THE USE OF ACID AND ALKALI-AIDED PROTEIN SOLUBILIZATION AND PRECIPITATION METHODS TO PRODUCE FUNCTIONAL PROTEIN INGREDIENTS FROM TILAPIA

By

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This thesis is dedicated to my loving grandparents.

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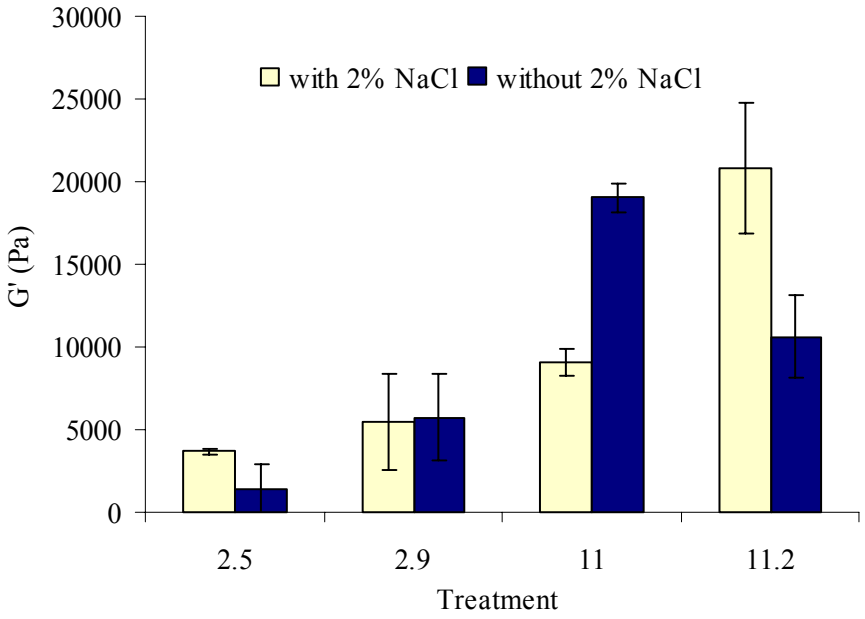

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There is great interest in increasing the use of fish muscle proteins, as food source and food ingredient, due to their functional and nutritional properties. Over the years several methods have been developed in order to isolate proteins from fish muscle, many of which cause loss of their functional properties. Therefore, processing methods that focus on retaining valuable functional properties of proteins are preferred. A good example of such a method is production of surimi. Surimi is a washed fish muscle used for manufacturing of imitation seafood products. The demand for surimi products is increasing, whereas the traditional resources for surimi making are limited. Other resources, preferably less expensive, have to be utilized, such as dark muscle species or byproducts. The use of such raw material has not been successful in conventional surimi processing until the development of the acid and alkali-aided processes which enables isolation of functional proteins. The processes are based on solubilization of muscle

proteins at low and high pH, separation of the soluble proteins via centrifugation and then precipitation of soluble proteins at their isoelectric pH.

The overall objective of this study was to investigate the use of acid and alkali-aided processing to recover functional proteins from tilapia white muscle. The effects of different low and high solubilization and precipitation pHs on solubility, viscosity and protein recovery were determined. In addition, quality of gels prepared from acid and alkali treated proteins was compared to gels prepared using washed tilapia muscle.

The use of the acid and alkali aided processes on tilapia muscle proteins in regard to isolation parameters showed that the type of proteins recovered using low and high pH was significantly different whereas the quantity of total protein recovered was not.

The ability of the protein isolates to form gels upon heating was compared to lab scale conventional surimi processing. Gels were prepared from acid and alkali treated protein isolates with and without the addition of 2% NaCl (w/w) at neutral pH and compared to gels prepared from washed muscle. Gel quality was determined using torsion, folding, oscillatory testing and water holding capacity. Hardness (shear stress) and elasticity of gels (shear strain) was improved using 2% NaCl (w/w) compared to treatments without salt. Overall, the acid treated proteins exhibited poorer gelling ability compared to alkali treated proteins. Total content of SH groups was measured before and after gelation and S-S bonding did not explain the difference in gel forming ability of different treatments. The results indicate that the alkali aided process can be used to produce high quality protein gels usable for manufacturing of imitation seafood products

CHAPTER 1
INTRODUCTION

There is a great interest in increasing the use of muscle proteins, as a food source and food ingredient, due to their functional and nutritional properties (*1*). Muscle proteins from fish are nutritive, easily digested and exhibit good functionality (*2;3*) which makes them desirable for various food applications. However, the use of fish proteins as a food or food ingredient, has been limited due to several reasons, such as, rapid bacterial spoilage, lipid oxidation, protein oxidation, low stability compared to mammalian and vegetable proteins and loss of functionality during processing (*4*). Over the years several methods have been developed in order to isolate proteins from fish muscle, many of which cause a loss of their functional properties. Many of them have been relatively harsh where conditions such as combination of very low or high pH in the presence of organic solvents and high heat have been used. These methods were highly unsuccessful since functionality and nutritional quality of the products were negatively affected (*5*). Processing methods that focus on retaining valuable functional properties of the proteins are preferred and have received increased attention. A good example of such a method is surimi processing which involves washing fish muscle and adding cryoprotectants prior to freezing to stabilize the proteins. Surimi is then used for manufacturing of imitation seafood products by heating. The popularity of surimi based products is gradually increasing both in the US and Europe. The surimi industry is largely based on the utilization of Alaska Pollack, which is now under pressure from over fishing and therefore other species have to be found (*4*).

Processing methods which could employ inexpensive raw materials to make a quality surimi would be highly desirable both to reduce the pressure of over fishing of currently used species and to reduce the cost of production. Potential raw materials could be; industrial fisheries presently exploited, seafood processing by-products and by-catch or unexploited/under-exploited stocks. Employing conventional surimi processing on these raw materials has been met with numerous problems (*6;7*). To address the problem of utilization of raw materials unusable for surimi processing, two novel processes were developed originally at the University of Massachusetts. These processes, which both work by the same principle, involve acid or alkaline solubilization and isoelectric precipitation of muscle proteins to give a highly functional and stable protein isolate from low value underutilized species and by-products (*1;7*). The new process has been shown to work well for various cold water species such as cod (*8*), herring (*9*), and Pacific whiting (*10*) but currently there is little data available for the potential of using these processes to produce functional proteins from warm water species such as tilapia.

Tilapia aquaculture is rapidly growing worldwide (*11*), generating large amounts of by-products (and primary products) which could be utilized for its protein content, provided the proper process is used. In addition, consumption of tilapia is increasing both in the US and globally. Conventional surimi processing from tilapia has been somewhat successful although the yields are fairly low. To the best of our knowledge the acid and alkali-aided processes have not been applied on tilapia muscle materials. Therefore, tilapia is a species of great interest to investigate. To reach the goal of full utilization of tilapia it is essential to investigate the use of the newly developed acid and alkali-aided processes on whole muscle before by-products can be utilized. The results

from this research are expected to give important information on the production of a high quality protein isolate from aquacultured tilapia.

CHAPTER 2
LITERATURE REVIEW

Preparation of Fish Protein Ingredients

Raw Material

Quality and characteristics of a protein ingredient or a finished product are highly dependent on the source of muscle protein and the processing procedures applied (*5;12*). Species with white flesh and low fat content are considered most suitable for surimi manufacturing, for example Alaska pollock, Pacific whiting, hoki, southern blue whiting, northern blue whiting and yellow croaker (*12*). The use of fish species with higher content of dark/red muscle and fat has met some complications such as low grade protein gels, color problems and lipid oxidation (*12*). The reason for poorer gelling ability of species with darker muscle has been related to its characteristics; higher proteolytic activity, lower muscle pH which can lead to more rapid protein denaturation, higher concentration of sarcoplasmic proteins, higher lipid content and high concentration of heme proteins in the muscle. All these factors have been reported as problems when producing high quality surimi from materials containing large amounts of dark muscle (*6*). Surimi manufacturers have resorted to several methods to alleviate this problem (e.g., by removing the dark muscle before surimi processing). The disadvantage of doing this is decreased protein recovery. The use of the acid and alkali aided processes has made it possible to produce good quality surimi from dark muscle species.

A major problem facing any protein extraction and recovery process is proteolysis by endogenous proteases. Postmortem fish muscle is very prone to proteolysis and the

problem varies greatly with species and season. The effect of proteolytic activity on muscle protein gels has detrimental effect on their quality due to rapid degradation of myofibrillar proteins, in particular myosin. Proteolytic activity and types of proteases vary among species. Yongsawatdigul et al. (*13*) reported that serine type protease(s) were most probably involved in proteolysis of tilapia surimi resulting in gel weakening. Gel weakening due to proteolysis has also been observed in Arrowtooth flounder, threadfin bream, Atlantic menhaden and lizard fish (*12*).

Proteolysis can be a significant problem during the acid and alkali-aided processes. The former process is thought to be more problematic since low pH's can activate contaminating gut enzymes (pepsin) and also certain lysosomal muscle enzymes (*14*). Undeland et al. (*9*) found that proteolysis occurred when herring proteins were held at pH 2.7, while no proteolysis occurred at pH 10.8. Choi and Park (*10*) also reported proteolytic degradation of muscle proteins during acid-aided processing of Pacific whiting.

Conventional Surimi Processing

Surimi was originated in Japan where it has been a traditional food source for centuries. It is a minced fish muscle washed with water and used as an ingredient for imitation seafood products, primarily crab substitutes. For many years the industry was dependent on supply and availability of fresh fish. The discovery of adding cryoprotectants to surimi in order to prevent protein denaturation during freezing, revolutionized the industry (*12*) which was no longer dependent on fluctuations in supply of fresh fish.

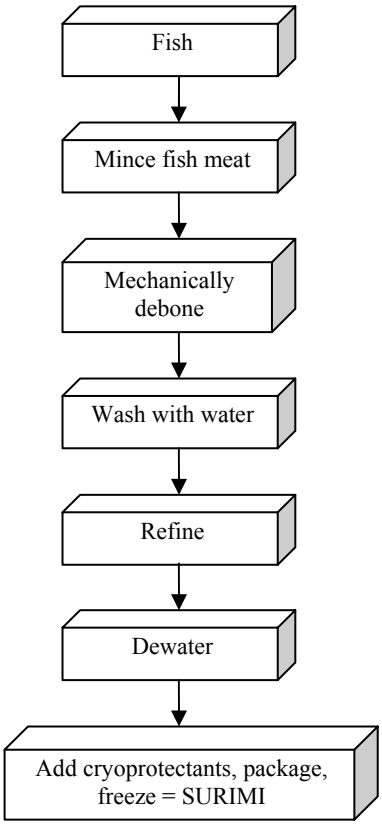


Figure 2-1. Conventional surimi processing. The fish (skinless fillets, fillets with the skin on, or headed and gutted fish) is minced, mechanically deboned, washed with water, reined, dewatered, cryoprotectants added ,the product packaged, and finally frozen in blocks until used. This product is termed "surimi" and is used in finished products, characterized as gels, formed by using heat.

In conventional surimi processing as shown in Figure 2-1, the raw material is minced and then mechanically deboned (bones, skins and scales are removed by pressing soft tissue through small holes in a screen). Washing with water concentrates the myofibrillar proteins, removes components that can have negative effects on gelation and compounds that can cause flavor, odor, stability and color problems. The amount of water and number of washes depends on the raw material, the desired final product and water availability. The temperature should be maintained low enough to prevent protein

denaturation which varies according to species (*15;16*). In the refining step, remaining bone pieces, skin, scales and connective tissue are removed. Dewatering is necessary because during the process water is absorbed (approximately 100% increase) due to repulsion of negatively charged proteins in the washed mince (pH ~ 6.4 to 7.0). The water minimizes the repulsion by separating the proteins and the addition of salt (0.1 to 0.3%), reduces the repulsive forces by shielding negative charges which allows the proteins to approach one another. Thus, expelling water and reducing the tendency of the tissue to absorb water. Finally cryoprotectants, usually sucrose (4%), sorbitol (4%) and sodium tripolyphosphate (0.2 to 0.3%) are added in order to protect the proteins from denaturation and aggregation during freezing which would result in reduced gelation ability of the proteins (*12*). Prior to freezing, proteolytic enzyme inhibitors are sometimes added along with cryoprotectants to prevent proteolytic degradation of proteins during heating. For example is the use of Pacific whiting for surimi manufacturing based on addition of enzyme inhibitors and application of fast heating rate to minimize proteolytic degradation of muscle proteins (*12;17*).

Generally, high quality surimi is produced from white fleshed fish such as Alaska pollock but to meet the increasing demand new resources have to be found. Utilization of dark fleshed underutilized species has often led to products with poor gelation properties. Recent studies show however that the use of warm water species like tilapia have resulted in better gel quality compared to more common surimi species. For example, Klesk and coworkers (*17*) compared the gel quality of tropical tilapia surimi to Alaska pollock and Pacific whiting and found that it was comparable to Alaska pollock and better than Pacific whiting (without enzyme inhibition) at when heated at 90°C for 15 min.

The Acid and Alkali-Aided Processes

The acid and alkali processes, originally developed by Hultin and coworkers are able to overcome some of the problems that are involved using dark muscle, small species or by-products for surimi production. The processes involve using acid or alkaline solubilization of muscle proteins, followed by isoelectric precipitation of soluble proteins to give highly functional and stable protein isolate used to produce quality surimi (*1;7*).

As outlined in Figure 2-2, the production is carried out by subjecting a diluted slurry of homogenized muscle tissue to either a very acidic (2.0 to 3.5) or alkaline (10.5 to 11.5) pH to solubilize the majority of muscle proteins via electrostatic repulsion. As a consequence of disruption of the muscle cell and solubilization of the proteins the viscosity of the protein solution is drastically lowered. The lowering of viscosity allows for separation of insoluble material, such as, bones, skin, connective tissue, cellular membranes and neutral storage lipids from the soluble proteins by centrifugation. The soluble proteins are collected after centrifugation and recovered by isoelectric precipitation (by adjusting the pH to 5.2 to 6.0) and then collected by centrifugation. The sediment (protein isolate) is kept and the supernatant discarded. Cryoprotectants are added to the protein isolate prior to freezing to protect the proteins from denaturation.

Protein gels made from protein isolates recovered with the new process from several species have shown to have equal and sometimes significantly better gelation properties than those produced using conventional surimi processing techniques (*6;9;10;18*). The process has also shown to improve other functional properties. The process has given excellent results for some cold water species as well as temperate and warm water species and is now in the route of commercialization for North Atlantic and

Pacific species as well as species in the US Southeast. A study done by Kristinsson and Hultin (*8*) on cod muscle proteins showed that the alkali treatment improved functional properties (emulsification and gelation) of cod myosin and myofibrillar proteins. This improvement was found to be directly linked to a unique partially unfolded structure of cod myosin after alkali-treatment.

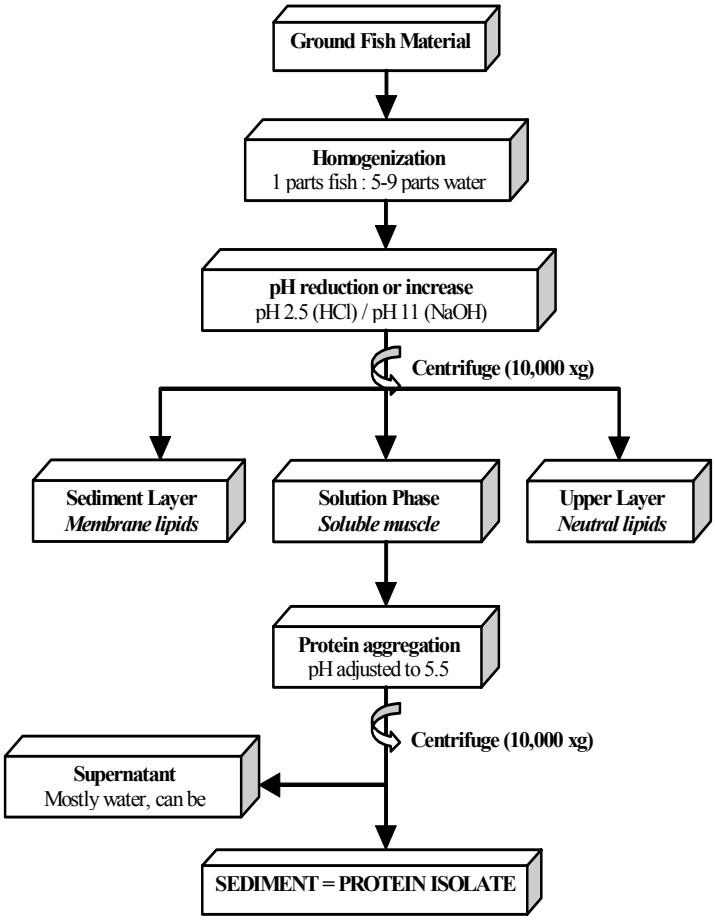


Figure 2-2. Schematic diagram of the acid and alkaline process used in the production of functional protein isolates.

A different response to the acid and alkali process can be expected for warm water species compared to cold water species in part because their proteins are more heat stable due to their living environment. A study performed on threadfin bream actomyosin indicated that aggregation underwent at higher temperatures than was seen for cold water

species such as cod and pacific whiting (*19*). A number of studies at the University of Florida have shown that several warm water species have a great potential to be utilized via these two processes. Kristinsson and Demir (*18*) compared the acid and alkali-aided processes to surimi on channel catfish, Spanish mackerel, croaker and mullet and demonstrated that the two processes led to significantly higher protein recoveries and lipid reduction than lab scale conventional surimi processing but also that the alkali process resulted in significantly improved gelling ability, color and oxidative stability (due to removal of heme proteins) compared to the acid treatment and surimi process. Theodore and Kristinsson (*20*) showed that using the alkali-aided processe on catfish led to protein gels of greater strength when compared to using acid-aided process and conventional surimi process over a wide range of pH (pH 6-8) and ionic strengths (0-600 mM NaCl). Results by Davenport and Kristinsson (*21*) indicate that there are major changes in myosin (from channel catfish) that contribute to the improved gel strength after alkali processing. In addition Kristinsson and Crynen (*22*) demonstrated that both myofibrillar and sarcoplasmic proteins of muscle (from channel catfish) are positively affected in terms of gelation ability after alkali-treatment, but negatively affected after acid-treatment. The molecular phenomena responsible for these differences are under investigation.

Studies on tilapia using conventional processing have shown promising results (*17;23-26*) whereas few studies have been conducted with tilapia muscle proteins using the acid and alkali processes to the best of the researchers' knowledge. Preliminary significantly greater gel forming ability of the proteins vs. untreated muscle proteins. Treating the tilapia muscle proteins to a low pH (2.5) followed by readjustment to pH 7

resulted in significantly poorer gel forming abilities compared to untreated proteins. The reasons behind these major differences are unknown and are important to determine to be able to successfully produce protein isolates from tilapia muscle and its by-products.

The acid and alkali-aided processes have several advantages over the conventional surimi process in regard to isolating functional and quality protein from fish muscle

- In the acid and alkali-aided processes protein recoveries are normally significantly higher due to recovery of both myofibrillar and a sizable amount of sarcoplasmic proteins. Recoveries using conventional techniques are usually lower due to loss of sarcoplasmic proteins (~ 30% of total protein) during washing steps.

- The acid and alkali-aided processes are simple and require less labor than surimi processes. For example, liquefaction of the material of interest makes it easier to move it around a processing plant compared to a more solid material such as in surimi processing.

- Whole fish with skin and bones and fatty fish can be utilized in the acid and alkali-aided processes because proteins are selectively separated and recovered from undesirable muscle components. This is not feasible using typical surimi processing without negatively affecting the recoveries and quality (*5*).

- Lipids and cellular membranes can be effectively removed in the acid and alkali-aided processes. This significantly increases the color and oxidative stability of the final product compared to proteins produced using surimi. Microorganisms are also effectively precipitated out during centrifugation.

- Removal of heme proteins are more effectively obtained with the alkali treatment compared to surimi processing resulting in a product that is whiter and more stable to lipid oxidation. Heme proteins are also protected from denaturation and autoxidation during high pH treatment. Acid treatment leads to heme protein denaturation and co-precipitation with muscle proteins, leading to color and oxidative problems.

- Functional properties are either retained, decreased (in few cases for the acid process) and often significantly improved (alkali process) using the process. The effect on functionality is highly dependent on species and type of functional property.

- Problems related to foaming and emulsification of lipids have been encountered in commercialization of the acid and alkali-process and have led to reduced protein recoveries.

- The use of high pH in combination of high heat can lead to formation of lysinoalanine. Acid hydrolysis can cause razemization of amino acids. This is normally not a problem in the acid and alkali-processing due to rapid process time and very low process temperature.

Functional Properties of Muscle proteins

Quality and stability of a final product are affected by functional properties of proteins (*27*). The most important functional properties when producing a functional muscle protein isolate for use in food are solubility, viscosity, water holding capacity and gelation (*1*). These properties are discussed in more detail below.

Solubility

Solubility of myofibrillar proteins is believed to play an important role in gelation and water holding capacity of muscle proteins (*28*). Therefore, solubility of muscle proteins has been the subject of much research.

A change in solubility can be obtained in various ways, for example, by varying ionic strength, ion types, pH, and/or temperature and thus affecting the hydrophobic and/or ionic nature of the proteins. For a long time it was a general belief that solubilization of myofibrillar proteins in high salt concentration (0.3 to 0.6 M) was required to form good fish gels. Studies have shown that this is not necessarily the case, Stefansson and Hultin (*29*) showed, that cod muscle myofibrillar proteins were soluble if the ionic strength was sufficiently reduced (< 0.3 mM) at both neutral and acidic pH. They suggested that the negative charge of myofibrillar proteins at neutral pH and/or in water or solutions of very low ionic strength, repulsive forces from negatively charged side chains are enough to drive the individual protein molecules apart when sufficient water is available (*29*).

