ABSTRACT

LEKSRISOMPONG, PHANIN. Effect of Precipitating Conditions on Floc Size, Recovery Yield and Gelation Properties of Meat Isolates Prepared by pH-Shifting. (Under the direction of Tyre C. Lanier.)

A new pH-shifting process for meat isolate manufacture involves solubilizing meat proteins at high or low pH followed by isoelectric precipitation, induced by acid or base addition. The alkaline-aided process produces a surimi with better gelling properties, and greater oxidative stability, than that produced by the acid-aided version. This thesis, therefore, focused almost exclusively on the alkaline pH shifting process for the production of meat protein isolates.

Although centrifugation has typically been used as the method of effecting solids/liquid separation for this process, industrial centrifuges are expensive, so we explored a less expensive, simpler alternative employing screens for these separations. For screening, a primary determinant of recovery yield is the size, shape and integrity of the precipitating protein flocs. This study focused on the effects of varying process parameters of the pH shifting method on protein flocculation, as well as their possible influence on heat-induced gelling properties of the isolate product; gelation being an important property for isolate's use as a protein ingredient particularly for the production of surimi seafoods (Park and Lin 2005).

There are two parts in this study, the first compared the effects of meat species (chicken breast, albacore tuna, or king mackerel), the use of fresh vs. frozen meat as starting material, and different process parameters (base/acid types, acid addition rate and mixing speed, and meat to water ratio) on gelling properties and recovery yield of the precipitated protein isolate. The second part further investigated the effects of several organic and inorganic acids on the floc size, gelling properties, and recovery yield of the precipitated protein isolate.

In the first study, freezing the raw material prior to processing had no effect on the gelation properties. The use of a stronger acid combined with slower agitation during its addition resulted in chicken isolate gels of poorer quality, likely due to localized damage to proteins near the incoming acid stream. No clear patterns were seen in regard to processing effects on protein yield of the pH shifting process. Chicken and tuna isolates made with acetic acid in the precipitation step had the best gelation properties compared to the other acids used in this study. However, the use of hydrochloric acid as the precipitant had opposite effects on chicken versus tuna protein isolates yielding chicken isolates with gelation properties greater than those for isolates made with the other acids, while tuna protein isolates made with HCl had the worst gelation properties compared to the other acids.

In the second study, the use of hydrochloric acid as the precipitant yielded chicken protein isolate with the worst gelation properties compared to all other acids trialed, and there was no significant effects due to acidulant type seen for gelation properties of tuna protein isolate. Floc sizes varied substantially in this study, but were not consistently reproducible for replicate treatments. It is thought that differences in gelation properties could be due to specific acid effects on primary particle morphology or on protein conformation.

Effect of Precipitating Conditions on Floc Size, Recovery Yield and Gelation Properties of Meat Isolates Prepared by pH-Shifting

By

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DEDICATION

For my parents and my grandparents, who always emphasized the value of education.

BIOGRAPHY

Phanin Leksrisompong, daughter of Phatanee and Vinai Leksrisompong, was born on December 28, 1983 in Bangkok, Thailand. She is the third of four daughters. She graduated from New International School of Thailand in Bangkok. She then moved to the United States to continue her academic education. She received the Bachelor of Science degree in Food Sciences in 2006 from North Carolina State University and decided to pursue a Masters of Science degree in Food Science under the guidance of Dr. Tyre Lanier at North Carolina State University.

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Introduction

Muscle proteins are widely used as a food source and food ingredient for human consumption due to their nutritive value and functional properties (Hultin and Kelleher 1999). Over the past decades, people have been more health conscious and started to consume more fish than red meat since protein from fish are healthy and easy to digest. There has also been an in increase in the use of muscle protein from fish as a food ingredient because of its excellent gelling properties, (Kristinsson and Rasco 2000); particularly in the form of surimi. Surimi is used to make so-called surimi seafood products (Park, 2002), mainly shellfish analogs, which became widely consumed in the US and Europe (Mackie 1994). The conventional process of making surimi involves water leaching of minced fish muscle and the addition of cryoprotectants to stabilize the proteins while stored and shipped in the wet, frozen state.

As of this writing, a twenty percent world-wide shortage of surimi has developed due to shrinking resources for surimi production despite growing demand; while the global demand for surimi is 600,000 tons, production will likely only reach 500,000 tons in 2008 (IntraFish 2008). The raw material price has increased as much as seventy percent thus far due to shortage of fish supply and increased costs of fish harvest because of high energy prices. To address this problem, alternative fishery resources must be explored, and/or means of increasing the yields of surimi from existing fisheries must be developed.

A new method of recovering a high-functionality meat protein from fishes and other meats or trimmings was introduced by Hultin and his co-workers (1999). The process has been termed the pH-shifting method, and takes one of two forms, either acid-aided or

alkali-aided. Both involve first the solubilization of proteins (either at very acidic or alkaline conditions), interim removal of connective tissues and lipids while the protein is soluble, followed by precipitation at the isoelectric point (about pH 5.5) with possible subsequent neutralization. Thus the process resembles closely that used in the manufacture of soy protein isolates (Nelson and Glatz, 1985; Fisher and others 1985; Rohani 1993).

Because the method yields a high-gelling protein material, and can effectively remove both fat and phospholipids, it has been suggested that the method could be used with non-conventional fish species to make a surimi-like, functional food ingredient (Hultin and others, 2005) Also, because this approach retains the sarcoplasmic fraction of meat that is discarded by the conventional surimi process, it is capable of increasing the recovery yield of functional protein from the same amount of starting meat or trimmings.

The alkaline version of the pH-shifting process has been shown to produce a material with the best gelling properties, and greater oxidative stability, than that produced by the acid version (Kristinsson and Liang, 2006; Yongsawatdigul and Park, 2004; Choi and Park, 2002). For this reason, the remainder of this thesis will focus almost exclusively on the alkaline pH shifting process, as applied to fish materials.

Most laboratory studies related to this process have employed centrifugation as the method of effecting solids/liquid separation, both in connective tissue removal while the other proteins are soluble and in liquid phase, and for protein recovery following the isoelectric precipitation step (Hultin and Kelleher 1999, 2000; Yongsawatdigul and park 2001; Undeland and others 2002). However, in industrial processes the use of centrifuges is more expensive; they also require highly trained personnel for supervision and maintenance,

and most are scaled to large plant throughput. A less expensive, simpler alternative would be to employ screens for these separations (Noelsoe and others 2007).

For screening, a primary determinant of recovery yield would be protein floc size, shape and integrity. Thus it is quite appropriate that studies be carried out to measure effects of process parameters associated with the pH shifting method which may impact the properties of the protein floc that influence retention during screening. These process parameters might also influence the functionality of the protein as a food ingredient; specifically its heat-induced gelling properties so important to the production of surimi seafoods (Park, 2005).

Thus the underlying hypothesis of the work to be undertaken is that properties of the protein flocs, produced upon isoelectric precipitation of the meat protein isolate produced by the alkaline version of the pH-shifting process, may be affected by certain process parameters in the pH shifting process, such that recovery yield and gelation properties of the isolate may in turn be affected.

Review of Literature

1 The pH shifting process as compared to the conventional surimi process

1.1 The conventional surimi process

The term 'surimi' has traditionally referred to the food ingredient made by fresh water leaching of minced white-fleshed fish, such as pollock or hake, which forms strong and deformable thermo-irreversible gels upon heating. It is an ingredient used in many products ranging from a variety of traditional Japanese fish-cake products, collectively termed 'kamaboko', to westernized surimi seafoods such as crab meat or other shellfish analogs (Park and Lin 2005). The excellent gelling properties of surimi had been thought to derive from its composition as primarily a concentrate of myofibrillar (salt-soluble, muscle derived) proteins. Light color, bland flavor, and low fat (<1%), connective tissue, and sarcoplasmic (water-soluble, muscle derived) protein contents are the characteristics of desirable surimi. Surimi was made by manual methods for centuries in Japan, and became an industrial process in the twentieth century for use in land-based plants and aboard factory ships (Park and Lanier 2002). Before 1960, surimi was used within a few days after manufacture since freezing deteriorated the gelling ability of the proteins. The discovery of the utility of cryoprotective additives allowed the product to become a world-wide commodity, and made possible the use of fishery resources far from the location of manufacturing the final surimi-based products (Park and Lin 2005).

The conventional process for surimi manufacture (Fig 1.1) starts with heading and gutting of the fish (these may be done by automated machinery or by hand), and then mechanical deboning of the meat in a rotary meat-bone separator (drum with perforated 3-5

mm holes against which the fish is pressed; meat going inside the drum while bone/skin largely remains on the outer drum surface). This is followed by several fresh water (usually a 3:1 water:meat ratio) leaching and dewatering steps to remove water-soluble sarcoplasmic proteins, such as colored myoglobin/hemoglobin and undesirable enzymes, etc.. The mince is then sent to a refiner, which uses a fine screen to remove smaller remaining impurities such as bits of skin, bone fragments, scales, connective tissues, and firmer red meat pieces. The leached, refined mince is then dewatered, first by rotary screens to remove excess water, then finally by a screwpress. Finer particles of meat escaping screens or screwpress may be recovered by a decanter centrifuge. At this point the moisture content is typically near 82%. Cryoprotectant additives (sucrose, sorbitol and polyphosphate) are added prior to freezing in order to stabilize the proteins to freeze-induced changes that would otherwise diminish their gelling ability (Carvajal and others 2005); this reduces the moisture content of the finished surimi to about 75%.

1.2 The pH shifting process

The pH shifting process has been suggested as an alternative method for manufacturing surimi, having been developed by Hultin and co-workers (Hultin and Kelleher 1999, 2000; Yongsawatdigul and Park 2001; Choi and Park 2002; Undeland and others 2002; Hultin and others 2005). This process produces what has been termed a meat protein isolate by first solubilizing the meat proteins at acidic or alkaline pH, then precipitating the proteins near the isoelectric point, followed by dewatering and neutralization.

As with the conventional surimi process, meat would typically be derived from headed and gutted fish, most likely as deboned mince from a meat-bone separator, or

possibly from trimmings that have been ground finely in a meat grinder (Fig 1.2). Water is then added to the meat, and this ratio may vary greatly; too much water limits the throughput of the process, whereas high viscosity and poor mixing can be a problem if the relative proportion of water in the meat:water mix is too low. For the acid pH shift process, the pH of the meat-water slurry is lowered to approximately 2.5 – 3.0, usually by addition of 2N HCl. For the alkaline pH shift process, the pH of the homogenized mixture is increased to approximately 10.5 - 11.0, usually by addition of 2N NaOH (Kristinsson and others 2005).

As the pH is increased or decreased away from the pI (pH 5.3-5.5), ionizable groups in the proteins become increasingly charged to a point where the charge repulsion causes the protein molecules to unfold (Dill and Shortle 1991). The proteins gain a net positive charge at acidic pH due to neutralization of the negative charges of the carboxylate side chains of aspartic and glutamic acids. The proteins gain a net negative charge at alkaline pH because of the deprotonation of basic groups such as the imidizole side chains of histidine, guanidyl side chains of arginine, and the amino side chains of lysine, and from deprotonation of the phenolic side chains (Kristinsson and others 2005; Hultin and others 2005). Both extremely high and low pH cause the proteins to repel each other to the extent that the muscle ultrastructure disintegrates and the individual muscle proteins are dispersed into solution (Kristinsson and other 2005; Wright 2007). At these extreme pH levels the viscosity of the solution increases due to the swelling and elongation (increased hydrodynamic volume) of the dispersed myofibrillar proteins (Kristinsson 2002, Undeland and others 2002). At this point in the process, remaining insoluble materials, such as cellular membranes and solids such as bone particles, scales and fat can be separated from the soluble proteins by

centrifugation or screening (Hultin and Kelleher 1999; Undeland and others 2002; Kristinsson and others 2005).

The pH of the soluble protein fraction is then adjusted to about pH 5.5, near the isoelectric point, to induce protein precipitation. At this point, most of the muscle proteins become insoluble. Minimum solubility at the pI is mainly due to lack of electrostatic repulsion which allows aggregation and precipitation of the proteins via hydrophobic interactions (Chang and others 2001a, b; Feng and Hultin 2001). Sarcoplasmic proteins, which are mostly leached away in the conventional surimi process, are largely precipitated and retained along with the myofibrillar fraction in the resulting meat isolate (Hultin and others 2005). This protein precipitation and aggregation (flocculation) facilitates subsequent dewatering by screening or centrifugation, which is needed to concentrate the recovered meat isolate (Kristinsson and others 2005).

Currently, such meat isolates are being commercially produced primarily for redispersion in water as marinades, to be used in injection or by other application into intact meat pieces of the same species. This has been found to enhance their succulence and cook yield, without the need for phosphate additives or non-meat protein (Deal 2008). But such meat isolates also have been shown to exhibit excellent gelling properties, equal or better to those of surimi produced by the conventional water-leaching process from the same raw materials (Choi and Park 2002; Kristinsson and Liang 2006; Yongsawatdigul and Park 2004) and thus may be considered as an alternative to conventionally produced surimi.

1.3 Comparison of meat isolates with conventional surimi

1.3.1 Gelation properties

Heat-induced gelation is a key functionality of surimi, and some meat isolates made by the pH shift method, particularly the alkaline-aided process, have been shown to gel as well as or better than conventional surimi made from the same raw material (Hultin and others 1999; Hultin and Kelleher 2000).

Several theories have been proposed to explain the enhanced gelling functionality of the pH shift meat protein isolates. One is that the pH shifting process disrupts the myofibrillar structure more completely than even a conventional surimi process, releasing individual proteins into dispersion and thus increasing the protein surface area for reactivity in gelation (Wright 2007). Another theory proposed by the same authors is that when protein is precipitated out of solution, a particular size or shape of aggregates are formed. The fractal geometry of the primary particles and aggregates (flocs) could secondarily influence the type of gel network subsequently formed. A third theory proposed by Kristinsson and Hultin (2003a) is that conformational changes in the proteins induced by the pH-shifting process favor their better gelation. Both acid and alkaline pH-shifting processes cause conformational changes in the globular head fraction of the myosin heavy chain that implicate a conversion to a molten globular configuration. Most of the myosin light chains are also lost during both extreme pH treatments. These unfolding and refolding processes which occur during both the alkali- and acid-aided processes seem thus to improve the gelling ability of the proteins (Kristinsson 2002; Kristinsson and Hultin 2003a, 2003b).

These structural changes, and the ability of the proteins released from structural restriction within myofibrils to be more easily dispersed, may also explain why isolates display excellent gel-forming properties even in the absence of added salts, unlike conventional surimi which is mainly composed of salt-soluble proteins that require 2-3% salt for initial protein solubilization (Chang and others 2001; Wright and Lanier 2005). In addition to the ability of this protein to form high quality gels, meat isolates have also been shown to have improved functional properties in other respects, such as emulsification, foaming, and water-holding capacity (Hultin and others 2005).

Myosin contains 2 identical heavy chains and 2 sets of light chains (Xiong 1997). One of these light chains is required for ATPase activity. In the study of surimi as a functional food ingredient, ATPase activity had become a preferred measurement to estimate the degree of protein denaturation, such that loss of ATPase activity showed the protein had been partially denatured. Choi and Park (2002) found no measurable ATPase activity in acid-treated isolate protein but their results show comparable gel breaking force and deformation to conventional surimi that retained ATPase activity. This illustrates that isolates gel well even though they have been partially denatured (i.e., lost their ATPase activity). Any such prior denaturation of conventional surimi, which tends to diminish ATPase activity, also is known to lower its gelling ability.

The pH-shifting process almost always produces isolates with better gelation properties than conventional surimi, according to many studies. Kristinsson and Liang (2006) found that the isolate produced by the alkali-aided process from frozen Atlantic croaker showed higher structural fracture stress and fracture strain than conventional surimi

gels. Perez-Mateos and others (2004) also found this to be true with the same species. Yongsawatdigul and Park (2004) found that the alkaline process produced stronger, more deformable gels from rock fish than did its conventional surimi and gave evidence that alkaline solubilization promoted the formation of more disulfide linkages than did the acid process. Hultin and Kelleher (1999) reported better gel forming ability of acid-aided isolates made from cod and Atlantic mackerel compared to conventional surimi. Kristinsson and Ingadottir (2005) also found that acid-aided isolate made from tilapia gelled better than conventional surimi from that species.

There have however been some reports that pH shifted meat isolates did not perform as well in gelation as did conventional surimi. Kristinsson and Demir (2003) reported poor gel forming ability of mackerel, catfish, and mullet acid-aided gel compared to surimi produced by the conventional process. Choi and Park (2002) and Yongsawatidgul and Park (2004) reported lower gel quality of acid-aided isolates of Pacific whiting and rock fish compared to conventional surimi, but suggested that this was due to greater proteolytic action occurring at the solubilization and precipitation pH, due to contamination by gut enzyme (pepsin) and lysosomal muscle enzymes, both of which are activated at low pH. They reported better gelation obtained from the alkali-aided isolate than from conventional surimi. Choi and Park (2002) also found that cathepsin L activity seems to bind to myofibrils. Being an acid protease, this likely explains why they found that gelation of isolates produced by the acid-aided process using Pacific whiting produced worse gels than by the alkali-aided process.

Thus, except for species and conditions that implicate acid protease activation by the acid pH shifting method, meat isolates typically gel better than conventional surimi from the same species. And, in almost every study comparing acid- and alkali-aided protein isolates, the alkali-aided isolate produced stronger gels (Kristinsson and Liang 2006).

Fish muscle protein is composed of 20-30% sarcoplasmic protein, 66-77% myofibrillar protein, and 3-5% stroma proteins (Suzuki, 1981). A prevailing theory has asserted that the better gelling properties of surimi as compared to unwashed mince is because the sarcoplasmic proteins are removed and the myofibrillar proteins concentrated by the conventional surimi process (Lanier and others 2005). Thus, it is interesting that isolates seem generally to gel as well as, or better than, conventional surimi, given that the sarcoplasmic proteins are not removed by the pH shifting process.

The role of sarcoplasmic proteins in regard to formation of myofibrillar protein gels is still not clear. Sarcoplasmic proteins were originally believed to interfere with gelation of the dominant myofibrillar fraction, in that the heat-coagulable sarcoplasmic proteins were thought to adhere to the myofibrillar proteins when fish muscle is heated and interfere with proper gelation (Shimizu and Nishioka 1974). This also was cited as a possible reason why it is difficult to make strong, elastic gels from pelagic fishes, since they are known to have higher sarcoplasmic protein content (Park and Lin 2005). In contrast, other recent studies have shown evidence that sarcoplasmic proteins can positively contribute to the gel formation of myofibrillar proteins (Morioka and Shimizu 1993).

Recently, Yongsawatdigul and Piyadhammaviboon (2007) conducted a study of the effect of gel-enhancing and protein cross-linking ability of tilapia sarcoplasmic proteins.

They found that sarcoplasmic proteins appeared to be a potential ingredient for improving textural properties of fish protein gels. This is because sarcoplasmic protein from tilapia contained transglutaminase activity, which when added as part of the sarcoplasmic protein to lizardfish surimi resulted in cross-linking of lizardfish actomyosin. They found that breaking force and deformation of lizardfish surimi gels increased 91.6% and 26.7%, respectively, when tilapia sarcoplasmic protein was added at 10 g kg^{-1} and pre-incubated at 37 °C for 1 h before heating at 90 °C for 10 min.

1.3.2 Enzymic activity

Included among the sarcoplasmic proteins are a host of cellular enzymes. Certain heat-stable proteinases can have a negative effect on the gelation of myofibrillar protein due to their ability to cleave proteins and thereby weaken the gel structure (Choi and others 2005). Alternatively, transglutaminase (TGase) is present which promotes protein cross-linking during low temperature pre-incubation of meat pastes, resulting in stronger textural properties when cooked. The occurrence and contents of these sarcoplasmic enzyme activities varies among fish species.

1.3.2.1 Heat-stable protease activity

Heat-stable proteinases of several types, most typically those activated during cooking in the range of 50-70ºC, are thought to be primarily responsible for the so-called 'modori' effect, or weakening of gels during cooking that occurs in surimi of some fish species. Cathepsin L has been implicated as the causative enzyme in many species (Choi and others 2005; Lanier and others 2005), but serine and other type proteases have also been implicated

(Choi and others 2005; Kinoshita and others 1990). Although cathepsin L is optimally active on myosin at pH 4.1 (Okitani and others 1980), it has clearly been shown to be active in surimi gels at much higher pH (Lanier and others 2005). Cathepsin L, unlike other cathepsins present in the fish muscle, is not removed during the water leaching steps of a conventional surimi process (An and others 1994) even though it is part of the water soluble (sarcoplasmic) fraction. Choi and Park (2002) also found that cathepsin L activity survives the washing steps. This indicates that the enzyme binds to the myofibrillar protein and could be closely associated with the myofibrils (Lanier and others 2005).

As mentioned previously, acid-aided meat isolates often do not perform as well in gelation as conventional surimi. Kristinsson and Demir (2003) conducted pH-shifting processes and conventional washing on four different species finding that alkali-aided isolate produced the best gels, followed by conventionally produced surimi and in all cases, the acid-aided isolate had the worst gel quality. Low protease activity during/after alkaline processing was their explanation of good producing gel for alkali-aided isolate.

Choi and Park (2002) found that cathepsin L activity seems to bind to myofibrils when investigating Pacific whiting. Cathepsin L being an acid protease, this likely explains why they found that gelation of isolates produced by the acid-aided process using Pacific whiting produced worse gels than by the three wash cycles conventional surimi. On the other hand, their results showed that acid-aided isolates produced gels with stronger properties than one wash cycle conventional surimi.

1.3.2.2. Tranglutaminase activity

Surimi pastes will gel at low temperature (0-40°C, dependent upon the habitat temperature of the fish; Lanier and others 2005) due to endogenous transglutaminase (TGase) activity which catalyzes the formation of non-disulfide covalent bonds between protein molecules (Seki and others 1990). These bonds form between the amino acids glutamine and lysine (ε-γ-glutaminyl-lysine cross-links]. This cross-linking results in the formation of myosin polymers with a concurrent decrease in myosin heavy chain monomers. Upon fully cooking of these gels, the strength will be increased as a result (Okada 1959; Niwa and Nakajima 1975; Lanier and others 1982). This gelation and textural strengthening of salted surimi paste at low temperatures has been termed "setting", or in Japanese, 'suwari' (Lanier 1986).

It might be expected that the pH-shifting process could result in greater exposure of reactive sites on myofibrillar proteins for TGase-induced crosslinking and other protein-protein interactions (Kristinsson and Hultin 2003a, b; Perez-Mateos and others 2004). In a study comparing the two pH shifting processes to conventional surimi for Atlantic croaker, Perez-Mateos and others (2006) showed a loss of endogenous setting ability due to the two isolate processes, but a very large increase in setting when microbial TGase (0.1%) was added to the gel formulations, particularly for the alkali-aided process. Perez-Mateos and Lanier (2006) examined the endogenous setting ability of surimi prepared by the same three methods in menhaden. SDS-PAGE showed similar amounts of crosslinking among all treatments although the gel fracture properties differed. It seems clear that TGase cross-linking can be

enhanced by the pH shifting process, but that the activity of endogenous TGase is greatly diminished or lost during the process.

1.3.3. Recovery yield

A high recovery yield is important to securing a reasonable economical benefit from the available raw materials. The conventional surimi process retains mainly the myofibrillar protein fraction, by removing sarcoplasmic proteins through several water leaching steps (Xiong 1997), whereas these are largely retained in the pH shifting process.

Laboratory studies by Kristinsson and others (2005) on channel catfish found that the acid-aided, alkali-aided and conventional surimi processes yielded 71.1%, 70.3% and 62.3%, respectively on a dry protein basis from the starting material. Kristinsson and Demir (2003) conducted a study on Spanish mackerel and found that acid-aided, alkali-aided and conventional surimi processes yielded 73.6%, 69.3%, and 54.1%, respectively. Ingadottir (2004) obtained similar yield results using tilapia muscle. Kristinsson and Liang (2006) reported 78.7% and 65.0 % yields for acid- and alkali-aided, respectively, of Atlantic croaker. Choi and Park (2002), using Pacific whiting, obtained 60% and 40% yields for acid-aided and conventional surimi, respectively, based on wet surimi yields as compared to starting fillet weight.

One explanation offered by Undeland and others (2002) for why the acid pH shifting process tends to produce higher yields than the alkaline is that there is greater solubility of the proteins under acidic conditions. They reported that 92% of herring light muscle was soluble at acidic pH (2.7) versus 88% soluble at alkaline pH (10.8). The higher recovery

yield of the pH-shifting process is clearly due to the additional recovery of sarcoplasmic proteins.

According to Choi and Park (2002), in the laboratory version of the pH shifting process the recovery yield can fluctuate up to 35% if centrifugation is not sufficient enough to separate a portion of soluble proteins from the bottom layer of insolubles. At higher speeds, these proteins can be separated into a middle soluble layer and retained during the first centrifugation. Nolsoe and others (2007) replaced first centrifugation (20 min/ 10,000xg) with screening (1 sq mm hole size screen) to determine effects on recovery yield and gelation properties of cod and haddock fish meat protein isolate prepared by an alkali-aided pH shifting process. Replacing the first centrifugation (when the slurry is at high pH; to remove insoluble materials) with screening increased the ultimate recovery yield from 71% to 90%. Thus Nolsoe and others (2007) results confirm the Choi and Park (2002) study; that some soluble protein can be retained by the centrifuge process and accidentally be discarded; but this protein is able to pass through an initial screening step and thus higher yields can be obtained.

1.3.4. Oxidative stability

Lipid reduction is a key step in the surimi making process since the raw materials are rich in triacylglycerols and membrane phospholipids, which are known to be a main substrate for oxidative reactions in fish muscle (Hultin 1995). In general it would be expected that the pH-shifting method might favor lipid reduction since, at extreme pH, the meat structure is completely solubilized, favoring release of the storage and membrane phospholipids. This should facilitate removal by flotation or centrifugation as the lipids are then released into the

aqueous environment and their particle density difference accentuated. The pH shifting process also favors removal of membranous material high in unstable phospholipids (from the top layer), and the removal of membrane lipids since they settle as sediment, which should yield a more stable product (Hultin and others 2005).

Several studies have shown that the pH-shifting process does more effectively remove lipids from the starting raw materials: Hultin and Kelleher 2000, Kristinsson and Demir 2003 (mackerel), Undeland and others 2002 (herring), Kristinsson and Demir 2003 (mullet, croaker), Dewitt and others 2002 (beef heart); Hultin and Kelleher 2002, Liang and Hultin 2003 (chicken); Yongsawatdigul and Park 2004 (rockfish); and Kristinsson and others 2005 (channel catfish).

Clearly, the important step in lipid reduction in the pH-shifting process is the first centrifugation step. Kristinsson and others (2005) conducted a study looking at lipid reduction on skinless channel catfish fillets between acid- and alkali-aided protein isolation and conventional surimi processing. The starting material had fat content ranging from 4.7% - 9.8% (w/w). For the pH-shifting process, two centrifugation steps were conducted at 10,000×*g* for 20 min. They reported that lipid reduction from the alkali-aided, acid-aided and conventional surimi processes was 88.6%, 85.4%, and 58.3%, respectively. If the first centrifugation was omitted, however, a huge decrease in lipid reduction occurred; lipid reductions for the alkali- and acid-aided processes were only 61.2% and 45.4%, respectively. They also noted that higher lipid reduction can lead to more protein loss. This protein loss likely occurs as proteins are centrifuged away from the soluble fraction due to emulsification with the lipids (Kristinsson and Demir 2003; Kristinsson and others 2005).

Kristinsson and Liang (2006) used thiobarbituric acid–reactive substances (TBARS) to measure secondary lipid oxidation of raw materials, conventional surimi, and isolates from Atlantic croaker. Immediately after production, alkali-aided isolates increased slightly in TBARS when compared to the initial ground raw material, whereas the TBARS of conventional surimi was reduced by about 50%, in agreement with Barrerro and Bello (2000). After 14 days storage, however both the surimi and the alkali-aided isolates exhibited low TBARS values, while the control oxidized significantly after just 3 days storage. Similar findings have been reported for isolates made from catfish, mullet, and Spanish mackerel (Kristinsson and Demir 2003; Kristinsson and others 2005; Petty and Kristionsson 2004). Kristinsson and Hultin (2004) found that acid-aided isolates attain much higher oxidation values as compared to alkaline-aided isolates.

1.3.5. Color

Consumers first judge food with their eyes, so if they do not like the way a food looks they will not purchase or consume it. Surimi products are expected to be white and slightly translucent in appearance; the requirement for slight translucency precludes the addition of excessive amounts of opaque whitening agents, such as calcium carbonate or titanium dioxide (Park 2005).

