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Isoelectric Solubilization/Precipitation as a Means To Recover Protein Isolate from Striped Bass (*Morone saxatilis*) and Its Physicochemical Properties in a Nutraceutical Seafood Product

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ABSTRACT: Excessive dietary intake of Na (i.e., NaCl) contributes to hypertension, which is a major risk factor for cardiovascular disease. Normally, NaOH and HCl are used to dissolve and precipitate, respectively, fish muscle proteins in isoelectric solubilization/precipitation (ISP), therefore contributing to increased Na content in the recovered fish protein isolates (FPI). Substitution of NaOH with KOH may decrease the Na content in FPI and, thus, allow development of reduced-Na seafood products. In this study, FPI was recovered with ISP using NaOH or KOH. In order to develop a nutraceutical seafood product, the FPI was extracted with NaCl or KCl-based salt substitute and subjected to cold- or heat-gelation. In addition, standard nutraceutical additives (ω-3 fatty acids-rich oil and dietary fiber) along with titanium dioxide (TiO_2) were added to FPI. Color, texture, dynamic rheology, Na and K content, and lipid oxidation of the FPI gels were compared to commercial Alaska pollock surimi gels. FPI gels had greater ($p < 0.05$) whiteness, good color properties ($L^*a^*b^*$), and generally better textural properties when compared to surimi gels. Although the ISP-recovered FPI and surimi developed similar final gel elasticity, the proteins in FPI and surimi had different gelation pattern. A reduction ($p < 0.05$) of Na content and simultaneous increase ($p < 0.05$) in K content of FPI gels was achieved by the substitution of NaOH with KOH during ISP and NaCl with the KCl-based salt substitute during formulation of the FPI paste. Although cooking and addition of NaCl during formulation of the FPI paste increased ($p < 0.05$) lipid oxidation in FPI gels, TBARS values were much below rancidity levels. These results indicate that KOH can replace NaOH to recover FPI from whole gutted fish for subsequent development of nutraceutical seafood products tailored for reduction of diet-driven cardiovascular disease.

KEYWORDS: *physicochemical properties, isoelectric solubilization/precipitation, protein isolates, functional food, nutraceutical food, product development, omega-3 polyunsaturated oil*

■ INTRODUCTION

Seafood consumption has been steadily increasing in the United States, and in 2007, Americans consumed 7.4 kg of fish and shellfish per person.[1] This trend is particularly relevant in light of a growing human population and recent forecasts indicating that some of the current fisheries may collapse by midcentury if they are not managed more sustainably.[1] Striped bass (*Morone saxatilis*) is a warm water species that can be aquacultured. The white flesh, mild taste, and low fat content of striped bass are major attributes sought by consumers.[2]

Processing of raw fish into food products generates large quantities of byproduct. It has been estimated that the value addition of human food developed from the byproduct will increase significantly in the future.[3,4] The byproducts are mainly rendered for animal feed. However, fish meat (i.e., muscle proteins) left over after processing could be recovered and used in human food products. In the present study, whole gutted striped bass was used as a model for fish processing byproduct, and it was the starting material for isoelectric solubilization/precipitation (ISP). The ISP processing allows selective, pH-induced water solubility of muscle proteins with concurrent separation of lipids and removal of materials not intended for human consumption such as bones, scales, skin, etc. Muscle proteins from fish have thus far been recovered using a batch mode ISP at the laboratory-[5−8] and pilot-scale.[9] The ISP processing has been applied to fish, beef, and chicken processing byproduct.[10−14] ISP allows high protein recovery yields from such difficult sources as fish processing byproduct, while significantly reducing fat content in the recovered protein isolates.[12,15] Recovered protein isolates retain functional properties and nutritional value.[16−21] Due to extreme pH shifts, ISP results in up to 5-log nonthermal microbial reduction.[22,23] ISP offers several benefits and may be a useful technology to recover functional and nutritious protein isolates from whole gutted fish or fish processing byproduct for subsequent application in nutraceutical/functional seafood products.

It is a general consensus that ISP at basic pH yields slightly better overall results. Protein solubilization at basic pH is normally induced with NaOH, while HCl is used for precipitation. However, NaOH and HCl result in accumulation of NaCl in the ISP-recovered protein isolates. Protein 3-D structure and, hence, protein functionalities undergo significant changes during ISP. These changes are influenced not only by the pH, but also by the type of anions and cations.[24] Hofmeister series ranks the ability of cations to stabilize (kosmotropic) or destabilize (chaotropic) proteins. Excessive dietary intake of Na (i.e., NaCl) is a risk factor in hypertension, leading to lower cardiovascular health. Therefore, this study

