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Compositional and Functional Characteristics of Materials Recovered from Headed Gutted Silver Carp (Hypophthalmichthys molitrix) By Isoelectric Solubilization and Precipitation Using Organic Acids

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ABSTRACT

Compositional and Functional Characteristics of Materials Recovered from Headed Gutted Silver Carp (Hypophthalmichthys molitrix) By Isoelectric Solubilization and Precipitation Using Organic Acids

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Silver carp (*Hypophthalmichthys molitrix*) is one of the main freshwater fish species with a high nutritional value; however, it is hard to process the fish due to its boney carcass. Therefore, isoelectric solubilization and precipitation (ISP) processing was applied to headed gutted silver carp to separate the protein from the bones, scales, skin, fins, etc. Different solubilization strategies (pH 2.5, 3.0. 11.5 and 12.0) using organic acids, either acetic acid (AA) or a 30% formic and lactic acid combination (F&L), were applied during ISP and the different effects of treatments on the materials recovered from the initial silver carp were compared. The recovered carp proteins were then used to make protein gels similar to surimi with the use of standard food additives. To further assess the effect of using different solubilization pH values and organic acids on the protein quality of the recovered fractions, the functional, texture and color properties were analyzed.

Proximate composition of the recovered proteins showed that processing at basic pH using AA was most effective at removing impurities (i.e. bones, scales, skin, fins) ($p<0.05$) and the impurities were effectively removed from recovered lipids regardless of processing pH or acid type. Functional properties of gels made from protein recovered by ISP processing using organic acids as the processing acid had typical gelation characteristics. Moreover, it was seen that isoelectrically recovered carp proteins were not denatured and retained functionality. Thermal denaturation and dynamic rheology of the protein gels revealed that using AA under acidic conditions and F&L with alkali treatments yielded improved gel structure. In addition to that, color analysis presented data showing that gels made using F&L were whiter for all solubilization conditions ($p<0.05$) and were similar to the whiteness of Alaska Pollock surimi gels under acidic treatments. Texture analyses highlighted that gels made from protein solubilized at basic pH values had firmer texture ($p<0.05$) and were harder and more cohesive, gummy and chewy ($p<0.05$) than proteins solubilized under acidic conditions. Moreover, gels made from proteins recovered using AA as the processing acid under basic conditions had similar shear stress responses as Alaska Pollock surimi.

This research shows that organic acids have the potential to recover protein and lipid from otherwise hard to process fish by ISP processing. The gels made from recovered carp protein show similar or improved functional, texture and color properties compared to Alaska Pollock surimi depending on the treatment and might be used for the development of restructured fish products for human consumption.

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INTRODUCTION

Fish contribute substantially to the world food supply, either as food for human consumption or as animal feed. According to the Food and Agriculture Organization of the United Nations (2010), of the 142 million tons of fish supplied by capture fisheries and aquaculture, 115 million tons were used as human food. Fish provides over 1.5 billion people with approximately 20 % of their protein intake, and 3.0 billion people with around 15% of protein (FAO, 2010). Fish and fish products are also significant in human nutrition due to their high, biologically valuable protein, fat and fat soluble vitamin content (Belitz et al., 2009). Other benefits of including fish in the human diet include the high concentration of omega-3 polyunsaturated fatty acids (PUFAs) like alpha linolenic acid (ALA), eicosapentenoic acid (EPA), and docosahexenoic acid (DHA) which are potentially beneficial in reducing the risk of various autoimmune (i.e. Alzheimer's disease), inflammatory and cardiovascular diseases (Kris-Etherton et al., 2002; Morris et al., 2003).

Though fish stocks are a naturally-renewable resource they are being depleted at a non-sustainable rate. The depleted or recovering stocks increased by 32% as of 2008 (FAO, 2010). The increasing trend towards over-exploited, depleted and recovering stocks arise concern. Therefore, the available sources need to be used more efficiently.

Carp is considered a delicacy in many countries and is one of the most abundant freshwater fish with a high growth rate and invasive characteristics (FAO, 2010). Silver Carp (*Hypophthalmichthys molitrix*), the most common carp species, is considered to be as nutritionally valuable as ocean fishes (Buchtová, et al., 2010). Although having a high

production rate, silver carp's commercial rate is low in the United States due to the boney carcass of the fish. Usually carp is processed with manual filleting and deboning; however, these methods are time and labor extensive procedures. Moreover, studies assessing the carp fillet yields showed a mean yield of 32- 41%, suggesting that the rest of the fish which is almost 60% of the initial weight is being discarded (Gela et al., 2003; Kocour et al., 2007; Bauer and Schlott, 2009). Therefore, it would be beneficial to explore other processing strategies to extract the nutritious protein from silver carp more efficiently and minimize losses.

Isoelectric solubilization and precipitation (ISP) processing is a protein recovery process where high or low pH conditions cause protein separation from insoluble fractions of the fish (i.e. bones, skin, scales, etc.) by solubilization. Using pH shifts, protein is recovered by precipitation and centrifugation. This process has been shown to provide efficient and favorable recovery rates when compared to other commercial processing techniques such as mechanical filleting or conventional methods involving separators and decanters (Taskaya et al., 2009). Traditionally, strong acids such as HCl are used in the process but organic acids have been shown to be more effective at reducing bacterial pathogens (Landsdowne et al., 2009a and 2009b, Otto et al, 2011a and 2011b). Therefore, the purpose of this experiment was to:

1. Compare the effects of the ISP process on total fat and protein recovery yields and proximate composition (% protein, % fat, % lipid and % ash) of recovered fractions of whole-gutted silver carp using different pH strategies and organic acid types; and
2. Characterize the compositional characteristics (fatty acid, amino acid and mineral profile) of recovered protein from the ISP-recovered protein from whole-gutted silver carp at different pH strategies and organic acid types; and

3. Measure differences in functional properties (thermal denaturation [T_{onset}, T_{max}, and ΔH]; viscoelasticity [G'], viscousity [G"]; texture properties [Kramer shear, Texture Profile Analysis, and torsion shear stress]; and color) of gels made from the ISP-recovered protein from whole-gutted silver carp at different pH strategies and organic acid types.

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CHAPTER II

REVIEW OF LITERATURE

Carp is considered a delicacy in many countries and is one of the most abundant and least expensive biomass marine foods in the world (Xu et al., 2012). Silver carp contains approximately 17.6% protein (Romvari et al., 2002); however, the nutritional potential has not been fully utilized mainly due to carp being a boney fish and the process of removing the small bones is not reliable. Therefore, more efficient methods to recover the nutritionally valuable protein and oil from carp need to be explored. Isoelectric solubilization and precipitation (ISP) processing is a method which allows efficient recovery of fish proteins and oil by exposure to high or low pH conditions that causes separation of protein from insoluble fractions of the fish (i.e. bones, skin, scales, etc.). When the pH is returned to the protein isoelectric point of pH 5.5, protein precipitation occurs and is recovered by centrifugation. Typically, strong acids are used to manipulate pH during ISP processing; however, recent studies have shown organic acids to be more effective at reducing bacterial pathogens (Lansdowne et al., 2009a and 2009b; Otto et al., 2011a and 2011b). The effects of organic acids on protein and lipid recovery, as well as protein composition and functionality have not been reported.

EFFICIENCY OF ISP PROCESSING ON THE RECOVERY OF PROTEIN AND LIPID IN SEAFOOD

The efficiency of the ISP process, as well as the nutritional and compositional characteristics of the recovered protein and lipids recovered from silver carp was investigated (Taskaya et al., 2009a). ISP was performed at four different pH values: 2.0, 3.0, 11.5, and 12.5 using HCl or NaOH to whole gutted ground silver carp. Protein, lipid, and insoluble fractions

were collected. Proximate analysis, mineral profile, fatty acid and amino acid assays were performed on the recovered fractions. Results showed that the basic processing pHs yielded more crude protein and lipids (94-97% and 88-89%, respectively) compared to acidic processing pHs with crude protein and lipid concentrations of 89-90% and 94-97%, respectively (Taskaya et al, 2009a). This was backed up by the data showing effective removal of minerals from the recovered proteins during ISP particularly with basic treatments. Kristinsson and Liang (2006) working with Atlantic croaker reported protein recovery yields at 78.7% and 65.0% for proteins recovered at solubilization pH values 2.50 and 11, respectively. This trend was confirmed by Chen et al. (2009) and higher yields at acidic pH compared to basic treatments were reported. Isoelectric solubilization and precipitation is effective at recovering myofibrillar proteins; however, only partially recovers water-soluble sarcoplasmic proteins depending on their ionic strength (Chen and Jaczynski 2007). Therefore, different solubilization treatments yield different recovery yields.

EFFECTS OF ISP PROCESSING ON THE COMPOSITIONAL CHARACTERISTICS OF RECOVERED FISH FRACTIONS

In the Taskaya et al. (2009a) study, whole gutted carp was determined to contain approximately 69% moisture, 52% protein, 36% fat, and 11% ash on dry basis; skinless and boneless carp fillet to contain 74% moisture, 67% protein, 28% fat, and 5% ash on dry basis; and Alaska Pollock surimi to contain 75% moisture, 61% protein, 1% fat, and 3% ash on dry basis (Taskaya et al., 2009a). After ISP was performed and the protein, lipid and insolubles were recovered; the proximate analysis showed that the protein section had a composition range of 89-91% moisture, 89- 95% protein, 1- 5% fat, and 4- 6% ash on dry basis; the lipid component had 50- 81% moisture, 3- 10% protein, 88- 97% fat, and 1- 2% ash on dry basis; and the insolubles

had 74- 95% moisture, 67- 42% protein, 12- 31% fat, and 17- 37% ash on dry basis (Taskaya et al., 2009a). The authors then concluded that the basic treatments seem to have yielded a more desirable composition of the recovered materials compared to the acidic treatments based on the compositional properties.

Another study performed to determine the compositional characteristics of whole Antarctic krill using ISP resulted in a protein recovery of 45-50%, moisture content of 72-82%, ash content of 4-6% on dry basis (Chen et al., 2009). The low protein recovery yield was also seen by Gigliotti et al. (2008) where the nutritional value and protein quality of krill protein concentrate isolated using ISP was studied. The researchers concluded that the protein recovery yield was approximately 46% on dry basis (Gigliotti et al., 2008). Proximate composition of the krill protein concentrate was 3.3% moisture, 77.7% crude protein, 8.1% lipid and 4.4% ash (dry basis) (Gigliotti et al., 2008).

A similar study investigating the effects of ISP on the protein recovery from rainbow trout processing byproducts determined that when different acidic and basic treatments (pH values 2.5, 3.0, 12.0, 12.5 and 13.0) were applied, the recovered trout proteins contained 75-80% moisture, 9- 19% lipid, 37- 54% protein and 1-2% ash on dry basis (Chen and Jaczynski, 2007). This resulted in a protein recovery of 78- 89%, where the greatest ($p<0.05$) recovery was seen at pH values 2.5 and 13.0. Moreover, it was determined that acidic treatments resulted in a higher protein recovery percentage than basic treatments. Furthermore, it was seen that the recovered protein had a relatively high lipid percentage for both basic and acidic treatments (Chen and Jaczynski, 2007).

A study focusing on the texture and color properties of the proteins recovered from whole gutted silver carp using ISP determined that the protein and lipid recovery were approximately 420-660 and 800-950 g kg^{-1} respectively. Moreover, it was concluded that the basic treatments yielded a lower ($p<0.05$) ash percentage in the recovered protein fraction which is consistent with the findings of other studies. In addition to that, protein recovery was higher ($p<0.05$) at basic treatments (Taskaya et al., 2009b).

Taskaya et al. (2009a) reported that basic solubilization conditions yielded more protein, lower fat and slightly lower ash percentage in the recovered protein fraction. Also, for the recovered lipid fraction, basic pH values yielded more fat, lower protein and lower ash percentage; and there was a higher percentage of protein lost in the insoluble fraction (Taskaya et al., 2009a). It was concluded in the same study that according to the data from the proximate analysis, the basic treatments resulted in a more favorable and efficient composition for the recovered components than the acidic treatments.

EFFECTS OF ISP PROCESSING ON THE AMINO ACID PROFILE OF RECOVERED FISH FRACTIONS

FAO/WHO/UNU requirements for essential amino acids in adults is 127 mg/g protein (WHO, 2002). Therefore, Taskaya et al. (2009a) examined the effect of ISP processing on amino acid profile of the recovered proteins. They reported that the recovered protein contained a range of 354- 397 mg/g protein essential amino acids, where the basic treatments had a higher ($p<0.05$) individual amino acid and essential amino acid content. The essential amino acid content of others were: whole gutted carp 196 mg/g protein, carp fillet 284 mg/g protein, Alaska Pollock surimi 270 mg/g protein, whole egg 536 mg/g protein, Similarly, Chen et al. (2009)

found that both essential amino acid and non-essential amino acid content of the recovered krill proteins were higher ($p<0.05$) than of whole krill. It was also determined by Gigliotti et al. (2008) that the total essential amino acid content of the protein concentrate was 531.5 mg/g protein whereas the essential amino acid content of whole krill was 212.1 mg/g protein. Additionally, when compared to the FAO/WHO/UNU requirements for adults, it was seen that all of the nine essential amino acids were found in sufficient amounts in the protein fraction (Gigliotti et al., 2008).

EFFECTS OF ISP PROCESSING ON THE FATTY ACID PROFILE OF RECOVERED FISH FRACTIONS

Fish and fish products are also significant in human nutrition due to their high concentration of omega-3 polyunsaturated fatty acids (PUFAs) like alpha linolenic acid (ALA), eicosapentenoic acid (EPA), and docosahexenoic acid (DHA). These essential fatty acids have potential health benefits which include reducing the risk of various autoimmune (i.e. Alzheimer's disease), inflammatory and cardiovascular diseases (Kris-Etherton et al., 2002; Morris et al., 2003). Therefore, Taskaya et al. (2009a) examined the effect of ISP processing on fatty acid profile of all recovered fractions. They reported that for all the recovered fractions, ALA, EPA, DHA and total omega 3 fatty acid concentrations were higher ($p<0.05$) at solubilization pH values of 3.0 and 11.5 compared to pH values 2.0 and 12.5. The data revealed that it was just the opposite for omega 6 fatty acids; LA and total omega 6 fatty acid content was higher ($p<0.05$) at pH values 2.0 and 12.5 compared to pH values 3.0 and 11.5. When the recovered carp components were compared to the Alaska Pollock surimi sample, it was seen that the surimi contained a higher ($p<0.05$) amount of DHA, EPA and total omega 3 fatty acids, but had lower amount of LA and omega 6 fatty acids than the carp components (Taskaya et al., 2009a).

Gigliotti et al. (2008) reported that the fatty acid composition of the lipid retained in ISP recovered krill protein concentrate contained 37% saturated fatty acids, 21% monounsaturated fatty acids, and 27% ω-3 PUFAs. Of this 27% portion, EPA made up 12.7%, ALA accounted for 1.5% and DHA made up 12.3%. As for the ω-6 PUFAs, 3.1% accounted for Linoleic Acid (LA), and 1.2% for arachidonic acid (ARA). On the other hand, whole krill contained 17.4% EPA, 1.1% ALA, 12.4% (ARA), 3.3% (LA) and 0.5% (ARA); Coho Salmon which is a fatty fish rich in ω-3 PUFAs contained 7.2% EPA, 2.6% ALA, 11.1% DHA, 6.5% LA and 2.2% ARA. When compared Coho Salmon, EPA and DHA content of the protein concentrate was found to be higher (Gigliotti et al., 2008).

EFFECTS OF ISP PROCESSING ON THE MINERAL PROFILE OF RECOVERED FISH FRACTIONS

Consuming food high in vitamin and mineral content plays a key role in human health. Minerals such as Calcium (Ca), Magnesium (Mg) and Phosphorus (P) are essential for healthy bone formation. It is also reported that up to 87% of the U.S. population do not get adequate levels of minerals such as Ca and Mg from their diet. It is also important to consume adequate levels of other minerals such as Iron (Fe) and limit the intake of others such as Sodium (Na). Therefore, Taskaya et al. (2009a) examined the effect of ISP processing on the mineral content of the recovered protein and insoluble fractions. The results showed that Mg, Ca and P contents were significantly reduced ($p<0.05$) in the recovered protein fraction compared to the initial carp sample. The recovered insolubles had greater ash content; therefore the high concentration of Ca and P was likely an indication that the bones were effectively separated from the protein and lipid fractions. Basic treatments yielded lower ($p<0.05$) Ca, Mg and P content in the recovered protein fraction, whereas, for the insolubles, the Ca, Mg and P content was increased with basic

treatments (Taskaya et al. 2009a). Similar to Taskaya et al. (2009a) findings, Chen at al. (2009) also determined that the mineral content of the recovered krill proteins were lowest ($p<0.05$) at basic treatments, and highest ($p<0.05$) in insolubles at acidic treatments.

EFFECTS OF ISP PROCESSING ON THE FUNCTIONAL PROPERTIES OF RECOVERED FISH PROTEIN

The structure and strength of gels are determined by the type and number of protein-protein interactions, aggregation and arrangement of unfolded proteins. Therefore, it is important to determine the effects of organic acids and ISP processing on the recovered protein fraction. Commonly, exposure to extreme pH conditions cause protein denaturation that have a negative effect on gelation; however, some studies reported higher gel functionality when protein was solubilized by acidic or basic treatments, as occurs during ISP processing, which may be attributed to the partially unfolded proteins being more flexible (Kristinsson and Hultin 2003; Ingadottir and Kristinsson, 2010). Using different organic acids would play a significant role in gel rheology since the acids have different ionic strengths and dissociation constants. For example, acetic acid is weaker than formic and lactic acids and has a higher dissociation constant (Tipping, 2002). Moreover, differences in acidic concentration of each treatment might have different effects on the level of protein denaturation.

Protein denaturation is any modification in its conformation that causes the three dimensional structure of the polypeptide chains to alter (Djikaev and Ruckenstein, 2009). This denaturation can be triggered by different factors such as change in pH, ionic strength, salt concentrations and hot or cold treatment. Therefore, thermal denaturation can be described as the protein conformational modification caused by increased temperature that leads to increased

flexibility and viscosity. Furthermore, as the temperature is increased, hydrogen bonds begin to break enabling increased water binding properties and gel network formation. These characteristics are important in determining the gel forming ability and thus the functionality of the recovered proteins.

The majority of muscle tissue in fish is made up of structural proteins such as actin, myosin and actomyosin (FAO, 2012). Myosin is the most significant component for thermal gel formation in aquatic products and therefore protein gelation is considered a controlled myosin denaturizing process (Kuwahara and Konna, 2010). Differential scanning calorimetry (DSC) is used to determine the thermal transitions (Tm) of proteins in muscle tissue. Typical DSC thermograms result in two distinct peaks: the first peak represents the temperature at which myosin denatures and the second peak represents the denaturation of actin (Taskaya et al., 2009b); however, up to five Tm have been reported for myosin, actin and sarcoplasmic proteins collectively (Thawornchinsombut and Park, 2007). Since most water soluble proteins should have been removed during ISP, it is not likely that the thermal curves reflect any denaturation of sarcoplasmic proteins in ISP recovered protein. Peak I represents the thermal transition due to denaturation of myosin, whereas peak II indicates the actin denaturation. Although actin is more resistant to denaturation due to thermal processes and is seen at higher temperatures, in some studies the actin denaturation curve was not detected on ISP recovered protein gels (Taskaya et al., 2009b; Thawornchinsombut and Park, 2007; Hastings et al., 1985).

Salt concentrations, ionic strength, and pH have an effect on protein denaturation. For example, Hastings et al., (1985) worked with salted herring that was cured with 14% salt and 7% acid, and did not observe a transition peak for actin. An actin peak was not detected in pH shifted fish protein isolates with NaCl controlled treatments made from pacific whiting; likely

due to the increased salt content of the protein homogenate followed by protein precipitation at the isoelectric point which may have had a significant effect in the denaturation of proteins (Hastings et al., 1995; Thawornchinsombut and Park, 2007). On the other hand, Taskaya et al. (2009b) detected an actin peak when protein gels were enhanced with functional additives. Moreover, the transition temperatures as well as the enthalpy presented in the Taskaya (2009b) study that used HCl to recover the carp proteins, were lower than the data presented in the present study. Therefore, organic acids may have played a role in shifting the myosin denaturation peak and increasing the thermal stability due to different protein concentration, lower ionic strength and less protein denaturation in the recovered protein fraction.

Enthalpy, or the net heat energy (ΔH), is indicative of both endothermic and exothermic events. Since the carp proteins undergo a series of exothermic measures such as aggregation during isoelectric solubilization and precipitation, this might have affected the endothermic entalpy (Taskaya et al., 2009b). In addition, the pH shifts would have caused the proteins to change their confirmation and in turn deprive the gels of endothermic transitions. Moreover, Liu et al. (2011) concluded that the extraction process that used either HCl or NaOH to recover peanut protein isolates changed the conformation of proteins and decreased the thermal stability shown by low enthalpy values. The authors (Yongsawatdigul and Park, 2004) attributed it to myofibrillar proteins undergoing denaturation due to being exposed to pH shifts.

