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Gelation of Protein Recovered from Whole Antarctic Krill (*Euphausia superba*) by Isoelectric Solubilization/Precipitation as Affected by Functional Additives

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This study demonstrated that the novel isoelectric solubilization/precipitation can be applied to recover functional muscle protein in a continuous mode from whole Antarctic krill. Protein recovered from whole krill had a much lower ash content than whole krill, suggesting good removal of inedible impurities (shell, appendages, etc.). Lipids were retained to a higher degree with krill protein solubilized at acidic rather than basic pH. The viscoelastic modulus (*G*′) showed that recovered krill protein failed to form heat-induced gel unless beef plasma protein (BPP) was added. Therefore, protease inhibitors are suggested for development of krill-derived products. Even with BPP, the *G*′ decreased between 45 and 55 °C. However, krill protein solubilized at acidic pH had a higher decrease of the *G*′ than the protein solubilized at basic pH, likely due to krill endogenous cathepsin L. Krill protein-based gels developed from protein solubilized at basic pH, especially pH 12.0, had better texture (torsion and Kramer tests and texture profile analysis) than acidic counterparts, possibly due to higher proteolysis and denaturation at acidic pH. Gels made from protein solubilized at acidic pH were brighter and whiter likely due to a higher lipid content.

KEYWORDS: Krill proteins; isoelectric solubilization/precipitation; storage modulus; bovine plasma protein; transglutaminase; texture and color properties

INTRODUCTION

Antarctic krill (*Euphausia superba*) (**Figure 1**) are small, shrimplike crustaceans with probably the largest biomass of any multicellular animal species on earth (*1*). Despite their small size, krill form surface swarms that reach densities of over 1 million animals per cubic meter of seawater; therefore, they are relatively simple for commercial capture (*1*). The animal size ranges from a few millimeters long to 15 cm for the largest deep sea species (*2*). However, only six species of krill are currently harvested commercially (*3*). Antarctic krill plays an important role in the Antarctic marine ecosystems (*4*) and has been shown to be pivotal to the Antarctic food web mainly due to the nutritional value of its protein (*5*).

Nicol and Foster (*6*) reported that the annual capture of Antarctic krill has been estimated at 0.1 million metric tons, while according to the Food and Agriculture Organization, the total annual capture of all fisheries including wild fisheries and aquaculture has been around 130 million metric tons since 2000 (*7*). In addition, the wild fisheries were forecasted as unlikely to increase in the future (*7*). However, the krill biomass has been estimated at 400−1550 million metric tons with sustainable annual harvest at around 70−200 million metric tons a year (*8*). Therefore, krill biomass that could be available for human food is comparable to the biomass of all of the other aquatic species currently harvested by humans.

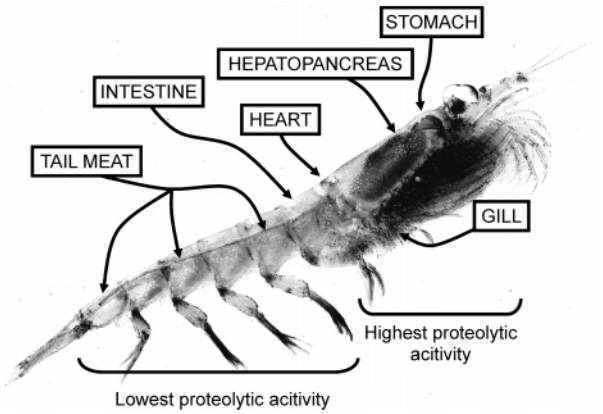

Figure 1. Appearance of krill whose sustainable biomass for human consumption has been estimated to be larger than the global annual human consumption of all other aquatic species.

Food deficiency due to the blooming human population on earth is an ongoing problem. Therefore, it is desirable to seek

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alternative food sources or further increase utilization of existing resources in order to meet human nutritional needs. The biological value (BV) of krill proteins has been reported as higher than the BV of other meat proteins and milk protein (i.e., casein) but lower than the BV of egg proteins (*8*). However, krill has primarily been used by reduction fisheries for the manufacture of fish feeds due to its high asthaxantin content and to a limited extent for human food products (*9*−*11*). Grantham (*12*) reported that krill contains 77.9−83.1% moisture, 0.4−3.6% lipids, 11.9−15.4% protein, and about 2% chitin and glucides. Therefore, considering the tremendous size of the sustainable krill biomass, an efficient protein recovery from krill could bring another source of high quality protein to the human diet. However, the digestive glands of krill produce hydrolytic enzymes such as proteases, carboxypeptidases, nucleases, and phospholipases (*13*). These enzymes are released immediately after the demise of krill and result in rapid autolysis. Autolysis leads to immediate spoilage and is a major hindrance to fully commercial processing of krill and further development of new food products. Therefore, these enzymes combined with the small animal size bring significant challenges for krill processing for human food. This is why most of the harvested krill is currently used as an animal feed.