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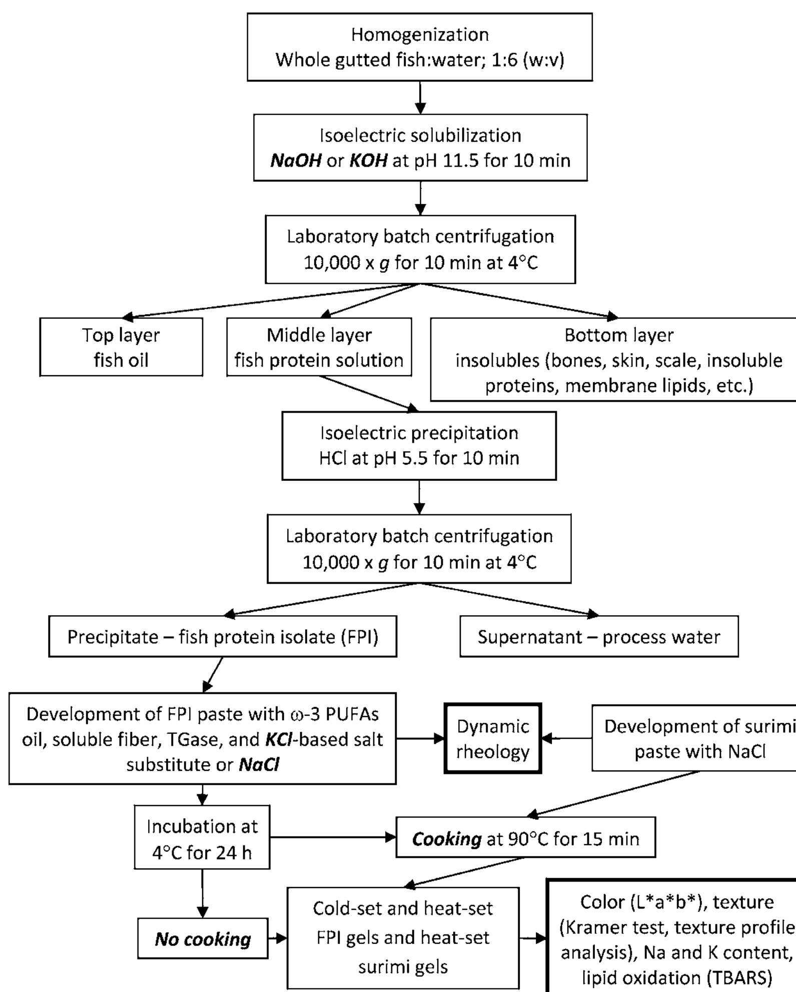

Figure 1. Experimental flow diagram. KOH or NaOH was used for protein solubilization during isoelectric solubilization/precipitation (ISP); NaCl or KCl-based salt substitute was used for protein extraction during paste formulation; and the paste was cooked or uncooked for heat-gelation or cold-gelation, respectively. TGase = microbial transglutaminase.

aimed at comparing the effects of two cations, Na^+ and K^+, on the physicochemical properties of protein isolates recovered from striped bass with ISP using NaOH or KOH, respectively.

Cooking is normally applied to fish proteins such as surimi to induce heat-gelation, resulting in the desired texture, flavor, and other sensory attributes. More importantly, though, cooking results in microbial reduction and, hence, acceptable microbial safety. Since ISP results in a 5-log microbial reduction, the cooking step may not be necessary. This may be beneficial in terms of energy and equipment savings as well as simplified processing. Therefore, this study also aimed at comparing physicochemical properties of gels made with ISP-recovered protein isolates using cold-gelation (i.e., "suwari" with exogenous transglutaminase) and heat-gelation.

Although ISP offers efficient recovery of functional and nutritious fish protein isolates (FPI) from whole gutted fish and fish processing byproduct, there are no food products on the market developed from FPI. Nutraceutical food products contain added, technologically developed ingredients with specific, well-documented health benefits.[25−28] Nutraceutical seafood products could be developed from FPI. Cardiovascular disease (CVD) has had an unquestioned status of the number one cause of death in the U.S. since 1921.[29] A 3-prong strategy is proposed to address the diet-driven CVD. Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), soluble dietary fiber, and salt substitution have well-documented cardiovascular benefits.

Therefore, the overall objective of this study was to develop a restructured, nutraceutical seafood whose main ingredient would be the ISP-recovered protein isolate from whole gutted striped bass (bone-in, head-on, skin-on, and scale-on) with added ω-3 PUFAs-rich oil, soluble dietary fiber, and salt substitute. An attempt was made to develop such a product that would have similar physicochemical properties to a respective product made from surimi, but without the additives. Specific objectives were to compare the effects of NaOH versus KOH during ISP and cold-gelation versus heat-gelation as well as addition of NaCl versus KCl-based salt substitute on physicochemical properties of gels made of the ISP-recovered protein isolate. The physicochemical properties were the following: (1) color, (2) texture, (3) dynamic rheology, (4) Na and K content, and (5) lipid oxidation of the gels.

MATERIALS AND METHODS

Sample Preparation and Protein Recovery. Whole gutted striped bass (bone-in, head-on, skin-on, and scale-on) were purchased from a local aquaculture farm. The fish were the input material for the isoelectric solubilization/precipitation (ISP) processing. Whole gutted bass were used as a model for fish processing byproduct. A processing flowchart for the recovery of fish protein isolate (FPI) and subsequent

development of heat-set and cold-set FPI gels is shown in Figure 1. The fish were ground (meat grinder model 812 with 2.3 mm grinding plates, Biro, Marblehead, OH) followed by homogenization with cold (4 °C) distilled deionized water (dd H_2O) at 1:6 ratio (ground fish:water, w:v) using a laboratory homogenizer (PowerGen 700, Fisher Scientific, Fairlawn, NJ) set at speed five for 5 min. During the entire ISP processing temperature was carefully controlled at 4 °C. The processing time did not exceed 60 min. The homogenization/mixing was continued with the PowerGen homogenizer set at speed three during subsequent pH adjustment steps.

