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Compositional Characteristics of Materials Recovered from Whole Gutted Silver Carp (*Hypophthalmichthys molitrix*) Using Isoelectric Solubilization/Precipitation

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Isoelectric solubilization/precipitation (ISP) at acidic and basic pH was applied to whole carp, yielding proteins, lipids, and insolubles. The objective was to characterize composition of recovered materials. Crude protein was concentrated to 89−90% in proteins recovered at acidic pH and to 94−95% at basic pH. Basic pH yielded proteins with more ($P < 0.05$) essential amino acids (EAAs). EAA content in recovered proteins met FAO/WHO/UNO requirements. ISP did not affect fatty acid (FA) composition. Lipids recovered at acidic pH contained 88−89% of total fat and at basic pH, 94−97%. Total fat in recovered proteins was low, with EPA and DHA at the highest ($P < 0.05$) percentage for pH 11.5. ISP, particularly basic pH, effectively removed impurities such as bones and scales from whole carp. This is indicated by 3.8−5.8% of ash in recovered proteins compared to 11.2% for whole carp and 5.4% for boneless/skinless carp fillets. Basic pH yielded less ($P < 0.05$) Ca, P, and Mg in recovered proteins. These minerals were more ($P < 0.05$) concentrated in insolubles recovered with basic pH. This study indicates that materials recovered from whole carp using ISP have high nutritional value and may be useful in the development of human food and animal feeds.

KEYWORDS: Fish proteins; isoelectric solubilization/precipitation; protein and lipid recovery; fish oil; mineral profile

INTRODUCTION

The global capture fisheries have remained fairly stable for the past several years at approximately 90 million tons and have been forecast as unlikely to increase in the future (*1*). Anchoveta and Alaska pollock represent the greatest production from capture fisheries, with 10.7 and 2.7 million tons, respectively. The world aquaculture production in 1950 was less than a million tons, but it reached nearly 50 million tons in 2005. The value of the world aquaculture production was estimated at U.S. $670.3 billion in 2004 compared with U.S. $653.8 billion in 2002, indicating a 31% increase.

According to an FAO report, carp species provide by far the greatest biomass of aquatic foods in the world, accounting for 18.3 million tons (*1*). Carp are known for their minimal growth requirements, yet rapid growth rates. Silver carp (*Hypophthalmichthys molitrix*) is the most common carp species. The FAO reported that silver carp is the cheapest fish (*2*). These statistics clearly indicate a global role of carp, particularly silver carp, providing a source of proteins and lipids to fulfill human nutritional needs.

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Silver carp typically contains 75.2% moisture, 17.6% protein, 5.5% lipids, and 1.3% ash (*3*). However, in the United States carp are generally considered to be unsuitable for human consumption. Various carp species have rapidly started populating major bodies of fresh waters in the United States to the extent that commercial processing is of interest. Typical mechanical means of meat recovery from carp is impractical due to the bony nature of the carp carcass. In addition to the processing challenges for carp associated with its bony characteristics, the proteolytic enzymes impose more technological difficulties. Hydrolytic enzymes, normally existing in the fish gastrointestinal tract, are released during fish processing, which may lead to proteolysis of muscle proteins and a loss of protein basic functional properties such as gel-forming ability. Enzymatic proteolysis is a typical and major issue associated with fish processing, contributing to the poor texture of fish food products. However, the enzymatic proteolysis can be suppressed by application of protease inhibitors (*4*−*6*).

It is desirable to develop a technique that would allow efficient recovery of muscle proteins and lipids from carp. Ideally, there would be no loss of the omega-3 polyunsaturated fatty acids (ω-3 PUFA) docosahexaenoic acid (DHA, 22:6n3) and eicosapentaenoic acid (EPA, 20:5n3) during processing, and the recovery technique would allow retention of essential amino acids (EAA). The recovered proteins would also need to retain gel-forming

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ability and, therefore, be a functional ingredient in the development of restructured value-added food products (*7*).

The recovered carp proteins may be processed into surimi seafood (i.e., crab-flavored seafood) (*8*), fish sausage (*9*), and similar final products. The recovered carp lipids may be used as dietary supplements or an ingredient in functional foods. The value-added food products developed from silver carp would not only enable the industry to diversify its product offerings but also offer another source of highly nutritious proteins and oil for human consumption.

Meinke and co-workers (*10*) as well as Meinke and Mattil (*11*) demonstrated the solubility of carp muscle proteins as a function of pH. An isoelectric solubilization/precipitation (ISP) has also been applied to fish muscle resulting in efficient recovery of fish muscle proteins and lipids (*12*, *13*). The recovered proteins retained gel-forming ability (*14*, *15*). Our research group has shown that functional muscle proteins can be recovered from whole Antarctic krill (*Euphausia superba*) and fish processing byproduct by using ISP (*16*−*21*).

Although ISP allows recovery of functional proteins, it is important to determine the amino acid, fatty acid, and mineral profiles of the proteins, lipids, and insolubles (such as bones, skin, fins, insoluble proteins, membrane lipids, etc.) recovered from carp using ISP. This information is critical to initiate the assessment of nutritional quality of carp proteins, lipids, and insolubles for the development of human food products and animal feeds. Therefore, the objectives of this study were to characterize (1) the amino acid profile (AAP) of the recovered proteins and insolubles, (2) the fatty acid profile (FAP) of the recovered lipids, proteins, and insolubles, and (3) the mineral profile (MP) of the insolubles and proteins recovered from whole gutted silver carp by ISP using basic (i.e., alkaline) and acidic pH treatments.

MATERIALS AND METHODS

Sample Preparation. Freshly caught silver carp (*H. molitrix*) were donated by Heartland Seafoods (Hollywood, SC). Fish were transported to the food science laboratory at West Virginia University in heavily insulated industrial-strength boxes filled with ice. The transportation time did not exceed 12 h. Upon arrival, the fish were manually eviscerated (head, skin, scales, fins, and bones were not removed), rinsed with cold tap water, ground (meat grinder model 812 with 2.3 mm grinding plates, Biro, Marblehead, OH), vacuum packed, and frozen at −80 °C. The storage time did not exceed 5 days.