Meat from krill is typically recovered by mechanical deshelling; however, the recovery yield is extremely low ranging between 10 and 15% by weight of whole krill (*14*). In fact, this type of processing generates significant amounts of byproducts. Suzuki and Shibata (*8*) reported research into the development of surimi from krill. Surimi processing is a commercial process that allows recovery of muscle proteins (primarily myofibrillar) from fish by using large amounts of water to leach impurities from the fish muscle. However, processing of 100 kg of raw fish using surimi technology recovers about 19.5 kg of proteins (i.e., surimi) (*15*). In addition to very active proteases, krill also contains a relatively high concentration of water-soluble proteins (*8*); therefore, surimi technology does not improve the low recovery yield from krill. This is probably why krill surimi has not been commercialized despite the krill abundance. Kolakowski and Gajowiecki (*16*) and Kolakowski and co-workers (*17*) developed a recovery technology based on optimal conditions for krill protein autoproteolysis, followed by thermal coagulation and precipitation of the hydrolysate. While this technology offered greatly improved recovery yields at approximately 80%, the proteolysis of krill proteins and their thermal coagulation resulted in a substantial loss of protein functional properties such as gelation and water-holding capacity (WHC). Likely, the recovered hydrolysate could be successfully applied as a protein additive to food products requiring water solubility; however, it would be highly unlikely to use the hydrolysate as a functional ingredient in the restructured food products requiring good gelation and WHC. Krill, due to its small size, is not destined for direct human consumption and for this purpose should be restructured (*18*).

Beef plasma protein (BPP) inhibits proteolytic activity; and therefore, improves texture of fish protein-based gels (*19*−*21*). Transglutaminase (TGase) is an enzyme catalyzing acyl-transfer reactions, producing nondisulfide covalent ϵ-(γ-glutamyl) lysine cross-links (*22*). 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 (*23*−*25*). The protease inhibitors and exogenous TGase are often used as functional additives in restructured food products and could likely be applied in krill-derived food products.

The isoelectric solubilization/precipitation of protein has been recently applied to fish muscle, which resulted in greatly improved recovery yield when compared to surimi processing (*26*, *27*). The proteins recovered by this novel approach also retain gel-forming ability, a critical functionality to form restructured products (*28*, *29*). Hence, isoelectric solubilization/precipitation of krill proteins may greatly improve the recovery yield of the functional (i.e., gelation and WHC) muscle protein from whole krill, while addition of BPP and TGase may reduce proteolysis of the recovered protein and induce the suwari effect, respectively, during gelation. Value-added restructured food products developed from krill will not only enable the krill industry to diversify its product offerings but also offer another source of highly nutritious protein for human consumption.

The objectives of this study were to (i) determine the feasibility of isoelectric solubilization/precipitation to recover krill muscle protein from whole Antarctic krill; (ii) investigate the effects of functional additives such as BBP, potato starch (PS), exogenous TGase, and polyphosphate (PP) on gelation of muscle protein recovered from Antarctic krill; (iii) compare the textural and color properties of the gels developed from Antarctic krill proteins solubilized at different pH values.

MATERIALS AND METHODS

Raw Material. Frozen whole Antarctic krill (*E. superba*) were obtained from Krill Canada (Langley, BC, Canada). The krill blocks were properly transported overnight to our laboratory in heavily insulated industrial strength boxes filled with dry ice. Upon arrival, the large krill blocks were sawed with a standard meat saw (model SA20-F, Lasar Manufacturing Co., Inc., Los Angeles, CA) while frozen into blocks that were small enough for a single experiment, followed by storage at −80 °C until used. The krill blocks were frozen during sawing in order to minimize autoproteolysis of krill proteins. Prior to an experiment, the smaller blocks were tempered overnight in a refrigerator at 2 °C resulting in partially frozen whole krill. According to AOAC methods (*30*), the average proximate composition of whole Antarctic krill was determined as 81.22% moisture and 12.12% total lipid (dry basis), 76.54% crude protein (dry basis), and 17.36% ash (dry basis).