A 6 L portion of the homogenate was transferred to a beaker, and the pH was adjusted to 11.50 ± 0.05 with 10 and 1 N NaOH or KOH.[11,12] 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). Once the desired pH was obtained, the solubilization reaction was allowed to take place for 10 min, followed by centrifugation at 10 000 × *g* and 4 °C for 10 min using a laboratory batch centrifuge (Sorvall Evolution RC refrigerated superspeed centrifuge equipped with Sorvall Fiber Lite rotor SLC- 6000, Sorvall Centrifuges, Asheville, NC). The centrifugation resulted in three layers: top, fish oil; middle, fish muscle protein solution; bottom, insolubles (bones, skin, scale, insoluble proteins, membrane lipids, etc.). The middle layer was collected, and its pH was adjusted to 5.50 ± 0.05 with 10 and 1 N HCl to isoelectrically precipitate muscle proteins. Once the desired pH was obtained, the precipitation reaction was allowed to take place for 10 min. The solution with precipitated proteins was dewatered by centrifugation as above. The centrifugation resulted in two layers: top, process water; bottom, precipitated and dewatered fish protein isolate (FPI). Final moisture of the FPI was adjusted to 82% by manual squeezing of the isolate wrapped in a cheese cloth. The isolate was used in the preparation of FPI paste.

Preparation of Fish Protein Isolate (FPI) Paste and Surimi Paste. FPI pastes and surimi paste were made using the procedure described by Jaczynski and Park.[30] The ISP-recovered FPI was chopped in a universal food processor (model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. An FPI paste was obtained by extracting myofibrillar proteins with 0.34 M of KCl using a KCl-based salt substitute (AlsoSalt sodium-free salt substitute, AlsoSalt, Maple Valley, WA) (hereafter called salt substitute) or 0.34 M of NaCl (noniodized Morton salt, Morton International Inc., Chicago, IL) and chopping at low speed for 0.5 min in the universal food processor. This level of salt substitute was found optimal and similar to salt (NaCl) in terms of texture and color development as well as protein gelation and reduction of water activity in heat-set fish protein gels.[31] The salt substitute contained 68% of KCl and L-lysine monohydrochloride and calcium stearate. According to the manufacturer, the patented L-lysine derivative masks the metallic bitter aftertaste of KCl. The concentration of 0.34 M of NaCl corresponds to 2% of NaCl (or 20 g of NaCl per 1 kg batch).

Final moisture content of the FPI paste was adjusted to 68% by adding functional additives at the following final concentrations (w:w): 10% of a mixture of two ω-3 PUFAs-rich oils (see below), 4% of soluble dietary fiber (Solca-Floc 900FCC, International Fiber Corporation, Urbana, OH), 0.5% of microbial transglutaminase (TGase) (Activa RM, Ajinomoto USA Inc., Teaneck, NJ), and 0.3% of polyphosphate (PP) (Kena FP-28, Innophos, Cranbury, NJ). The above levels of functional additives were previously found as optimal for gelation of FPI, and, consequently, texture development, as well as closely resembling commercial surimi-based seafood products.[32,33] A 0.5% of titanium dioxide (TiO_2) [titanium(IV) oxide, Sigma-Aldrich, Inc., St. Louis, MO] was also added to the FPI paste.[13,14] Up to 1% of TiO_2 is commonly added to food products as a whitening agent. The fiber, TGase, PP, and TiO_2 were in a dry powder form. The 10% of ω-3 PUFAs-rich oil added to the FPI paste in this study replaced ice/water that is normally added to a fish protein-based paste such as surimi paste.[32,33] Chopping at low speed for 1 min was applied to mix all of the ingredients with the FPI paste. Additional chopping was performed at high speed under vacuum (0.5 bar) for the last 3 min. The paste temperature was controlled between 1 and 4 °C during chopping. FPI and surimi pastes were prepared in 1 kg batches.

The following ω-3 PUFAs-rich oils were added to the FPI paste: (1) Flaxseed oil was obtained from Jedwards International, Inc. (Quincy, MA). (2) Fish oil (Omega Pure 8042TE) was obtained from Omega Pure (Reedsville, VA).

The oil added to the fish protein FPI paste was a mixture of flaxseed oil and fish oil (1:1, v:v). When oil is homogenized with a comminuted protein-based paste, it results in light scattering, and, therefore, improves whiteness of cooked gels.[33] This is why, besides nutraceutical benefits, 10% of the ω-3 PUFAs-rich oil was added to the FPI paste. Anderson and Ma[34] provided an up-to-date and comprehensive review of health benefits specific for α-linolenic (ALA, 18:3ω3), eicosapentaenoic (EPA, 20:5ω-3), and docosahexaenoic (DHA, 22:6ω3) FAs.

Frozen surimi grade A commercially prepared from Alaska pollock (*Theragra chalcogramma*) was obtained from Trident Seafoods Corp. (Seattle, WA). Surimi contained cryoprotectants (4% of sorbitol and 4% of sucrose), 0.15% of sodium tripolyphosphate, and 0.15% of tetrasodium pyrophosphate. Frozen surimi blocks (10 kg each) were shipped overnight in heavily insulated industrial strength boxes filled with ice. Upon arrival, surimi blocks were cut into approximately 800 g units, vacuum-packaged, and stored at −80 °C until needed. The moisture content of surimi was determined as 76.04%. Surimi paste was prepared in the same manner as the FPI paste except surimi was used instead of the ISP-recovered FPI, myofibrillar proteins were extracted with NaCl only, and no other additives were used. Since surimi paste did not contain added ω-3 PUFAs-rich oil, final moisture content was adjusted to 78% by adding cold distilled deionized water (dd H_2O).[32,33] FPI pastes and surimi paste prepared in this manner were immediately used in the dynamic rheology test.