Recovery of Muscle Proteins and Lipids from Whole Gutted Silver Carp. Figure 1 shows a processing flowchart for the recovery of muscle proteins and lipids from whole (head-on, skin-on, bone-in) gutted silver carp and subsequent analyses of the recovered fractions (proteins, lipids, and insolubles) as well as the starting material (i.e., whole gutted silver carp). Partially frozen, eviscerated, and ground silver carp were homogenized (PowerGen 700, Fisher Scientific, Fair Lawn, NJ) in cold (4 °C), distilled, and deionized water (dd H_2O) at 1:6 ratio (ground fish/water, w/v). Fifteen hundred milliliters of the homogenate was transferred to a beaker that was placed in ice slush. During the entire process the temperature was carefully controlled at 1−4 °C to reduce the activity of carp endogenous proteases and lipid oxidation. The processing time did not exceed 60 min. Homogenization/mixing was continued during the pH adjustment steps.

The pH of the homogenates was separately adjusted to pH 2.00 and 3.00 ± 0.05 as well as pH 11.50 and 12.50 ± 0.05 to isoelectrically solubilize carp proteins at acidic as well as basic (i.e., alkaline) pH ranges (*16*, *17*, *20*, *21*). The 10 and 1 N reagents were used for crude and fine pH adjustments during both protein solubilization and subsequent precipitation (pH 5.5) (see below). The pH-meter (model AB15, Fisher Scientific) was properly calibrated prior to pH adjustments. Once the desired pH was obtained, the solubilization reaction was allowed to take place for 10 min, followed by centrifugation at 10000*g* and 4 °C for 10 min using a laboratory batch centrifuge (Sorvall RC-5B Refrigerated Superspeed, Kendro Laboratory Products, Newtown, CT). Centrifugation resulted in three layers: top, carp lipids; middle, carp muscle protein solution; and bottom, insolubles (bones, skin, fins, insoluble proteins, membrane lipids, etc.). The lipids and insolubles were collected, and proximate analysis was performed. The recovered lipids were analyzed for fatty acid profile (FAP). The recovered insolubles were analyzed for FAP, AAP, and MP.

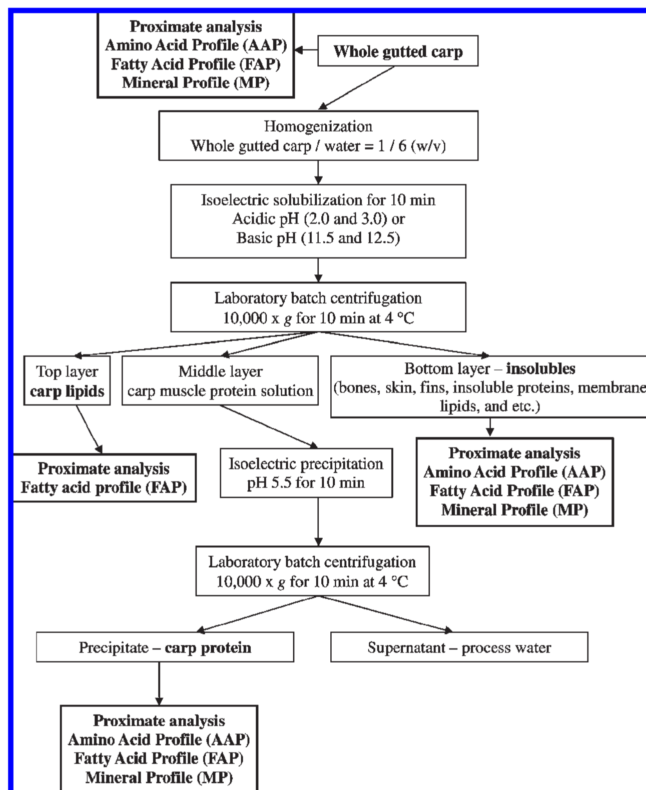

Figure 1. Flowchart for isoelectric solubilization/precipitation for whole gutted silver carp and subsequent analyses of the recovered fractions.

The carp muscle protein solution was collected and the pH adjusted to 5.50 ± 0.05 by either 10 and 1 N HCl or 10 and 1 N NaOH to isoelectrically precipitate carp proteins (*16*, *17*, *20*, *21*). HCl (10 and 1 N) was used for the carp muscle protein solutions that had been solubilized at basic pH, whereas NaOH (10 and 1 N) was used for those carp muscle protein solutions that had been solubilized at acidic pH. Once the desired pH was obtained, the precipitation reaction was allowed for 10 min. The solution with precipitated proteins was centrifuged as above. The centrifugation resulted in two layers: top, process water; and bottom, precipitated carp muscle proteins. The precipitated proteins were collected, and proximate analysis as well as AAP, FAP, and MP was performed.

Proximate Analysis of Materials Recovered from Whole Gutted Silver Carp. The moisture content, total fat, crude protein, and ash content were determined for the recovered proteins, lipids, and insolubles (bones, skin, fins, insoluble proteins, membrane lipids, etc.). For comparison, the same proximate analysis was also performed for whole gutted carp [i.e., starting material for isoelectric solubilization/precipitation (ISP)], skinless and boneless carp fillets (manual filleting), and Alaska pollock surimi grade AA. Surimi was donated by Pacific Coast Seafoods (Warranton, OR). Surimi contained a cryoprotectant mixture (4% sorbitol and 4% sucrose, w/w). Frozen surimi blocks were shipped overnight in heavily insulated industrial-strength boxes filled with ice. Upon arrival, surimi was stored at −80 °C. The storage time did not exceed 1 month.

Sample (2 g) was placed on an aluminum dish (Fisher Scientific), spread evenly across the dish, and oven-dried (105 °C for 24 h) (*22*). Total fat content was determined according to the Soxhlet extraction method (*22*) and expressed as percent (dry weight basis). Crude protein was determined by Kjeldahl assay (*22*) and expressed as percent (dry weight basis). Ash content was performed by incinerating samples in a muffle furnace at 550 °C for 24 h (*22*) and expressed as percent

(dry weight basis). All proximate analyses are reported as mean values of at least three replicates.