Changes in protein solubility can also be obtained by varying the pH of the solution, as is done in the acid and alkali-aided processes. By changing the pH, the protein acquires a net negative or net positive charge where hydration of the charged residues and electrostatic repulsion results in an increase in solubility (*30*). Conditions like lowering the pH near to the isoelectric point reduces the repulsive forces and allows the proteins to associate. Therefore, many proteins exhibit minimum solubility at the isoelectric point (pI) where the lack of electrostatic repulsion promotes aggregation between protein molecules. Due to aggregation of proteins at these conditions they can be removed from solution by appropriate centrifugal force.

In order to find the most appropriate pH to solubilize and recover proteins from a protein solution a solubility curve can be constructed (protein concentration vs. pH). For example, a study done by Choi and Park (*10*) showed that solubility of Pacific whiting proteins was lowest at pH 5.0 which indicated a suitable pH to precipitate the proteins. Maximum solubility was observed at pH 1.5 on the acidic side and at pH 11.0 on the alkali side. The solubility curve of channel catfish showed that most solubility was observed at pH 2.5 and 11 and least at pH 5.5, suggesting that the former two pH values would be suitable to solubilize the proteins and the latter pH to precipitate them from solution (*18*).

The ionic strength of a solution has a dramatic impact on the pH-dependent solubility profile of muscle proteins. For example, Dagher et al. (*31*) showed that solubility of a washed cod muscle mince increased dramatically between 8.9 and 9.2 at an ionic strength of 0.001 M. However the effects of pH at high salt concentration were not as dependent as when the salt concentration was low. A recent study by DeWitt et al.

(*32*) on using the acid-aided process to solubilize and recover proteins from beef heart showed that increasing the ionic strength at low pH led to a decrease in solubility and thus their extractability. This reduction in solubility stems from screening of electrostatic repulsive forces between the proteins (*33*).

Viscosity

The viscosity of muscle protein homogenates at low and high pH in the acid and alkali process is important since low viscosity is necessary to separate insoluble material from soluble proteins via centrifugation. Viscosity of a protein solution is believed to be affected by factors like protein concentration, pH, salt, and raw material processing which in turn can affect size, shape, flexibility and hydration of the proteins (*33*). Viscosity of protein solutions usually increases exponentially with protein concentration. This is due to increased interaction between the hydrated protein molecules. When two hydrated molecules are in close proximity, short range repulsive interaction forms where the strength depends on the degree of hydration (more hydration => stronger repulsion and longer range) (*34*). An increase in ionic strength usually decreases viscosity by affecting the hydration capacity of the proteins (*33*).

The two most important factors affecting viscosity of a protein solution according to Damodaran (*33*) are hydrodynamic size and shape of the protein molecules. Partial denaturation and/or heat induced polymerization, increases hydrodynamic size of proteins and thus increases viscosity. Most macromolecular solutions do not exhibit Newtonian behavior instead viscosity is decreased with increasing shear rate. This is called pseudoplastic behavior or shear thinning. This is due to the fact that the proteins align themselves in the direction of flow and weakly bound dimers and oligomers are dissociated into monomers (*30*).

Gelation

One of the most important functional properties of muscle proteins is their ability to form gels upon heating. A gel is an intermediate stage between a solid and a liquid where polymers (e.g., proteins) form a three-dimensional network that is capable of holding water and other low molecular compounds (*30*). Characteristics of protein gels are determined by the type and number of protein-protein interactions, aggregation and arrangement of unfolded proteins which are in turn affected by pH, ionic strength, protein concentration, and heating and cooling rates (*35*).

The pH and ionic strength are by far the most important determinants of strength and quality of muscle protein gels. Optimum pH for the gelation of muscle proteins has often been reported to be between 5.5 and 7.0, and depended on animal species, protein concentration, salt concentration, and instrument used to analyze gel strength. The use of salt (0.3 to 0.6 M) has been regarded a prerequisite in order for myofibrillar proteins to form good gels. Recently, this general belief was challenged as muscle proteins have been found to form excellent gels in the absence of salt provided that electrostatic repulsion is sufficiently high to create a strong osmotic pressure in the gel matrix (*18;36*).

The formation and characteristics of muscle gels are also highly dependent on the heating procedures. Different species respond very differently to the same heat treatment, which makes it important to determine the optimal heating and cooling scheme for good gel formation. As an example, gel strength can be improved for some gels by holding the protein paste at a temperature below 50°C before the final heat treatment at ca. 80 to 90°C, a process called setting. However, for some species this results in loss of gel quality if the temperature is held at 50 to 60°C for too long due to proteolytic degradation of muscle proteins (*5*). In a study performed by Klesk and coworkers (*37*),

tilapia formed the best gel when no setting was applied when compared to pollock and pacific whiting. Whereas, using temperature of 60°C during setting was found to reduce strain and stress values of the gels and indicated increased protease activity leading to the degradation of myosin heavy chain.

According to Lanier et al. (*38*) the setting of fish muscle proteins at temperatures below that at which rapid aggregation occurs (ca. 40°C) may be viewed as a process where partially denatured proteins begin to interact non-covalently to form a fine elastic network. Setting below 40° C would allow for slow ordering of the molecules and give gels with greater firmness and cohesiveness. Hermansson (*39*) also reported that denaturation of proteins prior to aggregation results in a finer gel structure, exhibiting greater elasticity than if random aggregation occurs prior to denaturation. Park et al. concluded that optimum setting temperature is highly related to habitat temperatures (*40*).

The proteins that have been found to be primarily responsible for gel formation and gel strength are the myofibrillar proteins most notably myosin and to some extent the complex of actin and myosin (actomyosin) (*41*). Grabowska and Sikorski (*42*) did a gel study on myofibrillar, sarcoplasmic and stroma fraction and the sarcoplasmic fraction showed no gelling ability. It has been a general believe that sarcoplasmic proteins have a negative effect on gelation. However recent studies point to that that sarcoplasmic proteins may actually improve gelation in some cases. Sarcoplasmic proteins are recovered in the acid and alkali-aided processes but not in surimi processing. Recently Kristinsson and Crynen (*22*) reported that a very complex interaction occurs between sarcoplasmic and myofibrillar proteins when mixed in different ratios under different pH and ionic strength conditions. In many cases the sarcoplasmic proteins had a positive

impact on the gel forming ability of myofibrillar proteins, especially when both had been treated at a high pH (11.0), as done in the alkali-aided process.

Gels are complex and their properties and the mechanism of their formation has been the subject of many studies. There are several ways available to study the properties of a gel and the mechanism of their formation. Rheology deals with flow and deformation of matter which is induced by applied force (*43*). There are mainly two quantities that rheology is concerned with; stress, which is the force applied divided by an area of matter that it is applied to and strain which is the deformation of a matter induced by the stress. One type of deformation is shear, which is when matter changes shape without changing volume (*44*). An important property of a gel is deformability, which is how a gel responds to strain without breaking. There are two main rheological studies available in order to obtain information on properties of gels and the mechanism of formation, small strain testing and large strain testing. The combination of these measurements can give valuable information on acceptability of a gel.

Small strain testing is a study where a sample is deformed without breaking the structure. Heating and cooling of a gel, using low frequency and small strain oscillatory experiments is one of the best suited methods to follow changes in physical properties relating molecular properties of a gel (*44;45*). The primary parameters of interest in small strain testing are; the storage modulus (G') which describes the elastic component of a protein gel, the loss modulus (G") a measurement of the viscous attribute of the gel and the phase angle (δ), where for a perfectly elastic material stress and strain are in phase or $\delta = 0$ and for a perfectly viscous material $\delta = 90$.

Large strain testing is when a sample is deformed until the structure is permanently broken. Large strain testing estimates fundamental properties of a gel and have shown to correlate with sensory texture. An example of large strain testing is puncture test and torsion (twisting) which was used in the experiments performed for this thesis (*44*). The torsion test is a typical method to test the hardness (shear stress) and elasticity (shear strain) of surimi gels. The advantages using the torsion over a compression test e.g. is that pure shear is applied, the volume is unchanged even if the sample is compressable and the shape is maintained during testing. To maintain accuracy of the measurements the cross-sectional diameter needs to have a constant length. It was observed that shear stress was extremely sensitive (40 to 70 kPa) to changes in gel diameter (0.90 to 1.10 cm) (*40*).

Water Holding Capacity

Water holding capacity (WHC) is an important factor in muscle protein gels as it not only affects the economics of their production but also their quality. Water holding capacity can be defined as the ability of a matrix (e.g., a protein gel to retain water against gravitational force) (*30*). The level of water retained in a gel is affected by much the same factors that affect the formation of a good gel matrix; pH and ionic strength (i.e., salt). Feng and Hultin (*36*) reported that gels with an evenly distributed gel structure showed improved WHC. A poor gel matrix on the other hand can lead to synerisis (i.e., a discharge of water from the gel). Gels prepared in the pH range of 6.4 to 7.4 had increasingly higher WHC as pH increased (*46*). An increase in WHC was shown to correlate well with increased negative charge on the muscle proteins. A comparison between the WHC of protein isolates prepared from the acid and alkali-aided processes

and those prepared using surimi processing has not been conducted, to the best of the writers knowledge.

Protein Denaturation

It is a commonly held view that denaturing fish muscle proteins has a detrimental impact on their functional properties. Denaturation often results in negative changes in protein functionality such as enzyme activity or loss of functional properties. In other cases, denaturation of the proteins can result in improvement of functional properties such as foaming and emulsification of egg albumin (*47*) or improved gelation. Loss of protein functionality has been correlated to loss in ATPase activity, a common indicator of muscle protein denaturation (*2;48*). Interestingly, ATPase activity is essentially lost in the acid and alkali processing where the proteins are partially denatured at low and high pH and then only partially refolded when the pH is readjusted (*49*). The fact that acid and alkali-treated proteins often have significantly improved functionality goes against common believe. Kristinsson and Hultin (*49*) have shown that the unique structure the proteins possess after pH-treatment is responsible for improved functional properties such as gelation, emulsification and solubility. For example, the partially unfolded/folded structure is more flexible and is able to form better protein networks on heating (gelation) and is able to adsorb more readily to interfaces and lower interfacial tension (emulsification). Results indicate that acid treatment has a different effect on the structure of the muscle proteins compared to the alkali treatment, also in a species dependent manner (*21;49*). It is of importance to understand what specific changes occur with the structure of the proteins during the process to optimize the functionality of these proteins.

Research Objectives

The overall objective of this study was to investigate use of acid and alkali-aided processing to recover functional proteins from tilapia white muscle. The effects of low and high solubilization pH and precipitation pH on solubility, viscosity and protein recovery was determined. In addition, quality of gels prepared from acid (pH 2.5 and 2.9) and alkali (pH 11.0 and 11.2) treated proteins was evaluated and compared to gels prepared using washed tilapia muscle.

CHAPTER 3
MATERIALS AND METHODS

Raw Material

Tilapia fillets were obtained fresh from a local supplier (Rain Forest Aquaculture, Sunrise, FL), transported on ice to the laboratory and stored in a cold room at 4°C until processed (within 24 hours). Red muscle was manually excised from the white muscle and discarded. The remaining white muscle was ground using a Scoville grinder (Hamilton Beach, Washington, NC) with 6 mm holes. All preparation of raw material and samples was performed in a cold room at 4°C or on ice when applicable to maintain temperature below 5°C.

Preparation of Muscle Homogenate

Ground muscle was mixed with cold (4°C) DI water (1:9, w:w) and homogenized for 60 sec (2x30 sec) with a Waring blender (Waring Products Division, New Hartford, CT) at 40% electrical output. The homogenate was carefully poured into a plastic beaker on ice and was adjusted to the appropriate pH by adding 2 M HCl or 2 M NaOH followed by stirring with a plastic spatula.

Protein Solubility

Solubility Curve

A protein solubility curve was constructed from pH 1.5 to 12.0 in 0.5 intervals. Two sets of homogenates were prepared from the same raw material. The first set was adjusted from the native muscle pH (6.5-6.9) to pH 1.5 with 2 M HCl and the second set was adjusted from the native muscle pH to pH 12.0 using 2 M NaOH. At each pH

approximately 30 g of homogenate was accurately weighed into centrifuge tubes (50 mL) and centrifuged at 10,000 G for 20 min in a Sorvall RC-5B using a SS-34 rotor (*29*), separating insoluble material from the soluble proteins.

Total protein was determined by taking a 2 g sample at each pH before centrifugation and diluting it 10 times with cold DI water at pH 11.0 to aid in solubilization of the proteins. The samples were homogenized with a hand held Tissue Tearor (Biospec Products, Inc, Bartlesville, OK) on speed 7 for 20 sec and then analyzed for protein content using the Biuret Method (see below). Soluble protein was determined by taking a 1 mL sample from the supernatant after centrifugation and diluting it 5-fold with cold DI pH 11.0 when applicable (pH 5 to 6 were undiluted).

Solubility Before Precipitation

From the previously constructed solubility curve, protein solubility at low pH (2.3 to 2.9) and high pH (10.8 to 11.4) was studied in more detail. Effects of different homogenization times (60, 90, and 120 sec) using these low and high pHs were investigated. Total protein before centrifugation and soluble protein after centrifugation were determined as previously described.

Solubility After Isoelectric Precipitation

Protein solubility after isoelectric precipitation, indicating loss of protein after 2nd centrifugation was determined using four solubilization pHs 2.5, 2.9, 11.0, and 11.2; and four precipitation pHs (5.1, 5.3, 5.5, or 5.7). After solubilization of the proteins, the homogenate was centrifuged at 1000 0g for 20 min using a GS-3 rotor. The supernatant, containing the soluble proteins, was recovered by pouring the content of the centrifuge bottle through a strainer covered with a double layer of cheesecloth, thereby separating the top layer and sediment from the supernatant. The supernatant was divided in two parts and the proteins

precipitated using pH 5.1 and 5.3, then centrifuged in Sorvall RC-5B at 10,000 G for 20 min using a SS-34 rotor. This was repeated for precipitation of pH 5.5 and 5.7. Soluble protein after the 2nd centrifugation was determined by taking a 1 mL sample from the resulting supernatant and analyzed for protein content using the Biuret Method (see below).

Protein Measurements

Protein content was determined using the Biuret Method (*50*) with the addition of 10% deoxycholic acid to reduce cloudiness due to lipids. Absorbance was read at 540 nm using an Agilent 8453 UV-VIS spectrophotometer (Agilent Technologies, Palo Alto, CA) and protein concentration estimated using a standard curve prepared with bovine serum albumin (BSA) in the range of 1-10 mg/mL.

Calculation of Solubility

Theoretical Protein Solubility: Defined as the fraction of the total protein soluble after centrifugation, assuming that no protein is lost in the top layer or sediment at centrifugation.

$$\%\ Soluble\ Protein = \frac{mg/mL\ soluble\ protein}{mg/mL\ total\ protein}$$

Actual Protein Solubility: Defined as the fraction of the total protein soluble after centrifugation based on the weight of the homogenate before centrifugation, the weight of the supernatant recovered after centrifugation and its protein concentration.

$$\%\ Soluble\ Protein = \frac{mg/mL\ soluble\ protein * mL\ supernatant}{mg/mL\ total\ protein * mL\ homogenate} * 100$$

Viscosity

Preparation of Samples

Preparation of viscosity samples was identical to the solubility samples, except centrifugation was skipped for construction of the viscosity curve for pH values between 1.5 and 12 and for viscosity of the solubilization pHs (2.3 to 2.9 and 10.8 to 11.4). The second centrifugation was skipped for preparation of the homogenates solubilized at pHs 2.5, 2.9, 11.0, and 11.2; and precipitated at pHs 5.1 to 5.7

Viscosity Measurements

The viscosity was determined using single gap cylinder geometry in an AR2000 Advanced Rheometer (TA Instruments, New Castle, DE). Homogenate sample size was approximately 15 mL. Measurements were performed at 5°C using an oscillatory time sweep program with frequency set at 0.1 Hz, oscillatory stress at 0.1809 Pa, temperature at 5°C and a run time of 2 min (*18*). Graphs were constructed using the final readings of viscosity (Pa*s).

Recovery of Proteins

For each solubilization pH (2.5, 2.9, 11.0 and 11.2) 3000 g of homogenate was prepared. The homogenate was divided in two parts, one was used for precipitation pHs 5.1 and 5.3 and the other for pHs 5.5 and 5.7. Waiting time for the second part of the homogenate before pH solubilization adjustment was approximately 30 min. After solubilization of the proteins the homogenate was centrifuged at 10,000 G for 20 min in a Sorvall RC-5B using a GS-3 rotor. The supernatant was recovered and divided in two parts before precipitation. Precipitated proteins were recovered by centrifugation as described for the solubilization step. Recovery of proteins was determined and reported

as *theoretical* and *actual recovery*: a) after 1st centrifugation b) after 2nd centrifugation and c) through the whole process.

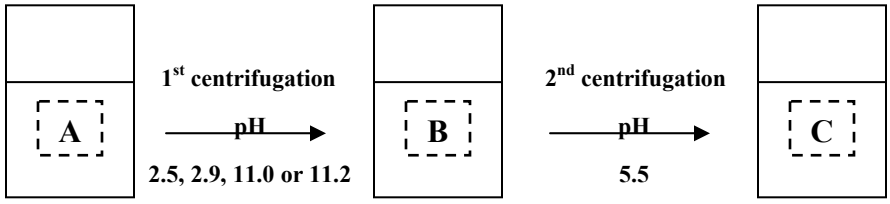


Figure 3-1. A: Soluble protein in initial homogenate (total protein), B: Soluble protein in supernatant after 1st centrifugation, C: Soluble protein in supernatant after 2nd centrifugation.

Protein Recovered after 1st centrifugation:

$$\% \ protein = \frac{B}{A} * 100$$

Protein recovered after 2nd centrifugation:

$$\% \ protein = 100 - \left(\frac{C}{B} * 100 \right)$$

Protein recovered through the whole process:

$$\% \ protein = \frac{B - C}{A} * 100$$

Equations for actual recovery are identical, except the weight of the supernatant and homogenate are multiplied with the protein concentration.

Electrophoresis

Proteins in the initial homogenate; top layer, supernatant and sediment after 1st centrifugation; supernatant and sediment after the 2nd centrifugation were separated according to the method described by Laemmli (*51*). Samples were prepared in small plastic vials by adding 170 µL of diluted protein samples to 330 µL of Laemmli sample buffer (BioRad Laboratories, Inc., CA), mixed well and 25 µL β-mercaptoethanol added. The vials were placed in boiling water for 5 min, cooled on ice and frozen at -30°C.

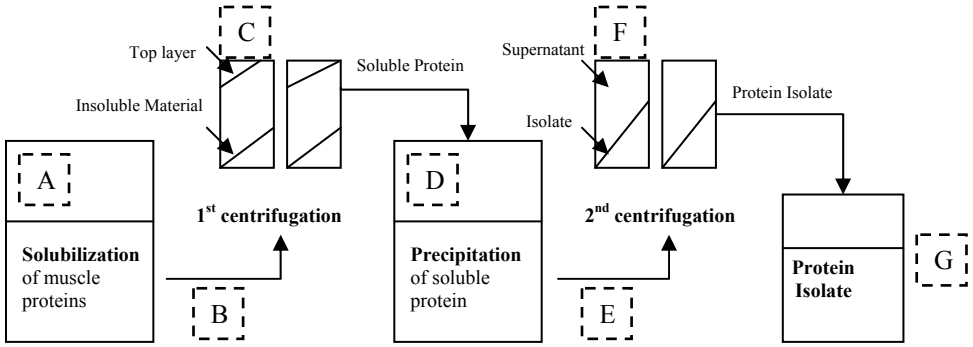
The samples (~3 mg/mL) were applied to precast gels 4 to 15% or 4 to 20% (BioRad Laboratories Inc., Hercules, CA) and run in a Mini PROTEAN 3 system (BioRad,

Laboratories Inc., Hercules, CA) using a constant current of 200mA (~45 min). A wide range (6.5 to 205 kDa) SigmaMarker™ molecular weight standard (Sigma Chemical Co., St. Louis, MO) was run for each gel. The wide marker contains thirteen proteins; mysosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), fructose-6-phosphate kinase (84 kDa), albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceradlehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14.2 kDa), aprotinin (6.5 kDa). The running buffer (pH 8.3) was prepared (500 mL) for each run by diluting a stock solution (30.3 g of Tris base, 144.0 g glycine and 10.0 g of SDS to 1000 mL) ten fold with cold DI water followed by thorough mixing. The protein bands were fixed in 12% TCA for 1 hour and stained overnight using an EZBlue™ Gel Staining Reagent (Sigma Chemical Co., St. Louis, MO).

Preparation of Protein Isolates

Ground muscle was mixed with cold DI water (1:9, w/w), homogenized for 60 sec (2x30 sec) with a Waring blender (Waring Products Division, New Hartford, CT) at 40% electrical output and carefully poured (to avoid foaming) into a plastic beaker on ice. The homogenates were adjusted to pHs 2.5, 2.9, 11.0 or 11.2 (~10 min) to solubilize the proteins, using 2 M HCl or 2 M NaOH. Then the homogenate was transferred to centrifuge bottles and centrifuged at 10,000 G for 20 min in a Sorvall RC-5B using a GS-3 rotor. Centrifugation resulted in the formation of 3 layers; the top layer containing mostly neutral lipids, the supernatant containing the soluble proteins and the sediment, containing insoluble material. The supernatant was separated from the top layer and the sediment by pouring the contents of the centrifuge bottles through a strainer covered with

two layers of cheesecloth. The top layer and sediment were discarded. The collected supernatant was subjected to isoelectric precipitation by adjusting the pH to 5.5, following centrifugation at 10,000 G for 20 min. The resulting supernatant was discarded but the sediment (protein isolate) was placed in a zip-lock bag and stored on ice in a cold room at 4°C overnight.



