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Protein Recovery from Rainbow Trout (*Oncorhynchus mykiss*) Processing Byproducts via Isoelectric Solubilization/Precipitation and Its Gelation Properties As Affected by Functional Additives

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Solubility of rainbow trout proteins was determined between pH 1.5 and 13.0 and various ionic strengths (IS). Minimum solubility occurred at pH 5.5; however, when IS = 0.2, the minimum solubility shifted toward more acidic pH. Isoelectric solubilization/precipitation was applied to trout processing byproducts (fish meat left over on bones, head, skin, etc.), resulting in protein recovery yields (Kjeldahl, dry basis) between 77.7% and 89.0%, depending of the pH used for solubilization and precipitation. The recovered protein contained 1.4–2.1% ash (dry basis), while the trout processing byproducts (i.e., starting material) 13.9%. Typical boneless and skinless trout fillets contain 5.5% ash, and therefore, the isoelectric solubilization/precipitation effectively removed impurities such as bones, scales, skin, etc., from the trout processing byproducts. The recovered proteins retained gel-forming ability as assessed with dynamic rheology, torsion test, and texture profile analysis (TPA). However, the recovered proteins failed to gel unless beef plasma protein (BPP) was added. Even with BPP, the recovered protein showed some proteolysis between 40 and 55 °C. Addition of potato starch, transglutaminase, and phosphate to the recovered proteins resulted in good texture of trout gels as confirmed by torsion test and TPA. Higher ($P < 0.05$) shear stress and strain were measured for gels developed from basic pH treatments than the acidic counterparts. However, proteins recovered from acidic treatments had higher ($P < 0.05$) lipid content than the basic treatments. This is probably why the gels from acidic treatments were whiter ($L^* - 3b^*$) ($P < 0.05$) than those from the basic ones. Our study demonstrates that functional proteins can be efficiently recovered from low-value fish processing byproducts using isoelectric solubilization/precipitation and subsequently be used in value-added human foods.

KEYWORDS: Trout proteins; isoelectric solubilization/precipitation; viscoelastic modulus; beef plasma protein; transglutaminase; texture and color properties

INTRODUCTION

Accordingto the U.S. Department of Agriculture's report, the total sale value of rainbow trout (*Oncorhynchus mykiss*) in the U.S. exceeded 68 million dollars in 2004, which was a 7% increase from 2003 (*1*). Trout farming is one of oldest forms of commercial fish production in the United States. Filleting fish for human consumption often requires removal of viscera, head, bones, skin, and fins. Commercial processing employs various mechanical means to remove these byproducts in order to recover marketable fish fillets. Mechanical filleting of 100 kg of rainbow trout typically recovers 40 kg of boneless skinless fillets, leaving 60 kg of the processing byproducts. The processing byproducts contain ~20 kg of fish meat and various amounts of fish oil (*2*). However, the fish meat is attached to bones, skin, head, etc., making it very difficult to recover with mechanical processing. Similar to trout processing, commercial filleting of fish such as cod, salmon, tilapia, seabream, pollack, etc., typically yields 60–70% of the byproducts and 30–40% of fillets (*2*). The fish processing byproducts are typically reduced to compost and animal feed or are landfilled. Overfishing, depletion of fish stocks, and environmental pollution associated with seafood processing have been often publicized. While it is not uncommon to just "grind and discard" the 60–70% of fish byproducts, this practice should be considered an ir-

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responsible utilization of natural resources, and the 60–70% should be used instead to fulfill human nutritional needs (*2*). Significant values could be added if protein and lipids were recovered from the fish processing byproducts for subsequent use in human food products. A food deficiency due to the blooming human population on earth is another ongoing problem. Therefore, it is desirable to develop a technology that would allow efficient recovery of food proteins from fish processing byproducts in order to meet human nutritional needs and reduce environmental stress associated with seafood processing.

Generally, rainbow trout contains 73–75% moisture, 19–20% protein, 1–4% lipids, and 1–1.5% ash (*3, 4*). Similar to other proteins of animal origin, the protein derived from trout processing byproducts is a complete protein as indicated by the presence of all nine essential amino acids (EAA) required by adults (*2*). Hydrolytic enzymes normally contained in the fish gastrointestinal track are released during trout processing, which may lead to autolysis of muscle protein and a loss of protein basic functional property gelation. The autolysis of fish proteins is a major hindrance to further develop new food products for human consumption. Therefore, these enzymes bring significant challenges to recover functional proteins from trout processing byproducts. Beef plasma protein (BPP) inhibits proteolytic activity and, therefore, reduces texture softening due to proteolysis in fish protein-based gels (*5–7*). Transglutaminase (TGase) is an enzyme-catalyzing acyl-transfer reaction, producing non-disulfide covalent ε-(γ-glutamyl)lysine cross-links (*8*). In addition, TGase naturally occurs in fish muscle (i.e., endogenous TGase) that is responsible for the "setting" phenomenon often referred to as "suwari", which results in more elastic gel (*9–11*). The protease inhibitors and exogenous TGase are often used as functional additives in restructured value-added food products and could likely be applied in food products developed from proteins derived from trout processing byproducts.

The growth of the aquaculture industry necessitates development of technologies that recover muscle proteins and lipids from the fish processing byproducts. The surimi technology could be a good alternative for recovery of functional proteins; however, surimi processing requires excessively large volumes of water and cannot be applied to recover muscle proteins from fish processing byproducts. An isoelectric solubilization/precipitation of proteins has been relatively recently applied to fish muscle, which resulted in greatly improved recovery yield compared to surimi processing (*12*). The proteins recovered by this novel approach also retained gel-forming ability (*13–15*). Therefore, the isoelectric solubilization/precipitation could likely be applied to efficiently recover functional (i.e., gelation) muscle proteins from trout processing byproducts for subsequent development of human food products with the aid of food additives such as BPP and exogenous TGase. Value-added restructured food products developed from the processing byproducts would not only enable the fish processing industry to diversify its product offerings but also offer another source of highly nutritious proteins for human consumption and alleviate environmental stresses due to seafood processing.

The objectives of this study were to (1) determine solubility of trout muscle proteins as affected by pH and ionic strength (IS), (2) determine feasibility of the isoelectric solubilization/precipitation to recover proteins from trout processing byproducts, (3) investigate the effects of functional additives such as beef plasma protein (BBP), potato starch (PS), exogenous transglutaminase (TGase), and polyphosphate (PP) on gelation of muscle proteins recovered from trout processing byproducts, and (4) compare the textural and color properties of the gels developed from the recovered proteins solubilized at different pH values.

MATERIALS AND METHODS

Sample Preparation and Determination of Solubility of Trout Proteins As Affected by pH and Ionic Strength (IS). Boneless and skinless, fresh rainbow trout (*Oncorhynchus mykiss*) fillets were minced in a laboratory blender (model 51BL31, Waring Commercial, Torrington, CT). The mince was homogenized with cold (1–3 °C) distilled and deionized water (ddH_2O) at 1:9 ratio (mince:water, wt:vol) using a laboratory homogenizer (PowerGen 700, Fisher Scientific, Fairlawn, NJ) set at speed three for 10 min. Temperature was carefully controlled and did not exceed 4 °C. The homogenate was centrifuged at 10000*g* and 4 °C for 10 min using a laboratory batch centrifuge (Sorvall RC-5B Refrigerated Superspeed, Kendro Laboratory Products, Newtown, CT). The centrifugation resulted in three phases: top phase, trout lipids; middle phase, water-soluble trout muscle protein; bottom phase, water-insoluble trout washed muscle. The middle phase was used to determine solubility of water-soluble trout protein, while the bottom phase was washed once more as described above and then used to determine solubility of water-insoluble trout washed muscle.

