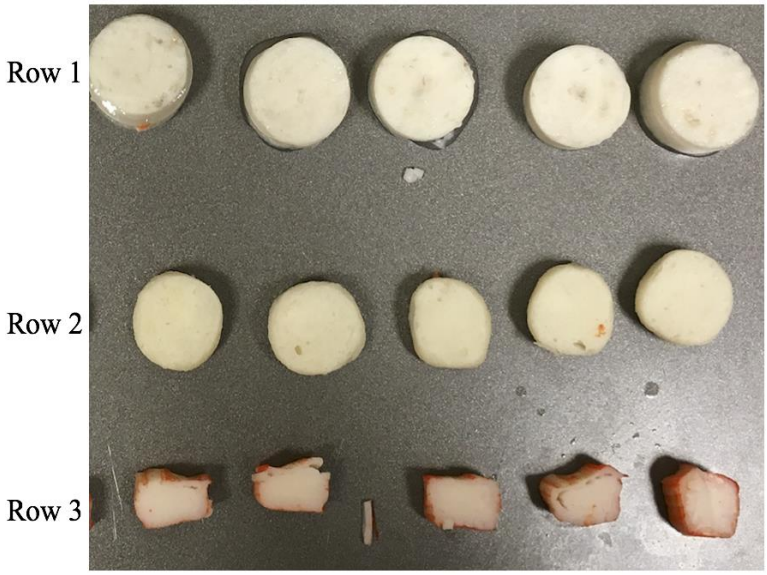

Figure 4.3 Catfish fillet surimi and commercial surimi samples

Row 1: Surimi made from catfish fillet; Row 2: Commercial fishball; Row 3: Commercial crabsticks

Figure 4.4 Surimi made from catfish head by alkaline extraction (Row 1) and salt extraction (Row 2).

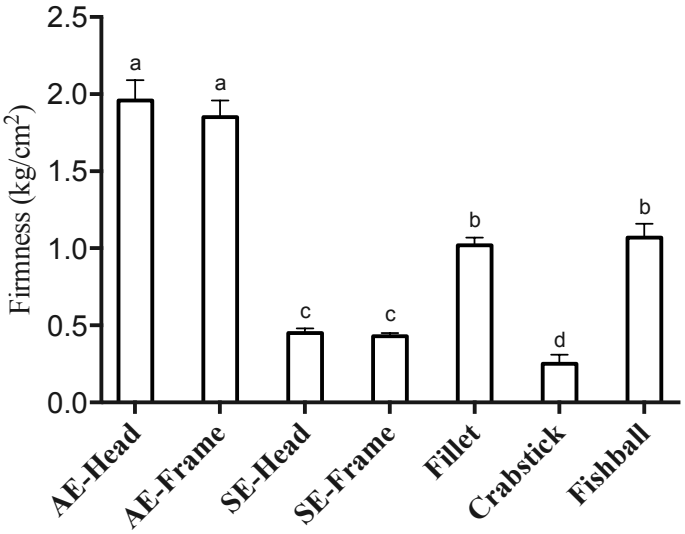

Figure 4.5 Puncture analysis (Instron) of surimi made from catfish by-products by alkaline extraction, salt extraction and commercial surimi products

Bars represents the standard deviation (n=3). Different lowercase letters indicate the significant difference ($P < 0.05$) between all samples.

4.6 Water holding capacity

Water holding capacity is one of the most important functional properties of heat induced protein gel (Puolanne and Halonen, 2010). The water holding capacity of surimi

made from catfish by-products by alkaline extraction and salt extraction are depicted in Figure 4.6. Alkaline processed surimi had significant higher ($P < 0.05$) water holding capacity than that of salt extracted surimi. Liu and others (2016) investigated the role of heat-denatured sarcoplasmic proteins in water holding capacity of myofibrils, and their results indicated that water holding capacity of myofibrils were improved proportionally with increasing amount of sarcoplasmic proteins. In this study, smaller amounts of sarcoplasmic protein was retained due to that more extensive washing of salt extraction compare to alkaline extraction. Hence, precipitated sarcoplasmic protein in protein isolate lowered the amount of water retained in gel matrix. These results are consistent with those obtained from other fish species. In 2013, Chanarat and Benjakul studied the effect of MTGase with various concentration on the gelling properties of Indian mackerel fish protein isolates. They showed that gel from alkaline extracted protein isolate without prewashing showed the highest expressible moisture content ($P < 0.05$), reflecting the poorest water holding capacity. Chaijian and others (2006) also reported that the alkaline processed gels of sardine and mackerel muscle had lower water holding capacity as compared with those from the conventional washing method.