Oscillatory Dynamic Rheology. Nondestructive gelation properties (elastic modulus, G′) of FPI pastes and surimi paste were measured as a function of temperature using a cone and plate attachment (4° and 4 cm diameter) mounted on a dynamic rheometer (Bohlin CVOR 200, Malvern Instruments Ltd., Worcestershire, U.K.) in oscillation mode. Once the sample was pressed by lowering the cone, excess sample was gently removed with a stainless steel spatula. The gap between cone and plate was 150 μm. A plastic cover supplied by the manufacturer was used to prevent moisture loss during measurements. Tests were conducted at a constant 1% strain and 0.1 Hz frequency, which was in the linear viscoelasticity range. The applied stress was also in the linear viscoelasticity range of the tested samples. The temperature ramp was programmed to increase from 25 to 90 °C at the rate of 1 °C/min.[11,12] The rheogram is reported as a mean value of three independent experiments (n = 3). In each experiment, three rheograms per treatment were generated and averaged.

Development of Fish Protein Isolate (FPI) Gels and Surimi Gels. Following paste formulation, FPI pastes and surimi paste were stuffed into stainless steel tubes (length = 17.5 cm, internal diameter = 1.9 cm) with screw end-caps. Since FPI paste was prepared with TGase, the tubes containing this paste were incubated at 4 °C for 24 h to allow for the formation of nondisulfide covalent ε-(γ-glutamyl)-lysine cross-links or the "suwari" effect. The tubes containing surimi paste were not incubated because they did not contain TGase. The tubes containing FPI paste were divided into two groups. One group was heated in a water bath at 90 °C for 15 min for heat-gelation, while the other group was not heated (i.e., cold-gelation only). Following heating, tubes were chilled in ice slush, and FPI gels were removed for analyses.

Color Properties of Fish Protein Isolate (FPI) Gels and Surimi Gels. Gel samples were equilibrated to room temperature for 2 h prior to the color measurement. Color properties were determined using a Minolta Chroma Meter CR-300 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan). The colorimeter was calibrated by using a standard plate supplied by the manufacturer. At least eight cylindrical gels (height =2.54 cm, diameter =1.90 cm) per treatment were used for color measurements. The values for the CIE (Commission Internationale d'Eclairage of France) color system using tristimulus color values, L^* (lightness), a^* (redness), and b^* (yellowness) were

Table 1. Color Properties[a] of Fish Protein Isolate (FPI) Gels and Surimi Gels[b]

	surimi NaCl Cook	KOH NaCl cook	KOH NaCl uncook	KOH KCl cook	KOH KCl uncook	NaOH KCl cook	NaOH KCl uncook
L^a	80.5 ± 0.5 b	87.0 ± 0.7 a	87.2 ± 0.7 a	87.1 ± 0.2 a	87.2 ± 0.2 a	87.1 ± 0.3 a	87.0 ± 0.1 a
a^a	−3.28 ± 0.13 d	−0.87 ± 0.03 c	−0.92 ± 0.04 c	−0.72 ± 0.03 b	−0.78 ± 0.09 b	−0.55 ± 0.03 a	−0.53 ± 0.03 a
b^a	4.24 ± 0.67 e	9.52 ± 0.27 ab	9.08 ± 0.34 c	9.76 ± 0.10 a	9.29 ± 0.14 bc	8.42 ± 0.08 d	8.13 ± 0.06 d
whiteness	79.8 ± 0.4 d	83.9 ± 0.5 c	84.3 ± 0.7 ab	83.8 ± 0.2 c	84.2 ± 0.2 bc	84.6 ± 0.3 a	84.7 ± 0.1 a

[a]Data are given as mean values ± SD (n = 3). Mean values in rows with different letters indicate significant differences (Fisher's Least Significant Difference test; $P < 0.05$). [b]Experimental flow diagram is shown in Figure 1. Legend: top row, KOH or NaOH indicates which base was used for protein solubilization during isoelectric solubilization/precipitation (ISP); middle row, NaCl or KCl (salt substitute) indicates which agent was used for protein extraction during paste formulation; bottom row, cook or uncook indicates whether the paste was heat-gelled or cold-gelled.