Different sources of protein show different thermogram peaks. For example, Ogawa et al. (1993) found that rabbit and horse mackerel myosin had only one major myosin peak; however, sardine stone flounder, walleye pollock, sea bream and carp had two myosin peaks, while rainbow trout, greenling, bigeye tuna and yellow tail showed three myosin peaks. Different Tm values have been reported for different species. Tm of myosin peak for Pacific Whiting (PW)

surimi (2% NaCl) was 33^{o}C (Esturk 2003), whole muscle PW was 45.5 ^{o}C (Hsu et al., 1993), fresh hake was 46.5 ^{o}C (Beas et al. 1990), blue whiting was 45 ^{o}C (Fernandez- Martin et al. 1998) and cod muscles was 44.5 ^{o}C (Hastings et al. 1985). Moreover, Tm values were not consistent within studies reporting the Tm for the same species (Esturk, 2003; Thawornchinsombut and Park, 2007). These inconsistencies may be due to different heating rates (10 ^{o}C/ min compared to 1 ^{o}C/ min) between studies (Thawornchinsombut and Park, 2007).

Functional additives, salt concentrations, ionic strength, and pH have an effect on protein denaturation. For example, Hastings et al., (1985) worked with salted herring that was cured with 14% salt and 7% acid, and did not observe a transition peak for actin. An actin peak was not detected in pH shifted fish protein isolates with NaCl controlled treatments made from pacific whiting; likely due to the increased salt content of the protein homogenate followed by protein precipitation at the isoelectric point which may have had a significant effect in the denaturation of proteins (Hastings et al., 1995; Thawornchinsombut and Park, 2007). On the other hand, Taskaya et al. (2009b) detected an actin peak when protein gels were enhanced with functional additives. Moreover, the transition temperatures as well as the enthalpy presented in the Taskaya (2009b) study that experimented on Alaska Pollock surimi enhanced with functional additives were similar to that of the carp gels made with AA pH 2.5 and F&L pH 12.0 presented in the current study.

It was observed that more protein unfolding was observed in protein isolates than conventional surimi even at lower NaCl levels and that the significant differences of protein characteristics were more influenced by salt concentration than pH (Thawornchinsombut and Park, 2007). Although Taskaya et al. (2009b) have not reported the salt concentration of the recovered protein fractions, having used HCl and NaOH in their study might have influenced the

salt content and the ionic strength of the proteins. Moreover, the macrostructure of the myosin molecule determine the DSC patterns of myosin. Extra stability due to the formation of filaments, just like the thick filaments in fish muscle, might explain higher transition temperatures (Wright and Wilding., 1984).

The functional properties of silver carp proteins recovered by ISP processing showed that typical endothermic transitions and increased viscoelasticity only when the recovered protein was enhanced with functional additives such as PS, BPP, TiGase and PP (Taskaya et al. 2009c). Studies also showed that salt concentrations play a significant role in the gel formation and stability (Thorarinsdottir et al., 2002; Schubring, 1999). Thawornchinsombut and Park (2007) concluded that protein gelation characteristics were more influenced by salt concentration than pH shifts. Therefore, protein gels made from recovered carp protein were analyzed by using differential scanning calorimetery (DSC) and rheometer methods to determine the differences in thermal transition temperatures, enthalpy, viscoelasticity [G´] and viscous modulus [G´´].

EFFECTS OF ISP PROCESSING ON THE TEXTURE PROPERTIES OF RECOVERED FISH PROTEIN

Texture is one of the major quality attributes of food products and is significant for consumer acceptance. Therefore texture properties were assessed by empirical tests being texture profile analysis and Kramer shear cell test as well as torsion shear stress at mechanical fracture which is a fundamental measurement for determining gel strength (Kim et al., 2005). In the study by Taskaya et al. (2009b), the recovered protein from whole gutted silver carp was made into a gel, texture profile analyses were conducted and the results were compared to Alaska pollock surimi grade AA. The protein gels made with the functional additives potato starch (PS), beef

plasma protein (BPP), transglutaminase (TGase) and polyphosphate (PP) had better ($p<0.05$) texture when compared to gels with no additives (Taskaya et al., 2009b). Moreover, the gels made from protein recovered by basic treatments had better texture. In addition to that, it was concluded that the protein solubilized at pH 11.5 resulted in a gel with similar texture to the Alaska Pollock surimi gels (Taskaya et al., 2009b).

Chen and Jaczynski (2007) looked at the gelation properties of the protein recovered from rainbow trout byproducts using ISP. The recovered protein was made into gels and texture profile analysis and torsion tests were performed to assess the gel forming ability. The researchers concluded that without the functional additive BPP, proteins did not form a gel, moreover even with the addition of BPP, the protein gels showed proteolysis between 40 and 55^{o}C. Well textured gels were formed with the addition of PS, TGase and PP and the shear stress of carp gels made with functional additives was lower ($p<0.05$) than that of surimi gels (Taskaya et al., 2009c). Both shear stress and strain were higher ($p<0.05$) when protein solubilized under basic treatments compared to acidic treatments (Taskaya et al., 2010).

The shear stress at fracture of both kamaboko and modori gels made from Pacific whiting surimi, which makes up for 20% of surimi products produced in U.S., was similar to the shear stress of the protein pastes made out of recovered carp protein (Rawdkuen et al., 2008). Rawdkuen et al. (2008) found that addition of chicken plasma to the surimi resulted in increased shear stress values. The highest shear stress was seen when 3.0% (w:w) chicken plasma was added to either kamaboko and modori gels and this data was similar to the shear stress achieved with protein gels made from isoelectrially recovered carp using basic treatments with AA. Another study measuring the shear stress of gels made out of pork, beef, surimi and turkey thigh (Hamann, 2006). When compared to current study results, the shear stress of surimi was

significantly lower while beef and pork gels were similar to the recovered carp protein gels. Only gels made from turkey thighs showed statistically higher shear stress compared to the carp gels.

The gels made from protein solubilized under basic conditions had higher shear force and stress, and were stronger (Taskaya et al., 2010). Other studies using HCL as the processing acid support these findings, concluding that gels made from protein recovered under basic conditions had better texture properties compared to acidic treatments (Chen and Jaczynski, 2007; Kristinsson and Hultin, 2003; Kristinsson and Liang, 2006; Taskaya, et al., 2009a and 2009b). Basic treatments were able to more effectively remove impurities and cause less protein denaturation compared to acidic solubilization treatments. It is likely that gels made from protein recovered using organic acids would have similar texture responses.

COLOR IMPROVEMENT FOR PROTEIN GELS

Color is a major quality attribute of food products and varies greatly depending on customer preferences for a specific product. Recovered protein fraction usually contains pigments that were retained causing the gels that are developed from recovered proteins to have poor color properties (Taskaya et al., 2010). The effect of titanium dioxide on color of protein gels made from protein recovered from whole silver carp using ISP showed the whiteness of carp gels were improved and were higher ($p<0.05$) than that of surimi gels when ≥ 0.2 g/ 100 g TiO_2 was added (Taskaya et al., 2010). In addition, TiO_2 did not affect viscoelasticity or texture of the gels (Taskaya et al., 2010). Protein solubilized under basic conditions produced the whitest gels (Taskaya et al., 2009b). Chen and Jaczynski (2007) also assessed the whiteness of the gels made from protein recovered from rainbow trout byproducts using ISP. It was concluded that the gels made out of protein recovered by acidic treatments were whiter ($p<0.05$) and less yellow

($p<0.05$); however, researchers suggest that it may be due to protein recovered by acidic treatments containing a higher lipid percentage (Chen and Jaczynski, 2007).

THE EFFECT OF ISP PROCESSING ON BACTERIAL LOAD REDUCTION IN FISH

Traditionally strong acids, like hydrochloric acid (HCl), are used to lower pH in the ISP process and is effective at significantly reducing *Escherichia coli* and *Listeria innocua* ($p<0.05$) in protein recovered from fresh rainbow trout at processing pH values of 2.0, 3.0, 11.5, or 12.5 (Lansdowne 2009a and 2009b). However, none of the pH values tested resulted in a pasteurization effect, defined as a 6-log reduction in microbial population. Organic acids are more effective at reducing bacterial pathogens during ISP processing (Otto et al., 2011a and 2011b). Otto et al. (2011b) studied the effects of using organic acids (acetic and citric acids) during ISP processing on the survival of non-pathogenic *Listeria inoccua* (a common surrogate for the pathogenic *Listeria monocytogenes*) in rainbow trout. Regardless of processing pH or acid type, significant reductions ($p<0.05$) were seen in microbial load. A 6.41 log CFU/g reduction of *Listeria inoccua* was seen in the recovered protein fraction when processed at pH 3.0 with acetic acid, compared to a 1.11 log CFU/g reduction when hydrochloric acid (HCl) was used as the processing acid (Lansdowne et al, 2009b). However, when the same organic acids and processing pHs were used to recover protein in *Listeria monocytogenes*-inoculated rainbow trout, the greatest microbial reduction was only 3.53 CFU/g (Otto et al., 2011a).

Using a combination of organic acids may prove to have better bactericidal properties. Blending citric and lactic acids at 2.5% were shown to be effective at reducing *Salmonella* spp. on whole chicken carcasses when sprayed on the carcasses and when dipped into the acid blend for 5 seconds (Laury et al., 2009). When acetic, lactic, propionic and formic acid were combined

1:1 at 1%, 1.5% and 2% concentrations and sprayed onto beef carcasses, significant reductions of *Staphylococcus aureus* were achieved (Mohammad et al., 2009). Moreover, it was shown that the most lethal combination was with 1:1 lactic and formic acids at all concentrations (Mohammad et al., 2009). These findings demonstrate the bactericidal potential of using organic acids and combinations of organic acids in the ISP process.

SUMMARY

Currently, there is no research on the effects of isoelectric solubilization and precipitation using organic acids, especially acetic and formic and lactic acid combination, on the compositional properties of the recovered protein, lipid and insolubles from silver carp, and the functional, textural and color aspects of the protein gels made from the recovered protein. Therefore, the goals of this study were to determine the effects of ISP on the fractions recovered from silver carp using either acetic acid or formic and lactic acid combination undergoing acidic or basic treatments by calculating protein and lipid recovery yields and measuring proximate composition (moisture, protein, lipid and ash) of the recovered fractions. The recovered proteins were made into gels using standard functional additives to examine the textural, functional and color properties. The data were compared to those of whole gutted silver carp, silver carp fillet and previous studies that used strong acids during the ISP process.

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CHAPTER III

Compositional Characteristics of Materials Recovered from Headed Gutted Silver Carp (*Hypophthalmichthys molitrix*) By Isoelectric Solubilization and Precipitation Using Organic Acids

Abstract

Protein may be recovered using isoelectric solubilization and precipitation (ISP), a process that allows protein from insoluble fish fractions (i.e. bones, skin, scales, etc.) to be salvaged by exposure to extremely high or low pH conditions. The effects of using organic acids during ISP processing on total protein and fat recovery yields, proximate composition (protein, fat, lipid and ash) and mineral analysis of recovered fractions (fat, protein and insolubles) of headed-gutted silver carp were compared. Four processing pH values were tested: 2.5, 3.0, 11.5 and 12.0 using glacial acetic acid (AA) or a 30% formic and lactic acid combination (F&L). Protein recovery (g/kg sample, dry basis) ranged from 567- 892 with AA and 175- 291 with F&L using acidic solubilization whereas 694- 728 with AA and 644- 657 with F&L when basic solubilization treatments were applied. Recovery was greatest ($p<0.05$) at solubilization pH 2.5 when using AA and lowest ($p<0.05$) when acidic treatments are used with F&L. Lipid recovery (g/kg, dry basis) ranged from 232- 338 with AA and 274- 975 with F&L using acidic treatments. Recovery was greatest at basic processing pH for AA and at pH 2.5, 11.5 and 12.0 for F&L. Proximate composition of the recovered proteins showed that processing at basic pH using AA was most effective at removing impurities such as bones, scales, skin, fins, etc. ($p<0.05$) and that impurities were effectively removed from recovered lipids regardless of processing pH or acid type. Mineral analysis of recovered protein when compared to the fish fillet revealed no

differences in Fe and Ca content ($p>0.05$) and less P ($p<0.05$). Na content was slightly increased ($p<0.05$) compared to the fish fillet. This research shows that organic acids have the potential to recover protein and lipid by ISP processing.

Keywords: Isoelectric solubilization and precipitation, protein recovery, lipid recovery, proximate composition, mineral profile, silver carp protein, organic acids

Introduction

Fish and fish products are significant in human nutrition due to their high, biologically valuable protein, fat and fat soluble vitamin content (Belitz et al., 2009). Other benefits of fish include the high concentration of omega-3 polyunsaturated fatty acids (Ω-3 PUFAs). Fish and fish products are rich especially in the Ω-3 PUFAs alpha linolenic acid (ALA), eicosapentenoic acid (EPA), and docosahexenoic acid (DHA) which may have positive health effects for chronic diseases (Tziomalos et al., 2007; Wall, et al. 2010). Fish and fish products represent a significant source of animal protein in the human diet; global consumption data indicate that since 2008, over 117.8 million tons of fish are consumed each year (FAO, 2010). Statistics highlight that most of the natural fish resources are either operating close to an optimal yield level or operating at levels that are not sustainable and at risk of stock depletion (FAO, 2004). Contribution of fish to the global food supply might decrease unless fish stocks are better managed; therefore, there is a need to find a reliable method to utilize the aquatic resources efficiently.

Catch trends show that cyprinids such as carp were the most abundantly caught fish group as of 2005. Carp production increased in the following years up to the point that freshwater fish production was dominated by carps in 2008, accounting for 20.4 million tons, or 71.1 percent of the freshwater fish market valued at $40.5 billion (FAO, 2012). Silver carp (*Hypophthalmichthys molitrix*), the most common carp species, is a fresh water fish and is considered to be as nutritionally valuable as ocean fishes (Buchtová, et al., 2010). In China, silver carp is one of the top 10 species of fish grown, with over 3.5 million tons grown annually (FAO, 2007). Collectively, carp represent the greatest amount of aquaculture produced fish in China because of their minimal growth requirements and rapid growth rates. This is the same reason why carp is a concern in the United States where they are considered an invasive species

because they compete with the indigenous aquatic life. Some species of carp are considered a delicacy in Asian countries; unfortunately, the commercial value of carp in the United States is low likely due to the boney nature of the carp carcass (Luo, et al., 2008). Carp is a boney fish, having two rows of small floating bones above and below the lateral line. Therefore, in order to produce a fillet, the floating bones need to be removed by grinding or scoring. This process is time consuming and removal of all the small bones is not reliable; which reduces the marketability of this type of fish; therefore the potential nutritional value of silver carp has not been fully utilized as a low-fat and high-protein food source (Luo, et al., 2008). More efficient methods to recover the nutritionally valuable protein and oil from these fish and its byproducts need to be explored.

Isoelectric solubilization and precipitation (ISP) processing is a method that allows separation of fish fractions (i.e. proteins, lipids and insolubles) by homogenization with water and either acids or bases. Centrifugation following homogenization causes separation of protein from insoluble fractions of the fish (i.e. bones, skin, scales, etc.). The pH of the protein slurry is then returned to the protein isoelectric point of pH 5.5 at which point protein precipitation occurs and is recovered by centrifugation. Typically, strong acids are used for pH shifts during ISP processing; however, recent ISP processing studies using organic acids have shown that weak acids are more effective than strong acids at bacterial reduction (Lansdowne et al., 2009a and 2009b; Otto et al., 2011a and 2011b). Therefore, it is possible that strong acids can be replaced with organic acids during ISP processing, however, the effect of organic acids on protein and lipid recovery, as well as protein composition and functionality, would need to be determined. Therefore, the objective of this study was to determine the impact of organic acids on nutritional

and compositional characteristics of protein and lipids recovered from silver carp during ISP processing.

Materials and Methods

Preparation of fresh silver carp. Fresh silver carp (*Hypophthalmichthys molitrix*) were headed and gutted and sanitized by submergence into a 50 ppm bleach solution for 10 sec (Clorox Regular Bleach, Clorox, Oakland, CA, USA). After draining for 10 sec, fish were ground into a thick paste using a sanitized food processor (Cuisinart ProClassic7-Cup Food Processor, Cuisinart Co., East Windsor, NJ, USA). All equipment was sanitized by cleaning with 70% ETOH and placing under UV light (254 nm) for 15 min (Otto et al. 2011a). The fish paste (255 g) was weighed and separated into freezer bags (Ziplock Freezer Bags, S.C. Johnson & Son, Inc., Racine, WI, USA) to be stored at -80°C until analyses were conducted.

Muscle protein recovery from headed and gutted silver carp. Protein was recovered using isoelectric solubilization and precipitation (ISP) processing methods described by Otto et al. (2011a & 2011b). Briefly, frozen fish paste was removed from -80°C storage and thawed for 24 h at 4°C. The recovery process of muscle proteins and lipids are shown in Figure 1. Thawed fish paste (250 g) was weighed and homogenized with 1500 mL distilled, deionized water in a glass beaker. The target pH values for protein solubilization were pH 2.5, 3.0. 11.5 and 12.0. The acids tested were glacial acetic acid (Fischer Scientific, Fairlawn, NJ, USA; AA) and a combination of 30% glacial L-Lactic acid (85%, EMD Chemicals, Netherlands) and formic acid (88%, Mallinckrodt Chemicals, Netherlands) (F&L) in sterile distilled water at a 1:1 ratio (Mohammad et al., 2009). The fish slurry was brought to the target pH (2.5, 3.0, 11.5 or 12.0) with the addition of the respective organic acid or 10 N sodium hydroxide (NaOH) and homogenized (PowerGen 700, Fisher Scientific, Pittsburgh, PA) until the pH stabilized (approximately 10 min). The homogenization process was continued for an additional 10 min to maximize protein solubilization. The homogenized fish slurry was centrifuged at 10,000 x G for

10 min at 4°C (Sorvall RC-SB Refrigerated Superspeed Centrifuge, Du Pont, Wilmington, DE, USA). After centrifugation, the top layer (fat) was collected for analyses and stored at -80^{o}C until further analyses was conducted. The supernatant was poured through a cheese cloth to remove the insoluble portions (scales, bones, etc.) of the solution; the insoluble fractions were retained for analyses. The pH of the supernatant was returned to the protein isoelectric point of pH 5.5 using the respective organic acid or 10 N NaOH and homogenized for 5 min. The homogenate was again centrifuged at 10,000 x G for 10 min at 4°C after which the supernatant (process water) was discarded and the protein, left in the centrifuge bottle as a pellet, was retained for analyses. The recovered fractions (protein, lipid and insoluble) were then placed in a freeze dryer (VirTis Genesis, SP Scientific, Gardiner, NY) until the probes read above 22^{o}C, indicating that the samples were dry. Dried samples were stored at 4^{o}C until further analyses were conducted.

Proximate analysis of recovered fractions of headed and gutted silver carp. Proximate composition (moisture, crude protein, fat, and ash) was determined on the initial fish paste, carp fillet and the recovered fractions (protein, lipid and insoluble) from ISP at each pH strategy according to the Association of Official Analytical Chemists (1995). Moisture content was determined prior to freeze-drying and reported on wet basis. All of the other tests were performed on the freeze-dried samples and reported on dry basis. Moisture was determined using the oven-drying method where each sample was spread on aluminum dishes and dried for 24 h at 105^{o}C. The Kjeldahl assay and the Soxhlet extraction method were used to determine crude protein and total fat content, respectively. Ash content was measured by incineration in a muffle furnace oven (Fisher Scientific, Pittsburgh, PA) at 550^{o}C for 24 hours. All proximate analyses were done in triplicate, reported as the mean value (±SD) and expressed as g kg^{-1}.

Protein recovery yield and fat recovery yield in ISP recovered fractions. The protein and fat recovery yields were calculated using the following equations (Chen and Jaczynski, 2007):

$$\text{protein recovery yield} = \frac{wt\ of\ recovered\ carp\ protein\ (g)(Kjeldahl, dry\ basis)}{wt\ of\ protein\ in\ raw\ carp\ (g)\ (Kjeldahl, dry\ basis)}$$

$$\text{lipid recovery yield} = \frac{wt\ of\ recovered\ carp\ fat\ (g)\ (Soxhlet, dry\ basis)}{wt\ of\ fat\ in\ raw\ carp\ (g)\ (Soxhlet, dry\ basis)}$$

Amino acid profile analysis. Amino acid analyses were conducted on headed-gutted carp, carp fillet and recovered proteins. The test was performed at the University of Missouri-Columbia according to the Association of Official Analytical Chemists methods (AOAC, 1995; AOAC 982.30 E). Samples were freeze-dried prior to analysis. Acid hydrolysis was conducted with 6 N HCl at 110 ^{o}C for 24 h, followed by oxidation with performic acid at 0-5 ^{o}C overnight. Acid hydrolysis was repeated with 6 N HCl at 110 ^{o}C for 24 h and concluded with alkaline hydrolysis with fresh 4.2 N NaOH at 110 ^{o}C for 22 h. Amino acid quantification was accomplished using a Beckman Amino Acid Analyzer (model 6300, Beckman Coulter, Inc., Fullerton, CA) using sodium citrate buffers as step gradients with the cation exchange postcolumn ninhydrin derivatization method following the hydrolysis processes (Taskaya et al., 2009).