A. Tilapia white muscle was homogenized for 60 sec using a Waring blender, transferred to a plastic beaker on ice and the muscle proteins solubilized by lowering the pH to 2.5 or 2.9, or increasing the pH to 11.0 or 11.2.
B. The pH adjusted homogenate was centrifuged for 20 min at 10,000 G in a Sorvall RC-5B using a GS-3 rotor.
C. After 1st centrifugation, three layers are formed; top layer, sediment and supernatant. The supernatant was recovered by pouring the contents through a strainer covered with two layers of cheesecloth. The top layer and sediment are discarded.
D. The soluble proteins are precipitated by adjusting the pH to 5.5
E. The precipitated proteins are centrifuged again for 20 min at 1,0000 G in a RC-5B using a GS-3 rotor.
F. Two layers are formed after 2nd centrifugation, supernatant (contains soluble proteins) which is discarded and the protein isolate that is recovered and used for subsequent experiments.
G. The recovered protein isolate is stored in a zip-lock bags on ice at 4°C.

Figure 3-2. A schematic showing protein isolation from tilapia white muscle.

Preparation of Washed Muscle

Ground muscle was mixed with cold DI water (1:3, w/w) and allowed to sit for 15 min, stirring every few minutes with a plastic spatula. The slurry was poured into a strainer covered with a double layer of cheesecloth and the water manually squeezed out. This was repeated 2 times, with the last wash water containing 0.2% NaCl to aid in the

dewatering of the tilapia white muscle proteins. The washed muscle was placed in a zip-lock bag and stored overnight on ice in the coldroom at 4°C before gel preparation.

Rheology

Preparation of Samples

To determine moisture content of the isolates and washed muscle a ~5 g sample was placed in a Cenco moisture balance (CSC Scientific Company, Inc., Fairfax, VA). Moisture content was adjusted to 90% by adding cold DI water based on formula (3). Tests however revealed that the samples usually required slightly less water than the formula indicated.

$$g\ H_2O = \frac{X\ g_{\ sample}\,(X_{moisture\ of\ sample} - X_{wanted\ moisture})}{X_{wanted\ moisture} - 1}$$

Protein isolate (25 or 30 g) was weighed into a 100 mL plastic beaker, homogenized with a hand held Tissue Tearor (Biospec Products, Inc, Bartlesville, OK). After homogenizing for 1 min on speed 6, 25 mM of sodium phosphate dibasic (pulverized with a mortar and pestle to reduce particle size) was added and the paste homogenized again for 2 min. Finally 2% NaCl was added (when applicable) and mixed well with a stainless steel spatula. The pH of the paste was adjusted to 7.1 to 7.2 with 2 M NaOH (~4-5*120 μL was needed of 2M NaOH to samples with salt while samples without salt needed ~3*120 μL) followed by mixing with a stainless steel spatula. After the pH adjustment the beaker was covered with parafilm and the paste allowed to sit on ice for 30 min before rheological measurements. Total sample preparation including the 30 min setting time was approximately 50 to 60 min.

Measurements

Viscoelastic changes on heating and cooling were determined using single gap geometry in an AR2000 Advanced Rheometer (TA Instruments, New Castle, DE). Approximately 20 g of sample were placed in the sample chamber at 5°C and the head set to a specified gap (4950 μm). After the head reaches the gap, excess sample was removed with a stainless steel spatula and a layer of mineral oil was placed on top of the sample to prevent evaporation on heating. The opening was covered with a metal moisture trap also to prevent evaporation. The samples were heated from 5 to 80° C at a rate of 2 °C/min and cooled from 80 to 5° C at the same rate and measurement conducted using a oscillatory mode with constant frequency set at 0.1 Hz and maximum strain at 0.01(*8*).

Gel Preparation

Protein isolates were adjusted to 83% moisture content by squeezing the water manually out of the isolates. The washed muscle was centrifuged at 10,000 G for 20 min in a RC-5B centrifuge using a GS-3 rotor to reach the right moisture content. Moisture was determined as described before. Approximately 130 g of isolate/washed muscle were accurately weighed and placed in a Mini Chopper (Sunbeam Products Inc., Boca Raton, FL) with 25 mM Sodium Phosphate Dibasic buffer added after blending for 20 sec and 2% NaCl added after ~1 min (when applicable). The pH was adjusted to 7.1 to 7.2 by adding 2M NaOH dropwise. The paste was mixed for a total of 4 min with all steps performed in a cold room at 4°C. The paste was then manually stuffed into steel tubes (diameter 19 mm) and the ends sealed with a rubber cap and fastened with a hose clamp. The paste was cooked for 30 min at 80°C in a Precision water bath (Precision Scientific, Winchester, VA) and cooled in ice water for 15 min. Gels were removed from the

stainless steel tubes and placed in zip-lock bags and stored in a cold room at 4°C for 48 hours prior to testing.

Gel Quality Analysis

Torsion Testing

After storing the gels at 4 °C for 48 hours they were cut into 28.7 mm long samples using a cutting motive. After reaching room temperature (~40 min) the gels were milled into a dumbbell shape with a minimum center diameter of 1.0 cm. Gels were tested using a modified Brookfield DV-II viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) or (Gel Consultants, Raleigh, NC) and the samples twisted at 2.5 rpm until structure failed. Shear stress (resistance to breakage) and shear strain (distance until breakage) of the gels were obtained using computer software linked to the viscometer.

A

B

C D

Figure 3-3. Modified Brookfield DV-II viscometer. A) Brookfield Engineering Laboratories, Inc., Stoughton, MA; or Gel Consultants, Raleigh, NC. B to D) Milling equipment.

Folding

Folding test was performed within 60 hours of storage at 4°C, according to the method of Kudo et al. (*52*). Approximately 3 mm slices were cut and folded by hand at room temperature. The ability of the gels to fold was graded using a five point system (Table 3-1).

Table 3-1. Grading system based on five-point system.

Grade	Description
5	No crack occurs even if folded in four
4	No crack when folded in two but forms a crack when folded in four
3	No crack when folded in two but splits when folded in four
2	Cracks when folded in two
1	Splits when folded in two

Water Holding Capacity (WHC)

Water holding capacity on cooking was determined by analyzing the moisture content of the paste before cooking and moisture content of the gels after cooking.

Moisture content was determined based on weight before and after drying the samples overnight at 106°C in an oven.

WHC of the gels on pressing was determined using the method of Feng (*36*) where pressing loss is defined as the water loss of a 3 mm thick slice under 3000 G pressure (using a 3L beaker full of water) for 1 min. The sample was sandwiched between five layers of Whatman filter paper (Whatman Inc., Clifton, NJ) which absorbed the expressible water. Weight before and after pressing was recorded and moisture content was determined using an oven as described before.

$$Expressible\,Water\,(\%) = \frac{Pre\ pressed\ -\ weight\,(g)\ -\ After\ pressed\ weight\,(g)}{Pre\ pressed\,Weight\,(g)} \times 100$$

$$WHC\,(\%) = \frac{Expressible\,Water\,Content\,(g)}{Total\ Moisture\ of\ \ Pre\ Pressed\ Sample\,(g)} \times 100$$

Sulfhydryl Content

Total sulfhydryl (SH) content was determined on the paste before cooking and on the gels after cooking by using the method of Choi and Park (*10*) with slight modifications. The pastes and gels were diluted 100 times to give a protein concentration between 1 and 2 mg/mL. A 0.25 mL sample of the protein solution was added to 2.5 mL of 8 M urea, 2% sodium dodecylsulfate (SDS) and 10 mM EDTA in 0.2 M Tri-HCl buffer at pH 7.1. To this solution 50 uL of 10 mM Ellman's reagent (10 mM 5,5'-dithiobis - (2-nitrobenzoic acid) was added, mixed and heated in a water bath at 40°C for 15 min. After the reaction the absorbance of the solution was measured at 420 nm using an Agilent 8453 UV-VIS spectrophotometer (Agilent Technologies, Palo Alto, CA) and total SH content was calculated using a molar extinction coefficient of 13600 mol/cm.

Statistics

Results are expressed as means ± SD. Analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$) between treatments. Transformation using arcsin was used for precipitation solubility. All results were analyzed by using the Statistical Analysis System (SAS).

CHAPTER 4
SOLUBILIZATION OF MUSCLE PROTEINS OVER WIDE PH RANGE

Solubility

The basis for utilizing the acid and alkali processes on tilapia white muscle is to solubilize the muscle proteins at low and high pH. In order to achieve high protein recovery it is important to obtain high solubility at certain pH values to separate the soluble proteins from undesirable constituents of muscle. Low solubility at certain pH values is also important to precipitate (i.e., recover) the solubilized proteins. Therefore, to understand how the muscle proteins react to different pH values a solubility curve was constructed ranging from pH 1.5 to 12.0 and changes in percent soluble protein were observed (Figure 4-1). Minimum protein solubility was observed between pH 5.0 and 6.0, which is in the range of the isoelectric point for the majority of proteins in muscle (*2*). Lowering the pH away from the isoelectric point resulted in a dramatic increase in protein solubility up to pH 4.0 where the proteins were ~94% soluble. Maximum solubility at low pH was at pH 2.5 or ~96% but decreased down to 90% at pH 1.5. This decrease could possibly be due to anion induced aggregation, since more HCl would increase the ionic strength of the solution and may reduce some of the electrostatic repulsion between the proteins (*53;54*). An increase in pH away from the isoelectric point resulted in a slight increase in protein solubility until pH 9.0 to 10.0 where it rapidly increased between pH 10.0 and 11.0; and reached a maximum ~99% at pH 12.0. This solubility curve is similar to what Undeland et *al*. (*55*) observed for herring and Kim et *al*. (*56*) observed for Pacific whiting.

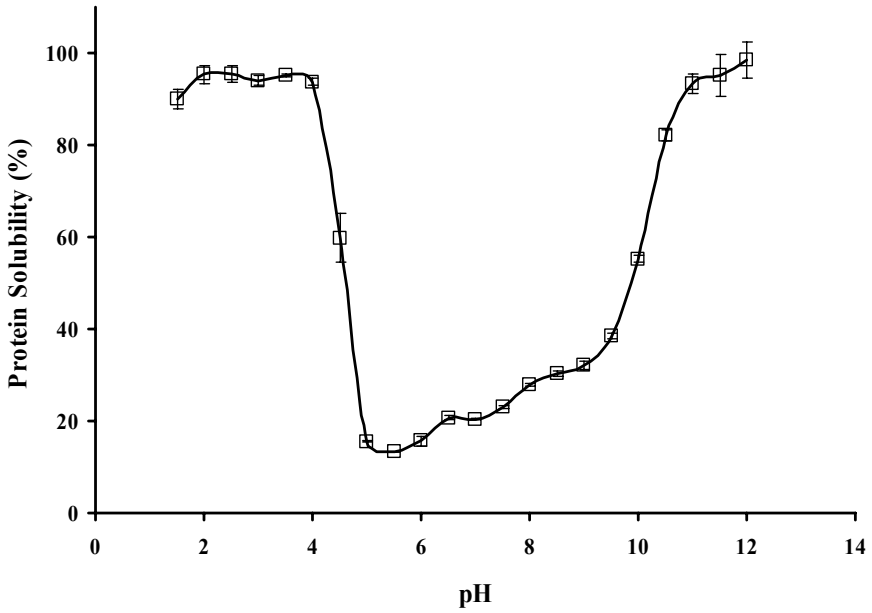


Figure 4-1. Shows protein solubility (%) of tilapia light muscle homogenate at pH 1.5 to 12.0. Protein content was determined using the Biuret method with the addition of 10% deoxycholic acid using a BSA standard curve. Protein solubility was defined as the fraction of the total protein soluble after centrifugation, assuming no protein was lost in the top layer or sediment. Results are mean ± SD.

As Figure 4-1 shows, approximately 20% of the proteins were soluble at physiological pH (6.5 to 7.0) at the 10 fold dilution commonly used in acid and alkali processes. Sarcoplasmic proteins which are soluble in water or solutions of low ionic strength usually constitute about 20 to 30% of fish muscle (*57*). The proteins which were soluble in the isoelectric point range were mostly sarcoplasmic proteins along with some dissociated actin (Figure 6-7). At the isoelectric pH (5.0 to 6.0) where minimum solubility was observed, lack of electrostatic repulsion between charged residues results in closer contact of the proteins which promotes hydrophobic interactions (protein-protein interaction) and aggregation. However, at pH below or above isoelctric pH, the proteins become positively or negatively charged which results in electrostatic repulsion between

the molecules and hydration of charged residues which in turn promotes solubility of the proteins (*30*). Mechanism of solubilization of myofibrillar proteins is believed to take place in two steps; first depolymerization of the thick filaments and then dissociation of actin from myosin (*58*). Addition of acid or base which raises ionic strength is not believed to have significant effects on protein solubility as suggested by Undeland et al. (*9*). The rapid increase in solubility at low pH compared to a more steady increase at higher pH might be attributed to more ionizable groups with pKa values between 2.5 and 7.0 than between 7.0 and 11.0 (*6;9*).

Viscosity

Low viscosity of the tilapia muscle homogenate is important in the acid and alkali process since it facilitates separation of soluble protein from insoluble material via centrifugation. As an example, Undeland et al. (*9*) showed that total lipid removal was reduced with increasing viscosity at low pH for homogenate prepared from herring light muscle. Low viscosity was also shown to be important to separate membrane phospholipids from muscle proteins, which could lead to increased oxidative stability of the final protein isolate (*6;9*).

A viscosity curve of homogenate prepared from tilapia white muscle was constructed in the pH range of 1.5 to 12.0 (Figure 4-2) in accordance to the solubility curve. The viscosity of the solubilization pHs of interest for the acid and alkali processes were low, or < 140 mPa*s. Two viscosity peaks were observed, at pH 5.0 and pH 9.5, respectively. At low pH, viscosity started to increase at pH 4.5, reaching a maximum ~2 Pa*s at pH 5.0. At high pH, the viscosity started to increase around pH 8.0 and reached a maximum ~ 10 Pa*s at pH 9.5. Homogenate prepared from catfish showed similar

results (*59*) whereas Undeland and coworkers (*55*) observed similar viscosity peaks at low and high pH for herring light muscle homogenate.

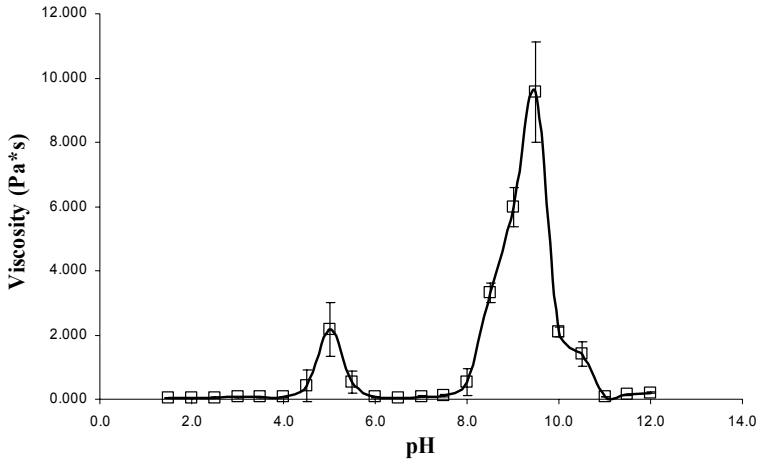


Figure 4-2. Shows viscosity (Pa*s) of tilapia light muscle homogenate at pH 1.5 to pH 12.0 in 0.5 increments. Viscosity was measured using oscillatory time sweep in an AR2000 Rheometer. Results are mean ± SD.

The large peak observed at pH 9.5 could be attributed to greater water binding capacities of proteins at pH 9.0 to 10.0 than at any other pH as a result of ionization of sulfhydryl and tyrosine residues (*30*). Interactions between hydrated residues are short range repulsive interactions that become stronger with increasingly hydrated molecules (*34*). This might contribute to increased hydrodynamic size of the proteins which in turn leads to an increase in viscosity.

Decreased water binding above pH 10 could be due partly to loss of positively charged ε-amino groups of lysyl residues and increased solubility of the proteins (*30*). Lower viscosity could also be contributed to large myofibrillar assemblies, which have a high hydrodynamic volume, but when they break up and the effective hydrodynamic volume significantly decreases. At pH 5.0 which is close to the isoelectric pH of the proteins a smaller peak was observed. Moving away from the isoelectric point increases

net charge and repulsive forces resulting in facilitation of the proteins to bind water and swell. Even just below to the isoelectric pH, proteins carry a net positive charge which results in repulsion and hydration of residues that could increase the hydrodynamic size of the proteins and cause an increase in viscosity (*30*). The drop in viscosity after pH 5.0 could be related to solubilization of the proteins which would result in lowering of viscosity. Figure 4-3 shows that at low pH, a peak in viscosity was observed just before a dramatic increase in solubility was observed. At high pH solubility was more gradually increasing with increasing pH and viscosity could not be directly related to increase in viscosity.

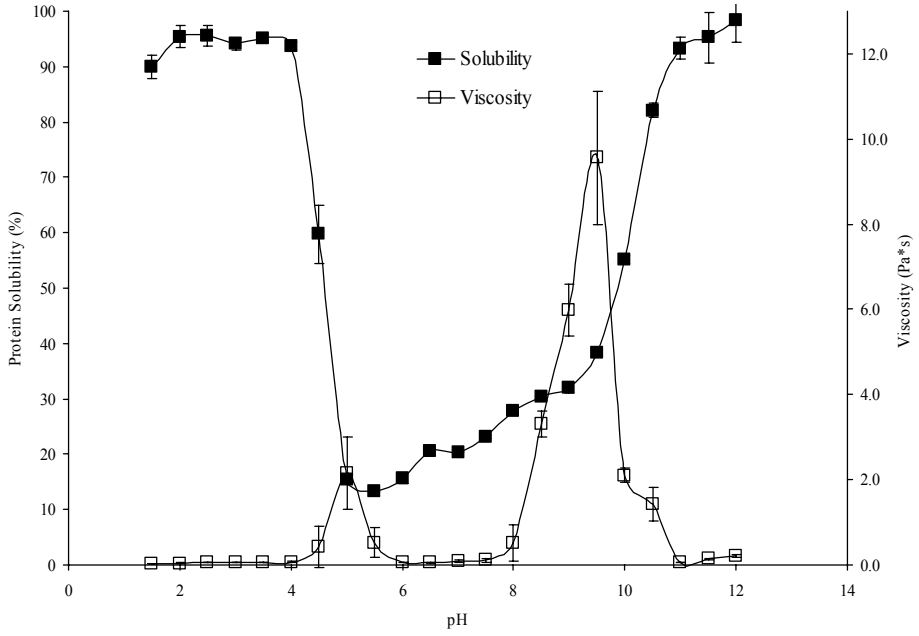


Figure 4-3. Shows solubility and viscosity curves previously demonstrated on the same graph. Results are mean ± SD.

CHAPTER 5
SOLUBILIZATION OF MUSCLE PROTEINS AT LOW AND HIGH PH BEFORE 1ST CENTRIFUGATION

Effect of Homogenization Times

It has been reported that different homogenization times may affect muscle protein solubility (*29*) which could in turn lead to variations in protein recovery. For this reason, effects of different homogenization times (60, 90, 120 sec) on protein solubility (%) were investigated at solubilization pHs of interest to the acid (pH 2.3 to 2.9) (Figure 5-1 A) and alkaline process (pH 10.8 to 11.4) (Figure 5-1 B). Overall, increasing the homogenization time did not have considerable effects on protein solubility. Longer homogenization times did not seem to result in lower solubility as Stefansson and Hultin (*29*) observed when homogenizing cod muscle for 120 sec. At low pH, using 90 sec at pH 2.3 led to significantly lower solubility ($p < 0.01$) compared to other pH values and homogenization times. For high pH, using 120 sec at pH 11.2 led to significantly higher protein solubility than was seen for other treatments ($p < 0.01$). The value obtained was above 100%, which could be in part be reflected by experimental error or deviation. Based on these results, further studies (solubility, viscosity and recovery) were performed by homogenizing the tilapia white muscle for 60 sec because of good stability in solubility and minimum foaming. To minimize the extent of protein denaturation due to shear force or temperature increase, which in turn leads to foaming (and less protein recovery), the tilapia muscle was homogenized in two steps of 30 sec at 4°C.

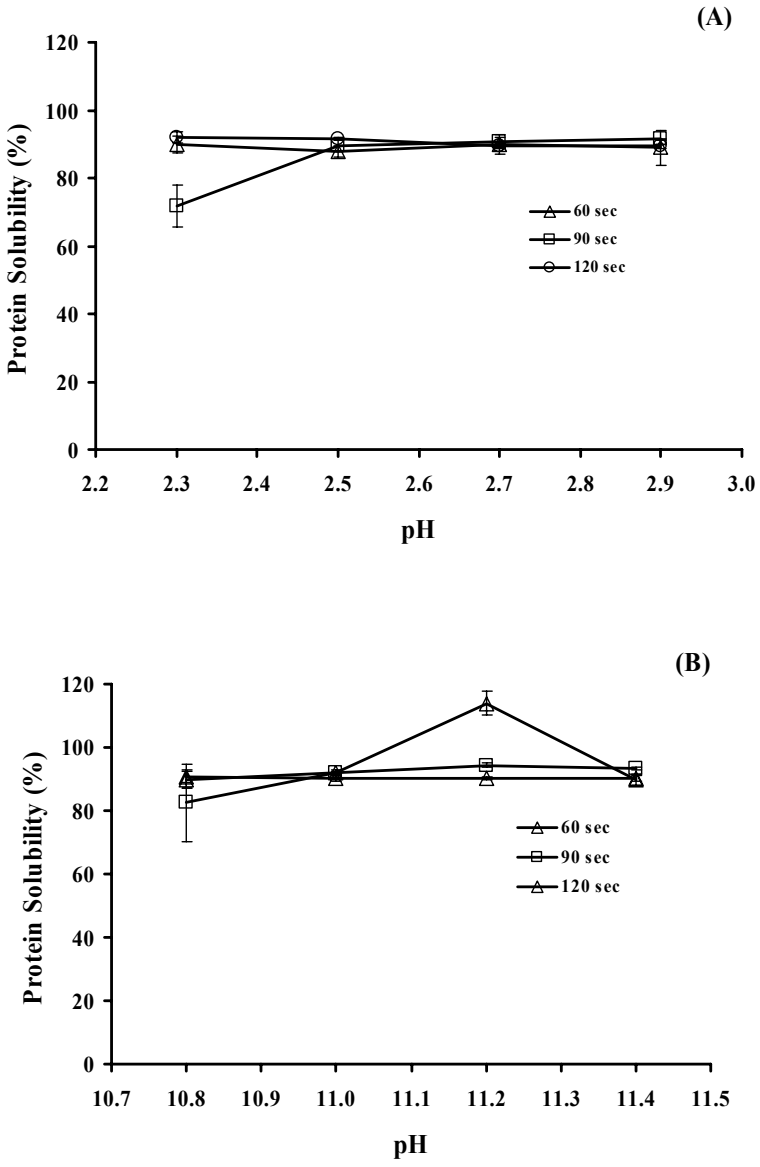


Figure 5-1. Effect of different homogenization times (60, 90 and 120 sec) on protein solubility (%) at low (A) and high (B) pH. The tilapia white muscle was homogenized at 4° C for 30 sec at a time with a 30 sec rest time in between. Results are mean ± SD.