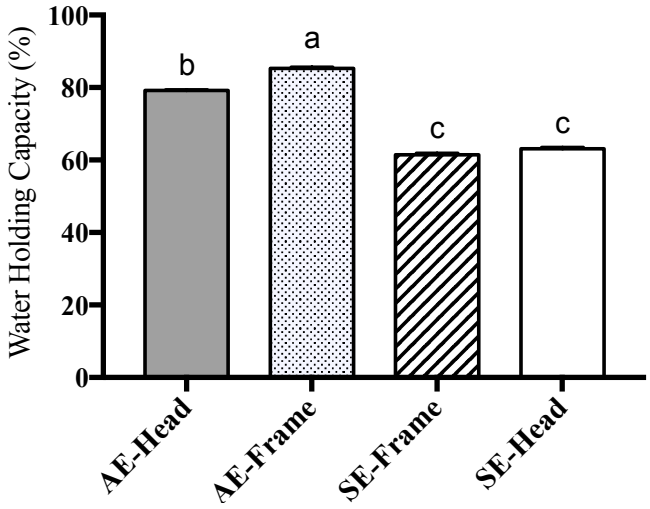

Figure 4.6 Water holding capacity of surimi made from catfish by-products by alkaline extraction and salt extraction

Bars represent the standard deviation (n= 3). Different lowercase letters indicate the significant difference ($P < 0.05$) among samples.

4.7 Extraction kinetic study of protein isolate

4.7.1 Effect of extraction time and solid/liquid ratio on protein recovery

The influence of extraction time and solid/liquid ratio was depicted in Figure 4.7. The extraction curves of catfish head indicated that there were no significant differences ($P < 0.05$) between the various mince/water ratio from 1:4 to 1:6. For protein recovered from catfish frames, however, the 1:5 and 1:6 mince/water ratios gave significantly higher yields than 1:4. This is probably due to that the difference of ionic strength. Lin and Park (1996) reported that a meat/water ratio at 1:5 or 1:6.7 would be sufficient to remove sarcoplasmic protein without loss of myofibrillar proteins. The extraction time for both protein recovery source indicated that 60 min was the minimum time period for recovering the proteins.

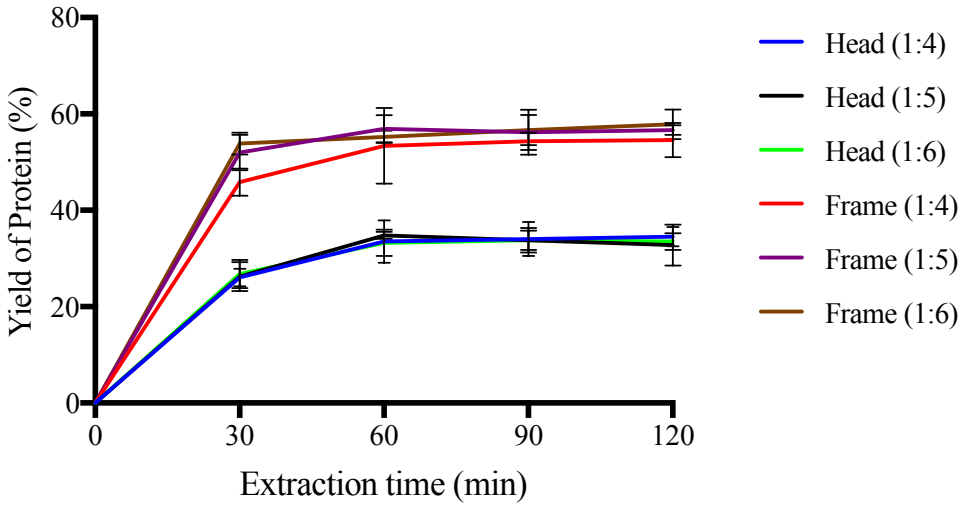

Figure 4.7 Recovery yield (%) of alkaline processed protein isolate extracted under various mince/water ratio and time

Bars represented the standard deviation (n=3).

4.7.2 Effect of extraction time and solid/liquid ratio on protein recovery

Based on the results (extraction time, solid/liquid ratio) above, the conditions with the highest protein recovery yield were selected for analyzing the influence of solubilization pH on recovery yield, which was shown in Table 4.5. The results indicated that the protein recovery yield was lowest at pH 10 and increased as pH was increased to 11 yielding significantly higher ($P < 0.05$) protein yield than others for both catfish heads and frames. Therefore, pH 11 were chosen as the optimal pH values for alkaline extraction in this study. Yongsawatdigul and Park (2004) investigated the effect of pH on protein solubility from Rockfish muscle. Their results indicated that the maximum solubility was achieved at pH 12 at about 80% solubility. Lin and Park (1998) reported that salmon myosin exhibited the minimum solubility at pH 4 to 5, and maximum solubility at pH 3 and 11.

Table 4.5 Effect of solubilization pH on protein recovery.

Sample	Protein Solubilization pH				
	10	10.5	11	11.5	12
Head	33.75 ± 2.02^{d}	34.28 ± 2.12^{c}	36.64 ± 1.31^{a}	35.46 ± 3.21^{b}	35.93 ± 1.42^{b}
Frame	56.02 ± 4.72^{a}	63.01 ± 1.92^{a}	55.68 ± 1.47^{a}	45.63 ± 2.49^{b}	43.75 ± 1.33^{b}

Values are means ± SD (n=3). Different superscript letters in the same row represent the significant difference ($P < 0.05$).