In separate beakers, 90 mL aliquots of the water-soluble protein were used, while 2 g of the washed muscle was mixed with 90 mL of cold ddH_2O followed by a pH adjustment between 1.50 and 13.00 $\pm$ 0.05 with 0.50 increments using 1 N cold NaOH or HCl. Final volume was adjusted to 100 mL. Once the desired pH was obtained, the water-soluble protein and washed muscle mixtures were continuously stirred for 10 min and centrifuged as above.

The solubility of water-soluble protein at each pH was calculated as a ratio of protein concentration in the supernatant following centrifugation to the protein concentration in the mixture prior to the pH adjustment and is reported as a percent of water-soluble protein (*16*). The solubility of water-insoluble washed muscle was determined as a protein concentration in the supernatant following centrifugation and is reported as g of soluble washed muscle/L (*17*). The Bradford dye-binding method was used to determine protein concentration (Bio-Rad protein assay kit, Bio-Rad Laboratories, Hercules, CA), and absorbance was measured at 595 nm using a UV/vis spectrophotometer (model DU530, Beckman Instruments, Fullerton, CA). The protein concentration was calculated by comparing the absorbance to a standard curve constructed with bovine serum albumin. Three separate experiments were performed ($n = 3$). All samples were in duplicates and mean values for protein concentration are reported.

Ionic strength (IS) plays an important role in fish protein solubility (*16*). Myofibrillar proteins (primarily myosin and actin) are the major component of fish muscle. These proteins are largely water-insoluble at the fish physiological IS ($\sim$0.05 for rainbow trout), but they are water-soluble at extremely low IS (*16*) or above 0.5 (*18*). Another major component of fish muscle, sarcoplasmic proteins, are mainly water-soluble. However, their water solubility decreases with an increase of IS (*19*). At the same time, the addition of acid and base during isoelectric solubilization/precipitation alters the IS. Therefore, in one set of the solubility experiments of the washed trout muscle at various pH (i.e., 1.50–13.00 $\pm$ 0.05 with 0.50 increments), the IS was monitored but not adjusted. The highest IS of nearly 0.2 was recorded for pH = 1.50. Therefore, the second set of solubility experiments of the same washed muscle at the same series of pH treatments was conducted, but the IS was adjusted to 0.2 with NaCl. The solubility experiments of the water-soluble trout muscle protein were conducted without the IS adjustment except for when the pH = 5.50 $\pm$ 0.05 (i.e., the isoelectric point of fish muscle). When the pH of the water-soluble trout muscle protein solution was adjusted to 5.50, the IS was modified between 0.01 and 2.91 with NaCl, followed by determination of protein solubility as described above. The IS was defined as an equivalent molar (M) concentration of NaCl, and the IS was determined by comparing the conductivity of a protein sample to a standard curve constructed with NaCl. The IS is reported as an equivalent % NaCl. The conductivity

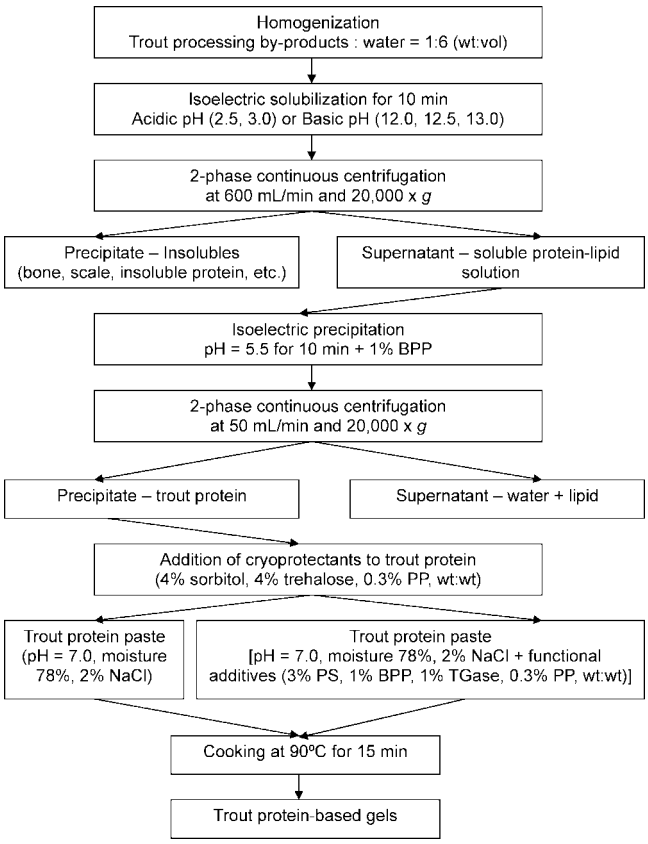

Figure 1. Flowchart for development of protein gels from trout processing byproducts.

of a protein sample was measured with a conductivity meter (AB30, Fisher Scientific, Pittsburgh, PA) that was properly calibrated prior to the experiments.

Raw Material for Protein Recovery. Farm-raised rainbow trout were mechanically filleted in a commercial processing facility (High Appalachian, Sophia, WV). The initial steps of filleting involve removing the head and viscera followed by mechanical recovery of the boneless fillets. The remaining material (hereinafter called the byproducts) contained bones with leftover fish meat, skin, scales, and fins. The byproducts were transported to our food science laboratory at West Virginia University in heavily insulated industrial strength boxes filled with ice. Upon arrival, the byproducts were rinsed with cold tap water, vacuum-packed, and frozen at −80 °C until used in our experiments. Based on AOAC methods (*20*), the average proximate composition of the trout processing byproducts was 71.3% moisture, 71.5% crude protein (dry basis), 15.2% total lipid (dry basis), and 13.9% ash (dry basis).

Protein Recovery from Trout Processing Byproducts. A flowchart describing recovery of muscle proteins from trout processing byproducts is shown in **Figure 1**. Partially frozen trout processing byproducts were homogenized with cold (4 °C) tap water at 1:6 ratio (byproducts:water, wt:vol) in a pilot-scale continuous meat homogenizer (Stephan Microcut MCH-10, Stephan GmbH & Co., Hameln, Germany) equipped with a recirculating flow and cutting ring that reduced particle size to below 0.2 mm. A 1500 mL of the homogenate was transferred to a beaker that was placed in ice slush. During the entire process to recover trout muscle proteins, temperature was carefully controlled at 1–4 °C to reduce the activity of trout endogenous proteases. In order to additionally minimize the proteolysis, the processing time did not exceed 60 min. The homogenization/mixing was continued with a laboratory homogenizer (PowerGen 700, Fisher Scientific, Fairlawn, NJ) set at speed three during subsequent pH adjustment steps.