Fatty acid profile analysis. Fatty acid analyses were conducted on headed-gutted carp, carp fillet and recovered protein and lipids. The analysis was performed at the University of Missouri-Columbia according to the Association of Official Analytical Chemists (1995) methods. All samples were freeze-dried prior to analysis. Fatty acid extraction was conducted using the acidic hydrolysis technique (AOAC 996.06). Oxidative degradation of fatty acids was minimized by the addition of pyrogallic acid. Fat was extracted into ether followed by

methylation to fatty acid methyl esters (FAMEs) with boron trifluoride and methanol. FAMEs were quantified by capillary gas chromatography against the internal standard (triglyceride, triundecanoin (C11:0)). A gas-liquid chromatography (Agilent Technologies, Santa Clara, Calif., U.S.A., Model 7890A) equipped with a 7683B series Injector was used to analyzed the samples. The carrier gas, Helium, was used at a 0.75 mL/flow rate. Separations began at 100^{o}C. After 4 min, the temperature was increased to 240^{o}C at 3^{o}C/min for 15 minutes. The injector and detector temperatures were 225 and 285^{o}C, respectively. The retention times were compared with the references to determine the peaks. Total fat was determined as the sum of individual fatty acids that were expressed as their triglyceride equivalents (Simmons et al., 2011).

Mineral profile analysis. The mineral profile of recovered protein samples, carp fillet and ground fish samples were determined. The glassware and the crucibles used in the analyses were kept in a 10% HCl solution overnight. Samples (5 g) were ashed in the muffle furnace (Fisher Scientific, Pittsburgh, PA) at 550^{o}C overnight and then dissolved in 2 ml of 70% nitric acid. After the acidification step, distilled water was added to the samples. The solution containing the samples were filtered through Whatman no.1 filter paper and diluted with distilled water in a 50 ml flask. Major minerals being calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe) and sodium (Na) were analyzed using inductively coupled plasma optical emission spectrometry (model P400; Perkin-Elmer, Shelton, CT) (Taskaya et al., 2009).

Statistical Analysis. Except where indicated, the experiment was replicated three times and all analyses were conducted in triplicate for each solubilization pH value and treatment. The fatty acid and amino acid profile assays were conducted on products from one replication and therefore statistical analysis was not conducted on the results. The mineral profile assay was performed in duplicates for each treatment. Data were analyzed by one-way analysis of variance

and Tukey-Kramer's honestly significant differences test ($P<0.05$) (JMP 9, SAS Inst., Cary, N.C., USA).

Results and Discussion

Proximate Analysis. The proximate compositions of headed gutted carp (starting material) and skinless and boneless carp fillets (manual filleting) were determined and are shown in Table 1. Headed gutted carp and boneless carp fillets contained 202.7 g kg^{-1} and 54.2g kg^{-1} ash (dry basis), respectively. The high ash content of the headed gutted carp was to be expected as ash is an indicator of impurities in the starting material (Taskaya et al., 2008). The headed gutted carp was ground with head, skin, bones, scales, fins, etc. which contributed to the ash content. The skinless and boneless carp fillet also had relatively high ash content. Carp are notoriously difficult to fillet because of having intramuscular pin bones that are a challenge to remove; therefore it is likely that pin bones were present in the filleted muscle.

Ash content is also of interest because it serves as an indicator of how well proteins and lipids were separated from the impurities during the ISP process. By determining ash content of the recovered components, the effectiveness of each acid and pH combination can be assessed. Data in Table 1 shows that using organic acids under acidic conditions were not very effective at separating the impurities from the protein. Ash content in protein solubilized at pH 2.5 and 3.0 using AA and 30% F&L ranged from 265.8 – 638.3 g kg^{-1}, which is significantly greater than the ash content of the skinless and boneless carp fillet ($p<0.05$). The only organic acid processing strategy that yielded ash values consistent with those reported when HCl was used as the processing acid was when NaOH was used to solubilize the protein at basic conditions and AA was used to precipitate it. In this case, the average ash content of the recovered protein was 32 g kg^{-1}, compared to 55 and 37 g kg^{-1} when HCl was used as the processing acid at acidic and basic solubilization conditions, respectively (Taskaya et al., 2009).

Proximate analysis shows that the organic acids used in this study were not as effective as HCl during ISP processing at separating the protein, lipids and insoluble fractions. This can be attributed to the acid dissociation constants (pKa) of organic acids when compared to strong acids such as HCl. HCl dissociates completely in aqueous solutions; however, AA and F&L have different dissociation constants and depending on the pH, have a lower dissociation percentage (Hasan, 2009). As the acid dissociates, hydronium ions are released. Increased positive surface charge leads to protein-water electrostatic interaction, and as the protein molecules become more charged, their solubility increases (Gehring et al., 2009). The amount of acid in its dissociated form depends on the pH of the slurry. The pKa of AA is 4.76 meaning that it will be in half dissociated and half non-dissociated form at pH 4.76 (Zhang et al., 2009; Tipping et al., 2002). At a lower solubilization pH value, there will be more non-dissociated AA. Similar to AA, formic acid and lactic acid have a pKa of 3.76 and 3.83, respectively (Zhang et al., 2009). Therefore, organic acids used in the study will only have complete dissociation at solubilization pH values above 4.76 whereas strong acids such as HCl will fully dissociate at all pH treatments (Tipping et al., 2002). Unfortunately, this pH is not sufficient to effectively solubilize protein which may explain why the acidic treatments using organic acids have lower protein concentrations in the protein fraction. Solubilization under basic conditions resulted in better protein concentrations ($p<0.05$); however, the protein concentrations in the recovered protein fraction were less than those reported when HCl was used as the processing acid (Takaya et al., 2009). Strategies to increase the solubility of protein may include increased concentration of organic acid combination, increased centrifugation time after protein solubilization and increased dilution rate of ground fish to water.

The recovered protein fraction also contained lipids. It is desirable to have less amount of lipid in the protein fraction due to the lipid related oxidation and possible rancidity issues. Similar to the other studies (Taskaya et al., 2009; Kristinsson et al., 2005), recovered proteins had lower lipid when AA is used with basic treatments. On the other hand, the lowest lipid retained in the protein fraction was seen when F&L was used at pH 3.0. Centrifugation after solubilization will let the membrane phospholipids sink to the bottom pellet in the centrifuge tube, causing the neutral lipids to rise to the top of the tube. The removal of these neutral lipids are easier compared to the membrane phospholipids which are only partially separated (Kristinsson and Liang, 2006). The lower lipid content of the protein fraction when solubilized using AA with basic treatments can be explained by the higher emulsification ability of the lipids at alkali pH values compared to acidic conditions.

The lipid concentration in the recovered lipid fraction was the greatest at basic solubilization conditions, regardless of processing acid tested (Table 1). The insoluble fraction contained 5.9- 226.4 g kg^{-1} of lipid throughout treatments. The lipid retained in the insolubles may have contained membrane lipids such as phospholipids, and due to their apolar nature, they may have been preserved in spite of centrifugation following solubilization. It is interesting to note that the insoluble fraction also contained a high amount of protein likely because when pH was shifted using weak acids, there was insufficient acid dissociation, and therefore insufficient protein-water electrostatic interaction. This would result in limited solubility of the myofibrillar proteins, which would remain with the rest of the insoluble components when centrifuged. Moreover, the reported protein concentrations of the insoluble fraction may be misleading because the Kjeldahl method only measures nitrogen containing compounds; therefore, it is possible that the protein concentration in the insoluble fraction may include non-protein nitrogen

compounds that are water-soluble especially since fish contains 9- 18 % of non-protein nitrogen compounds of the total nitrogen (FAO, 2012).

Protein and Lipid Recovery Yield. Protein recovery yield is an important aspect of determining ISP efficiency. Overall, the greatest protein recovery yields occurred when acetic acid was used, with the maximum recovery of 892 g kg^{-1} happening when protein was solubilized at pH 2.5 ($p<0.05$) (Figure 1). When ISP processing was applied to Antarctic krill and Atlantic croaker using HCl as the processing acid, the highest recovery yields were seen at both extremely low (pH 2.0) and basic solubilization pH values (pH ≥11) (Chen et al., 2009; Kristinsson and Liang, 2006). The average recovery yield when protein solubilization occurred under basic conditions was 711.41 g kg^{-1} for AA and 650.49 g kg^{-1} for F&L; there were no significant differences ($p>0.05$) in recovery yields regardless of basic solubilization pH or organic acid used. When ISP processing was applied to silver carp using HCl as the processing acid, the protein recovery yields ranged from 420 – 660 g kg^{-1} (Taskaya et al., 2009b) which is less than the yields reported in this current study. The major components of fish muscle are myofibrillar and sarcoplasmic proteins. Myofibrillar proteins make up the major portion of protein recovered during ISP processing because although they are largely water-insoluble, they will solubilize at extreme pH. On the other hand, sarcoplasmic proteins readily solubilize into the process water and are not typically recovered during the ISP process. It is possible that the greater protein recovery yields in this present study are due to insufficient separation of proteins from the rest of the fish components resulting in an increase of sarcoplasmic protein in each fraction that otherwise would have been discarded with the ISP processing water.

Studies show that lipid recovery yield, similar to protein recovery yield, is more efficient when ISP is conducted at basic treatments compared to acidic conditions (Liang and Hultin

(2005a, 2005b; Taskaya et al., 2009b; Kristinsson et al., 2005). In correlation with the previous studies, the lipid recovery yield was greater when basic solubilization treatments were applied using either organic acid with the exception of F&L under pH 2.5 (Figure 2). Taskaya et al. (2009b) showed a lipid recovery yield ranging from 800- 950 g kg^{-1} for whole gutted carp using ISP. The current study shows that using organic acids with basic treatments with the exception of F&L 2.5, accomplished similar results as when HCl was used as the processing acid during ISP.

Amino Acid Profile Assay. The amino acid composition of the recovered protein fraction was assessed since crude protein was concentrated in the recovered protein. Basic treatments resulted in higher amounts of individual essential amino acids (EAA) as well as total EAA compared to acidic treatments, regardless of acid used (Table 2). Similar results were reported by Taskaya et al. (2009a) for recovered silver carp protein using HCl. Moreover, the total EAA for all processing conditions except AA at basic solubilization treatments exceeded the amount of headed gutted carp because protein was more concentrated in the recovered protein fraction. The total EAA of recovered protein when AA was used at basic solubilization pHs was greater than the EAA content of silver carp fillets. The total EAA amounts of recovered protein using either acid under either treatment exceed the level (141 mg EAA/g protein) recommended by WHO (2002) for adults; however, are much lower than the amount in whole egg (536 mg EAA/g protein) which is considered the gold standard. The amino acid composition of soluble and insoluble protein powders from arrowtooth flounder fillets (Sathivel et al., 2004) were reported to be slightly higher than the current results; however, when compared to Alaska Pollock surimi with 270.4 mg EAA/g protein (Taskaya et al., 2009a), protein recovered with basic treatments using either organic acid was higher.

The ratio of total EAA to total amino acids was similar to the data presented by Taskaya et al. (2009a). The recovered protein yielded a higher total EAA to total amino acid ratio compared to that of headed gutted carp regardless of solubilization pH or acid type; however, only the basic treatments using either acid were up to the same ratio as carp fillets. Recovered protein using either acid only under basic treatments had a similar ratio of total EAA to total amino acids as Alaska Pollock surimi. Chen et al. (2007) also showed that basic pH treatments showed higher ($p < 0.05$) total EAAs compared to acidic solubilization pHs.

The liberation of amino acids is accomplished by two different hydrolysis; non-enzymatic (pH induced) and enzymatic (endogenous proteases) hydrolysis causing partial hydrolyzation of protein. Moreover, polar amino acids such as partially hydrolyzed carp protein and free amino acids are soluble in water. Therefore, acidic solubilization conditions resulted in less amino acid retention than basic solubilization treatments likely due to the increased protein recovery when solubilization was achieved using basic pHs. In addition to that, using AA with basic solubilization conditions yielded the highest individual and total EAA which is in correlation with the compositional characteristics data (Table 1) showing highest protein content and purity with AA at alkali treatments.

Fatty Acid Profile Assay. Fish and fish products are significant in human nutrition due to their high concentration of polyunsaturated fatty acids (PUFAs) like the ω-3 and ω-6 series of fatty acids. The ω-3 fatty acids of interest are alpha linolenic acid (ALA; 18:3ω-3), an essential fatty acid, and its metabolites eicosapentenoic acid (EPA; 20:5ω-3) and docosahexenoic acid (DHA; 22:6ω-3); which are potentially beneficial in reducing the risk of various autoimmune (i.e. Alzheimer's disease), inflammatory and cardiovascular diseases (Kris-Etherton et al., 2002;

Morris et al., 2003). The ω-6 fatty acids of interest are linoleic (LA; 18:2ω-6), an essential fatty acid, and its metabolite arachidonic acid (ARA, 20:4ω-6). The fatty acid profile of ISP-recovered protein and lipid fractions were analyzed and reported in Table 3. The highest total ω-3 level was seen when protein was recovered at pH 2.5 followed by pH 3.0 using AA and pH 11.5 using F&L. This is similar to the findings by Taskaya et al. (2009a) where total ω-3 level was highest when protein was solubilized at pH 3.0 and 11.5 using HCl. This might be due to the increased ionic strength at either extremely low or extremely high pHs causing increased hydrophobic reactions between carp protein, triglycerides and phospholipids. There are two major groups of lipids present in teleost fish species: phospholipids and triglycerides (Ababouch, 2005). Phospholipids have amphiphilic characteristics and emulsifying properties, allowing them to interact with apolar triglycerides as well as charged molecules like proteins and water (Chen and Jaczynski, 2007). It is harder to remove phospholipids from the protein section that get trapped inside the folding and unfolding proteins. Therefore, the rapid unfolding of the carp protein under extremely low and extremely high solubilization conditions followed by the rapid folding of proteins during precipitation might have resulted in increased amount of fatty acid entrapment.

Total ω-3 in headed gutted carp was lower than that of recovered protein using AA under pH 2.5, 3.0 and 11.5, and F&L with basic treatments. Total ω-3 in the recovered protein using either treatment was higher compared to silver carp fillets. The highest values of total ω-3 in the lipid fraction were seen at basic treatments using either acid. Similar to the protein fraction data, the total ω-3 content in the lipid fraction was higher than that of carp fillets with either treatment; however, only using basic treatments yielded higher value than the starting material. Chen and Jaczynski (2007) showed that acidic treatments resulted in higher lipid retention in the recovered

krill protein compared to basic solubilization conditions when HCl was used as the processing acid. This was explained by free fatty acids being more readily removed with alkaline processing compared to acidic conditions (Nawar, 1996) which might explain the high ω-3 lipid content in the lipid fraction.

Total ω-6 amounts of the protein and lipid fractions showed the same trend as the total ω-3 levels. The highest levels of total ω-6 in the lipid fraction were seen with recovery under basic treatments. As for the protein fraction, the highest levels were seen with acidic treatments using AA and basic treatments with F&L. This might be attributed to increased ionic strength when AA is used with acidic conditions and when F&L is used with basic conditions leading to increased hydrophobic interactions between phospholipids, triglycerides, protein and water.

It is interesting to note that there was no ALA or EPA detected in the fish fillets; implying that a significant source of these fatty acids is in the insoluble fractions of the fish which otherwise would have been discarded, likely the skin. Unfortunately, this is another example of the ineffectiveness of AA and 30% F&L to separate protein from the other fractions of the fish. Perhaps increasing the concentration of the F&L solution would yield better separations. On the other hand, retention of ω-3 in the protein may be beneficial to the nutritional quality of the recovered material, especially when one considers the ω-6 to ω-3 ratio. Western diets are deficient in ω-3 fatty acids while having high amounts of ω-6 fatty acids. Excessive ω-6 and a high ω-6 to ω-3 ratio are suspected to promote cardiovascular diseases. A ω-6 to ω-3 ratio closer to 1 is associated with a healthier diet whereas the ratio in Western diets is approximately 15/1 to 16.7/1 (Simopoulos, 2002). All the treatments and the initial material showed values below 1 for ω-6 to ω-3 ratio (Table 3).

Mineral Profile Assay. Consuming foods high in vitamin and mineral content plays a key role in human health. Minerals such as Calcium (Ca), Magnesium (Mg) and Phosphorus (P) are essential for healthy bone formation. It is also reported that up to 87% of the U.S. population do not get adequate levels of minerals such as Ca and Mg from their diet. It is also important to consume adequate levels of other minerals such as Iron (Fe) and limit the intake of others such as Sodium (Na). Therefore, Ca, Mg, P, Fe and Na were assessed in the recovered protein fractions.

The mineral contents of the headed gutted fish and carp fillets were lower than the Recommended Daily Allowance (RDA) for adults; therefore, the treatments also resulted in a mineral content lower than the RDA. The Ca and P content of recovered protein at pH 2.5 using F&L was higher ($p< 0.05$) than the other treatments (Figure 4). This may be due to the insoluble fish components, most likely bone, were not entirely removed from the protein fraction. The lowest Na content was seen when protein was recovered using AA at pH 11.5. Although the mineral contents of the recovered protein fractions were lower than the initial starting material; they were comparable to the mineral content of fish fillet. The initial starting material, headed gutted carp, was processed through the grinder with its bones and previous studies have shown that silver carp bones are rich in minerals (Wu et al., 2012; Chen et al., 2006).

Conclusion

Protein recovery was greatest at basic processing pH for F&L and at pH 2.5, 11.5 and 12.0 for AA. At the same solubilization pH, using AA as the processing acid yielded greater protein recovery compared to F&L. Lipid recovery was also increased when basic solubilization treatments were applied except for pH 2.5 using F&L.

Proximate composition showed that basic treatments using AA was most effective at removing impurities such as bones, scales, skin, fins, etc. ($p<0.05$). Furthermore, basic treatments using AA yielded a more advantageous composition of essential amino acids followed by basic solubilization conditions using F&L. The fatty acid composition of isoelectrically recovered protein using either treatment was more beneficial compared to the carp fillet; however, for the most part there weren't any significant differences between isoelectrically recovered protein and carp fillet when iron, magnesium, phosphorus and calcium amounts were compared. Sodium amount was slightly increased during ISP especially when F&L was used as the processing acid.

Overall, ISP using organic acids especially with basic solubilization treatments has the potential to recover protein and lipid effectively.

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Table 1. Proximate composition[a] (g kg^{-1}, dry basis) of recovered carp proteins, lipids and insolubles that were solubilised at different pH values and solubilization strategies.