4.8 Effect of MTGase on gelation property of alkaline processed protein isolate

4.8.1 Cooking loss

Figure 4.8 depicts the cooking loss of surimi processed under various MTGase concentrations and reaction times. The addition of MTGase at various concentration (0.2 u/g, 0.5 u/g and 1 u/g sample) resulted in a significant increase ($P < 0.05$) in cooking loss, reflecting the poorer water binding capacity. This result was in agreement with Kaewudom and others (2013) who reported that surimi gels from threadfin bream surimi containing added MTGase (0.4 u/g, 0.8 u/g and 1.2 u/g surimi) induced significant increases ($P < 0.05$) in expressible moisture content. According to Chanarat and Benjakul (2013), the greater protein-protein interaction was associated with the denser network, which might lead to the lower water binding sites of proteins. Increased cooking loss were observed with level of MTGase for reaction times of 0, 0.5 h and 2 h ($P < 0.05$). However, when reaction time is 1 h, the addition of 1 u/g MTGase induced the marked decrease of cooking loss of gel when compared with 0.5 u/g MTGase were added. For surimi gels added with the same amount of MTGase, reaction time from 0 to 0.5 h induced the significant increase of cooking loss ($P < 0.05$). However, decreased cooking

loss was observed for surimi added with 0.2 u/g and 1u/g MTGase from 0.5 h to 2 h. A similar behavior was reported by Ramirez-Suarez and Xiong (2003), who studied the effect of MTGase on gelation of myofibrillar/soy protein mixture under various reaction times (2 min, 5 min, 15 min, 30 min, 60 min, 120 min and 240 min). They speculated that the enzyme-catalyzed 'intramolecular association' in the mixture was reversible. The reversibility phenomenon might stemmed from certain proteolytic/plastein (reversible cross-linking) activity which could be present in the MTGase preparation (Ramirez-Suarez and others, 2001). The results suggested that water holding capacity of gel matrix was dependent upon type of protein substrates, level and reaction time of ingredients incorporated.

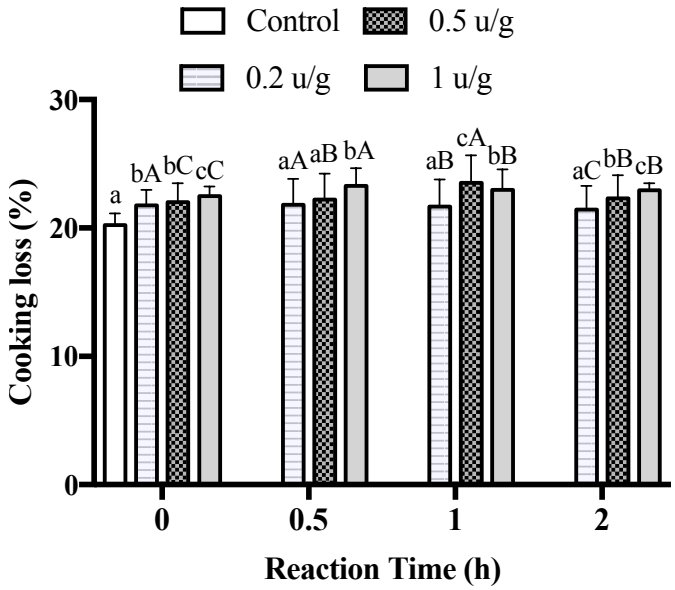

Figure 4.8 Cooking loss (%) of surimi under various MTGase concentration and reaction time

Bars represent the standard deviation (n= 3). Different lowercase letters within the same reaction time indicate significant difference ($P < 0.05$). Different uppercase letters within the same concentration of MTGase indicate significant difference.

4.8.2 Puncture analysis by Texture Analyzer

Breaking force and deformation of surimi gels from catfish protein isolates added at different reaction times (0, 0.5 h, 1 h and 2 h) in the presence of MTGase at various concentrations (0, 0.2 unit/g, 0.5 unit/g and 1 unit/g sample) are presented in Figure 4.9. Generally, breaking force of the gel was positively correlated with gel strength, whilst the deformation represented the elasticity of the gels (Chanarat and Benjakul, 2013). Under reaction time from 0.5 h to 2h, breaking force and deformation of gels increased as MTGase at higher levels was incorporated ($P < 0.05$). MTGase is able to induce the acyl transfer between acyl donors to acyl acceptor, in which the ε-(γ-glutamyl) lysine linkage can be formed (Benjakul and others, 2008). Hence, the non-disulfide covalent bonds enhanced the gel matrix. For surimi added with MTGase at the same level, the increases ($P < 0.05$) in breaking force and deformation were obtained when reaction time from 0-0.5 h. Also a significant increase was observed at 1 u/g from 0.5 to 1 h. However, there was a significant decrease ($P < 0.05$) of breaking force and deformation from 1-2 h of reaction time. Sakamoto and others (2006) reported that the excessive formation of ε-(γ-glutamyl) lysine cross-links would inhibit a uniform development of the protein network and lower the improvement of gel strength.