The pH of the homogenates was separately adjusted to 2.50 and 3.00 ± 0.05 with 10 and 1 N HCl as well as 12.00, 12.50, and 13.00 ± 0.05 with 10 and 1 N NaOH in order to isoelectrically solubilize trout proteins at acidic and basic pH ranges, respectively. The 10 and 1 N reagents were used for crude and fine pH adjustments, respectively, during both protein solubilization and subsequent precipitation (pH = 5.5) (see below). The pH meter (model AB15, Fisher Scientific, Fair Lawn, NJ) was properly calibrated prior to the pH adjustments. Once the desired pH was obtained, the solubilization reaction was allowed to take place for 10 min, followed by centrifugation at 20000*g* and a flow rate of 600 mL/min using a continuous, temperature-controlled (4 °C) centrifuge system equipped with vacuum pump designed to reduce frictional heat and containment of aerosols and foam in the separating bowl (Powerfuge Pilot Separation System CSMA-22478, Kendro Laboratory Products, Newtown, CT). Our preliminary experiments (data not shown) showed that the flow rate of 600 mL/min resulted in excellent separation of the insolubles (bone, skin, fin, insoluble protein, etc.) from the supernatant. Protein content in the supernatant was analyzed using the Bradford dye-binding method. Lipids were not separated because the centrifuge system allowed liquid/solid two-phase separation.

The supernatant was collected and the pH was adjusted to 5.50 ± 0.05 by either 10 and 1 N HCl or 10 and 1 N NaOH in order to isoelectrically precipitate trout proteins. Once the desired pH was obtained, spray-dried beef plasma protein (BPP) (Proliant Inc., Ankeny, IA) was added at 1% (wt:wt) of trout proteins and the precipitation reaction was allowed for 10 min followed by centrifugation (same as above). However, preliminary experiments (data not shown) showed that the best separation of the precipitated protein pellet from water–lipid solution was achieved at a flow rate of 50 mL/min.

The proteins recovered from trout processing byproducts were mixed with a cryoprotectant mixture [4% D-sorbitol (Sigma Aldrich Inc., St. Louis, MO), 4% crystalline dihydrate trehalose (Cargill Inc., Wayzata, MN), 0.3% phosphate (PP) (Kena FP-28, Innophos, Cranbury, NJ); wt:wt] in a temperature-controlled food processor (Sunbeam Mixmaster model 2350, Sunbeam Products Inc., Boca Raton, FL) followed by a 1-day refrigerated storage (2 °C). The pH of the recovered proteins was adjusted to 7.00 ± 0.05 with 1 N NaOH. Trout proteins prepared in this manner were used the next day to develop protein gels as well as to determine moisture content, total fat, crude protein, and ash content.

Proximate Analyses of Protein Recovered from Trout Processing Byproducts. Two grams of the recovered trout proteins was placed on an aluminum dish (Fisher Scientific Co., Fairlawn, NJ) and spread evenly across the dish. The moisture content of trout proteins was determined by the oven-drying method (105 °C for 24 h) (*20*). Total fat content in was determined according to the Soxhlet extraction method (*20*). The sample size was 5 g, and extraction with petroleum ether was performed for 16 h at a drip rate of 10 mL/min. Total fat content was determined on a gravimetric basis and expressed as percent (dry weight basis). Crude protein was determined by Kjeldahl assay (*20*) and expressed as percent (dry weight basis). Ash content was performed by incinerating a sample in a muffle furnace at 550 °C for 24 h (*20*) and expressed as percent (dry weight basis). All proximate analyses are reported as the mean value of at least three replicates.

Development of Trout Protein-Based Gels. The recovered trout proteins were divided into two parts (i.e., experimental treatments): (1) addition of 2% (wt:wt) NaCl only; (2) addition of 2% (wt:wt) NaCl and functional additives [1% BPP (wt:wt), 1% exogenous transglutaminase (TGase) (wt:wt) (Avtiva RM, Ajinomoto USA Inc., Teaneck, NJ), 3% potato starch (PS) (wt:wt) (Penbind 1000 modified potato startch, Penford Food Ingredients Corp., Centennial, CO), and 0.3% PP (wt:wt)].

The trout protein-based gels for both experimental treatments (i.e., with and without functional additives) were developed separately by using a procedure as described by Chen and Jaczynski (*21*). The recovered trout proteins mixed with cryoprotectants were chopped in a universal food processor (model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. Salt (2%, wt:wt) was added, followed by chopping at low speed for 0.5 min, which resulted in a trout protein paste. The functional additives were added only to one experimental treatment group, while the functional additives were not added to the other group. Final moisture content was adjusted to 78% by adding ice to the paste, followed by chopping at low speed for 1 min. Additional chopping was performed at high speed under vacuum (0.5 bar) for the last 3 min. Final pH 7.00 ± 0.05 of the trout protein

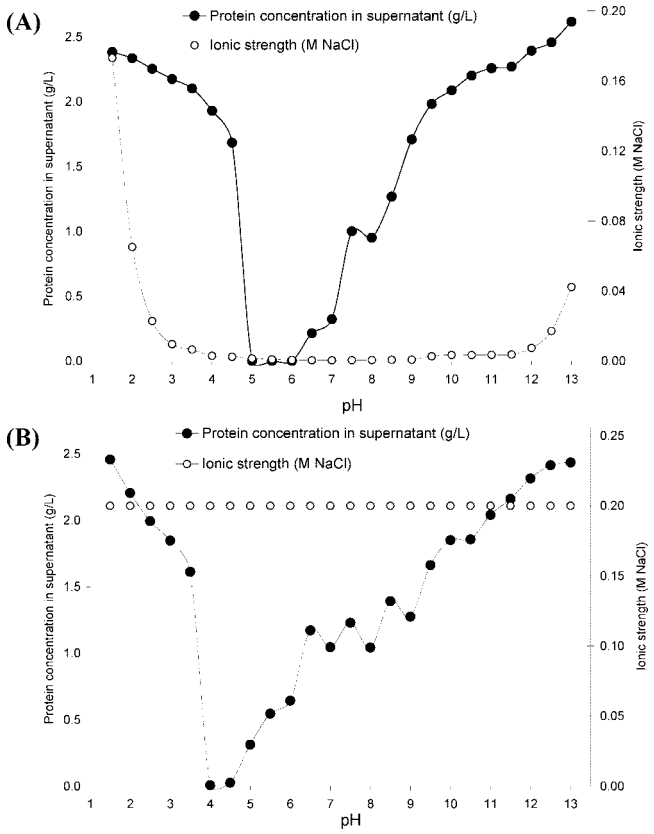

Figure 2. Protein solubility of trout washed muscle as affected by pH and ionic strength (IS): (**A**) the IS was not adjusted, but monitored, as the pH was altered during isoelectric solubilization; (**B**) the IS was adjusted to 0.20 with NaCl. The standard deviation bars were omitted in the figures to maintain clarity.

paste was verified with a pH meter and adjusted as needed. During chopping, the paste temperature was controlled between 1 and 4 °C.

Following chopping, trout protein paste was stuffed into (1) stainless steel tubes (length = 17.5 cm, inner diameter = 1.9 cm) for determination of color and texture properties with tristimulus color values ($L^*a^*b^*$) and texture profile analysis (TPA), respectively, and (2) dumbbell premolded stainless steel torsion tubes (length = 17.5 cm, end diameter = 1.9 cm, midsection diameter = 1.0 cm) for determination of texture properties with torsion test. The tubes were heated in a water bath set at 90 °C for 15 min. However, tubes that contained trout protein paste with the functional additives (i.e., exogenous TGase) were refrigerated (4 °C) for 24 h prior to heating in order to allow development of non-disulfide covalent bonds. Careful attention was paid to the torsion gel samples to avoid "skin" formation on the joints of torsion tubes. Gel samples without the "skin" were only used in the torsion test. Following heating, tubes were chilled in ice slush, and the gel samples were stored at 5 °C for 24 h.