Recovery of Muscle Proteins From Whole Krill. A processing flowchart for recovery of muscle protein from whole krill is shown in **Figure 2**. Cold (1 °C) distilled and deionized water (ddH_2O) was added to partially frozen whole krill at a 1:3 ratio (krill:water, wt:volume), followed by homogenization in a laboratory blender (model 51BL31, Waring Commercial, Torrington, CT) for 1 min. A 1500 mL amount of the homogenized sample was transferred to a beaker that was placed in ice slush. Because of extreme activity of krill endogenous proteases, the temperature during the entire processing to recover krill muscle protein was carefully controlled at 1−4 °C. To additionally minimize the proteolysis, the protein recovery 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 2.00, 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 krill proteins at acidic as well as 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 was properly calibrated prior to pH adjustment. Once the desired pH was obtained, the solubilization reaction was allowed 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

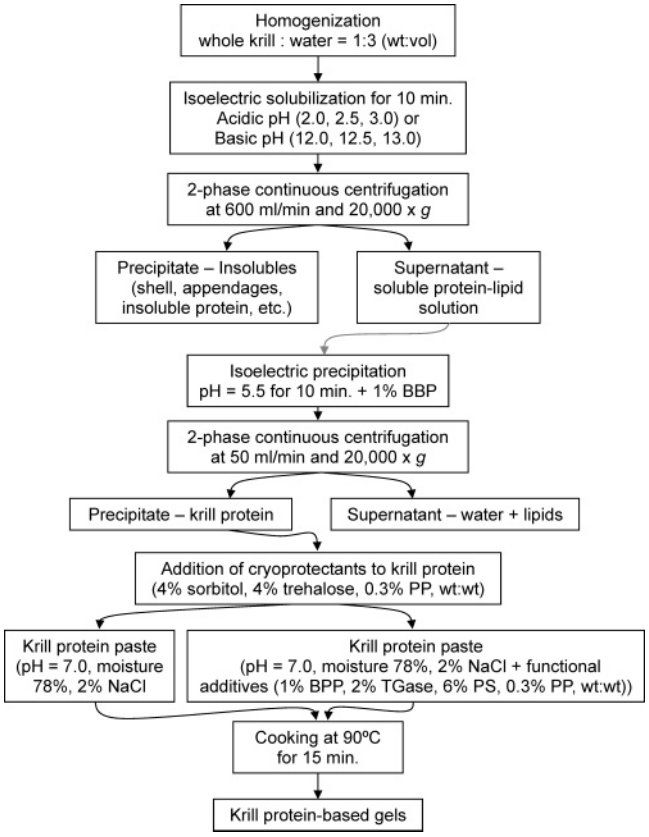

Figure 2. Flowchart for processing of whole krill into krill protein-based gels.

preliminary experiments (data not shown) showed that the flow rate of 600 mL/min resulted in excellent separation of the insolubles (shell, appendages, insoluble protein, etc.) from the supernatant containing a high concentration of krill protein. The protein content in the supernatant was analyzed using Bradford dye-binding method (*31*). 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 either by 10 and 1 N HCl or 10 and 1 N NaOH in order to isoelectrically precipitate krill proteins. The acid was used for the supernatants with krill protein solubilized at basic pH, while base was used for those supernatants at acidic pH. Once the desired pH was obtained, spray-dried BPP (Proliant Inc., Ankeny, IA) was added to the precipitating protein at 1% (wt:wt) of krill protein and the precipitation reaction was allowed for 10 min. The precipitated protein was centrifuged by the continuous centrifuge system (same as described above for separation of the insolubles from solubilized protein solution). However, preliminary experiments (data not shown) showed that the best separation of precipitated protein pellet from water−lipid solution was achieved at a flow rate of 50 mL/min.

The protein isolated from whole krill was mixed with a cryoprotectant mixture [4% D-sorbitol (Sigma Aldrich Inc., St. Louis, MO), 4% crystalline dihydrate trehalose (Cargill Inc., Wayzata, MN), and 0.3% 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 1 day of refrigerated storage (2 °C). The pH of the recovered krill protein was adjusted to 7.00 ± 0.05 with 1 N NaOH. Krill protein prepared in this manner was 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 Whole Krill. Two grams of recovered krill protein was placed on an aluminum dish (Fisher Scientific Co.) and spread evenly across the dish. The moisture content of krill protein was determined by the oven-drying method (100 °C for 18 h) (*30*). The total fat content in krill protein was determined according to the Soxhlet extraction method (*30*). The sample size was 5 g, and extraction with petroleum ether was performed for 16 h at a drip rate of 10 mL/min. The total fat content was determined on a gravimetric basis and expressed as percent (dry weight basis). Crude protein was determined by Kjeldahl assay (*30*) and expressed as percent (dry weight basis). Ash content was performed by incinerating a krill protein sample in a muffle furnace at 550 °C for 24 h (*30*) and expressed as percent (dry weight basis). All proximate analyses of krill protein were reported as the mean value of at least three replicates.