Viscosity

Examination of viscosity for low and high solubilization pHs showed that at low pH viscosity was significantly higher ($p < 0.05$) for pH 2.9 compared to pH 2.3 and 2.5 (Figure 5-2). At high solubilization pH there was no significant difference ($p > 0.05$) in

viscosity (Figure 5-3). Lower viscosity was observed for certain high pH values over the range tested compared to viscosity at low pH. There was also more variation in viscosity at low pH compared to high pH. Adjustment to low pH (2.3 to 2.9) after storage of homogenate over night resulted in a dramatic lowering of viscosity, down to similar values as were seen for alkali solubilized protein (data not shown). This decrease could possibly have been attributed to limited hydrolysis which was observed at low pH (Figures 6-8 and 6-9, lanes 3 to 4).

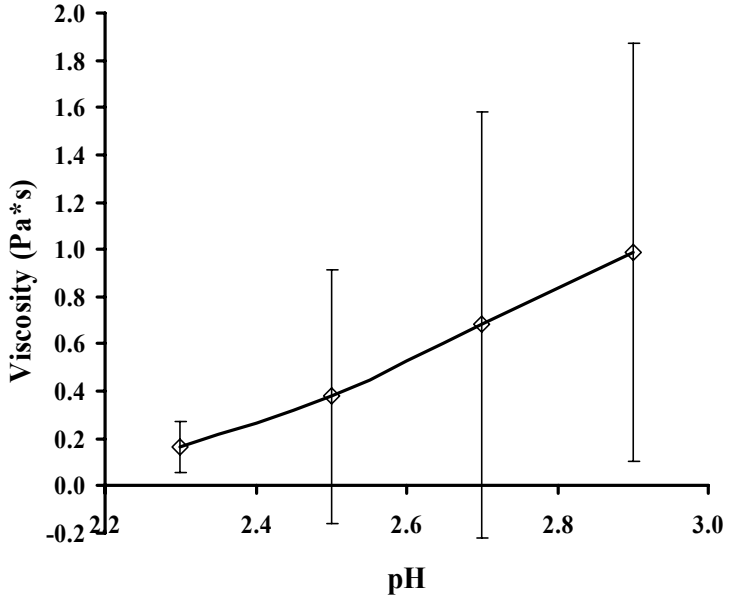


Figure 5-2. Shows viscosity (Pa*s) of tilapia homogenate at low solubilization pH (2.3 to 2.9) determined with oscillatory time sweep with controlled stress in an AR2000 Rheometer. Results are mean ± SD.

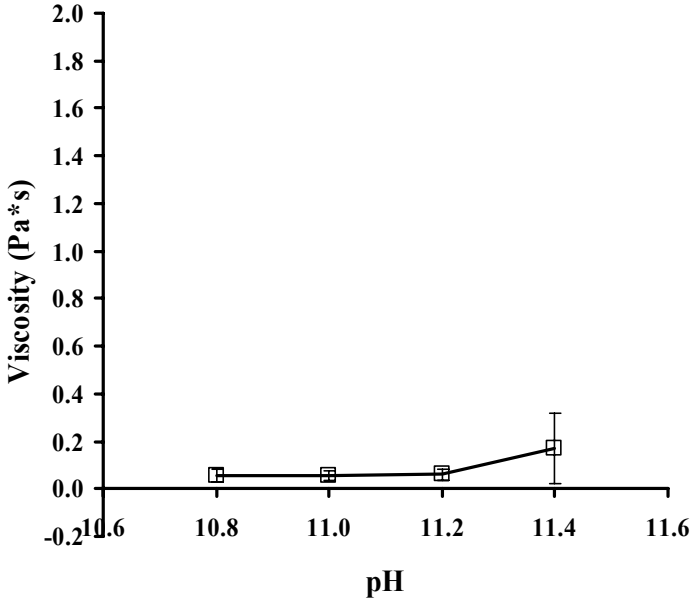


Figure 5-3. Shows viscosity (Pa*s) for tilapia homogenate at high solubilization pH (10.8-11.4) determined with oscillatory time sweep with controlled stress in an AR2000 Rheometer. Results are mean ± SD.

At extreme low and high pH the proteins are partially denatured. Partial denaturation of proteins can increase their hydrodynamic size and thus result in an increase in viscosity (*33*). It has been suggested that proteins at extreme low pH are more extensively denatured than proteins at extreme high pH (*49*) and work on herring light muscle homogenate (*55*) and cod myosin (*49*) supported that suggestion. Kristinsson and Hultin's work with myosin suggested that at pH 2.5 the myosin heavy chains were dissociated whereas at pH 11.0 the heavy chains were still associated, which led to a higher viscosity of myosin at low pH compared to high pH. Increased hydrodynamic volume of the myosin molecule at low pH could contribute to the high viscosity observed at low pH.

When a protein unfolds, hydrophobic groups buried in the interior of a protein are exposed to the water surrounding them. The water tries to minimize contact to the non-polar groups resulting in strong attractive forces between non-polar groups (*60*). The

proteins could form “aggregates” or other elongated structural assemblies and resulting in an increase in their hydrodynamic volume (*34*) resulting in higher viscosity at pH 2.9 compared to pH 2.3. With increasing charge these forces are reduced and the viscosity is lowered as was seen for pH 2.3.

The large variation in viscosity at low pH was interesting to note. When proteins are denatured at low or high pH they can take on many different structural states, depending pH and other solution conditions (*61*). These different states may thus have different viscosities. It has also been hypothesized that at low pH proteins may take on more numerous structural states than at high pH, and there may be rapid conversions between one state to another (*61*). This could be one explanation between the great variations seen in viscosity at low pH in contrast to high pH. Time might also contribute to the variability seen at low pH compared to high pH. Undeland and coworkers (*55*) showed that viscosity was reduced when acidified herring homogenate was stored on ice for up to 25 min.

More volume was needed of 2 M HCl than 2 M NaOH to adjust the pH. The chloride ion (1.81Å) has twice the radius of the sodium ion (0.98Å) and this size difference as well as larger volume could possibly contribute to increased volume of the proteins and therefore viscosity (*60*).

The trend towards decrease in viscosity at low pH with decreasing pH could be due to dilution of the solution when 2 M HCl is added. Diluting the homogenate may result in decreased interactions between hydrated protein molecules and their ability to absorb water and swell which in turn results in a decrease in viscosity (*33*).

Recovery of Soluble Proteins

The amount of proteins recovered at low and high pH was studied by comparing two acid solubilization pHs (2.5 and 2.9) and two alkali solubilization pHs (11.0 and 11.2). The basis for the selection of these solubilization pHs was as follows: pH 10.8 was very difficult to work with due to instability, pH 11.4 was considered too high due to amount of base needed to adjust the pH and possible deamination of the proteins (e.g. indicated by the smell). For the low pH solubilization pHs, pH 2.5 and 2.9 were selected because one of the most widely used pH in the acid aided process is pH 2.5 (*10;18*) and pH 2.9 has been used with good results in other labs (*62*), pH 2.3 was excluded due to very large quantities of acid needed to lower the pH (*55;56*).

In Figure 5-4, results are summarized and expressed as theoretical and actual protein recovery (%) after 1st centrifugation based on total protein in the homogenate. The difference between theoretical and actual recovery lies in difference in calculation and representation of the results. Theoretical protein recoveries are calculated based on the concentration of protein present in the supernatant after centrifugation compared to before centrifugation. On the other hand, actual recovery takes into account proteins that are lost in the separation process of soluble proteins from insoluble material, e.g. proteins lost in the fat layer and in the cheesecloth that covers the strainer used for separation. The bottom layer containing the insoluble material can be reprocessed to recover some of the proteins that are lost during separation especially when using low solubilization pH (*63*).

After the 1st centrifugation, actual recovery for alkali treated proteins was significantly higher ($p < 0.01$). Theoretical recovery of proteins was higher than actual protein recovery and ranged from 89% for pH 11.0 to 95% for pH 11.2. Actual recovery

ranged from 62% for pH 2.5 to 73% for pH 11.2. In regard to actual recovery, no significant difference was observed within the acid or alkali treated proteins. There was no significant difference ($p > 0.05$) in regard to theoretical recovery between low and high solubilization pH.

A difference between the acid and alkali treated proteins was observed using electrophoretic separation of the proteins. Hydrolysis of the myosin heavy chain (~205 kD) was observed of supernatants at pH 2.5 (Figure 5-8, lanes 3 to 4) and 2.9 (Figure 5-9, lanes 3 to 4) after 1st centrifugation. This was not seen for the supernatants recovered after 1st centrifugation for solubilization pHs 11.0 (Figure 5-10, lanes 3 to 4) and 11.2 (Figure 5-11, lanes 3 to 4). Apart from that similar polypeptides were recovered after 1st centrifugation using different solubilization pHs.

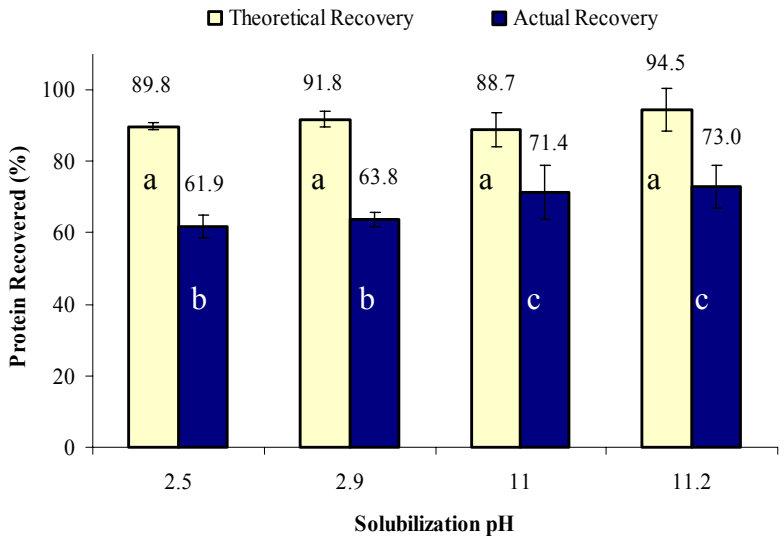


Figure 5-4. Theoretical and actual % protein recovery after 1st centrifugation for four different solubilization pHs (2.5, 2.9, 11.0, 11.2). Results are mean ±SD. Different letters indicate significant difference ($p < 0.05$) between treatments.

CHAPTER 6
PRECIPITATION OF MUSCLE PROTEINS

Solubility

Soluble proteins which are separated from neutral fat and insoluble material in 1st centrifugation are subjected to isoelectric precipitation in the acid and alkali processes to recover the proteins (*1*). Therefore, it is of great interest to investigate how different solubilization pHs respond to various precipitation pHs and to determine the combination which gives the highest recovery. Based on results from the solubility curve (Figure 3-1) where minimum solubility was observed at pH 5.0-6.0, four pHs (pH 5.1, 5.3, 5.5, and 5.7) were selected to precipitate the proteins.

Precipitation of proteins solubilized at low pH values resulted in significantly ($p < 0.01$) lower amount of soluble protein after 2nd centrifugation (Figure 6-1) compared to high solubilizing pH values. This is in agreement with results for catfish reported by Kristinsson and Demir (*18*). At high solubilization pH (11.0 and 11.2), the use of precipitation pH 5.5 and 5.7 was not significantly different ($p > 0.05$). Proteins precipitated at pH 5.1 contained more soluble protein after 2nd centrifugation for all treatments except for pH 11.0 probably because pH 5.1 is further away from the isoelectric point.

The large difference seen between low and high solubilization pHs in the precipitation is likely to be partly explained by different degree of denaturation of the proteins at low and high pH and then subsequently different degree of refolding as pH is readjusted to pH 5.1 to 5.7. A more extensively denatured protein has more hydrophobic

areas exposed and is better able to form more and stronger protein-protein interactions. Work with trout hemoglobin by Kristinsson and Hultin (*64*) demonstrated that the protein was more extensively denatured at low pH compared to high pH, and was also less able to refold on pH readjustment to 5.5 and 7, leaving more exposed hydrophobic groups. This led to substantially more protein aggregation for hemoglobin refolded from low pH compared to high pH, and thus less hemoglobin remained in solution after centrifugation, in accordance with that seen here.

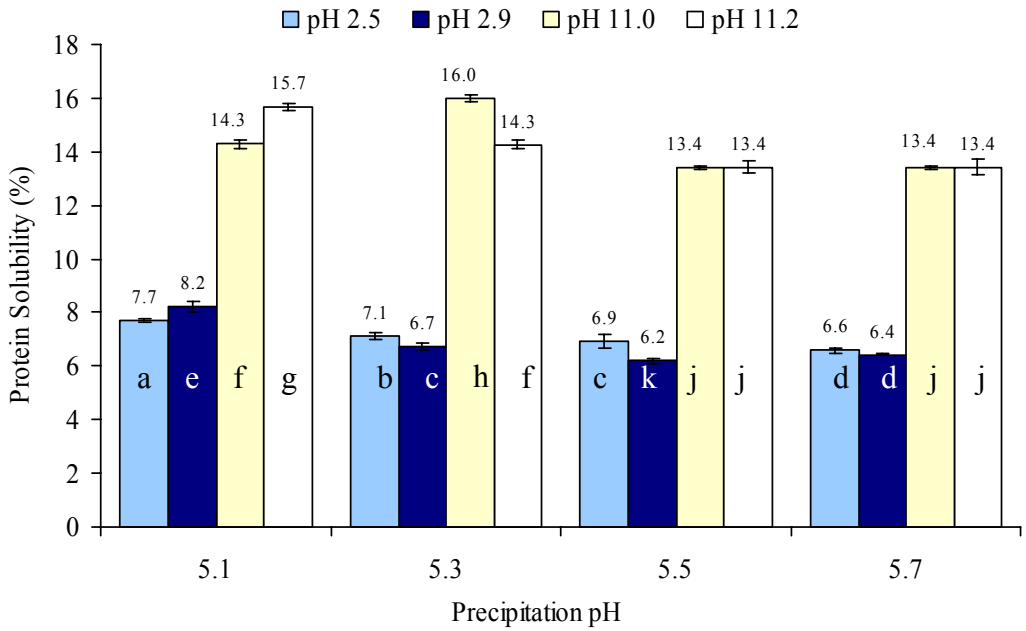


Figure 6-1. Soluble protein (%) in supernatant after the 2nd centrifugation, using four different precipitation pHs. Protein solubility was defined as the fraction of the total protein soluble after centrifugation, assuming no protein was lost. Results are mean ± SD. Different letters indicate significant difference ($p < 0.05$).

Viscosity

Differences in viscosity between low and high pH using various precipitation pHs before 2nd centrifugation were not as evident as was observed in solubility after precipitation (Figure 6-1). This indicated that the differences seen in solubility after

precipitation were not necessarily due to differences in viscosity. However, pH 2.9 did seem to give significantly higher viscosity at precipitation pHs 5.1 and 5.3 ($p < 0.05$). This difference did not reflect the results seen for precipitation solubility. Viscosity of precipitated proteins solubilized at pH 2.9 formed larger units than when solubilized at the other pH values, presumably due to different protein-protein interactions which resulted from unfolding and refolding for that pH treatment. The viscosity values after readjusting the proteins to pH 5.1 to 5.7 resulted in an enormous increase in viscosity values compared to native protein at similar pH values (Figure 3-2). The reason for this dramatic increase is probably due to the fact that the proteins have not refolded to their original state leading to increased interaction and aggregation, leading to an increase in hydrodynamic volume and therefore increase in viscosity. These changes were also observed in a study performed on acidified and alkalized sarcoplasmic proteins from herring white muscle did not show an increase in viscosity indicating that the myofibrillar proteins are probably responsible for this changes in viscosity (*55*). Then it can be concluded that the use of different solubilization pH (low vs. high) has more impact on protein conformation than the use of different precipitation pHs.

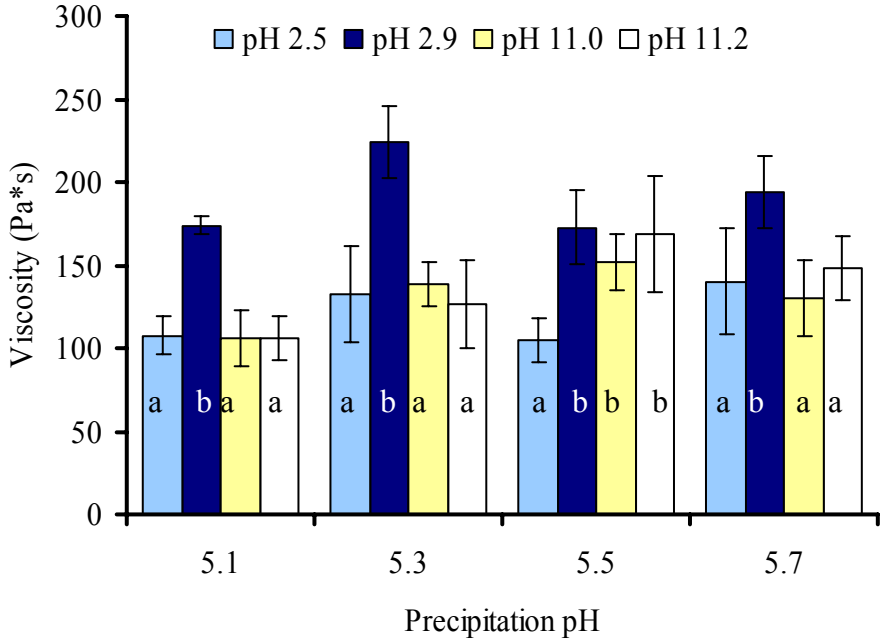


Figure 6-2. Viscosity, (Pa*s) of tilapia muscle proteins at different precipitation pH before the 2nd centrifugation. Tilapia white muscle proteins were solubilized using four different pHs (2.5, 2.9, 11.0, and 11.2) and precipitated using four different pHs (5.1, 5.3, 5.5, and 5.7). Results are mean ± SD. Different letters WITHIN each precipitation pH indicate significant difference ($p < 0.05$).

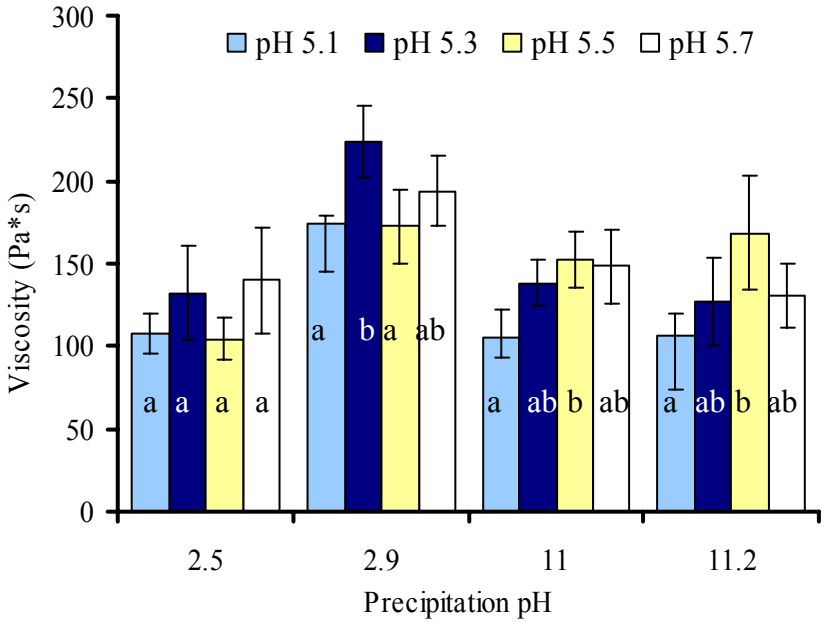


Figure 6-3. Shows the same as Figure 6-2, but in a different arrangement, categorized based on solubilization pH not precipitation pH. Results are mean ± SD. Different letters WITHIN each solubilization pH indicate significant difference ($p < 0.05$).

Protein Recovery Between 1st and 2nd Centrifugation

Protein recovery after 2nd centrifugation using different precipitation pHs was expressed as proteins recovered after 2nd centrifugation as a fraction of total soluble protein after 1st centrifugation. Theoretical and actual protein recovery (%) were calculated (Figure 6-4). Opposite to what was seen after 1st centrifugation, recoveries were higher for the acid treated proteins, or 92 to 94% whereas recovery for the alkali treated proteins ranged from 84 to 89%. These data are in direct agreement with the data on the amount of soluble proteins after precipitation. Where the proteins treated at low pH resulted in lower amount of soluble protein in the supernatant after 2nd centrifugation compared to proteins treated at high pH, indicating higher amount of protein recovered in the protein isolate. The reason for the higher level of precipitation can be explained as

before that the proteins adjusted to low pH were more extensively denatured and less refolded and thus had a higher level of aggregation. The use of different precipitation pH therefore does not seem to have impact on protein recoveries since similar percentages are observed for all precipitation pHs. This finding indicates that selecting a specific precipitation pH is not critical for tilapia muscle when using the acid and alkali processes.