Dynamic Rheology. The trout protein paste was tested to determine gel-forming ability using oscillatory rheology with a Bohlin rheometer (Bohlin CVOR 200, Malvern Instruments Ltd., Worcestershire, U.K.). At least three samples of trout protein paste per treatment (with the functional additives and without the additives) were used to determine storage modulus (G') as a function of temperature. An oscillation measurement was conducted at 1% strain and 0.1 Hz frequency with temperature increasing from 5 to 100 °C at the rate of 1 °C/min (*13*, *14*, *21*).

Texture and Color Properties of Trout Protein-Based Gels. The recovered trout proteins without the functional additives failed to gel; therefore, the texture and color properties were not determined. Two different methods were employed to determine texture, torsion test, and texture profile analysis (TPA). Although these two texture measurements are commonly employed for determination of texture properties, each method provides slightly different information. Torsion test is considered a fundamental test for texture, while TPA is an empirical test. Likely, the most comprehensive understanding of texture properties is provided by a combination of the fundamental and empirical tests. Therefore, we employed these two different tests in our experiments.

Torsion test of cooked trout protein-based gels with the functional additives was performed according to Jaczynski and Park (*22*). Surimi gels were equilibrated to room temperature for 2 h prior to the measurement. At least six dumbbell cylindrical gels (length = 2.87 cm, end diameter = 1.90 cm, and midsection diameter = 1.0 cm) per treatment were glued to plastic discs and subjected to torsional shear using a Hamman gelometer (Gel Consultant, Raleigh, NC) set at 2.5 rpm. Shear stress and shear strain at mechanical fracture were measured to determine gel strength and gel cohesiveness, respectively.

Texture profile analysis (TPA) of cooked trout protein-based gels was performed according to Cheret and co-workers (*23*). The gel samples at room temperature were subjected to two-cycle compression at 50% using the texture analyzer (Model TA-HDi, Texture Technologies Corp., Scarsdale, NY) with a 70 mm TPA compression plate attachment moving at a speed of 127 mm/min. From the resulting force–time curves, hardness, springiness, cohesiveness, gumminess, chewiness, and resilience were determined. The definitions of the TPA parameters are as follows: (1) hardness indicates the maximum force required to compress a sample; (2) springiness indicates the ability of a sample to recover its original form after the deforming force is removed; (3) cohesiveness corresponds to the extent to which the sample can be deformed before rupture; (4) gumminess is the force required to disintegrate a semisolid sample to a steady state of swallowing (hardness × cohesiveness); (5) chewiness is related to the work needed to chew a solid sample to a steady state of swallowing (springiness × gumminess); (6) resilience indicates how well a sample resists to regain its original position. At least 15 cylindrical gels (length = 2.54 cm, diameter = 1.90 cm) per treatment were used for the TPA measurement.

The color properties of cooked trout protein-based gels were determined using a Minolta Chroma Meter CR-400 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan). A CIE color system using $L^*a^*b^*$ tristimulus color values was determined. Whiteness of gels was calculated using $L^* - 3b^*$ (*24*). At least 20 cylindrical gels (length = 2.54 cm, diameter = 1.90 cm) per treatment were used for color measurement.

Determination of Protein Recovery Yield from Trout Processing Byproducts. The homogenization, protein solubilization, and precipitation steps were performed in the same manner as described above in "Protein Recovery from Trout Processing Byproducts" except the two centrifugation steps. Following solubilization and precipitation, the solution was poured into six 250 mL tubes and centrifuged at 10000*g* and 4 °C for 10 min (Sorvall RC-5B Refrigerated Superspeed, Kendro Laboratory Products, Newtown, CT).

The pH of the homogenates was separately adjusted to 2.50 and 3.00 ± 0.05 as well as 12.00, 12.50, and 13.00 ± 0.05. The solubilized proteins were precipitated at 5.00, 5.50, and 6.00 ± 0.05. The recovery yield was calculated by determining the weight of the recovered trout protein (Kjeldahl, dry basis) as a percentage of the total protein weight (Kjeldahl, dry basis) in the raw material used (i.e., trout processing byproducts) (*25*). The protein recovery yield was calculated by the following equation:

$$\text{protein recovery yield (\%)} = \frac{\text{wt of recovered trout protein (g) (Kjeldahl, dry basis)}}{\text{wt of protein in raw material (g) (Kjeldahl, dry basis)}} \times 100 \quad (1)$$

Statistical Analysis. The experiment was conducted using a completely random design (CRD) (*26*). Data were analyzed using analysis of variance (ANOVA) (*27*). A significant difference was used at 0.05 probability level, and differences between treatments were tested using the least significant difference (LSD) test (*28*). All statistical analyses of data were performed using SAS (*27*).

RESULTS AND DISCUSSION

Solubility of Trout Protein As Affected by pH and Ionic Strength (IS). The solubility profile as a function of pH for

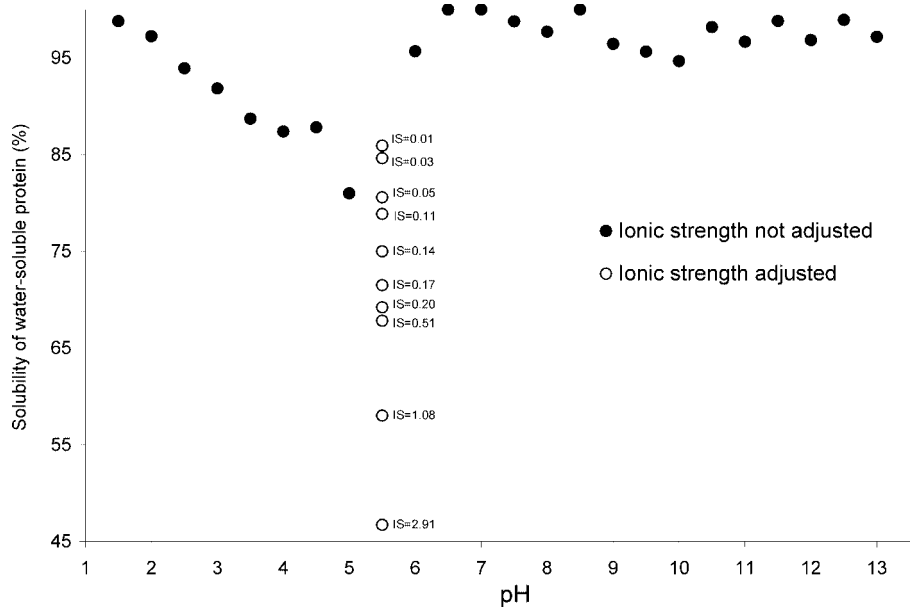

Figure 3. Protein solubility of trout water-soluble proteins as affected by pH (filled circles) and ionic strength (IS). The IS was only adjusted at pH = 5.50 (open circles). The numbers next to each data point indicate IS at pH = 5.50. The standard deviation bars were omitted in the figure to maintain clarity.

the water-insoluble proteins in trout washed muscle exhibited a characteristic parabolic shape with a minimum solubility (or maximum precipitation) between pH 5.0–6.0 (**Figure 2A**). The protein solubility increased dramatically below pH 5.0 and more gradually above pH 6.0. Therefore, the muscle proteins contained in the trout processing byproducts can be selectively solubilized at acidic or basic pH, while the insolubles such as bones, scales, fins, skin, insoluble proteins, etc., can be separated from the solubilized protein solution via for example centrifugation. Following the separation of insolubles, the solubilized proteins can be selectively precipitated at its minimum solubility (pH 5.0–6.0) followed by their separation from water. Stefansson and Hultin (*16*) showed very similar parabolically shaped solubility curve for myofibrillar proteins from cod muscle between pH 4.0 and 8.0 with a minimum solubility at pH 5.5. Okada and Morrissey (*29*) determined solubility of sardine muscle protein between pH 5.0 and 5.9. They pinpointed the isoelectric point (pI) at pH 5.5. Okada and Morrissey (*29*) also demonstrated that the solubility increased much faster at acidic than basic pH range. Meinke and co-workers (*30*) and Meinke and Mattil (*31*) also demonstrated similar solubility profiles for muscle proteins of carp, golden croaker, and mullet. Solubility profiles determined in our research for protein in washed muscle isolated from rainbow trout are in accordance with the current literature.