		Recovered Proteins			
Solubilization pH	**Acid**	**Moisture (wet basis)**	**Lipid**	**Protein**	**Ash**
2.5	AA	950.6 ± 7.9 ^{a}	54.7 ± 23.5 ^{ab}	321.6 ± 11.5 ^{cd}	402.7 ± 25.0 ^{b}
3	AA	902.6 ± 6.1 ^{ab}	80.3 ± 14.9 ^{a}	427.9 ± 62.8 ^{bc}	311.9 ± 27.5 ^{bc}
11.5	AA	874.5 ± 5.8 ^{b}	18.4 ± 12.7 ^{bc}	817.3 ± 1.7 ^{a}	30.8 ± 12.0 ^{d}
12	AA	887.1 ± 7.8 ^{ab}	19.6 ± 6.2 ^{bc}	766.1 ± 94.4 ^{a}	33.6 ± 10.4 ^{d}
2.5	F&L	814.0 ± 8.7 ^{c}	93.4 ± 41.2 ^{a}	517.4 ± 21.0 ^{b}	265.8 ± 29.2 ^{c}
3	F&L	891.3 ± 18.9 ^{ab}	0.6 ± 0.3 ^{c}	200.6 ± 12.4 ^{d}	638.3 ± 34.3 ^{a}
11.5	F&L	803.9 ± 16.2 ^{c}	48.5 ± 10.7 ^{abc}	506.4 ± 89.0 ^{b}	259.3 ± 82.3 ^{c}
12	F&L	833.1± 6.3 ^{c}	63.4 ± 5.7 ^{ab}	803.8 ± 55.5 ^{a}	363.3 ± 48.3 ^{bc}
		Recovered Lipids			
Solubilization pH	**Acid**	**Moisture (wet basis)**	**Lipid**	**Protein**	**Ash**
2.5	AA	831.9 ± 7.7 ^{b}	644.6 ± 38.0 ^{c}	149.5 ± 18.6 ^{bc}	50.0 ± 11.0 ^{ab}
3	AA	822.9 ± 6.7 ^{b}	452.9 ± 39.7 ^{d}	432.4 ± 112.3 ^{a}	76.7 ± 36.2 ^{a}
11.5	AA	671.1 ± 5.2 ^{cd}	899.9 ± 20.5 ^{a}	69.3 ± 18.9 ^{c}	8.4 ± 3.5 ^{c}
12	AA	600.6 ± 11.2 ^{e}	851.1 ± 71.3 ^{ab}	71.6 ± 12.2 ^{c}	21.4 ± 9.3 ^{bc}
2.5	F&L	895.0 ± 15.9 ^{a}	381.5 ± 49.3 ^{d}	296.0 ± 10.5 ^{ab}	55.0 ± 7.8 ^{ab}
3	F&L	937.7 ± 17.1^{a}	160.4 ± 46.2 ^{e}	343.5 ± 51.1 ^{a}	68.5 ± 6.5 ^{a}
11.5	F&L	683.5 ± 44.6 ^{c}	600.4 ± 24.5 ^{c}	162.9 ± 27.8 ^{bc}	57.4 ± 48.9 ^{ab}
12	F&L	624.0 ± 23.9 ^{de}	730.6 ± 64.5 ^{bc}	21.3 ± 0.4 ^{c}	66.5 ± 5.0 ^{a}
		Recovered Insolubles			
Solubilization pH	**Acid**	**Moisture (wet basis)**	**Lipid**	**Protein**	**Ash**
2.5	AA	927.7 ± 12.6 ^{a}	35.4 ± 10.4 ^{cd}	481.8 ±91.9 ^{ab}	133.5 ± 28.4 ^{bcd}
3	AA	919.7 ± 11.1 ^{a}	107.0 ± 18.5 ^{b}	645.5 ± 106.1 ^{a}	132.7 ± 22.3 ^{bcd}
11.5	AA	696.6 ± 10.7 ^{b}	70.8 ± 8.5 ^{bc}	643.4 ± 24.7 ^{a}	266.9 ± 98.9 ^{b}
12	AA	708.0 ± 3.5 ^{b}	95.6 ± 13.1 ^{b}	488.8 ± 71.5 ^{ab}	141.8 ± 64.2 ^{bcd}
2.5	F&L	923.2 ± 7.0 ^{a}	175.1 ± 39.0 ^{a}	434.5 ± 17.7 ^{b}	79.7 ± 14.0 ^{d}
3	F&L	933.5 ± 1.0 ^{a}	226.4 ± 24.8 ^{a}	582.7 ± 95.2 ^{ab}	92.1 ± 12.9 ^{cd}
11.5	F&L	915.3 ± 29.2 ^{a}	5.9 ± 2.6 ^{d}	390.6 ± 31.6 ^{b}	638.0 ± 63.8 ^{a}
12	F&L	919.4 ± 15.2 ^{a}	15.1 ± 0.5 ^{cd}	562.4 ± 70.4 ^{ab}	233.1 ± 26.6 ^{bc}

[a] Data are given as mean ± standard deviation (n = 3). Mean values in a column with different letters are significantly different (Tukey's honestly significant difference test, $p<0.05$).

For comparison, proximate analysis of headed gutted silver carp: moisture 721.2 (wet basis), crude protein 504.1 (dry basis), total fat 245.2 (dry basis) and ash 202.7 (dry basis) g kg^{-1}; boneless skinless carp fillet: moisture 796.3 (wet basis), crude protein 801.2 (dry basis), total fat 5.8 (dry basis) and ash 54.2 (dry basis) g kg^{-1}.

Table 2. Essential amino acid (EAA) content (milligrams per gram of protein, dry basis) in the protein recovered from headed gutted carp using isoelectric solubilization at different pH values with different organic acids and precipitation at pH 5.5 as compared to headed gutted carp and carp fillets.

pH	Acid	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Histidine	total EAA	total non-EAA	total AA	ratio EAA/ non-EAA	ratio EAA/ total AA
2.5	AA	17.3	27.2	30.9	9.6	14.2	13.7	4.6	18.0	7.3	142.8	182.8	325.6	78.1	43.9
3	AA	24.2	38.9	42.3	14.0	19.9	20.3	5.7	25.3	10.4	201.0	257.0	458.0	78.2	43.9
11.5	AA	44.1	72.7	82.4	26.2	35.0	37.7	10.9	47.8	20.7	377.5	453.8	831.3	83.2	45.4
12	AA	41.6	69.3	78.0	25.1	33.3	35.8	10.1	45.4	19.7	358.3	431.3	789.6	83.1	45.4
2.5	F&L	18.8	29.3	30.4	10.9	15.3	15.4	4.9	20.1	8.0	153.1	213.7	366.8	71.6	41.7
3	F&L	24.9	39.5	41.6	14.5	20.3	20.7	6.0	26.6	11.1	205.2	270.7	475.9	75.8	43.1
11.5	F&L	34.5	56.0	60.5	20.0	28.4	28.3	7.5	36.3	15.2	286.7	345.9	632.6	82.9	45.3
12	F&L	35.4	61.5	68.5	21.8	30.6	32.4	9.3	38.5	18.4	316.4	383.0	699.4	82.6	45.2
Ground carp		30.7	49.7	57.8	18.5	25.1	26.7	6.3	33.5	17.0	265.3	392.5	657.8	67.6	40.3
Fillet		39.9	65.5	75.9	23.0	33.6	34.7	9.8	43.0	24.1	349.5	420.9	770.4	83.0	45.4

Table 3. Fatty acid (FA) content (milligrams per gram of sample, dry basis) in the protein and lipid recovered from headed gutted carp using solubilization at different pH values with different organic acids and precipitation at pH 5.5 as compared to headed gutted carp and carp fillets

		Recovered Proteins									
pH	**Acid**	**LA**	**ALA**	**EPA**	**DHA**	**n3**	**n6**	**SFA**	**UFA**	**ratio n6/n3**	**Ratio %**
2.5	AA	28.2	54.6	47.5	42	181.1	50.4	310.9	613.9	0.28	27.83
3	AA	27.1	43.9	25.1	17.5	109.3	37.7	340.8	578.5	0.34	34.49
11.5	AA	16	20.2	14.1	12.2	62.7	25.2	406.3	490.5	0.40	40.19
12	AA	12.9	11.3	3.1	2.9	22.8	14.5	404.5	495.8	0.64	63.60
2.5	F&L	8.1	6.5	1.9	2.2	23.1	9.7	425.3	497	0.42	41.99
3	F&L	8.6	9.7	3.6	3.3	31	10.7	409.5	487.2	0.35	34.52
11.5	F&L	25.8	35.4	27.3	21.8	105.6	41.8	371.5	560.9	0.40	39.58
12	F&L	16.9	22	23.9	22.9	89	27.9	392.1	518.7	0.31	31.35
		Recovered Lipids									
pH	**Acid**	**LA**	**ALA**	**EPA**	**DHA**	**n3**	**n6**	**SFA**	**UFA**	**ratio n6/n3**	**Ratio %**
2.5	AA	7.8	7.3	2.9	2.5	29.9	9.2	410	493.1	0.31	30.77
3	AA	5.7	5	1.5	1.3	24.2	6.5	443.1	393.1	0.27	26.86
11.5	AA	26.9	51	26.3	15.2	119.4	34.9	315.1	582.8	0.29	29.23
12	AA	26.2	45.6	19	10.4	96.2	32.6	324.6	577.4	0.34	33.89
2.5	F&L	8.1	9.1	2.9	2.3	28.1	9.4	420.2	437	0.33	33.45
3	F&L	6.3	7.8	3.1	2.7	25.2	7.7	420.6	460.1	0.31	30.56
11.5	F&L	30.6	66.3	34.6	20	153.9	39.9	301.4	596	0.26	25.93
12	F&L	23.9	46	30	19.7	124.4	31.8	316.8	583.1	0.26	25.56
		Ground Carp									
		LA	**ALA**	**EPA**	**DHA**	**n3**	**n6**	**SFA**	**UFA**	**ratio n6/n3**	**Ratio %**
		22.6	28.2	8.2	5.5	52.4	27.3	369.4	545.6	0.52	52.10
		Fillet									
		LA	**ALA**	**EPA**	**DHA**	**n3**	**n6**	**SFA**	**UFA**	**ratio n6/n3**	**Ratio %**
		6.6	0	0	2.6	19.5	9.6	482.8	379.8	0.49	49.23

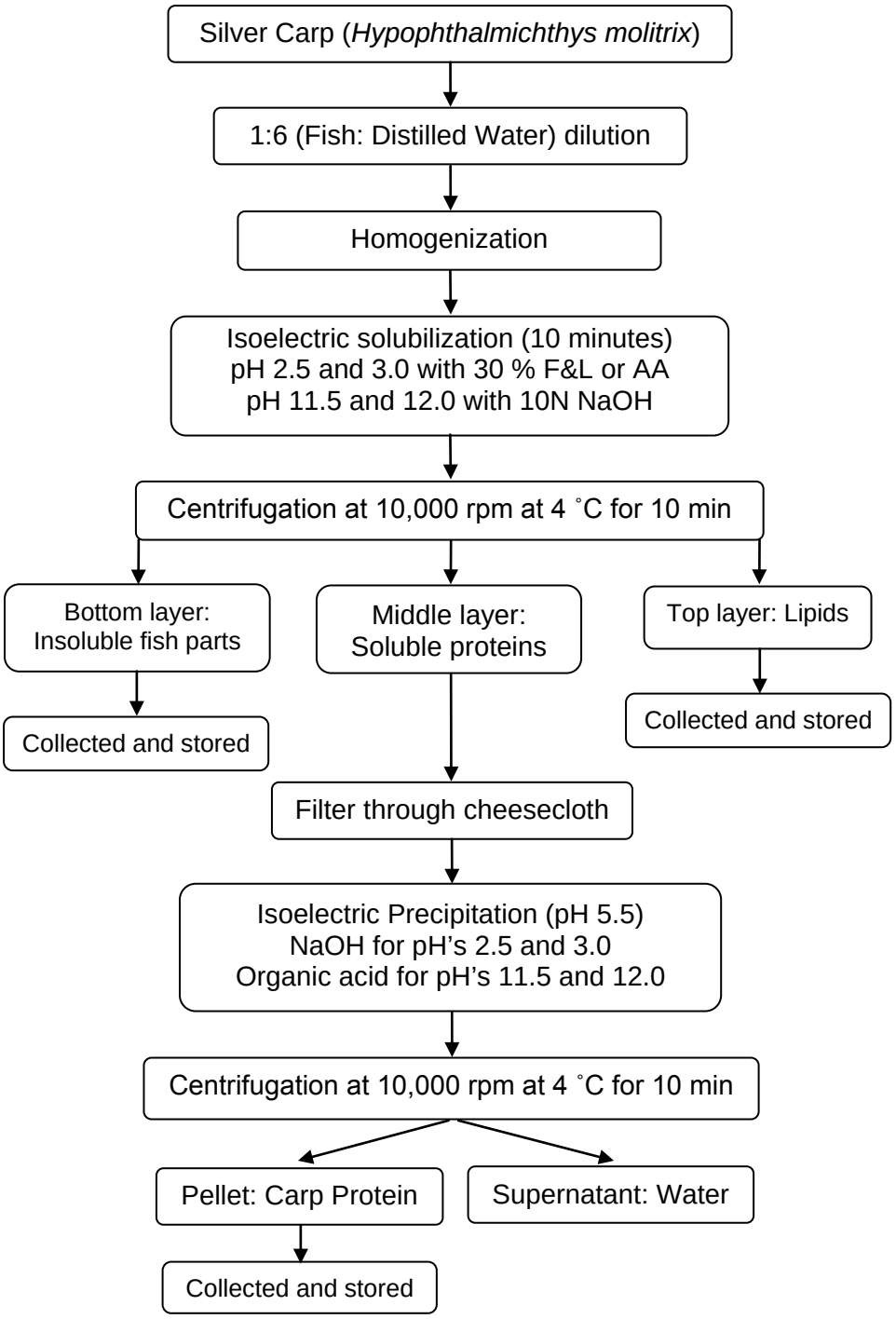

Figure 1. Isoelectric Solubilization and Precipitation Flow Chart

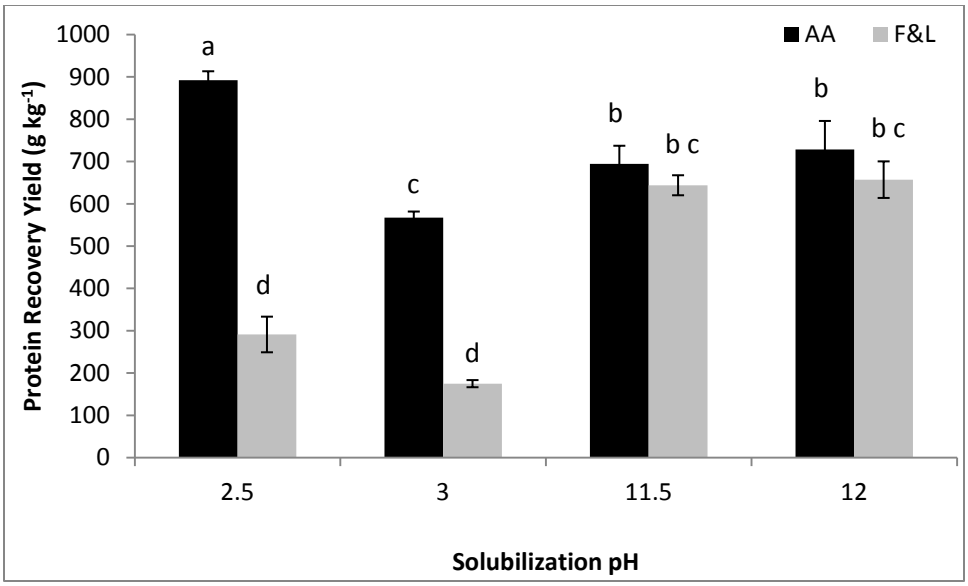

Figure 2. Protein recovery yield (g kg^{-1} dry basis, Kjeldahl) from silver carp at different pH values using different acids during isoelectric solubilization and precipitation. Small bars on the data bars indicate standard deviation. Different letters on the top of data bars indicate significant differences (Tukey's honestly significant difference test, $p<0.05$, $n = 3$) between mean values. Levels not connected by the same letter are significantly different.

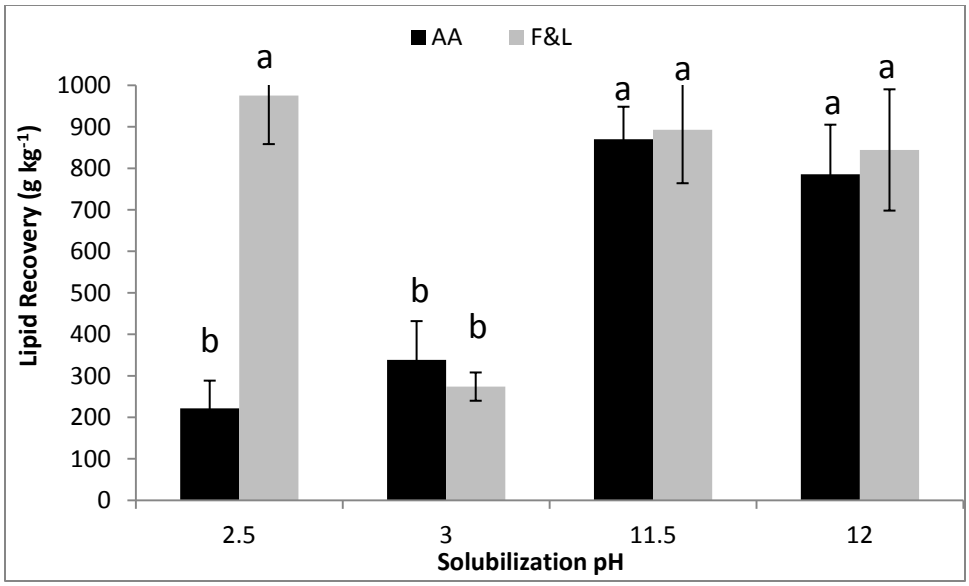

Figure 3. Lipid recovery yield (g kg^{-1} dry basis, Soxhlet) from silver carp at different pH values using different acids during isoelectric solubilization and precipitation. Small bars on the data bars indicate standard deviation. Different letters on the top of data bars indicate significant differences (Tukey's honestly significant difference test, $P<0.05$, n= 3) between mean values. Levels not connected by same letter are significantly different.

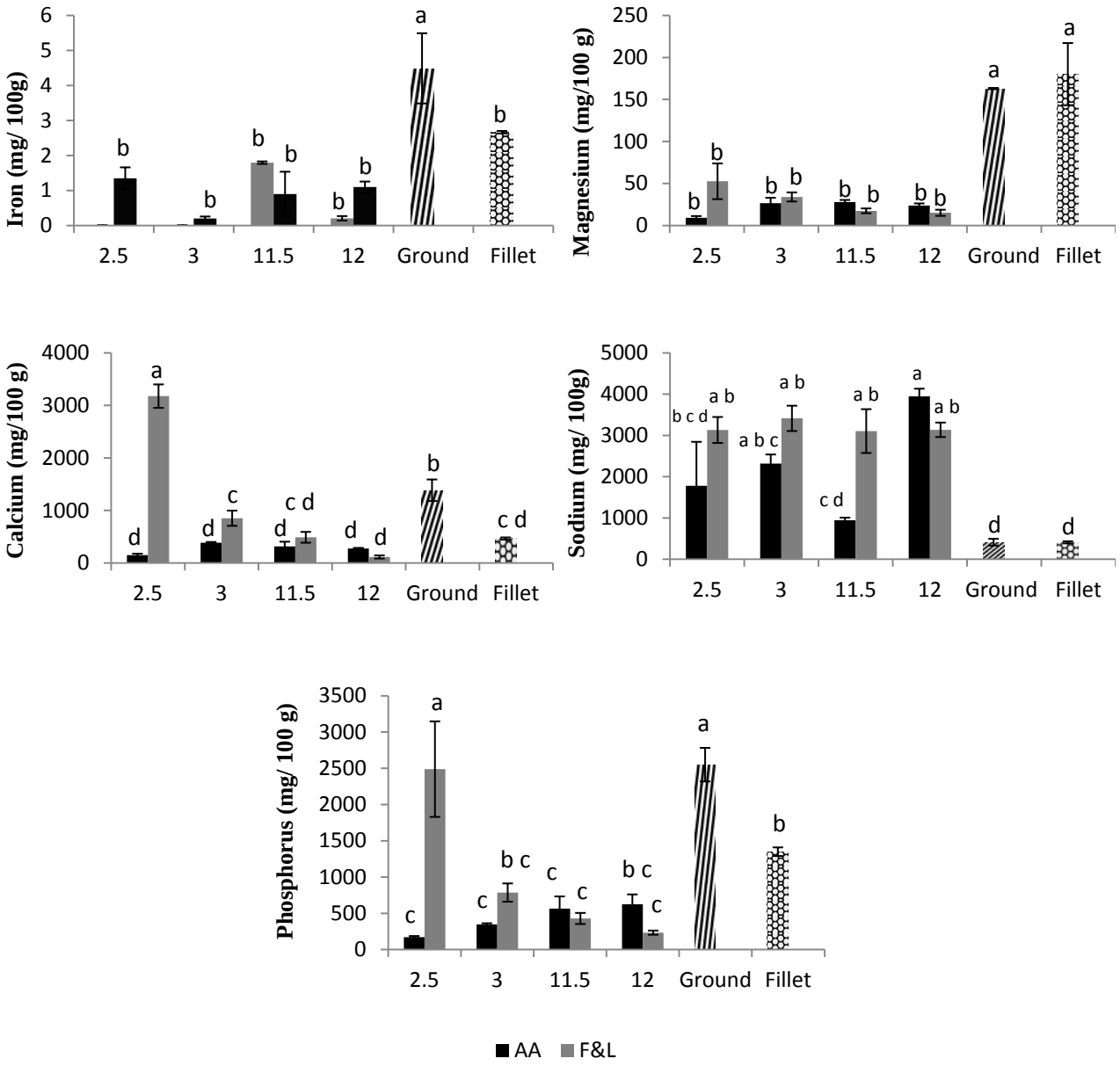

Figure 4. Mineral (Fe, Mg, Ca, Na, P) contents[a] (milligrams per 100 grams) in the protein recovered from headed gutted carp using solubilization at different pH values using different organic acids and precipitation at pH 5.5. [a] Data are given as mean values ± SD (n = 2). Different letters on each column indicate significant differences (Tukey's honestly significant difference test, $p<0.05$) between mean values.