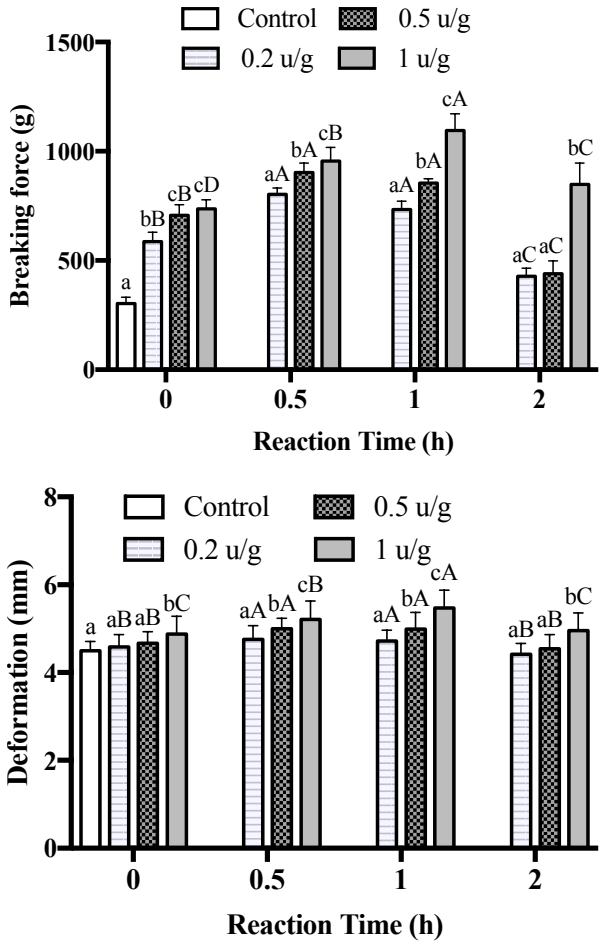

Figure 4.9 Breaking force and deformation of gels from catfish alkaline processed protein isolate added with MTGase at different concentrations (0-1 units/g sample)

Bars represent the standard deviation (n= 3). Different lowercase letters within the same reaction time indicate significant difference ($P < 0.05$). Different uppercase letters within the same concentration of MTGase indicate significant difference.

CHAPTER V

CONCLUSIONS

In summary, compared with catfish head, catfish frame has higher protein recovery yield and color similarity with commercial products. With respect to the extraction method, both are able to (AE and SE) extract muscle proteins from catfish by-products, and AE method has significant higher ($P < 0.05$) protein recovery yield than SE. The SDS-PAGE patterns of the proteins extracted from catfish by-products indicate that the SE method yield protein that are comparable with commercial surimi products. Texture profile results indicate that protein extracted by AE method have higher gel strength but lower water holding capacity than that by SE method. Thus, catfish frame and AE method shows more promise and feasible for applying to industrial catfish by-products utilization. Results of our study on extraction yield of protein isolate indicate that the protein recovery can reach to 60% as the extraction process were optimized. The addition of MTGase in protein isolate could improve the gel forming ability of catfish effectively.

CHAPTER VI

SUMMARY OF ACHIEVEMENTS, CHALLENGES AND FUTUTRE RESEARCH NEEDS

In this study, we analyzed the effect of materials, methods and various extraction condition on protein yield. Optimal extraction methods, condition and sources have been established based on the results. However, whether the final protein product developed from catfish waste would be acceptable by consumers depends greatly upon its functional properties include color, texture and flavors. For the texture profiles of the recovered proteins, we incorporated various doses of MTGase to analyze its effect on gelation properties. However, other ingredients such as protein additives and starch has not been explored for their influence on the formation of gel matrix. In addition, color properties of the recovered proteins have not meet the criteria of the surimi products on the market. Hence, future research needs include improving gel matrix by incorporating protein additives and whitening agents. Dr. Chang's group has screened more than 100 soybean lines for their total trypsin inhibitory activity (include both Kunitz inhibitor and Bowman-Birk inhibitor, which also contains chymotrypsin inhibition activities), and those crude enzymatic extract of soy flour with the highest trypsin inhibitory activity may be used to control the degradation of fish proteins (Li and others, 2016). Various types of starch (native starch and modified starch with high-amylose or high-amylopectin content) may be used in future research to investigate its effect on gel formation abilities. To improve

color properties of the recovered proteins, more extensive washing may be used to refine the final products. After the optimal types and concentrations of protein additives and whitening agents were selected to add when making surimi gels, which will subject to sensory evaluation by trained panelist to test its acceptability. If those recovered proteins are compatible with the existing product specifications, market acceptance should not be a problem. However, if the recovered solids result in new products, market research may be necessary to determine if there is a demand for these products (Ismond, 2003). Catfish protein isolated from the by-product can help alleviate protein storage problem, and hence help reduce problems associated with world hunger and malnutrition.

REFERENCES

AOAC INTERNATIONAL. (2015). Official Methods of Analysis, 19th Ed., Association of Official Analytical Chemist, Washington, DC.

Arason, S. (2002). Utilization of Fish Byproducts in Iceland. In P. J. Bechtel (ed.), Advances in Seafood Byproducts: 2002 Conference Proceedings. Alaska Sea Grant College Program, University of Alaska Fairbanks, Fairbanks, 43-62.

Arnesen, J. A., Gildberg, A. (2006). Extraction of muscle proteins and gelatine from cod head. Process Biochemistry, 41, 697- 700.

Aspmo, S. I., Horn, S. J., Eijsink, V. G. H. (2005). Hydrolysates from Atlantic cod (Gadus morhua L.) viscera as components of microbial growth media. Process Biochemistry, 40, 3714-3722.