In addition to pH, the IS also affects protein solubility (*16*). As the strong acid or base such as HCl or NaOH dissociates in a solution, the conductivity, and hence ionic strength (IS), increases accordingly. Therefore, the IS was monitored, and it is shown in **Figure 2A**. The IS started increasing above pH 11.5 and below pH 4.0 and reached maximum of 0.17 at pH 2.0. Therefore, we also conducted solubility experiments for washed muscle with the adjustment of IS to 0.2 using NaCl (**Figure 2B**). When the IS was adjusted to 0.2, the minimum of protein solubility was shifted toward more acidic pH and occurred at pH 4.0–4.5 (**Figure 2B**). However, the general parabolic shape of the solubility curve was unaltered, and similarly to solubility without IS adjustment (**Figure 2A**), the solubility increased rapidly below pH 4.0 and more steadily above pH 4.5. Thawornchinsombut and Park (*32*) determined solubility of Pacific whiting muscle protein between pH 2.0 and 12.0 and IS of 0.6 and 0.01. The minimum solubility was determined at pH 5.5 and 3.5 when the IS was adjusted to 0.01 and 0.6, respectively. Thawornchinsombut and Park (*32*) attributed this shift in minimum solubility at increased IS to protein aggregation as confirmed by SDS-PAGE data. Aluko and Yada (*33*) determined solubility of cowpea protein as a surface response function of pH between 3.0 and 8.0 and IS between 0.5 and 2.0. They observed a decline of protein solubility when the IS was increased using NaCl at all of the pH tested. The decline was attributed to the increased protein–protein interactions (i.e., aggregation) due to decreased electrostatic repulsions as a result of Cl^- binding with positively charged protein at acidic pH and consequently leading to the masking of the protein charges. In our experiments the IS was 0.2. However, our data show a similar trend and confirm the shift of minimum solubility for trout protein toward more acidic pH when the IS was increased. The shift of pH at which the minimum solubility occurred has significant implications. It would be desirable to recover proteins using isoelectric solubilization/precipitation of fish processing byproducts in a continuous mode instead of batch. Besides several advantages of a continuous system over a batch mode, water recycling and high recovery yields seem the most obvious. However, as acid and base are added during isoelectric solubilization/precipitation and the processing water is recycled in a continuous system, a salt buildup would likely occur and result in increased IS. The increased IS would need to be considered in order to account for the shift of protein minimum solubility toward more acidic pH and not at pH = 5.5.

The water-soluble proteins isolated from trout muscle also exhibited a similar parabolic shape of the protein solubility profile (**Figure 3**) when compared to the solubility profile of the washed muscle (**Figure 2A**,**B**). However, the minimum solubility without IS adjustment was not as profound as for proteins in the washed muscle (**Figure 2A**,**B**) and approached 80% solubility at pH 5.0–5.5. Water-insoluble proteins (mainly myofibrillar) constitute a vast majority of the total proteins in fish muscle, and the water-soluble proteins (mainly sarcoplasmic) contribute much less (*18*). The minimum solubility for

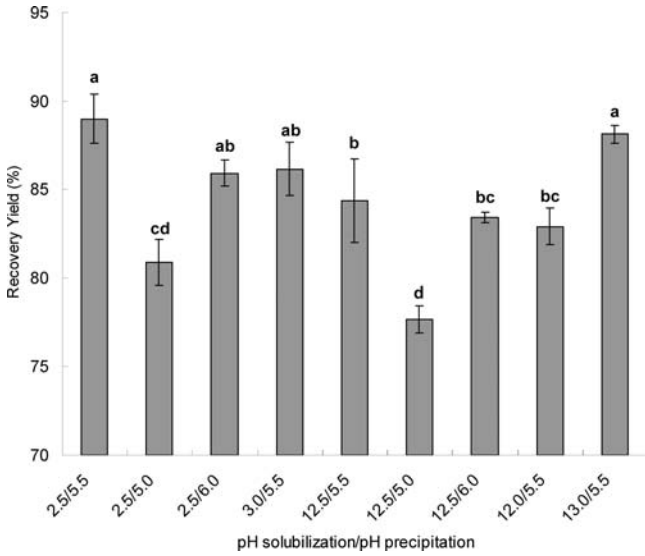

Figure 4. Protein recovery yields (% dry basis, Kjeldahl) from trout processing byproducts at different pH during isoelectric solubilization/precipitation. Small bars on the data bars indicate standard deviation. [a]Different letters on the top of data bars indicate significant differences (least-squares difference test, $P < 0.05$, $n = 3$) between mean values.

water-insoluble proteins from trout washed muscle was determined in our experiments at pH = 5.50, and therefore, this pH results in highest removal of the majority of muscle proteins due to their pH-induced precipitation. This is why we conducted solubility experiments of the water-soluble proteins with IS adjustment between 0.01 and 2.91 only at pH = 5.50 (**Figure 3**). If the water-soluble proteins are not removed, they likely contribute to high biological oxygen demand (BOD) of the processing water that is commonly discarded. Thereby, the water-soluble proteins are often responsible for environmental issues associated with seafood processing. In addition, the sarcoplasmic proteins appear to contribute better gel texture (*19*), although literature shows some controversy (*18*). It has also been shown that the recovery yield of the isoelectric solubilization/precipitation results in higher protein recovery than conventional surimi processing due, in part, to higher removal of the sarcoplasmic proteins with the former technique (*14, 15*). It is, therefore, desirable to recover water-soluble proteins. Although the fish sarcoplasmic proteins are generally considered water-soluble (*18*), Kim and co-workers (*19*) demonstrated that the solubility of sarcoplasmic proteins isolated from rockfish (*Sebastes flavidus*) decreases with increasing the IS. While at 0% addition of NaCl the solubility of sarcoplasmic proteins was very slightly lowered at pH 5.0–6.0, the addition of 0.1 and 0.5 M NaCl significantly decreased solubility of these proteins, especially at acidic pH (*19*). Our solubility data are very similar and confirm that solubility of water-soluble proteins isolated from trout decreases with increasing IS.