Amino Acid Profile Analysis. The recovered proteins and insolubles were analyzed for a full AA profile according to AOAC method 982.30 E (a, b, c) (*22*). For comparison, AAP was also determined for whole gutted carp (i.e., starting material for ISP), skinless and boneless carp fillets (manual filleting), and Alaska pollock surimi grade AA. The samples were freeze-dried and subjected to the following three types of hydrolysis: acid hydrolysis with 6 N HCl at 110 °C for 24 h, performic acid oxidation at 0−5 °C overnight followed by acid hydrolysis (6 N HCl at 110 °C for 24 h), and alkaline hydrolysis with fresh 4.2 N NaOH at 110 °C for 22 h. Following the hydrolysis, amino acids were quantified using a Beckman Amino Acid Analyzer (model 6300, Beckman Coulter, Inc., Fullerton, CA) employing sodium citrate buffers as step gradients with the cation exchange postcolumn ninhydrin derivatization method. The data are reported as mean values of at least three replicates, and the mean values are expressed as milligrams of amino acid per gram of sample (dry weight basis).

Fatty Acid Profile Analysis. The recovered lipids, proteins, and insolubles were analyzed for FAP. For comparison, FAP was also determined for whole gutted carp (i.e., starting material for ISP), skinless and boneless carp fillets (manual filleting), and Alaska pollock surimi grade AA. Lipids were extracted for FAP analysis using the methodology described by Folch and co-workers (*23*). Fatty acids were transmethylated by the addition of 4 mL of 4% (w/v) methanolic H_2SO_4 and heated in a 90 °C water bath for 60 min according to the method of Chen and co-workers (*24*, *25*). The mixture was saponified by transfer through a Na_2SO_4-filled glass Pasteur pipet and subsequently dried under N_2 in a 60 °C water bath for 60 min. The fatty acid methyl esters (FAMEs) were resuspended in filtered iso-octane. The FAMEs were analyzed by using a gas chromatograph (model CP-3800; Varian Analytical Instruments, Walnut Creek, CA) and a flame ionization detector fitted with a WCOT-fused silica capillary column (50 m length, 0.25 mm inside diameter; Varian Analytical Instruments). Injection and detection temperatures were maintained at 220 °C, and column temperature was 190 °C. The stationary phase was CP-Silica 88 (Varian Analytical Instruments). Nitrogen was the carrier gas, and a split ratio of 10:1 was used. The fatty acids were identified by comparing their retention times with those of known standards and references (*26*). Peak area and the amount of each fatty acid were computed by an integrator using the Star GC workstation version 6 software (Varian Analytical Instruments). The data are reported as mean values of at least three replicates, and the mean values are expressed as percent of a fatty acid in total fatty acids.

Mineral Profile Analysis. The recovered proteins and insolubles were analyzed for MP according to the method of Chen and co-workers (*20*, *21*). For comparison, MP was also determined for whole gutted carp (i.e., starting material for ISP), skinless and boneless carp fillets (manual filleting), and Alaska pollock surimi grade AA. All glassware was washed overnight in a solution of 10% HCl in distilled and deionized water (dd H_2O, v/v) prior to use. Ashed samples were dissolved in 2 mL of 70% nitric acid. The acidified samples were neutralized in 5 mL of dd H_2O and filtered through Whatman no. 1 paper. Samples were diluted to volume with dd H_2O in a 50 mL volumetric flask. All samples were analyzed in duplicate, and three separate experiments were performed. Major minerals, calcium (Ca), phosphorus (P), and magnesium (Mg), as well as iron (Fe) were determined using inductively coupled plasma optical emission spectrometry (model P400; Perkin-Elmer, Shelton, CT).

Statistical Analysis. The study was conducted using a completely random design (CRD) (*27*). All samples were analyzed in at least triplicate, and at least three separate experiments were performed (n = 3). The data were analyzed using analysis of variance and are reported as mean values (ANOVA) (*28*). A significant difference was used at 0.05 probability level, and differences between treatments were tested using the least significant difference (LSD) test (*29*). All statistical analyses of data were performed using SAS (*28*).

RESULTS AND DISCUSSION

Proximate Analysis of Materials Recovered from Whole Gutted Silver Carp. Whole gutted carp were used as the starting material. Whole carp contains a high proportion of impurities (i.e., bones, skin, scales, and fins) when compared to boneless and skinless fillets. Therefore, it is of interest to assess how well these impurities were separated from the recovered proteins and lipids during the ISP processing. Ash content (dry basis) was determined as an indicator of how well the impurities were removed because most of the minerals are located in the impurities.

As shown in **Table 1**, regardless of the pH used during protein solubilization, the recovered proteins contained 3.80−5.83% of ash (dry basis) and the recovered lipids contained 0.74−2.13% of ash as compared to 5.39% for boneless and skinless carp fillets (manual filleting), 11.16% for whole gutted carp (the starting material), and 2.91% for Alaska pollock surimi. Solubilization at basic pH (pH 11.5 and 12.5) resulted in lower ($P < 0.05$) ash content than at acidic pH (pH 2.0 and 3.0) in the recovered proteins and lipids. In the insolubles, ash was concentrated to a greater ($P < 0.05$) extent with the basic pH than with the acidic treatments. The data from the present experiments are consistent with those of Anderson and co-workers (*30*), who demonstrated that basic treatments yielded optimal demineralization of Antarctic krill when compared to the acidic treatments. Therefore, ISP of whole gutted carp, particularly at basic pH, yielded proteins and lipids with lower ash content and the majority of ash was retained with the recovered insolubles.