Table 2. Texture Properties[a] (Kramer Shear Force and Texture Profile Analysis) of Fish Protein Isolate Gels (FPI) and Surimi Gels[b]

	surimi NaCl cook	KOH NaCl cook	KOH NaCl uncook	KOH KCl cook	KOH KCl uncook	NaOH KCl cook	NaOH KCl uncook
shear force (N/g)	187 ± 11 c	315 ± 9 a	226 ± 12 b	174 ± 3 d	126 ± 2 f	161 ± 4 e	121 ± 3 f
hardness (N)	1642 ± 50 d	2944 ± 262 a	2161 ± 150 c	2805 ± 101 a	2249 ± 79 c	2522 ± 235 b	1579 ± 113 d
cohesiveness	0.66 ± 0.02 d	0.70 ± 0.01 b	0.79 ± 0.02 a	0.67 ± 0.01 cd	0.78 ± 0.01 a	0.68 ± 0.06 bc	0.78 ± 0.01 a
springiness	2.01 ± 0.01 b	2.00 ± 0.01 b	1.91 ± 0.02 e	2.03 ± 0.01 a	1.94 ± 0.01 d	2.03 ± 0.01 a	1.96 ± 0.01 c
gumminess	1094 ± 66 e	2057 ± 184 a	1713 ± 112 c	1911 ± 27 b	1739 ± 53 c	1767 ± 159 c	1236 ± 81 d
chewiness	2177 ± 88 f	4117 ± 375 a	3254 ± 222 d	3823 ± 101 b	3378 ± 95 cd	3503 ± 302 c	2413 ± 163 e
resilience	0.34 ± 0.00 c	0.33 ± 0.01 d	0.46 ± 0.01 a	0.31 ± 0.01 e	0.45 ± 0.01 b	0.33 ± 0.01 d	0.44 ± 0.00 b

[a]Data are given as mean values ± SD (n = 3). Mean values in rows with different letters indicate significant differences (Fisher's Least Significant Difference test; $P < 0.05$). [b]Experimental flow diagram is shown in Figure 1. Legend: top row, KOH or NaOH indicates which base was used for protein solubilization during isoelectric solubilization/precipitation (ISP); middle row, NaCl or KCl (salt substitute) indicates which agent was used for protein extraction during paste formulation; bottom row, cook or uncook indicates whether the paste was heat-gelled or cold-gelled.

determined. The gel whiteness was calculated by the following equation:[36,37]

$$\text{whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Texture Properties of Fish Protein Isolate (FPI) Gels and Surimi Gels. Two different methods were employed to determine texture: Kramer shear test and texture profile analysis (TPA). Although these texture measurements are commonly employed for determination of textural properties, each method characterizes different textural parameters.[38] Likely, the most comprehensive understanding of textural properties is provided by a combination of these methods. Therefore, these two different tests were employed in the present study.

Gel samples were equilibrated to room temperature for 2 h prior to the texture measurement. At least five cylindrical gels (height = 8.0 cm, diameter = 1.9 cm) per treatment were subjected to Kramer shear test using a texture analyzer (Model TA-HDi, Texture Technologies Corp., Scarsdale, NY) with a Kramer cell attachment.[13,15,26] The Kramer shear cell consisted of five 3.0-mm thick and 70-mm wide shear blades passing through a cell having a corresponding number of slots. Individual gel samples were weighed and placed under the blades in the Kramer cell. Shear force was measured at a 127 mm/min crosshead speed and expressed as maximum peak force (N peak force/g of gel sample).

TPA was performed according to Cheret et al.[39] At least eight cylindrical gels (height = 2.54 cm, diameter = 1.90 cm) per treatment were subjected to two-cycle compression (50% compression) using the texture analyzer equipped with a round 70-mm diameter TPA plate attachment moving at a speed of 127 mm/min. From the resulting force−time curves, hardness, cohesiveness, springiness, gumminess, chewiness, and resilience were determined.

Sodium and Potassium Content of Fish Protein Isolate (FPI) Gels and Surimi Gels. All glassware was washed overnight in a solution of 10% HCl in deionized distilled water (dd H_2O) 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 #1 paper (Whatman International Ltd., Maidstone, U.K.). Samples were diluted to volume with dd H_2O in a 50 mL volumetric flask. Na and K content were determined using inductively coupled plasma optical emission spectrometry (model P400, Perkin-Elmer, Shelton, CT). The measurements were performed in triplicate, and mean values ± standard deviation are reported.

Lipid Oxidation of Fish Protein Isolate (FPI) Gels and Surimi Gels. Oxidative rancidity of the gel samples was measured by a 2-thiobarbituric acid reactive substances (TBARS) assay of malondialdehyde (MDA) as previously described.[40,41] The absorbance was measured at 535 nm using an UV−vis spectrophotometer (model DU530, Beckman Instruments, Fullerton, CA). The TBARS values were calculated using molar absorptivity of MDA (156 000 M^{-1} cm^{-1}) at 535 nm. The TBARS values are reported as mean values ± standard deviation of at least three replicates. The mean values are expressed as mg of MDA per kg of gel samples.

Statistics. The experiments were independently triplicated (n = 3). In each triplicate at least three elastic modulus measurements, eight color measurements, five Kramer tests, eight TPA, three Na and K content measurements, and three TBARS assays were performed. Data were subjected to one-way analysis of variance (ANOVA). A significant difference was determined at 0.05 probability level, and differences between treatments were tested using Fisher's Least Significant Difference (LSD) test.[42] All statistical analyses of data were performed using SAS.[43]

RESULTS AND DISCUSSION

One important parameter when comparing different processing methods is the color of a protein isolate or gel made of this isolate. The market is in general most interested in isolates that are white.[44,45] The color of a fish protein isolate (FPI) can be affected by the amount of dark muscle, blood, and pigments such as melanin. Melanin is present in eyes, skin, and the black lining around fish belly. This is why the color becomes a particularly important parameter when FPI is recovered from whole fish or processing byproduct.