Benhabiles, M. S., Abdi, N., Drouiche, N., Lounici, H., Pauss, A., Goosen, M. F. A. and Mameri, N. (2012). Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit. Materials Science and Engineering, C, 32, 922-928.

Benjakul, S., Phatcharat, S., Tammatinna, A., Visessanguan, W. and Kishimura, H. (2008). Improvement of gelling properties lizardfish mince as influenced by microbial tranglutaminase and fish freshness. Journal of Food Science, 73, S239-S246.

Benjakul, S., Visssanguan, W. and Tanaka, M. (2005) Properties of phenoloxidase isolated from the cephalothorax of kuruma prawn (Penaeus japonicus). Journal of Food Biochemistry, 29, 470-485.

Bechtel, P. (2003). Properties of different fish processing by-products from pollock, cod and salmon. *Journal of Food Processing and Preservation, 27(2),* 101-116.

Blenford, D. E. (1994). Protein hydrolysates: functionalities and uses in nutritional products. International Food Ingredients, 3, 45.

Byun, H. G., Kim, S. K. (2001). Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska pollack (Theragra chalcogramma) skin. Process Biochemistry, 36, 1155-1162.

Cai, Y. and Wang, L. (2009). Method for recycling processing waste of Ictalurus punctatus. Hubei Institute of Fishery Sicnece, China, 8.

Cao, M. J., Hara, K., Tachibana, K., Izumi, T., and Ishihara, T. (1999). Myofibril-bound serine proteinase (MBP) and its degradation of myofibrillar proteins. Journal of Food Science, 64, 644- 647.

Campo, L., Tovar, C. (2008). Influence of the starch content in the viscoelastic properties of surimi gels. Journal of Food Engineering, 84, 140-147.

Chanarat, S., Benjakul, S. (2013). Impact of microbial transglutaminase on gelling properties of Indian mackerel fish protein isolates. Food Chemistry, 136, 929-937

Chaijian, M., Panpipat, W. and Benjakul, S. (2010). Physicochemical and gelling properties of short-bodied mackerel (Rastrelliger brachysoma) protein isolate prepared using alkaline-aided process. Food and Bioproducts Processing, 88(2).

Chen, Y. C., Jaczynski, J. Protein recovery from rainbow trout (Oncorhynchus mykiss) processing by-products via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. (2007). Journal of Agriculture and Food Chemistry, 55, 9079-9088.

Cheftel, C., Ahern, M., Wang, D. I. C. and Tannenbaum, S. R. (1971). Enzymatic solubilization of fish protein concentrate: Batch studies applicable to continuous enzyme recycling processes. Journal of Agriculture and Food Chemistry, 19, 155.

Cremades, O., Parrado, J., Alvarez-Ossorio, M. C., Jover, M., Collantes de teran, L., Gutierrez, J. F. and Bautista, J. (2003). Isolation and characterization of carotenoproteins from crayfish (Procambarus clarkii). Food Chemistry, 82, 559-566.

Davenport, M. P., Kristinsson, H. G. (2011). Channel catfish (Ictalurus punctatus) muscle protein isolate performance processed under different acid and alkali pH values. Journal of Food Science,76, 240-247.

Duangmal, K., Taluengphol, A. (2010). Effect of protein additives, sodium ascorbate, and microbial transglutaminase on the texture and color of red tilapia surimi gel. International Journal of Food Science and Technology, 45, 48-55.

Dufossea, L., De La Broise D, Guea Rard, F. (2001). Evaluation of nitrogenous substrates such as peptones from fish: a new method based on Gompertz modeling of microbial growth. Current Microbiology, 42, 32-38.

D. Kelleher, S., O. Hultin, H. (1991). Lithium Chloride as a preferred extractant of fish muscle proteins. Journal of Food Science, 56, 315-317.

FAO. (2007). The state of world fisheries and aquaculture 2006.

Fisheries of the United States, 2013, NOAA Office of Science and Technology, 101.

Froese, R., Pauly, D. (2000). FishBase 2000 (www.fishbase.org). Concepts, design and data sources, retrieved April 2005 from the World Wide Web: http://www.fishbase.org/manual/english/contents.htm

Fukazawa, T., Yasui, T., Hashimoto, Y. (1961). Effect of some proteins on binding quality of an experimental sausage. Journal of Food Science, 26, 541-9.

Fukazawa, T., Yasui, T., Hashimoto, Y. (1961). Effect of storage condition on some physicochemical properties in experimental sausage prepared from fibrils. Journal of Food science, 26, 331-36.

Gehring, C. K., Davenport, M. P., Jaczynski, J. (2009) Functional and nutritional quality of protein and lipid recovered from fish processing by-products and underutilized aquatic species using isoelectric solubilization/precipitation. Current Nutrition & Food Science, 5, 17-39.

Gehring C. K., Gigliotti J. C., Moritz J. S., Tou J. C. and Jaczynski J. (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: a review. Food Chemistry, 124, 422-431.

Gildberg, A. (2002). Enhancing returns from greater utilization. In H. A. Bremner (Ed.), Safety and quality issues in fish processing. Cambridge: Woodhead Publishing Limited, 425-449.