The insolubles had relatively high protein and fat contents (**Table 1**). The myofibrillar and sarcoplasmic proteins become water-soluble when the pH is altered. However, the proteins recovered in the insolubles did not become water-soluble; therefore, they likely were nonmuscle nitrogenous compounds. The lipids in the insolubles were likely phospholipids that were

Table 1. Proximate Analysis (Dry Basis) of the Recovered Carp Proteins, Lipids, and Insolubles That Were Solubilized at Different pH Values and Precipitated at pH 5.5[a]

	recovered proteins				recovered lipids				recovered insolubles			
solublization pH	% moisture	% fat	% protein	% ash	% moisture	% fat	% protein	% ash	% moisture	% fat	% protein	% ash
2.0	89.86 b	3.38 b	89.86 c	5.83 a	80.75 a	88.55 c	10.18 a	2.13 a	94.94 a	15.51 c	67.09 a	16.67 d
3.0	91.01 a	4.93 a	89.21 c	5.14 b	79.64 a	88.02 c	9.61 b	1.50 b	94.72 a	12.00 d	65.42 b	21.68 c
11.5	90.50 ab	1.73 c	93.51 b	3.80 c	50.50 c	94.06 b	5.48 c	0.74 c	74.40 c	18.75 b	44.35 c	36.58 a
12.5	88.50 c	0.72 c	95.12 a	3.93 d	52.54 b	96.91 a	3.03 d	0.78 c	85.09 b	31.18 a	42.40 d	26.91 b

	% moisture	% fat	% protein	% ash
whole gutted silver carp	68.88 ± 0.68	35.83 ± 1.05	52.38 ± 1.12	11.16 ± 0.43
skinless and boneless carp fillets	74.34 ± 0.41	27.71 ± 0.59	66.61 ± 0.66	5.39 ± 0.05
Alaska pollock surimi	75.01 ± 0.63	1.29 ± 0.24	60.77 ± 0.27	2.91 ± 0.03

[a] For comparison, proximate analysis (dry basis) of whole gutted silver carp, skinless and boneless carp fillets (manual filleting), and Alaska pollock surimi are also listed. Data are given as mean values (n = 3). Mean values in rows with different letters indicate significant differences ($P < 0.05$).

retained despite centrifugation at 10000*g* for 10 min. Due to their polarity, phospholipids can bond with water dipoles and hydrophilic moieties of proteins. Therefore, unlike neutral (apolar) lipids, centrifugation alone may not result in simple separation of phospholipids. Hultin and co-workers (*31*) as well as Hultin and Kelleher (*12*, *13*) stated that the insolubles contain membrane lipids (i.e., phospholipids) among other components.

Lipid removal from the recovered proteins is desirable because fish lipids are prone to oxidation, which leads to rancidity. Therefore, it is interesting to note that the proteins recovered from basic treatments retained less ($P < 0.05$) total fat (dry basis) (pH 11.5, 1.73% fat; pH 12.5, 0.72% fat) than acidic treatments. Consequently, this resulted in higher ($P < 0.05$) concentration of crude protein (dry basis) at 93.51% for pH 11.5 and 95.12% and for pH 12.5 (**Table 1**). Similar results have been reported for channel catfish (*32*), herring (*33*), Atlantic croaker (*15*), and Antarctic krill (*16*). During oil processing to obtain a soapstock, free fatty acids are more readily removed with alkaline processing than with acidic treatment (*34*). This may explain the lower fat content obtained with basic treatments during processing in the present study. In the lipids recovered from basic treatments, total fat was more ($P < 0.05$) concentrated (pH 11.5, 94.06% fat; pH 12.5, 96.91% fat), whereas the crude protein was lower ($P < 0.05$) (pH 11.5, 5.48% protein; pH 12.5, 3.03% protein) than from the acidic treatments (**Table 1**). On the basis of the proximate analysis, the basic treatments appear to have resulted in a more desirable composition of the recovered materials compared to the acidic treatments.

Amino Acid Profile Analysis. Proximate analysis showed that ISP of whole gutted carp concentrated crude protein in the recovered proteins (**Table 1**). The recovered insolubles contained a considerable amount of crude protein, and the recovered lipids had relatively low content of crude protein. This is why AAP was determined for the recovered proteins and insolubles only.

The carp proteins recovered by basic pH treatments had higher ($P < 0.05$) content of all of the individual AAs and total EAAs than the acidic treatments (**Table 2**). This may have been due to less pH-induced (nonenzymatic) hydrolysis of carp proteins during protein solubilization at the basic compared to acidic pH. The pH-induced and enzymatic (endogenous proteases) hydrolysis might have led to partially hydrolyzed carp proteins that liberated free amino acids (AAs). Partially hydrolyzed fish proteins and free AAs liberated from these proteins are typically water-soluble (*35*, *36*). Lysine and threonine exhibit high water solubility (*37*). More ($P < 0.05$) EAAs, including lysine and threonine, were retained with the recovered proteins that had been solubilized at basic rather than acidic pH (**Table 2**). Therefore, the EAAs may have been recovered to a lesser degree with the acidic treatments in the present study, explaining why the basic pH treatments resulted in the greatest ($P < 0.05$) total EAAs.

To our knowledge, there are no published studies reporting AA analysis of proteins recovered via ISP. Sathivel and co-workers (*36*) reported AA composition of freeze-dried protein powders recovered from various fish by heating at 85 °C for 60 min and centrifugation at 2560*g*. The total content of EAAs ranged from 260.3 to 397.6 mg/g for herring gonad protein powder and arrowtooth flounder protein powder, respectively. The values reported by Sathivel and co-workers (*36*) are higher than the total content of EAAs in whole gutted carp (196.5 mg/g) (**Table 2**). However, the total EAAs in the recovered proteins in the present study ranged from 354.4 to 396.7 mg/g, which is in the upper range of the values reported by Sathivel and co-workers (*36*).

The total EAAs in the recovered proteins and in whole gutted carp constituted approximately 45.1 and 41.3% of the total AA, respectively (**Table 2**). The ISP resulted in an increased ($P < 0.05$) total EAAs in the recovered proteins as well as increased ($P < 0.05$) ratio of EAAs to AAs (EAA/AA) when compared to whole gutted carp. The EAA/AA ratios in the present study were 41.3% for whole gutted carp and 44.9−45.9% for the proteins recovered at different pH values. Sathivel and co-workers (*36*) reported EAA/AA ratios from 26.2% for herring gonad protein powder to 39.9% for arrowtooth flounder protein powder. However, they did not use ISP to develop the powders, which may account for the lower values compared to the present study.

Total EAAs in the recovered carp proteins were higher ($P < 0.05$) than in whole gutted carp, carp fillets, and surimi (**Table 2**), but lower than in egg protein (*38*), commonly used as a reference protein due to its high nutritional quality. Lysine is often the limiting AA. It is important to note that the proteins recovered from whole gutted carp had a higher content of lysine (80−87 mg/g) than whole egg (70 mg/g). When compared to the biological value (BV) of soybean protein concentrate (*38*) and milk protein, namely, sodium caseinate (*39*), the BV for the proteins recovered from whole gutted carp appears to be higher.