Development of Krill Protein-Based Gels. The recovered krill protein was divided into two parts (i.e., experimental treatments). NaCl at 2% (wt:wt) was added to both parts, but functional additives [1% BPP (wt:wt), 2% exogenous TGase (wt:wt) (Avtiva RM, Ajinomoto USA Inc., Teaneck, NJ), 6% PS (wt:wt) (Penbind 1000 modified potato startch, Penford Food Ingredients Corp., Centennial, CO), and 0.3% PP (wt:wt)] were only added to one part. The krill protein-based gels for both experimental treatments (i.e., with and without functional additives) were developed separately by using the procedure as described by Jaczynski and Park (*32*). The recovered krill protein mixed with cryoprotectants was chopped in a universal food processor (model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min, resulting in a krill protein paste. Salt (2%, wt:wt) was added, and the krill protein paste was chopped at low speed for 0.5 min. The functional additives were added only to one experimental treatment group, while the functional additives were not added to the other group. The 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. The final pH of 7.00 ± 0.05 of the krill paste was verified with a pH meter and adjusted as needed. During chopping, the temperature of krill paste was controlled between 1 and 4 °C.

Following chopping, krill paste was stuffed into (i) stainless steel tubes (length = 17.5 cm, inner diameter = 1.9 cm) for determination of color and texture properties with tristimulus color values ($L^*a^*b^*$), Kramer test, and texture profile analysis (TPA) and (ii) dumbbell premolded stainless steel torsion tubes (length = 17.5 cm, end diameter = 1.9 cm, and 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 krill paste with TGase were refrigerated (4 °C) for 24 h prior to heating in order to allow development of nondisulfide covalent bonds. Careful attention was paid to the torsion gel samples to avoid "skin" formation on the torsion tubes joints. 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 krill paste was tested to determine gel-forming ability using oscillatory rheology with a Bohlin rheometer (Bohlin CVOR 200, Malvern Instruments Ltd., Worcestershire, United Kingdom). At least three samples of krill 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 (*20*, *28*, *29*).

Texture and Color Properties of Krill Protein-Based Gel. The recovered krill protein without the functional additives failed to gel; therefore, the texture and color properties were not determined. Three different methods were employed to determine texture, torsion test, Kramer shear, and TPA. Although these three texture measurements are commonly employed for determination of texture properties, each method provides slightly different information. The torsion test is considered a fundamental test for texture, while Kramer shear and TPA are empirical tests. Likely, the most comprehensive understanding of textural properties is provided by a combination of the fundamental and empirical tests. Therefore, we employed these three different tests in our experiments. The torsion test of cooked krill protein-based gels with the functional additives was performed according to Jaczynski and Park (*32*). 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.

At least eight cylindrical gels (length = 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. 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 cross-head speed and expressed as maximum peak force (g peak force/g of gel sample).

TPA of cooked krill protein-based gels was performed according to Cheret and co-workers (*33*). The gel samples at room temperature were subjected to two-cycle compression at 50% using the texture analyzer (model TA-HDi, Texture Technologies Corp.) with a 70 mm TPA compression plate attachment moving at a speed of 127 mm/min. From the resulting force−time curves, hardness, springiness, cohensiveness, gumminess, chewiness, and resilience were determined. The definitions of the TPA parameters are as follows: (i) Hardness indicates the maximum force required to compress a sample; (ii) springiness indicates the ability of a sample to recover its original form after the deforming force is removed; (iii) cohesiveness corresponds to the extent to which the sample can be deformed before rupture; (iv) gumminess is the force required to disintegrate a semisolid sample to a steady state of swallowing (hardness × cohesiveness); (v) chewiness is related to the work needed to chew a solid sample to a steady state of swallowing (springiness × gumminess); and (vi) resilience shows 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.

The color properties of cooked krill 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 were determined. The whiteness of gels was calculated using $L^* - 3b^*$ (*34*). At least 20 cylindrical gels (length = 2.54 cm, diameter = 1.90 cm) per treatment were used for color measurement.

Statistical Analysis. The experiment was conducted using a completely random design (*35*). Data were analyzed using analysis of variance (*36*). A significant difference was used at 0.05 probability level, and differences between treatments were tested using the least significant difference (LSD) test (*37*). All statistical analyses of data were performed using SAS (*36*).

RESULTS AND DISCUSSION

Proximate Analysis of Protein Recovered from Whole Krill. Following protein recovery from whole Antarctic krill and addition of cryoprotectants (**Figure 2**), a proximate analysis was performed to determine % moistrure, total fat, crude protein, and ash content (**Table 1**). Because whole animals (including shell, appendages, and other inedible impurities) were used, it was of our interest to assess how protein recovery processing could remove these impurities. Ash content is a relatively good indicator that can be used to estimate how these impurities are retained in the recovered protein fraction. **Table 1** shows that lower ash content was obtained in the recovered krill protein than that in whole krill. On average, regardless of the pH treatment, the recovered krill protein contained approximately 5% of ash as compared to about 17% in the whole animal. Therefore, processing of whole krill to recover krill protein by using acid or basic treatments followed by centrifugation and isoelectric precipitation removes most of the inedible impurities. Kolakowski and Gajowiecki (*16*) and Kolakowski and co-workers (*17*) also used centrifugation as a means to remove the inedible impurities during processing of whole krill based on krill autoproteolysis. The removal of impurities reported by those investigators was similar to ours. The ash content was highest in proteins recovered with the 2.0 and 13.0 pH treatments (**Table 1**). These most extreme pH treatments in our experiments might have solubilized some of the minerals from krill, which resulted in higher ash content than other treatments.