Protein Recovery Yield from Trout Processing Byproducts. Our protein solubility data show that when muscle proteins were isolated from rainbow trout fillets and solubilized by acidic or basic pH treatments followed by isoelectric precipitation, most proteins could be recovered (**Figures 2** and **3**). This is in accordance with published research (*14, 15*). Therefore, we applied nine different pH combinations to determine recovery yields of proteins isolated from trout processing byproducts (**Figure 4**). The greatest ($P < 0.05$) protein recovery approaching 90% (Kjeldahl, dry basis) was achieved when trout processing byproducts were solubilized at pH 2.5 or 13.0 followed by their precipitation at pH 5.5 (**Figure 4**). The isoelectric point (pI) hinders protein–water interaction and favors protein–protein bonds, which leads to low protein solubility and their gradual precipitation. Therefore, the pH during precipitation is important to achieve the maximum protein recovery. Generally, the pI of fish muscle proteins ranges between pH 5.0 and 6.0 (*13–15, 34*). Our recovery yield data show that pH 5.5 is the most optimal for precipitation of trout proteins. However, when proteins were precipitated at pH 6.0 and 5.0, the recovery yield drastically decreased for pH 5.0 regardless of solubilization pH, which correlated well with the rapid increase in solubility of trout proteins at acidic pH when compared to the basic counterparts (**Figure 2**). Okada and Morrissey (*29*) tested precipitation of sardine protein muscle between pH 5.0 and 6.0 with increments of 0.1 pH unit. They concluded that the pI was at pH 5.5, which is in accordance with our recovery yield data. Kristinsson and Liang (*14*) reported recovery yields at 78.7% and 65.0% for proteins recovered from Atlantic croaker at pH 2.5 and 11.0, respectively. Although our recovery yields confirm the same trend for higher recovery yields at acidic pH when compared to basic treatments, the recovery yields for trout proteins are slightly higher than those for Atlantic croaker (*14*). The slight differences may likely be attributed to different methods used to determine protein concentration. In addition, trout is a freshwater species and Atlantic croaker is a sea fish, which may also have contributed to a lower protein recovery yield from croaker due to inherent salinity (i.e., increased IS). Sathivel and co-workers (*25*) developed freeze-dried protein powders (FPP) from various fish processing byproducts by heating them at 85 °C for 60 min, followed by protein separation via centrifugation at 2560*g* and subsequent freeze-drying. The protein recovery yields ranged from 13.6 to 30.6% (dry basis) depending on the starting material. However, Sathivel and co-workers (*25*) did not use the isoelectric solubilization/precipitation of the protein and used much lower *g* force for centrifugation, which likely accounts for the differences between our and their recovery yields. Our recovery yield data demonstrated that muscle proteins can be isolated from fish processing byproducts at the recovery yield approaching 90% using optimal conditions for isoelectric solubilization/precipitation. Likely, even higher protein recoveries may be achieved with implementation of a recovery system in a continuous mode and water recycling. However, for applications of the recovered proteins to develop human food products, it is critical to determine their functionalities such as gelation (see below).

Table 1. Proximate Analysis[a] of the Recovered Trout Proteins That Were Solubilized at Different pH Values and Precipitated at pH = 5.50[b]

treatment (pH value)	moisture (%)	lipid (% dry basis)	protein (% dry basis)	ash (% dry basis)
2.5	78.07 ± 0.31 b	18.98 ± 1.75 a	36.78 ± 2.52 c	2.14 ± 0.10 a
3.0	80.50 ± 0.30 a	18.08 ± 1.60 a	53.81 ± 0.51 a	1.60 ± 0.16 b
12.0	75.49 ± 0.29 c	8.80 ± 0.92 b	45.42 ± 1.81 b	1.61 ± 0.05 b
12.5	76.94 ± 0.64 b	10.99 ± 0.39 b	44.28 ± 1.65 b	1.37 ± 0.12 b
13.0	77.62 ± 0.41 b	9.89 ± 0.47 b	49.34 ± 2.11 ab	2.14 ± 0.22 a

[a] Data are given as mean ± SEM ($n = 3$). Mean values in a vertical column with different letters were significantly different (least-squares difference test; $P < 0.05$). [b] The cryoprotectants (4% sorbitol, 4% trehalose, 0.3% phosphate, wt:wt) were added following protein precipitation, and then the proximate analysis was performed. Proximate analysis of trout processing byproducts: 71.3% moisture, 71.5% crude protein (dry basis), 15.2% total lipid (dry basis), and 13.9% ash (dry basis).

Proximate Analysis of Proteins Recovered from Trout Processing Byproducts. Trout processing byproducts (including bones, scales, skin, and other impurities) were used in our experiments as a starting material. Therefore, it was of our

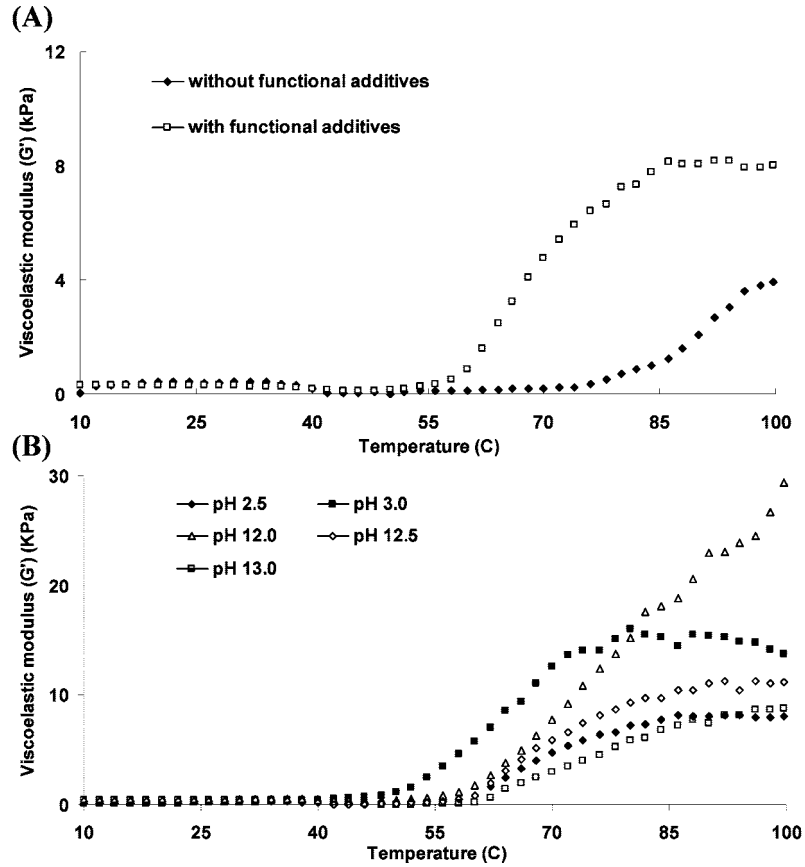

Figure 5. Viscoelastic modulus (G') of trout proteins as affected by (**A**) functional additives (3% PS, 1% BPP, 1% TGase, and 0.3% PP) and (**B**) different pH treatments during solubilization. Trout proteins in (**A**) were solubilized at pH 2.5. All of the trout proteins samples in (**B**) contained functional additives. The pH of trout proteins samples in both (**A**) and (**B**) was adjusted to 7.0 prior to the measurement.

interest to assess the removal of these impurities during isoelectric solubilization/precipitation. Since minerals are mainly in fish bones, scales, etc., ash content was used as an indicator of how well these impurities were removed from the recovered proteins.