CHAPTER IV

Functional Properties of Protein Gels Made from Silver Carp (*Hypophthalmichthys molitrix*) Protein Recovered by Isoelectric Solubilization and Precipitation Using Organic Acids

Abstract

Isoelectric solubilization and precipitation (ISP), a protein recovery process, was applied to ground, headed and gutted silver carp (*Hypophthalmichthys molitrix*) at both acidic and basic solubilization pH values using either glacial acetic acid (AA) or a 30% formic and lactic acid combination (F&L). The goal of this study was 1) to examine the functional properties of the gels made from the recovered proteins using standard additives and 2) to compare the effects of different organic acids and solubilization pH values on the protein gels. Thermal denaturation (temperature of onset $\{T_{onset}\}$, maximum temperature $\{T_{max}\}$ and net heat energy ΔH), viscoelasticity (G´) and viscous modulus (G´´) of the protein gels were measured. Protein gels presented with typical endothermic reactions. Using AA to solubilize protein at pH 2.5 and F&L at pH 12.0 resulted in T_{onset}, T_{max} and ΔH required for carp proteins to form a thermally-induced gel network similar to Alaska Pollock surimi. Viscoelastic properties were elevated in gels made under basic solubilization conditions using F&L and under acidic treatments using AA. The results of this study suggest that gels made from recovered carp protein show similar or improved functional properties compared to Alaska Pollock surimi and might be used for the development of restructured fish products.

Key words: carp proteins, isoelectric solubilization and precipitation, organic acids, protein gels, thermal denaturation, viscoelasticity

Introduction

Carp is considered a delicacy in many countries and is one of the most abundant and least expensive aquatic food sources due to its feeds and fertilizers being readily available at low cost (FAO, 2012). Since production costs are lower than other aquatic sources and most consumers can afford to include it in their diet regularly, further increase in production is expected (FAO, 2012). Silver carp (*Hypophthalmichthys molitrix*) is one of the top three species grown in China with production exceeding 3.5 billion tons per year (FAO, 2007). It is also one of the main protein sources for human consumption in China and has a high market value (Zhang et al., 2010a). Although silver carp is abundant and a nutritious source of protein, the potential value of this fish has not been fully utilized in the global market.

It has been reported that herbivorous fish like carp are more tasteful than carnivorous fish (Pandey and Shukla, 2005); regardless, carp are not widely accepted as a food source in United States likely due to the boney structure of the fish and the process of removing the small bones is not reliable. In production plants, manual filleting and deboning are time and labor extensive procedures and therefore are generally handled by using simple, single purpose machinery. However, manual processing yields better protein recovery results when dealing with fish that have pin bones (Bykowski and Dutdiewicz, 1996). More efficient methods to recover the nutritionally valuable protein and oil from carp need to be explored.

Isoelectric solubilization/precipitation (ISP) is a method that allows efficient recovery of fish proteins and oil by exposure to high or low pH conditions that cause separation of protein from insoluble fractions of the fish (i.e. bones, skin, scales, etc.). Using ISP technique on aquatic sources is shown to result in high protein recovery with similar or improved functionality of

protein gels compared to the ones obtained by using the traditional surimi process (Corte´s Ruiz et al., 2008; Kristinsson and Hultin, 2003). Typically, strong acids are used in the ISP process but organic acids have been shown to be more effective at reducing bacterial pathogens likely due to their ability to diffuse through a cell membrane in a nondissociated form and dissociating upon entering the cytosol (Piper, 2011). Currently, there are no studies observing the functional characteristics of gels made from isoelectrically recovered carp protein with the use of organic acids.

Gelation is an important functional property of protein involving partial denaturation of followed by irreversible aggregation that causes the three dimensional network formation. The muscle tissue of fish majorly is made up of structural proteins such as actin, myosin and actomyosin (FAO, 2012). Myosin is the most significant component of thermal gel formation in aquatic products and protein gelation is a controlled myosin denaturizing process (Kuwahara and Konna, 2010). The thermal denaturation of myosin is followed by soluble aggregate formation from unfolded proteins that are then associated to form gel networks (Meng and Ma, 2001; Mori et al., 1986). The protein aggregation and the level of protein denaturation is extremely important in the network structure of a heat induced globular protein gel that is highly dependent on the balance between attractive and repulsive forces among protein molecules. It is shown that gel network formation process is affected by many parameters such as pH, ionic strength, protein concentration, and heating conditions (Zhang et al., 2010b; Xiong and Blanchard, 1994).

Since the functionality and the structure of gels are determined by the type and number of protein-protein interactions, aggregation and arrangement of unfolded proteins, it is important to determine the effects of organic acids and ISP on the protein fraction that is used to make the carp protein gels. Commonly, pH shifts are believed to cause more protein denaturation which

would affect gelation adversely; however, Kristinsson and Hultin (2003) observed improved functional properties related to gelation, emulsification and solubility following acidic or alkali treatments likely due to the partially unfolded proteins being more flexible (Kristinsson and Hultin 2003; Ingadottir and Kristinsson, 2010). The effect of ISP plays an important role in the protein concentration of the recovered protein fraction and the pH shifts encountered during the process effect the denaturation of protein. In addition to that, using different organic acids influence the gel rheology due to their different ionic strengths and dissociation constants. In the present study, the effects of acetic acid versus a 30% lactic and formic acid combination were assessed. Acetic acid being a weaker acid compared to formic and lactic acids due to its dissociation constant being higher, does not release its ions readily and therefore has lower ionic strength in the solution (Tipping, 2002). On the other hand, due to the differences in the acid concentration of each treatment, different levels of protein denaturation might be observed. This will lead to differences in the gel formation, structure and functionality which will in turn affect texture properties of the gels; and therefore should be investigated. The objectives of this study were to determine and compare the effects of using different organic acids (acetic acid and a 30% formic and lactic acid combination) and solubilization pH values (pH 2.5, 3.0, 11.5 and 12.0) on the functional properties of the gels made from recovered carp protein by using rheometer and differential scanning calorimetery (DSC) methods.

Materials and Methods

Fish preparation. Headed and gutted fresh silver carp (*Hypophthalmichthys molitrix*) were sanitized by submergence into a 50 ppm bleach solution for 10 sec (Clorox Regular Bleach, Clorox, Oakland, CA, USA) and drained. The fish were ground into a thick paste using a sanitized food processor (Cuisinart ProClassic7-Cup Food Processor, Cuisinart Co., East Windsor, NJ, USA). The equipment was sanitized by cleaning with 70% ETOH and drying under UV light (254 nm) for 15 min (Otto et al. 2011a). The fish was ground twice (through coarse and fine filters) and the paste was weighed. The fish pate was then separated into freezer bags (Ziplock Freezer Bags, S.C. Johnson & Son, Inc., Racine, WI, USA) each containing 255 grams of ground fish and stored at -80°C.

Isoelectric solubilization and precipitation (ISP). Frozen fish paste was removed from -80°C storage and thawed for 24 h at 4°C. The recovery process of muscle proteins and lipids using isoelectric solubilization and precipitation are shown in Figure 1. Thawed fish paste (250 g) was weighed and homogenized with 1500 mL distilled, deionized water in a glass beaker. The target pH values for protein solubilization were pH 2.5, 3.0. 11.5 and 12.0. The acids tested were glacial acetic acid (Fischer Scientific, Fairlawn, NJ, USA) and a combination of 30% glacial L-Lactic acid (85%, EMD Chemicals, Netherlands) and formic acid (88%, Mallinckrodt Chemicals, Netherlands) in sterile distilled water at a 1:1 ratio (Mohammad et al., 2009). The solution was brought to the target pH (2.5, 3.0, 11.5 or 12.0) with the addition of the respective organic acid or 10 N NaOH and homogenized (PowerGen 700, Fisher Scientific, Pittsburgh, PA) for 10 min while the pH stabilized. Once stabilized, the homogenization process was continued for an additional 10 min to maximize protein solubilization. The homogenized fish mixture was then transferred to centrifuge bottles and centrifuged at 10,000 x G for 10 min at 4°C (Sorvall

RC-SB Refrigerated Superspeed Centrifuge, Du Pont, Wilmington, DE, USA). After centrifugation, the top layer (fat) was discarded. The supernatant was poured through a cheese cloth into a glass beaker to remove the insoluble portions (scales, bones, etc.) of the solution. The pH of the supernatant was returned to the protein isoelectric point of pH 5.5 using the respective organic acid or 10 N NaOH and homogenized for 5 min. The homogenate was again centrifuged at 10,000 x G for 10 min at 4°C. After centrifugation, the supernatant (process water) was discarded and the protein, left in the centrifuge bottle as a pellet, was collected.

Development of protein paste. Protein pastes were made using a modified procedure described elsewhere (Tahergorabi et al. 2011; Taskaya et al., 2009b; Chen and Jaczynski, 2007). The recovered carp proteins were chopped in a universal food processor (model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. Salt (2 g/100 g) was added and the mixture was chopped at low speed for an additional min. Functional additives were added to the protein paste. The additives included: 1 g/100 g potato starch (Penbind 1000 modified potato starch, Penford Food Ingredients Corp., Centennial, CO); 0.3 g/100 g polyphosphate (Kena FP-28, Innophos, Cranbury, NJ); 0.5 g/100 g titanium dioxide (TiO2) (Titanium (IV) oxide, Sigma-Aldrich, Inc., St. Louis, MO); and 0.5 g/100 g transglutaminase (TGASE) (Taskaya et al., 2009b). Final moisture was adjusted to 80 g/100 g by adding cold, distilled water and mixed for an additional min. The pH of the mixture was pH 7.0, confirmed using a pH/ion analyzer (Oakton, Eutech Instruments.; Singapore) and paste temperature was kept between 1 and 4 °C throughout the chopping process. Chopping was continued for an additional 3 min under vacuum (50 kPa). The protein paste was then vacuum packaged and a small portion of it was used to evaluate functional properties of the protein gels using differential scanning calorimetry (DSC) and rheometric measurements immediately.

Differential scanning calorimetry (DSC). The thermal transitions of fish proteins during gelation were measured on the protein pastes using differential scanning calorimetry (DSC) method. Approximately 15 mg of gel was spread into an aluminum pan (Instrument Specialists Inc. 4mm crimp/en cap) and hermetically sealed. The pans were placed in the differential scanning calorimeter (DSC Infinity Series F5010, Instrument Specialists, Inc., Spring Grove, IL) and scanned from 15^{o}C – 90 ^{o}C at a heating rate of 10 ^{o}C/min (Taskaya et al, 2009b). The DSC thermograms provided by the Infinite Software were used to determine the net heat energy (enthalpy, ΔH), the onset (T_{onset}) and maximum (T_{max}) temperatures. Data were collected from five samples per treatment.

Dynamic rheology. The viscoelasticity of the protein pastes (elastic modulus (G´) and viscous modulus (G´´)) were measured as a function of temperature using oscillatory rheology with a Bohlin rheometer (Bohlin CVOR 200, Malvern Instruments Ltd.,Worcestershire, UK). Testing was conducted at 1 % strain and 0.1 Hz frequency and the temperature was increased from 25^{o}C to 90^{o}C at a rate of 1^{o}C/min (Taskaya et al., 2009b). Three samples were tested per treatment, and the mean (SD) was reported.

Preparation of the protein gels. After chopping, the protein pastes were individually stuffed into dumbbell pre-molded stainless steel torsion tubes (length ¼ 17.5 cm, end diameter ¼ 1.9 cm, midsection diameter ¼ 1.0 cm). The tubes were lightly sprayed with cooking spray (PAM Original cooking spray, ConAgra Foods, USA) prior to stuffing to prevent sticking. The filled tubes were stored under refrigeration (4^{o}C) for 24 h to allow gel formation. After refrigeration, the tubes were placed in a 90^{o}C water bath (Precision, Jouan Inc, Wincester, Virginia) for 15 min and chilled on ice for 15 min. The gels were removed from the tubes and cut

into smaller pieces for further analysis. The analyses were run immediately after cutting the gels and the gels were kept at room temperature during the analyses.

Statistical Analysis. The rheometer analyses were replicated three times, DSC experiments were performed in quinaries. Rheometer data was presented as mean (± SD). Out of the five replications per treatment performed for the DSC analysis, one data set representing a standard endothermic curve was selected and presented.

Results and Discussion

Thermal denaturation of carp proteins. The majority of muscle tissue in fish is made up of structural proteins such as actin, myosin and actomyosin (FAO, 2012). Myosin is the most significant component for thermal gel formation in aquatic products and therefore protein gelation is considered a controlled myosin denaturizing process (Kuwahara and Konna, 2010). Differential scanning calorimetry (DSC) is used to determine the thermal transitions (Tm) of proteins in muscle tissue. Typical DSC thermograms result in two distinct peaks: the first peak represents the temperature at which myosin denatures and the second peak represents the denaturation of actin (Taskaya, 2009b); however, up to five Tm have been reported for myosin, actin and sarcoplasmic proteins collectively (Thawornchinsombut and Park, 2007). The results of DSC for ISP protein gels are shown in Table 1 and Figure 2. Myosin denaturation is represented by Peaks I and II, where the 1st transition from 27- 46 ^{o}C is shown to be due to an initial association of denatured myosin heads (Egelandsdal et al., 1986) and the latter transition between 50- 61 ^{o}C due to the association of myosin tails (Wright and Wilding 1984). Actin is more resistant to thermal denaturation and occurs at higher temperatures (Taskaya et al, 2009b), as indicated by the 3rd peak. Since most water soluble proteins should have been removed during ISP, it is not likely that the thermal curves reflect any denaturation of sarcoplasmic proteins in ISP recovered protein. However high protein recovery yields were reported (Paker, 2012); therefore, it is likely that sarcoplasmic proteins were not entirely removed from the protein fraction and the 4th peak possibly represents the endothermic transitions for sarcoplasmic proteins.

Functional additives, pH, ionic strength, and salt concentrations all have an effect on protein denaturation. Taskaya et al. (2009b) was able detect an actin peak when carp protein gels

were enhanced with functional additives. Moreover, the carp gels in this current study made with functional additives and protein isolated under AA pH 2.5 and F&L pH 12.0 conditions had transition temperatures and enthalpy similar to Alaska Pollock surimi enhanced with functional additives (Taskaya et al., 2009b).

Solubilization under pH 11.5 with AA yielded the highest protein percentage in the protein fraction (Paker, 2012); therefore, the gels made from the recovered protein showed the highest enthalpy for the actin peak. Actin and myosin ratio influences the stability of milkfish actomyosin (Jiang et al., 1989) and thermal gelation of carp actomyosin (Sano et al., 1989). Moreover, free myosin versus bound myosin ratio will affect gelation properties (Benjakul et al., 2001). Tang et al (2002) showed that myosin-I, being the single-headed member of the myosin family, is associated with lipid membranes. Reported proximate composition showed that the lipid content in the recovered protein fraction was highest for the carp protein treated with F&L under pH 2.5 (Paker, 2012), which may explain why the endothermic transition and enthalpy are the lowest for protein solubilized at pH 2.5 under F&L for the myosin Peak I.

Ionic strength of the organic acids during ISP may have affected the protein functionality as well. Increased ionic strength enables myofibrillar proteins to solubilize and induce free myosin associated with actin to form actomyosin, as indicated by lower endothermic transition and enthalpy (Kiowski and Mast, 1988). The lowest transition temperatures in this current study were seen in protein gels made from protein recovered at pH 2.5 with F&L. Although all of the acids used in this study are weak acids, formic and lactic acid have greater ionic strength compared to AA as seen by the differences in their dissociation constants (Tipping, 2002). Formic and lactic acids have dissociation constants (pKa) of 3.8 and are stronger than acetic acid (pKa= 4.8) because they are 10 times more rapidly releasing their protons in solution (Tipping,

2002). F&L will increase the ion concentration in solution faster than AA; however, since F&L was used at a 30% concentration compared to 99.7 % pure glacial AA, AA had greater ionic strength. This likely resulted in more structural changes in the protein.

The enthalpy and peak temperatures (Table 1) for myosin peak presented in the current study were higher than the ones reported for gels made from silver carp protein recovered using HCl (Taskaya et al., 2009b). HCl, a strong acid, can be completely ionized at any one time in the solution to produce hydroxonium and chloride ions. On the other hand, the organic acids used in this study are weak acids that only partially dissociate and the hydrogen ions concentrations are much less than the total concentrations (Tipping, 2002). A study by Paredi et al. (1994) showed that as the ionic strength increased, the peak temperatures decreased. This was likely because at low ionic strength myosin molecules aggregate to form filaments with better stability than the individual molecules which exist at high ionic strength (Samejima et al., 1983). Moreover, the peak temperatures reported in this study were lower than the ones reported for cod muscle protein (Hastings et al., 1985), squid (Hastings et al., 1985), hake (Beas et al., 1990), Molina (Paredi et al., 1994), and sea bream (Howell et al., 1991), none of which were exposed to acidic or basic extraction treatments. The current study shows that organic acids may have played a role in shifting the myosin denaturation peak and increasing the thermal stability due to different protein concentration, lower ionic strength and less protein denaturation in the recovered protein fraction (Thorarinsdottir et al., 2002). Although Luo et al (2001) showed that the gel forming ability of silver carp, grass carp and common carp surimi was not up to the standards of Alaska Pollock surimi, this study shows that gels made from isoelectrically recovered carp protein using organic acids resulted in similar or increased gel forming ability as Alaska Pollock surimi (Taskaya et al., 2009b).

The salt content of the recovered protein was higher than that of the starting material (headed, gutted carp) (Paker, 2012) which may have affected the transition temperatures of the recovered protein. Studies have shown that salt content decreases transition temperatures as well as peak areas (Thorarinsdottir et al., 2002; Schubring, 1999; Hastings et al., 1985) and results from this current study support these findings. The protein recovered using AA at solubilization pH 2.5 and 11.5 had the lowest salt content of all the treatments and was similar to that of headed gutted silver carp (Paker, 2012). The gels made with this recovered protein had higher transition temperatures than the rest, and gels made from protein recovered using AA at pH 11.5 showed the highest actin peak enthalpy. Functional additives, pH, ionic strength, and salt concentrations all have an effect on protein denaturation; future research is needed to better determine processing conditions for retaining optimum protein functionality.

Dynamic rheology of the carp proteins. Dynamic rheology is a way to determine the elastic and viscous behavior of protein gels (Liu et al., 2007). Therefore, the gel transition properties of the protein gels were assessed by testing the viscoelastic properties (G' and G") shown in Figure 3 and 4. A typical gelation curve for Alaska Pollock surimi samples has an initial increase in viscoelasticity at around 35- 45 ^{o}C followed by a steep increase at 55 ^{o}C (Taskaya et al., 2009b). As shown in Figure 3, the viscoelastic curve of gels made from protein recovered using AA at solubilization pH 2.5 is very similar to a typical surimi pattern. As for the other treatments, viscoelasticity increased around 45-60 ^{o}C, indicative of a more thermal denaturation resistant protein composition. The rapid increase in G´ indicated that a gel forming network structure was formed, whereas the following decrease around 70 ^{o}C (for treatments AA with pH 2.5 and F&L with pH 11.5) may be explained by the rate of bond disruption caused by thermal denaturation being surpassed by bond formation as a result of protein aggregation (Stone

and Stanley, 1994). This suggests that reorganization and realignment of the protein molecules resulted in increased thermal denaturation (Smyth and O'Neill, 1997).

The carp proteins that were solubilized at 11.5 and 12.0 using F&L showed better gelation and higher gel quality due to having higher G´ (Thawornchinsombut and Park, 2007). This data is in agreement with Chen and Jaczynski (2007) where the best gelation is seen at solubilization pH 12.0 for rainbow trout protein gels. Thawornchinsombut and Park (2007) also concluded that alkaline treatment during pH shifts yielded higher quality gels. Previous research studying pH shifts (Underland et al. 2002; Kim et al. 2003; Kristinsson and Hultin, 2004) concluded that acidic treatments caused myofibrillar protein denaturation more rapidly than the basic treatments. This might explain why the gels made from recovered protein using basic treatments resulted in a higher gel quality.

Protein gels made with recovered carp protein solubilized at pH 3.0 using AA also resulted in high G´ values. A study by Underland et al. (2002) experimenting on pH shifts at pH 2.7 and 10.8 using herring protein reported that gels prepared from protein usings either treatment had equal gel qualities. Moreover, other studies assessing the effects of acidic and basic treatments on gelation properties found that gels prepared at pH 11 had superior gel quality, followed by gels prepared at pH 2 (Choi and Park 2002; Kim et al. 2003). Ionic strength of the environment is recognized as the most important factor in gel development and final structure of the gel network (Thawornchinsombut and Park, 2007).

As shown in Figure 3 and Figure 4, all the curves showed an initial increase followed by a slight decrease and then a steeper incline. The first increase of viscoelastic curves was attributed to the cross-linking of myosin, followed by a decrease due to the denaturation of

meromyosin which causes increased fluidity (Yongsawatdigul and Park, 2004). Then, the second major increase is due to the final bonding of myosin filaments (Taskaya et al., 2009b). In the present study all treatments, except AA pH 11.5 and F&L pH 3.0, showed typical patterns as reported by other studies observing the rheological characteristics of myofibrillar proteins of different protein sources (Yongsawatdigul and Park, 2004). The curve that resembles the surimi elastic and viscous properties most was seen with the solubilization pH 2.5 using AA. Therefore, the protein recovered using AA with solubilization pH 2.5 showed similar characteristics as fish protein that was not exposed to pH shifts. It can be concluded that using organic acids especially AA during ISP causes less protein denaturation yielding high quality protein gels.