Guerard, F., Dufossea, L., De La Broise D, Binet, A. (2001). Enzymatic hydrolysis of proteins from yellow fin tuna (Thunnus albacares) wastes using Alcalase. Journal of Molecular Catalysis B-Enzymatic, 11, 1051-1059.

Halldorsdottir, S. M., Kristinsson, H. G., Sveinsdottir, H., Thorkelsson, G., Hamaguchi, P.Y. (2013). The effect of natural antioxidants on haemoglobin-mediated lipid oxidation during enzymatic hydrolysis of cod protein. Food Chemistry, 141, 914-919.

Hanson, T. and Sites, D. (2015). 2014 Catfish Industry Review and 2015 Outlook.

Haper, A., Barbut, S., Lim, L-T., Marcone, M. F. (2012). Microstructural and textural investigation of various manufactured collagen sausage casings. Food Research International, 49, 494-500.

Harnedy, P. A., FitzGerald, R. J. (2012). Bioactive peptides from marine processing waste and shellfish: a review. Journal of Functional Foods 4, 6–24.

Hathwar, S. C., Bijinu, B., Rai, A. K. and Narayan, B. 2011. Simultaneous recovery and lipids and proteins by enzymatic hydrolysis of fish industry waste using different commercial proteases. Applied Biochemistry and Biotechnology,164, 115-24.

He, S., Franco, C., Zhang, W. (2013). Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Research International, 50, 289-297.

Huang, Y. W., Abdel-aal, H. A., Awad, A. H. (1995). Properties of surimi made from tilapia, in Nineteenth and Twentieth Annual Conferences of Tropical and Subtropical Seafood Science and Technology Society of the Americans, Humacao, Puerto Rico, Nov. 5-8, 273-289.

Hultin, H. O., Kristinsson, H. G., Lanier, T. C. and Park, J. W. (2005) Process for recovery of functional proteins by pH shifts. In J. W. Park (Ed), Surimi and Surimi Seafood (Second Edition) New York, USA: CRC Press, pp. 107-139.

Hultin, H. O. and Kelleher, S. D. (1999). Process for isolating a protein composition from a muscle source and protein composition. US Patent No. 6,005,073.

Hultin, H. O., Kelleher, S. D., Feng, Y., Kristinsson, H. G., Richards, M. P., Undeland, I. A. and Ke, S. (2004). High efficiency alkaline protein extraction. US Patent Application No. 10/363,612.

Hultin, H. O. and Kelleher, S. D. (2000). Surimi processing from dark muscle fish. In J. W. Park Surimi and Surimi Seafood, New York, Marcel Dekker, 59-77.

Ibrahim, S. M. (2009). Evaluation of production and quality of salt-biscuits supplemented with fish protein concentrate. World Journal of Dairy and Food Sciences, 4(1), 28-31.

Irianto, H. E., Giyatmi, S. (2009). Teknologi Pengolahan Hasil Perikanan. [Fishery Products Processing Technology]. Penerbit Universitas Terbuka. Jakarta. [Bahasa Indonesia].

Irineu, B. (1999). Recovery of proteins from fish waste products by alkaline extraction. European Food Research and Technology, 210, 84- 89.

Ismond, A. (2003). The impact of food safety and competitive markets on by-product recovery strategies. In: Advances in Seafood Byproducts. 2002 Conference Proceedings. PJ Bechtel, ed. Fairbanks, AK: Alaska Sea Grant College Program, University of Alaska, 2003.

Jae, J. Y., Park, P. J., Kwon, J. Y. and Kim, S. K. (2004). A novel angiotensin converting enzyme inhibitory peptide from Alaska pollack (Theragra chalcogramma) frame protein hydrolysate. Journal of Agricultural and Food Chemistry, 52, 7842-7845.

Jung, W. K., Park, P. J., Byun, H. G., Moon, S. H. and Kim, S. K. (2005b). Preparation of hoki (Johnius belengerii) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. Food Chemistry, 91, 333-340.

Kaewudom, P., Benjakul, S. and Kijroongrojana, K. (2013). Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. Food Bioscience, I, 39-47.

Kelleher, S. D., Feng, Y., Kristinsson, H., Hultin, H. O. and Mcclements, D. J. (2004). Functional fish protein isolates prepared using low ionic strength, acid solubilization/precipitation. In: More Efficient Utilization of Fish and Fisheries Products. New York, Elsevier Science, 407-414.

Kim, J. M., Liu, C. H. and Eun J. B. (1996). Surimi from fillet frames of channel catfish. Journal of Food Science, 61, 428- 431.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Kishimura, H. and Shahidi, F. (2005). Characterization of acid-soluble collagen from skin and bone of bigeye snapper (Priacanthus marcracanthus). Food Chemistry, 89, 363-372.

Kristinsson, H. G., Lanier, T. C., Halldorsdottire, S. M., Park, J. W. (2014). Chapter 6. Fish protein isolate by pH shift. In the book of "Surimi and Surimi Seafood" 3rd ed. Edited by Park, 169-192. CRC Press, Taylor and Francis Group. Boca Raton, FL.