Table 2. Essential Amino Acid (EAA) Content[a] (Milligrams per Gram of Protein, Dry Basis) in the Protein Recovered from Whole Gutted Carp Using Solubilization at Different pH Values and Precipitation at pH 5.5 As Compared to Whole Gutted Carp, Carp Fillets, and Alaska Pollock Surimi

	proteins recovered from whole gutted carp at different treatments (mg/g of protein)								
EAA	pH 2.0	pH 3.0	pH 11.5	pH 12.5	whole gutted carp (mg/g of protein)	carp fillets (mg/g of protein)	Alaska pollock surimi (mg/g of protein)	whole egg (mg/g of protein)	FAO/WHO/ UNU 1985 adults (infants) (mg/g of protein)
isoleucine	40.4 b	39.4 b	44.1 a	44.2 a	21.9 d	31.9 c	31.0 c	63	13 (46)
leucine	70.3 b	69.8 b	75.4 a	78.2 a	36.1 d	53.5 c	53.0 c	88	19 (93)
lysine	80.5 b	79.7 b	85.7 a	87.4 a	42.7 d	62.4 c	62.2 c	70	16 (66)
methionine[b]	32.1 b	32.6 b	36.2 a	37.4 a	18.5 e	26.2 d	27.4 c	56	17 (42)
phenylalanine[c]	64.1 bc	62.7 c	69.8 ab	71.2 a	33.4 f	49.7 d	45.7 e	98	19 (72)
threonine	35.0 c	35.4 c	38.4 b	40.1 a	18.4 e	27.0 d	26.8 d	49	9 (43)
tryptophan	9.1 b	8.7 bc	10.5 a	10.8 a	6.1 e	7.3 de	7.5 cd	16	5 (17)
valine	45.4 b	43.8 b	49.7 a	49.3 a	24.6 d	35.5 c	32.9 c	72	13 (55)
histidine	20.7 b	19.8 b	22.6 a	22.3 a	13.0 c	19.6 b	13.4 c	24	16 (26)
total EAA	359.8 b	354.4 b	389.7 a	396.7 a	196.5 e	284.4 c	270.4 d	536	127 (460)
total non-EAA	438.0 b	435.6 b	459.0 a	469.8 a	279.5 e	350.3 c	331.4 d	NA[d]	NA[d]
ratio EAA/non-EAA	45.1 a	44.9 a	45.9 a	45.8 a	41.3 b	44.8 a	44.9 a	NA[d]	NA[d]

[a] Data are given as mean values ($n = 3$). Mean values in rows with different letters indicate significant differences ($P < 0.05$). Standard deviation (±SD) was omitted to maintain table clarity. The EAA content of whole egg and the recommended values for adults and infants are provided. [b] Methionine + cysteine. [c] Phenylalanine + tyrosine. [d] Not applicable.

As shown in **Table 2**, regardless of pH during protein solubilization, the recovered carp proteins and carp fillets met the recommended intakes for the EAAs for adults. Due to the more stringent recommendations for infants (*38*), none of the carp samples met the EAA recommendations with the exception of lysine in the recovered carp proteins.

According to **Table 3**, the insolubles recovered at basic pH had lower ($P < 0.05$) EAAs and non-EAAs compared to the acidic pH treatments and, consequently, lower EAA/AA. These data indicate that basic solubilization results in higher ($P < 0.05$) concentration of EAAs in the recovered proteins, but less ($P < 0.05$) EAAs in the insolubles when compared to the acidic treatments. The total content of EAAs in the recovered insolubles was lower than in the protein powders reported by Sathivel and co-workers (*36*); however, the EAA/AA for the recovered insolubles was relatively comparable.

On the basis of the results from the present study, ISP, particularly at basic pH, is a useful technique for recovering proteins from whole gutted carp to be used in food products for adults that require a high content of EAAs, whereas the insolubles may potentially be used in animal feeds.

Fatty Acid Profile Analysis. Eicosapentaenoic (EPA, 20:5ω-3), docosahexaenoic (DHA, 22:6ω-3), and linolenic (ALA, 18:3ω-3) acids are the main ω-3 polyunsaturated fatty acids (PUFAs), whereas linoleic (LA, 18:2ω-6) and arachidonic (AA, 20:4ω-6) acids are the main ω-6 PUFAs in fish fillets (*25*). Several studies have indicated that dietary ω-3 PUFAs are beneficial to human health, particularly for the cardiovascular system (*40−42*). However, PUFAs are susceptible to oxidation, which results in rancidity. Therefore, it is desirable to prevent oxidation during the recovery of fish lipids to be used in food products and dietary supplements.

Proximate analysis showed that all of the recovered fractions (i.e., proteins, lipids, and insolubles) contained fat (**Table 1**). **Figure 2** shows that ω-3 PUFAs (ALA, EPA, and DHA) and, consequently, total ω-3 were higher ($P < 0.05$) for the least extreme pH (i.e., pH 3.0 and pH 11.5) than for pH 2.0 and 12.5. In contrast, the less extreme pH treatments resulted in lower ($P < 0.05$) LA (ω-6 PUFA) and total ω-6 in the recovered lipids. It is important to note that although Alaska pollock surimi contains very little fat (**Table 1**), it had higher ($P < 0.05$) percentages of DHA, EPA, and total ω-3 but lower ($P < 0.05$) LA and total ω-6 when compared to carp samples. On the basis of the total ω-3, ω-6, saturated, and unsaturated FAs, it does not appear that ISP affects FA composition of the recovered carp lipids when compared to the starting material and carp fillets.

Okada and Morrissey (*43*) extracted oil from skin-on sardine fillets by adjusting the pH of the mince to the isoelectric point (pH 5.5) followed by centrifugation at 10000g for 20 min.