Table 1. Proximate Analysis[a] of Krill Protein That Was Solubilized at Different pH Values and Precipitated at pH 5.50[b]

		% dry basis		
treatment (pH value)	moisture (%)	lipid	protein	ash
2.0	78.19 ± 0.24 a	11.58 ± 0.51 b	49.92 ± 0.23 b	5.98 ± 0.18 a
2.5	74.29 ± 0.27 b	12.47 ± 0.88 b	50.32 ± 0.90 b	4.32 ± 0.20 c
3.0	74.04 ± 0.87 b	19.02 ± 1.23 a	56.57 ± 0.92 a	4.01 ± 0.01 c
12.0	74.27 ± 0.27 b	8.09 ± 1.46 c	54.35 ± 1.06 a	4.88 ± 0.08 b
12.5	74.23 ± 0.35 b	7.22 ± 1.47 c	49.77 ± 1.81 b	5.71 ± 0.05 a
13.0	73.64 ± 0.47 b	7.47 ± 0.43 c	50.09 ± 0.26 b	5.74 ± 0.02 a

[a] Data are given as means ± differences (least squared difference test; $P < 0.05$). [b] The cryoprotectants (4% sorbitol, 4% trehalose, and 0.3% phosphate; wt:wt) were added following protein precipitation, and then, the proximate analysis was performed.

When fish muscle protein is solubilized by acid or basic treatments followed by centrifugation and isoelectric precipitation, most sarcoplasmic proteins can be recovered in addition to the myofibrillar proteins. Therefore, this technique results in higher protein recovery than the conventional surimi process (*29*, *38*). In addition, according to Hultin and Kelleher (*26*) and Hultin and co-workers (*27*), this technique allows significant reduction of concentration of fish lipids in the recovered protein. Adjusting the pH of the krill homogenate to 2.0 resulted in the highest ($P < 0.05$) moisture content of recovered krill protein as compared to other pH treatments, while recovered krill protein from acid treatments had higher ($P < 0.05$) lipid contents than those from alkaline treatments (**Table 1**). However, both basic and acidic pH treatments resulted in a relatively high retention of lipids in the protein recovered from whole krill. We used a liquid/solid two-phase continuous centrifuge that following protein solubilization separated the insolubles (shell, appendages, etc.) from the protein−lipid solution. Following protein precipitation at pH 5.50, the same centrifuge was used to separate the precipitated protein from the water−lipid solution. However, the lipids were finally separated from water by decanting following their flotation to the top. Cod muscle protein undergoes reversible unfolding when subjected to pH 11.0 and 2.5, followed by refolding when the pH is adjusted to neutral (*28*). These phenomena are accompanied by increased surface hydrophobicity of the cod muscle protein (*28*). In addition, about 65% of fatty acids in krill are incorporated into phospholipids, while most of the fatty acids in fish are incorporated into apolar triglycerides (*39*, *40*). Phoshpolipids due to their amphiphilic characteristics have emulsifying properties, which allow them for interactions with apolar triglycerides and charges molecules such as water and proteins. Therefore, as the krill proteins were unfolding during protein solubilization, they also increased surface hydrophobicity due to exposure of hydrophobic regions of the protein that had been buried in the protein interior prior to protein solubilization. This increased hydrophobicity likely resulted in a formation of hydrophobic interactions between krill protein and triglycerides along with the phoshpolipids. During subsequent separation, only the insolubles (shell, appendages, etc.) were separated from protein−lipid solution by the liquid/solid centrifuge and the lipids were retained by the solubilized protein phase. As the krill protein was refolding approaching