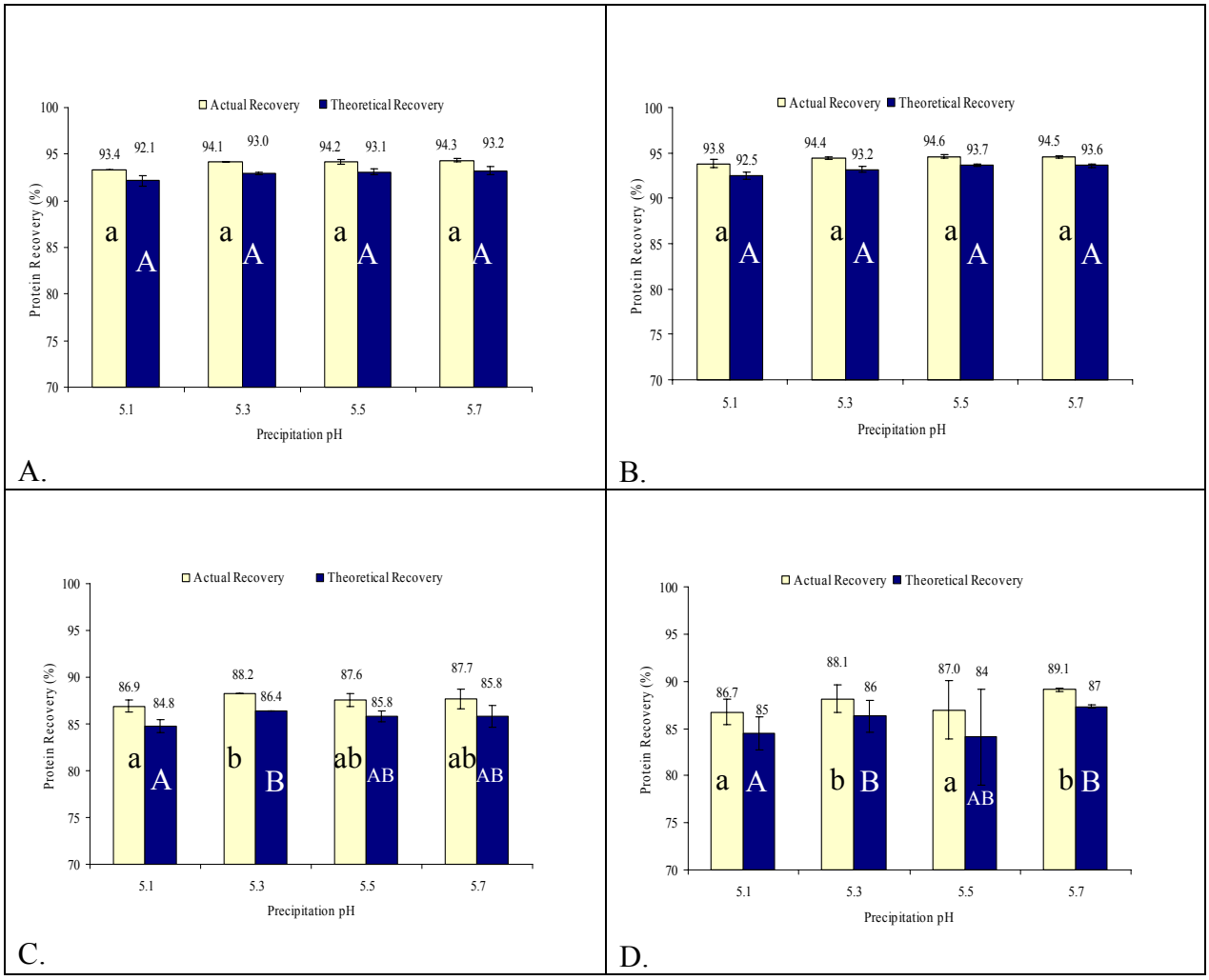


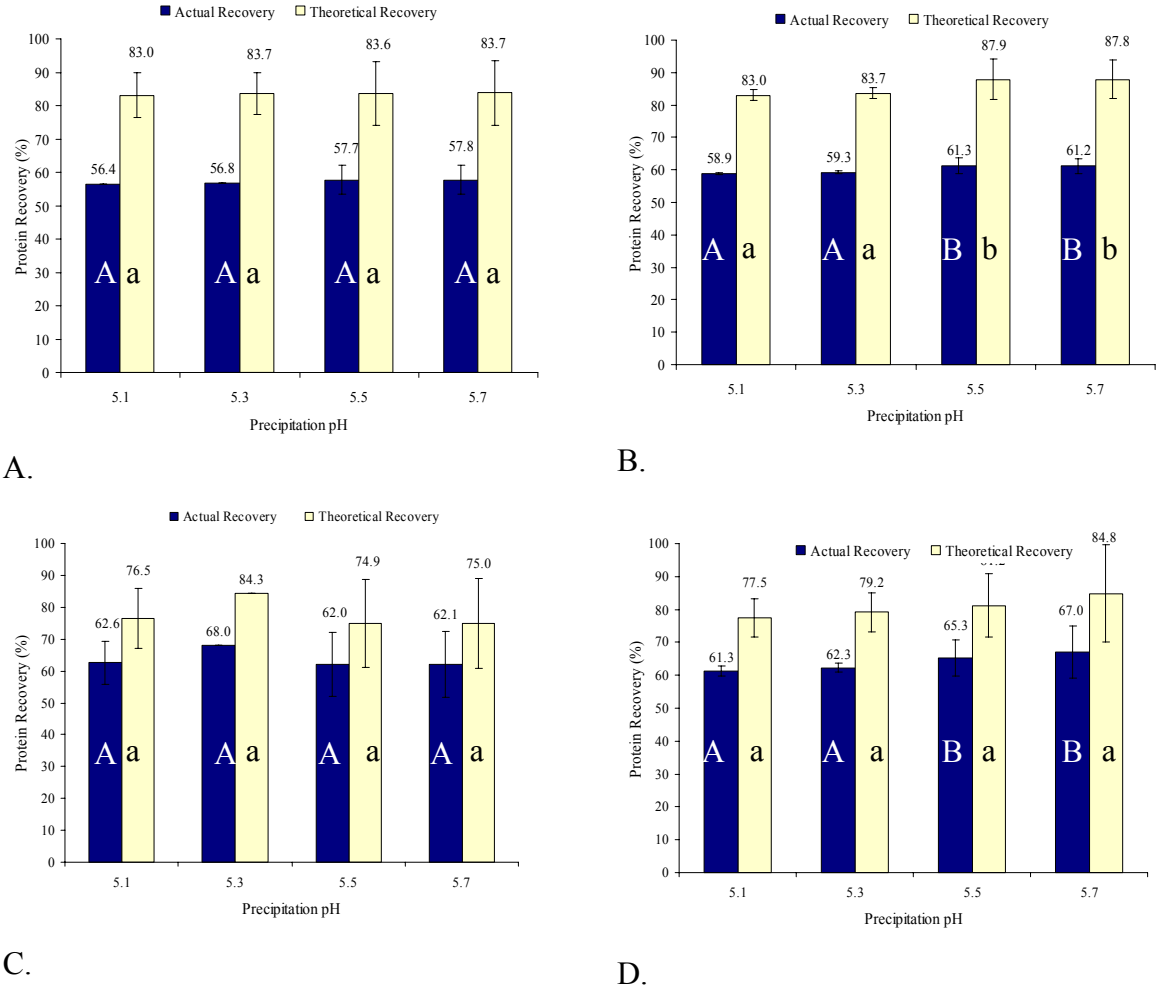
Figure 6-4. White muscle tilapia proteins were solubilized at pH 2.5 (A), 2.9 (B), 11.0 (C) and 11.2 (D) and precipitated at four different pHs (5.1, 5.3, 5.5, and 5.7). Theoretical and actual protein recovery (%) after 2nd centrifugation were expressed as the percentage of total soluble protein recovered after 1st centrifugation. Different capital letters indicate significant difference ($p < 0.05$) for actual recovery. Different small letters indicate a significant difference for theoretical recovery.

Total Protein Recovery

Protein recoveries through the whole process were determined for all treatments including recoveries from washed muscle which was used as a control. The washed muscle mimics the current industry standard of making surimi. Figure 6-55 summarizes the results and showed that actual recoveries were lower than theoretical recoveries, as would be expected. No one treatment gave statistically significantly (>0.05) better results than other treatments. Theoretical recoveries ranged from 83% to 88% for low solubilization pH but more variability was observed at high solubilization pH where it ranged from 75 to 85%. This was also observed for herring light muscle (*9*) as well as catfish, mackerel, mullet and croaker (*18*). Lower recoveries (theoretical) using the acidic treated proteins could be explained by larger amount of proteins lost in the 1st centrifugation. Kristinsson and Hultin (*8*) found that emulsification ability of pH-treated treated proteins was significantly improved compared to untreated proteins due to higher hydrophobicity. The acid treated proteins may thus have emulsified with lipids in the tilapia and therefore less was recovered on the first centrifugation.

Actual recoveries for high solubilization pH were on the other hand slightly higher when compared to acidified proteins, or ranging from 61 to 68% compared to 56 to 61%, respectively. There were however no statistically significant ($p > 0.05$) differences between low and high solubilization pHs. That could be explained by the fact that after 1st centrifugation more proteins are lost during the acidic treatment than alkali but after 2nd centrifugation more proteins are lost in the alkali treatment which evens out the difference. Protein recoveries from washed fish muscle were 65.1% which was similar to the results obtained for high solubilization pHs. Preparation of washed muscle involves

washing most of the sarcoplasmic proteins (*2*) possibly along with some of the myofibrillar proteins (*65*).



A. B.

C. D.

Figure 6-5. White muscle tilapia proteins were solubilized at pH 2.5 (A), 2.9 (B), 11.0 (C) and 11.2 (D) and precipitated at 4 different pHs 5.1, 5.3, 5.5 and 5.7. Theoretical and actual protein recovery (%) in final protein isolate was based on total protein in initial homogenate. Results are mean ± SD. Different capital letters indicate significant difference ($p < 0.05$) for actual recovery. Different small letters indicate a significant difference ($p < 0.05$) for theoretical recovery.

An SDS-PAGE analysis was performed on samples collected during washing of the muscle (Figure 6-6). The final washed muscle (lane 2) showed that the washed muscle mainly contained protein bands tentatively identified as myosin heavy chain or MHC (~205 kD), actin (~43 kD) and possibly some tropomyosin (35.5 kDa). During washing

of the muscle a large amount of actin was washed out a long with proteins with tentative weights; ~100 kDa, ~69 kDa, ~56 kDa (desmin), ~41 kDa (troponin T), ~38 kDa (tropomyosin-beta) and ~17 kDa (unidentified).

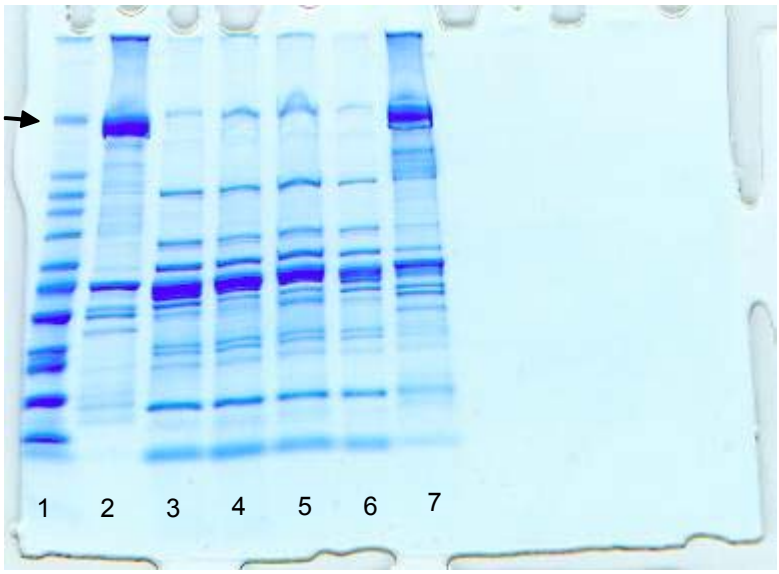


Figure 6-6. SDS page for washed tilapia muscle. Lane 1: sigma wide marker, lane 2: washed muscle after 3 washes, lane 3: 1st wash, lane 4: 2nd wash, lane 5: 3rd wash (0.2% NaCl), lane 6: skip, lane 7: initial sample.

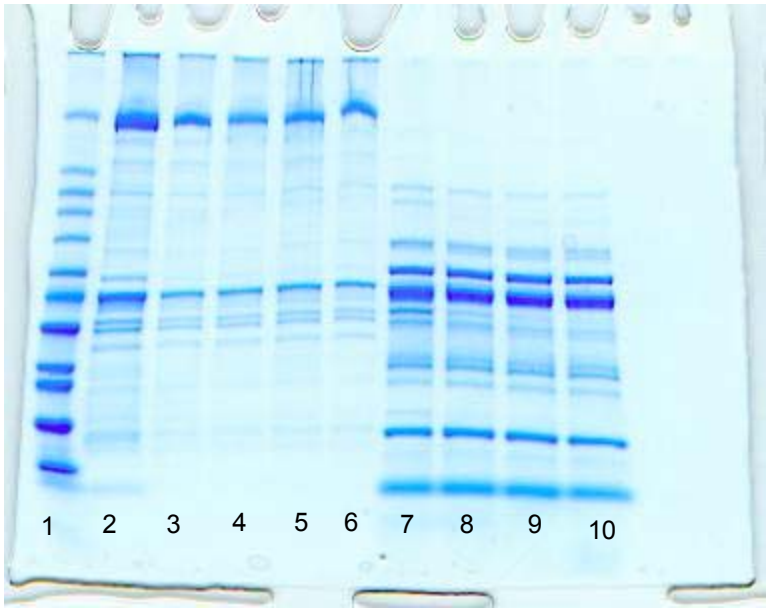


Figure 6-7. Precipitation of proteins at pH 5.1-5.7 without solubilization. Lane 1: Sigma wide marker, 2: Initial homogenate, lanes 3-6: Protein isolate precipitated at pHs 5.1, 5.3, 5.5, 5.7, lanes 5 to 7: supernatants after centrifugation at pHs 5.1, 5.3, 5.5, and 5.7.

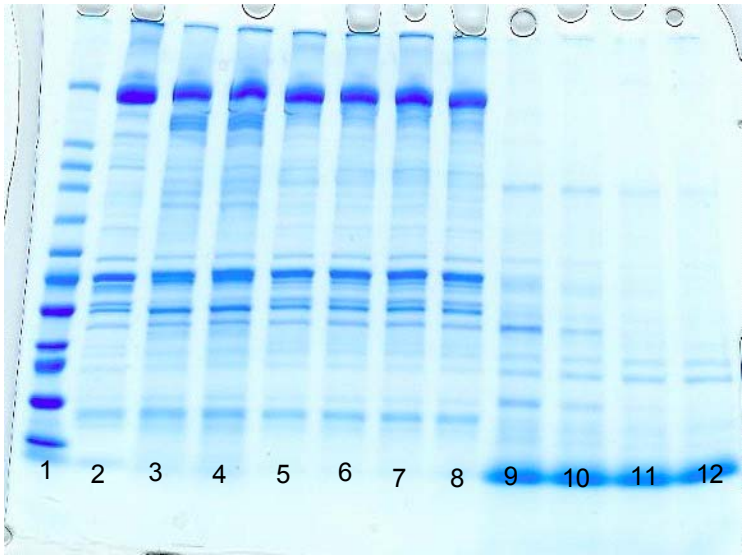


Figure 6-8. Lane 1: Wide Sigma Marker, lane 2: Initial homogenate at pH 2.5, lane 3: supernatant at pH 2.5 after 1st centrifugation, lane 4: supernatant at pH 2.5 after 1st centrifugation, lanes 6-8: isolates precipitated at pH 5.1, 5.3, 5.5 and 5.7, respectively. Lanes 9-12: Supernatants after precipitation and 2nd centrifugation at pH 5.1, 5.3, 5.5, 5.7.

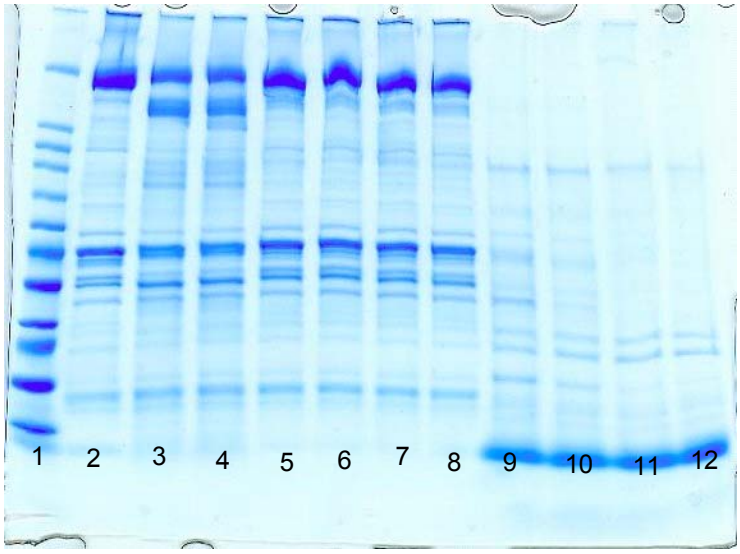


Figure 6-9. Lane 1: Wide Sigma Marker, lane 2: Initial homogenate at pH 2.9, lane 3: supernatant at pH 2.9 after 1st centrifugation (used for precipitation pH 5.1 & 5.3), lane 4: supernatant at pH 2.9 after 1st centrifugation (used for precipitation pH 5.5 & 5.7), lanes 6-8: isolates precipitated at pH 5.1, 5.3, 5.5 and 5.7, respectively. Lanes 9-12: Supernatants after precipitation and 2nd centrifugation at pH 5.1, 5.3, 5.5, 5.7.

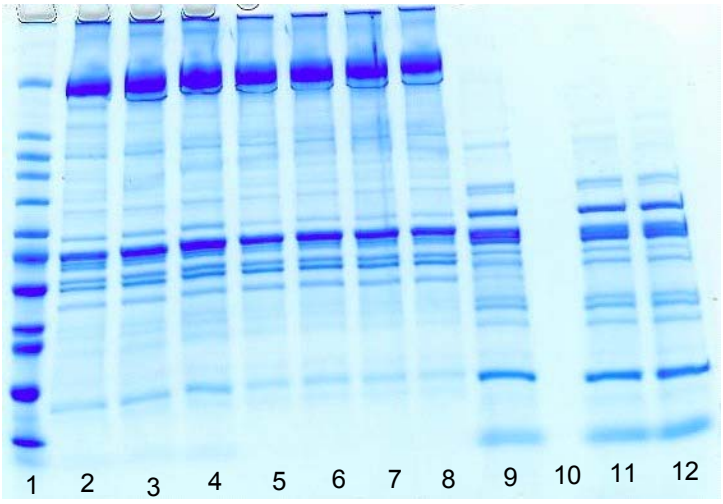


Figure 6-10. Lane 1: Wide Sigma Marker, lane 2: Initial homogenate at pH 11.0, lane 3: supernatant at pH 11.0 after 1st centrifugation (used for precipitation pH 5.1 & 5.3), lane 4: supernatant at pH 11.0 after 1st centrifugation (used for precipitation pH 5.5 and 5.7), lanes 6-8: isolates precipitated at pH 5.1, 5.3, 5.5, and 5.7, respectively. Lanes 9-12: Supernatants after precipitation and 2nd centrifugation at pH 5.1, 5.3, 5.5, and 5.7.

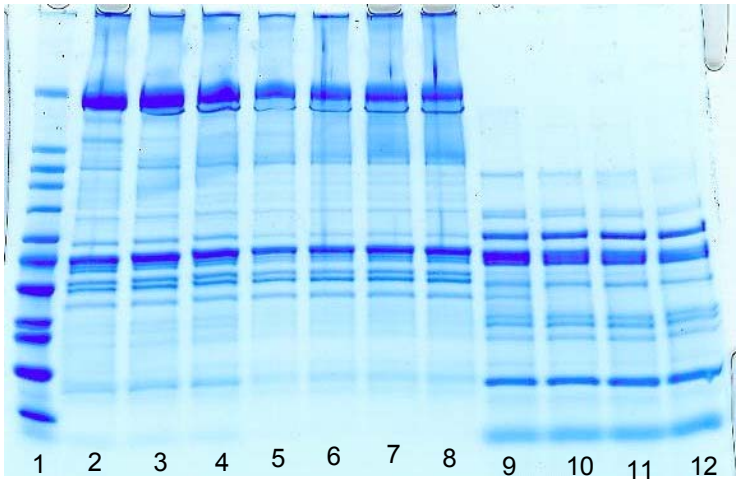


Figure 6-11. Lane 1: Wide Sigma Marker, lane 2: Initial homogenate at pH 11.2, lane 3: supernatant at pH 11.2 after 1st centrifugation (used for precipitation pH 5.1 and 5.3), lane 4: supernatant at pH 11.2 after 1st centrifugation (used for precipitation pH 5.5 and 5.7), lanes 6-8: isolates precipitated at pH 5.1, 5.3, 5.5, and 5.7, respectively. Lanes 9-12: Supernatants after precipitation and 2nd centrifugation at pH 5.1, 5.3, 5.5, and 5.7.

CHAPTER 7
GELATION

Gel forming ability of tilapia white muscle protein isolates prepared with the acid and alkali processes were determined and compared to washed tilapia white muscle (analog to conventional surimi processing). Gel characteristics were determined using torsion, rheology (viscoelastic changes), folding and water holding capacity. Gels were prepared with and without addition of 2% NaCl (w/w) at pH 7.1 to 7.2. These are common gelation conditions for fish muscle gels. Adjusting the pH of the protein isolates from pH 5.5 to slightly above neutrality leads to increases electrostatic repulsion between proteins, thus giving a more even distribution of proteins in the gel matrix resulting in good gel quality (*36;66*). The use of 2.0% NaCl (w/w) is commonly used in gelation since it partially solubilizes the muscle proteins. Previous work with washed tilapia muscle has shown higher strength and deformation of gels prepared with 2% NaCl (w/w) or higher compared to gels with 0.5 to 1.5% NaCl added (*24*). Recently, salt free muscle protein gels at pHs above neutrality have also been found to have good gelation properties (*8*), which made it of interest to study these conditions for the tilapia muscle proteins.