Table 3. Essential Amino Acid (EAA) Content[a] (Milligrams per Gram, Dry Basis) in the Insolubles (Bones, Skin, Fins, Insoluble Proteins, Membrane Lipids, Etc.) Recovered from Whole Gutted Carp Using Solubilization at Different pH Values and Precipitation at pH 5.5

	insolubles recovered from whole gutted carp at different treatments (mg/g of protein)			
EAA	pH 2.0	pH 3.0	pH 11.5	pH 12.5
isoleucine	23.9 a	19.9 b	8.9 c	9.2 c
leucine	39.2 a	33.9 b	13.9 c	15.5 c
lysine	46.6 a	39.8 b	17.7 c	18.7 c
methionine[b]	18.9 a	17.1 b	10.9 c	10.7 c
phenylalanine[c]	37.8 a	32.8 b	15.3 c	16.1 c
threonine	23.6 a	21.4 b	12.3 c	13.0 c
tryptophan	4.5 a	3.7 b	2.2 c	0.9 d
valine	27.9 a	23.3 b	11.6 c	11.9 c
histidine	14.0 a	11.5 b	4.6 c	5.0 c
total EAA	216.6 a	185.7 b	91.5 c	94.7 c
total non-EAA	390.8 a	346.0 b	299.9 c	298.6 c
ratio EAA/non-EAA	35.7 a	34.9 a	23.4 b	24.1 b

[a] Data are given as mean values ($n = 3$). Mean values in rows with different letters indicate significant differences ($P < 0.05$). Standard deviation (±SD) was omitted to maintain table clarity. [b] Methionine + cysteine. [c] Phenylalanine + tyrosine.

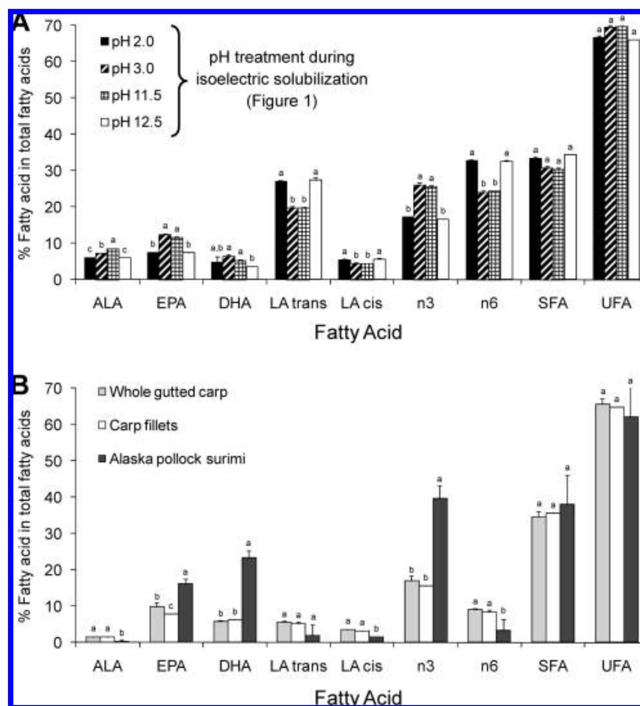

Figure 2. Major fatty acids (percent fatty acid in total fatty acids) in the lipids recovered from whole gutted carp using solubilization at different pH values and precipitation at pH 5.5 (**A**) as compared to whole gutted carp, carp fillets, and Alaska pollock surimi (**B**). Data are given as mean values ± SD ($n = 3$). Different letters on data bars indicate significant differences ($P < 0.05$) between mean values. ALA, linolenic acid (18:3ω-3); EPA, eicosapentaenoic acid (20:5ω-3); DHA, docosahexaenoic acid (22:6ω-3); LA, linoleic acid (18:2ω-6); n3, total omega-3 fatty acids (ω-3 FA); n6, total ω-6 FA; SFA, total saturated FA; UFA, total unsaturated FA.

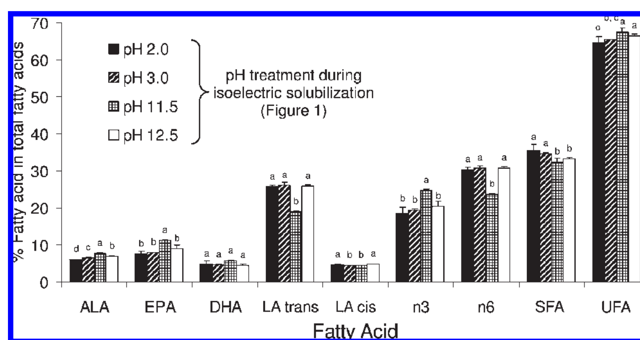

Figure 3. Major fatty acids (percent fatty acid in total fatty acids) in the insolubles (bones, skin, fins, insoluble proteins, membrane lipids, etc.) recovered from whole gutted carp using solubilization at different pH values and precipitation at pH 5.5. Data are given as mean values ± SD ($n = 3$). Different letters on data bars indicate significant differences ($P < 0.05$) between mean values. ALA, linolenic acid (18:3ω-3); EPA, eicosapentaenoic acid (20:5ω-3); DHA, docosahexaenoic acid (22:6ω-3); LA, linoleic acid (18:2ω-6); n3, total omega-3 fatty acids (ω-3 FA); n6, total ω-6 FA; SFA, total saturated FA; UFA, total unsaturated FA.

Similar to the present study, there was no change in ω-3 PUFA levels. However, unlike in the present experiments, Okada and Morrissey (*43*) did not apply a protein solubilization step. Liang and Hultin (*44*, *45*) demonstrated that ISP of cod muscle at basic and acidic pH allows efficient recovery of lipids. However, they did not present the FA composition of the recovered lipids.

The FAP of the recovered insolubles indicates that ω-3 and ω-6 PUFAs are retained in this fraction (**Figure 3**). However, the pH during protein solubilization does not appear to have a significant effect on the FA composition. Although the content of total fat in the recovered proteins is low, EPA, DHA, and total ω-3 were at the highest ($P < 0.05$) percentage for pH 11.5 (**Figure 4**). This indicates that ISP at basic rather than acidic pH may be more beneficial to the nutritional quality of the recovered materials.