The recovered trout proteins contained 1.4–2.1% of ash as compared to 13.9% in the trout processing byproducts (i.e., starting material) (**Table 1**). For comparison, boneless and skinless trout fillets have been reported to contain 5.5% ash (dry basis) (*2*). Therefore, the isoelectric solubilization/precipitation of the processing byproducts removed most of the impurities from the recovered trout proteins. However, proteins that were solubilized at pH 2.5 and 13.0 had higher ($P < 0.05$) ash content than other treatments (**Table 1**). Higher amount of minerals might have been solubilized from the processing byproducts in these most extreme pH treatments, resulting in their retention with the recovered proteins.

Lipid removal from the recovered proteins is often desirable because fish lipids are prone to oxidation, which leads to rancidity development commonly associated with a fishy odor. Hultin and Kelleher (*12*) demonstrated that the isoelectric solubilization/precipitation allows significant reduction of fish lipids in the recovered proteins. Acidic treatments resulted in higher ($P < 0.05$) lipid retention in the recovered trout proteins than the basic counterparts (**Table 1**). Similar results have been reported for channel catfish (*15*), herring (*34*), Atlantic croaker (*14*), and Antarctic krill (*21*). During oil processing to obtain a soapstock, free fatty acids are more readily removed with alkaline processing than acidic treatment (*35*), which may in part explain why lower lipid content was obtained with basic treatments during our processing. However, both basic and acidic pH treatments resulted in a relatively high retention of lipids in the recovered trout protein probably because we used a two-phase continuous centrifuge (*21*).

Rheological Properties of Proteins Recovered from Trout Processing Byproducts. Viscoelastic properties (G') of the proteins recovered from trout processing byproducts were assessed (**Figure 5**). Linear heating of trout protein-based pastes resulted in a typical rheogram as for other meat systems (*11, 21, 36, 37*). A greater viscoelasticity (G') was measured for the recovered trout proteins when the functional additives were added than that without the additives (**Figure 5A**). In fact, when the additives were not present, trout proteins failed to gel. Therefore, addition of beef plasma protein (BPP) or other protease inhibitor to the proteins recovered from trout processing byproducts seems necessary to inhibit heat-induced enzymatic proteolysis and consequently gel softening. BPP shows a good inhibition against fish proteases and enhances gelation of surimi, especially in protease-laden species (*38–40*). Instead of fillets, we used processing byproducts that inherently have greater chances than the fillets for contamination with proteolytic enzymes during processing; hence, the processing byproducts should also be considered protease-laden. Therefore, the failure to form a gel when the functional additives were absent was likely due to the proteolytic breakdown of the recovered proteins by endogenous proteases present in the trout processing byproducts.

When the functional additives were added to the trout proteins that were solubilized at different pH values followed by precipitation at pH = 5.5, the viscoelastic modulus (G') started a significant increase at approximately 56–60 °C (**Figure 5B**). Since the addition of protease inhibitor to trout proteins seems

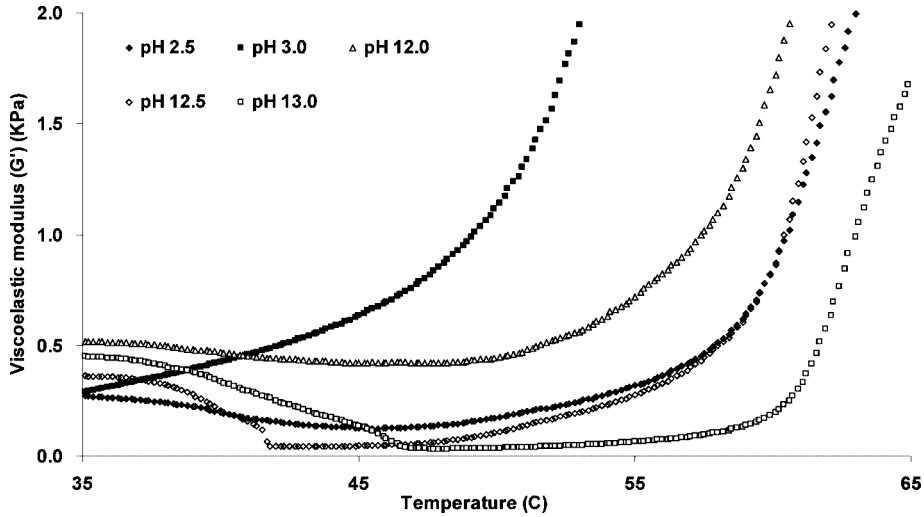

Figure 6. Decrease in viscoelasticity of trout proteins at temperature range between 35 and 65 °C. The pH of trout proteins samples was adjusted to 7.0 prior to the measurement, and all of the samples contained functional additives.

necessary to suppress proteolysis, the BPP was added to all of the trout protein-based pastes presented in **Figure 5B**. The trout proteins that were solubilized at pH 12.0 had higher G' when compared to other treatments, indicating better gelation. Kristinsson and co-workers (*15*) suggested that the alkali-aided processing led to less denaturation than the acid-aided process. Chen and Jaczynski (*21*) reported similar results for proteins recovered from Antarctic krill using basic treatments. However, Raghavan and Kristinsson (*41*) recently have directly measured denaturation of isolated fish myosin as affected by acid solubilization and correlated it with G'. They concluded that the G' of myosin increased with an increase in myosin denaturation and that G' and denaturation was the highest at the lowest pH tested.

Although pH 12.0 resulted in lower ($P < 0.05$) protein recovery yield than other pH treatments (**Figure 4**), the proteins solubilized at pH 12.0 probably experienced the least pH-induced irreversible denaturation and formed the best gel.

Figure 6 shows viscoelastic properties of the recovered trout proteins at temperatures 35–65 °C. The functional additives were added to all of the samples presented in **Figure 6**. Despite the presence of BPP in the trout protein paste, a significant decreases of G' occurred between 40 and 55 °C, except for pH 3.0 and 12.0 treatments, which were the two least extreme pH treatments in our experiments. The decrease of G' at this temperature range is likely an indicator of proteolytic activity, which has been reported for several fish species. In Pacific whiting, the acid-aided processing enhanced the cathepsin activity (*42*), whereas the alkali-aided processing did not eliminate, but minimized, this activity, particularly cathepsin type L (*17*). Although the lysosomal cathepsin types B and H are removed from fish muscle by extensive washing during surimi manufacture, cathepsin L has been shown to be retained with surimi, contributing to poor gelation (*38*). Cathepsin L that was retained in Pacific whiting surimi had the highest activity at 55 °C and pH = 5.5, but it also readily degraded the myofibrillar proteins at neutral pH (*43*). The isoelectric solubilization/precipitation uses less water than conventional surimi processing, and our starting material was trout processing byproducts. Therefore, cathepsin L seems to have been retained with the trout protein recovered in our experiments similarly to surimi processing (*38*, *43*). This is likely why the G' for trout proteins recovered in our experiments decreased at the temperatures between 40 and 55 °C. Proteins solubilized at pH 3.0 and 12.0 were degraded by cathepsin L to a lower degree than at other pH treatments,