Most animals have both light and dark muscle fibers; the light fibers are largely devoid of heme pigments (Xiong 1994). Fish muscle differs from that of homeotherms because the light muscle fibers are usually clearly separated from the red/dark meat, whereas the muscle fiber types of homeotherms within muscle cell types are usually more mixed. A

large problem when attempting to produce surimi from pelagic species is the greater content of red/dark meat, and the difficulty in separating this from the lighter meat which can be leached to remove some of the heme pigments (Hultin and others 2005). Even when mainly light colored meat is used to make surimi, however, residual blood and some remaining dark meat can impart color to the minced meat. Heme proteins are undesirable not only from a color standpoint; they are also the predominant catalyst or mediator of lipid oxidation in fish muscle (Hultin and others 2005).

Thus a primary purpose for the water-leaching step of the conventional surimi process is to leach out the heme pigments from the meat. Given that the conventional surimi process usually consists of two or more separate leaching steps, each followed by partial dewatering, it would seem likely that surimi from this process would be lighter in appearance than that produced by pH shifting, which has a single dewatering step and, if conducted at a relatively low water:meat ratio, would effect much less leaching of heme pigments from the meat. Likewise since most sarcoplasmic proteins are precipitated along with myofibrillar proteins in the pH shifting process, this too could contribute to greater color retention (Lanier and others 2005; Choi and others 2005).

Thus it is not surprising that Kristinsson and Liang (2006) reported that conventional surimi gels exhibited greater lightness than those made from a pH-shifted isolate of Atlantic croaker. However, earlier Kristinsson and others (2005) had studied channel catfish to compare acid-aided, alkali-aided and conventional surimi processing and found that the conventional surimi raw paste had a lower whiteness and higher redness compared to both protein isolates. Kim and others (1996), however, had reported higher whiteness values for

raw conventional surimi of catfish than were reported by Kristinsson and others (2005). Measuring the color of raw surimi paste does not always predict color of the cooked paste, however, because entrained air bubbles can falsely lighten the color.

Kristinsson and Liang (2006) found that the lightness of conventional surimi cooked gels was whiter than for the Atlantic croaker protein isolates. This finding agrees with Yongsawatdigul and Park (2002) and Choi and Park (2002) who studied isolates vs. surimi made from rock fish and Pacific whiting; respectively.

1.3.6. Process time

Time is money, and the pH-shifting approach to surimi manufacture can reduce the process time (increase product throughput in the plant). The solubilization of proteins comprising the very fine meat particles, when exposed to alkaline conditions of the pH-shift process, occurs much faster than does the removal of soluble materials (sarcoplasmic proteins) from the larger meat particles processed during conventional surimi production. The extraction rate for leaching depends on the size of the particles being leached, and the diffusion rate for soluble materials (sarcoplasmic proteins) to move out of the meat particles. Hultin (unpublished observation) noticed that it takes approximately 20 min to extract less than 80% of soluble protein of ground mackerel muscle. Leaching is also usually a two- or three-step process, with a dewatering phase required between leaching steps, whereas the pH-shifting process is a single step of adding and removing water.

1.3.7. Dewatering

Dewatering of isolate is very much easier than dewatering of surimi. As the ionic strength is reduced with successive washes in the conventional process, the meat progressively absorbs more water and swells, becoming quite difficult to dewater. To assist the process, up to 0.4% NaCl is added, and a screwpress must be used to assist the dewatering process. Standard decanter centrifuges, when used as an alternate method of dewatering conventionally processed surimi, must be altered to incorporate a dam within the unit that helps retain material in the high gravitational centrifugal field for a longer period of time so that smaller particles can be retained and the yield improved. Isolates produced by pH shifting are quite easy to dewater with a standard decanter centrifuge, so that throughput is greatly increased and the cost of such expensive equipment can be better justified. Alternatively, relatively good dewatering of the isoelectric meat slurry can be accomplished by simple screening, usually augmented by screwpressing (screening augmented by pressure application).

A caveat to this, however, is that screens can only accommodate a relatively narrow range of material properties (particle size distribution, density, shape, water-holding ability) and thus cannot accommodate much change in these parameters if good throughput is desired while controlling finished product moisture content within an acceptable range. Decanter centrifuges, however, can easily be adapted to accommodate a wider range of particle properties while maintaining the desired water content of the finished product. A decanter used to dewater isolate would not require the dam or internal buffle needed to properly dewater conventional surimi.

2. Effects of process parameters of the pH-shifting process on properties of the recovered protein isolates

The proteins of meat at the beginning of the pH-shifting process, if the meat has been kept cold and handled properly up to this point, can be considered to be in a near-native state, in that little prior denaturation or degradation should have occurred post-mortem. Several process parameters associated with the pH-shifting process can however induce dramatic changes in the protein molecules, which can affect the properties and functionality of the meat isolate proteins recovered from the process.

2.1 Protein solubilization step

Very little has been documented as to the effects of process parameters of the pH-shifting process as applied to meat isolates, but the conditions of solubilization of the meat proteins has been studied to some extent. Kim and others (2003) and Davenport and Kristinsson (2005) reported effects of ultimate solubilization pH on subsequent gelation properties for both acid- and alkaline-aided isolate processes, as applied to Pacific whiting and catfish, respectively. Kim and others (2003) reported that solubilization at pH 11 or 2 produced isolates with the best gelation properties, followed by isolates solubilized at pH 10.5 or pH 3. Isolates solubilized at pH 12 produced gels of poor quality; these heated pastes were described as "coagula" rather than as gels. Davenport and Kristinsson (2005) also varied not only ultimate pH but also the time that proteins were held in the solubilized state. They found that alkaline-aided isolates were not very affected by changes in solubilization pH over the range 10.5 - 11.5, nor by times held at these pH levels. However, protein

isolates solubilized at pH lower than 3 suffered molecular changes that led to poor gelation properties.

Thawornchinsombut and Park (2007) investigated the effects of ionic strength (NaCl levels of 10, 150 or 400 mM) in combination with solubilization pH (3.0 or 11.0) on Pacific whiting isolate. They found that gels made from isolates solubilized at either pH with 150 mM NaCl had better gelation properties than isolates solubilized at lower or higher ionic strength. These effects did not appear to be related to their measurements of protein solubility, surface hydrophobicity or total sulfhydryl content.

Although only these few studies have been conducted on meat isolate processing variables, the soy industry has used a similar protein isolation process for several decades. It might be expected that, despite the differences in the nature of the proteins being isolated, possibly some effects of the processing parameters that affect soy isolate precipitation could similarly affect meat isolate precipitation. These will be discussed relative to the alkaline solubilization – acid-induced precipitation of both soy and meat proteins.

2.2 Effects of agitation rate

When proteins precipitate in a stirred vessel, the protein that initially separates from solution appears as small (microscopic) spherical 'primary particles' that tend to grow into larger (visible) aggregated clusters known as flocs. The frequency of collision and likelihood of attachment govern the growth rate for these particles, while the strength of attachment relates to the ultimate aggregate (floc) size. The process is dynamic, such that a balance will exist between the progress of particle growth and aggregation (dependent upon interparticle

cohesion due to primarily van der Waals forces and hydrophobic interactions plus the structural arrangement of the particles) and disruption caused by fluid shear in the system (Tomi and Bagster 1978; Glasgow and Luecke 1980).

The growth of the primary particles to form larger flocs can occur by molecular diffusion (molecular diffusion; Brownian motion or perikinetics; Parker and Dalgleish 1977) but is greatly aided by orthokinetic mechanisms (shear flow). The latter depend on the particle and fluid properties (Akers 1975). Thus, to a point at least, greater fluid shear can contribute to larger aggregate size since the rate of aggregation increases due to increasing collision frequency.

Foster and others (1976), as well as Virkar and others (1982), found that mixing conditions can have a profound effect on the kinetics of precipitation and on the primary particle size distribution of precipitated soy proteins. They showed an increase in the initial growth rate with higher turbulence but a decrease in final particle size due to the break up of aggregates during the process. They also looked at the consequences of adding all of the precipitating acid at once rather than at a constant slower speed and found larger equilibrium particle size when the acid was added instantaneously. Fisher and others (1986) also used this technique on soy proteins and found that rapid acid addition with no stirring during the first minute produced a significantly larger primary particle size than a steady slower acid addition with continuous stirring.

Some proteins could conceivably be damaged by excessive shear; however Thomas and Dunnill (1979) and Virkar and others (1981) conducted studies which suggested that the globular proteins of soy can tolerate high shear itself, but when there is rapid renewal of gas-

liquid interfaces (such as can lead to foaming), protein denaturation usually occurs presumably induced by oxidation.

Most proteins can be irreversibly denatured when local extremes in pH occur causing alteration in their precipitation behavior (Fisher and Glatz 1986). To reduce the likelihood of local extremes in pH, agitation is usually applied to insure a more uniform exposure of protein to the acid precipitant; that is, a more rapid equilibration of the solution pH once acid is introduced.

2.3 Protein concentration effects

Higher protein concentration certainly contributes to higher viscosity, which can make mixing and fluid transfer processes difficult. Higher protein concentration might also be expected to contribute to a more rapid and complete precipitation of proteins during the adjustment of pH to the isoelectric point, as higher protein concentration should favor more frequent collisions of proteins to form primary particles, and of primary particles which lead to formation of visible flocs

Working with soy protein and sulfuric acid as the precipitating acid, Virkar and others (1982) showed a very slight increase in the mean equilibrium particle diameter (sample taken after 300 s) from 6 to 6.5 um when the protein concentration was increased from 2 to 30 kg/m^3. Nelson and Glatz (1985) also found a slight increase in primary particle size as soy protein concentration was increased.

2.4. pH, ionic strength, and ion type effects

Most protein precipitation models describe the effective protein-protein interactions as being mediated by salt ions and water structuring (Chiew and others 1995; Fornaseiro and others 1999; Malfoiss and other 1996; Piazza and others 1999; Poon and others 1997; Rosenbaum and others 1996).

At low ionic strength (<0.5M), ions in the solution serve to neutralize charges at the surface of proteins. This charge screening affects solubility in one of two ways, depending on the characteristics of the protein surface. A decrease in solubility occurs for proteins that contain a high percentage of non-polar patches, such as soy proteins, due to an increase in hydrophobic interactions. For proteins that have a more hydrophilic surface, such as β-lactoglobulin, an increase in solubility occurs through the process called "salting-in". The ions react with the charges of proteins and decrease the electrostatic attraction between opposite charges of their neighboring molecules. Moreover, the solvation connected with these ions serves to increase the hydration of the protein and thereby increase their solubility.

On the other hand, higher salt concentrations often decrease protein solubility ('salting out'; Cohn, 1943). Once a certain ionic strength is reached, the water that hydrates the protein may alternatively be used to hydrate the salts instead, causing the protein to fall out of solution. This is an example of the phenomenon known as "salting out".

Various solutes, at high enough concentration (>1.0 M), can affect solvent quality. Thus, in turn they also affect the stability of proteins and may exert specific effects on their solubility and aggregation (von Hipple and Schleich 1969; Walstra 2003). Solute cations or anions are grouped in the Hofmeister, or lyotropic, series (Hofmeister 1888) according to

their effect on solvent quality and the resulting effects on protein solubility and stability. This occurs because different ions, according to their size and charge density, affect the hydrogen-bonding of water, and this in turn affects how water interacts with protein, and the strength of hydrophobic interactions within the protein molecule (Domodaran and Kinsella 1982; Schnepf 1992). Thus cations and anions present in the solution can affect the conformational structure of a protein molecule, affecting its surface properties which govern protein association/precipitation. In this way, cations or anions of various acids might also affect the size, shape and density of protein flocs formed during isoelectric precipitation.

The Hofmeister series for those anions commonly used in foods is given as (Walstra, 2003):

$$SO_4^{2-} > HPO_4^{2-} > CH_3CO_2^{-} > C_3H_5O(COO)_3^{3-} > Cl^- > NO^{3-} > I^- > SCN^-$$

The anions listed on the left-hand side of the Cl^- are known as kosmotropes. These anions are strongly hydrated causes hydrophilic structure around them and increase surface tension of water. These anions stay away from apolar surfaces, which enhance internal hydrophobic bonding of protein. This increases protein stability and decrease their solubility, therefore, less protein is precipitate.

The anions listed on the right hand side of the Cl^- ion are known as chaotropes. They are less hydrated and not so greatly different from apolar solute in their effect on water making the local water structure more hydrophobic. They destabilize proteins by decreasing hydrophobic s within proteins causing them to unfold. As proteins open up, they are more reactive with one another and thus precipitate.

Chloride is approximately neutral with respect to its effects on protein stability and effects on water structure. It has little effect on water structure and is approximately neutral with respect to lyotropic effects (Creighton 1993; Curtis and others 2002; Walstra 2003)

Salt and others (1982) studied the effect of varying agitation speed (Reynolds number 2,800 – 28,000) during various acid precipitations of soy proteins and found that the protein modification after precipitation was dependent on the acid anion following the inverse of the Hofmeister series. The destabilizing effect of the anion specific effects of acids ran inverse to the series order of Hofmeister, which has been explained on the basis of competitive water reorganization between the ions, protein non-polar groups and peptide groups (von Hipple and others 1973).

Resch and others (2005) studied the effects of acidulant type (hydrochloric, lactic, citric, and phosphoric acids) on the rheological properties of beta-lactoglobulin gels (fracture stress and fracture strain) and dried powders (viscosity and water holding capacity) derived from these gels. They observed variability of gelation properties when using different acidulants during acid induced gelation, thus suggesting that specific anion effects may lead to important differences in protein gel formation and in turn the functionality of the subsequently dried powder. The specific effectiveness of various anions in stabilizing the structure of proteins and other macromolecules tended in their study to follow the Hofmeister series. Optimum thickening and water holding was observed in the powders that derived from strong, translucent gels created in systems utilizing lactic and hydrochloric acid. An opaque coagulum formed with the use of citric acid (a kosmotrope) resulting in a powdered ingredient with very poor functionality.

Nelson and Glatz (1985) found that the primary particle size of isolated soy proteins was affected by the acid used for isoelectric precipitation, in that sulfuric acid caused the formation of slightly smaller primary particles than did hydrochloric acid. This also followed the Hofmeister series since sulfuric acid stabilized and precipitated less protein than hydrochloric acid.

Thus the Hofmeister series can be potentially useful in explaining the effects of ions on stability of proteins because hydrophobic interactions play a major role in determining protein conformation. However, electrostatic interactions, hydrogen bonding, hydration interactions, and van der Waals forces also can be contributing factors (Bryant and McClements 1998).

2.5 Effects of rate of pH change

Local extremes in pH can cause irreversible denaturation of the proteins (Bell and others 1983), which could alter the precipitation behavior (Fisher and Glatz, 1986). Fisher and others (1986) studied the two extremes in the speed of acid addition during precipitation of soy protein and found that changing the pH more rapidly (by rapid acid addition) resulted in larger primary particles and larger, stronger aggregates.

Zhong and others (2004) studied the cooling effects on model rennet casein gel system. Cooling is a critical process; it is a final stage of cheese processing. During cooling, fluid-like processed cheese approximately 85 °C forms into a gel while cooling to a storage temperature (4 °C), creating a microstructure that dictates textural and functional properties. A slower cooling process had been observed to yield a firmer product (Carić and Kaláb

1993). They found that a slower cooling rate produced a larger number of smaller uniform flocs, and attributed the stronger gel formed to the aggregation of these smaller flocs.

By analogy, it might be concluded that smaller primary particles might form if the rate of pH change during the precipitation step were slower. These primary particles likely would survive the readjustment to above pH 7 and comminution required to make heat-induced gels, since it has been shown by Hultin and others that the proteins, once isolated by this pH shifting process, do not recover much of their initial salt-solubility. They are, however, highly dispersible. Primary particles of precipitated proteins are hydrocolloids; highly dispersible but not soluble when centrifuged.

3. Factors affecting protein flocculation from solution

To properly understand the flocculation of proteins from solution, we first must understand why proteins can attain a soluble state. Somewhat similar to the soluble state is the protein dispersion.

3.1 Protein solubility

Protein solubility refers to the amount of total muscle protein that goes into solution under specified conditions (Zayas 1997). From a thermodynamic standpoint, solubilization refers to maximizing the interaction between protein and solvent molecules (Cheftel and others 195). Thus, a thermodynamic equilibrium exists between protein-protein and protein-solvent interactions; e.g.:

Protein-Protein + Solvent-Solvent <=> Protein-Solvent

The solubility of proteins in aqueous media can range up to about 35% by volume. The degree of solubility depends upon intrinsic and extrinsic factors (Damodaran 1996).

Intrinsic factors: The solubility of proteins is highly dependent on their surface properties; that is, the interactions of the protein groups that contact the solvent (usually water). Proteins with more non-polar groups on the surface generally have poorer solubility in water, as hydrophobic interactions favor protein-protein interactions over protein-water interactions. Conversely, proteins with more charged groups at their surface are more soluble, since this promotes protein-water interactions. Surface charge diminishes protein-protein interactions for two reasons: 1) electrostatic repulsion between similarly charged protein molecules occurs as the pH is moved away from the isoelectric point, the pI; and 2) hydration shells form around the charged protein surfaces, which also results in steric repulsion (Damodaran 1996).

Extrinsic factors: The solubility also depends on solvent composition (such as the pH, ionic strength, the type of solvent), duration of extraction and temperature and temperature of the system.

Protein solubility in part determines the functional properties of proteins; thickening, foaming and, emulsifying depend upon the protein being in the soluble state. Gelling ability has also thought to be highly affected by protein solubility (Lee 1984,1986; Sano and others 1990) but others have shown that only good dispersion of the proteins is needed (Chang and others 2001a, b; Feng and Hultin 2001). There are only limited uses in foods for totally insoluble proteins (Damodaran, 1996).

3.1.1 Effects of added salts on protein solubility

It has been widely stated and believed that optimum gelation upon heating can be obtained only when myofibrillar proteins are first made soluble (for example, Lee 1984, 1986; Sano and others 1990). Except for the extreme case (that is practically quite difficult to achieve) of high solubility exhibited by myofibrillar proteins at near-zero ionic strength (Cheftel and others 1985), these 'salt-soluble' proteins therefore require the addition of 2% - 3% NaCl or other salts to become soluble (Lee 1984, 1986; Sano and others 1990). Thus, the first step in preparing fine-particle, gelled meat products always includes comminution of the muscle tissue with salt (Foegeding 1996). Salt effects solubilization of the myofibrillar proteins by disrupting ionic bonds, both intermolecular and intramolecular, that stabilize the native muscle ultrastructure (Hamm 1986; Niwa 1992; Munasinghe and Sakai 2004). The proteins are thus more evenly dispersed as a thick sol or paste prior to being heated to induce their gelation. This even dispersion of the proteins prior to gelation is evidently important to forming optimally strong, heat-set gels (Sato and Tsuchiya 1992).

The ionic strength of a solution is given by (Damodaran 1996):

$$\mu = \frac{1}{2}\Sigma c_i z_i^{\,2}$$

Where: C_i – concentration

Z_i - Valence

At lower ionic strength, ions in the solution serve to neutralize charges at the surface of proteins. This charge screening affects solubility in one of two ways, depending on the characteristics of the protein surface. A decrease in solubility occurs for proteins that

contain a high percentage of non-polar patches, such as soy proteins, due to an increase in hydrophobic interactions. For proteins that have a more hydrophilic surface, such as β-lactoglobulin, an increase in solubility occurs through the process called "salting-in". The ions react with the charges of proteins and decrease the electrostatic attraction between opposite charges of their neighboring molecules. Moreover, the solvation connected with these ions serves to increase the hydration of the protein and thereby increase their solubility.

Chloride ions are known to selectively neutralize positively charged sites on the protein surface, which effectively shifts the pI to a lower value. This then enhances protein solubility at the existing pH (Hamm 1986). Thus chloride ion (from NaCl) effects a "salting in" of myofibrillar proteins, depolymerizing the thick filaments of the myofibrils in meats (Munasinghe and Sakai 2004). Many researchers have come to the conclusion that for myofibrillar proteins, 0.3-0.6 M NaCl is the prerequisite for solubilization (Goodno and Swenson 1975; Sano and other 1990).

At higher ionic strength ion specific effects of salts on protein solubility become more apparent. Once a certain ionic strength is exceeded the water that hydrates the protein may alternatively be used to hydrate the salts instead, causing the protein to fall out of solution, a phenomenon known as "salting out".

3.1.2. Effects of pH change on protein solubility

As the pH is shifted further below or above the pI, the proteins carry a net positive or negative charge, respectively. Electrostatic repulsion between like-charged protein molecules, plus the hydration of the charged residues at the surface of proteins, both

contribute to their solubilzation. Increasing charge not only repels proteins from one another, to inhibit clumping; internal charge repulsion also causes myofibrils to expand at extreme pH and/or high ionic environments. In particular, the myosin tail is composed of many charged residues; therefore, the functionality of this protein is very sensitive to changes in pH and ionic strength (Galluzzo and Regenstein 1978).

When the solubility of given protein is plotted as a function of pH, roughly a V- or U-shaped curve, centered around the pI, results (Fig 1.3). At the isoelectric point, proteins are electrically neutral, because the net positive and negative charges on the surface are equal, but the surface is still highly charged. The protein thus may be soluble at the pI if the surface hydrophobicity is relatively low such that the hydration repulsion forces arising from these charged surface residues are greater than the tendency for protein-protein hydrophobic interactions. For example, a few food proteins, such as β-lactoglobulin (pI 5.2) and bovine serum albumin (pI 5.3) are highly soluble at their pI, due to their high ratio of hydrophilic residues non-polar groups at the protein surface (Damodaran 1996).

However, most protein molecules show minimal interactions with water at pH close to the isoelectric point since their net charge is small, allowing polypeptide chains to approach each other. At this point some aggregates are formed and this may lead to protein precipitation. The rate of precipitation is enhanced when the bulk density of aggregates differ greatly from that of the solvent, and when the diameters of the newly formed aggregates are large (Cheftel and others 1985).

3.2 Protein dispersion

Protein gels which exhibit a uniform gel matrix are stronger and more cohesive than irregular, coagulative-type structured gels (Foegeding 1996). Sato and Tsuchiya (1992) using transmission electron microscopy (TEM) found that a more homogeneous dispersion of myofibrillar proteins always corresponded with stronger and more deformable gels made from surimi.

A combination of high shear and the addition of salts and sodium phosphate/polyphosphates during commution, which together promote disruption of the myofibril structure and solubilization of myofibrillar proteins, has been the commonly applied method to ensure good dispersion of proteins when preparing gelled meat products (Niwa 1992; Stefansson and Hultin 1994; Munasinghe and Sakai 2004). Thus it has been widely stated and believed that optimum gelation upon heating can be obtained only when myofibrillar proteins are first made soluble, which clearly should promote good protein dispersion (for example, Lee 1984, 1986; Sano and others 1990).

However, Stefansson and Hultin (1994) proposed that obtaining an optimal heat-induced gel does not require that the proteins be truly soluble, but only well dispersed. Chang and others (2001a, b) and Feng and Hultin (2001) demonstrated this to be true. Strong, deformable heat-induced gels, of good water-holding capacity, were shown to be formed by the myofibrillar constituents of meat when good protein dispersion was obtained upon swelling of proteins at a pH away from the pI.

There are two factors will primarily determine whether colloidal suspensions will be well dispersed rather than aggregated/ flocculated, these being the particle interaction energy

and particle concentration. Many factors, such as pH, surfactant and salt additives, and particle size impact on interparticle interaction energy (Aubert and Cannell 1986). In the case of meat proteins it must also be kept in mind that the myofibrillar proteins must first be freed from the myofibril structure; this requires more than just mechanical disruption by comminution. The pH-shifting process promotes complete disruption of the myofibril structure by fully solubilizing the myofibrillar proteins. Their good dispersibility is thus insured despite these proteins being less than fully soluble after subsequent isoelectric precipitation and resuspension at near-neutral pH.

3.3. Flocculation of proteins

Flocculation or precipitation is a common and effective technique used to isolate and recover proteins from solution.

3.3.1. Definitions

There is some confusion in the literature as to the proper terms to use when describing the formation of visible colloidal clumps from a solution of that colloid; the process may be referred to by such terms as aggregation, agglomeration, agglutination, coagulation, or flocculation. These terms are used in different ways depending on the area of application and on the supposed mechanism (Gregory 1989). The term ‘aggregation’ can properly be used to denote either coagulation or flocculation. Coagulation denotes a strong aggregation that is essentially irreversible (Cornwell and Bishop 1983; Walstra 2003) whereas flocculation is the process whereby coagulated colloids (“primary particles”) weakly

aggregate to form larger flocs (Klimpel and Hogg 1991; Gregor and others 1997; Walstra 2003).

3.3.2. Protein flocs

Protein flocs are highly porous, irregularly structured and loosely connected aggregates composed of smaller primary particles formed initially upon precipitation of the proteins from solution (Dolfing 1981; Huang 1994; Kim and others 2001). These primary particles exhibit diameters between 1 nm – 1 μm, within the colloidal size range (Gregory 1989). Colloidal particles are subject to significant diffusion (Brownian motion) because of their small size, and under ordinary gravity they may settle very slowly if at all. Only when they associate to form protein flocs is protein precipitation apparent visually.

The size, density, and structure of the flocs are of interest because these can be important to the operation of industrial unit processes (Waite 1999). The formation of aggregates and flocs usually occurs in stirred tank reactors where the physico-chemical conditions during the operation affect the properties of both the primary particles and the protein flocs, such as size, density, and strength (Zumaeta and others 2005). Flocculation is an important step in many solid-liquid separations; protein precipitation and flocculation is a well known technique used to separate solid protein from bulk water. Subsequent unit operations such as flotation, sedimentation, filtration/screening, and centrifugation become more effective as the size and density of the flocs increase (Gregory 1989).

Thus the physical characteristics of the flocs can be vital in determining the separation efficiency. For example, in the treatment of wastewater to remove protein

contaminants, large compact flocs have a high settling rate that results in a treated water of low turbidity (Wilen and others 2003). Large and porous flocs aid filtration/screening for water removal due to their greater size relative to the screen or filter plus their high permeability to water flow (Bushell and others 2002). For these reasons, it would be desirable to have a much better understanding of the factors which influence the structure of protein flocs. However, evaluation and quantification of floc structural characteristics is difficult because of their very irregular three dimensional structure, the weak, delicate nature of their particle adhesion, and their relatively high porosity.

3.3.3. Morphology of protein flocs

In earlier theory the shapes of colloidal particles in water are considered to be evenly spherical. More recently it has become recognized that such colloidal particles have distinct morphological characteristics, which has spawned the study of flocculation morphology. This encompasses the effects of such factors as colloid stability, flocculation kinetics, and flocculation process efficiency on the morphological characteristics of the flocs produced. These floc characteristics include their shape, size/diameter, size distribution and spatial structure. Properties of both the flocs, and the primary particles of which the flocs are composed, are of interest.

The shape of the primary particles plays an important role in the flocculation process since the force distribution that surrounds the particles is affected by their shape. Besides the variable force distribution that surrounds non-symmetrical primary particles, the hydration

film can also vary in thickness along the surface and therefore affect the way in which the particles associate to form a protein 'primary floc'.

In a flow field where a velocity gradient exists, the orientation of non-symmetrical particles can accelerate the rate of flocculation. This is because the larger axes of the non-symmetrical particles usually orient along the direction of flow, resulting in a decrease in the distance between particles. When no flow is present, irregular Brownian movement prevents such close orientation. (Jiang and Guan 2006).

3.3.4. Forces important in protein flocculation

The aggregation of protein particles is controlled by colloidal interactions. Traditional Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, used to describe the stability of colloidal systems, considers that the balance between van der Waals attractions and electrical repulsion are the primary factors that controls colloid stability (Walstra 2003). This theory however cannot explain many events that occur during the flocculation of proteins in water (Jiang and Guan 2006). To apply DLVO theory in practice, the particle size distribution and shape of the particles has to be known in order to predict the stability. In protein flocculation, the primary particles are nonspherical and irregular such that particles can encounter each other in a number of orientations. To calculate the degree of aggregation the Hamaker constant is also needed; but since these primary particles are inhomogeneous, the value of the Hamaker constant used may be uncertain (Walstra 2003). Zukoski and others (1996) suggested that solvation/structural interactions can dominate over van der Waals and electrostatic interactions during precipitation reactions unlike what is assumed in classical colloid stability. The main forces regulating aggregation of proteins are 1) van der

Waals attractions, 2) covalent bonding, 3) hydrophobic interactions, 4) electrostatic interactions, and 5) hydrogen bonding (Walstra 2003; Clark and others 1981; Clark 1998; Zielgler and Foegeding 1990).