Torsion

In Figure 7-1, it can be seen that the shear stress (i.e., resistance to breakage) of gels prepared using 2% NaCl (w/w) was significantly higher ($p < 0.01$) than that seen for gels without added NaCl, except for gels prepared from washed muscle. For the gels with 2% added salt, the lowest shear stress, thus least resistance to breakage, was obtained for washed muscle gels (32.1 ± 4.3 kPa). Gels from isolates made using the pH 2.5, 11.0, and

11.2 treatments gave the highest shear stress values, ranging from 80 to 84 kPa. There was however no significant difference between these gels ($p > 0.05$). For the gels without added salt, the highest stress value (69.1 ± 12.0) was obtained for the isolates made using pH 11.0 treatment while the lowest stress value was obtained for the isolates made using pH 2.9 treatment and the washing procedure (30.4 ± 7.0 and 26.0 ± 2.0, respectively.

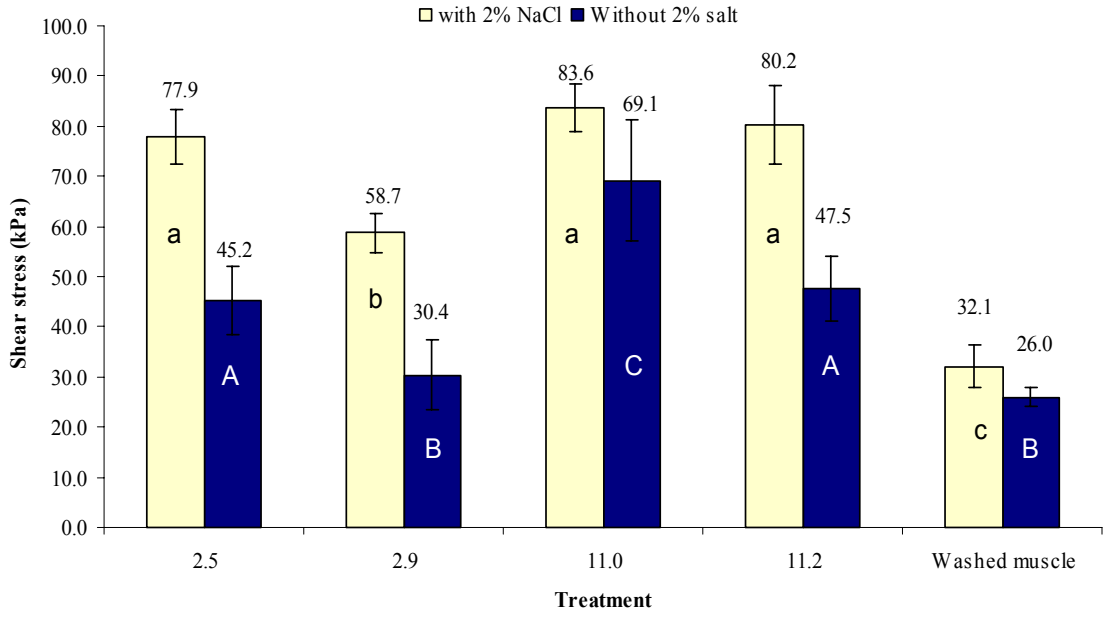


Figure 7-1. Shows shear stress values (kPa) of gels produced from white muscle proteins of tilapia. The use of low and high solubilization pH treatment was compared to a three cycle washing treatment (control). Gels were cooked in steel tubes at 80°C for 30 min. The gels were stored in a cold room at 4° C for 48 hours prior to testing with a Torsion Gelometer. Results are mean ± SD. Different capital letters indicate significant difference ($p < 0.01$) for treatments without 2% NaCl. Different small letters indicate a significant difference for treatments with 2% NaCl. For each treatment gels with 2% NaCl (w/w) had a significantly higher stress value, except for washed muscle.

Resistance of the gels to deformation or shear strain showed that the addition of 2% NaCl (w/w) resulted in significantly higher ($p < 0.01$) strain values for all treatments compared to gels without added NaCl (Figure 7-2). For samples without added salt, isolates made using pH 11.0 and 11.2 treatments gave significantly ($p < 0.05$) higher strain

values (1.6 ± 0.1 and 1.5 ± 0.2, respectively) compared acid treated proteins and washed muscle. On the other hand, isolates made using pH 2.9 and the washed muscle had the lowest shear strain values (1.1 ± 0.1 and 1.2 ± 0.1, respectively), which is in line with the results seen for shear stress. If isolates made using low pH treatment are compared it is clear that pH 2.5 treatment with and without 2% NaCl (w/w) gave significantly higher strain values than pH 2.9 treatment, thus being significantly more elastic.

The strain values obtained for the treatments were lower or borderline for the ideal shear strain values for surimi from fish muscle, which are expected to be between 2 and 3. However, the reason for lower values could be explained by the absence of cryoprotectants and setting which are used in surimi production. Bakir et al. (*67*) observed that gels prepared from Atlantic mackerel and Bluefish without using cryoprotectants resulted in significantly lower strain values possibly due to the preventive action towards the proteins during heating which could result in improved gelation ability.

Shear stress and strain values were significantly higher for samples with 2% added NaCl (w/w). Salt is believed to improve gelation ability and water retention by solubilizing myofibrillar proteins (*2*). Addition of salt above 300 mM (~1%) solubilizes the myofibrillar proteins by breaking up the interactions between myosin in the thick filament and actin in the thin filaments, along with other cytoskeletal proteins (*68*). In a concentrated gel paste however, the osmotic pressure is probably too high to obtain completed solubilization of the myofibrillar proteins. Uniform dispersion of partially solubilized proteins is likely to be more important in a gel paste. Addition of salt is also

believed to contribute to a more elastic gel by dispersing the proteins more evenly, which is a consequence of partial or full solubilization.

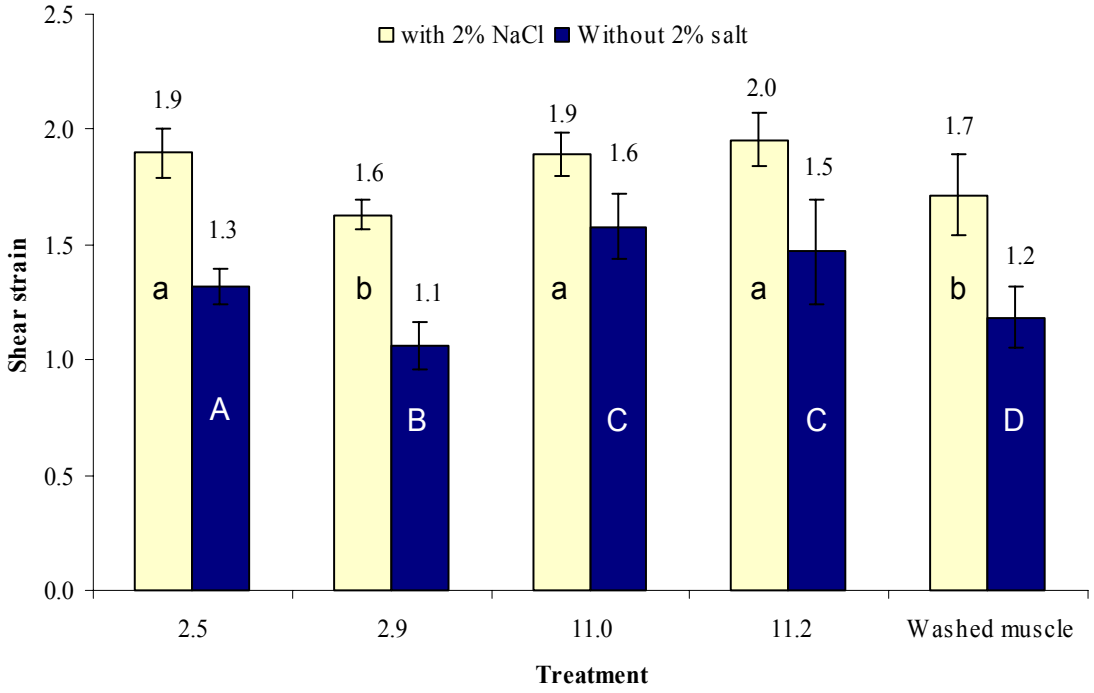


Figure 7-2. Shows shear strain values of gels produced from white muscle of tilapia . The use of low and high solubilization pH treatment was compared to a three cycle washing treatment (control). Gels were cooked in steel tubes at 80°C for 30°min. The gels were stored in a cold room at 4°C for 48 hours prior to testing with a Torsion Gelometer. Results are mean ± SD. Different capital letters indicate significant difference ($p < 0.01$) for treatments without 2% NaCl. Different small letters indicate a significant difference for treatments with 2% NaCl. For each treatment gels with 2% NaCl (w/w) had a significantly higher strain value when compared to gels without 2% NaCl (w/w).

It was interesting to note the difference between the two low-pH treatments. A difference in gel performance between different low pH treatments has been seen with other species. A study performed by Kim et al. (*56*) on Pacific whiting showed that deformation (mm) of gels (contained 1.5% beef plasma protein) using a puncture test were higher for proteins treated at pH 2.0 compared to pH 3.0. This was in part

explained by the authors by increased hydrophobicity of the proteins at low pH. Davenport and coworkers (*69*) also found a significant difference in gel forming ability among three different low pH treatments which could on the other hand not be explained by hydrophobicity changes or changes in protein conformation using tryptophan as a structural probe.

The pH 2.9 treatment in particular resulted in the formation of weak gels. During isoelectric precipitation of proteins solubilized at pH 2.9 it was observed that they seemed to form larger aggregates compared to pH 2.5 and pH 11.0. Larger aggregates do not form as ordered three dimensional structures and thus form a weaker gel. To many protein-protein interactions might result in an hard and inelastic gel whereas to many protein-water interactions might result in a soft and fragile gel (*63*). Hydrolysis of the myosin heavy chain (Figures 6-8 and 6-9) was observed for low pH, which could in part explain the reduced gel forming ability of the protein isolates made with acid treatment. This is in agreement with results by Undeland et al. (*9*) who observed significantly higher stress values for gels prepared from alkali processed isolates (with cryoprotectants and 2% NaCl (w/w)) from herring light muscle compared to acid produced isolates. The authors suggested that reduced amount of myosin heavy chain (possibly due to proteolysis) contributed to lower stress values at acidic conditions. It is interesting to note however that the pH 2.5 treatment with tilapia proteins was better than the pH 2.9 treatment. It is possible the proteases in question were more active at pH 2.9 than pH 2.5. Differences in protein conformation between the two treatments may also be causing the difference, as discussed above.

It is also worth noting that the washed muscle, acid isolates and alkali isolates had different protein composition, which may contribute to the different gel forming ability of these treatments (Figures 6-6 to 6-11). Interestingly the alkali process and the washing process removed similar proteins but had significantly different gel forming abilities. The very low stress values obtained for the washed muscle compared to the acid and alkali treated proteins indicates that conformational differences contribute to these differences.

Folding

The fold test is a common test used in the surimi industry for a quick evaluation of gel quality. The fold test was performed on 3 mm slices of gels which were subjected to the strain and stress tests mentioned before. All treatments with 2% added NaCl exhibited excellent folding ability and received the highest score available, or 5 (Table 7-1). Gels prepared using alkali treated proteins without 2% NaCl (w/w) exhibited excellent folding ability and were double folded without breaking. On the other hand, the salt free gels prepared using acid treated proteins and washed muscle performed very poorly. The lowest folding score was obtained for gels prepared from isolates made with pH 2.9 treatment which split in two during the first fold (score 1). Gels prepared from washed muscle and pH 2.5 cracked without splitting during the first fold and received a score of 2.

Table 7-1. Quality of protein gels as assessed by the fold test. Gel quality was estimated by folding approximately 3 mm thick gel slices by hand at room temperature and grading the quality using a five point system., 5: No crack occurs even if folded in four, 4: No crack when folded in two but forms a crack when folded in four, 3: No crack when folded in two but splits when folded in four, 2: Cracks when folded in two, 1: Splits when folded in two. Results are mean ± SD. Different letters within each column indicate significant difference ($p < 0.05$). Different numbers within each row indicate significant difference ($p < 0.05$).

Added NaCl	2.5	2.9	11	11.2	Washed Muscle
2%	$5.0 \pm 0.0^{a, 1}$	$5.0 \pm 0.0^{a, 1}$	$5.0 \pm 0.0^{a, 1}$	$5.0 \pm 0.0^{a, 1}$	$5.0 \pm 0.0^{a, 1}$
0%	$2.0 \pm 1.5^{b, 1}$	$1.0 \pm 0.0^{b, 2}$	$5.0 \pm 0.0^{a, 3}$	$5.0 \pm 0.0^{a, 3}$	$2.0 \pm 1.2^{b, 1}$

Rheology

Small strain oscillatory rheological testing was used to follow changes in viscoelastic properties of gels during heating and cooling. Initial and final storage modulus (G') during gelation was determined for all treatments in two separate experiments (replicate 1 and 2). The results for initial G' for both replicates are summarized in Figure 7-3. Due to large variation in data obtained for final G' between the replicates, the results are represented in two separate bar graphs (Figure 7-4 and Figure 7-5).

All treatments exhibited higher initial G' (Pa) in the absence of NaCl (Figure 7-3) compared to samples with 2% NaCl (w/w) added. A higher G' translates to a more rigid system. The protein paste was at pH 7.1 to 7.2 which would give the muscle proteins a substantial negative charge (*70*). This negative charge creates strong repulsion forces between the proteins, creating more space for water to enter, and a more expanded and rigid system. When 2% NaCl (w/w) is added the ions screen some of these repulsive forces bringing the proteins closer together and decreasing the space available for water to enter (*46*) and therefore decrease hydration and make the system less expanded. This

explains the higher initial G' for samples without added salt. There were some differences in initial G˝ between treatments. For example, the washed muscle showed highest value among the samples that contained salt. This is likely due to more structure in the washed muscle, since some of the myofibrils would still be intact for that system.

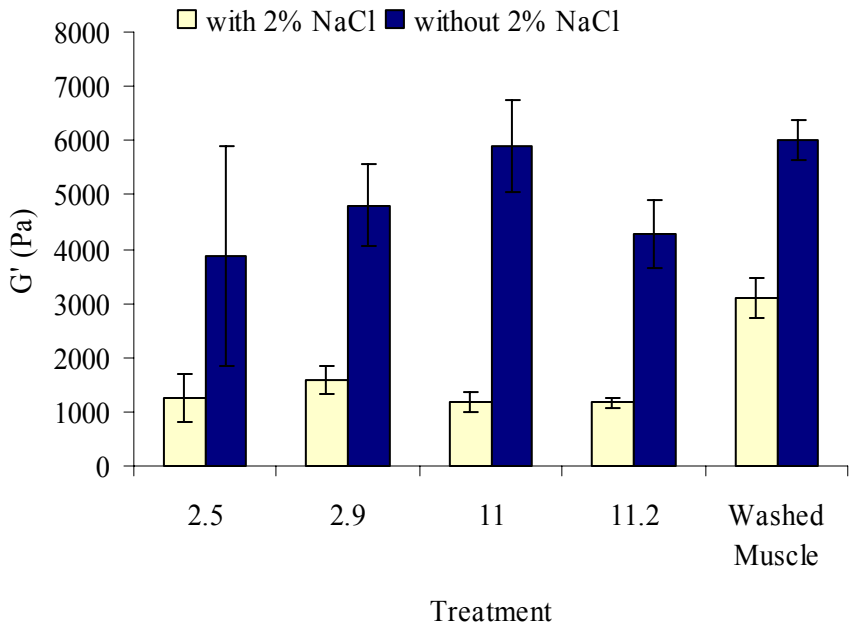


Figure 7-3. Storage modulus (G˝) of protein pastes at 5°C before gelation. Results are mean ± SD.

The final G' represents samples that have undergone thermally induced gelation along with setting on cooling (Figure 7-4 and Figure 7-5). The results for final G' showed that the acid treated samples exhibited better gelling ability in replicate 2 than in replicate 1, indicating significant variations, which could be attributed to the procedures used, the isolate or the raw material. There is however evidence that the acid process may lead to larger variations in gelation when using small strain oscillatory testing

compared to the alkali process (*20*). The reason for this is unknown, but is hypothesized to be due to unstable structural protein conformations that can form at low pH, thus leading to different refolded structures at pH 5.5, where the proteins are precipitated. Although variations were large, the results indicate that the proteins from the alkali process have the ability to form stronger gels in both replicates compared to the proteins from the acid process, which partly agrees with the torsion results.

In contrast to the torsion results where samples containing 2% NaCl (w/w) exhibited better gelling ability, samples without salt seemed to form stronger gels, especially in replicate 2, except for the isolates made with the pH 2.9 treatment. The small scale oscillartory testing and the torsion testing are not necessarily expected to go hand in hand, since they are different tests done at different protein concentrations. The small scale oscillatory testing gives more insight into the gel forming mechanism and protein-protein interaction potential of the muscle proteins at lower concentrations, while the torsion test measures gel strength and quality at high protein concentration. It is interesting to note the higher G' for the isolates from the alkaline process, suggesting it has substantially more protein-protein interactions and higher gel forming potential at lower protein concentrations than higher concentrations compared to the isolates from acid process which performed poorer at lower concentrations compared to higher concentrations. The rheology results also indicated that the washed muscle performs better relative to the alkali isolates, at low compared to high protein concentrations.

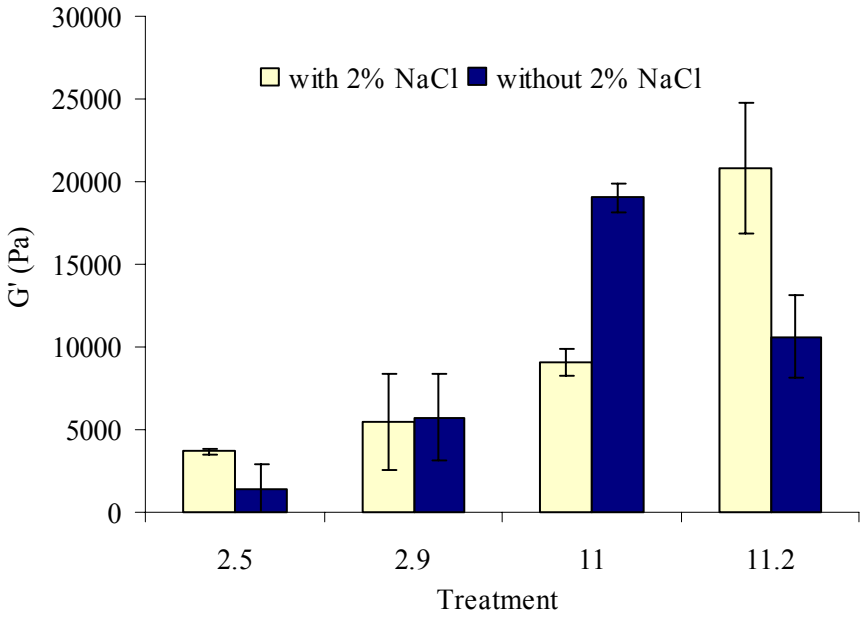


Figure 7-4. Storage modulus (G´) of protein pastes at 5°C after gelation. Data represent the first replicate. The final G' for gels was obtained by heating the protein samples from 5 to 80 °C followed by cooling from 80 to 5°C at a rate of 2°C/min using a small strain oscillatory procedure. Results are mean ± SD.

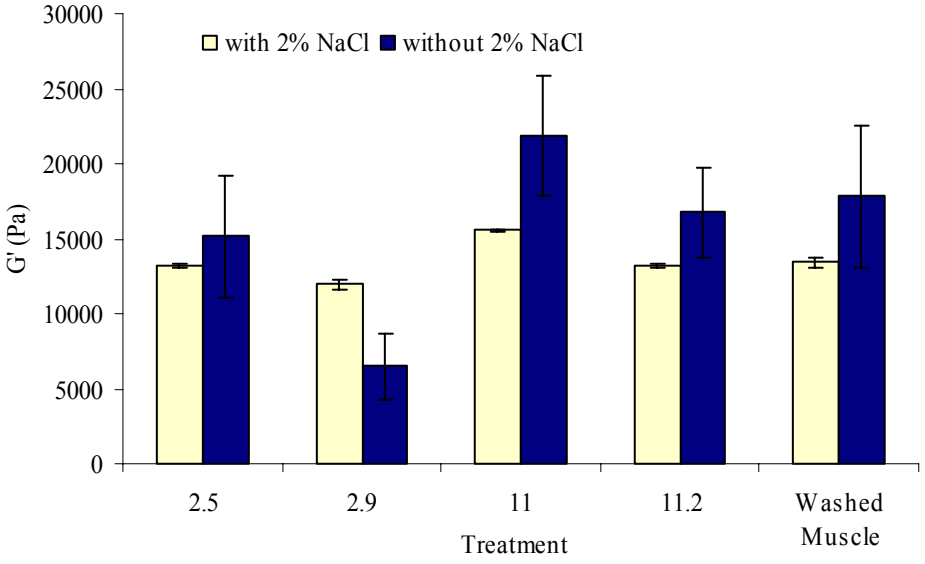


Figure 7-5. Storage modulus (G′) of protein pastes at 5°C after gelation. Data represent the second replicate. The final G' for gels was obtained by heating the protein samples from 5 to 80 °C followed by cooling from 80 to 5 °C at a rate of 2°C/min using a small strain oscillatory procedure. Results are mean ± SD

It was of interest to observe the changes in G' during heating and cooling for the acid and alkali treated proteins and washed tilapia white muscle to gain an insight into possible differences in gel forming mechanisms between the samples (Figures 7-6 to 7-15).