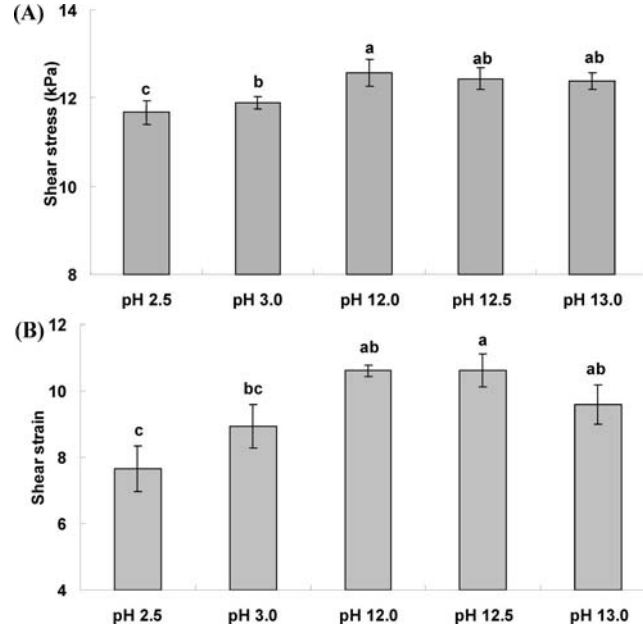

Figure 7. Shear stress (**A**) and strain (**B**) of trout protein-based gels developed from trout processing byproducts solubilized at different pH. All of the gels contained functional additives. Small bars on the data bars indicate standard deviation. Mean values in each pH value with different letters were significantly different (least-squares difference test, $P < 0.05$, $n = 6$).

resulting in respectively higher G' between 40 and 55 °C. The proteins recovered from trout processing byproducts and Antarctic krill seem to face the same problem with proteolytic enzymes; however, the extent of degradation as assessed with dymanic rheology is higher for krill (*21*). Therefore, whenever good gelation is required, effective protease inhibitors should to be added to the recovered trout proteins similarly to krill proteins.

Texture and Color Properties of Trout Protein-Based Gels. The shear stress and strain of trout protein-based gels are shown in **Figure 7**. Since the trout protein without functional additives failed to gel, the texture and color properties were not determined. Trout proteins recovered from basic treatments had higher ($P < 0.05$) shear stress and strain values than those from acidic treatments, indicating better gel strength and cohesiveness, respectively. Although the proteins recovered from

Table 2. Texture Profile Analysis[a] (TPA) of Trout Protein-Based Gels Developed from Trout Processing Byproducts Solubilized at Different pH[b]

treatment (pH value)	hardness (N)	springiness	cohesiveness	gumminess (N)	chewiness (N)	resilience
2.5	5.68 ± 0.12 bc	0.91 ± 0.01 b	0.53 ± 0.03 c	5.07 ± 0.38 a	4.66 ± 0.35 ab	0.32 ± 0.01 c
3.0	5.49 ± 0.11 c	1.19 ± 0.07 a	0.85 ± 0.01 a	4.67 ± 0.12 ab	5.63 ± 0.48 a	0.58 ± 0.01 a
12.0	5.87 ± 0.20 b	1.03 ± 0.07 a	0.72 ± 0.06 b	4.11 ± 0.29 bc	4.42 ± 0.53 b	0.46 ± 0.04 b
12.5	6.32 ± 0.10 a	0.96 ± 0.00 a	0.70 ± 0.02 b	4.41 ± 0.13 bc	4.24 ± 0.13 b	0.41 ± 0.01 b
13.0	5.53 ± 0.10 bc	0.97 ± 0.00 a	0.71 ± 0.09 b	3.94 ± 0.09 b	3.80 ± 0.09 b	0.43 ± 0.01 b

[a] Data are given as mean ± SEM ($n = 15$). Mean values in a vertical column with different letters were significantly different (least-squares difference test; $P < 0.05$). [b] All of the gels contained functional additives.

Table 3. Color Properties[a] of Trout Protein-Based Gels Developed from Trout Processing Byproducts Solubilized at Different pH[b]

treatment (pH value)	L^*	a^*	b^*	whiteness $L^* - 3b^*$
2.5	97.58 ± 0.06 bc	−0.05 ± 0.01 ab	1.42 ± 0.03 c	93.31 ± 0.08 b
3.0	97.39 ± 0.08 c	−0.04 ± 0.01 a	1.09 ± 0.07 d	94.11 ± 0.19 a
12.0	97.80 ± 0.07 a	−0.05 ± 0.02 ab	1.87 ± 0.05 b	92.20 ± 0.14 c
12.5	97.70 ± 0.11 ab	−0.08 ± 0.01 b	1.79 ± 0.07 b	92.34 ± 0.20 c
13.0	97.04 ± 0.05 d	−0.18 ± 0.01 c	2.10 ± 0.05 a	90.74 ± 0.15 d

[a] Data are given as mean ± SEM ($n = 20$). Mean values in a vertical column with different letters were significantly different (least-squares difference test; $P < 0.05$). [b] All of the gels contained functional additives.

basic treatments had better gel quality, acidic treatments resulted in generally higher recovery yields (**Figure 4**). The results from torsion test correlated well with the rheograms (**Figures 5** and **6**). Despite addition of BPP, a decrease of protein viscoelasticity (G') at temperature range 40–55 °C was still measured, especially for the most extreme pH treatments (**Figure 6**), which probably contributed to the poorer texture of the respective gels (**Figure 7**). In addition, Kristinsson and co-workers (*15*) suggested that the alkali-aided processing led to less denaturation than the acid-aided process. The lower denaturation of trout proteins solubilized at basic pH might also have contributed to better texture.

A texture profile analysis (TPA) of the trout protein-based gels is summarized in **Table 2**. Generally, the higher hardness of the gels developed from trout proteins that were solubilized at basic pH was in agreement with the rheogram (**Figure 5B**) and torsion test (**Figure 7**). In summary of the six TPA parameters (**Table 2**), the gels developed from trout proteins that were solubilized at pH 3.0 and 12.0 exhibited better texture properties than the gels made from proteins solubilized at other pH values.

The gels developed from proteins recovered from acidic treatments were whiter ($P < 0.05$) and less yellow ($P < 0.05$) than their basic counterparts (**Table 3**). It has been demonstrated that an oil addition to surimi results in brighter (L^*) and whiter ($L^* - 3b^*$) surimi gels (i.e., imitation crabmeat). This whiteness enhancement has been attributed to light scattering that results from the emulsion created when oil is comminuted with fish muscle proteins and water (*44*). It is likely, therefore, that the gels developed from trout proteins solubilized at acidic pH exhibited whiter color due to higher oil content (**Table 1**).

CONCLUSIONS

Trout muscle proteins exhibited similar pH solubility to other muscle proteins with minimum solubility at pH 5.5 which increased rapidly and more moderately when the pH was altered toward acidic and basic range, respectively. Increased ionic strength (IS) shifted the minimum solubility of the trout muscle proteins toward more acidic pH. Therefore, muscle proteins can be efficiently isolated from low-value trout processing byproducts (fish meat left over on bones, head, skin, etc.) at protein recovery yields (Kjeldahl, dry basis) approaching 90% by isoelectric precipitation/solubilization. The recovered protein retained gel-forming ability. However, dynamic rheology suggested proteolysis at temperature range between 40 and 55 °C. Therefore, an addition of protease inhibitors seems necessary to suppress the proteolytic activity during heat-induced gelation of the recovered proteins. Addition of other common functional additives such as starch, transglutaminase, and phosphates to the recovered proteins resulted in good texture properties of the gels. Generally, the firmer gels were obtained from proteins solubilized at pH 12.0 than those at other pH treatments, possibly due to less proteolysis and denaturation at basic pH. Because of the higher ($P < 0.05$) lipid content in the proteins recovered at acidic pH, the gels had higher ($P < 0.05$) whiteness ($L^* - 3b^*$) values.

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