Surimi has a more elastic and less viscous nature during thermal processing (Smyth and O' Neill, 1997). A similar gel structure was seen with all treatments as indicated by higher elastic modulus (G') than viscous (G") modulus values. The viscous modulus (G´´) patterns of the recovered protein showed typical gelation profiles. The decline in G″ curves indicates protein denaturation while an increase points out bond formation (Yin et al., 2011). Protein gels made from carp protein recovered using basic treatments with F&L and acidic treatments with AA show elevated G″ values at higher temperatures and therefore, have strong protein bonds. In correlation with that, the viscous modulus values of basic treatments using AA and acidic treatments using F&L were lower and showed less magnitude as well as peaks. This mainly indicates that the protein structure was denatured and that the viscous properties did not change as much as the other treatments due to the increased temperature. The effect of ionic strength on the gelation properties may explain these results. AA is the weaker of the organic acids used in the current study and is fully dissociated only at basic treatments, possibly causing more protein denaturation during alkali processing. On the other hand, F&L is more rapidly dissociated at

lower solubilization pH values compared to AA leading to faster protein denaturation during acidic processing as indicated by the decreased G´´ values when acidic treatments are used with F&L.

Conclusion

Functional properties of gels made from protein recovered by ISP processing using organic acids as the processing acid had typical gelation characteristics. Isoelectrically recovered carp proteins were not denatured and retained functionality; therefore, has the potential to be used as a high quality protein source.

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Table 1. The onset (T_{onset}) and maximum (T_{max}) temperatures for the endothermic transitions and net heat energy (enthalpy, ΔH) required for these transitions of recovered protein from headed gutted silver carp by isoelectric solubilization and precipitation using different solubilization treatments and organic acids.

		Peak 1			Peak 2			Peak 3			Peak 4		
Acid	**pH**	**T_{onset}**	**T_{max}**	**Enthalphy (J/g)**	**T_{onset}**	**T_{max}**	**Enthalphy (J/g)**	**T_{onset}**	**T_{max}**	**Enthalphy (J/g)**	**T_{onset}**	**T_{max}**	**Enthalphy (J/g)**
AA	2.5	39.1	46.06	1.07	48.93	53.48	0.25	67.89	71.77	0.01	77.41	80.57	0.52
AA	3	29.4	36.33	1.03	44.77	49.84	1.58	53.46	58.07	1.77	71.52	81.54	0.08
AA	11.5	30.21	36.65	0.31	46.98	51.03	0.47	53.65	68.63	16.16	80.04	84.87	0.33
AA	12	24.49	30.82	0.36	45.37	57.8	5.13	63.35	67.63	0.32	70.53	74.31	4.4
F&L	2.5	17.29	26.86	0.18	35.85	44.98	1.58	53.01	56.36	0.03	78.62	82.53	0.08
F&L	3	21.36	29.59	0.06	55.05	60.53	1.91	63.03	68.51	3.04	74.58	79.15	2.21
F&L	11.5	25.78	31.96	0.47	36.15	51.84	1.17	55.38	61.14	1.25	N.d.	N.d	N.d
F&L	12	38.85	44.5	0.93	48.87	61.54	0.76	63.5	66.03	0.21	71.31	76.22	1.11

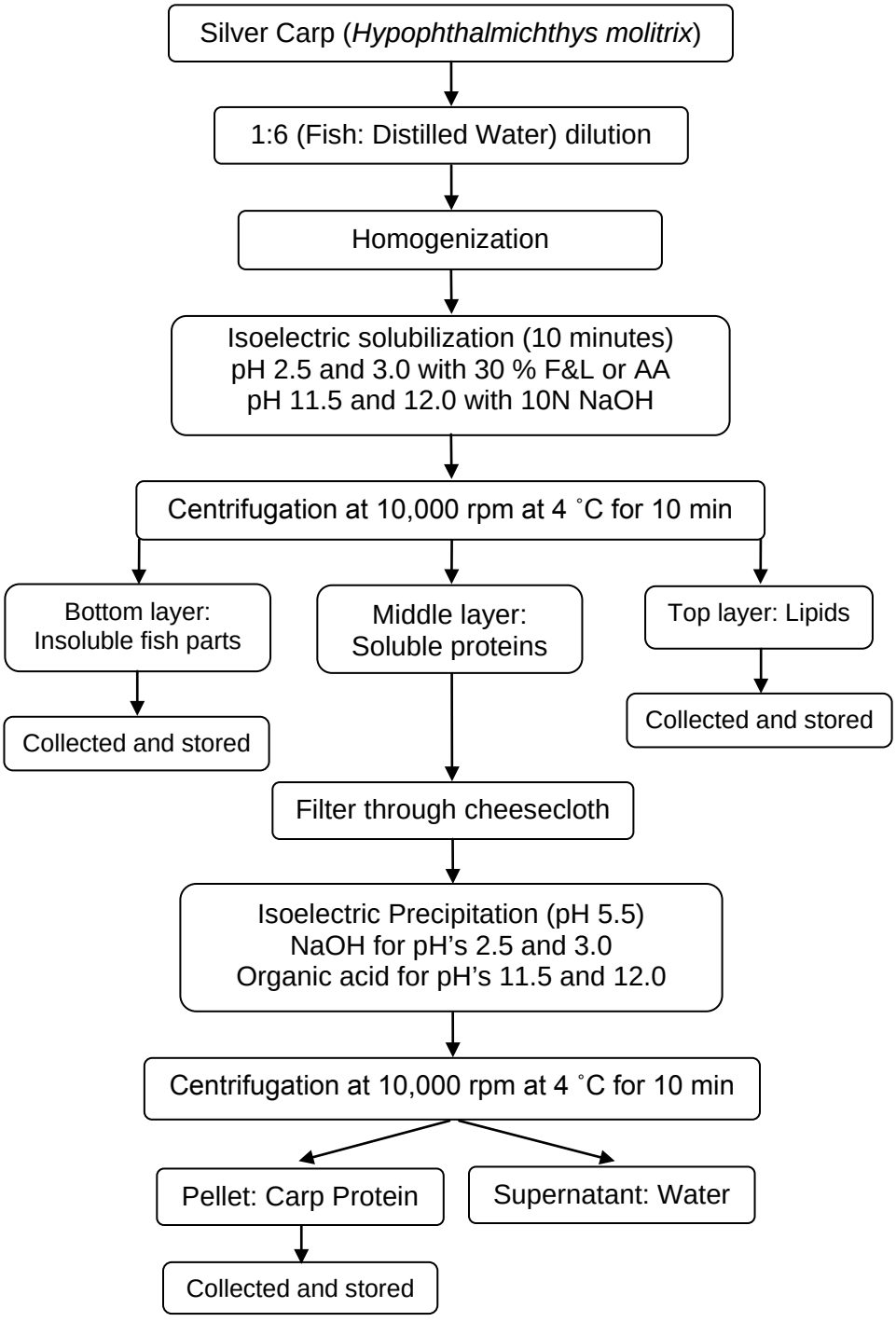

Figure 1. Isoelectric Solubilization and Precipitation Flow Chart

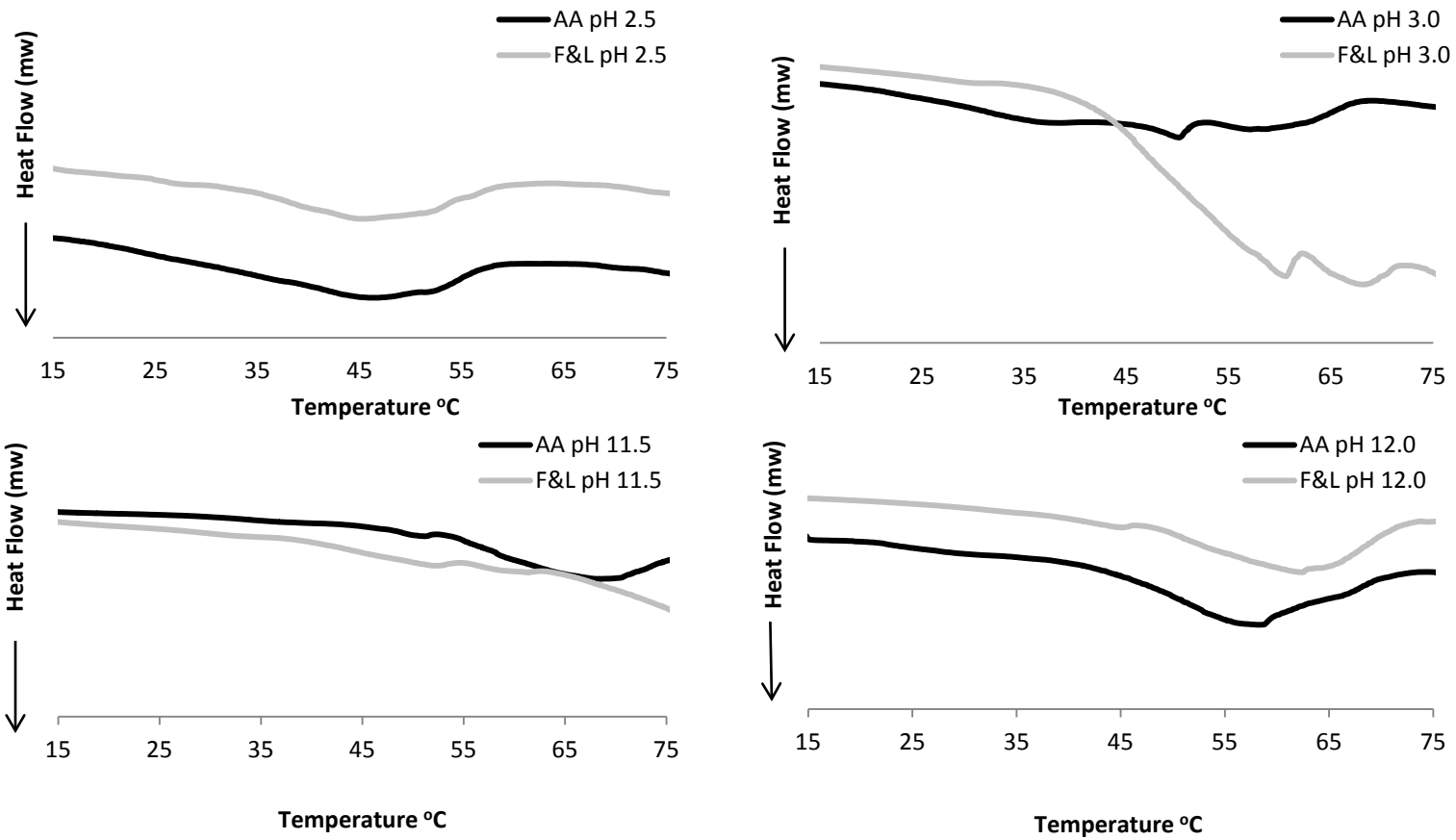

Figure 2. Differential scanning calorimetry (DSC) thermograms of protein pastes made from recovered carp protein using isoelectric solubilization and precipitation with different organic acids and solubilization pH values.

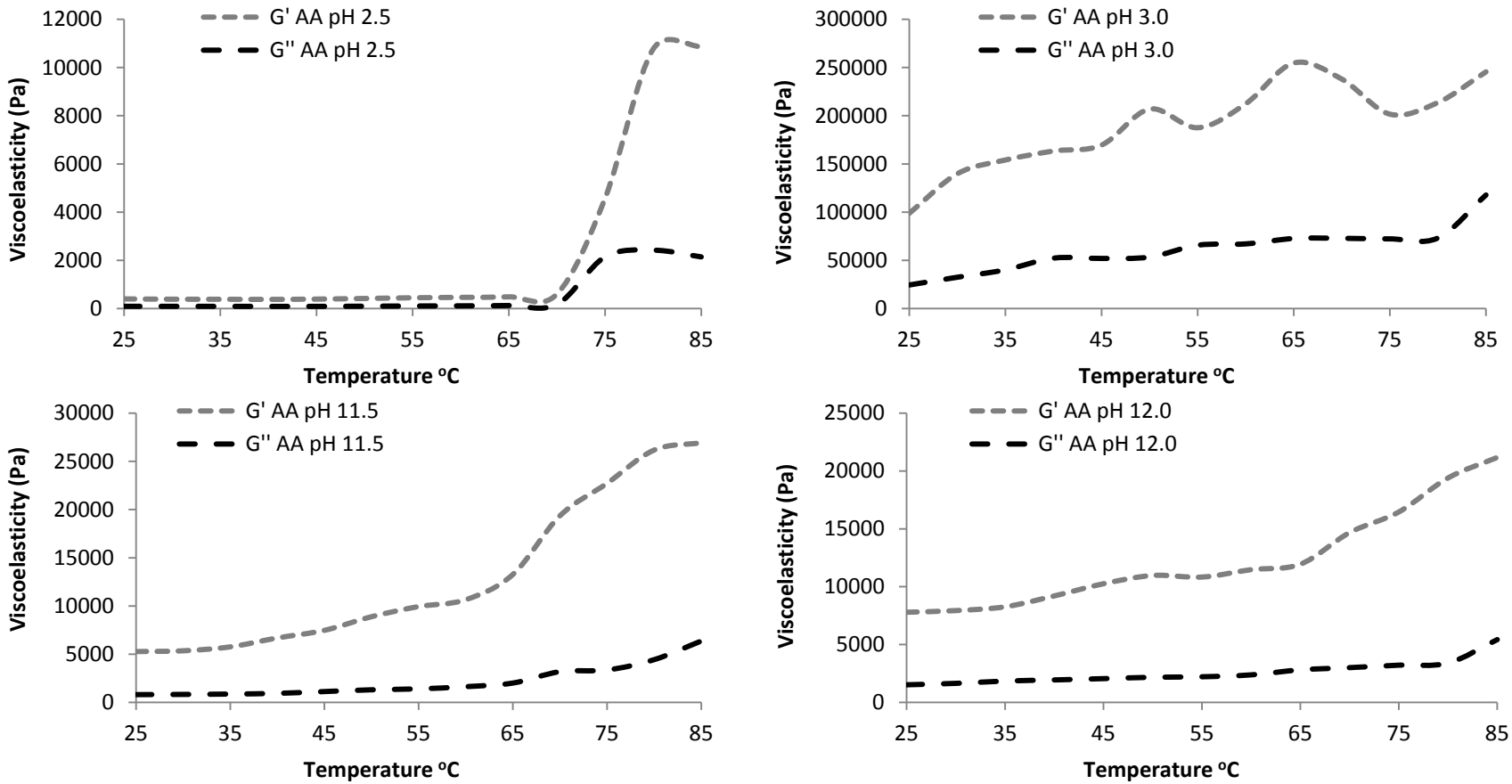

Figure 3. Viscoelastic properties shown by the elastic or storage modulus (G´) and viscous or loss modulus (G´´) of protein gels recovered using different solubilization strategies with AA.

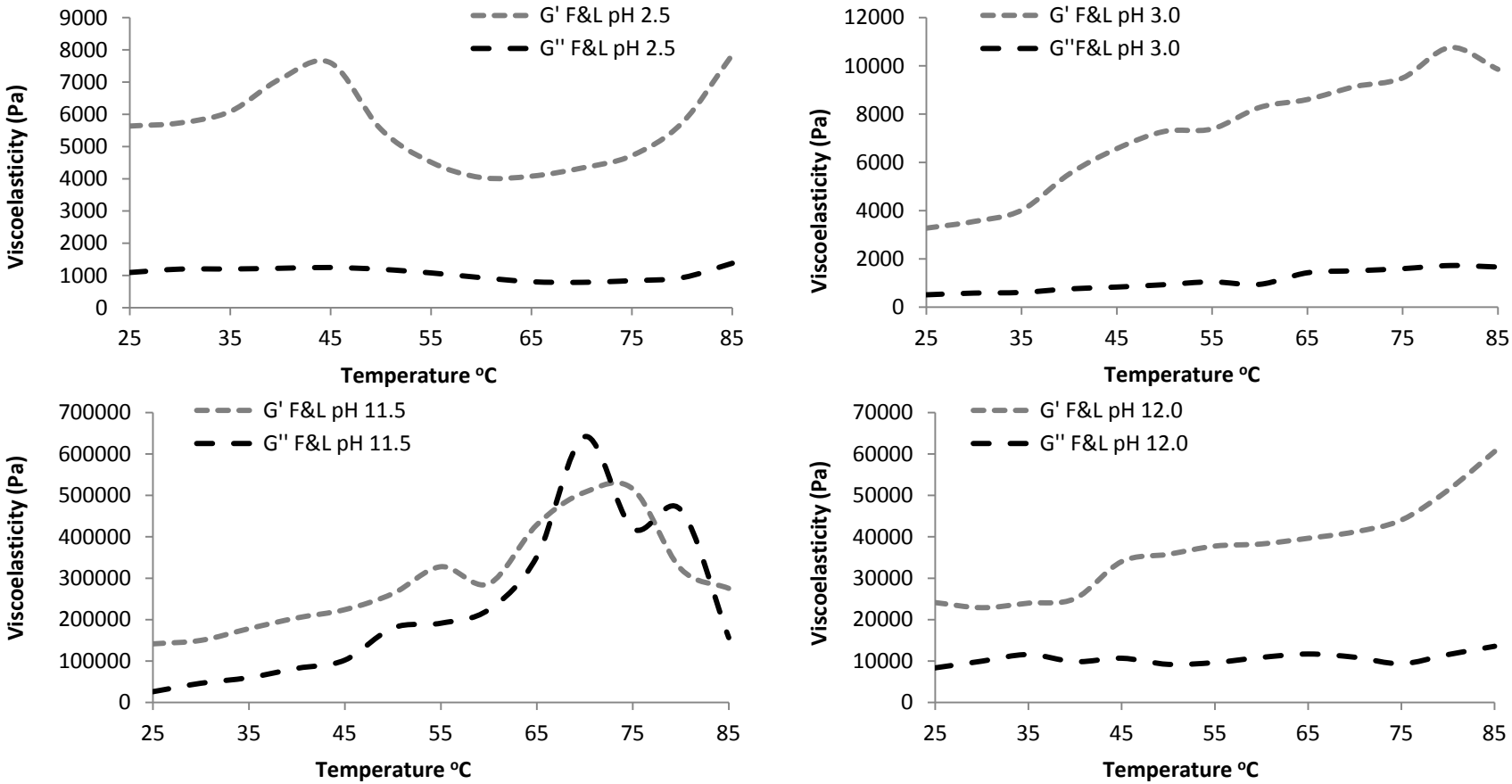

Figure 4. Viscoelastic properties shown by the elastic or storage modulus (G´) and viscous or loss modulus (G´´) of protein gels recovered using different solubilization strategies with F&L.

CHAPTER V

Texture and Color Properties of Protein Gels Made From Silver Carp (*Hypophthalmichthys molitrix*) Protein Recovered by Isoelectric Solubilization and Precipitation Using Organic Acids

Abstract

Silver carp (*Hypophthalmichthys molitrix*), one of the most common carp species, is a fresh water fish considered to be as nutritionally valuable as ocean fishes; however, in the U.S. commercial value of silver carp is low possibly due to its boney carcass. Isoelectric solubilization and precipitation (ISP) processing uses pH shifts to separate protein from fish frames which may increase the commercial viability of the carp protein as a functional ingredient in value-added foods. The aim of this study was to compare the texture and color properties of protein gels made from ISP-recovered protein from silver carp at different pH strategies and organic acid types. ISP was applied to headed-gutted carp using 10N sodium hydroxide (NaOH) and either glacial acetic acid (AA) or a 30% formic and lactic acid combination (F&L). Protein gels were made with the concentrated protein and functional additives such as potato starch, polyphosphate, titanium dioxide and transglutaminase. Protein gels made from recovered protein solubilized under acid conditions were whiter than gels made from recovered protein solubilized under basic conditions ($p<0.05$). Moreover, gels made when F&L was used as the processing acid were whiter for all solubilization conditions ($p<0.05$) and under acidic solubilization conditions the gels were similar to the whiteness of Alaskan Pollock surimi gels. Texture profile analysis and Kramer shear test showed that protein gels made from protein solubilized at basic pH values had firmer texture ($p<0.05$) and were harder and more cohesive,

gummy and chewy ($p<0.05$) than proteins solubilized under acidic conditions. Shear stress, also a measurement of gel texture, was the highest ($p<0.05$) for gels made from proteins recovered using AA at each solubilization pH except for pH 3.0. Gels made from proteins recovered using AA under basic conditions had similar shear stress responses as Alaska Pollock surimi. The shear strain was lowest ($p<0.05$) for gels made from proteins recovered using F&L at solubilization pH 3.0. The results of the present study indicate that gels made from ISP-recovered protein are similar in texture and color of Alaskan Pollock surimi gels and have potential for use in the development of restructured aquatic products for human consumption.