Washed tilapia muscle with 2% (w/w) added NaCl (Figure 7-6) had a stable G', from 5 to 38°C, where it started to increase until peaking at approximately 44°C and then declining down to a minimum at 53°C. The G' then rose steadily until 80°C was reached. A similar curve for heating was observed by Klesk and coworkers (*37*) who studied the effect of state of rigor on the gel-forming ability of washed tilapia muscle. The increase up to 44°C has been attributed to cross-linking of myosin and the drop in

G' after that to denaturation of light meromyosin, leading to increased fluidity. The second increase in G´ has been attributed to the formation of permanent cross-linked myosin filaments. A rapid increase in G' was observed during cooling of the gel indicating formation of a firm gel structure

Myofibrillar proteins, especially myosin and actin are believed to be largely responsible for gelation. Therefore, gelation of myosin, actin and their complex actomyosin has been the subject of many studies. For myosin, the heavy chain is believed to be the main subunit involved in the gelation. The role of the light chain seems to be dependendent on ionic strength where at high ionic strength they are not very important. However, at low ionic strength, removal of the light chains the rigidity of myosin gels was substantially lowered (*2*). Results from these studies have been used to try to elucidate gelation mechanism of complex mixture of myofibrillar proteins (*2*). The increase in G' that was observed for the washed muscle on heating is attributed to dissociation of light chains from the heavy chains resulting in conformational changes to the molecule. Cross-linking of myosin is also believed to be responsible for the increase in G' since it forms a strong elastic network. The subsequent drop is postulated to relate to dissociation of the actin-myosin complex and unfolding of the alpha-helix portion of the myosin rod (*35*). This transformation leads to a large increase in fluidity and might disrupt some protein networks that have already formed (*35*). All these structural changes lead to rapid aggregation and formation of gel networks resulting in a steady increase in G' (*2*).

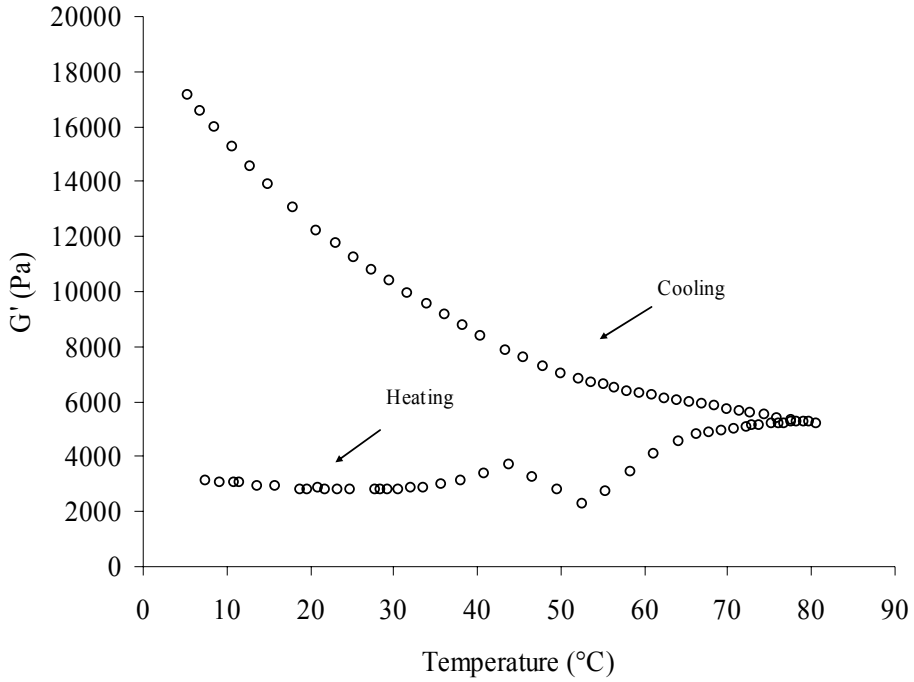


Figure 7-6. Example of a typical rheogram obtained for washed tilapia muscle with 2% NaCl (w/w) that shows storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

Acid treated proteins with 2% NaCl (w/w) behaved quite differently from the washed muscle. The pH 2.5 treated proteins showed a continuous increase in G' which started around 36°C and leveled off at approximately 65°C (Figure 7-7). A similar pattern was observed for the heating phase of the pH 2.9 treatment (Figure 7-8). The onset of gelation was thus at a significantly lower temperature that that seen for washed tilapia muscle. This could be due to a more unfolded structure of the acid treated proteins, thus requiring a lower temperature to further unfold and interact on heating. The same

was seen for cod muscle proteins by Kristinsson and Hultin (*8*). The large difference observed between replicates of the acid treated proteins has been discussed in the previous section and maybe attributed to unstable protein structures at low pH leading to different refolded structures which can affect the gelation properties.

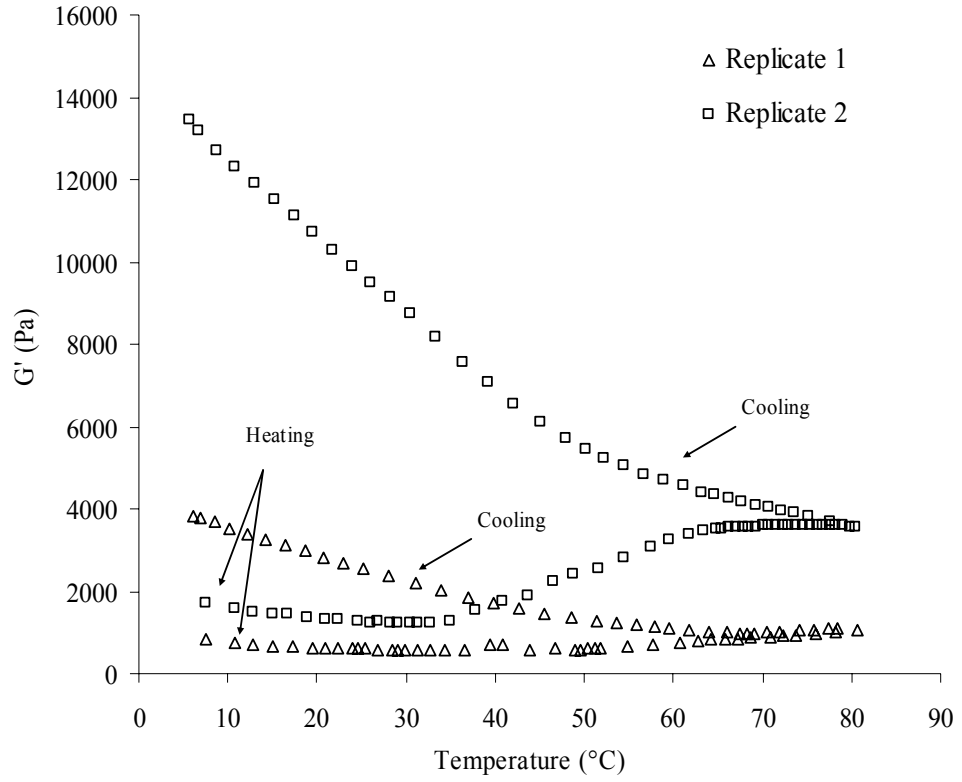


Figure 7-7. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 2.5 with 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

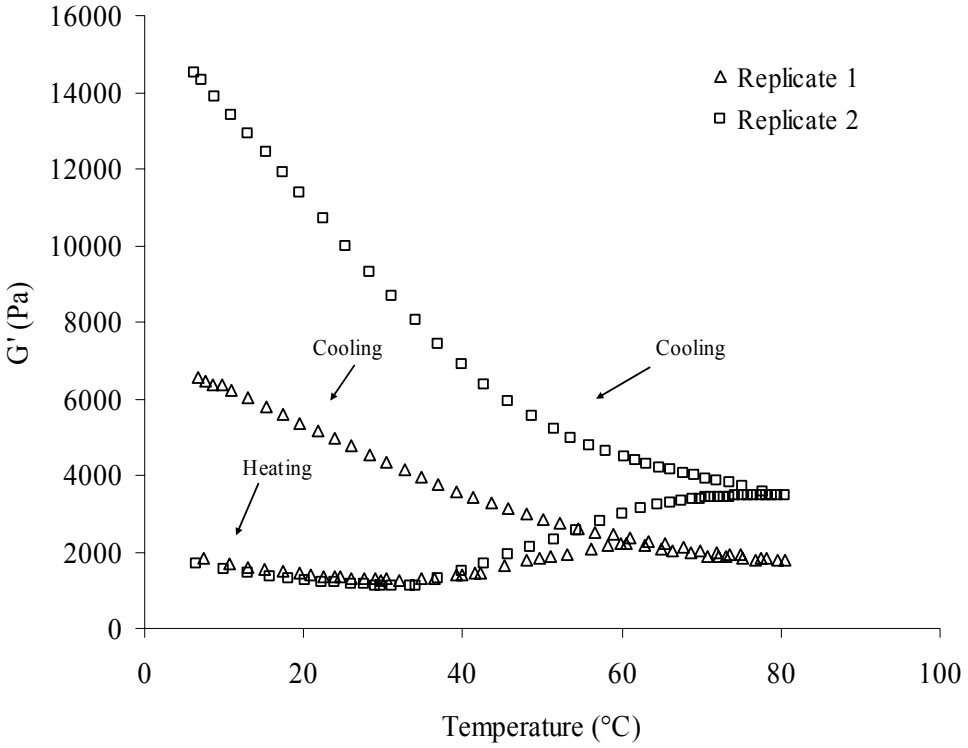


Figure 7-8. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 2.9 with 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

The alkali treated proteins showed a similar viscoelastic curve on heating as the acid treated proteins. The G' started to gradually increase around 40°C but as presented previously had a larger final G' indicating the formation of a firmer gel compared to the acid treated proteins (Figure 7-9 and Figure 7-10). The slightly higher point of gelation for the alkali treated proteins compared to acid treated proteins suggested differences in conformation. The higher onset temperature for the alkali treated proteins may suggest that they are more refolded than the acid treated proteins. The protein composition

differences between the two isolates might also partly account for the differences in temperature sensitivity.

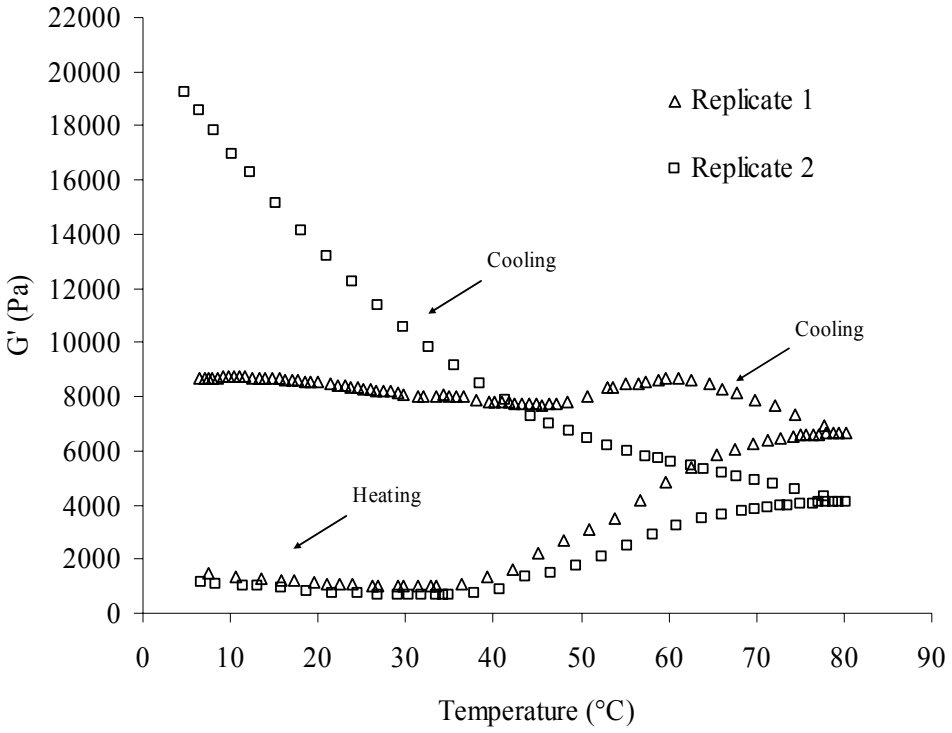


Figure 7-9. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 11.0 with 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

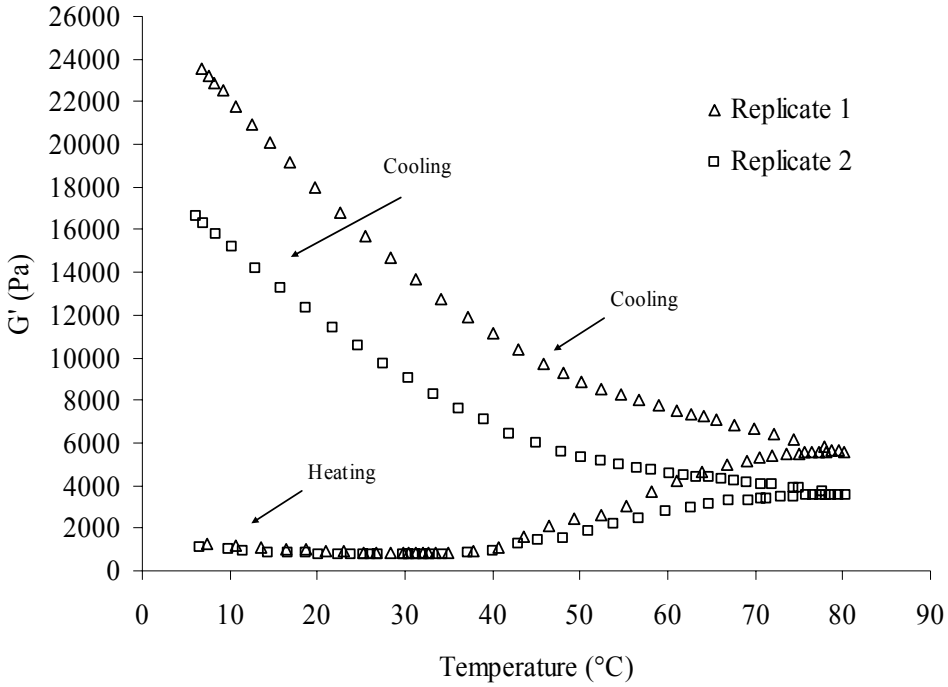


Figure 7-10. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 11.2 with 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) were adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

The acid and alkali treated proteins with added 2% NaCl (w/w) did not show the same transitions as were observed for washed muscle possibly due to denaturation of the muscle proteins during processing. It has been found that major changes occur with myosin on acid and alkali treatment (*8*). It was for example found that acid treatment led to complete dissociation of cod myosin while alkaline treatment only led to the dissociation of the light chains from the myosin head. As a result the proteins had different viscoelastic behavior on heating and cooling compared to a washed cod muscle

(*8*). It was believed that the absence of light chains led to protein aggregation and cross-linking at lower temperatures due to a more exposed myosin head group. Yongsawatdigul and Park (*71*) also observed that acid and alkali treated proteins from rockfish were denatured during treatment while still retaining ability to form gels. The lower gelation ability of the acid-treated proteins compared to the alkali treated could be due to different conformational changes; partly because of loss of myosin heavy chain during processing, or because of unfavorable conformation of the proteins during acidic treatments (e.g., too many hydrophobic groups, leading to bigger aggregates and a less ordered gel). Another explanation for the poor gelling ability of the acid-treated proteins could be the presence of denatured sarcoplasmic proteins, many which were retained in the acid process but not in the alkali and washed process. Crynen and Kristinsson demonstrated with catfish proteins that the acid process not only negatively affect the myofibrillar proteins but also lead to changes in the sarcoplasmic proteins that, when mixed with the myofibrillar proteins, have a very detrimental effect on gelation.

Washed muscle without 2% added NaCl (Figure 7-11) exhibited different viscoelastic behavior on heating compared to the washed muscle containing salt. Initial G' was higher and decreased with increasing temperature until it reached a minimum at 46°C, which was 7°C lower than the minimum observed for washed muscle with 2% NaCl (w/w). This decrease suggests that protein complexes may have dissociated instead of forming cross-links as in the system with salt. A steady increase, similar to the one seen for the proteins in salt, was then observed with increasing heating excluding a small bump at approximately 56°C. The increase in G' on cooling was similar for both washed muscle in the presence and absence of salt.

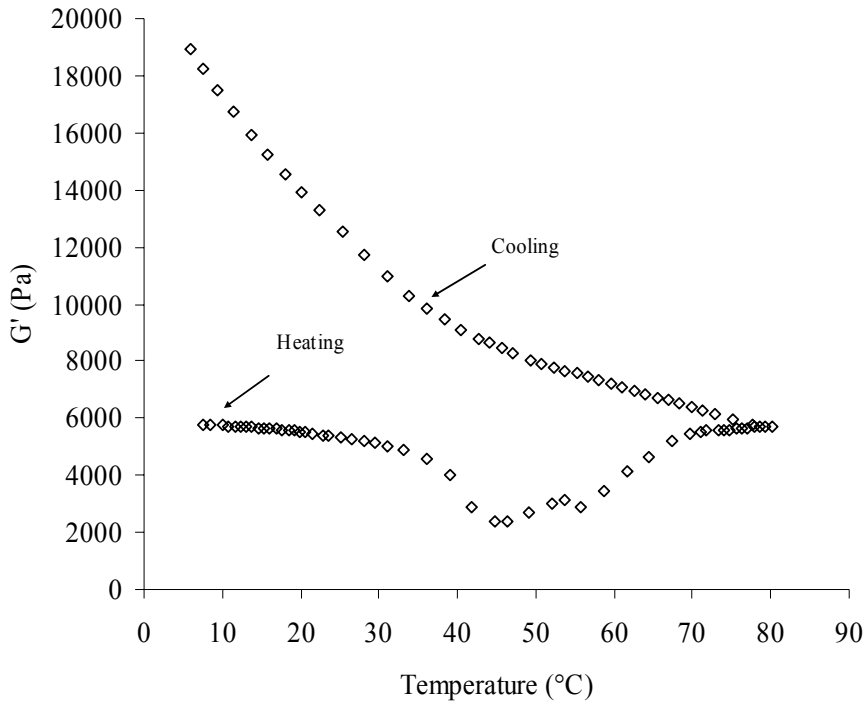


Figure 7-11. An example of a typical rheogram obtained for washed tilapia muscle without 2% NaCl. (w/w) that shows storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) were adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

When the viscoelastic curves for samples without added NaCl were compared it to samples with 2% NaCl (w/w) they were quite different and resembled the curves for washed tilapia muscle. The acid treated proteins showed a slight decrease in G' on heating until they reached approximately 40°C, where the G' dropped down to a minimum at 47°C. The G' then increased again and leveled off at higher temperatures. Similar to the samples containing salt, the acid treated proteins without added salt showed large variability and only one curve from both replicates was usable for the isolates made

with the pH 2.5 treatment (Figure 7-12). Protein isolates made with the pH 2.9 treatment showed a very similar trend, however with a lower final G' (Figure 7-13). The alkali treated proteins without added salt showed the same trend as the acid treated proteins except for a higher final G' and less variability (Figure 7-14 and Figure 7-15).