The color properties of gels made of surimi and ISP-recovered FPI are presented in Table 1. L^*, a^*, and b^* of FPI gels were higher ($p < 0.05$) than surimi gels. Application of KOH or NaOH during ISP, addition of NaCl or salt substitute during formulation of the FPI paste, and heat- or cold-gelation

did not ($p > 0.05$) affect L^* and generally did not have an effect on a^* and b^*. Although the FPI gels had higher ($p < 0.05$) b^* than surimi gels, their higher ($p < 0.05$) L^* and a^* resulted in greater ($p < 0.05$) whiteness. The higher a^* and b^* were likely caused by various pigments including residual heme and melanin retained with the ISP-recovered FPI.[46] The ISP processing resulted in the removal of most pigments from FPI, while the added TiO_2 and ω-3 PUFAs oil increased L^* of the FPI gels. As a result, the FPI gels had higher ($p < 0.05$) whiteness than surimi gels without chalky, artificial appearance. Kristinsson and Demir[47] compared color properties of gels made from the ISP-recovered FPI and surimi. The best L^* was for gels made from alkali-produced FPI, followed by surimi and acid-produced FPI. However, they did not add TiO_2 or ω-3 PUFAs oil.

Kramer shear force and texture profile analysis (TPA) are considered empirical tests that are often used to evaluate texture properties of gelled meat products including surimi-based seafood.[38,48,49] Table 2 shows TPA and Kramer shear force for FPI gels in comparison to surimi gels. The application of KOH during ISP and addition of NaCl during formulation of the FPI paste resulted in generally better texture (i.e., TPA and Kramer shear force) when compared to respective treatments with NaOH and salt substitute. Heat-gelation (i.e., 90 °C for 15 min) when compared to cold-gelation (i.e., TGase only) of the FPI resulted in higher ($p < 0.05$) values for the gel textural parameters as assessed with TPA and Kramer shear force except for cohesiveness and resilience. In the present study 0.5% of TGase was added to the ISP-recovered FPI during formulation of the FPI paste to induce "suwari".[50−58] However, TGase was not added to surimi paste. This likely accounts for a good texture of FPI gels that was generally comparable or better than surimi gels.

Dynamic rheology has extensively been applied to study heat-induced gelation of fish myofibrillar proteins.[59,60] Because elastic modulus (G') measures the energy recovered per cycle of sinusoidal shear deformation, its increase indicates the formation of elastic gel network. Hence, the changes in G' have been used to monitor protein thermal gelation.[61] The elastic moduli (G') of the FPI recovered with KOH or NaOH and surimi are shown in Figure 2. Surimi paste with added NaCl showed a typical pattern for protein gelation as a function of temperature. The G' had an initial increase at approximately 45 °C followed by a pronounced increase of elasticity until approximately 75 °C. Although FPI gelled differently than surimi, the application of KOH or NaOH during ISP did not appear to have a major effect on rheological behavior of the FPI. Elastic moduli for FPI pastes were not different from surimi paste during initial heating at 25−35 °C, but the FPI pastes started gelation at a lower temperature (approximately 35 °C) than surimi. The initial increase of G' indicates the onset of gel network formation due to partial unfolding of myosin. The G' data in the present study suggests that myosin in FPI is less thermally stable because it starts unfolding at lower temperature. The elastic moduli of FPI pastes did not exhibit as pronounced an increase starting at 45 °C as surimi paste. Instead, the FPI pastes showed a steady increase of the G' from the initial increase at 35 °C. However, all three pastes completed gelation with relatively the same final elasticity. The G' data suggests that the ISP-recovered FPI and surimi develop similar final gel elasticity, but proteins gel differently in FPI and surimi under conditions applied in the present study. Gelation of surimi proteins was complete at approximately 75 °C, which confirms previous observations. Several studies concluded that conformational changes in the supercoiled α-helix of the myosin rod above 55 °C are the most crucial because actomyosin gels do not attain appreciable gel strength below this temperature.[62−65]

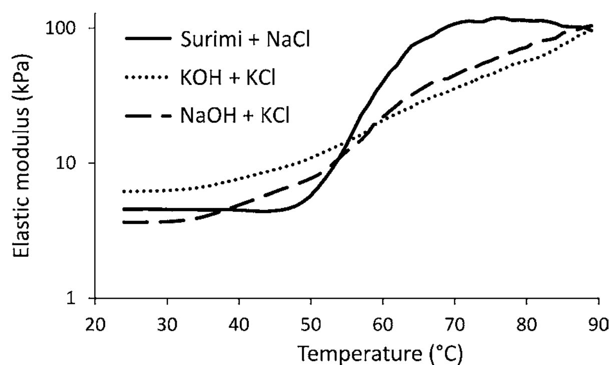

Figure 2. Elastic modulus of fish protein isolate (FPI) paste and surimi paste. Experimental flow diagram is shown in Figure 1. Legend: surimi + NaCl , surimi was used as a protein source and NaCl was used to extract proteins during paste formulation; KOH + KCl, KOH was used for protein solubilization during isoelectric solubilization/precipitation (ISP) and KCl was used to extract proteins during paste formulation; NaOH + KCl, NaOH was used for protein solubilization during isoelectric solubilization/precipitation (ISP) and KCl was used to extract proteins during paste formulation.