Key words: carp proteins, isoelectric solubilization and precipitation, organic acids, protein gels, texture properties, color properties, whiteness

Introduction

Aquaculture is the most rapidly growing animal food producing industry. In order to supply for the growing population demands, the per capita supply increasing from 0.7 kg in 1970 to 7.8 kg in 2008, with an average annual growth rate of 6.6%. Moreover, fish made up 16% of the animal protein consumption globally and 6% of total protein consumed as of 2007. Out of all the species, the production of freshwater fish was dominated by carps by 20.4 million tons making up for 71.1% as of 2008 (FAO, 2010).

Carp is a freshwater fish native to Asia but is imported to many countries all around the world. Silver carp (*Hypophthalmichthys molitrix*) is one of the most abundant carp species and is one of the major freshwater fish in aquaculture production since it has a rapid growth rate, easy cultivation, high feed efficiency ratio and is highly nutritional (Xu et al., 2012). Silver carp is one of the top species grown in China and recent markets include the surimi industry (Asgharzadeh et al., 2010). In the wild, carp is usually found in muddy waters; therefore, it has a mild, mossy flavor to its white flesh (Dong et al., 2008). In addition to having a mild flavor, carp is a hard to process fish due to having a boney carcass. The filleting process has gained significance since consumers prefer convenience foods that require less preparation time. Moreover, a benefit to carp fillet producers is that without intramuscular bones, the price of the final product is higher (Bauer and Schlott, 2009). The study conducted by Bauer and Schlott (2009) assessed the carp fillet yields and found a mean yield of 34.0–39.0% without any correlation between the fillet yield and the weight of the initial carp. This data is similar to previous findings (38.7%, Oberle et al., 1997; 34.6%, Cibert et al., 1999; 31.4–35.4%, Gela et al., 2003; 32.1- 41.1%, Kocour et al., 2007), suggesting that the rest of the fish making up to approximately 60% of weight, is not

being used and is being discarded. It is necessary to find method to effectively recover the otherwise discarded nutritious carp protein and make it into a marketable food product.

Isoelectric solubilization and precipitation (ISP) processing is a method that is shown to effectively recover the nutritious protein from aquatic sources by exposing the starting material to extremely low or high pH values to separate protein from bones, skin, scales, etc., and then returning the solution to the isoelectric point to facilitate protein precipitation and recovery (Chen et al., 2007a; Chen et al., 2009; Taskaya et al., 2009a). Typical ISP processing uses HCl as the acid and NaOH as the base for shifting the pH; however, organic acids are more effective at reducing bacterial pathogens (Landsdowne et al, 2009a and 2009b; Otto et al., 2011a and 2011b). Therefore, the effects of using weak acids on the texture and color properties of protein gels made with isoelectrically recovered protein need to be explored.

Texture and color are the major quality attributes of food products and vary greatly depending on customer preferences for a specific product. Three common texture analyses are: texture profile analysis (TPA), Kramer shear cell test and torsion analysis. Texture of sausage style meat products are commonly analyzed using the two bite test mimicking mastication as known as Texture Profile Analysis (TPA) (Rosenthal, 2010). This analysis measures six primary characteristics (hardness, springiness, cohesiveness, gumminess, chewiness and resilience of the product being tested; therefore, enabling researchers to assess the mechanical differences in products. In addition to TPA, Kramer shear cell test measures texture using a multiple slot and blade fixture to determine firmness. Stress at fracture measured by Kramer cell test as well as TPA's hardness are correlated with mechanical sensory perception of firmness. In order to have a more comprehensive understanding of the texture, Torsion test measuring shear stress and shear strain was also performed. Kramer shear test and TPA are empirical tests and Torsion is a

fundamental test defining texture properties of protein gels (Pietrowski et al., 2011). Moreover, torsional shear stress and shear strain are highly correlated with the textural properties; firmness and cohesiveness (Taskaya et al., 2010).

Studies showed that increased lipid percentage in the composition of gels increase firmness and chewiness; however, the texture of the gel is also affected by pH, salt content and rate of heating (Cakir et al., 2012). Also, functional additives such as transglutaminase, starch, and phosphates are proven to enhance textural properties of gels made from recovered protein (Taskaya et al., 2009c; Chen and Jaczynski, 2007a). Another major finding was that acidic solubilization treatments using HCl caused increased protein denaturation compared to basic conditions; therefore, gels made with protein recovered at basic treatments ended up having a firmer texture (Chen and Jaczynski, 2007a). Therefore, the effects of using organic acids with different solubilization treatments on the texture properties of carp protein gels were assessed in this study.

Color of recovered gels is another important aspect when it comes to consumer acceptability. ISP recovered protein usually contains pigments resulting in gels with poor color properties; however, the addition of titanium dioxide is an effective whitening agent (Taskaya et al., 2010). Therefore, the purpose of this study was to measure color and texture properties [Kramer shear, Texture Profile Analysis, and torsion shear stress] of gels made from ISP-recovered protein from whole-gutted silver carp at different pH strategies and organic acid types.

Materials and Methods

Fish preparation. Headed and gutted fresh silver carp (*Hypophthalmichthys molitrix*) was chopped into pieces and sanitized by submergence into a 50 ppm bleach solution for 10 sec (Clorox Regular Bleach, Clorox, Oakland, CA, USA). The fish were drained and then ground using a sanitized food processor (Cuisinart ProClassic7-Cup Food Processor, Cuisinart Co., East Windsor, NJ, USA); the resulting fish paste was packaged into freezer bags (Ziplock Freezer Bags, S.C. Johnson & Son, Inc., Racine, WI, USA). All the equipment that was used to prepare the fish paste was sanitized by cleaning with 70% ETOH and drying under UV light (254 nm) for 15 min (Otto et al. 2011a). In order to have a homogenous ground fish, grinding was performed twice (through coarse and fine filters) and the final paste was portioned into freezer bags and stored at -80°C.

Isoelectric solubilization and precipitation (ISP). ISP was performed on thawed (24 h at 4°C) fish paste. The ISP process is shown in Figure 1. The thawed fish paste (250 g) was weighed and homogenized with distilled, deionized water at a 1:6 ratio in a glass beaker. The selected target solubilization pH values were pH 2.5, 3.0. 11.5 and 12.0. Glacial acetic acid (Fischer Scientific, Fairlawn, NJ, USA) and a combination of 30% glacial L-Lactic acid (85%, EMD Chemicals, Netherlands) and formic acid (88%, Mallinckrodt Chemicals, Netherlands) in sterile distilled water at a 1:1 ratio (Mohammad et al., 2009) were used as the processing acid. The target pH was achieved with the addition of the respective organic acid or 10 N NaOH while being homogenized (PowerGen 700, Fisher Scientific, Pittsburgh, PA) for 10 min for pH stabilization. The homogenization process continued for an additional 10 min after the stabilization of pH to maximize protein solubilization. Following the homogenization process, the fish slurry was transferred to centrifuge bottles and centrifuged at 10,000 x G for 10 min at 4°C (Sorvall RC-SB Refrigerated Superspeed Centrifuge, Du Pont, Wilmington, DE, USA).

Following centrifugation, the top layer containing fat was discarded and the supernatant containing solubilized protein and some insolubles was poured through a cheese cloth into a glass beaker. The filtering step allowed the insoluble fractions (scales, bones, etc.) to be taken out of the solution. The precipitation of the protein fraction was achieved by returning the pH of the solution to the protein isoelectric point (pH 5.5) using the respective organic acid or 10 N NaOH and homogenizing for 5 min. Following precipitation, the homogenate was again transferred to centrifuge bottles and centrifuged at 10,000 x G for 10 min at 4°C. After centrifugation, the supernatant (process water) was discarded and the protein that formed a pellet in the centrifuge bottle was collected.

Development of protein paste. The procedure of making protein pastes were described elsewhere (Tahergorabi et al. 2011; Taskaya et al., 2009b; Chen and Jaczynski, 2007a). A universal food processor (model UMC5, Stephan Machinery Corp., Columbus, OH) was used for chopping the recovered carp proteins at low speed for 1 min. After adding salt (2 g/100 g), the chopping process was continued at low speed for an additional min. Functional additives including: 1 g/100 g potato starch (Penbind 1000 modified potato starch, Penford Food Ingredients Corp., Centennial, CO); 0.3 g/100 g polyphosphate (Kena FP-28, Innophos, Cranbury, NJ); 0.5 g/100 g titanium dioxide (TiO2) (Titanium (IV) oxide, Sigma-Aldrich, Inc., St. Louis, MO); and 0.5 g/100 g transglutaminase (TGASE) were added to the chopped protein paste (Taskaya et al., 2009). Adjusting the final moisture to 80 g/100 g was achieved by addition of cold, distilled water and the paste was mixed for an additional min. The pH of the mixture was set to pH 7.0- 7.2 by adding the respective organic acid of 10 N NaOH, and confirmed using a pH/ion analyzer (Oakton, Eutech Instruments.; Singapore). Protein paste temperature was kept between 1- 4 °C throughout the chopping process. After continuing the chopping for 3 more min

at high speed under vacuum (50 kPa), the paste was collected into a vacuum bag. The protein pastes were vacuum packaged prior to being stuffed into steel tubes to avoid air particle formation which might interfere with the texture analysis results.

Preparation of the protein gels. The protein pastes were individually stuffed into stainless steel tubes (length 17.5 cm, inner diameter 1.9 cm) and dumbbell pre-molded stainless steel torsion tubes (length ¼ 17.5 cm, end diameter ¼ 1.9 cm, midsection diameter ¼ 1.0 cm). Prior to stuffing, the tubes were lightly sprayed with cooking spray (PAM Original cooking spray, ConAgra Foods, USA) to avoid sticking. To allow gel formation, the filled tubes were stored under refrigeration (4^{o}C) for 24 h before being cooked. After 24 h, the tubes were placed in a water bath (Precision, Jouan Inc, Wincester, Virginia) that was preset to 90^{o}C, cooked for 15 min and chilled on ice for another 15 min. The gels were then removed from the tubes and cut into pieces for texture and color analysis. Analyses followed the cutting process after the gels equilibrated to room temperature.

Color analysis. Color was measured on the protein gels using a colorimeter (Minolta Camera Co. Ltd, Osaka, Japan) calibrated with a standard white plate No.21333180 (CIE L* 93.1; a* 0.3135; b* 0.3198). Values of L* (lightness; scale: 0 - 100), a* (intensity in red color; scale: -60 - +60), and b* (intensity in yellow color; scale: -60 - +60) were measured and whiteness was calculated using the following equation:

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

Texture properties of protein gels. Texture profile analysis, Kramer shear test and Torsion analysis were conducted on the protein gel samples. Texture profile analysis (TPA) was performed as described by Taskaya et al. (2009c). At least 8 cylindrical gel samples (length 2.54 cm, diameter 1.9 cm) per treatment were compressed under a 70 mm TPA compression plate

attachment for two cycles. Textural properties of hardness, springiness, cohesiveness, gumminess, chewiness and resilience were determined from the resulting force – time curves (Texture Expert Exceed version 2.64, Stable Micro Systems, 2003).

The Kramer shear test was applied to at least 4 cylindrical gel samples (length 8 cm, diameter 1.9 cm) per treatment using a texture analyzer (Model TA-HDi, Texture Technologies Corp., Scarsdale, NY) with a Kramer cell attachment. Individual gel samples were weighed prior to testing, and then placed in the Kramer cell of the texture analyzer where five shear blades (3 mm thick and 70 mm wide) cut through them (Taskaya et al., 2009c). The shear force (g peak force g^{-1} gel sample) was measured at 127 mm min^{-1} crosshead speed and the shear stress is calculated as the shear force divided by the area of the sample.

A torsion test to measures shear stress and shear strain at mechanical fracture was used to determine gel strength and gel cohesiveness, respectively. At least 8 hour-glass shaped gels were fixed to disks and torsional shear was applied using a Hamman Gelometer (gel Consultant, Raleigh, NC).

Statistical Analysis. The texture profile analysis was replicated at least nine times, Kramer shear test was replicated a minimum of four times, torsion analyses were performed for at least eight times and color analysis was conducted in at least five sets for each pH value and treatment. The data was presented as mean (± SD) and analyzed by one-way analysis of variance. The means were separated using Tukey-Kramer's honestly significant differences test ($P<0.05$) (JMP 9, SAS Inst., Cary, N.C., USA).

Results and Discussion

Color. Color properties are an important factor for consumer acceptance, with whiter fish fillets being more desirable (Taskaya et al., 2009c). Color (L*, a*, b*) of gels made from recovered carp protein was measured (Table 1) and whiteness was calculated (Figure 2). L* value measures the lightness of a sample and is the main attribute that affects whiteness (Moayedi et al., 2010). Table 1 shows that the L*values of the proteins solubilized under acidic conditions excluding AA under pH 3.0, were greater than values under basic conditions. In general, whiter gels were seen when processed under acidic conditions (excluding AA under pH 3.0) as compared to basic conditions, regardless of processing acid. Chen and Jaczysnki (2007a) also reported that krill protein gels were brighter (higher L*) ($p< 0.05$) and whiter when protein recovered at acidic treatments were used compared to basic treatments.

Protein recovered using F&L as the processing acid resulted in significantly whiter gels ($p<0.05$) for all solubilization conditions except pH 2.5 where there was no difference in whiteness ($p<0.05$). Yellowness, shown by positive b* values, were the highest ($p<0.05$) for protein recovered at basic pH values using AA. This may explain why gels made with protein recovered at basic conditions using AA were not as white. The starting material of silver carp is associated with yellow pigments (Taskaya et al., 2009c) which may not have been completely removed from the protein fraction when using AA with basic treatments.

The whiteness of the gels, irrespective of treatment type, was higher than that of Alaska Pollock surimi (Taskaya et al., 2010) and was also whiter than the gels made from protein recovered using HCl (Taskaya et al., 2009c). Taskaya et al. (2009c) explained the low whiteness to be due to increased yellowness (b∗) and attributed this to the pigments from whole gutted carp

that have leached and were retained in the recovered proteins. It can be concluded that pigment removal was more effective when organic acids were used compared to HCl.

Texture.

Texture Profile Analysis (TPA). TPA is commonly used for the determination of a number of textural attributes of muscle foods and surimi gels (Park, 2005). Therefore, this empirical test was performed on the protein gels made from isoelectrically recovered carp protein to assess the effects of using different treatments. Gels made from protein solubilized under basic treatments resulted in harder, gummier and chewier texture ($p<0.05$) (Figure 3). Similar findings were reported when protein pastes were made from recovered carp protein, krill and trout byproducts (Taskaya et al., 2009c; Chen and Jaczynski 2007a and 2007b). The textural properties of protein paste can be attributed to gelation characteristics which are based on protein denaturation. Acidic solubilization treatments lead to more protein denaturation compared to basic treatments (Kristinsson et al., 2005); therefore, the protein gels made out of protein that was recovered using basic treatments likely caused less protein denaturation which resulted in firmer and chewier ($p< 0.05$) gels. It is interesting to note that when AA was used as the processing acid at pH 2.5 solubilization pH, the resulting gels presented with significantly lower ($p<0.05$) texture properties (hardness, springiness, gumminess, chewiness and resilience). This may be due to the protein denaturation caused by exposure to extremely low solubilization pH. Furthermore, compositional characteristics highlighted that solubilization at pH 2.5 using AA as the processing acid yielded a protein fraction with a higher ($p< 0.05$) ash content compared to solubilization at the same pH using F&L (Paker, 2012). The higher ash content indicating impurities might have affected the gel network adversely, resulting in weaker texture properties. In correlation with this, gels made from protein recovered at solubilization pH 3.0 using F&L

showed lower ($p<0.05$) hardness, gumminess and chewiness compared to gels made from protein recovered at the same solubilization pH using AA. The proximate composition of the protein fractions reflected that solubilization at pH 3.0 using F&L as the processing acid contained higher ash than using AA at the same solubilization pH (Paker, 2012). Therefore, it is likely that acidic solubilization conditions induce more protein denaturation than basic conditions and the amount of impurities in the protein fraction have an effect on the texture properties.

For the most part, the texture properties (hardness, gumminess and chewiness) of protein gels made from carp protein recovered using organic acids yielded much higher values for all treatments when compared to gels made from carp protein recovered using HCl and also Alaska Pollock surimi (Taskaya et al., 2009c); however, the samples in this present study were less cohesive which may be due to the ineffective removal of the insolubles (i.e. bones, skin, scales) from the protein fraction. The compositional analysis of the recovered protein showed that there was a large portion of ash in the recovered protein which implies that using AA and 30% F&L as a processing acid is not as effective as HCl at separating the impurities (bones, scales, etc.) from the protein fraction (Paker, 2012). Impurities, lipids and other compounds lower the myofibrillar protein concentration in the recovered protein fraction and the lower amount of actin and myosin which will lead to poor gel network formation (Pietrowski et al., 2012). Therefore, the high concentration of impurities may have prevented the formation of a more cohesive gel.

There was no difference ($p>0.05$) in cohesiveness based on processing acid type (AA or 30% F&L); however, resilience values differed ($p<0.05$). When AA was used as the processing acid for protein solubilization at pH values 2.5, 3.0 and 12.0 there was significantly lower resilience ($p<0.05$). Interestingly, hardness, springiness, gumminess and chewiness were also significantly lower when AA was used at solubilization pH 2.5 ($p<0.05$). However, hardness,

gumminess and chewiness was improved at solubilization pH 3.0 using AA. There were no statistical differences in the texture properties (hardness, springiness, cohesiveness and gumminess) under basic treatments using either acid. Vardhanabhuti et al. (2010) showed that there was a difference in gel swelling when whey protein gels were exposed to different acids including hydrochloric, phosphoric and citric acid. Gels equilibrated in phosphoric acid showed more swelling compared to the others; on the other hand, the gels equilibrated in citric acid yielded the lowest gel swelling which was attributed to the different dissociation constants of acids (Vardhanabhuti et al., 2010). This can explain why the protein gels made using protein recovered by 30% F&L at solubilization pH 2.5 resulted in harder, springier, chewier and gummier characteristics compared to that of AA. The solubility of the protein molecules depends on the surface charges leading to the protein-water electrostatic interaction and therefore, the dissociation percentage of the organic acids play a significant role in protein solubilization (Gehring et al., 2009). AA has a higher dissociation constant compared to both formic and lactic acids (Tipping, 2002). This means that at as the acidity of the solution decreases so does the protein solubility of F&L. Therefore, at pH 2.5 using F&L may result in increased texture attributes; however, using AA as the processing pH increases may also be beneficial. This also explains why basic treatments yield improved texture properties. Both acids used in this study will be 100% dissociated at the basic pH treatments and will accomplish higher protein solubility leading to higher texture attributes. Moreover, the concentration of F&L was much lower than that of AA, which might also have an effect on protein solubility. Even at the same concentration, the type of equilibrating acid would have a major influence on the degree of gel swelling which would impact texture properties (Vardhanabhuti et al., 2010). Therefore, different solubilization treatments might yield protein gels with preferable texture properties.

Future studies assessing the effects of using organic acids at different concentrations on the texture properties of protein gels might be useful.

Kramer Shear Cell. Kramer shear force and shear stress were measured for the carp gels (Figure 4 and 5). The gels made from protein solubilized under basic conditions had higher shear force and therefore were harder (Sigurgisladottir et al., 1999); these findings are supported by previously reported experiments where HCl was used as the processing acid (Taskaya et al., 2010; Tahergorabi et al., 2011). For the most part, there were no statistically significant differences in the gel strength (i.e. shear stress) of gels made with protein solubilized at the same pH using either acid (Figure 5); however, shear force varied depending on processing acid used (Figure 4). The shear force was higher when F&L was used under pH 2.5 and 12, whereas using AA under solubilization pH 3.0 resulted in a higher force. Almost all treatments resulted in a higher shear force than that of Alaska Pollock surimi (Taskaya et al., 2010). Another study showed that both breaking force and deformation of acidified actomyosin gels increased when pH was decreased from 6.3 to 4.6 and the higher gel strength was attributed to the extensive formation of hydrogen bonds and hydrophobic interactions (Xu et al., 2012). Fretheim et al. (1985) also showed a decrease in gel strength below pH 4- 4.5. The reduced gel strength due to lowering the pH far below the isoelectric point was explained by the increased electrostatic repulsive interactions (Xu et al., 2012). The present data is supported by the TPA findings and can be attributed to the rate of acid dissociation which would affect protein denaturation depending on the solubilization pH. Dunajski (1979) suggested using Kramer shear cell test to examine the tenderness of fish muscle and the present study shows that the protein recovered at basic solubilization conditions are harder and stronger regardless of processing acid, and

therefore would yield improved texture properties compared to gels made from protein solubilized under acidic conditions.

Torsion. The torsion test is a fundamental test for texture, while the Kramer shear test and TPA are empirical tests (Pietrowski et al., 2011). Some advantages of the torsion test over the empirical tests are that its measurements are not influenced by stress measurements, it measures "pure shear" which is a stress condition that does not modify the specimen volume, and the specimen shape is maintained during the test. Moreover, shear stress and shear strain at fracture measured by torsion are major factors for determining the shelf-life and stability of the protein gels (Park, 2005).