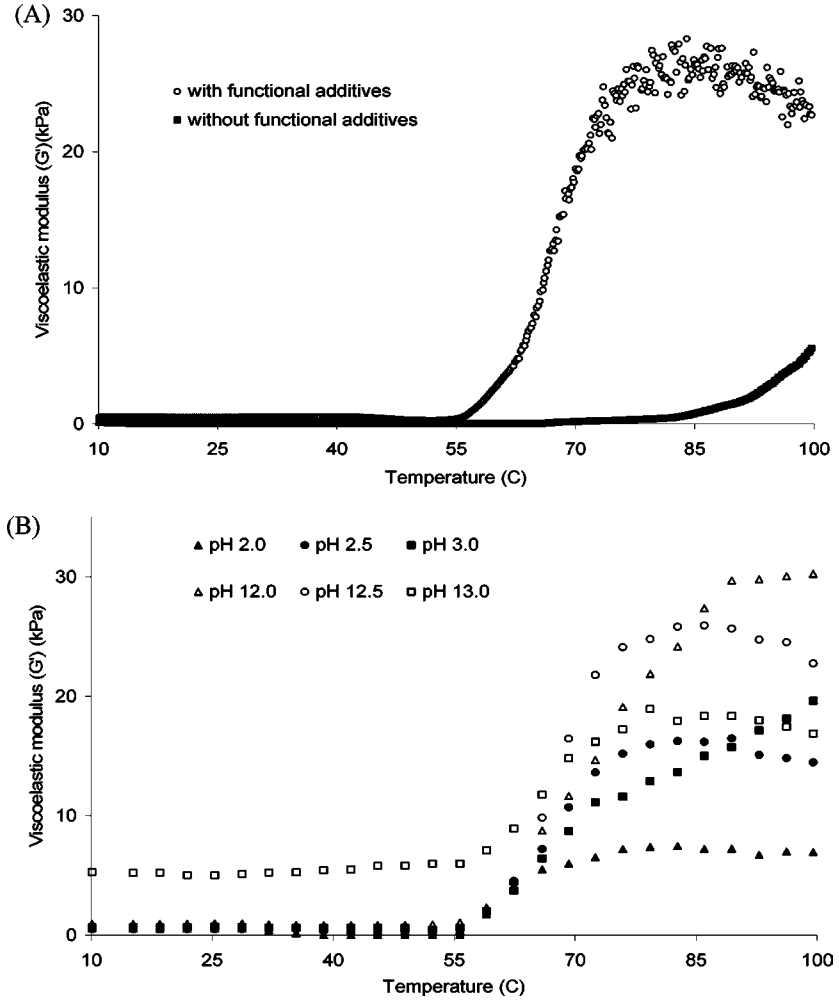

Figure 3. Viscoelastic modulus (*G*′) of krill protein as affected by (**A**) functional additives (1% BPP, 2% TGase, 6% PS, and 0.3% PP) and (**B**) different pH treatments during solubilization. Krill protein in **A** was solubilized at pH 12.5. All of the krill protein samples in **B** contained functional additives. The pH of krill protein samples in both **A** and **B** was adjusted to 7.0 prior to the measurement.

pH 5.5 during the precipitation step, the lipids were probably trapped in the interior of the refolding protein due to interactions between the protein's hydrophobic regions and the lipids that had been formed at basic or acidic pH during the solubilization step. Subsequently, the precipitated krill protein was separated from the water−lipid solution by the liquid/solid centrifuge. Therefore, the difference between krill and fish lipid composition along with different separation technique may partly explain the lipid retention in the recovered krill protein. In addition, acidic treatment resulted in higher lipid retention in the recovered krill protein than the basic treatment (**Table 1**). During oil processing to obtain a soapstock, free fatty acids are more readily removed with alkaline processing than acidic treatment (*41*), which may explain why lower lipid contents were obtained with basic treatment during our krill processing.

Bunea and co-workers (*42*) investigated the effect of krill and fish oil on hyperlipidemia using human subjects. These authors found that krill oil significantly reduced triglycerides and low-density lipoproteins (LDLs), while increasing the high-density lipoproteins. However, the fish had no effect on triglycerides and LDL. Krill oil also resulted in higher reduction of cholesterol than the fish oil. Bunea and co-workers (*42*) concluded that krill oil was more effective in terms of improving human blood lipids and lipoproteins than fish oil. Although retention of krill lipids in the recovered protein may cause oxidative changes if proper antioxidative strategies (antioxidants, vacuum packaging, temperature, etc.) are not implemented, the human cardiovascular health benefits of the resultant krill food products may be a very important attribute. Therefore, the retention of krill lipids in the recovered protein from whole krill should be considered beneficial.

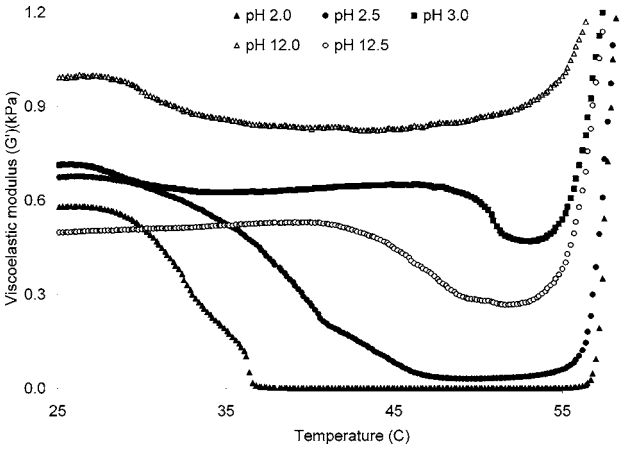

Figure 4. Decrease in viscoelasticity of krill protein at a temperature range between 35 and 55 °C. The pH of krill protein samples was adjusted to 7.0 prior to the measurement, and all of the samples contained functional additives.