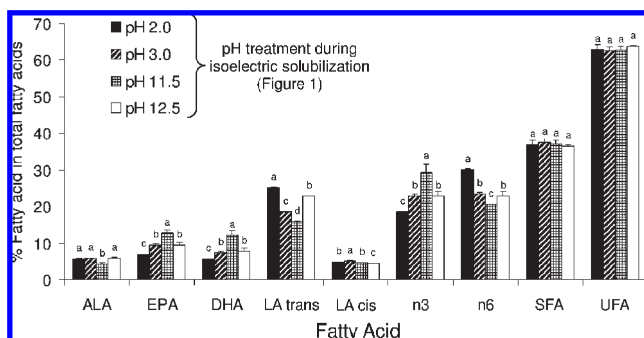

Figure 4. Major fatty acids (percent fatty acid in total fatty acids) in the protein recovered from whole gutted carp using solubilization at different pH values and precipitation at pH 5.5. Data are given as mean values ± SD (n = 3). Different letters on data bars indicate significant differences ($P < 0.05$) between mean values. ALA, linolenic acid (18:3ω-3); EPA, eicosapentaenoic acid (20:5ω-3); DHA, docosahexaenoic acid (22:6ω-3); LA, linoleic acid (18:2ω-6); n3, total omega-3 fatty acids (ω-3 FA); n6, total ω-6 FA; SFA, total saturated FA; UFA, total unsaturated FA.

Mineral Profile Analysis. The major minerals, Ca, P, and Mg, are involved in bone health. The trace mineral, Fe, is the most commonly deficient nutrient worldwide. Inadequate intake of dietary Fe results in anemia. Whole gutted carp contain bones, skin, scales, etc., making them rich in minerals and, hence, a potential good source of these minerals. The proximate analysis showed that ash was concentrated in the recovered insolubles, whereas recovered proteins had low ash content (**Table 1**).

The Ca and P contents of the recovered insolubles exceeded recommended dietary allowances (RDA); however, Fe and Mg were below the RDA (*46*, *47*). These minerals were under the RDA in the recovered carp proteins as well as whole gutted carp, carp fillets, and surimi. The lower content of Fe and Mg in the starting material (i.e., whole gutted carp) explains the Fe and Mg content being below the RDA in the recovered proteins (**Figure 5**) and insolubles (**Figure 6**). **Figure 5** shows that regardless of the pH treatments during protein solubilization, Mg, Ca, and P contents in the recovered proteins were greatly reduced ($P < 0.05$) when compared to the starting material. These minerals were likely removed from the proteins due to the centrifugation following protein solubilization (**Figure 1**) as indicated by the low ash content in the recovered proteins (**Table 1**).

The effect of pH during protein solubilization on the content of Ca, Mg, P, and Fe in the recovered proteins and insolubles was also investigated. The acidic treatments yielded higher ($P < 0.05$) contents of Mg, Ca, and P in the recovered proteins

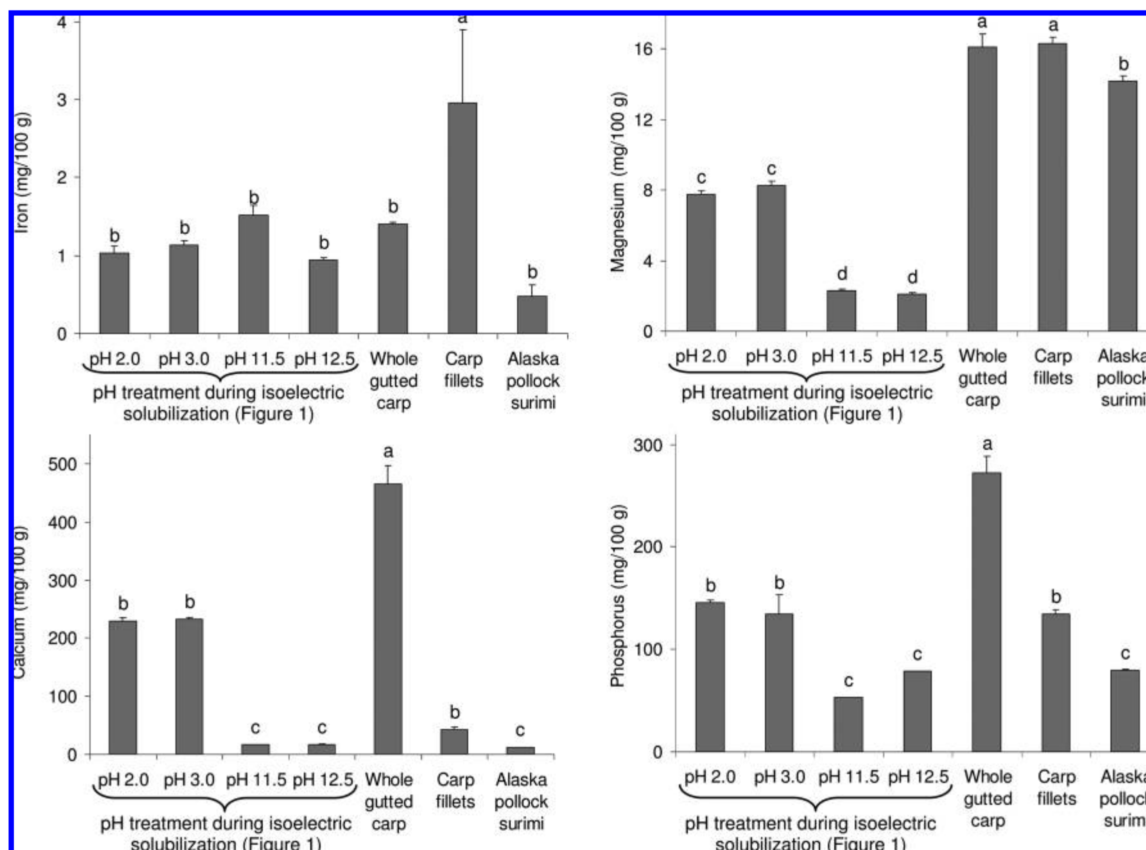

Figure 5. Fe, Mg, Ca, and P contents (milligrams per 100 g, dry basis) in the protein recovered from whole gutted carp using solubilization at different pH values and precipitation at pH 5.5 as compared to whole gutted carp, carp fillets, and Alaska pollock surimi. Data are given as mean values ± SD (n = 3). Different letters on data bars indicate significant differences ($P < 0.05$) between mean values.