The shear stress and shear strain values of protein gels are presented in Figure 6 and 7. Shear stress, highly correlated with firmness, was statistically highest ($p<0.05$) in gels made out of recovered protein when basic treatments were used with AA during ISP, and the lowest value was seen when acidic treatments were used with F&L. All of the treatments aside from the gels made from recovered protein using F&L at acidic treatments, resulted in having statistically similar textural qualities compared to high quality Alaska Pollock surimi which is shown to have excellent gel forming abilities as well as texture (Taskaya et al., 2009c). Shear strain, on the other hand, indicates the cohesiveness of gels. The data presented in Table 2 shows that all treatments (excluding F&L with solubilization pH 3.0) resulted in high shear strain. Gels made from proteins recovered using F&L under pH 3.0 as the processing acid had the least amount of lipid and protein and highest amount of ash than the other treatments (Paker, 2012). The lower cohesive properties of the resulting gels may be due to the greater amount of insolubles (i.e. bones, scales and skin) and lower amount of myofibrillar protein.

Conclusion

Results of this study demonstrated that the protein gels made from recovered carp protein by ISP processing with organic acids, especially at basic protein solubilization conditions, resulted in gels that were as firm and cohesive as traditional surimi and gels made from ISP recovered proteins when HCl was used as the processing acid. Results also showed that gels made from protein recovered under basic conditions had improved texture and color properties compared to protein recovered under acidic conditions (Chen and Jaczynski, 2007a and 2007b; Kristinsson and Hultin, 2003; Kristinsson and Liang, 2006; Taskaya, et al., 2009b and 2009c) likely because when protein is solubilized under basic conditions, impurities are removed more effectively and less protein denaturation is caused compared to acidic solubilization conditions (Paker et al., 2012). Overall, using ISP with organic acids resulted in efficient protein recovery. The protein gels made with the isoelectrically recovered protein showed similar or improved functional, texture and color properties compared to conventionally prepared surimi products. Therefore, this study shows that using organic acids during ISP may be beneficial in producing a value added food product for human consumption.

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Table 1. Color Properties of Protein Gels made from recovered carp protein

Acid Type	Solubilization pH	L*	a*	b*
AA	2.5	85.69±1.69 ab	-0.07±0.27 d	9.52±0.25 bc
AA	3.0	82.43±0.80 e	090±0.10 a	9.57±0.26 c
AA	11.5	81.85±1.09 e	0.41±0.16 c	11.12±0.88 a
AA	12.0	80.61±0.84 f	0.74±0.18 b	10.99±0.38 a
F&L	2.5	85.01±1.19 bc	-0.42±0.07 e	9.72±0.32 bc
F&L	3.0	87.03±1.09 a	-0.32±0.09 de	7.44±0.40 d
F&L	11.5	83.88±0.82 cd	0.72±0.15 ab	9.90±1.45 bc
F&L	12.0	82.18±0.96 de	0.64±0.14 b	10.87±0.68 ab

Data are given as mean ± standard error of mean. Mean values in a column with different letters are significantly different (Tukey's least significant difference test, $p<0.05$).

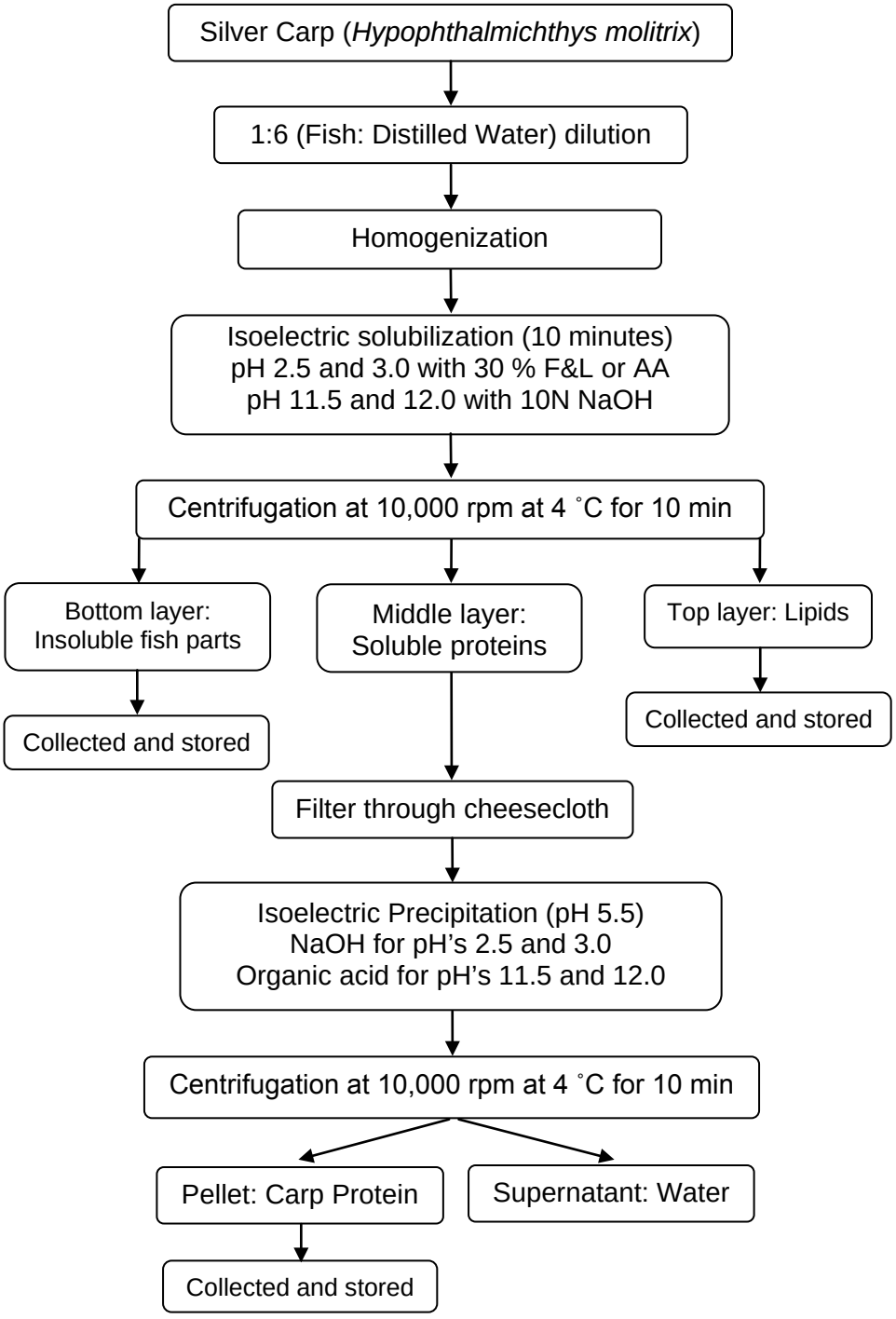

Figure 1. Isoelectric Solubilization and Precipitation Flow Chart

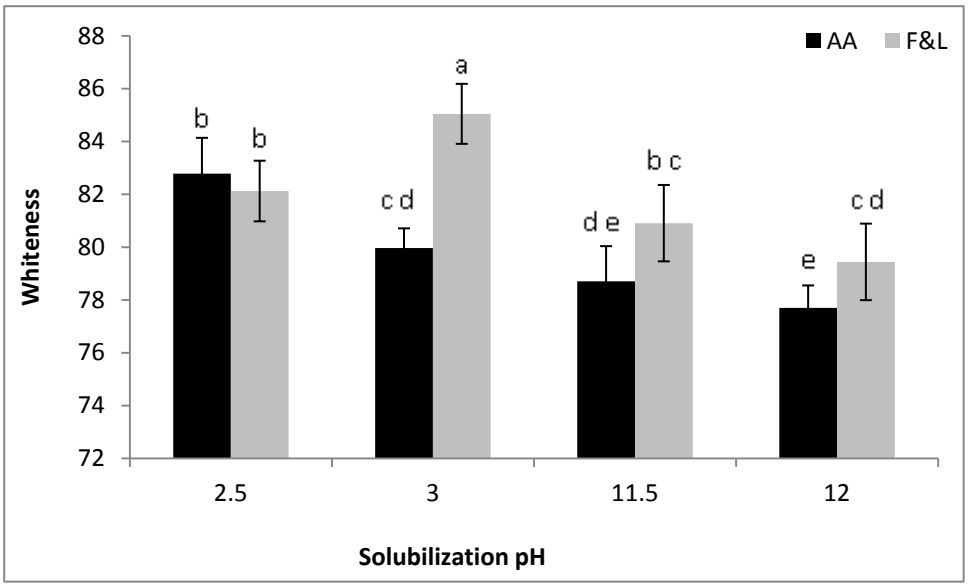

Figure 2. Whiteness of carp protein gels. Data are given as mean ± standard deviation. Mean values in each treatment with different letters are significantly different (Tukey's least significant differences test, $p<0.05$)

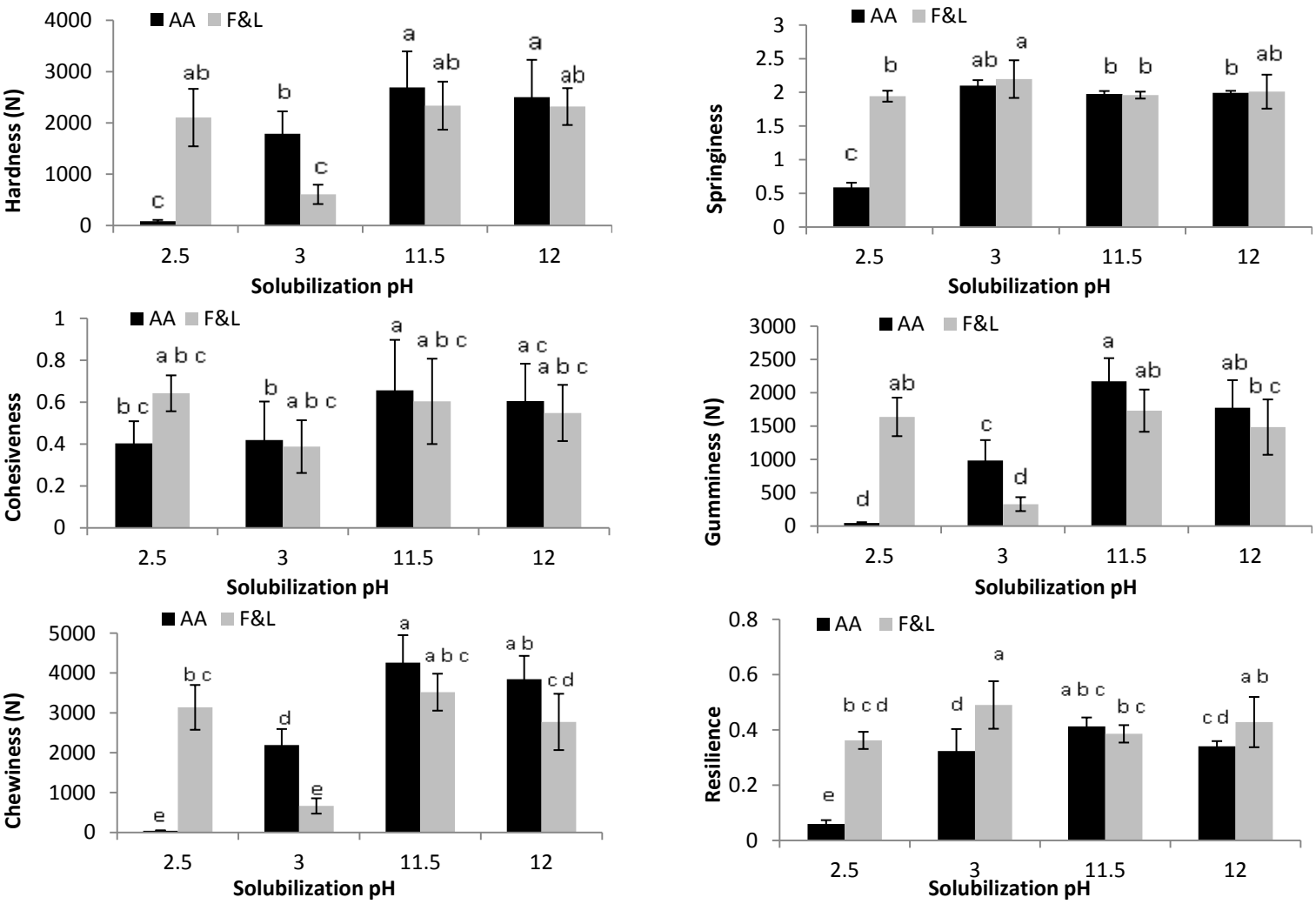

Figure 3. Hardness, Springiness, Cohesiveness, Gumminess, Chewiness and Resilience (texture profile analysis) of recovered carp protein. Data are given as mean ± standard deviation. Mean values in each treatment with different letters are significantly different (Tukey's honestly significant differences test, $p < 0.05$).

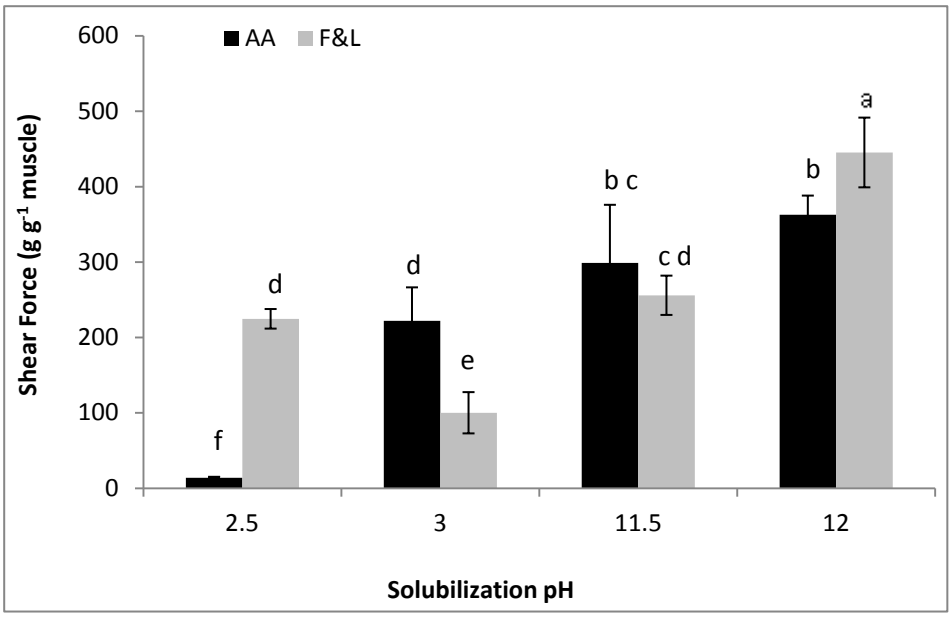

Figure 4. Kramer shear force of recovered protein gels. Data are given as mean ± standard deviation. Mean values in each treatment with different letters are significantly different (Tukey's honestly significant differences test, $p < 0.05$).

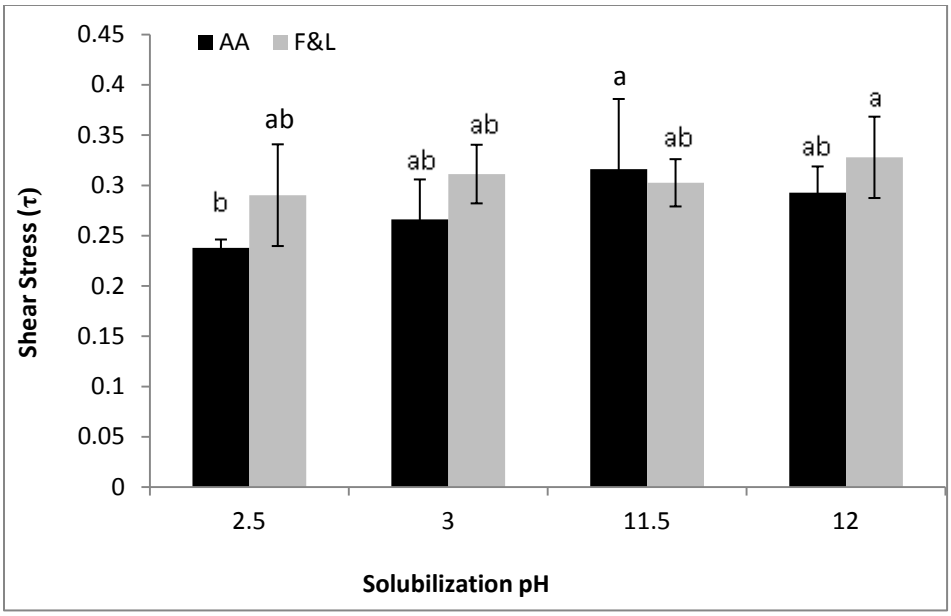

Figure 5. Kramer shear stress (τ) of recovered protein gels. Data are given as mean ± standard deviation. Mean values in each treatment with different letters are significantly different (Tukey's honestly significant differences test, $p < 0.05$)

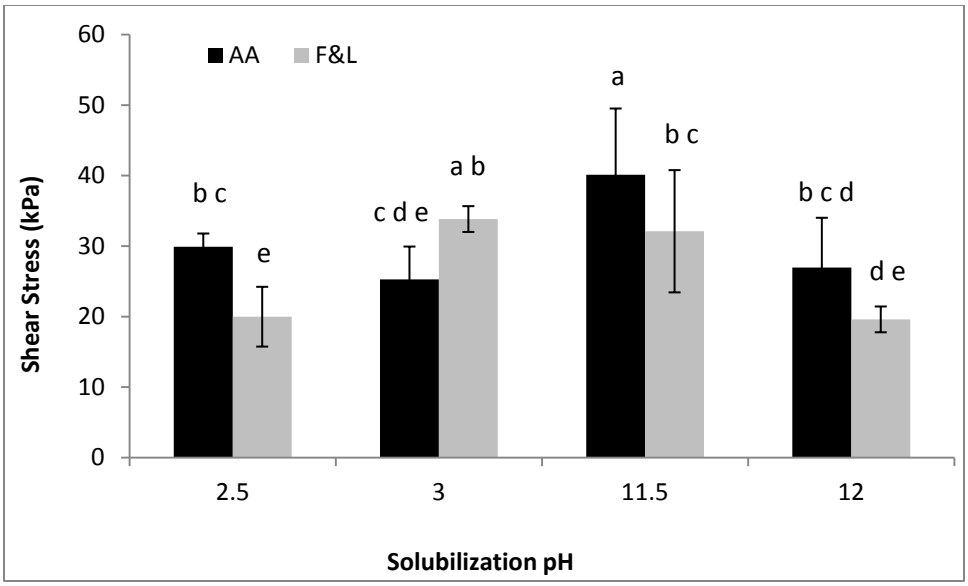

Figure 6. Torsion shear stress (kPa) of gels made from recovered carp protein using isoelectric solubilization and precipitation with different organic acids and solubilization pH values. Data are given as mean ± standard deviation. Mean values in each treatment with different letters are significantly different (Tukey's honestly significant differences test, $p < 0.05$).

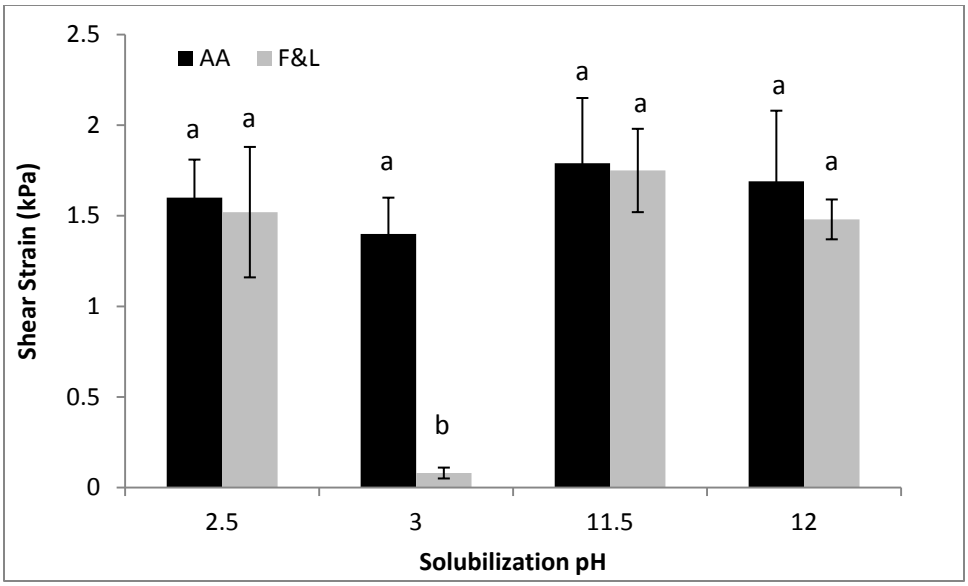

Figure 7. Torsion shear strain (kPa) of gels made from recovered carp protein using isoelectric solubilization and precipitation with different organic acids and solubilization pH values. Data are given as mean ± standard deviation. Mean values in each treatment with different letters are significantly different (Tukey's honestly significant differences test, $p < 0.05$).