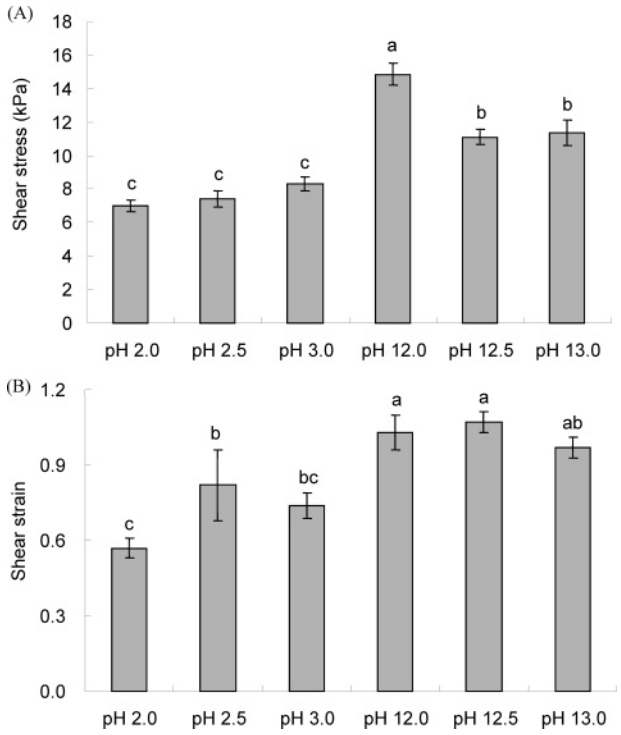

Figure 5. Shear stress (**A**) and strain (**B**) of krill protein-based gels developed from krill protein solubilized at different pH values. All of the gels contained functional additives. For shear stress and strain, mean values in each pH value with different letters were significantly different (least squared difference test, $P < 0.05$, $n = 6$).

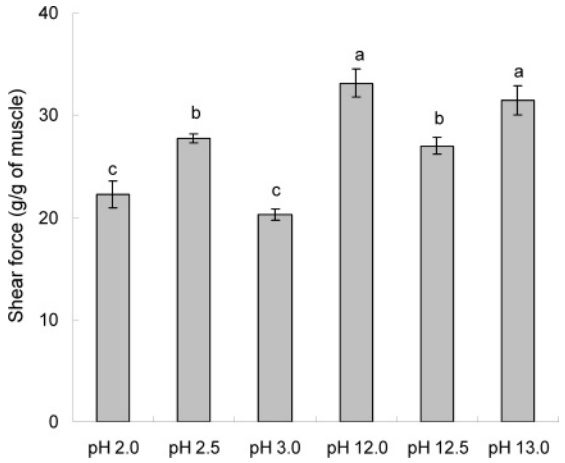

Figure 6. Kramer shear force of krill protein-based gels developed from krill protein solubilized at different pH values. All of the gels contained functional additives. For shear force, mean values in each pH value with different letters were significantly different (least squared difference test, $P < 0.05$, $n = 8$).

Dynamic Rheology Properties of Krill Proteins. Viscoelastic properties of the protein recovered from whole krill are shown in **Figure 3**. Linear heating of krill protein-based paste resulted in a typical rheogram as other meat systems (*25*, *43*, *44*). **Figure 3A** shows that the typical gelation pattern occurred only when the functional additives were added to the krill protein. The viscoelastic modulus (G') started a significant increase at approximately 56 °C. When the additives were not present, the krill protein failed to gel. Therefore, addition of BPP or other protease inhibitor to the recovered krill protein seems necessary for proper gel development. The BPP has been shown to inhibit proteolysis of fish muscle protein; therefore, BPP enhances gelation of surimi recovered from protease-laden species (*44*−*48*). It has also been demonstrated that addition of exogenous TGase enhances gelation of surimi-based gels (*25*). Therefore, the failure of krill protein to form a gel when the functional additives were absent was likely due to the hydrolytic breakdown of krill protein by krill endogenous proteases.