There are three main steps in the flocculation process; the first is the coagulation of proteins to form primary particles, and the latter steps are the ways by which these particles can associate to form larger flocs.

Step 1: *Protein + Protein* → *Primary particle*

During acid-induced (isoelectric) precipitation, addition of precipitants alters the soluble protein environment (such as the pH and ionic strength). This causes a change in the thermodynamic condition which generates supersaturation and electrostatic forces affecting the surface of protein molecules. The removal of hydration or electrostatic barriers facilitates collision of protein molecules causing a decrease in protein solubility, such that they come out of solution and nucleate/associate (cannot remain suspended during centrifugation). Protein molecules associate and their subsequent diffusion results in the formation of a strong, dense solid phase which appears as small spherical, so-called 'primary particles' of solid protein, which are themselves composed of many protein molecules (Nelson and Glatz, 1985; Rohani and Chen 1993; Bogush and Zukoski 1991; Look and Zukoski 1995; Chow and Zukoski 1994)

The time scale of the formation of primary particles is much smaller than the overall precipitation process (Grabenbauer and Glatz 1981). Their growth occurs initially by molecular diffusion (Parker and Dalgleish 1977) and subsequently by perikinetic (Brownian motion) and/or orthokinetic (shear flow) mechanisms which are dependent on the particle

and fluid properties (Akers 1975). Primary particles will continue to grow by diffusion until they reach a size at which shear-induced collisions between growing particles limit their growth (Grabenbauer and Glatz 1981; Petenate and Glatz 1983b; Hoare 1982; Bell and others 1983, Glatz and others 1986).

Step 2: *Primary Particle + Primary particle → Primary floc (Cluster)*

On a longer time scale, the primary particles then weakly aggregate as a result of convective transport to form primary flocs. These aggregates continue to increase in size until they encounter a disruptive force (Gregory and others 1997; Walstra 2003).

Step 3: *Primary floc (Cluster) + Primary floc (Cluster)* →

OR *Ultimate floc*

Primary floc (Cluster) + Primary particle →

The first type of interaction leading to growth of floc size growth is termed "cluster-cluster aggregation", which usually results in flocs displaying a more open aggregate structure than the latter "cluster-particle" type aggregation.

When there is significant repulsion between particles, only a small fraction collisions result in permanent attachment. This allows particles to detach and rearrange after particles have first attached. The ultimate size of protein flocs will be determined by the balance between the progress of aggregation (dependent upon interparticle cohesion and the structural arrangement of the particles) and disruption caused by shear (Tomi and Bagster 1978; Glasgow and Luecke 1980).

3.3.5 Methods of measuring floc size

Many different measurement techniques such as microscopy, light scattering, settling, and photography/ image analysis have been studied to quantitate the floc size. However, it is very hard to determine floc size due to the irregular size and shapes of flocs. They are highly irregular 3D structures, very delicate, and the ultimate floc size can easily change depending on physical and chemical conditions prevailing (Farrow and Warren 1989).

The simplest measurement of floc size is longest single dimension. A more common approach is to measure the longest dimension of the floc in both the horizontal and vertical planes (Farrow and Warrebm 1989; Manning and Dyer 1999).

However, often an "equivalent diameter" measurement is made (Cousin and Ganczarcyk 1998). This defines a non-symmetrical particle as a sphere or a circle that is in some way equivalent to that particle, and thus allows comparisons to be made between very irregular forms.

It is difficult to get representative size data from a single measurement when measuring complex and irregular 3D flocs with 2D measurement techniques. The results depend on the orientation of the flocs at the solvent interface (Allen 1997). The particle dimension that is perpendicular to the viewing plane is usually the smallest and is often not measured. This can cause a major error in analysis. Thus, diameters based upon 2D images are known as statistical diameters, and they are only a valid indication of particle size distribution when enough measurements are made (625 particles per treatment required according to statistical criteria of British standard).

Image analysis by computer requires that photographs (macro or microscopic) have good contrast between flocs and the background, yielding pixel area and equivalent radius values, where the equivalent radius is given by

$$\text{Equivalent radius} = \sqrt{\frac{pixels}{\pi}}$$

Light scattering is considered to be one of the most powerful methods to analyze fractal structures of colloidal aggregates **(**Ikeda and others 1999). When light is passed through a solution of particles, some of the light is absorbed by the particles and some is scattered, and the remainder of the light passes straight through the suspension. This can thus be used to determine the particle size and the nature of the particles and the suspending medium in the solution (Farrow and Warren 1993).

3.3.6. Density of flocs

The density of a floc is simply the mass of particles plus the included water divided by the 'envelope' volume. To determine the 'effective'density of the floc, the density of water is subtracted from the density of the aggregate (Gregory 1989). The rate of sedimentation is determined by the size and density of the flocs (Tambo and Watanabe 1979).

The density of the protein flocs is thus important because the recovery of precipitated protein by centrifugation affected by the floc density. Larger sized flocs also contribute to a faster rate of sedimentation, but large flocs tend to be lower in density. Since the size factor predominates over the density factor in Stoke's Law, the net effect of larger, less dense flocs is of course a greater sedimentation rate.

Another aspect of solid-liquid separation processes, whether by centrifugation or especially by screening (filtration), is the porosity of the cake that builds as filtration/centrifugation proceeds. Movement of water through this cake is faster when the flocs are compact, since there is less solid surface in contact with water, therefore, less drag. Open floc structures also are more susceptible to restructuring under pressure which results in compression and can lead to considerable blocking of pores, in turn reducing the flow rate through the cake (Gregory 1989).

3.3.7. Floc strength and fragility

Floc strength is a particularly important operational parameter in solid/liquid separation techniques for the efficient removal of aggregated particles, since the disintegration of a floc leads to smaller floc size and thus a slower sedimentation rate in centrifugation, or the possibility of passing though the holes of a screen. Either situation would lead to a lower recovery yield. In operations where high shear is prevalent it is hard to minimize floc breakage (McCurdy and others 2004).

Floc strength is directly related to floc structure; therefore, it is highly dependent upon the floc formation process. Aggregation of primary particles to form flocs is considered to be a two stage process of particle transport and particle attachment (Amirtharahah and O'Melia 1990): primary particles must first collide with each other and secondly must adhere upon collision. The resulting strength of the flocs formed then depends on the interparticle forces and the number of particle-particle contacts during collision. The number of particle-particle contacts in an aggregate will be greater for more compact (higher density) structures

and therefore these aggregates should be stronger than those that are less compact (lower density) (Gregory 1989).

The flocculation model of Firth and Hunter (1976) shows that floc strength is determined by the number of bonds/area, $n_{c,}$, the attractive force, F, of each bond, the particle size, d_1, and the solid fraction of the floc, ϕ:

$$n_c = \frac{\Phi}{d_1^2}$$

In shear conditions, flocs do not just continue to grow until they reach a steady-state size. The rate of aggregation is considered a balance between floc formation and floc breakage since the floc growth is held in check by floc breakage (Francois 1987; Spicer and Pratsinis 1996; Ducoste and Clark 1998; Biggs and Lant 2000). Thus, the stability of flocs in suspension depends on how easy they are to break with aggregate breakage being directly related to the strength and number of the bonds holding the floc together.

3.3.7.1. Measurement of floc strength

There have been a number of different approaches taken in measuring floc strength. For some researchers, floc strength is indicated by the size as a floc reaches the end of its growth phase, whereas for others floc strength has been determined as the force required to break already formed flocs. Most research has concentrated on exposing flocs to increased shear rate in a containing vessel by the application of stirring, ultrasonification or oscillation. More recently microscopic techniques have been developed that relate the energy required to pull apart or compress individual flocs until breakage (Francois 1987; Fitzpatrick and others 2003; Leentvaar and Rebhun 1983)

3.4 Unit processes of floc-water separation

As has been explained in some detail, the size, shape, and density of the protein flocs are important to recovery of precipitated proteins during the solids-liquids separation step, whether carried out by centrifugation or by screening. Before concluding this discussion of flocculation and its importance in protein recovery, a discussion of the mechanics of these two separation processes, relative to floc properties, is appropriate.

3.4.1. Centrifugation

In a surimi-type process, centrifugation may be used only to recover the finer insoluble particles from the liquid which has passed through screens or screwpresses, or alternatively it may be used to replace screens and screwpressing, being used to collect the full range of particle sizes (Park and Lin 2005).

Stoke's law governs the sedimentation rate of particles under gravity (g) or during centrifugation (some multiple of g in the equation below) (Lamb 1994):

$$V_s = \frac{2}{9}\frac{r^2 g(\rho_p - \rho_f)}{\eta}$$

where:

V_s is the particles' settling velocity (m/s) (vertically downwards if $\rho_p > \rho_f$, upwards if $\rho_p < \rho_f$),
r is the Stokes radius of the particle (m),
g is the standard gravity (m/s^2) (or a multiple if centrifugation is used),
ρ_p is the density of the particles (kg/m^3),
ρ_f is the density of the fluid (kg/m^3), and
η is the fluid viscosity (Pa s).

Thus the velocity of particle or floc separation is affected by four variables: 1) the density differential between phases (between the protein flocs and water); 2) the viscosity of

the solution (which should approach that of pure water in the pH shift process); 3) the amount of centrifugal acceleration (usually expressed as a factor of gravitational acceleration, e.g., xG); and 4) the particle size (effective diameter).

Of these variables, Jaczynski and Taskaya (2007) surmised that only particle (protein floc) size is controllable during an alkaline pH shifting process. Since the particle settling velocity will increase by the square of the particle size, certainly this factor can be quite important to dewatering by centrifugation, given that flow-through (continuous) decanter-centrifuges, having a fixed acceleration rate typically less than 4000 x G and a very short dwell time of material, are typically used for surimi processing (Jaczynski and Taskaya 2007). In their study, flocculating agents were used to increase floc size and size consistency

Nelson and Glatz (1985) also believed that, in addition to maximizing the size of protein aggregates, it might be possible to increase their density to aid in the separation of recovered protein solids from solution.

3.4.2. Screening

A drawback to the use of centrifugation for solid/liquid separation is that this operation requires expensive equipment and the attention of more highly trained personnel (Nolsoe and others 2007). For this reason, screening equipment, typically rotary- or inclined planar-type screens, are often used in surimi manufacture for bulk water removal, and are typically augmented by screwpresses for final dewatering.

During screening, the separation of solids from liquids will depend primarily upon the shape and size of protein particles/flocs. Shape becomes important if particles are fibrillar

or elongated in nature, in which case only a portion may be retained by a screen whose hole size is just slightly less than the maximum dimension of the floc or particle. A typical hole size (may be circular or square) will have maximum diameter openings of about 0.6 mm, typically using sheet metal or woven screens which are about 0.4 mm thick, with a distance between the centers of the hole being about 1.2 mm.

3.5 Relationship of floc properties to gelation properties of recovered protein isolates

The heat-induced gelation properties of the meat proteins ultimately determine the texture, water- and fat -binding properties of food products made from surimi (Totosaus and others 2002; Kim and others 2005). The intra- and inter-molecular forces that stabilize native protein conformation and myofibrillar ultrastructure are the same forces that facilitate the gelation upon heating (Wright 2007). Processing and compositional factors that affect flocculation and floc properties may directly, or independently, affect the gelation properties of the recovered protein. That is, the effects on protein conformation and aggregation which influence the size, shape and/or density of the protein flocs may also ultimately affect the gelling properties of the proteins under the conditions to which this material is subjected during the making of surimi seafoods, directly or independent of the effects on the floc properties.

As has already been discussed, acidulants can have direct effects on gelation properties of the protein, irrespective of their effect on protein floc properties. Venugopal and others (2004) explored the acid-induced gelation of washed, collagen-free shark myofibrillar proteins and found that organic acids facilitated the gelation of the proteins, whereas

inorganic acids only caused precipitation out of solution. They attributed this to a more marked denaturation of the proteins caused by the inorganic acids.

Several workers (Nash and others 1971; Wolf 1977; Lillford and Wright 1981) found that acid addition, to effect isoelectric precipitation, causes irreversible damage to soy proteins such that they lose solubility. Resch and others (2005) similarly saw differing effects of different acidulants on the gelation of whey proteins.

Alternatively, it is conceivable that the floc properties might directly affect the subsequent gelling properties of the proteins, irrespective of the conditions that led to formation of the flocs which display these particular properties. Wright (2007) hypothesized that the enhanced gelling functionality of meat protein isolates produced by pH shifting could be affected by the particular size or shape of the primary aggregates formed upon isoelectric precipitation, since it is known that those particles are only subsequently dispersed, not fully dissolved. That is, the shape and size of the swollen primary particles (the particles would swell as the pH is raised for preparing pastes for heat-induced gelation) might influence the structure of the gel formed by their interactions during heating. Likely the properties of the flocs formed by these particles would not influence the subsequent heat-induced gels, since high shear comminution and salt/base addition are used for the paste preparation, factors that would tend to disintegrate the flocs.

Of course many other factors may affect the gelling properties of proteins other than the primary particle properties leading into the gelation process; these will be discuss in the next section of this review.

4. Heat gelation properties and chemistry of meat proteins

4.1 Protein Gels as Foods

Gels are an intermediate phase between solid and liquid (Damodaran 1996). The definition given by Ferry (1961) is "A substantially diluted system which exhibits no steady state flow". Gels have a yield stress and show mechanical properties that are characteristic of solids.

Nearly all food gels are man-made to create specific physical properties appropriate to storage, handling, eating, and physical stability with respect to their mechanical properties (Walstra 2003). Protein gelation is the key to many processed food applications such as cooked sausages, luncheon meats, and surimi seafoods. The gel structure determines the texture, water, and fat binding properties of these food products (Totosaus and others 2002). Thus, protein gelation is a model system used to understand the mechanisms responsible for the properties of processed meat products (Asghar and others 1985).

4.1.1 Types of food gels

1) Polymer gels

Many gelled food products are polymer gels; these are typically made from carbohydrate hydrocolloids like gums or starches, but also include gelatin from animal collagen. They typically consist of long cross-linked polymer molecules forming junctions involving weak physical bonds; the resulting gels are typically either brittle or strong, depending on the gel architecture and the number and strengths of the interpolymer bonds. Stacks of helices or other straightened polymer sections are known as microcrystallites.

Most of these polymer gels have thermo-reversible characteristics, that is, they melt upon heating and reform upon cooling. However, some anionic polymer gels such as those made from alginates with calcium cross-junctions do not melt at high temperature.

2) Particle gels

Casein gels are an example of an ideal particle gel which is usually formed by fractal aggregation. These gels can be formed by either acidification or renneting. Initially small fractal aggregates are formed which change into more compact particles consisting of 4-40 primary particles. Once the gel is formed, long term rearrangement can occur under some conditions. This leads to straightening of strands of particles in the gel and a decrease in fracture strain (gel becomes more brittle). However, the fracture stress (strength) and gel permeability are not really affected. The structure of particle gels is determined by the volume fraction of the particulate material, the size of the primary particles, and their fractal dimensionality.

3) Heat-induced protein gels

Protein gels are typically thermo-irreversible; that is, they do not melt upon heating and do not reform upon cooling. The main forces that stabilize heat-induced protein gels are hydrophobic and covalent bonds, although hydrogen and ionic bonds also play a role to some extent (Damodaran 1989). The traditional molecular model for heat-induced protein gelation is a 3-step process:

Step 1: Initial unfolding of protein (denaturation) - high temperature induces unfolding of protein causing the reactive sites (hydrophobic groups) to expose for intermolecular binding.

Step 2: Subsequent formation of protein-protein interactions - caused by intermolecular hydrophobic interactions and covalent bonding.

Step 3: Aggregation - which leads to formation of the three dimensional gel network. (Bigelow 1967; Catsimpoolas and Meyer 1970; Niwa 1992).

Heat-induced formation of muscle protein gels is the main focus of this thesis. Disruption and dispersion of myobrillar proteins is a critical step in maximizing the gelling properties prior to the heating step. This can be done by mechanical chopping to expose more surface area of myofibrillar proteins. This accompanied by the addition of salts such as sodium chloride and polyphosphates (Barbut and others 1996) with sufficient water to solubilize myofibrillar proteins further disrupts the muscle structure.

4.2 Intermolecular forces involved in heat-induced gelation of muscle proteins

The molecular forces involved in protein gelation depend first on overcoming those forces involved in muscle/protein structure to effect solubilization/dispersion, and the molecular forces involved in muscle protein heat-induced aggregation. (Acton and Dick 1989). These are basically the same, and consist mainly of covalent, ionic, hydrogen, and hydrophobic bonds/interactions (Totosaus and others 2002; Lanier and others 2005).

1). Covalent bonds are the strongest formed, typically during heating at temperatures greater than 40°C by disulfide bonding or, in certain cases involving specific enzyme action, like ε-(γ-glutamyl) lysine cross linking catalyzed by Transglutaminase in surimi (Lanier and others, 2005). Their bond strength is dependent upon the number of electrons

shared and is normally in the range of 25-110 kcal/mol. These bonds, once formed, are thermally irreversible.

2) Ionic interactions between and within proteins are of lesser strength (10-20 kcal/mol) than covalent bonds (Action and Dick 1989). At higher pH and salts contents, the ionic interactions can be minimized to maximize dispersion of proteins (Lanier and others 2005). These bonds are not influenced by temperature, but being weak may be overcome by thermally induced molecular motion. Electrostatic interactions of muscle proteins are most important in the proper dispersion of proteins prior to gelation; because gelation of meat proteins is highly pH and salt-sensitive (Xiong 1997).

3) Hydrogen bonds are quite weak (1-5 kcal/mol; Creighton 1993; Acton and Dick, 1989), formed primarily upon cooling of a paste or gel. Thus these bonds are essentially thermo-reversible, and thus gels will exhibit different rheological properties dependent upon how temperature affects the internal hydrogen bonding (Howe and others 1994).

4) Hydrophobic bonds/interactions are similarly weak as hydrogen bonds (1-3 kcal/mole; Acton and Dick 1989). It is a result of the hydrophobic portions of proteins associating in the presence of water to achieve less exposure and structuring (lowering of entropy) of water molecules. Heating actually increases the amount of hydrophobic bonding since it destroys the native structure of the protein and exposes the hydrophobic regions to the aqueous solution; also hydrophobic interactions become stronger as the temperature is increased to about 60°C (Chan and others 1992). Thus hydrophobic bonds are considered to be thermo-irreversible for all practical purposes.

A distinguishing aspect of protein gelation as compared to that of carbohydrates (including the protein gelatin) is that heating induces formation of covalent and hydrophobic bonds, thus making these gels thermoirreversible. Yet ionic and hydrogen bondings also contribute to the texture of the gels and thus must be considered for their contributions.

4.3 The mechanism of heat-induced gelation of muscle proteins

Myosin is the most predominant protein in meat sarcomeres, comprising approximately 45% of myofibrillar proteins (Foegeding and others 1996). Myosin is considered to be the major protein contributing to formation of heat-induced meat gels (Fukazawa and others 1961a; b). Therefore much of the research conducted into the mechanisms of meat gelation have focused on the gelation of myosin.

Myosin has an elongated rod shape with a thickened portion at one end, called the "head", and a long/thin section which forms the backbone of the thick filament, called the "tail" or "rod". The head and tail portions play different roles during heat-induced gelation of muscle minces. As heating proceeds, the head portions aggregate and form a three dimensional network as they undergo irreversible aggregation involving hydrophobic interactions and the oxidation of –SH groups (formation of disulfide bonds). The tail portion undergoes a partial irreversible helix-to-coil transition and also then participates in the formation of the three dimensional network (Foegeding and others 1996).

4.4 Salt and pH effects on gelation of myofibrillar proteins

Two of the most important factors that affect gelation of myofibrillar proteins are salt concentration and pH. Many studies have shown that the application of approximately 0.3-0.6 M NaCl is a prerequisite to achieve adequate protein solubilization and dispersion, leading to proper gelation of myofibrillar proteins (Goodno and Swenson 1975; Sano and others 1990). The optimum pH for gelation seems to be between 5.5 and 7.0, depending on the protein concentration and the species from which the salt-soluble proteins are derived (Foegeding 1987; Yamamoto and others 1988; Xiong and Brekker 1991; Chung and others 1993)

However, Kim and Park (2002) found a negative effect of salt addition on the gelation of acid- and alkali-aided fish protein isolates. They suggested that NaCl did not solubilize the myofibrillar proteins prepared by pH shifting, but instead contributed to their aggregation. Ke and Hultin (2005) also found that adding salt up to 50 mM decreased the water holding capacity and gel strength of acid-induced meat isolate gels. These works suggest that NaCl addition may not be required if myofibrillar proteins are already well dispersed by having been solubilized and released from the myofibril structure.

On the other hand, Wright (2007) showed that the microstructure of salt-containing gels of mackerel protein isolate showed greater solubility of proteins, evidenced as "spiderweb-like" structures in electron micrographs of the gels. These gels also showed greater protein dispersion when salt was added. Gels made without added salt were more fibrous and long stranded, while gels made with added salt displayed aggregated proteins that

were more spherical and bead-like. Additionally, the spaces between the fibers/aggregates appeared to be more filled with solubilized protein when gels were made with added salt.

4.5 Rheological methods of monitoring gel formation and measuring gel properties

Protein gels are known to be viscoelastic, since they exhibit properties of both fluids (viscous) and solids (elastic) (Steffe 1996). There are two main types of gel testing employed by rheologists; those which involve large strain, and typically fracture the sample, and those which utilize oscillatory small strain to evaluate rheological properties in a non-destructive manner. Usually fracture methods are employed on cooked, cooled gels, whereas oscillatory testing is used to monitor gels during the cooking process.

Large strain testing may be conducted by torsional testing, which yields more fundamental measurements of fracture properties (stress, strain; Hamann and MacDonald 1992) or by empirical methods that simulate biting the sample. The most common empirical method employed with meat gels is the punch test, using a 5 mm ball-tipped probe (Kim and others 2005); this also yields measurements related to fracture force and deformation. These two parameters can then be plotted to form a so-called 'texture map' that roughly relates these measurements of the mechanical properties of the gels to their sensory terms (Fig 1.4).

Small strain oscillatory testing is most often used to produce plots of rigidity (G' and G'') versus temperature during the heating-induced gelation process. G' is the storage modulus and reflects elasticity (solid-like nature) of the material, while G'' is the loss modulus and reflects the viscous nature of the material. A gel is considered to have formed when the curve of G' crosses over that of G'' (Steffe 1996).

5.0 Factors other than processing parameters that may impact flocculation and gelation properties of meat isolates formed by pH shifting

5.1 Compositional effects

Meats contain approximately 15-22% protein. Myofibrillar proteins make up 60-70% of all protein in muscle, and myosin makes up approximately 45% of myofibrillar protein (Foegeding and others 1996). Because myofibrillar proteins can interact with each other and other non-protein ingredients, they play the major functional role in the processing of meat and meat derived products (Xiong 1997). Thus any processing step in the conversion of meats to finished product must consider the effects produced on the quality and quantity of myofibrillar proteins, especially myosin.

5.2 Chemical and physical changes postmortem

When animals are slaughtered, muscles do not suddenly stop all their living functions. The conversion of muscle to meat is a gradual degradative process; usually physical and chemical changes occur over a period of several hours or days depending on the size of the animal. Once the respiratory system ceases to function, there is no further oxygen intake. As the stored oxygen supply is depleted, aerobic metabolism through the TCA cycle and the electron transport chain begins to fail. In order to try to maintain homeostasis, anaerobic metabolism continues but with less energy in the form of ATP produced. In living animals, pyruvate (the end product from anaerobic metabolism) either feeds into the TCA cycle (aerobic metabolism) to be converted into ATP or is converted into lactic acid which is transported from the muscles to the liver where it gets re-synthesized into glucose and

glycogen or to the heart where it gets metabolized to CO_2 and water. This is similar to when oxygen becomes limiting in living muscle tissue during periods of heavy exercise.

In non-living muscle, all of the pyruvate is converted into lactic acid because the TCA cycle is no longer working (Aberle and others 2001). During the postmortem period, this lactic acid remains in the muscles and increases in concentration because the circulatory system is no longer capable of removing metabolites from tissues of exsanguinated animals. This causes the muscle pH to decline. The rate and extent of muscle pH decline after an animal has been exsanguinated are highly variable.

The accumulation of muscle lactic acid early in the postmortem period therefore determines the ultimate pH of the meat. Declining pH is important because acidic pH causes denaturation of proteins which leads to lower quality of meat; i.e. poor color stability due to loss in intensity of muscle pigment coloration, poor water holding capacity/juiciness, poor texture, loss of protein solubility, and poor gel-forming ability (Aberle and others 2001). The gel-forming ability of surimi is known to decreases as a function of the final pH of the fish meat used for its manufacture (Hashimoto and Arai 1978).

5.3. Species effects

Tissues from different species, and even different muscles within species, can exhibit large differences in protein composition, such as percent of myofibrillar proteins which consequently can affect protein functionality (Smith 1991). The strength of gels is quite sensitive to the content and quality of myofibrillar proteins (Foegeding 1987; Dudziak and others 1988). To a much smaller extent, gel deformability is also affected by the concentration of myofibrillar proteins.

The sensitivity to protein denaturation of the muscles also varies from species to species. Fish muscle proteins are more heat sensitive than mammalian muscles, largely because the animal is acclimated to function at lower body temperatures (Ikariya and others 1981; Kato and Konno 1993). The meat of different fishes will display different thermal stabilities based upon the habitat temperature of the fish.

Pelagic fishes, those species that are migratory and surface feeding, usually contain a higher percentage of dark muscle. This dark muscle contains higher quantities of glycogen because they are needed for sustained swimming, which causes greater post-mortem drop in muscle pH (increase in acidity). The drop in pH can dramatically affect the heat gelation properties of surimi by accelerated denaturation of the protein (Hashimoto and Arai 1978).

Thus the functional properties of surimi cannot always be predicted strictly from compositional analysis (Park and Lin 1992). Besides differing in thermal stability, many fish species are known to have higher contents of proteolytic enzymes that can degrade myosin during heating; for example, Pacific whiting, which has a heat-stable protease content associated with a parasite infestation (An and others 1994).

5.4 Animal age effects

Animal age can also affect meat protein quality. Older animals produce meat with more intense flavor and darker color since these tend to contain more myoglobin. Older animals also tend to have tougher meat since the amount of collagen cross-linking increases in mammals, and the total amount of collagen increases in fishes (Foegeding and others 1996).

5.5 Freezing, frozen storage, and thawing effects

The freshness and quality of fish are very time/temperature dependent (Park and Lin, 2005). For example, rapid chilling of a carcass can help control protein denaturation of more acidic pH meats. Freezing is an excellent technique for storage of meats for extended periods, but it does not fully prevent deterioration (Foegeding and others 1996; Haard 1992).

During frozen storage, myofibrillar proteins, especially myosin, can denature and aggregate, causing the meat proteins to lose water holding ability. When myosin unfolds, non-polar amino acids are exposed that can lead to hydrophobic interactions and protein aggregation (Jasra and other 2001). Low temperature slows down but does not stop enzymatic and chemical reactions in frozen meat (Foegeding and others 1996). For example, in fish, cathepsin protease activity decreases during frozen storage but is not fully inactivated (Jiang and others 1997).

Meat quality during frozen storage depends on how the freezing process was conducted as well as upon the characteristics of the starting material. Fish proteins are more sensitive to changes in frozen storage; thus typically cryoprotective additives such as sucrose and sorbitol are immediately mixed with wet proteins to help stabilize the proteins to denaturation and aggregation and protect them from many other deleterious influences during freezing and storage (Carvajal and others 2005).

Using correct packaging is also important during storage of meat proteins. Freezer burn, or the dehydration of the surface of animal tissue during frozen storage, can occur if poor packing is used. Freezer burn occurs when the equilibrium water vapor pressure of the air is lower than that at the surface of the meat, causing ice crystals to sublime. The result of

freezer burn is the oxidation and darkening of heme pigments and accelerated denaturation and aggregation of the myofibrillar proteins.

The water holding properties of the muscle tissues and cells can be affected by the rate of thawing (Bell and others 2000; Farkas and others 2003). A proper thawing process is also needed with some fishes to prevent the acceleration of autolysis of muscle proteins.