Kristinsson, H. G., Rasco, B. A. (2000). Biochemical and functional properties of Atlantic salmon (Salmon salar) muscle proteins hydrolyzed with various alkaline proteases. Journal of Agriculture and Food Chemistry, 48, 657-666.

Kristinsson, H. G., Rasco, B. A. (2000). Fish protein hydrolysates: production, biochemical and functional properties. Critical Reviews in Food Science and Nutrition, 40, 43-81.

Kristinsson, H. G. (2002). Conformational and functional changes of hemoglobin and myosin induced by pH: Functional role in fish quality. Ph.D dissertation. University of Massachusetts at Amherst.

Kristinsson, H. G. and Rasco, B. A. (2002). Fish protein hydrolysates and their potential use in the food industry. In M. Fingerman and R. Nagabhushanam, Recent Advances in Marine Biotechnology, Vol. 7, Enfield, NH, Science Publishers, Inc., 157-181.

Kristinsson, H. G., Theodore, A. E., Demir, N. and Ingadottir, B. (2005). The recovery of channel catfish muscle proteins using acid and alkali-aided processing vs. surimi processing. Journal of Food Science, 70, C298-C306.

Kumar, D. and Bhalla, T. C. (2003). Bacillus sp. APR-4 proteases in protein recovery from waste bones. National Academy Science Letters (India), 26, 332-335.

Kumazawa, Y., Nakanishi, K., Yasueda, H., Motoki, M. (1996). Purification and characterization of transglutaminase from walleye pollack liver. Fish Science 62, 959-64.

Lansdowne, L., Beamer, S., Jaczynski, J., Matak, K. E. (2009). Survival of Escherichia coli after isoelectric solubilization/precipitation of fish. Journal of Food Protection, 72(7), 1398-1403.

Lansdowne, L., Beamer, S., Jaczynski, J., Matak K. E. (2009). Survival of Listeria innocua after isoelectric solubilization and precipitation of fish protein. Journal of Food Science, 74(4), M201-M205.

Lin, T. M., Park, J. W. (1996). Extraction of proteins from pacific whiting mince at various washing conditions. Journal of Food Science, 61, 432-438.

Lin, T. M., Park, J. W., Morrissey, M. T. (1995). Recovered protein and reconditioned water from surimi processing waste. Journal of Food Science, 60, 4-9.

Liu, H. and Guo, S. (2009). Method for producing lipid-containing protein powders from Ictalurus punctatus head with protease. Jiangnan Universiy, China, 4.

Liu, J., Arner, A., Puolanne, E., Ertbjerg, P. (2016) On the water-holding of myofibrils: Effect of sarcoplasmic protein denaturation. Meat Science, 119, 32-40.

Macfarlane, J., Schmidt, G., Turner, R. (1977). Binding meat pieces: A comparison of myosin, actomyosin, and sarcoplasmic proteins as binding agents. Journal of Food Science, 42, 1603-5.

Mackie, I. M. (1974). Proteolytic enzymes in recovery of proteins from fish waste. Process Biochemistry, 12, 12.

Mackie, I. M. (1982). Fish protein hydrolysates. Process Biochemistry, 17, 26-32.

Messina, C. M., Bono, G., Renda, G., Barbera, L. L. (2015). Effect of natural antioxidants and modified atmosphere packaging in preventing lipid oxidation and increasing the shelf-life off common dolphinfish (Coryphaena hippurus) fillets. LWT-Food Science and Technology, 62, 271-277.

Montecalvo JR, J., Constantnides, S. M., Yang, C. S. T. (1984). Optimization of processing parameters for the preparation of flounder frame protein product. Journal of Food Science, 49, 172-176.

Moskowitz, R. W. (2000). Role of collagen hydrolysate in bone and joint disease. Seminar in Arthritis and Rheumatism 30, 87–99.

Munasinghe D. M. S., Sakai, T. (2004). Sodium chloride as a preferred protein extractant for pork lean meat. Meat Science, 67, 697-703.

Nazar, D. S., Persson, G., Olin, T., Waters, S., Decken, A.V. (1991). Sarcoplasmic and myofibrillar proteins in white trunk muscle of salmon (Salmo salar) after estradiol treatment. Biochem. Physiol, 98, 109-114.

Okada, M. (1964). Ashi and its reinforcement. In Kneaded Seafood Products, M. Okada (Ed.), Koseisha-Koseikaku Publishing Co., Tokyo, Japan, 189-211.

Park, J. W. (2014). Surimi and Surimi Seafood, Third Edition, 108.

Park, J. W. (1995). Surimi gel colors as affected by moisture content and physical conditions. Journal of Food Science, 60, 15-18.

Park, J. W., Lin, T. M. J. (2005). Surimi: manufacturing and evaluation. J.W. Park (Ed.), Surimi and Surimi Seafood (ed.), CRC Press, Baca Raton, FL, USA (2005), 33-37.

Pietrasik, Z., Li, C., E. C. Y. (2002). Binding and textural properties of beef gels as affected by protein, carrageenan and microbial transglutaminase addition. Food Research International, 35, 91-98.

Puolanne, E., Halonen, M. (2010). Theoretical aspects of water-holding in meat. Meat Science, 86, 151-156.