Although myofibrillar proteins in the ISP-recovered FPI undergo pH-induced misfolding during ISP processing, salts such as NaCl and KCl neutralize and shield the negative charges generated at alkaline pH, thereby protecting myosin from extensive and irreversible damage.[66] Surimi proteins are not subjected to extreme pH during manufacturing, and therefore, myosin retains its native structure. Application of NaOH or KOH during ISP did not seem to have a major effect on FPI gelation; however, G' pattern for FPI recovered with NaOH was slightly more similar to surimi. This is in accordance with Raghavan and Kristinsson[67] who studied how the application of KOH and NaOH during ISP affects gelation and physicochemical properties of catfish muscle proteins. According to the Hofmeister series, K^+ typically better stabilizes protein structure than Na^+.[68] In the present study, however, the G' for the FPI recovered with KOH when compared to NaOH seemed slightly less similar to surimi paste. This is opposite to the Hofmeister series, suggesting that during ISP K^+ stabilized FPI to the lesser degree than Na^+. A reverse Hofmeister effect is based on electroselectivity series, which has been attributed to the ion valence, charge, and its size.[69] The Na^+ ions are smaller than K^+, facilitating their stronger interaction/binding with myosin and consequently neutralizing/shielding the negative charges on myosin during ISP at alkaline pH, thereby decreasing the degree of myosin unfolding during ISP. Due to their smaller size, Na^+ ions most likely stabilized myosin structure better during ISP than K^+ in the present study. In addition to K^+/Na^+ effects, contributions of Cl^- to protein gelation should be considered. The excessive unfolding of myosin structure during ISP exposes its hydrophobic interior, promoting premature inter- and intramolecular hydrophobic interactions prior to cooking of the FPI, thereby changing the gelation pattern of the FPI during cooking.

Although salt (NaCl) has several important functions in muscle food products, the high dietary intake of Na contributes to hypertension. The hypertension is a major risk factor for

cardiovascular disease (CVD) and both forms of stroke (ischemic and hemorrhagic). Current average intake of salt (NaCl) in Western populations is 9−12 g/day (3500−4700 mg Na/day).[70] According to most public health recommendations aimed at lowering hypertension, this excessive intake should be reduced to 4−6 g/day (1500−2300 mg Na/day).[70] The most common strategy to decrease Na content in processed food products is salt substitution. During ISP NaOH is typically used to dissolve myofibrillar proteins. Following separation of insolubles, HCl is added to precipitate the proteins. NaCl is inevitably generated, contributing to increased Na content in the ISP-recovered FPI. In this study we hypothesized that replacing NaOH with KOH would decrease Na content while increasing K content in the final gels made of the FPI. K has an antihypertensive effect.

Na and K contents of FPI gels and surimi gels are presented in Figure 3. The Na and K contents in the FPI gels were

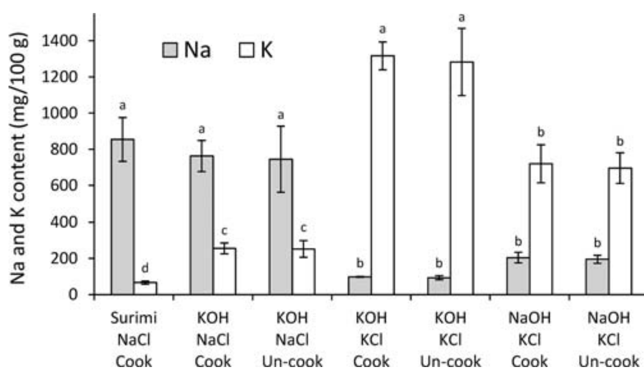

Figure 3. Na and K content of fish protein isolate (FPI) gels and surimi gels. [Data are given as mean values ± SD (n = 3). The small bars of the top of data bars indicate SD. Different letters on the top of SD bars indicate significant differences between mean values within Na or K content (Fisher's Least Significant Difference test; $P < 0.05$).] Experimental flow diagram is shown in Figure 1. Legend for the horizontal axis: top row, KOH or NaOH indicates which base was used for protein solubilization during isoelectric solubilization/precipitation (ISP); middle row, NaCl or KCl (salt substitute) indicates which agent was used for protein extraction during paste formulation; bottom row, cook or uncook indicates whether the paste was heat-gelled or cold-gelled.

reduced ($P < 0.05$) and increased ($P < 0.05$), respectively, by the substitution of NaOH with KOH during ISP and NaCl with the KCl-based salt substitute during formulation of the FPI paste. A fish muscle contains a variety of salts resulting in ionic strength of 0.11−0.15 M NaCl.[71,72] In addition, small amounts of various forms of Na phosphates are added to surimi with cryoprotectants. Surimi is the main and functional ingredient for various surimi-based seafood products. Although low levels of Na are expected in these products, the Na content increases greatly due to salt addition during formulation. The addition of 2% of NaCl to surimi in the present study resulted in 854 ± 121 mg Na/100 g and 66 ± 9 mg K/100 g of surimi gels (wet or "as is" basis). However, FPI recovered with KOH instead of NaOH and subsequent addition of salt substitute instead of salt resulted in 93−98 mg Na/100 g and 1283−1317 mg K/100 g of FPI gels (wet or "as is" basis). This represents approximately 9 times less Na and 20 times more K in the FPI gels compared to surimi gels. Replacement of NaOH with KOH during ISP processing resulted in reduction of Na in the FPI gels from 195 to 204 to 93−98 mg Na/100 g. According to the Dietary Reference Intakes from the Institute of Medicine, the Adequate Intake of K is 4700 mg/day.[73] It has been estimated that a 600 mg/day increase of dietary K intake lowers blood pressure by 1.0 mmHg, while increasing current K intake to the recommended level reduces stroke and heart disease mortalities by 8−15% and 6−11%, respectively.[74] This is of similar magnitude to what can be achieved by lowering dietary Na intake and highlights the importance of dietary strategies focusing on a simultaneous reduction of Na intake and increase of K intake.