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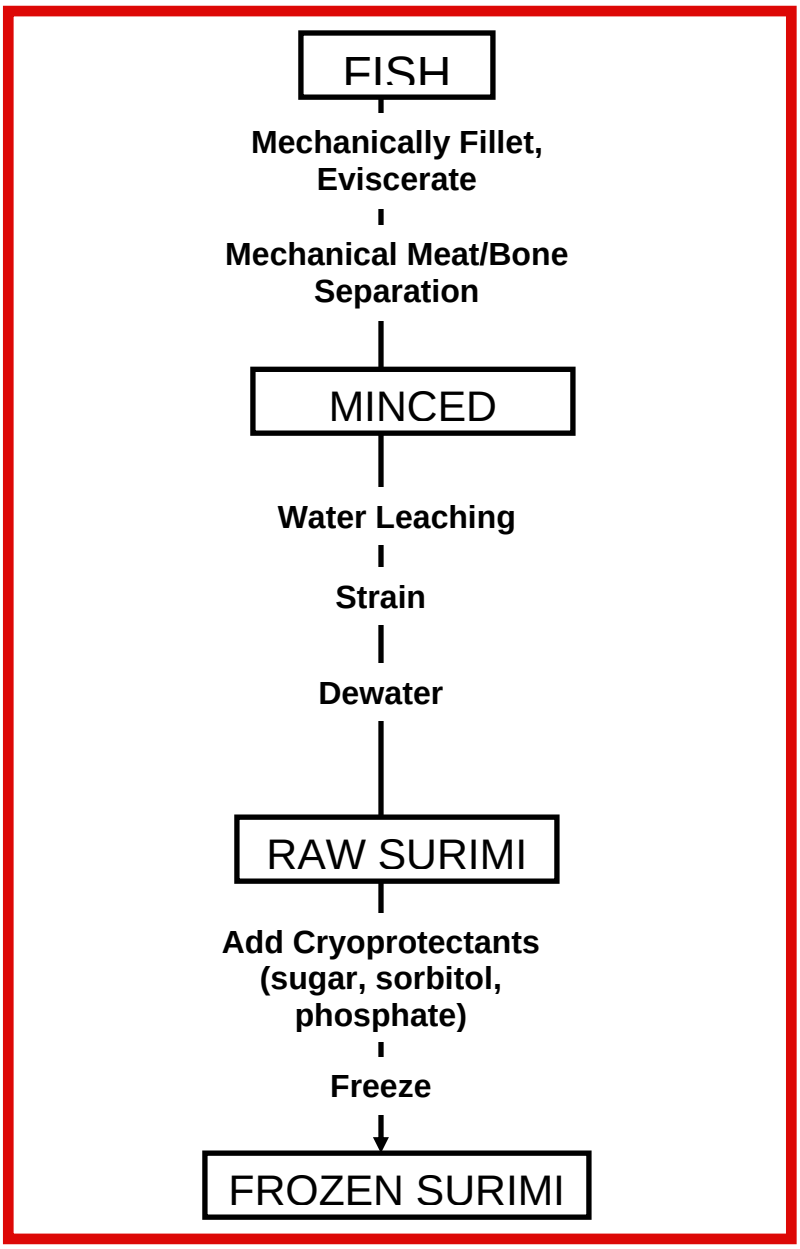

Figure 1.1 Conventional surimi manufacturing process

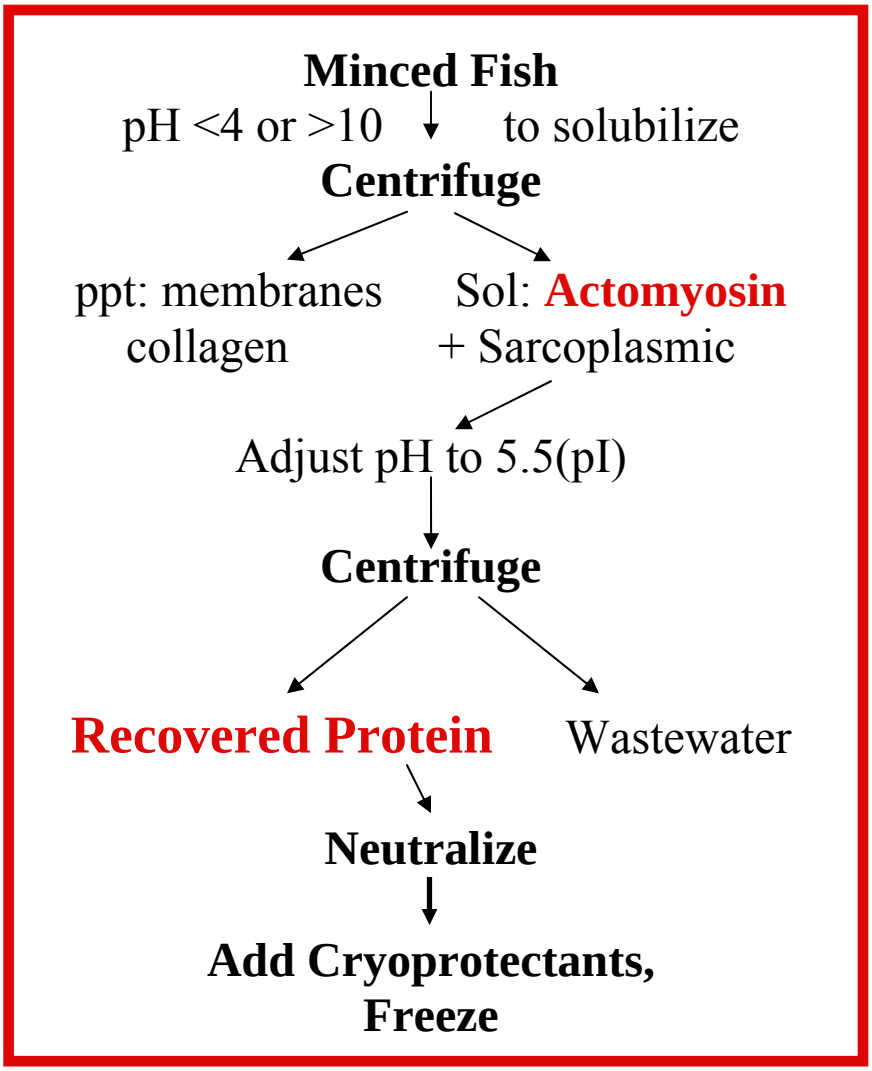

Figure 1.2 Alkali and acid-aided surimi manufacturing process

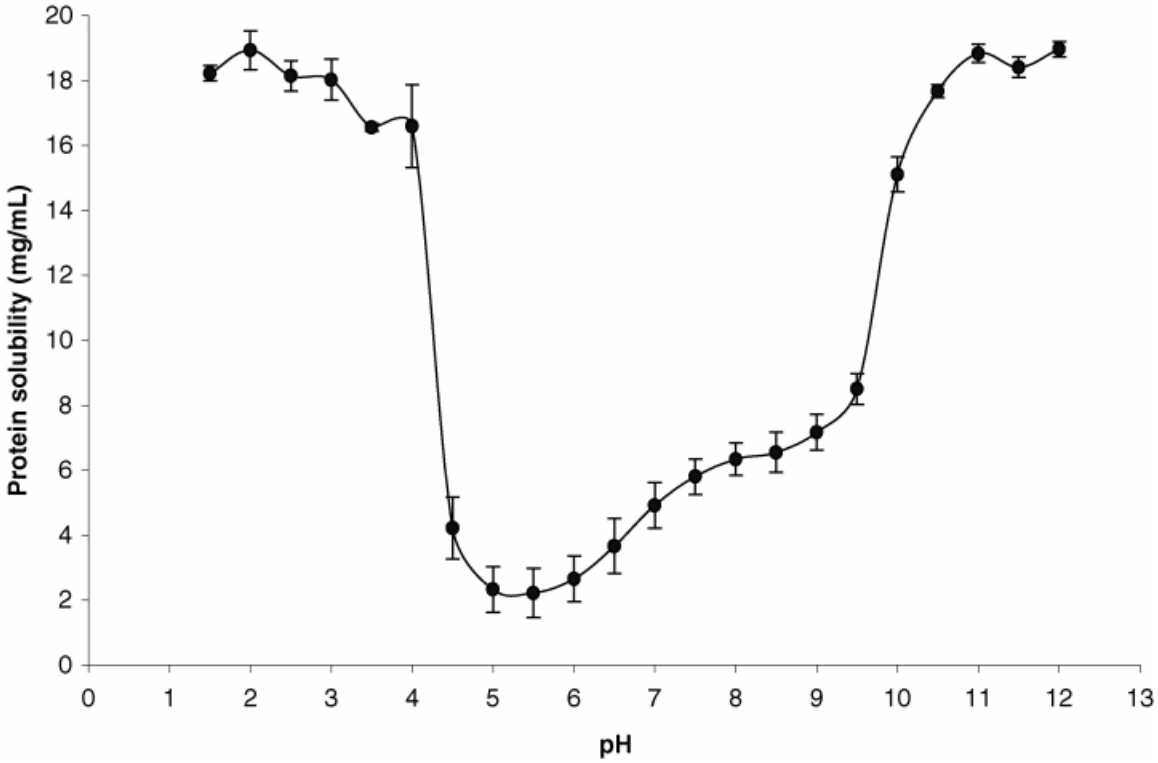

Figure 1.3 The solubility of Atlantic croaker muscle proteins from pH 1.5 to 12. Muscle tissue was homogenized with 9 volumes of deionized water, and the pH was adjusted using 2 M HCl and 2 M NaOH (Kristinsson and Liang 2006)

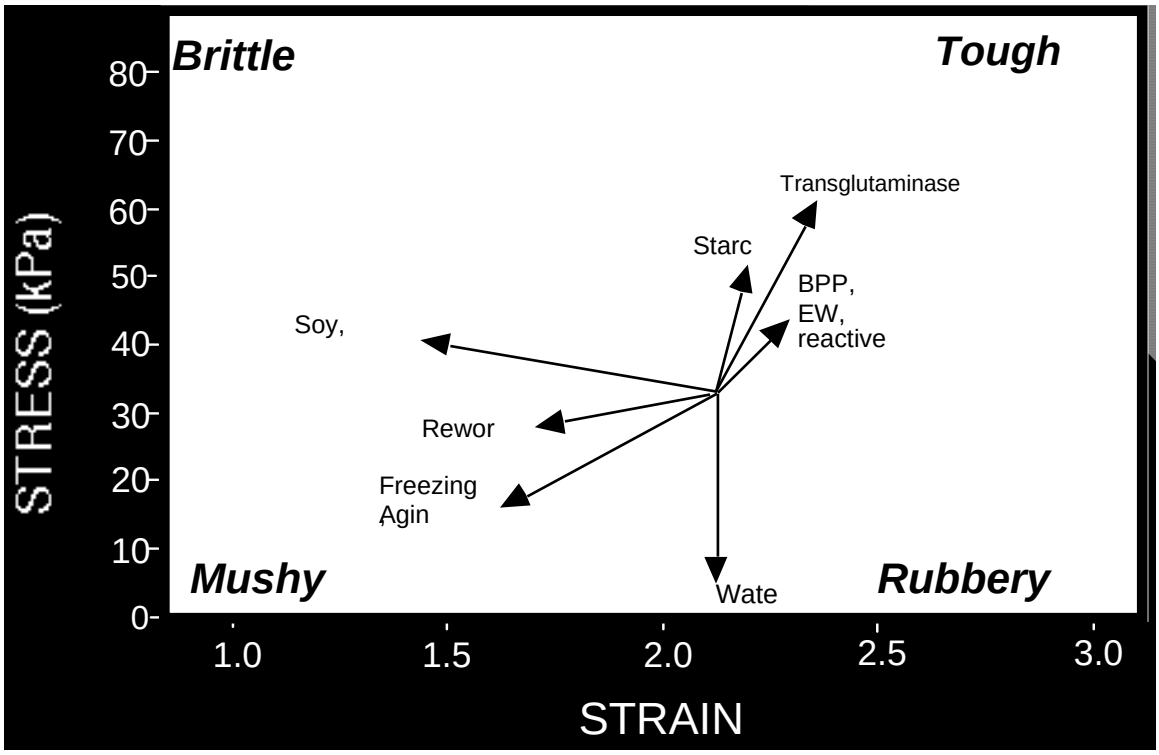

Figure 1.4 Texture map illustrating the general sensory attributes associated with changing in fracture stress and fracture strain (Adapted from Hamman and MacDonald 1992).

Manuscript I to be submitted for publication

Effect of Processing Parameters on Gelation Properties of Chicken and Fish Protein Isolates Prepared by pH-Shifting

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ABSTRACT

A new pH-shifting process for meat isolate manufacture involves solubilizing the meat proteins at high pH followed by isoelectric precipitation, induced by acid addition. This study compared the effects of meat species (chicken breast, albacore tuna, or king mackerel), the use of fresh vs. frozen meat as starting material, and different process parameters (base/acid types, acid addition rate and mixing speed, and meat to water ratio) on gelling properties and recovery yield of the precipitated protein isolate.

Acidulant type and species had the most significant effects on gelation properties of the meat isolates. Gels made from isolates prepared using acetic acid had the highest gel strength for both chicken and tuna. Hydrochloric and sulfuric acids induced opposite effects on gelation for these species. The best gels were produced using chicken breast as the starting meat. Freezing the raw material prior to processing had no effect on the gelation properties of mackerel or chicken isolates, indicating that strictly fresh, unfrozen raw material is not favored. The use of a stronger acid (HCl) concentration combined with slower agitation during its addition resulted in chicken isolate gels of poorer quality, likely due to localized damage to proteins near the incoming acid stream. No clear conclusions could be drawn from the effects of these parameters on protein yield of the pH shifting process.

Key words: Tuna, Chicken, King mackerel, Protein Isolate, pH-Shift, Processing Parameters, Fish, Alkaline

INTRODUCTION

Hultin and Kelleher (1999, 2000a) proposed a pH-shifting method for producing protein isolates from meats (usually, trimmings and frame meats are the starting materials). Sarcoplasmic and myofibrillar proteins are first solubilized at high pH whereupon the stroma (connective tissue) fraction is removed by centrifugation or screening. The solublized proteins are then precipitated near the isoelectric point by the addition of acid, which facilitates solids/liquid separation to recover the refined meat isolate. This process can yield meat protein isolates that exhibit good heat-induced gelling properties, similar to or better than surimi as commercially produced by a water-leaching process (Hultin and Kelleher 2000; Undeland and others 2002; Kristinsson and Hultin 2003; Perez-Mateos and others 2004; Perez-Mateos and Lanier 2006; Kristinsson and others 2005; Kristinsson and Liang 2006), but at higher yield (the sarcoplasmic fraction is conserved) and increased lipid stability (Kristinsson and others 2005).

This pH shifting approach to produce meat protein isolates closely follows the process scheme for production of soy protein isolates which has been commercially practiced for decades. Several reports have explored the effects of varying the raw material and process parameters used in the soy isolate process so as to achieve better control of the process and properties of the finished isolates (Nelson and Glatz 1985, Salt and others 1982, Fisher and others 1986). The present work was undertaken as an initial attempt to determine how varying the raw material meat species and several process parameters might affect the gelation properties and process yield of meat isolates produced by the alkaline pH shifting process.

MATERIALS AND METHODS

Raw Materials

Skinless, boneless chicken breasts were obtained from a local poultry processor. King mackerel (*Scomberomorus cavalla*) was purchased fresh from a local market. Frozen albacore tuna loins (*Thunnus alalunga*) were air shipped from an offshore commercial canning plant. Frozen tuna were partially thawed, then meats from each species were cut into 2 in cubes and the same-species pieces mixed to minimize differences due to individual animal variations. Except for meat isolates made from fresh chicken or mackerel, the meat cubes were then bagged in zipper-type freezer bags and stored at -23.3 °C until used (1 week to 2 months). Meats were subsequently thawed by floating the bagged cubes in ice water for approximately 1 h. All chemicals used were reagent grade and obtained from either Fisher Scientific (Waltham, MA) or Sigma-Aldrich Co. (St. Louis, MO).

Preparation of meat isolates

Fresh or previously thawed meats were finely chopped in a Stephan vertical cutter-mixer (Model 2889, UMC 5 electronic, Stephan Machinery Corp., Columbus, OH) for 1 min with 1 part water at 2400 rpm. The homogenized meat slurry was then transferred to a large drum (40 cm in diameter and 50 cm height) for further dilution with either 5 or 8 parts of ice water.

To solubilize myofibrillar and sarcoplasmic meat proteins, the pH of the muscle protein homogenate was increased to pH 11 with 2M NaOH (or KOH) added at 8.62 mL/s with a lab pump (Model PM6014, FMI Lab Pump, Fluid Metering Inc., Syosset, NY), while

mixing at 275 rpm using a 10 cm x 20 cm paint mixer rotor attached to an adjustable speed drill connected to a rheostat. The pH was periodically measured using a double junction gel pH probe (model PhTestr10; Oakton Instruments; Vernon Hills, IL) and verified with litmus paper as the junction occasionally clogged due to protein buildup. The homogenate was then screened through 1 coarse (1.7 sq mm mesh size) and 2 fine (1.3 sq mm mesh size) plastic square mesh screens to remove connective tissue, skin, and other non-soluble large particulates.

To precipitate the proteins from solution, acid solution (varied by type and normality) was then added to the solubilized meat slurry at 8.62 mL/s, while mixing with the same rotor at 70 rpm (termed "Medium" agitation), or 275 rpm (termed "Fast" agitation), or while hand stirring slowly (approximately 30 strokes/m) with a 11 cm x 13 cm blade plastic paddle (termed "Slow" agitation) until the solution pH reached pH 5.5 (near the isoelectric point). A sample of slurry with suspended precipitated protein particles (flocs) was collected to determine its density. The flocculated proteins were then dewatered through an organza fabric screen (0.3 mm mesh size) by drawing the fabric into a "bag" and manually squeezing, to attain a final moisture content of about 73%.

Dewatered meat isolates were then packed in a 1" layer in 1 gal zipper-type freezer bags, weighed to determine yield, then quick frozen on the cement floor of a -23.3 °C walk-in freezer. Samples of each isolate were oven dried to measure moisture content (AOAC 1995).

Measurement of Gelling Properties

Gel forming ability of isolates was measured according to Wright (2007) with slight modifications. The isolate samples were chopped with or without 2% NaCl and enough chilled (~4°C) water to yield a final moisture content of 78% or 80% depending on the applicable salt content. During chopping, NaOH was used to adjust pH to 7.3. Samples were vacuum packaged, then extruded into stainless steel tubes 19 mm dia and 150 mm length which were capped and cooked by submersion in a water bath. Tuna and king mackerel were cooked at 90°C for 15 min and chicken was cooked at 70°C for 20 min. The cooked gels were immediately cooled in an ice bath for 3 min, bagged, and refrigerated at 5°C

The following day gels were equilibrated to room temperature and prepared for fracture testing by torsion. Gels were cut into 28.7 mm length pieces with notched plastic disks glued to each end using cyanoacrylate glue. The cylinders were mounted on a modified milling machine and ground into capstan shapes with a center diameter of 1 cm, then mounted on the Hamann Torsion Gelometer to measure fracture stress and fracture strain of the gels (Foegeding 1992).

Experimental Design (Factorial design)

Experiment 1. Processing parameters varied during the first experiment included 2 concentrations of HCl (1M and 2M) as the precipitating acid for both fresh and frozen meat from 2 species (chicken breast and king mackerel). In addition, test gels were made with and without the addition of 2% NaCl. This experiment was expanded to include 2 different agitation speeds ("Slow" and "Medium" described previously) during the precipitation step for chicken breast.

Experiment 2. The second experiment performed on frozen chicken breast and frozen albacore tuna expanded pH adjustment variables to include a treatment where all of the HCl was added at once with very rapid stirring (275 rpm) with the electric mixer. This variable ("Fast" agitation) was trialed along with the "Medium" and "Slow" agitation rates from the first experiment. Test gels in this experiment were made with 2M HCl, with or without the addition of 2% NaCl.

Experiment 3. The third experiment performed on frozen chicken breast and frozen albacore tuna loin compared two different ratios of meat to water (1:6 and 1:10).

Experiment 4. The final experiment compared 6 different base/acid treatments. Five different precipitating acids; citric, tartaric, hydrochloric, and acetic acids along with sodium bisulfate—all 2 M, were used with 2M NaOH as the solubilizing base and the sixth treatment used 2M KOH as the solubilizing base in conjunction with HCl as the precipitation acid.

Statistical Analysis

Data were analyzed as individual experiments (corresponding to each figure) using the general linear models procedure of SAS software version 9.1 (SAS Institute, Cary, NC). All statistical inferences reported are at the 5% or lower probability level. Differences between treatment combinations were determined using paired comparisons (Student's t) on the least square means.

RESULTS

Experiment 1

The addition of less concentrated HCl (1M versus 2M) when precipitating the protein while making chicken protein isolate (CPI) generally resulted in increased (~10-15 kPa) fracture stress (strength) in gels made from CPI, but had no effect on the fracture strain (deformability) of these gels (Fig 2.1). For mackerel protein isolate (MPI), the concentration of the HCl used for precipitation had no effect on either gel property (Fig 2.2). The use of fresh versus frozen meat when making the isolate also did not affect the gel properties for either species. It should be noted, however, that these meats were only held 2 weeks in frozen storage as this experiment was intended more to judge whether protein isolate must be made at the processing facility or whether frozen product could be shipped to a different location for isolate production.

CPI gels prepared with 2% added NaCl had higher fracture stress than gels made without NaCl; however when data were adjusted for the difference in moisture content caused by the addition of salt (changing from 80% without NaCl to 78% with NaCl), these differences were not significant. For MPI gels, no change in the fracture stress of gels was observed when NaCl was included in the gel formulation. Fracture strains for gels made from both species were also unaffected by NaCl addition. The different processing variables used did not affect % solids yield for either species (Figs 2.3 and 2.4).

After finding significant differences in gel properties when different concentrations of HCl were used in CPI preparation, the experiment was expanded to include a slower rate of agitation in combination with differing HCl concentration. The slower agitation speed

resulted in decreased gelation properties for CPI (both fracture stress and strain; Fig 2.5), with fracture stress particularly affected when 2M HCl was used. It is likely that HCl was concentrated in the localized area where the acid was pumped into the slowly stirred solution, causing permanent damage to these local proteins which would in turn lead to the loss of functional protein for gelation.

Experiment 2

To further test the effects of agitation rate and rate of pH adjustment on protein isolate properties, the next experiment introduced a very "Fast" agitation rate (275 rpm with a paint mixer rotor) in combination with a very fast rate of HCl addition (all HCl added at one time) in addition to the previous (from experiment 1) "Medium" agitation rate of 70 rpm with the paint mixer rotor and "Slow" agitation rate of 30 strokes/min with the paddle; all with addition of HCl at the single 2M concentration. In this experiment, only frozen meat was used and albacore tuna was used instead of king mackerel. Since the results from experiment 1 showed that use of frozen meat was likely not a factor in this process, we chose to continue our experiments with only frozen meats. Frozen albacore tuna was more available than mackerel and additionally gave us a second fish species to compare to the first.

Gelation properties for CPI made with fast adjustment of pH in combination with fast agitation were statistically similar to those made with the medium agitation rate only when NaCl was included in the batter formulation (Fig 2.6). Without the addition of NaCl, CPI gels made with the "Fast" rates of agitation and pH adjustment had lower fracture stress and strain values than those made with the medium pH/agitation treatment (with and without NaCl), and these were similar to those for gels made from CPI prepared with the slow

agitation and pH adjustment. Water loss was greatest for the "Slow" and "Fast" CPI treatments in gels made without NaCl (Fig 2.7).

There were no differences in gelation properties for tuna protein isolate (TPI) made with the different pH adjustment methods, either with or without the addition of NaCl during gel paste preparation (Figs 2.8 and 2.9).

The total yield of CPI was greatest when pH was adjusted quickly (all acid added at once) with 'Fast" agitation, followed by CPI made with a "Medium" agitation rate. Yield was significantly lower when the slow agitation rate was used to mix the HCl into the solubilized protein solution. For TPI, yields were not affected by the agitation speed used during its preparation (Fig 2.10).

Experiment 3

Preparing isolates from frozen chicken breast or frozen tuna using a greater water to meat ratio (10:1 compared to 6:1) did not result in changes to the isolate properties (Figs 2.11 and 2.12). Gels made from this CPI and TPI had the same fracture stress, strain and water loss as gels made from the isolates using a 6:1 water to meat ratio. Yields were similar also (Fig 2.13).

Experiment 4

When different precipitating acids were used in making CPI and TPI, results were not easy to interpret. Overall, isolates made with acetic acid as the precipitating acid resulted in gels of the best quality (Figs 2.14, 2.15, 2.16 and 2.17). For CPI made with acetic acid, the

addition of NaCl to the paste improved the fracture stress and strain of the gels, while for TPI made with acetic acid; the gels formulated without NaCl had higher fracture stress but equal fracture strain as those made with NaCl. For CPI the use of other precipitating acids resulted in gels of lesser but good quality with no significant differences between them, while for TPI, those made with sulfuric acid produced gels with excellent gelation properties while the three other precipitating acids (HCl, phosphoric and sodium bisulfate) produced TPI with much poorer gelation properties. The use of KOH as the solubilization base (HCl was the precipitating acid) resulted in gelation properties equal to those of protein isolates made with NaOH for both chicken and tuna. However, water loss for KOH/CPI gels made without NaCl was significantly greater than for gels made from NaOH/CPI prepared without NaCl (Figs 2.18 and 2.19).

Isolate yield was greater for CPI made with the new acids compared with that made with HCl, but for TPI yields were greatest when HCl, acetic acid, sulfuric acid or sodium bisulfate were used (Fig 2.20). Solids yields for TPI made with KOH/HCl or phosphoric acid were lower than for the other preparations made from tuna.

Discussion

Background: gelation mechanisms of meat isolates. Several theories have been proposed to explain the enhanced gelling properties of meat protein isolates made by the pH shifting process. One theory is that the pH shifting process disrupts the myofibrillar structure more completely than even a conventional surimi process, thus dispersing the released proteins more homogenously; a factor known to enhance gel properties (Sato and Tsuchiya 1992)

presumably by increasing the protein surface area available for reactivity in gelation (Wright 2007). Wright (2007) alternatively suggested that, based on work with milk proteins when proteins are precipitated out of solution, the particular size or shape of aggregates formed are of a fractal nature building upon a single type of primary particle and that this fractal geometry could secondarily influence the type of gel network (Marangoni and others 2000; Ould Eleya and others 2004; Zhong 2003). A third theory, proposed by Kristinsson and Hultin (2003), was that conformational changes in the myofibrillar proteins induced by the pH-shifting favor better gelation (the protein surfaces are made more available or attractive for protein-protein interactions). They showed that the pH-shifting processes causes conformational changes in the globular head fraction of the myosin heavy chain leaving it in a molten globule configuration (Hultin and others 2005). Additionally, most of the myosin light chains usually associated with the head region are lost during both extreme pH treatments leaving exposed thiol groups in the head region for later intermolecular bonding when heated (Kristinsson 2001; Kristinsson and Hultin 2003).

Thus, any processing parameters that will 1) disrupt myofibril microstructure, leading to more homogeneous dispersion of myofibrillar proteins, and/or; 2) produce a fractal arrangement of proteins, or aggregated primary particles when proteins precipitate out of solution, that could secondarily influence the type of gel network formed, and/or; 3) cause conformational changes to proteins that provide more reactive groups at the surface; will likely enhance the gelation properties of meat proteins.

Effects of Added NaCl. Protein isolates produced by the pH shifting process have been shown to exhibit excellent gel forming ability even when no salt is added (Chang and others 2001; Wright 2007). Usually, however, the first step in preparing fine-particle gelled meat products is the comminution of the muscle tissue with salt (Foegeding and others 1996). Sodium chloride is added to solubilize the myofibrillar proteins (Hamm 1986; Niwa 1992) by disrupting ionic bonds that stabilize muscle ultrastructure (both intermolecular and intramolecular), thus disrupting the proteins' native state (Munasinghe and Sakai 2004) and yielding a thick sol or paste. Subsequently heat induces individual myofibrillar proteins to unfold and bond to form a gel (Foegeding and others 1996).

While protein isolates have been shown to form strong and deformable heat-induced gels without added NaCl, in our experiments there were generally positive effects of added salt on gelation properties. This was also noted by Wright (2007) in similarly prepared gels made from king mackerel. The microstructure of the salt-added treatment showed greater solubility of proteins, evidenced as "spiderweb-like" structures in electron micrographs of the gels. These also showed greater protein dispersion when salt was added. Gels made without added salt were more fibrous and long stranded, while gels made with added salt displayed aggregated proteins that were more spherical and bead-like. Additionally, the spaces between the fibers/aggregates appeared to be more filled with solubilized protein when gels were made with added salt.

Fresh vs. Frozen Starting Material. In the present study, the effect of using fresh versus frozen meat as the starting raw material was trialed for chicken breast and king mackerel.

Raw material protein quality is known to play an important role in finished product quality and functionality (Aberle and others 2001). Freezing is an excellent technique used to preserve meats over longer periods of time. However, this method does not fully prevent deterioration and does not completely conserve meat quality in that, during frozen storage, myofibrillar proteins, and especially myosin, can denature and aggregate causing the proteins to lose gelling or other functionality (Connell 1960; 1962; Foegeding and others 1996; Haard 1992; Jasra and others 2001; Park and others 1987; 1993).