Regenstein, J. M., Stamm, J. R. (1980). Factors affecting the sodium chloride extractability of muscle proteins from chicken breast, trout white and lobster tail muscles. Journal of Food Biochemistry, 3, 191-204.

Regenstein, J. M. (2004). Total utilization of fish. Food Technology, 58, 28-30.

Rodger, G., Wilding, P. (1990). Muscle proteins. In: Food Gels. Harris, P., ed. London, UK: Elsevier Applied Science Publishers, 361.

Sakamoto, H., Kumazawa, Y., Toiguchi, S., Seguro, K., Soeda, T. and Motoki, M. (2006). Gel strength enhancement by addition of microbial tranglutaminase during onshore surimi manufacture. Journal of Food Science, 60, 300-304.

Sanmartin, E., Arboleya, J, Villamiel, M., Moreno, F. (2009). Recent advance in the recovery and improvement of functional proteins from fish processing by-products: Use of protein glycation as an alternative method. Comprehensive Reviews in Food Science and Food Safety, Vol. 8.

Shahidi, F., Kamil, Y. V. A. (2001). Enzymes from fish and aquatic invertebrates and their application in the food industry. Trends in Food Science and Technology, 12, 435-464.

Shahidi, F., Metusalach and Brown, J. A. (1998). Carotenoid pigments in seafoods and aquaculture. Critical Review in Food Science and Nutrition, 38, 1-67.

Skea G. L., Moutfort D. O., Clements K. D. (2005). Gut carbohydrases from the New Zealand marine herbivorous fishes Kyphosus sydneyanus (Kyphosidae), Aplodactylus arctidens (Aplodactylidae) and Odax pullus (Labridae). Comparitive Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 140, 259-269.

Snyder, D. G. (1967). Bureau of commercial fisheries program. Food Technology, 21(9), 70.

Sikorski, Z. E., Kolakowska, A. and Pan, B. S. (1990). The nutritive composition of the major groups of marine food organisms. In: Seafood: Resources, Nutritional Composition and Preservation (Ed. Sikorski, Z.E.). CRC Press, Boca Raton, FL.

Stine, J. J., Pedersen, L., Smiley, S., Bechtel, P. J. (2011). Recovery and utilization of protein derived from surimi wash-water. Journal of Food Quality, 35, 43-50.

Sun, F., Huang, Q., Hu, T., Xiong, S., Zhao, S. (2014). Effects and mechanism of modified starches on the gel properties of myofibrillar protein from grass carp. International Journal of Biological Macromolecules, 64, 17-24.

Sun, T., Pigott, G. M. and Herwig, R. P. (2002). Lipase assisted concentration of n-3 polyunsaturated fatty acids from viscera of farmed Atlantic salmon (Salmo salar L.). Journal of Food Science, 67, 130-136.

Tahergorabi, R., Matak, K., Jaczynski, J. (2014). Fish protein isolate: Development of functional foods with nutraceutical ingredients. Journal of Functional Foods, 18, 746-756.

Taheri, A., Sabeena Farvin, K.H., Jacobsen, C., Baron, C.P. (2014). Antioxidant activities and functional properties of protein and peptide fractions isolated from salted herring brine. Food Chemistry, 142, 318-326.

Taniguchi, A., Takano, K., Kamoi, I. (2001). Purification and properties of lipase from tilapia stomach: digestive enzyme of tilapia-VII. Nippon Suisan Gakkaishi, 67, 96-101.

USDA United States Department of Agriculture (2015). Catfish Production reports from National Agricultural Statistics Service.

USDT, American fisheries act. www.marad.dot.gov/ships_shipping_landing_gape/afa_home/afa_home.htm (Accessed February 15. 2012)

Vareltzis, K., Soultos, N., Zetou, F., Tsiaras, F. (1990). Proximate composition and quality of a hamburger type product made from minced beef and fish protein concentrate. LWT- Food Science and Technology, 23(2), 112-115.

Venugopal, V., Chawla, S. P. and Nair, P. M. (1996). Spray dried protein powder from threadfin beam: preparation, properties and comparison with FPC type-B. Journal of Muscle Foods, 7, 55.

Yuge, W., Nozaki, H. Evolution of surimi seafood products in Japan-then and now. Presented at 1st Surimi Forum Japan. (July 20-21), Tokyo, Japan, 2010.

APPENDIX A

UTILIZATION OF SEAFOOD BY-PRODUCTS IN MEDICAL AND PHARMACEUTICAL PRODUCTS

It has been an ancient tradition of utilizing bioactive compounds of the seafood by-products in medical and pharmaceutical applications. In some areas, by-products from marine species poses stronger biological activities than their land-based counterparts. Advances in marine biotechnology have increased the efficiency and profitability of the seafood industry. Genetic engineering is already looking into designing and biosynthesizing analogs of bioactive compounds in short supply. Elastin and squalamine are two prototype products for which laboratory synthesis and biological engineering of analogs are already underway (Brunel and others, 2005; Girotti and others, 2004). Protamine bioactivity is mostly associated with the repeated chains of arginine. Hence, biosynthesis of polyarginine molecules may provide a way to replace bioseparation of the polypeptide from aquatic sources.