Flaxseed oil used in this study contained over 40% of ALA, but negligible amounts of EPA and DHA. While fish oil contained trace levels of ALA, it had approximately 11% and 10% of EPA and DHA, respectively.[35] Thus, the percentages of particular FAs and their ratios per serving size can be readily calculated for the FPI gels developed in this study. The addition of 10% of the ω-3 PUFAs-rich oil (5% flaxseed oil + 5% fish oil) resulted in approximately 2000 mg of ALA, 550 mg of EPA, and 500 mg of DHA per 100 g (approximate size of 1 serving) of the FPI gel. Specific health benefits of ALA, EPA, and DHA have recently been reviewed.[34] Lipid oxidation is a major quality indicator of processed muscle food products.[75,76] When ISP is conducted at acidic pH, heme is activated and acts as a pro-oxidant increasing lipid oxidation when compared to ISP at alkaline pH.[77] Surimi gels had the lowest ($p < 0.05$) TBARS value (Figure 4). A good oxidative stability of surimi gels is

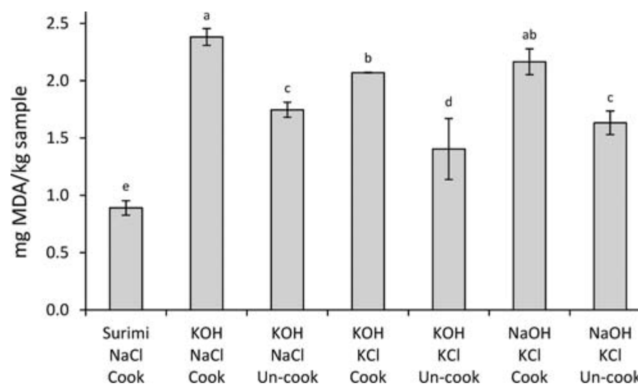

Figure 4. Lipid oxidation (thiobarbituric acid reactive substances = TBARS) in fish protein isolate (FPI) gels and surimi gels. [Data are given as mean values ± SD (n = 3). The small bars of the top of data bars indicate SD. Different letters on the top of SD bars indicate significant differences between mean values (Fisher's Least Significant Difference test; $P < 0.05$).] Experimental flow diagram is shown in Figure 1. Legend for the horizontal axis: top row, KOH or NaOH indicates which base was used for protein solubilization during isoelectric solubilization/precipitation (ISP); middle row, NaCl or KCl (salt substitute) indicates which agent was used for protein extraction during paste formulation; bottom row, cook or uncook indicates whether the paste was heat-gelled or cold-gelled.

mainly due to the low fat content and efficient removal of heme from surimi. In addition, unlike ISP, surimi processing does not apply pH shifts, and therefore, even the residual heme in surimi is not activated to become a pro-oxidant.[19] Cooked samples had higher ($p < 0.05$) TBARS than the uncooked samples. Heating inactivates antioxidants, releases Fe from myoglobin, and disrupts muscle cells allowing easy access of O_2, and thus, it accelerates lipid oxidation.[76,78−81] The increase ($p < 0.05$) in TBARS was more pronounced when salt was used to extract FPI during paste formulation compared to salt substitute,

regardless of the type of base used for protein solubilization during ISP. Salt has been shown to have a pro-oxidant effect in muscle food systems.[82−87] It has been proposed that the maximum level of TBARS indicating good quality of seafood products is less than 5 mg MDA/kg, but they may be consumed up to 8 mg MDA/kg.[88] In the present study, TBARS for all of the samples were much lower than the proposed limit. The TBARS data indicates that cooking and NaCl are the two factors inducing lipid oxidation in ISP-recovered FPI gels.

Isoelectric solubilization/precipitation (ISP) can be used to recover a fish protein isolate (FPI) from whole gutted striped bass without prior removal of bones, skin, scales, etc. FPI gels made with nutraceutical additives (ω-3 fatty acids-rich oil, dietary fiber, and salt substitute) along with transglutaminase (TGase) and titanium dioxide (TiO_2) resulted in greater ($p < 0.05$) whiteness, good color properties ($L^*a^*b^*$), and generally better textural properties when compared to surimi gels. The gelation data suggests that the ISP-recovered FPI and surimi develop similar final gel elasticity, but proteins gel differently in FPI and surimi. A reduction of Na content and simultaneous increase ($p < 0.05$) of K content in FPI gels was achieved by the substitution of NaOH with KOH during ISP and NaCl with the KCl-based salt substitute during formulation of the FPI paste. Although cooking and addition of NaCl during formulation of the FPI paste increased ($p < 0.05$) lipid oxidation in FPI gels, TBARS values were much below rancidity levels. These results indicate that KOH can replace NaOH to recover FPI from whole gutted fish for subsequent development of nutraceutical seafood products tailored for reduction of diet-driven cardiovascular disease.

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Notes

The authors declare no competing financial interest.

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