It is evident, however, that the short frozen storage time used in this study (two weeks) was not sufficient to effect any significant change in gelling properties of the meat isolates, suggesting that isolate production need not depend upon strictly fresh meat supplies for raw material, as does the commercial production of surimi from most fish species (Park and Lin 2005). Longer term frozen storage could contribute to lowering the quality of proteins isolates however.

Effects of agitation and pH adjustment rates. In the isolate making (pH shifting) process, as the acid is added to precipitate the soluble protein, local changes in pH that occur in the immediate vicinity of the acid stream are greater than the global pH change for the entire batch. Most proteins are known to be irreversibly denaturated/damaged at extremes in pH (Salt and others 1982; Bell and others 1983). To reduce the possibility of this localized damage, mixing/agitation is usually applied during acid addition. However, very fast agitation could also cause protein damage due to the high shear forces (but mainly only when aeration/foaming occurs simultaneously; Fisher and others 1986). An additional concern

with fast agitation is the greater probability of floc breakage, leading to smaller flocs (Tomi and Bagster 1978; Glasgow and Luecke 1980) and possibly lower yields; these decreasing with increased speeds.

The results from experiment 1 showed that "Slow" agitation resulted in CPI gels of poorer quality than those made with "Medium" agitation. This effect was more pronounced when using the 2M HCl, suggesting that local concentrations of strong acid did indeed damage the proteins in CPI when "Slow" agitation was used.

Nelson and Glatz (1985) studied effects of agitation rate on primary particles and final aggregate properties of soy protein during acid precipitation. Their results showed that agitation rate did not have an influence on the primary particle size; therefore, it did not influence the final aggregates either. On the other hand, a study by Rohani and Chen (1993) found that primary particle size and growth rate increased with increased stirring speed when conducting an isoelectric precipitation of canola protein.

Fisher and others (1986) conducted acid precipitation of soy protein with two extremes in pH adjustment (very rapid with no mixing in the first minute vs. very slow via acid dialysis with complete mixing). They proposed that when all of the acid was added at once to the solubilized soy solution a large number of nucleation sites were created since the interactions of acid with protein molecules would be greatly increased compared to the slow release of acid from a dialysis bag. Although they found differences in primary particle size (larger for the instantaneous introduction of acid), they did not find any differences in yield or microstructure in aggregates between the two pH adjustment treatments.

If larger primary particle sizes favor a fractal arrangement that in turn influences the type of gel network that will form upon heating, then rapid acid addition (if it does not damage the protein) could increase the gelation properties of the protein. However, our results from experiment 1 found the opposite to be true; that CPI adjusted with 1M HCl (Slow) had better stress (gel strength) than CPI adjusted with 2M HCl (medium). Also, results from our experiments did not show an effect on gelation properties between the two higher agitation rates used.

Ke (2006) studied the rate of acidification during the pH shifting process with albacore tuna and used sodium polyphosphate and malic acid to provide buffering capacity in the protein solution during the acidification so that tuna proteins were not exposed to strong acids. He found that when alkaline solubilized protein was neutralized with diluted HCl slowly (approximately 1.5 hour acidification time in 50g tuna mince suspended in 9 parts of water), a fine ultimate floc formed. The ultimate flocs were small enough to pass through a 1 mm screen. In contrast, when a predetermined amount of 2 N HCl was quickly poured into the solublized protein isolate, large flocs formed which were retained by the 1 mm screen. The observed results were consistent over several trials. Fisher and others (1986) also found that rapidly changing the pH produced larger and stronger flocs. Therefore, higher recovery yield would be expected from fast pH adjustment since the recovery yield for both centrifugation (based on Stokes' Law; Stokes 1888) and screening is primarily based on floc size. In experiment 2 "Slow" agitation rate/pH adjustment resulted in lower solids recovery yield for CPI than those made with a "Medium" and "Fast" rate of agitation/pH adjustment. However, no effect of agitation/pH adjustment rate was seen in percent solids recovery for

TPI. Possibly, the mean ultimate flocs produced by "Slow" pH adjustment were small enough to go through our screen during dewatering, whereas mean ultimate floc sizes were larger than the screen mesh in other treatments.

Effect of protein concentration during precipitation. Protein concentration of soluble soy has been shown to have an effect on aggregate size developed during acid precipitation in that the rate of aggregation increased due to increasing collision frequency at higher protein concentrations (Nelson and Glatz, 1985), also resulting in a slight increase in primary particle size. Virkar and others (1981) also found that the mean aggregate size increased when the protein concentration was increased. Rohani and Chen (1993) reported similar results for isolates produced from canola protein.

However, in Experiment 3 there was no clear conclusion on the effect of protein concentration in the making of meat isolates since gelation properties of CPI and TPI responded oppositely to protein concentration differences.

Effects of acidulants or bases. Hultin (2007) noticed that changing the acid used to precipitate the proteins seemed to affect the size of protein flocs. In the present study, the use of acetic acid as the precipitant yielded CPI and TPI with the best gelation properties compared to all other acids trialed. Other than this similarity, different precipitants used in the isolate making process affected gelation properties variably. Most interesting was the difference between the two meat species when HCl and sulfuric acids were used in the isolate

making process. For CPI, the use of sulfuric acid resulted in gels of poor quality, and the use of HCl resulted in gels of excellent quality, but for TPI the exact opposite was true.

Salt and others (1982) studied the effect of varying acids on recovery of native proteins from soy. They found that the use of concentrated hydrochloric acid to induce precipitation caused damage to the proteins at lower mixing speeds while the use of concentrated sulfuric acid had only a small effect on protein native structure (measured by quantitative PAGE). Nitric acid induced little loss of native structure, while both phosphoric and trichloroacetic acids caused more marked damage to soy proteins, particularly to the glycinin subunit.

Nelson and Glatz (1985) found that the size of the primary particle aggregates was affected by the precipitating agent, in that when hydrochloric acid was used, slightly larger primary particles formed than when using H_2SO_4 or calcium acetate as the precipitating agent.

With respect to the effects of the base used for solubilization, Raghavan and Kristinsson (2008) studied changes in the conformation of catfish myosin as affected by the base used; the ultimate pH attained, and salt addition. During solubilization, the pH was shifted to 11.0, 11.5, and 12.0 using either NaOH or KOH. As soon as the pH target was reached (myosin presumably maximally unfolded), the pH was immediately adjusted back to 7.3 to refold the myosin. This unfolding/refolding process increased the gel rigidity (G') of thermally treated myosin. Greater denaturation but stronger gelling ability (G') was seen when using KOH than when NaOH was used to unfold the protein. In our results, however,

similar gelation properties were obtained with both KOH and NaOH as the solubilization base.

Species Differences Gels made with CPI consistently gelled better than gels made from either fish meat isolate. The sensitivity of proteins to denaturation varies from species to species; some muscle proteins are more sensitive than the others, primarily based on body temperature (which for fishes is largely dependent on habitat temperature. Thus chicken would be expected to be the more robust of the species tested. Moreover, the tuna had been frozen for an indeterminate time (most likely several months) which would favor poorer functionality. Yet the more fresh mackerel meat did not produce isolates that gelled better than the tuna.

CONCLUSIONS

High acid concentration seemingly damaged the protein gelling functionality. Thus, agitation rate during precipitation is important to reduce the possibility of this localized damage of protein by exposure to high local concentration of acid during its addition. Isolates prepared using acetic acid, the weakest acid trialed in this study, produced gels which exhibited the best gelation properties for both chicken and tuna; possibly this reflects less damage to the protein caused by this acid. However, isolates prepared using hydrochloric and sulfuric acids induced opposite effects on gelation properties of chicken vs. tuna. Thus the effect of acidulant type is not straightforward and should be further investigated.

Freezing for up to two weeks showed no effect on gelling properties of chicken isolate. This would indicate that isolates do not have to be made only when the raw material is fresh, such that frozen raw materials could be accumulated and/or shipped from a different location for isolate production. This could be a key advantage for the isolate process as compared to a conventional surimi process, though the present study did not compare these two methods in this regard.

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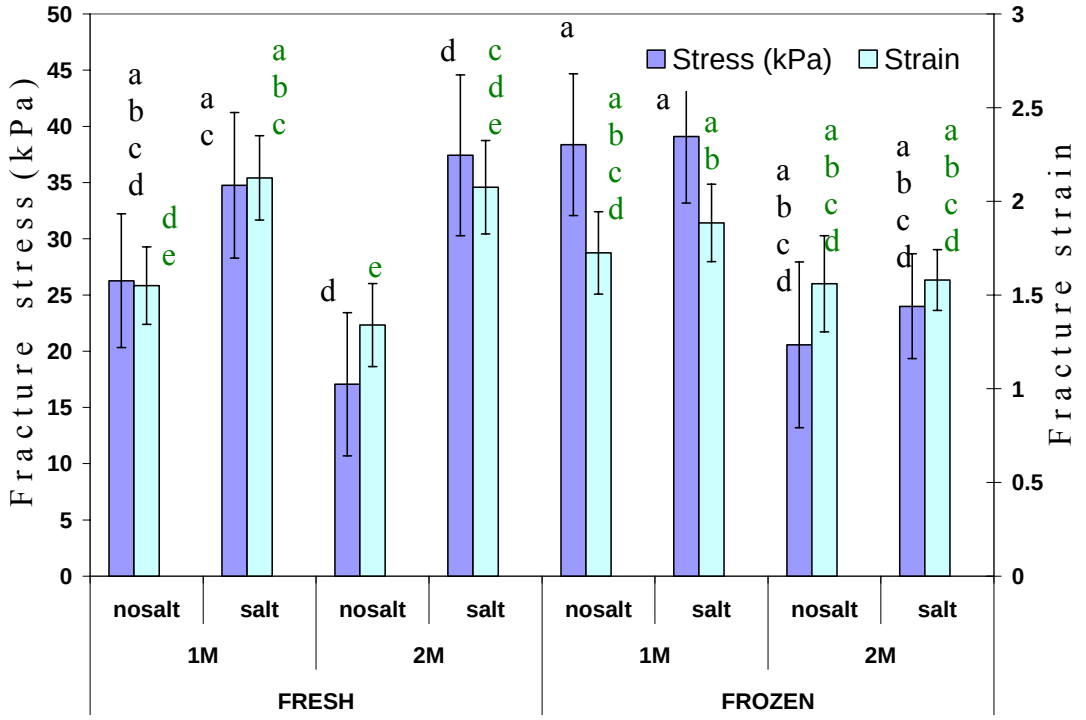

Figure 2.1 Fracture stress and strain of gels made from **chicken** protein isolate prepared with a **medium** agitation rate. Error bars are the standard error for the least squares means analysis. Bars with different letters are statistically different at the $p<0.05$ level.

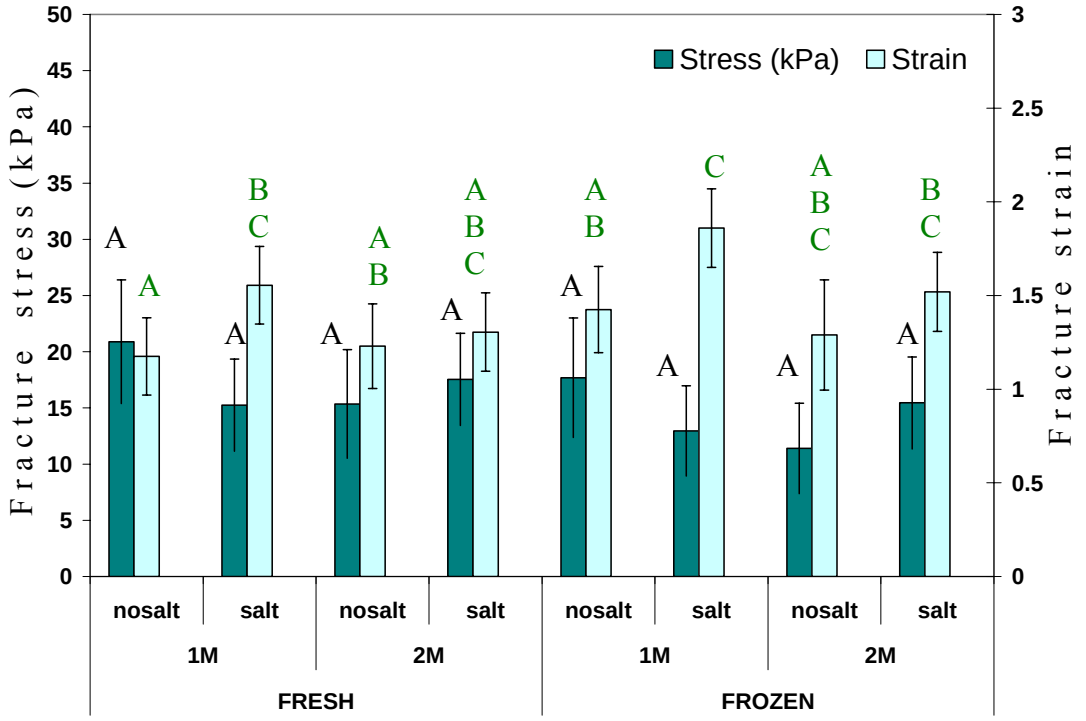

Figure 2.2 Fracture stress and strain of gels made from **king mackerel** protein isolate prepared with a **medium** agitation rate. Error bars are the standard error for the least squares means analysis. Bars with different letters are statistically different at the $p<0.05$ level.

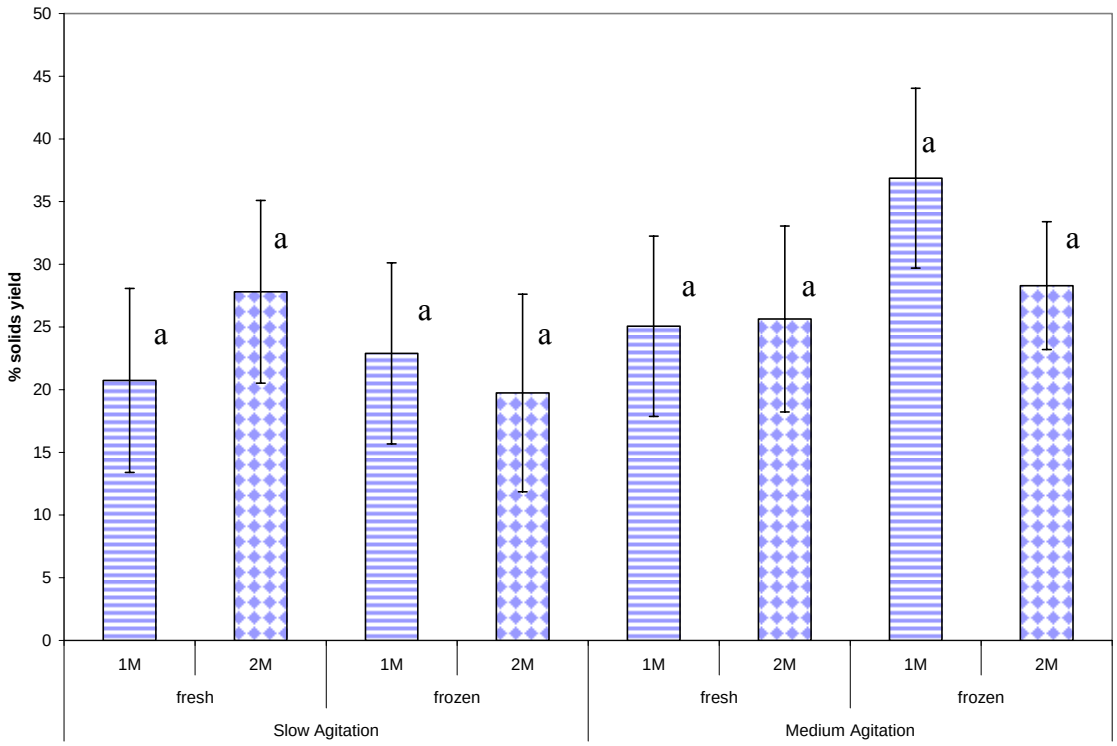

Figure 2.3 Percent solids yield of chicken. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

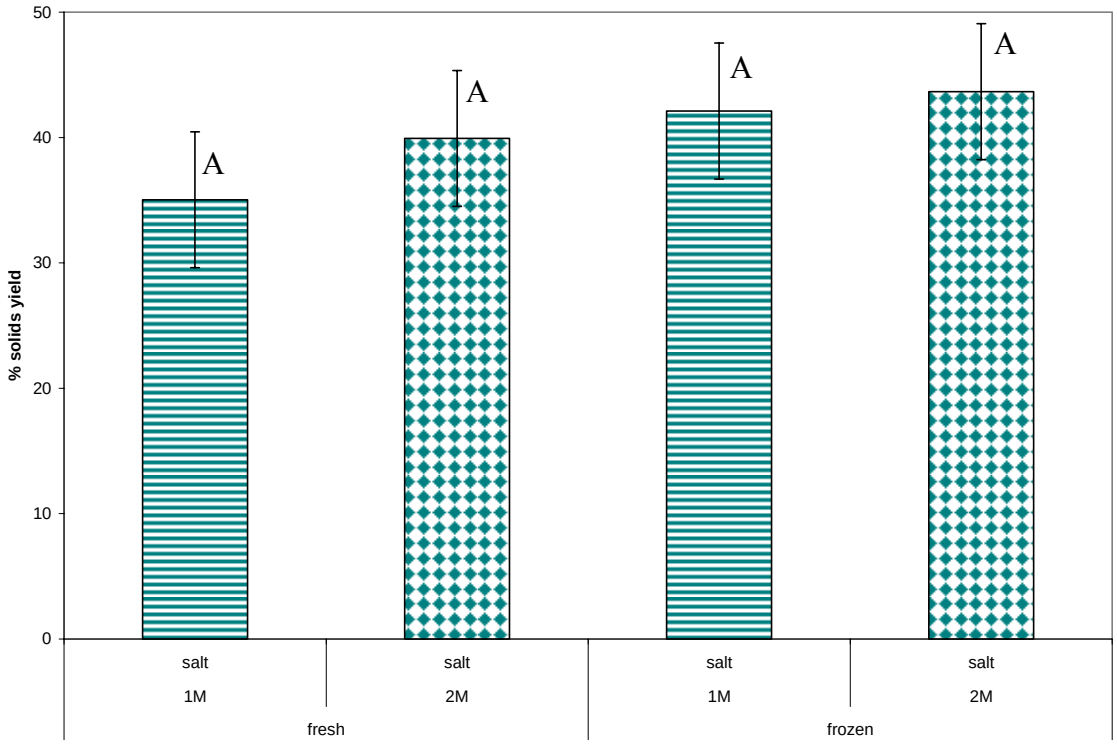

Figure 2.4 Percent solids yield of king mackerel. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

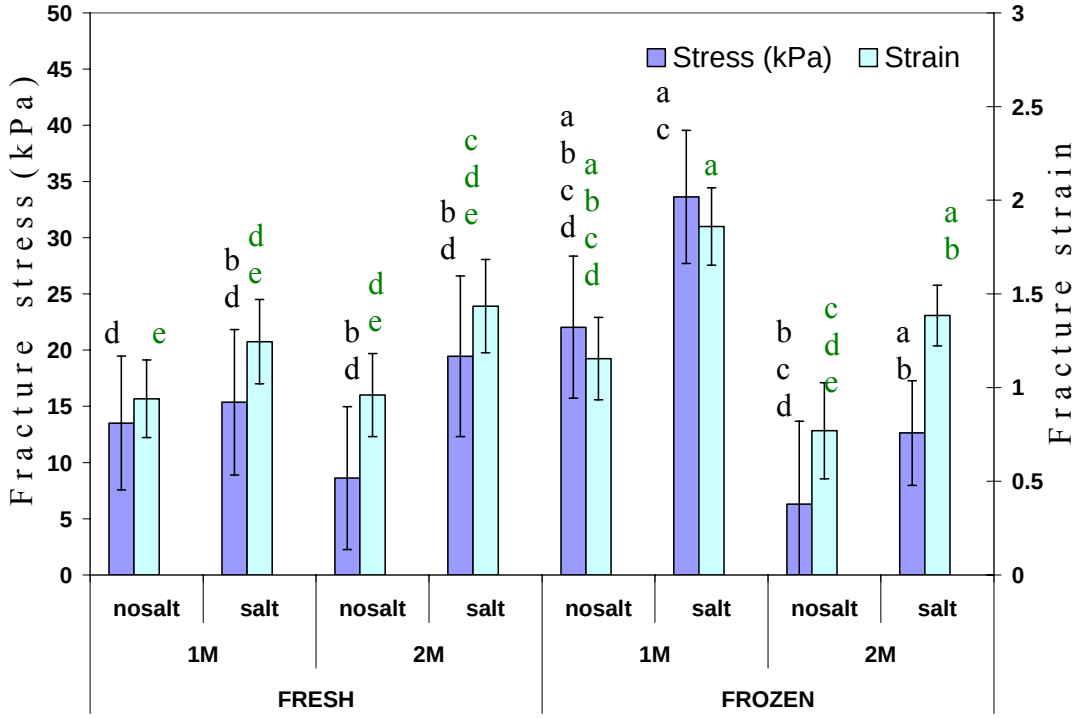

Figure 2.5 Fracture stress and strain of gels made from **chicken** protein isolate prepared with a **slow** agitation rate. Error bars are the standard error for the least squares means analysis. Bars with different letters are statistically different at the $p<0.05$ level.

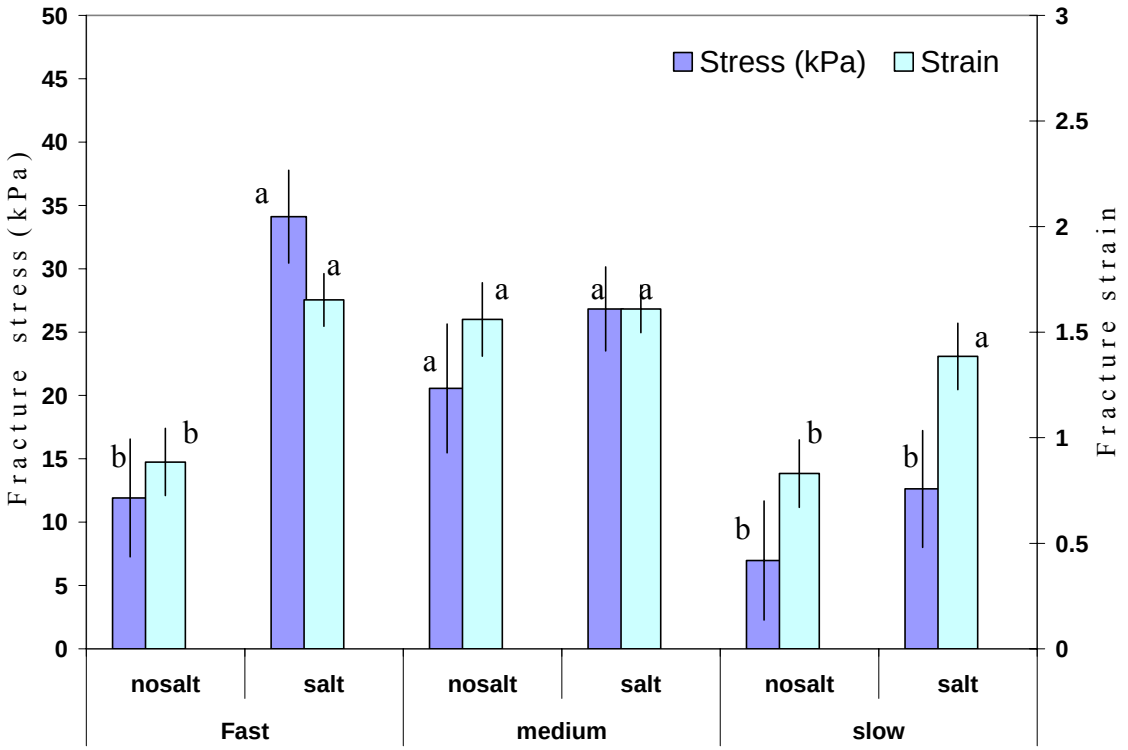

Figure 2.6 Fracture stress and fracture strain of chicken gel prepared by three different speeds of acid addition. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

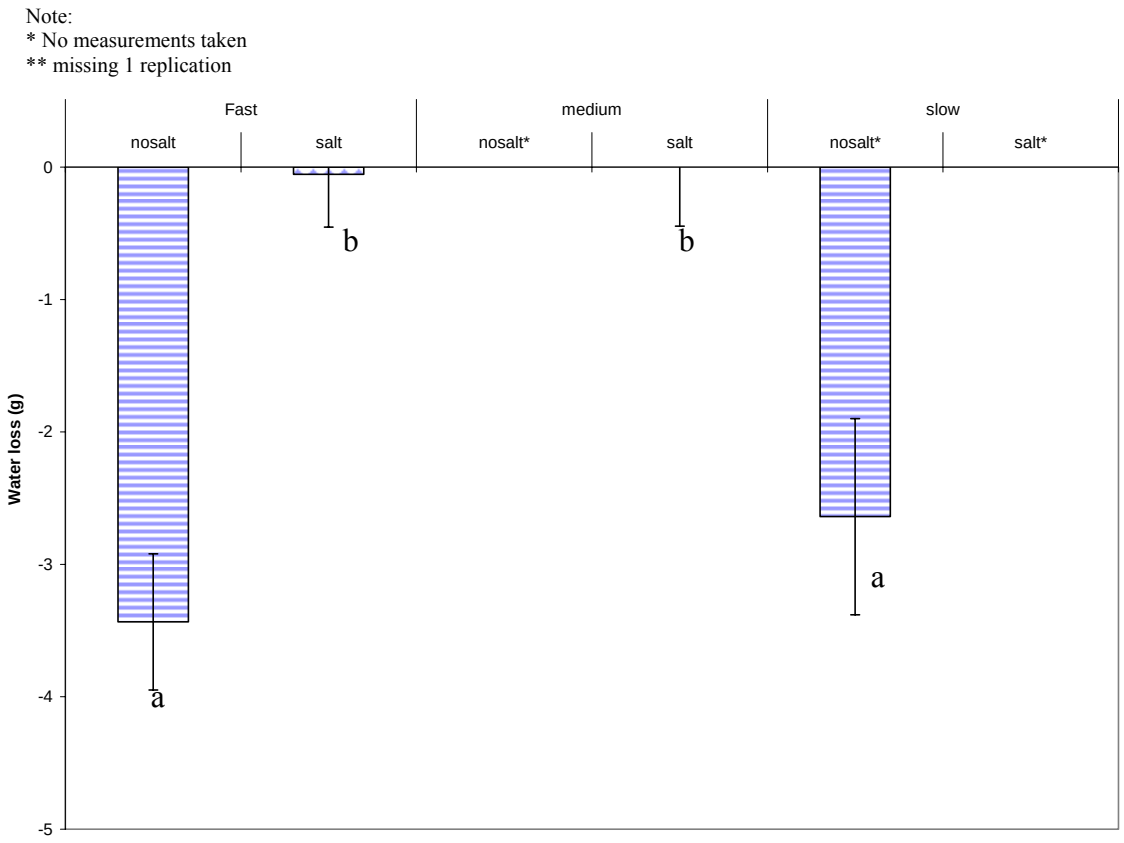

Figure 2.7 Water loss of chicken gel prepared by three different speeds of acid addition. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