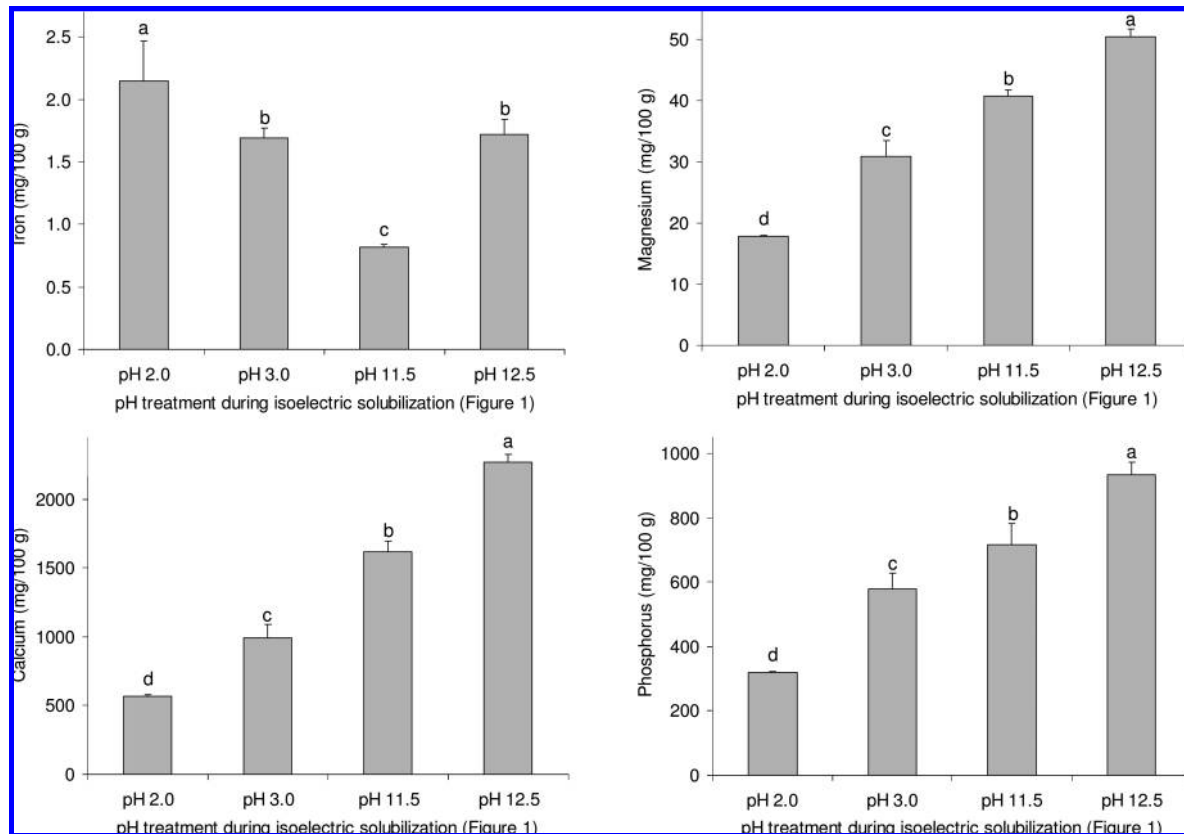

Figure 6. Fe, Mg, Ca, and P contents (milligrams per 100 g, dry basis) in the insolubles (bones, skin, fins, insoluble proteins, membrane lipids, etc.) recovered from whole gutted carp using solubilization at different pH values and precipitation at pH 5.5. Data are given as mean values ± SD ($n = 3$). Different letters on data bars indicate significant differences ($P < 0.05$) between mean values.

(**Figure 5**). In the insolubles, Mg, Ca, and P were more ($P < 0.05$) abundant when basic rather than acidic treatments were applied (**Figure 6**). Therefore, these results demonstrate that the basic treatments of whole gutted carp facilitated recovery of proteins with less of the insolubles (bones, skin, scales, fins, etc.) than the acidic treatments. The data in the present study are in accordance with a krill study by Anderson and co-workers (*30*), who showed that basic treatments resulted in greater demineralization when compared to the acidic treatments. Sathivel and Bechtel (*48*) determined the mineral content of freeze-dried protein powders recovered from Alaska pollock byproduct by heating at 85 °C for 60 min and centrifugation at 2560*g*. The Fe, Mg, Ca, and P contents of the powders were 110, 110, 40, and 1060 mg/100 g, respectively. The Fe, Mg, and P contents in the proteins recovered from whole gutted carp were much lower than those reported by Sathivel and Bechtel (*48*) (**Figure 5**). As opposed to the present study, Sathivel and Bechtel (*48*) did not use ISP for protein recovery. Therefore, the differences are likely due to the pH treatments, the much higher *g* force during separation used in the present experiments, and different starting materials.

ISP, particularly the basic treatments, effectively removed minerals from the recovered proteins even without removal of the bones, skin, scales, fins, etc., prior to processing. Major minerals in whole gutted carp were concentrated in the insolubles recovered with basic treatments. The insolubles recovered with ISP may potentially be used as a mineral supplement in human and/or animal diet. The Ca, Mg, and P contents in the insolubles were of particular interest given their role in maintaining bone health and prevention of osteoporosis.

Torres and co-workers (*49*) proposed a continuous bioreactor system for the recovery of muscle proteins and lipids from fish processing byproduct and whole fish. The flow rate in the bioreactor system was controlled to allow solubilization and precipitation reactions for 10 min. The bioreactors were equipped with built-in pumps for various processing additives such as antifoams, coagulants, and flocculants. The flocculants enabled more efficient separation of the precipitated fish muscle proteins from the process water (*18*) and, consequently, could facilitate process scale-up from laboratory to pilot scale. In addition to the possibility of a higher protein recovery yield due to flocculants, the bioreactor system allowed separation of insolubles (bones, skin, scales, etc.) as well as a continuous mode of processing, which facilitated water reusage (*49*). The bioreactor system could likely be applied to pilot- and commercial-scale processing of whole gutter silver carp.

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