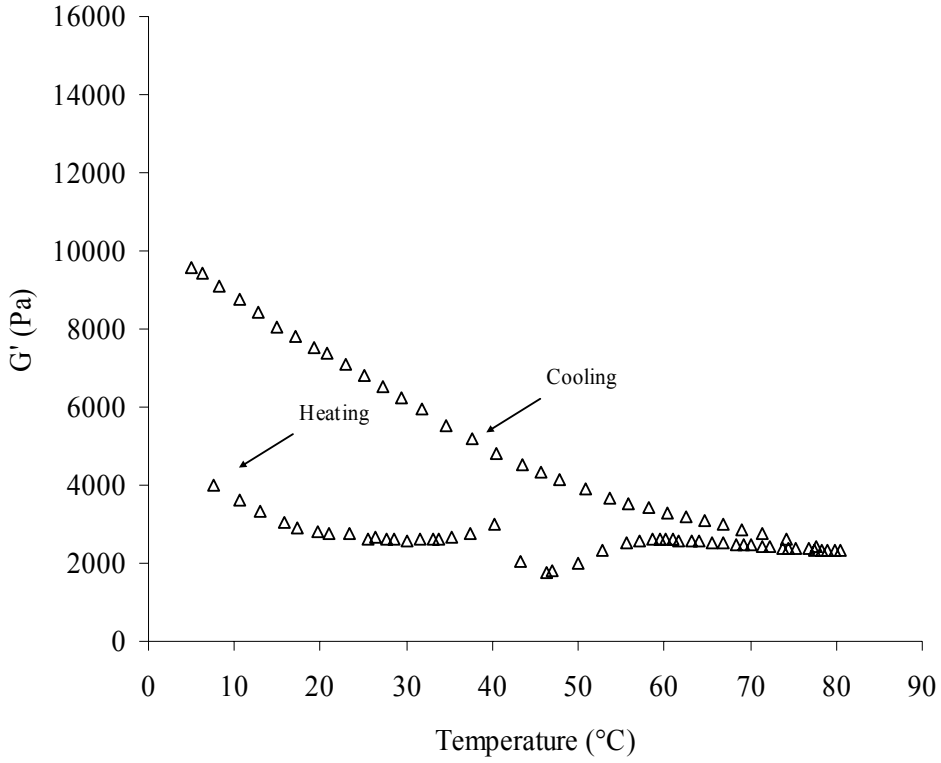


Figure 7-12. Examples of typical rheograms from obtained for proteins treated at pH 2.5 without 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

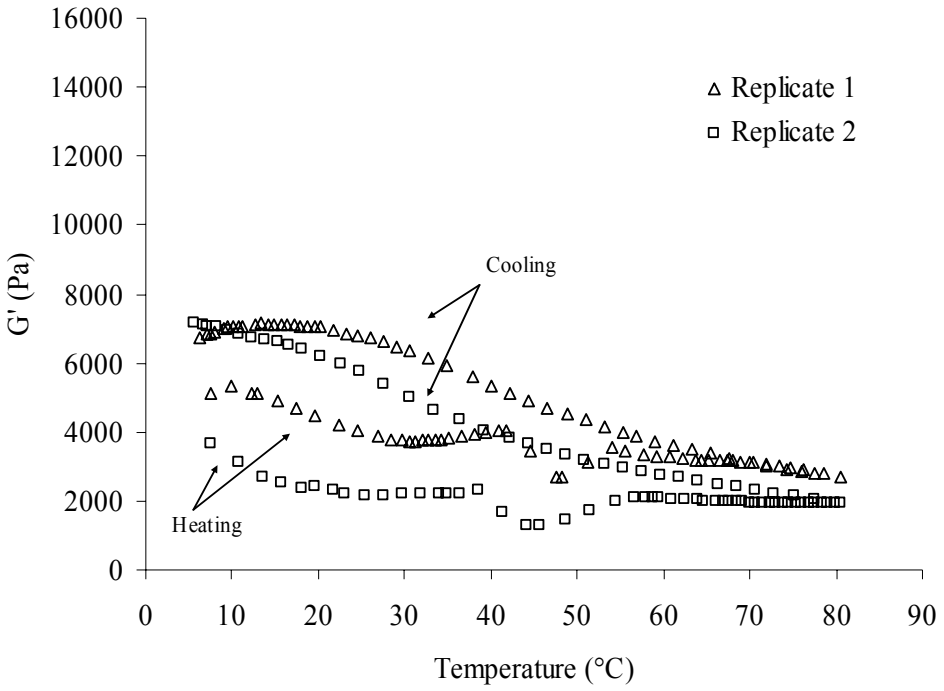


Figure 7-13. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 2.9 without 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

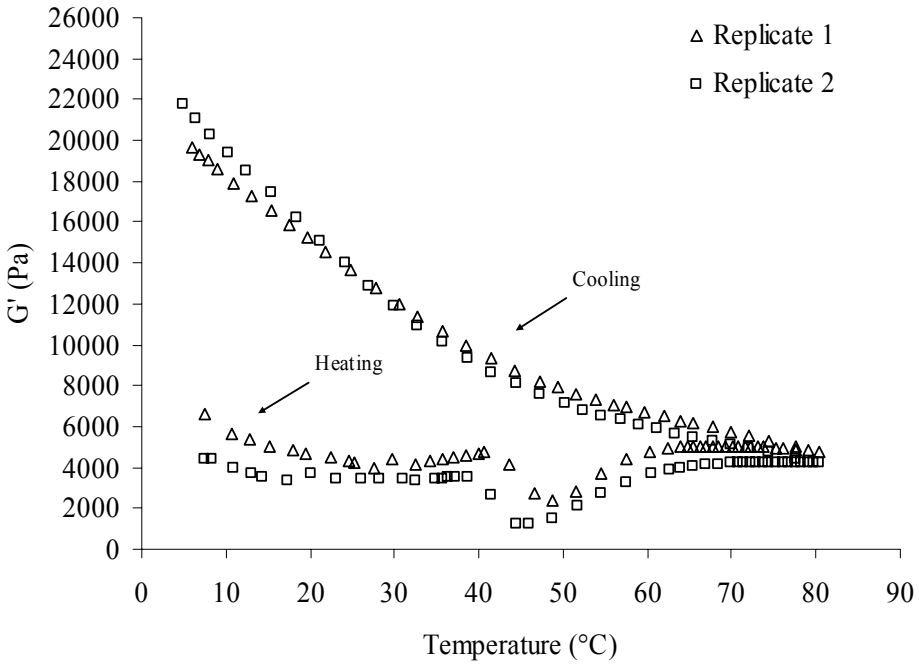


Figure 7-14. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 11.0 without 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

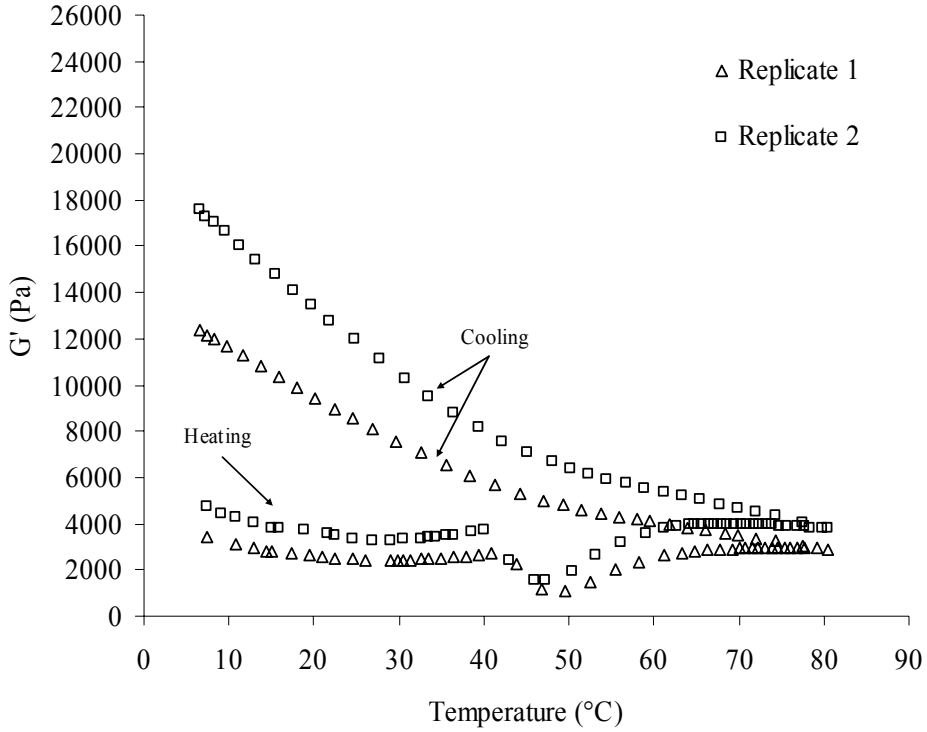


Figure 7-15. Examples of typical rheograms from two independent experiments (replicate 1 and 2) obtained for proteins treated at pH 11.2 without 2% NaCl (w/w). The rheograms show storage modulus (G') during heating at 5 to 80°C followed by cooling from 80 to 5°C at 2°C/min. Samples (~10% protein (w/w)) adjusted to pH 7.1 to 7.2 were allowed to sit for 30 min on ice before heating.

Not many gelation studies have been performed with fish proteins in the absence of salt, in part due to the long held believe that salt was necessary for gelation. The results here show that gels can form in the absence of salt, and higher final G´ values were in most cases seen in the absence of salt. The absence of salt leads to more electrostatic repulsion between the proteins at pH 7.1 to 7.2 compared to samples with salt, which may explain the higher G'. The onset of thermal gelation was also different for samples in the absence of salt. For example the rheological curve of washed muscle without salt

exhibited a similar pattern reaching a minimum 7°C lower than compared with salt. Another observation was that the rheology curves for acid and alkali treated proteins without salt exhibited all similar drop in G' around 40°C. This drop could be explained by a breakup of protein aggregates with higher temperatures, and the subsequent increase in G' due to proteins denaturing and forming permanent cross-links at higher temperatures. Washed muscle consists mainly of myofibrillar proteins because much of the sarcoplasmic proteins are removed during the washing step (*12*). The acid-produced protein isolate contained a sizable amount of sarcoplasmic proteins, while the alkali isolate had significantly less of the sarcoplasmic proteins. The types of proteins recovered therefore does not seem to explain this similar behavior of the acid and alkali isolates on heating. The above emphasizes that the differences between the two systems are far from trivial and more investigation is needed to understand the underlying mechanisms for gelation in salt and no salt.

Water holding Capacity

Water Holding Capacity on Cooking

The same gels which were tested with the Torsion Gelometer were analyzed for water loss upon cooking (Figure 7-16). All treatments gave gels with good water-holding properties. Addition of 2% NaCl (w/w) significantly reduced ($p < 0.05$) water loss for protein isolates made with the pH 2.9 and pH 11.0 treatment compared to other treatments. Most water loss or ~1% was observed for acidified proteins at pH 2.9 without the addition of 2% NaCl (w/w) which is still quite good. A study performed by Feng and Hultin (*36*) on washed chicken muscle protein at pH 7.0 with 0.15M NaCl resulted in 1.7% loss of water during cooking. Another study done by Kristinsson and

Hultin (*46*) on chicken breast muscle showed that samples without salt lost more water on cooking compared to samples containing salt.

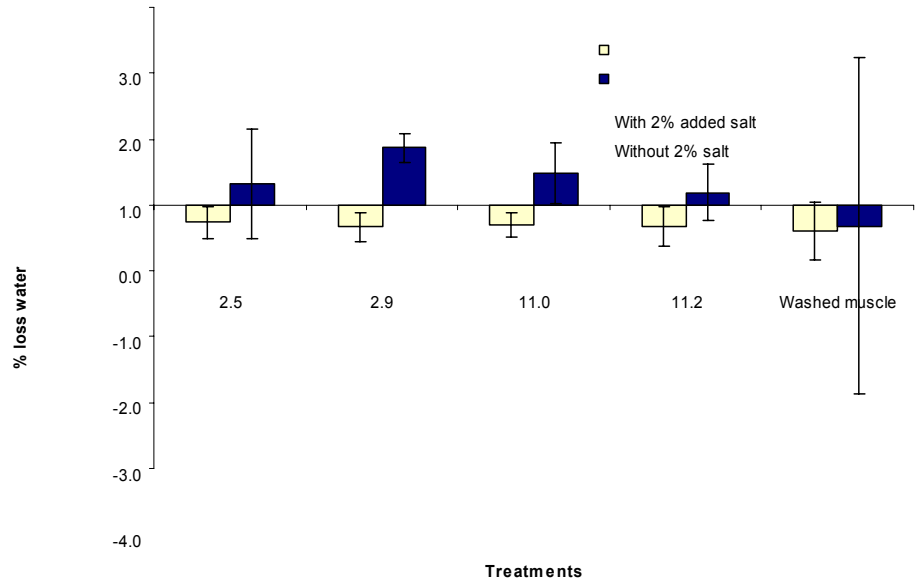


Figure 7-16. Water loss (%) of tilapia muscle protein pastes on cooking at 80°C for 30 min. Results are mean ± SD.

Water Holding Capacity on Pressing

The same connection between salt concentration and water holding capacity was seen for the gels when they were subjected to a press test (i.e., gels without 2% salt lost more water than those containing salt, except for gels from the washed muscle where no difference was seen). Again, the gel prepared from proteins treated at pH 2.9 lost most water on pressing than any other treatment. The gel without added salt from the other pH-treatments had similar water loss. The gel from the washed muscle without added salt lost the least amount of water upon pressing. A smaller difference between treatments was seen for samples containing 2% NaCl (w/w).

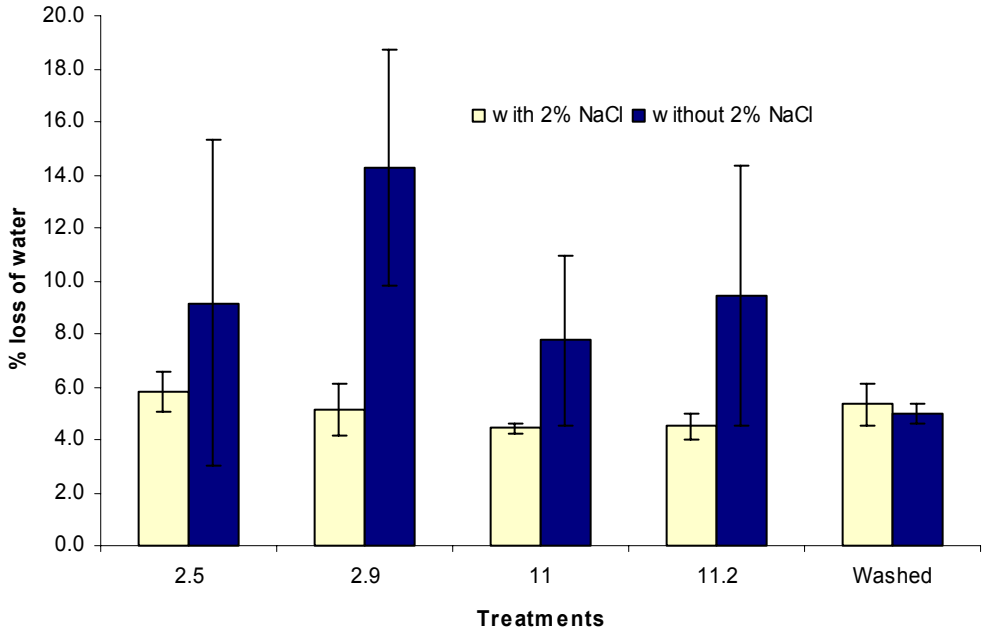


Figure 7-17. Water loss (%) of heat induced tilapia muscle protein gel with and without 2% NaCl under pressure (3kg) pressing. Results are mean ± SD.

Gel structure is believed to greatly influence the ability of a gel to hold water. In a well ordered gel, movement of water might be restricted compared to a less ordered structure (*45*). This is partly due to strong capillary forces within the ordered structure and also high gel pressure if water is being held due to strong repulsive forces between negative charges in the protein matrix (*46*). Between pH 7.1 and 7.2 there are strong repulsive forces between muscle proteins and these repulsive forces strengthen even more in the absence of salt (*46*). The water-holding results agree with the gelation results, in that the weaker and more brittle gels had lower water-holding abililty. Formation of localized aggregates is believed to contribute to lower gel forming ability (*36*) and a correlation between a gel elasticity and water holding capacity has been seen for chicken breast gels (*46*). For example the gels from the pH 2.9 treatment had the poorest gel

forming ability (especially in the absence of salt), thus having a poorer gel structure, and also had the least ability to hold water than the other treatments. The salt free gels in general had poorer gel forming ability than the gels with salt, and these did also have worse water-holding ability. The addition of salt to the proteins thus appears to have led to a better ordered structure than the absence of salt. The salt is able to partially solubilize the muscle proteins, and this could have been important for the formation of a ordered structure since proteins would have been dispersed better prior to gelation.

SH-Content

Changes in free sulfhydryl (SH) groups and disulfide bonds (S-S) are often associated with gelation of muscle proteins. It is also known that SH groups become more reactive at the high pH values representative of the alkaline process (*71*). It was thus of interest to see if the processes led to a change in SH and S-S groups. Therefore, total SH content of protein pastes before cooking and gels after cooking was determined by unfolding the proteins using urea and thus exposing SH groups that might be located in the interior of the proteins (Figures 3-1 and 3-2). Washed muscle exhibited the highest total SH content before cooking for samples without added NaCl, whereas washed muscle with 2% NaCl (w/w) had significantly lower ($p < 0.05$) total SH content. There were no significant differences ($p > 0.05$) between gels with no added salt and added salt between the other treatments. There were also no significant difference ($p > 0.05$) in total SH-groups after cooking between treatments and salt concentrations. It was also interesting to see that there were no significant ($p > 0.05$) difference in total SH-groups before and after gel formation.

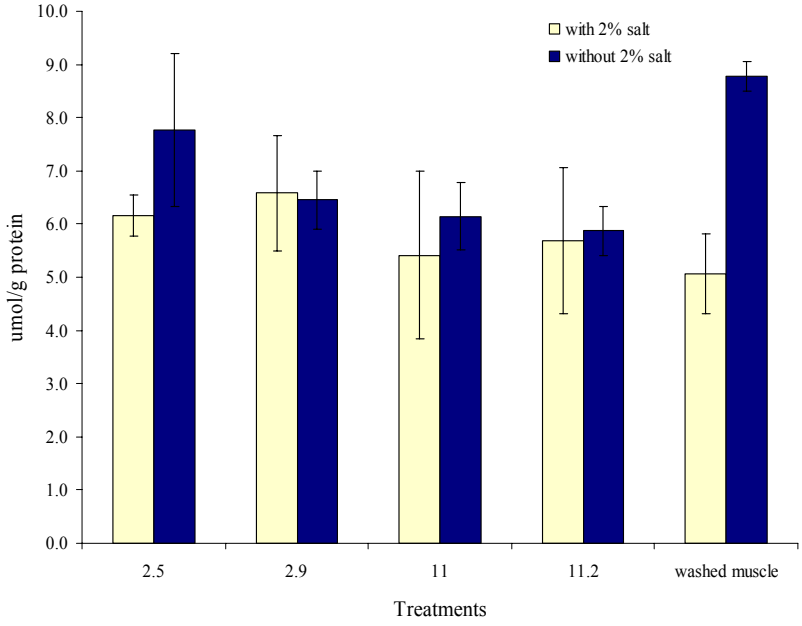


Figure 7-18. Shows total SH group content in tilapia muscle protein paste before cooking. Results are mean ± SD.

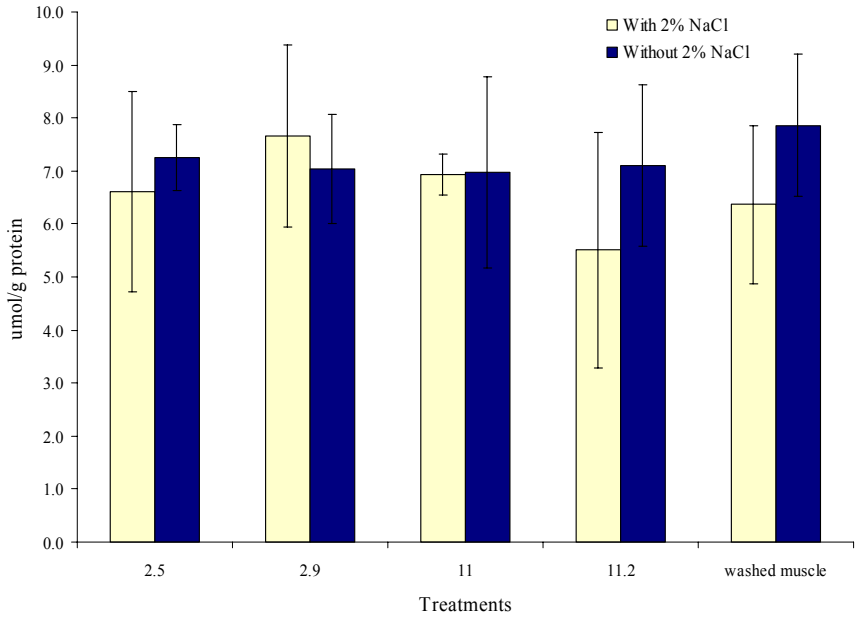


Figure 7-19. Shows total SH group content in tilapia muscle protein gel after cooking. Results are mean ± SD.

A reduction in total SH groups indicates the formation of S-S bonds between proteins, and has been found on many occasions on gelation of muscle proteins (*68*). A significant difference between the low and high pH treatments was not found with the tilapia protein isolates. Other studies with the acid and alkali processes have either reported differences or no differences in reactive and total SH-groups. Kristinsson and Hultin (*49*) showed that alkali treated myosin had more reactive SH groups than acid treated myosin, and also had better get forming ability. A study by Yongsawatdigul and Park (*71*) performed on rockfish muscle showed that washed muscle had lower content of total SH compared to unwashed muscle. These authors observed a higher amount of total SH groups in proteins made with the alkaline process compared to acid process, thus suggesting S-S bonds formed during the acid process. The proteins made with the alkali process had the largest decrease in SH groups, suggesting that more S-S bonds were formed during gelation for that treatment compared to other treatments. This was not seen with the tilapia muscle proteins. Another study performed on Pacific whiting showed a significantly higher total SH content of isolates made at low pH compared to high pH, suggesting SH groups became more reactive on alkali treatment. Even though differences were seen in SH groups with Pacific whiting proteins there were no link between these and gel forming ability. A study performed on threadfin bream showed that total sulfhydryl groups were stable up to 30°C but decreased from 40 to 80°C which indicates formation of disulfide linkages during heating (*19*). Again, the data with the tilapia proteins indicate that no significant S-S bonds were formed on heating and thus differences in SH groups could not explain the different functionality of the proteins.

CHAPTER 8
CONCLUSIONS

The use of the acid and alkali aided processes on tilapia muscle proteins regarding isolation parameters showed that the type of proteins recovered using low and high pH was significantly different whereas the quantity of total protein recovered was not different.

Gel forming ability of the acid and alkali treated proteins was determined and compared to washed tilapia muscle. Using large strain testing (torsion) showed that gels with 2% added NaCl were stronger and more elastic compared to samples without 2% NaCl. The alkali treated proteins also gave better results when compared to acid treated proteins and washed muscle. The use of small strain oscillatory testing gave very interesting results. Samples without 2% NaCl performed in some cases better than samples with 2% NaCl. Variability at low pH was great possibly due to a more unstable structure of the proteins. The small and large strain testing are not necessarily expected to go hand in hand because they are different tests performed at different protein concentration. Torsion determines fundamental gel quality and has been related to acceptability of a product texture whereas oscillatory small strain testing gives more insight in gel forming ability of the proteins at lower protein concentration. More investigation is needed to understand the underlying mechanism and the molecular properties involved in gelation of tilapia muscle proteins.

The findings from this research indicate that alkali treated tilapia muscle proteins can form strong and elastic gels upon heating.

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BIOGRAPHICAL SKETCH

Bergros Ingadottir was born and raised in Reykjavik, Iceland. Bergros graduated from the University of Iceland in June 2002 where she received a B.Sc. in food science. She was accepted in the Department of Food Science and Human Nutrition at the University of Florida in August 2002, where she was awarded a research assistanceship. There she pursued a master's degree in food science, specializing in protein biochemistry under the supervision of Dr. Hordur G. Kristinsson. While at the University of Florida she was invited to join Phi Tau Sigma, the honorary society of food scientists. Bergros graduated in 2004 and returned to Iceland.