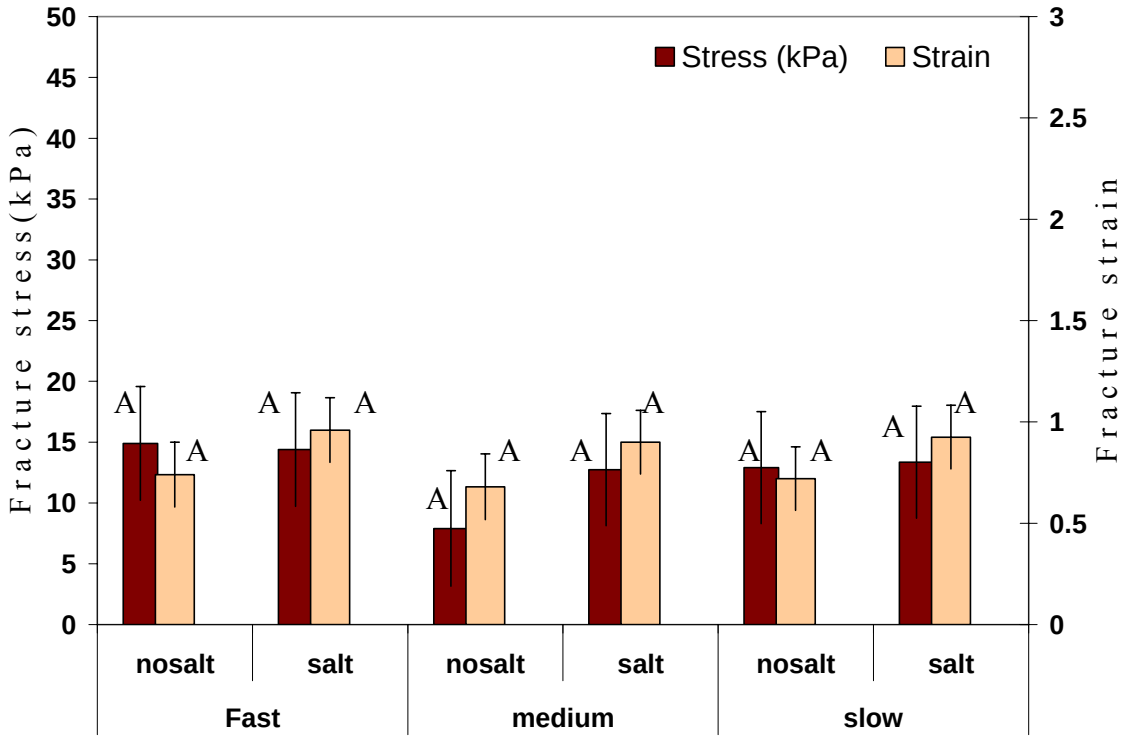

Figure 2.8 Fracture stress and fracture strain of tuna gel prepared by three different speeds of acid addition. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

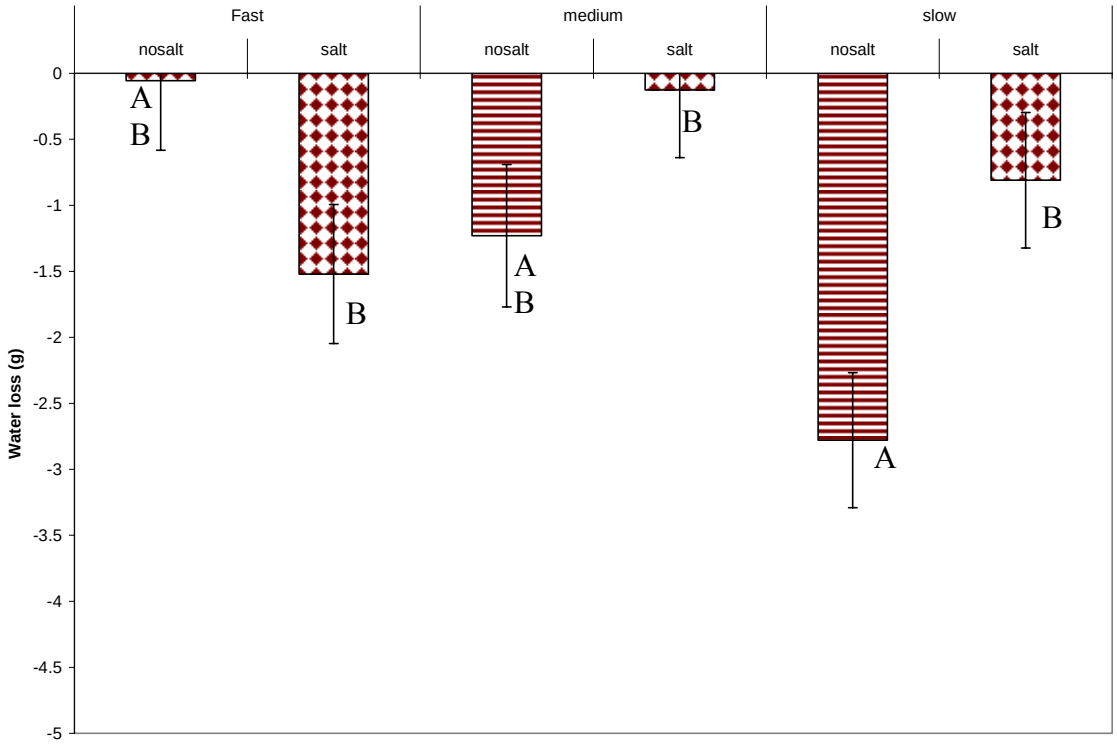

Figure 2.9 Water loss of tuna gels prepared by three different speeds of acid addition. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

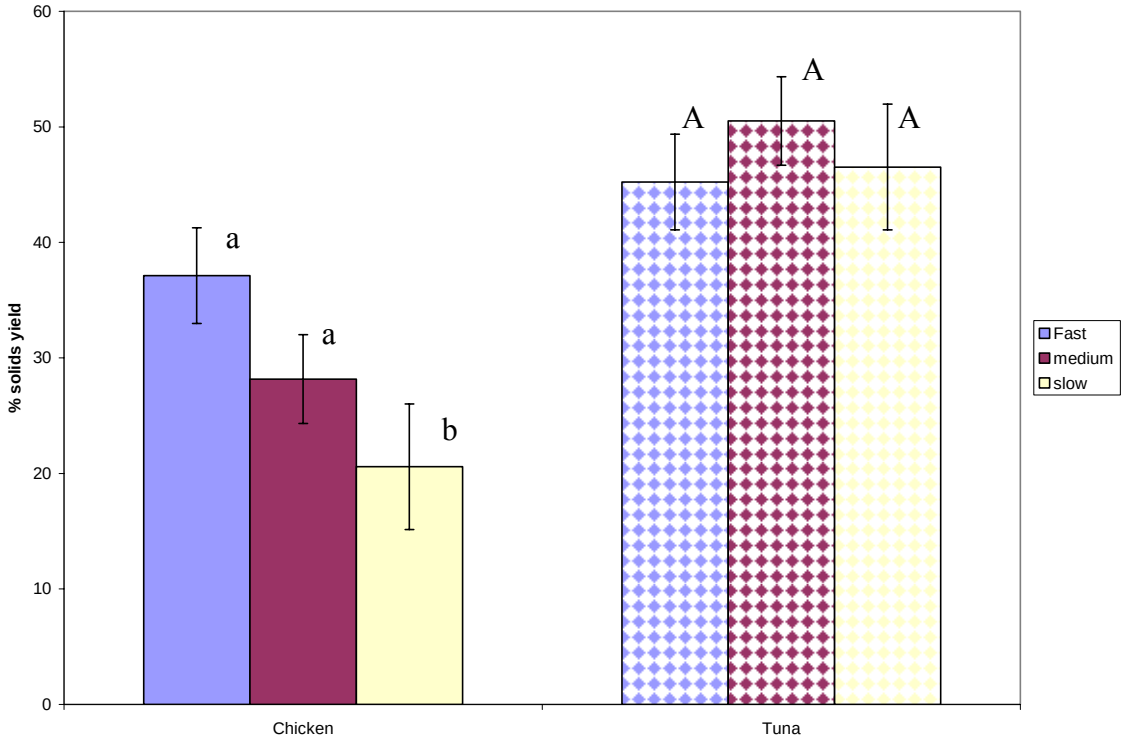

Figure 2.10 Percent solids yield at 5.5 of chicken and tuna isolate prepared by three different speeds of acid addition. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

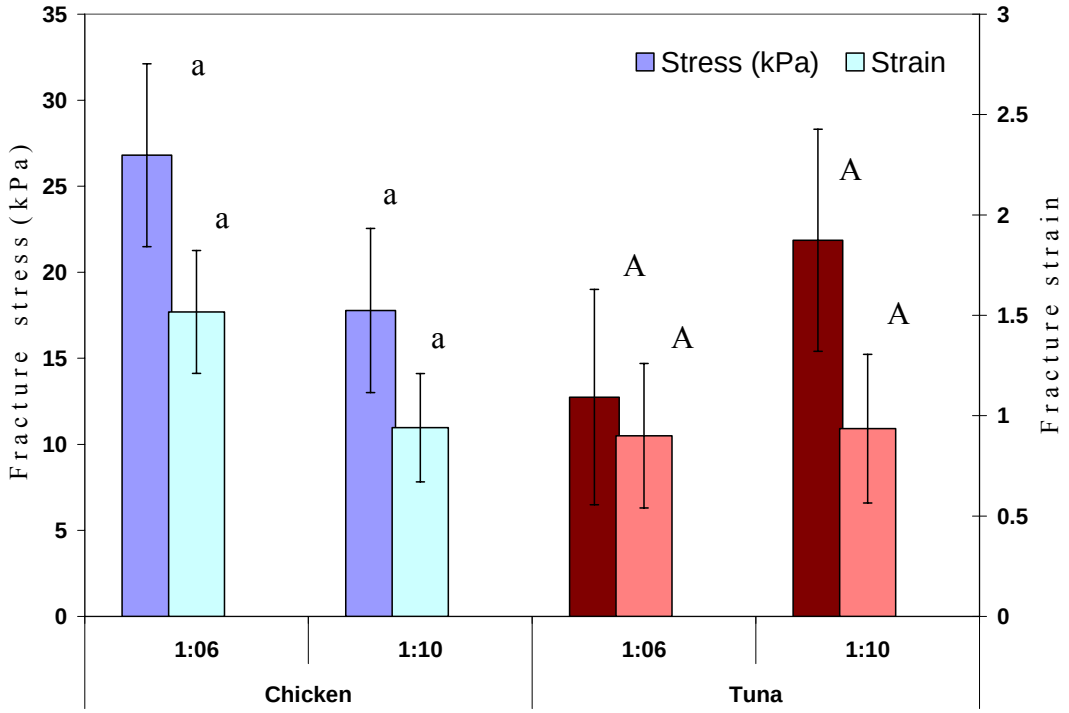

Figure 2.11 Fracture stress and fracture strain of gels made from chicken and tuna protein isolate prepared with either 1:6 or 1:10 meat to water ratio. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

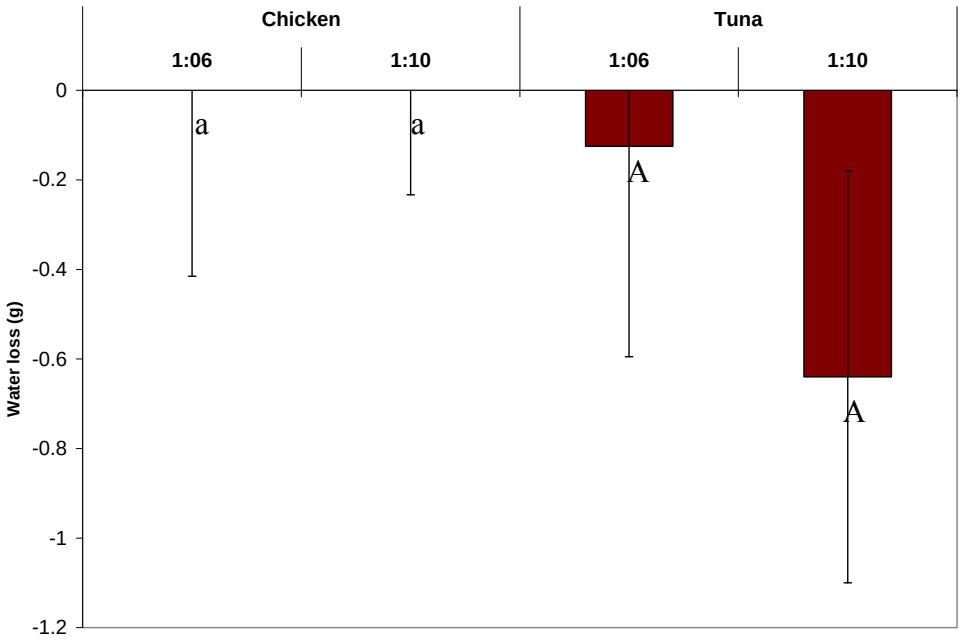

Figure 2.12 Chicken and tuna protein isolate density at pH 5.5 prepared with either 1:6 or 1:10 meat to water ratio. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

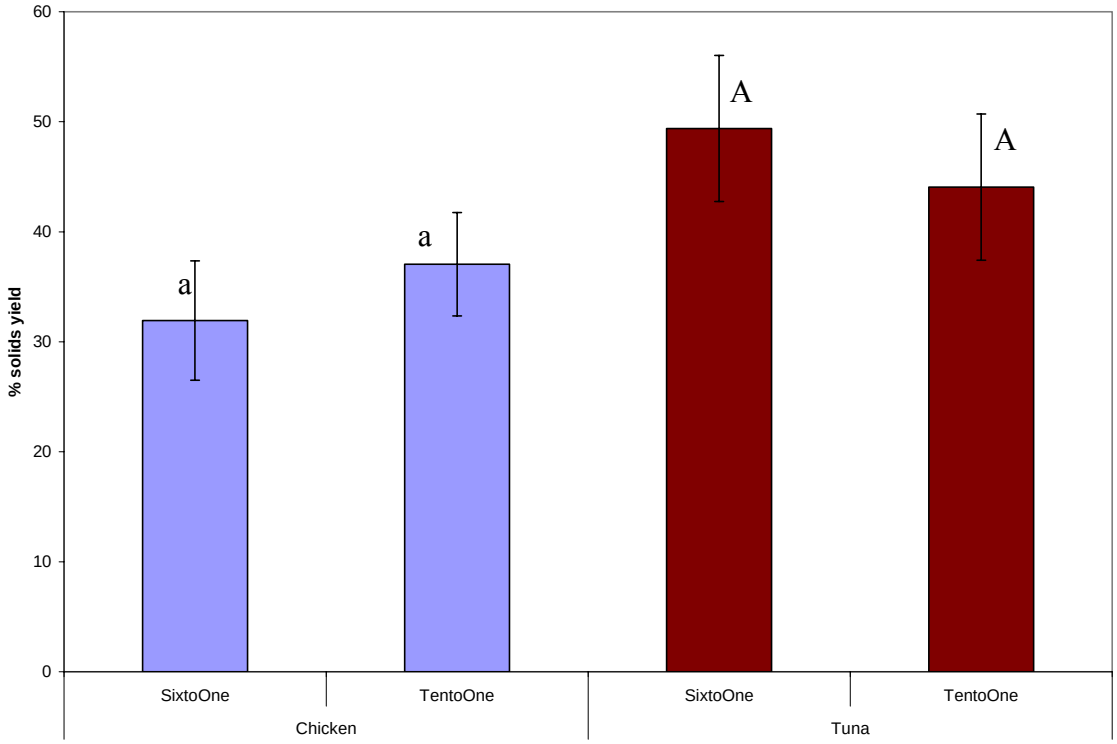

Figure 2.13 Chicken and tuna protein isolate yield prepared with either 1:6 or 1:10 meat to water ratio. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

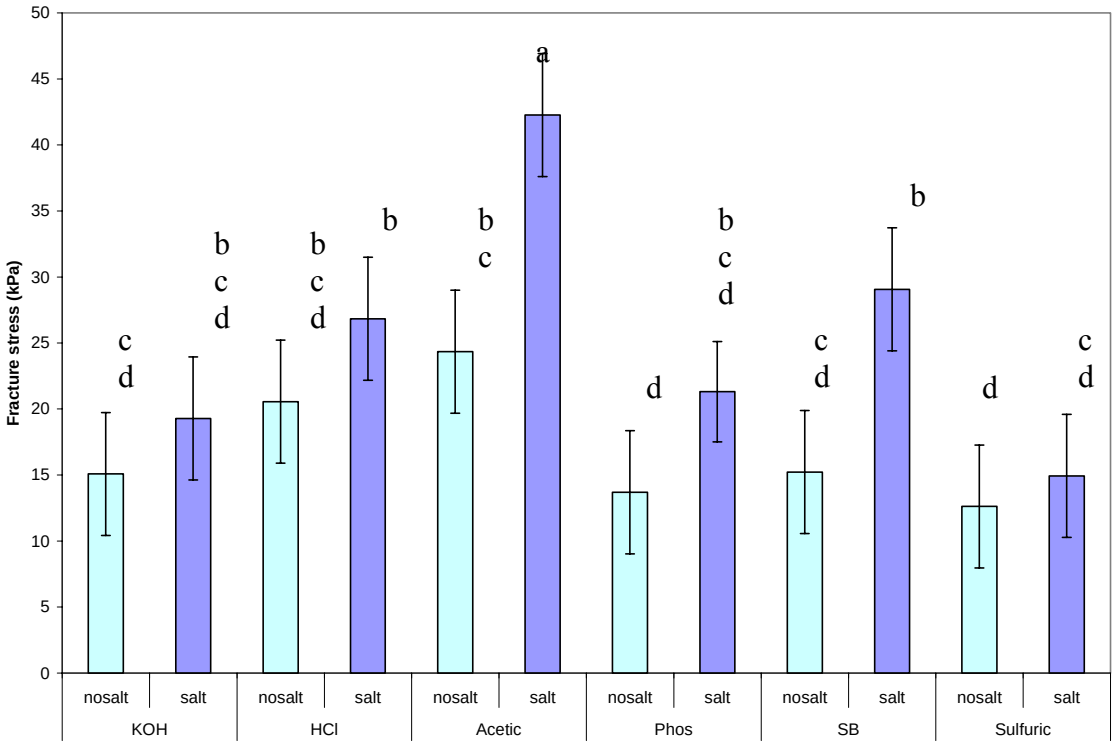

Figure 2.14 Fracture stress of gels made from chicken protein isolate prepared with a different acids and base. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

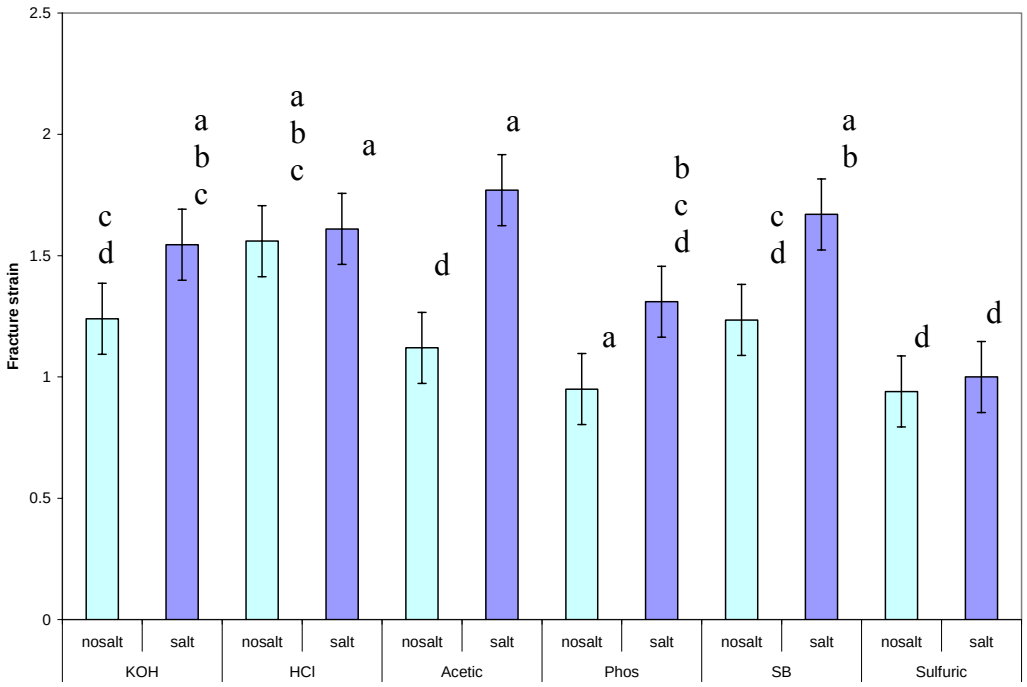

Figure 2.15 Fracture strain of gels made from chicken protein isolate prepared with different acids and base. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

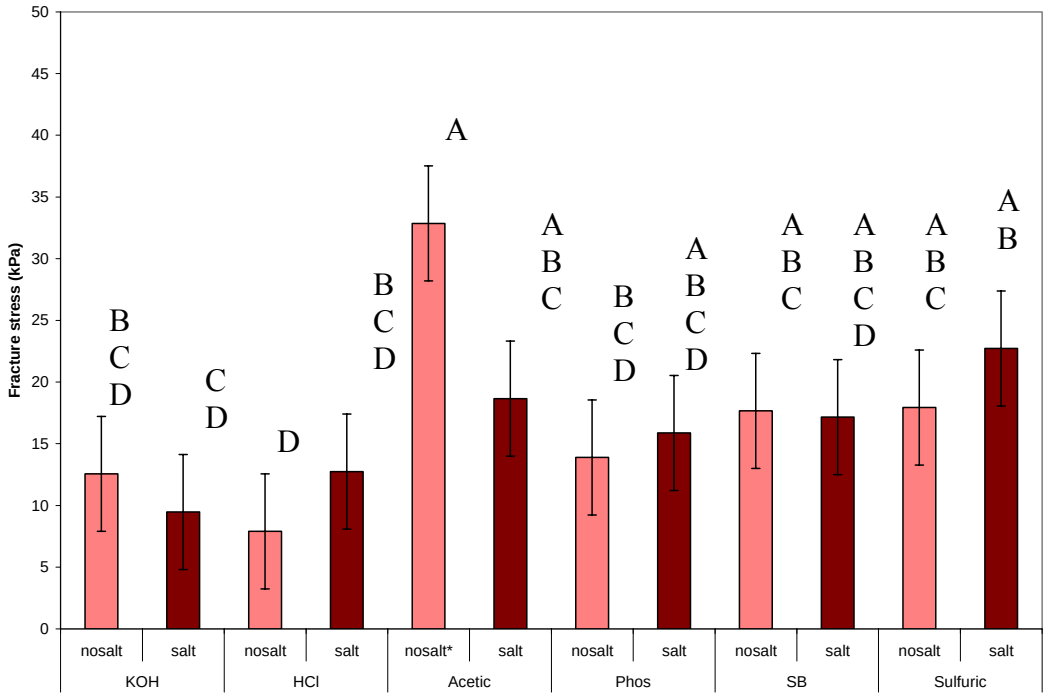

Figure 2.16 Fracture stress of gels made from tuna protein isolate prepared with a different acids and base. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

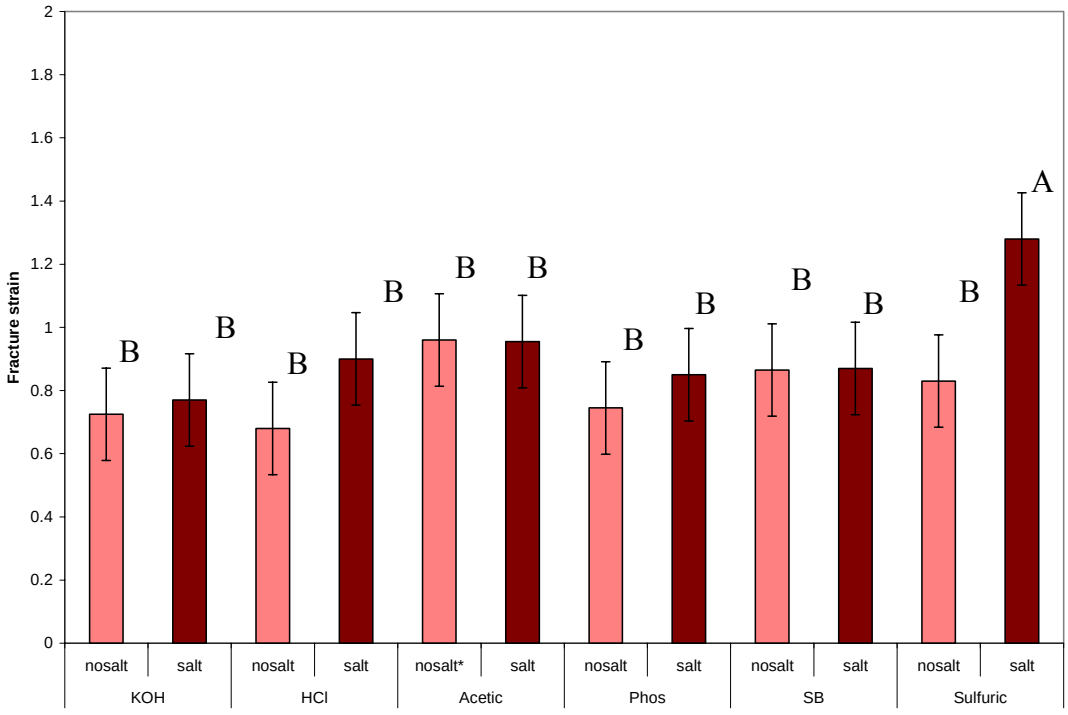

Figure 2.17 Fracture strain of gels made from tuna protein isolate prepared with different acids and base. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

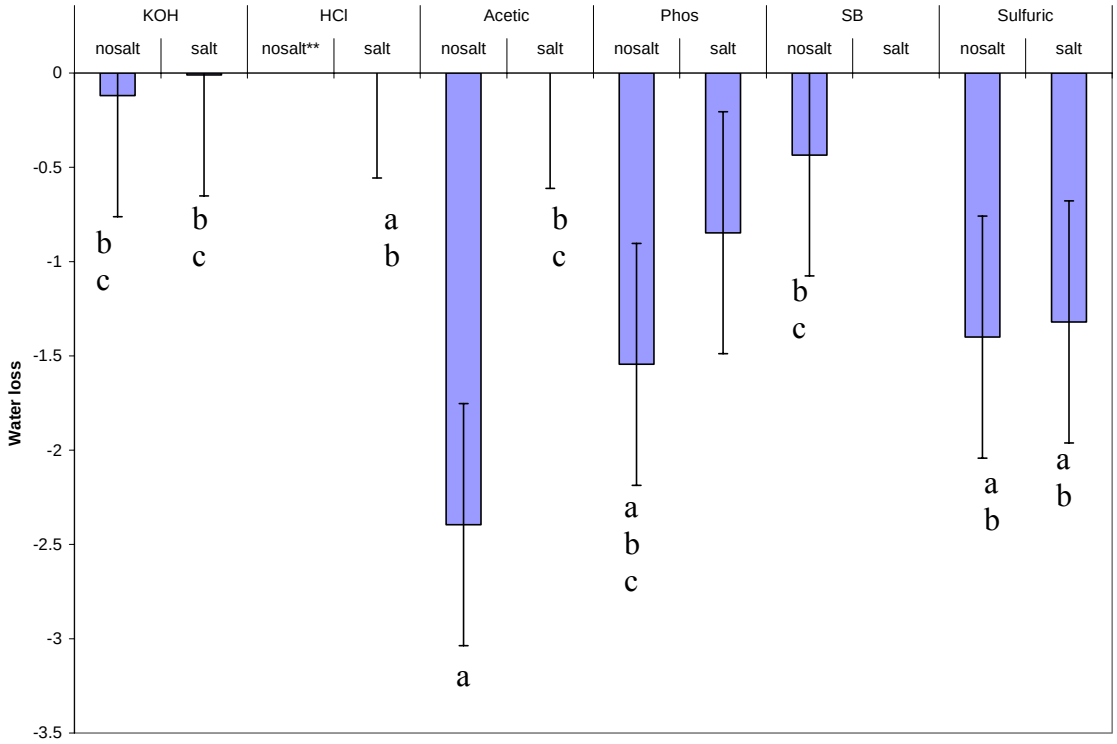

Figure 2.18 Water loss of gels made from chicken protein isolate prepared with different acids and base. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

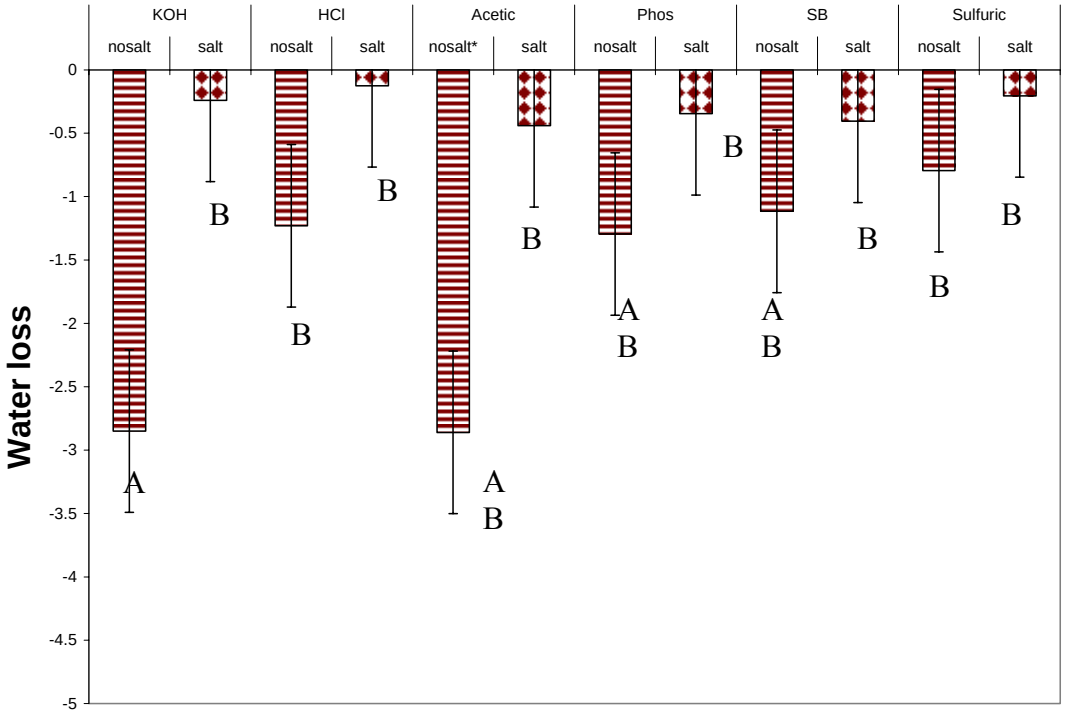

Figure 2.19 Water loss of gels made from tuna protein isolate prepared with different acids and bases. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

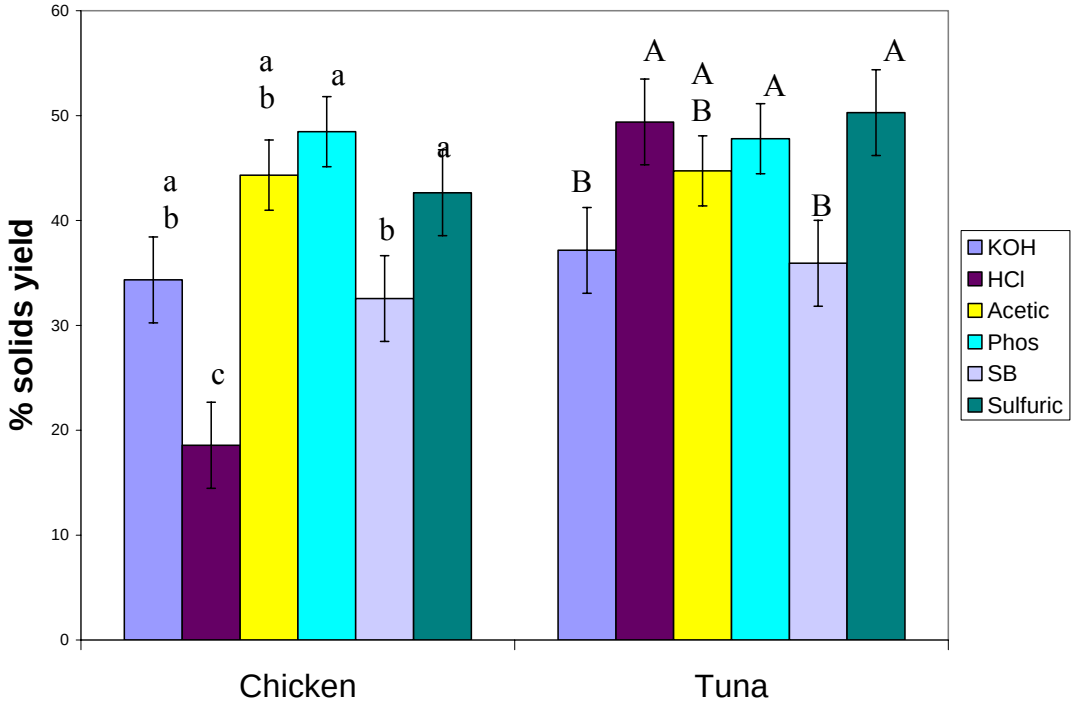

Figure 2.20 Percent solids yield of chicken and tuna protein isolate prepared with different acids and base. Error bars are the standard error for the least squares means analysis. Bars with different letters are significantly different ($p<0.05$) from each other.

Manuscript II to be submitted for publication

Effect of Precipitating Acid on Floc Size, Recovery Yield and Gelation Properties of Chicken and Tuna Meat Isolates Prepared by pH-Shifting

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ABSTRACT

The pH-shifting process for functional meat isolate manufacture involves solubilizing the meat proteins at high pH followed by isoelectric precipitation, induced by acid addition. This study compared the effects of several organic and inorganic acids on the floc size, gelling properties, and recovery yield of the precipitated protein isolate. Acids trialed were hydrochloric, phosphoric, sulfuric, citric, acetic, formic, and tartaric acids plus sodium bisulfate, added at constant rate during "medium" mechanical stirring. Although floc sizes varied substantially during the study, they were not consistently reproducible. Visually large floc sizes generally gave higher recovery yield on a stainless screen mesh (1mm mesh size), more representative of commercial rotary screens used in surimi manufacture, whereas flocs size had no effect on recovery yield on a fabric screen (0.3mm mesh size). Yields on the stainless screen were 10-20% lower than by use of the fabric. The use of hydrochloric acid as the precipitant yielded chicken protein isolate with the worse gelation properties compared to all other acids trialed. No effect of acidulant type was seen on gelation properties tuna protein isolate. Primary particle morphology or specific acid effects on protein conformation may account for the effects of particular acids on gelling properties of meat isolates.

Key words: Tuna, Chicken, Protein Isolate, pH-Shift, Acidulants, Flocculation, Alkaline, Fish

INTRODUCTION

Fish meat isolates made by a pH-shifting method (protein solubilization at high or low pH plus reprecipitation near the isoelectric point; (Hultin and Kelleher 1999, 2000, 2001, 2002, 2004; Hultin and others 2003) have been considered as a possible substitute for conventional surimi because the isolate process offers certain potential advantages. The protein recovery yield is theoretically higher in the isolate process due to the recovery of both myofibrillar and sarcoplasmic proteins, protein isolates have been shown with some fish species to produce stronger gels than conventional surimi, and, if a membrane-removal step is included, isolates would likely exhibit better oxidative stability (Hultin and Kelleher 1999, 2000; Yongsawatdigul and Park 2004; Choi and Park 2002; Undeland and others 2002; Kristinsson and others 2005). The alkaline version of the pH shift isolate manufacturing process (alkaline solubilization + acid precipitation) has been shown to produce isolates which gel more strongly and are more stable to oxidative rancidity development in frozen storage than isolates made by the acid version of the process (Hultin and Keheller 1999; 2000; Kristinsson and Hultin 2004; Kristinsson and Liang 2006).

Salt and others (1982) found that varying the acid used to flocculate soy proteins during isolate production influenced the protein structure and functional quality. They reported that the modification of protein native structure after precipitation was dependent on the acidulant anion following the inverse of the Hofmeister series. Similarly, Resch and others (2005) found that the functional properties of powdered, pre-gelled whey proteins were affected by varying the acid used to induce their gelation. They reported that ion specific effects were generally follows the Hofmeister series of the acid anions. That is, the

powder derived from strong, translucent gels prepared by hydrochloric and lactic acids exhibit maximum viscosity and water holding.

Venogupal and others (1994) conducted an experiment on acid-induced gelation of shark myofibrillar proteins and observed that organic acids led to gelation of a fairly dilute myofibrillar protein solution, whereas the stronger inorganic acids could not induce gelation. They suggested that this was because the stronger acids denatured the meat proteins more. But, it could be that the weaker acids produced bigger and looser protein flocs such that they could form a weak gel, whereas the stronger acids produced tightly associated flocs that instead precipitated as they described.

The size and the structure of the precipitated protein flocs are important to the operation of industrial unit processes related to solid/liquid separations by either centrifugation or screening (Waite 1999; Wilen and others 2003). We recently reported preliminary evidence that acidulant type affected the recovery yield and gelling properties of meat isolates made from chicken and tuna by the alkaline pH shifting method (Leksrisompong and others, to be published).

The objective of this research was to study the effect of varying the precipitating acid on the resulting floc size, recovery yield and gelation properties of meat protein isolates prepared by pH-shifting.

MATERIALS AND METHODS

Isolate making procedure

Raw Material

Skinless, boneless chicken breasts were obtained from a local poultry processor. Frozen albacore tuna (*Thunnus alalunga)* loins were air shipped from an offshore commercial canning plant. Fresh or partially thawed meats were cut up into 2 inches cubes and mixed to minimize differences due to individual animal variations, then bagged in zipper-type freezer bags and stored at -23.3 °C until used (up to 2 months). Meats were subsequently thawed by floating the bagged cubes in ice water for approximately 1 h. Chemicals used were reagent grade and obtained from either Fisher Scientific (Waltham, MA) or Sigma-Aldrich Co. (St. Louis, MO)

Preparation of meat isolates; Floc sizes, Recovery yield, and Gelation

Previously thawed meats (1500±5 grams chicken or 1000±5 grams tuna) were finely chopped in a Stephan vertical cutter-mixer (Model 2889, UMC 5 electronic, Stephan Machinery Corp., Columbus, OH) for 1 min with 1 part water at 2400 rpm. The homogenized meat slurry was then transferred to a large drum (40 cm in diameter and 50 cm height) for further dilution with 5 parts of ice water.

To solubilize myofibrillar and sarcoplasmic meat proteins, the pH of the muscle protein homogenate was increased to pH 11 with 2N NaOH added at 8.62 mL/s with a lab pump (Model PM6014, FMI Lab Pump, Fluid Metering Inc., Syosset, NY), while mixing at 275 rpm using a 10 cm x 20 cm paint mixer rotor attached to an adjustable speed drill

connected to a rheostat. The pH was periodically measured using a double junction gel pH probe (model PhTestr10; Oakton Instruments; Vernon Hills, IL) and verified with litmus paper as the junction occasionally clogged due to protein buildup. The homogenate was then screened through 1 coarse (1.7 sq mm mesh size) and 2 fine (1.3 sq mm mesh size) plastic square mesh screens to remove connective tissue, skin, and other non-soluble large particulates.

To precipitate the proteins from solution, 2N acid solutions (hydrochloric, phosphoric, acetic, sulfuric, sodium bisulfate, citric, tartaric, or formic acid) were then pumped into the solubilized meat slurry at a rate of 8.62 ml/sec, again using the FMI Lab Pump while mixing at a slower rate (70 rpm) with the same mixer, until the solution pH reached pH 5.5 (near the isoelectric point). A sample of precipitated protein solution was collected to determine its floc size.

Floc size determination

To qualitatively compare the size of protein flocs, the flocculated dispersion was diluted 1:1 with glycerin to disperse the flocs, then poured into a clear plastic container (45 mm dia., approx. 3 mm in thickness) and photographed. The size distribution of aggregates (ultimate floc sizes) from the photos were to be determined by image analysis using the method of visual texture evaluation using color primitives (Balaban 2008).

Yield determination

The flocculated proteins were partially dewatered on one of two different screens: a stainless steel mesh screen of 1 sq mm opening size, or a fine organza fabric screen of 0.3 sq mm hole size. The precipitated protein dispersion was poured onto either screen for draining

while gently scraping across the surface of the screen with a large spoon to assist draining and minimize pore clogging. Material was left on the screens to drain until the moisture content was reduced to approximately 90%. The samples on the screen were analyzed for solids content by oven drying for 16 hours (AOAC 1995).

In each case, the material remaining on the screen was further dewatered by gathering up and manually squeezing the resulting organza fabric 'bad' to attain a final moisture content of about 73%. This squeezing step was used to mimic action of a screwpress, which would be required to attain a usable moisture content in the isolate for its desired end product applications. In this case the finished moisture content, is lower than that of conventional surimi following screwpressing due to the higher water-holding ability of traditionally washed mince at its higher pH (near neutral).

Dewatered meat isolates were then packed in a 1 in layer in 1 gal zipper-type freezer bags, weighed to determine yield, then quick frozen on the cement floor of a -23.3 °C walk-in freezer to facilitate temperature control during subsequent comminution for testing of heat-induced gel-forming properties (no cryoprotectants were added since the freezing time was short). Samples of each isolate were oven dried to measure moisture content by oven drying for 16 hours (AOAC 1995) to calculate recovery yields and to adjust moisture content for gel testing.

Mass Balance

A mass balance was also conducted on the entire process used to prepare isolates from tuna (screening first on the SS screen, then transferring to organza for final dewatering),

which included collection of fractions remaining on all screens used to remove connective tissues.

Gelation properties

Gel forming ability of isolates was measured according to Wright (2007) with slight modifications. The frozen isolate samples were chopped with 2% NaCl and enough chilled (~4°C) water to yield a final moisture content of 78%. During chopping, 2N NaOH was used to adjust the pH to 7.3. Temperature was kept to below 10 °C by use of frozen isolates and addition of ice instead of water. The pastes were vacuum packaged, a corner of the package opened and placed in a hand sausage stuffer, and then extruded into stainless steel tubes 19 mm dia. and 150 mm length which were capped and cooked by submersion in a water bath. Tuna was cooked at 90°C for 15 minutes (typical of surimi gel preparation for testing to simulate the cooking of surimi crabstick products under commercial processing (Park and Lin 2005). Chicken was cooked at 70°C for 20 min to simulate the cooking method for most commercial chicken products. The cooked gels were immediately cooled in an ice bath for 3 minutes, bagged, and refrigerated overnight at 5 °C.

Rheology – Large-Strain Torsional Fracture Testing

Chilled gels in zipper type plastic bags were equilibrated to room temperature and prepared for fracture testing by torsion. The cylinders were ground on a modified milling machine into capstan shapes with a center diameter of 1 cm as described by Foegeding (1992). The gels were cut into 28.7 mm lengths and glued to plastic disks using

cyanoacrylate glue. A torsional gelometer was used to measure fracture stress and fracture strain of the gels (Wright, 2007).

Statistical Analysis

All experiments were repeated at least two times. Data were analyzed using the general linear models procedure of SAS software (SAS Institute, Cary, NC). All statistical inferences reported were at least at a 5% probability level. Differences between treatment combinations were determined using paired comparisons (pdiff option) on the least square means.

RESULTS AND DISCUSSION

Acidulant effects on protein floc size

Despite attempts to exercise extreme care in controlling the rate of acid addition and agitation of the mixture during acid addition, there was poor reproducibility in the sizes of flocs obtained within treatments (Figs 3.1 a-g and 3.2 a-g), with the possible exception of the initial tuna lot. In that lot, and to some extent in other meat lots used, organic acids tended to produce larger sized flocs than did inorganic acids (Figs 3.3a-d). Certainly the size of protein flocs did vary among treatments, so it is apparent that some, as yet unidentified, factors must be working in concert to affect ultimate floc sizes. We thus found it unnecessary to attempt quantitation of floc sizes by image analysis nor to consider whether Hofmeister effects of acid anions might be influencing the floc sizes. The great difference in effects of acids on floc size obtained for the two different lots of tuna would suggest that some compositional/biochemical parameters of the meats themselves could have a strong influence on floc size formation. At the present time all the perturbing factors cannot be both identified and manipulated to the extent that the floc size distribution of precipated proteins could be reliablycontrolled in an industrial process.

Acidulant effects on recovery yield

Across both meat species tested there was no clear pattern in how acidulants affected recovery yield (Figs 3.4 and 3.5). For example, the citric acid treatment exhibited a high recovery yield for tuna but a low recovery yields for chicken. Acetic acid produced one of the highest yields for chicken isolates, but one of the lower yields for tuna.

A qualitative examination of Figs. 3.4 and 3.5, and the other photographs of protein flocs not shown, seemed to generally support that larger floc sizes observed visually did roughly correlate with higher yields obtained on the larger SS screen. It is of course intuitive that this might be so, and points up the fact that lack of the ability to control floc size in production of meat protein isolates could seriously affect recovery yield if only screening techniques were used for solids-liquids separations (Choi and others 1981).

Overall the solid recovery yields for tuna were higher than for chicken. From the discussion of the mass balance work that follows, it seems likely that this species effect likely also relates to a relatively smaller particle size distribution of the flocs for this species, for what reason is as yet not understood.

Mass balance to investigate yield losses

Recovery yields for both species used in this study were low as compared to some other studies that employed the alkaline pH shifting method, but which employed centrifugation rather than screening for all separations (Undeland and others 2002; Kim and others 2003; Kristinsson and others 2005). The mass balance was conducted on tuna isolate processing. This revealed a loss of about 14% of the total initial solids during the first screening step (solids removal). The material retained by the first screen accounted for 10% solids loss, while the material on the bottom of this screen and the material adhering to the second two of the three screens used for the initial screening step (insolubles removal) accounted for about 4% of the total solids introduced to the process initially. These two screens were slightly smaller in hole size than the first (1.3 vs. 1.7 sq mm), and most of the material remaining on these two screens did not appear to be connective tissue.

Liquid which had passed through both the SS and organza fabric screens was subsequently centrifuged and an additional 31-32% of solids was recovered. While this amount of solids would represent a loss if screening equipment were commercially used for dewatering, it is possible that a decanter centrifuge could be employed to recover these fine particles from the wastewater, as is typically practiced in many conventional surimi plants today (Park and Lin 2005). According to Lanier and others (1992), protein recovery yields for a conventional surimi process employing only rotary screens and screw presses can lose up to 50% more yield than when decanter technology is also employed in final recovery in conventional surimi production.

Nelson and Glatz (1985) noted the utility of maximizing the size and density of aggregates for the separation of solids from a pH 8 soy solution. According to Stoke's law, the particle settling velocity will increase directly in proportion to particle density, and by the square of the particle diameter (Jaczynski and Taskaya 2007). However, because of the similar densities of the protein precipitate and the aqueous phase, the particle size of the precipitated protein likely plays the more important role in effectiveness of separation. There is evidence that small particle size may be responsible for poor yields associated with some precipitation and recovery techniques (Choi and others, 1981).

In general, centrifugation requires more expensive equipment and qualified personnel (Nolsoe and others 2007). However, over the long term centrifugation likely would prove more economically beneficial if protein recovery could be substantially increased by its adoption. In the mass balance experiment conducted to investigate yield losses, residues remaining on the 2nd and 3rd screens of the initial screening step were incorporated back

into the meat slurry just prior to acid precipitation. Fine particles escaping the fabric screen on the final dewatering were recovered by centrifugation and also added back to isolate retained by the fabric screen. The increased yield in the process effected by the reincorporation of these fractions (the largest being the centrifuge retentate of the screening permeate) was found to cause no significant change in the fracture properties of gels made from this combined material (Figs 3.6).

Nolsoe and others (2007) actually found no difference in total process yields for the alkaline pH shifting process when they tried to further investigate the possibility for replacing centrifugation of precipitated proteins by filtration (500mL filter flask connected to a precision Vacuum Pump 25L/min) as a final dewatering step. They used a series of screen mesh size ranges from 0.02-0.42 mm.

This finding is in contrast to our results, when we replaced screens with a laboratory centrifuge, the percent solids recovery increased approximately 31-32%. Possibly, it is because we hand squeeze for the final dewatering step whereas their study used vacuum dewatering through filters. When using vacuum, such small screens would be quite prone to clog giving, thus, making screen mesh size even smaller. Also their smallest screens meshes are much smaller than the organza effectively thus their recovery yields were the same with filtering or centrifugation. Using such a small mesh size would be difficult to implement in an industrial solids-liquids separation process for proteins.

Acidulant effects on gelation properties

Varying the acid precipitant used to prepare isolates from tuna had no significant effect on fracture properties of gels made from these isolates (Fig. 3.7), though the lowest fracture stress values and second lowest fracture strain value measured were of gels made from isolate using HCl. Also, only fracture stress and strain of the chicken isolate made with HCl was significantly (negatively) affected by acidulant type (Fig. 3.8).

In contrast, our previous study (Leksrisompong and others to be published) showed that the use of acetic acid as the precipitant yielded chicken protein isolate and tuna protein isolate with the best gelation properties compared to all other acids trialed (phosphoric, sulfuric, sodium bisulfate and hydrochloric). Moreover, the two meat species responded differently when HCl and sulfuric acids were used in the isolate making process. For CPI, the use of sulfuric acid resulted in gels of poor quality, and the use of HCl resulted in gels of excellent quality, but for TPI the exact opposite was true.

Thus it does not appear that there were ion specific effects on subsequent gelation properties of the isolates, according to classical Hofmeister series behavior. Resch and others (2005) did seem to observe acid anion effects of acidulant type on the rheological properties of beta-lactoglobulin gels and the reconstituted powders derived from these gels. Their gels, however, were actually induced by acid addition, whereas in the present case gels were heat-induced after subsequent neutralization (also with comminution and heating) of the acid-precipitated proteins. The apparent negative effect of hydrochloric acid precipitation on subsequent gelation of isolates could possibly be attributed to effects on the morphology of the primary particles formed during protein precipitation (Fisher and others 1986; Nelson and

Glatz 1985; Zukoski and others 1996). Because it has been shown that gelation of meat isolates is not dependent upon solubility of the previously precipitated proteins (Chang and others 2001a, Undeland and others 2002), it seems reasonable that whatever primary particles form during precipitation, though they may swell upon upward adjustment of the pH, they likely do not dissolve during commution and therefore their size and shape might influence gel structure development. This theory was proposed by Wright (2007) based on work previously conducted with milk proteins (Marangoni and others 2000; Ould Eleya and others 2004; Zhong and Daubert 2004). Kristinsson and Hultin (2003a,b) suggested that unfolding and refolding processes which occur during the alkali-aided process also seem to affect the gelling ability of the proteins, and it is conceivable that HCl might have a specific effect compared to the other acids upon protein conformation.

CONCLUSIONS

There was poor reproducibility of acid effects on protein floc size across all meat lots tested, despite clear indications with one meat lot (tuna) that employing organic acids favored larger floc size. It seems likely, given the care taken in this study to control the rates of acid addition and agitation, that control of floc size in an industrial process, and dependence upon such control in design and operation of the system, is not presently possible. However, some food grade directly-interacting protein flocculant additive might be identified which could consistently induce larger flocs and thereby add more control to such a screen-based process (Jaczynski and Taskaya 2007).

Recovery yields were variably affected by the type of acid precipitant used, but the effects were not the same across species and did not follow Hofmeister theory. All recovery yields measured in this study, obtained by screening, were low as compared to other studies which all employed centrifugation, or much finer screens, for final dewatering. The greater percentage of solids lost were as fine particles in the final screening permeate, which when recovered by centrifugation were found to not affect gelation of the total (screened + centrifuged) meat isolate recovered. Thus, results of this study would indicate that the use of centrifugation for final dewatering of acid precipitated meat protein isolates is highly recommended to maximize the recovery yield.

Gelation properties were variably affected by the acid used to effect isoelectric precipitation of the meat proteins. Acid type had no significant effect on gelation of tuna isolates, and only hydrochloric acid had a significant (negative) effect on gelation of chicken isolates. However, hydrochloric acid is relatively low in cost, tasteless, and readily available. Also its use presently (with dilute sodium hydroxide to raise pH) results only in formation of sodium chloride as a residual, and thus favors cleaner labeling of the finished meat isolate or foods made with it.

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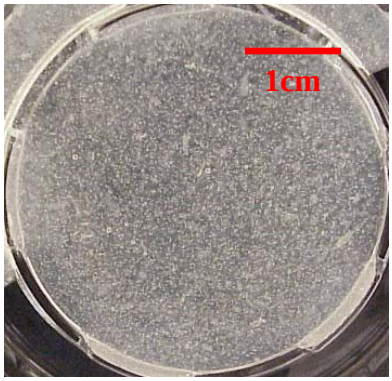
Figure 3.1

a. Effect of **citric** acid on floc



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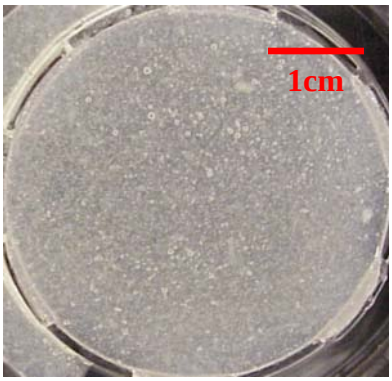

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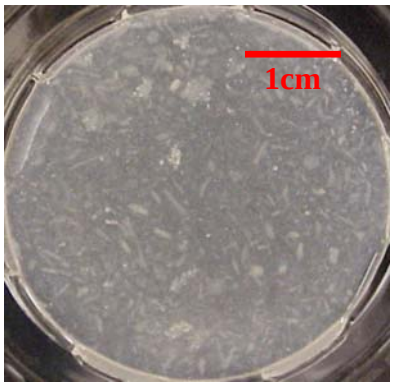

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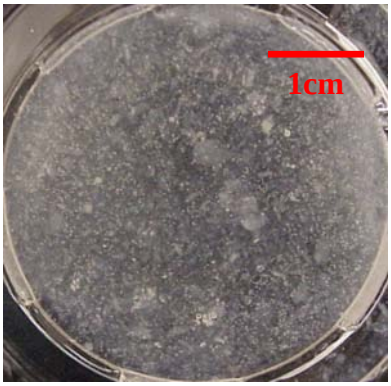

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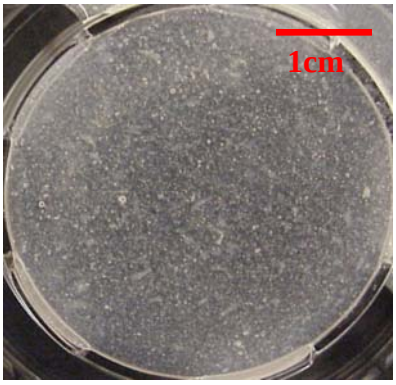

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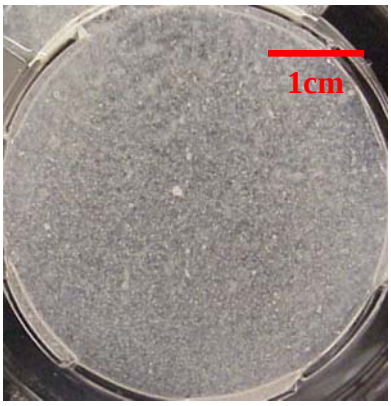

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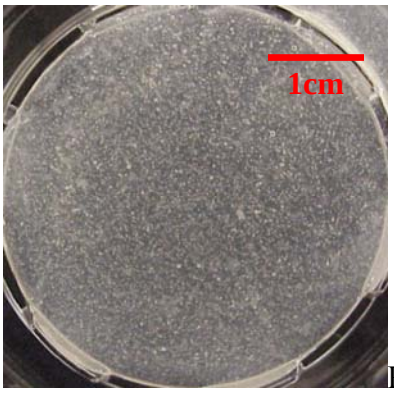
d. Effect of **tartaric** acid on chicken floc



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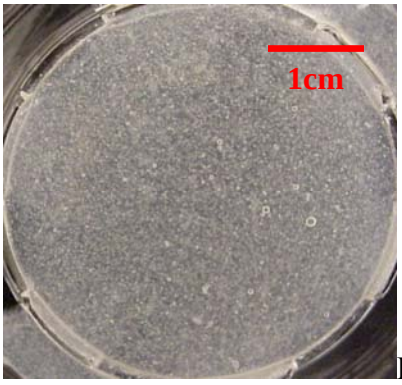

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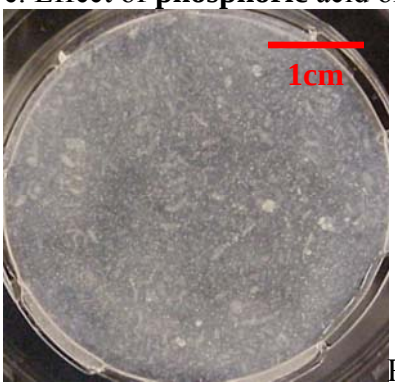
e. Effect of **phosphoric** acid on chicken floc



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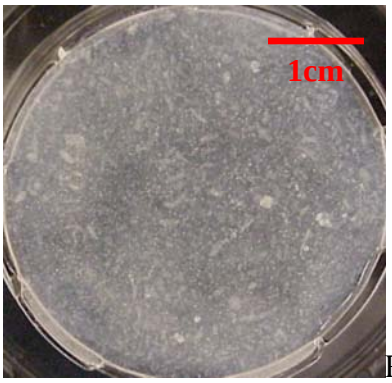

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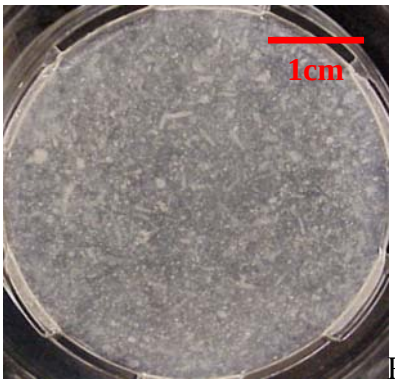
f. Effect of **sulfuric** acid on chicken protein floc



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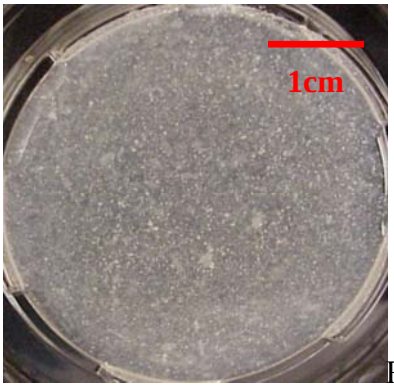

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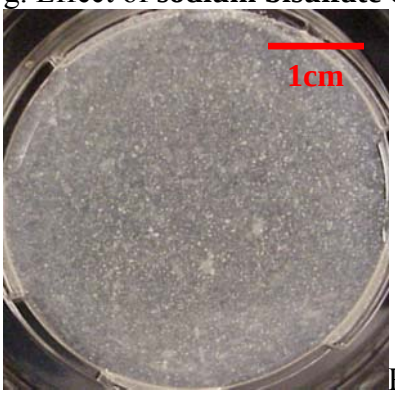

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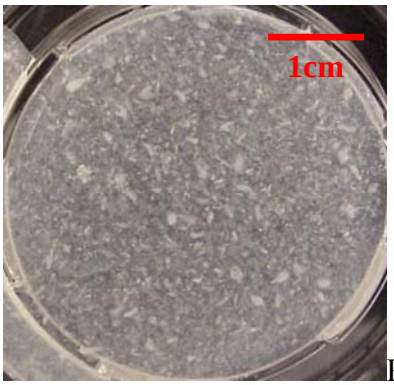

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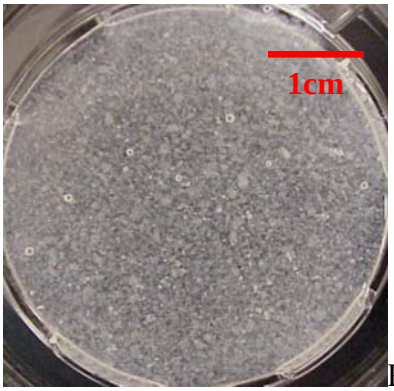
h. Effect of **hydrochloric** acid on chicken protein floc



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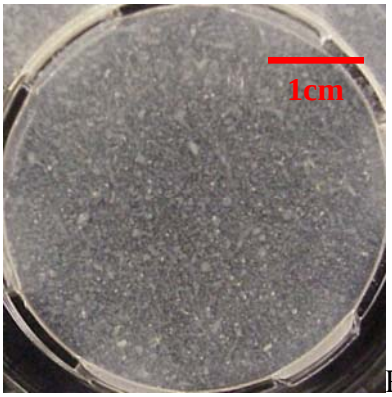
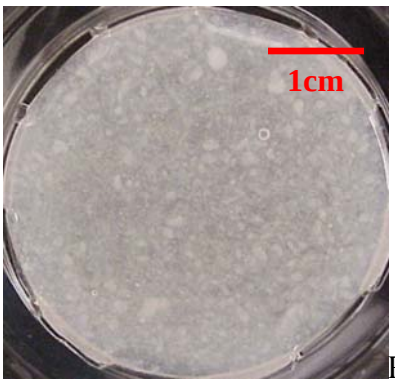


Replication 2

Figure 3.2

a Effect of **citric** acid on floc size



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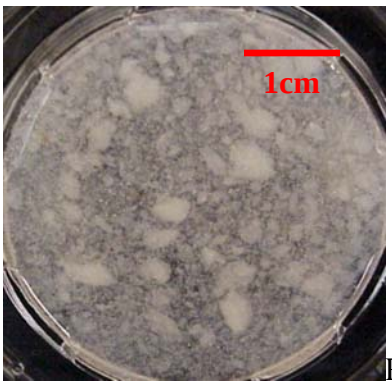
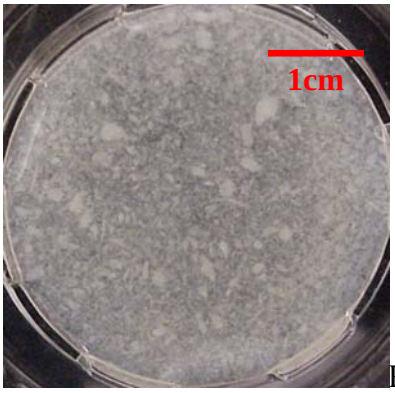


Replication2

b. Effect of **acetic** acid on tuna protein floc size



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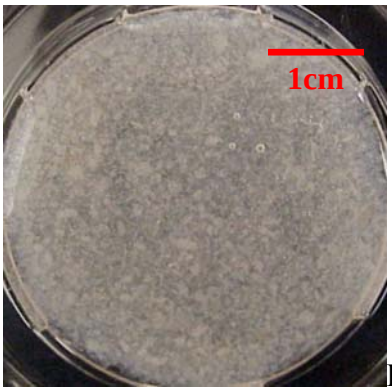
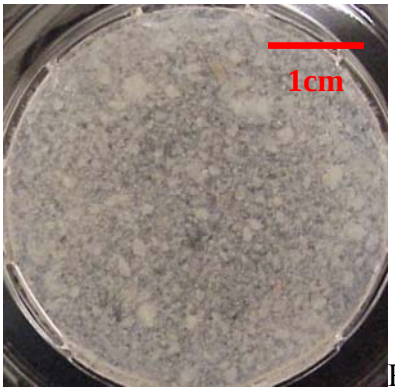


Replication 2

c. Effect of **formic** acid on tuna protein floc size



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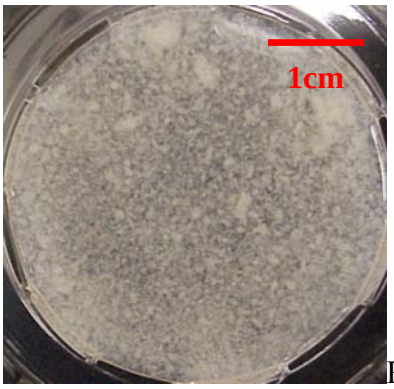
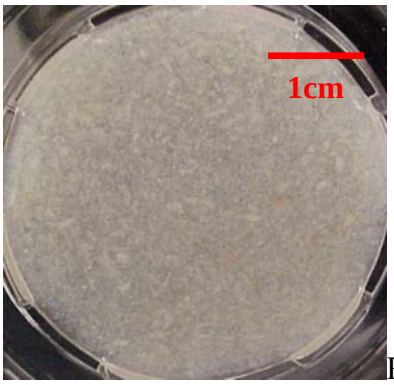


Replication 2

d. Effect of **tartaric** acid on tuna protein floc size



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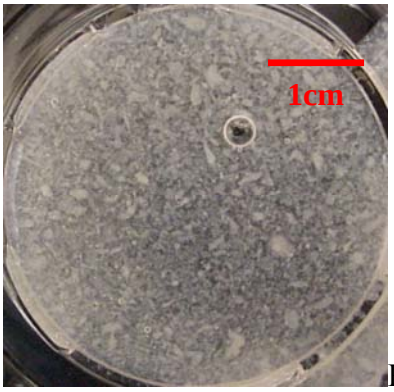
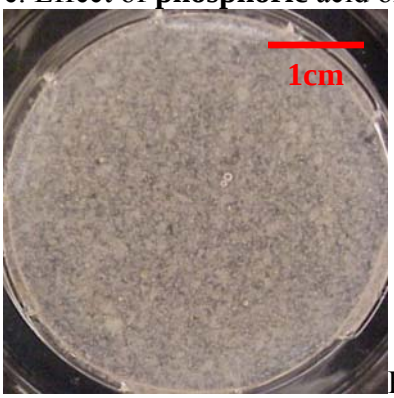


Replication 2

e. Effect of **phosphoric** acid on tuna protein floc size



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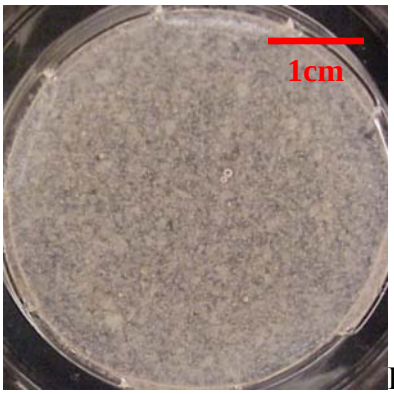

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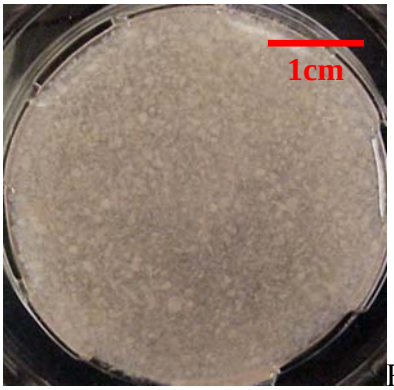
f. Effect of **sulfuric** acid on tuna protein floc size



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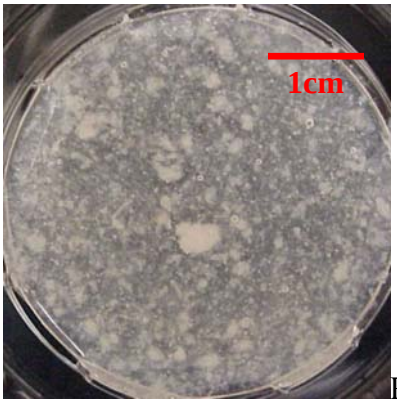
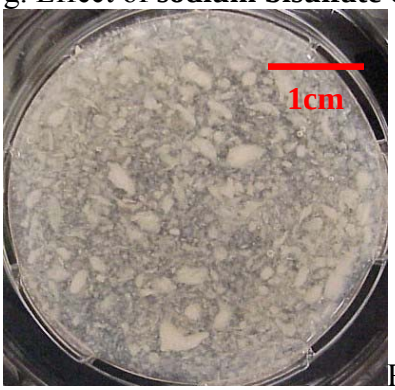


Replication 2

g. Effect of **sodium bisulfate** on tuna protein floc size



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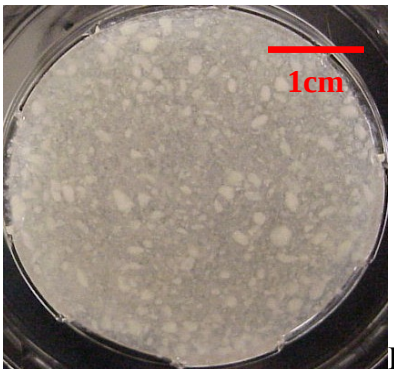
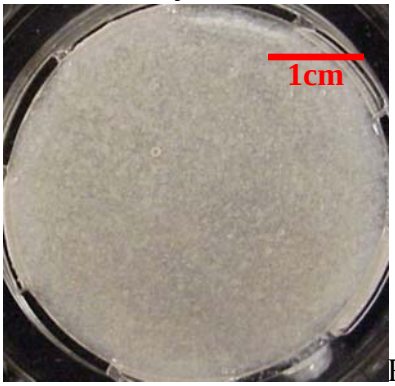


Replication 2

h. Effect of **hydrochloric** acid on tuna protein floc size



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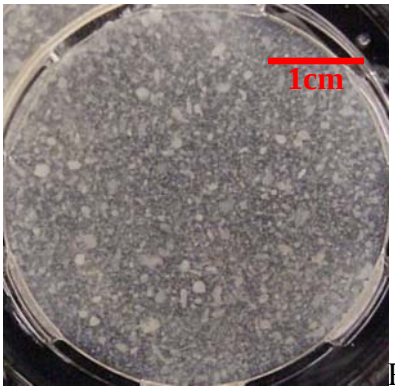
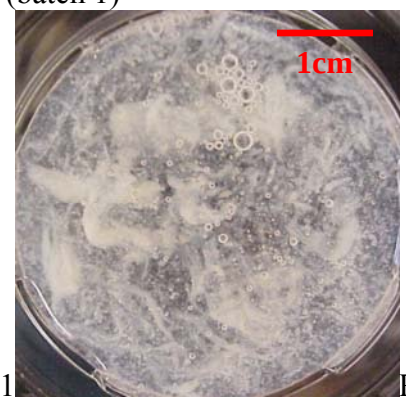


Replication 2

Figure 3.3

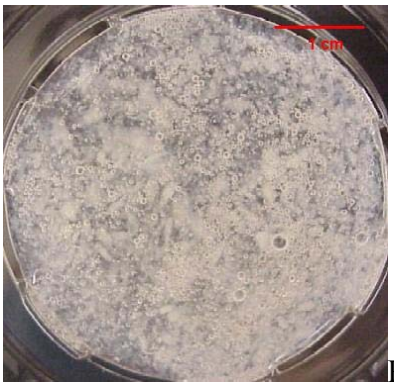
a. Effect of **citric** acid on floc size of tuna (batch 1)

Replication 1



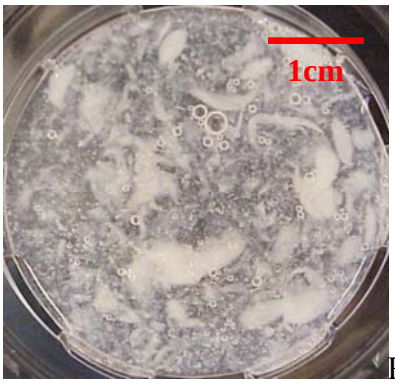
Replication 2

b. Effect of **formic** acid on floc size of tuna (batch 1)



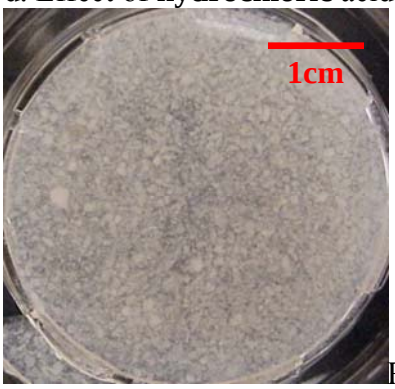
Replication 1

c. Effect of **tartaric** acid on floc size of tuna (batch 1)



Replication 1

d. Effect of **hydrochloric** acid on floc size of tuna (batch 1)



Replication 1

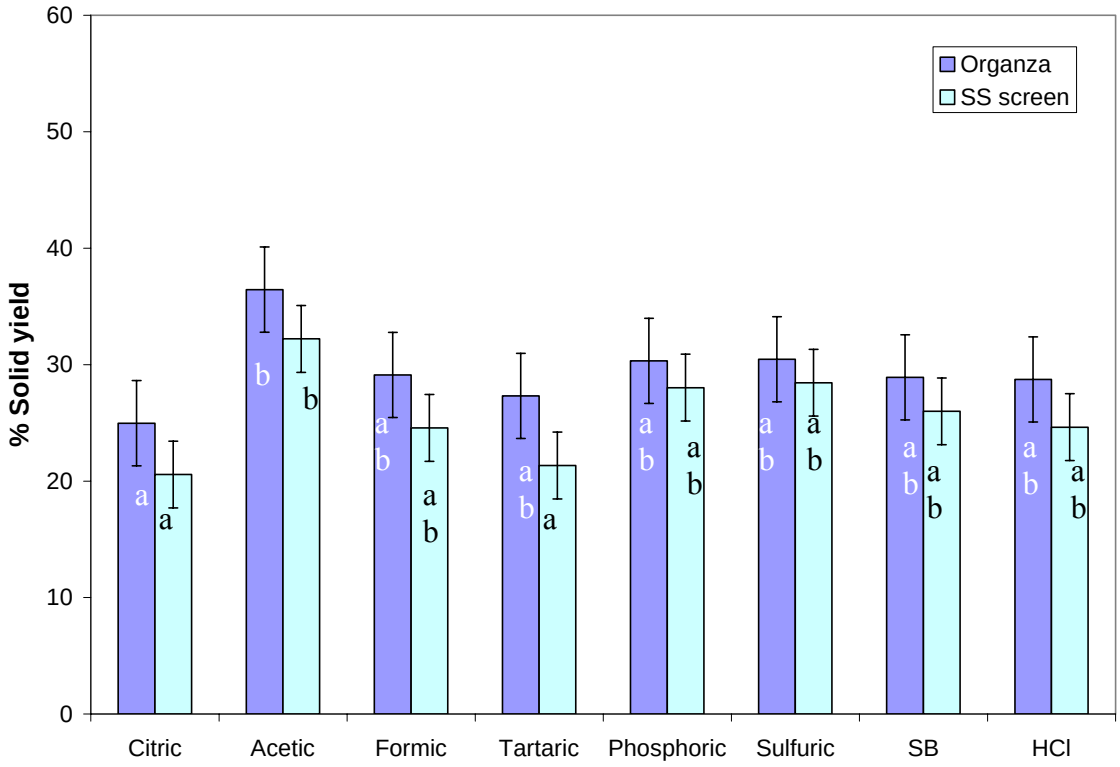


Figure 3.4. Effect of acid type on percent solids recovery yield of chicken isolate on SS and organza screens. Error bars are standard errors from the analysis of variance. Bars with different letters are significantly different ($p<0.05$) from each other.

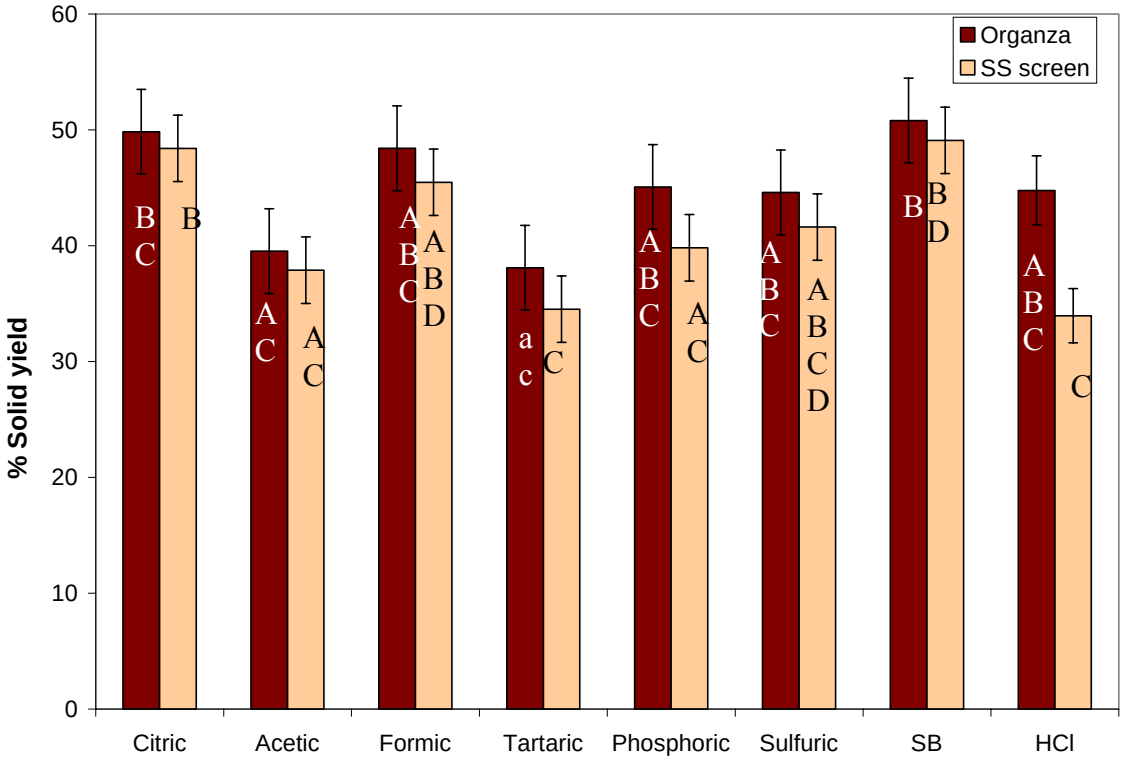


Figure 3.5. Effect of acid type on percent solids recovery yield of tuna isolate on SS and organza screens. Error bars are standard errors from the analysis of variance. Bars with different letters are significantly different ($p<0.05$) from each other.

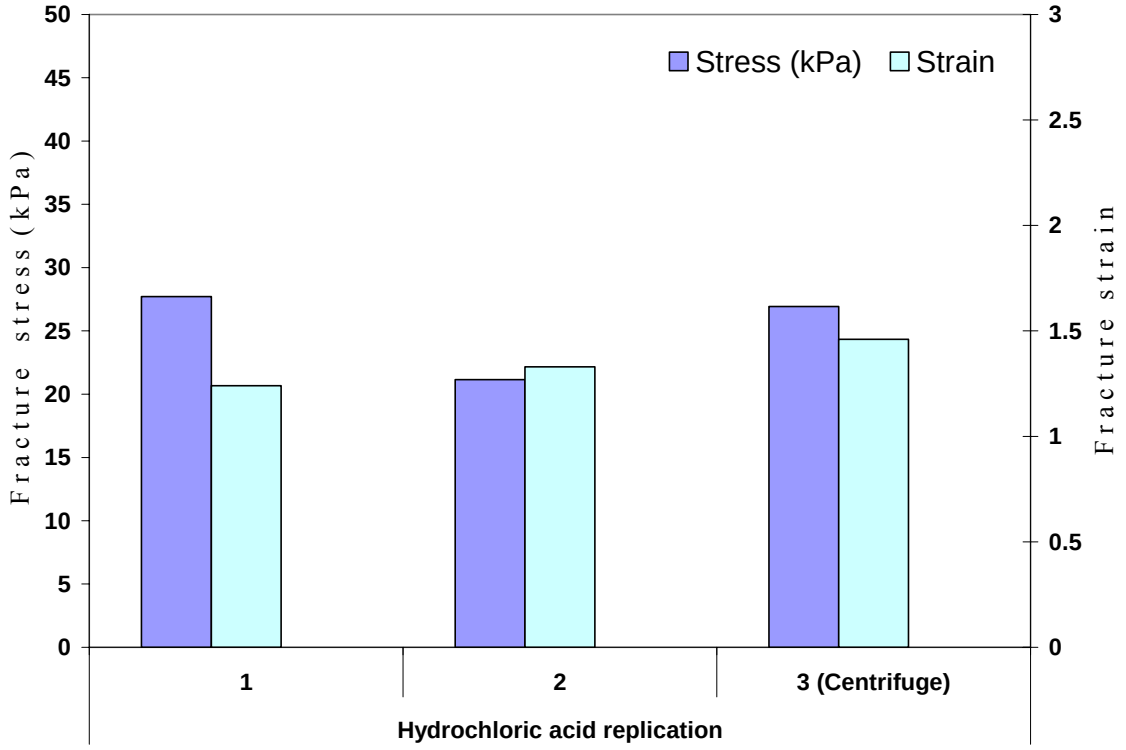


Figure 3.6 Fracture stress and strain of chicken gels prepared from isolate made with hydrochloric acids from 3 different trials. Trial 3 shows fracture stress and fracture strain of gel made when reincorporate solid fraction from waste water (recovered by using centrifuge) back into the initial recovered isolate prior of making gel .

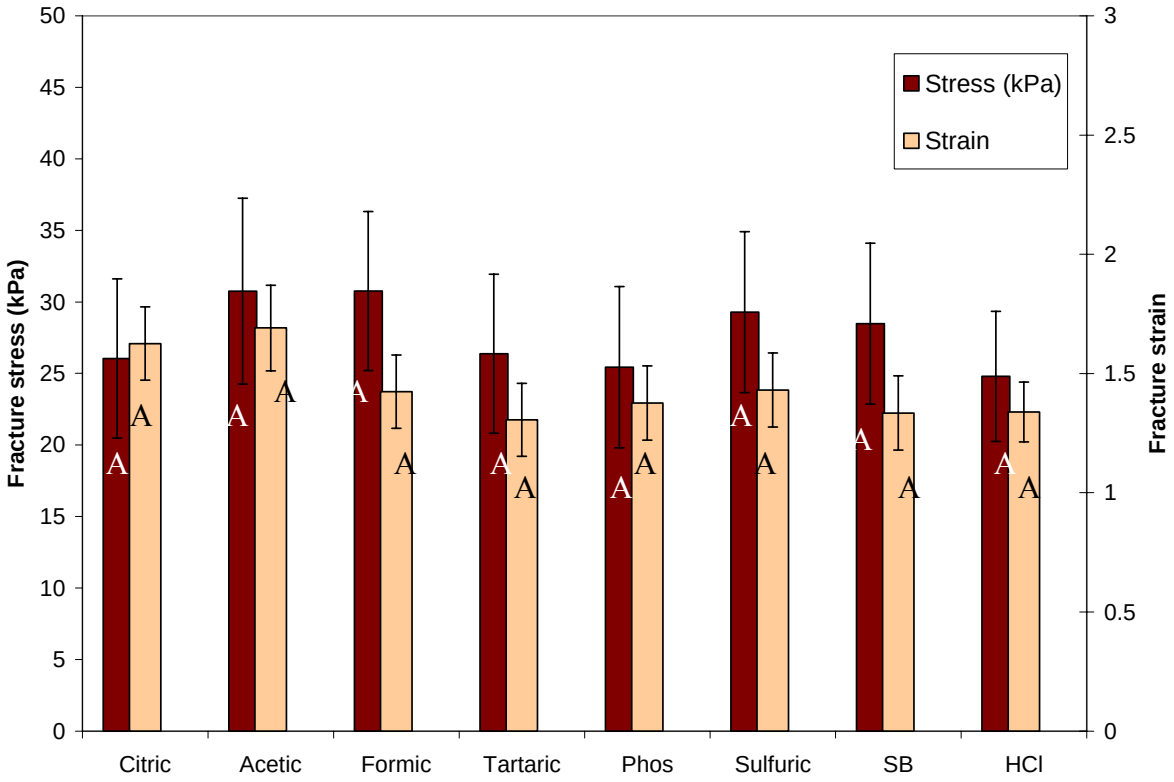


Figure 3.7 Fracture stress and strain of tuna gels prepared from isolate made with different acids. Error bars are standard errors from the analysis of variance. Bars with different letters are significantly different ($p<0.05$) from each other.

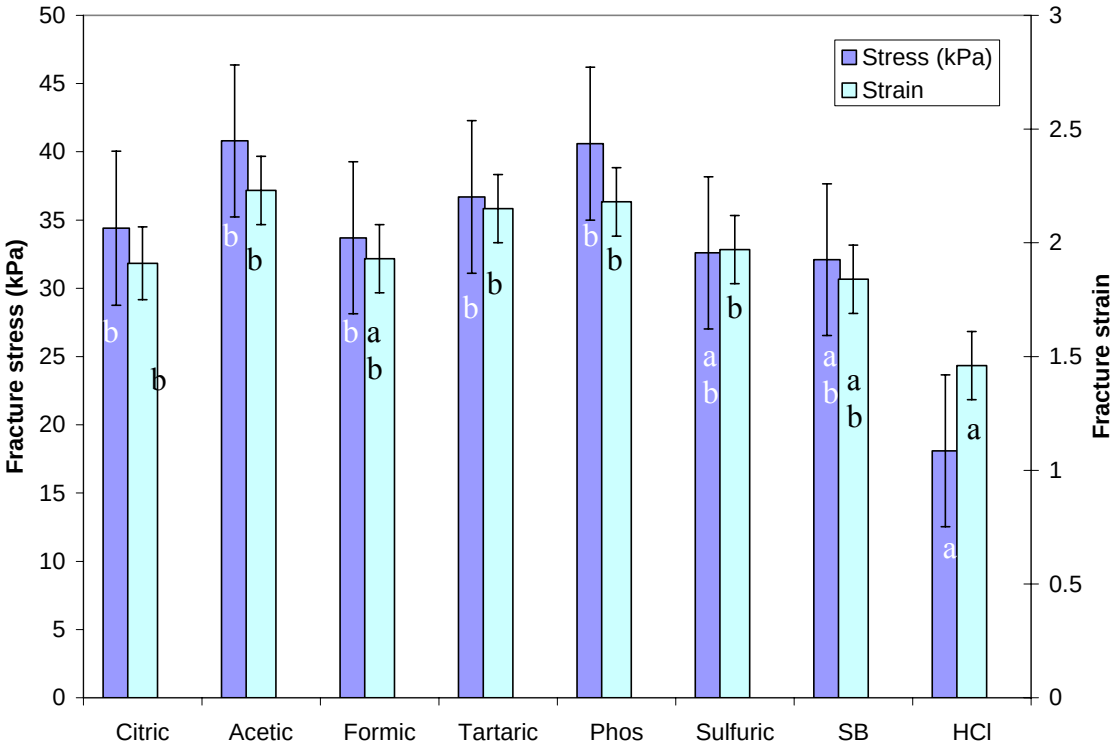


Figure 3.8 Fracture stress and strain of chicken gels prepared from isolate made with different acids. Error bars are standard errors from the analysis of variance. Bars with different letters are significantly different ($p<0.05$) from each other.