Figure 3B shows viscoelastic properties of krill proteins that were solublized at different pH values followed by precipitation at pH 5.5. Because the addition of protease inhibitor to krill proteins seems necessary to suppress proteolysis, the BPP was added to all of the krill protein-based pastes presented in **Figure 3B**. The viscoelastic modulus (G') for all of the krill protein pastes started increasing at about 55−60 °C. The krill pastes recovered from basic treatments had higher G' values when compared to those from acid treatments, indicating better gelation of protein recovered with basic treatments. Kristinsson and co-workers (*48*) suggested that the alkali-aided processing led to less denaturation than the acid-aided process. Therefore, krill protein solubilized at basic pH likely experienced less denaturation, resulting in higher G'.

Figure 4 shows viscoelastic properties of the recovered krill protein at temperatures up to 60 °C. The functional additives were added to all of the samples presented in **Figure 4**. Despite the presence of BPP in the paste, a significant decrease of G' occurred between 45 and 55 °C, likely indicating proteolytic activity. However, the decrease of the G' for krill proteins that were solubilized at acidic pH was more profound than that for the proteins at basic pH. Kubota and Sakai (*49*) determined that the optimal temperature for krill autolysis was 45−50 °C. Kawamura and co-workers (*50*) concluded that the main proteases responsible for the autolysis of krill were cathepsins (B, H, and L) and trypsinlike proteases at acidic pH and neutral pH, respectively. Choi and Park (*51*) suggested that the acid-aided processing enhanced the cathepsin activity in Pacific whiting, whereas the alkali-aided processing significantly reduced this activity, particularly cathepsin type L (*52*). The lysosomal cathepsin types B and H are removed from fish muscle by extensive washing during surimi manufacture; however, cathepsin L is retained in surimi (*53*). Although less water is used with isoelectric solubilization/precipitation than conventional surimi processing, cathepsin L seems to have been retained with the krill protein recovered in our experiments similarly to surimi processing (*53*). 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 (*54*). This is probably why krill protein solubilized at acidic pH was degraded by cathepsin L to a higher degree than at basic pH, resulting in more pronounced decrease of G' between 45 and 50 °C. Therefore, efficient protease inhibitors will have to be added to krill protein recovered with isoelectric solubilization/precipitation. Alternatively, gelation of krill protein could likely be induced with ohmic, radio frequency, or microwave heating (*21*, *55*, *56*). These heating techniques offer heating rates that would probably be sufficiently rapid to counteract heat-induced texture degradation between 35 and 55 °C of krill protein-based food products.

Texture and Color Properties of Krill Protein-Based Gel. Krill paste without functional additives failed to gel; therefore, texture and color data were not determined. The shear stress and strain of krill protein-based gels are shown in **Figure 5**. The gels developed from krill protein recovered from basic treatments had higher ($P < 0.05$) shear stress and strain values

Table 2. TPA[a] of Krill Protein-Based Gels Developed from Krill Protein Solubilized at Different pH Values[b]

treatment (pH value)	hardness (N)	springness	cohensiveness (N)	gumminess (N)	chewiness (N)	resilience
2.0	2.90 ± 0.14 e	0.82 ± 0.02 a	0.41 ± 0.03 ab	1.21 ± 0.10 d	1.02 ± 0.11 cd	0.19 ± 0.01 c
2.5	3.88 ± 0.09 d	0.79 ± 0.02 a	0.44 ± 0.02 a	1.69 ± 0.11 bc	1.40 ± 0.12 bc	0.22 ± 0.01 ab
3.0	3.77 ± 0.11 d	0.81 ± 0.02 a	0.43 ± 0.02 a	1.61 ± 0.11 c	1.33 ± 0.12 bcd	0.21 ± 0.01 bc
12.0	6.88 ± 0.19 a	0.84 ± 0.02 a	0.47 ± 0.02 a	3.29 ± 0.21 a	2.79 ± 0.24 a	0.25 ± 0.01 a
12.5	4.77 ± 0.10 b	0.79 ± 0.02 a	0.42 ± 0.01 a	2.02 ± 0.01 b	1.63 ± 0.12 b	0.20 ± 0.01 bc
13.0	4.27 ± 0.14 c	0.64 ± 0.04 b	0.36 ± 0.02 b	1.33 ± 0.16 d	0.93 ± 0.17 d	0.14 ± 0.01 d

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

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

treatment (pH value)	L^*	a^*	b^*	whiteness $L^* - 3b^*$
2.0	96.21 ± 0.42 b	0.09 ± 0.13 bc	2.04 ± 0.18 cd	90.08 ± 0.57 ab
2.5	98.17 ± 0.27 a	0.37 ± 0.13 b	1.69 ± 0.32 d	92.64 ± 0.98 a
3.0	98.17 ± 0.24 a	–0.19 ± 0.16 c	2.08 ± 0.23 cd	91.93 ± 0.81 a
12.0	95.80 ± 0.30 bc	0.30 ± 0.08 bc	2.47 ± 0.14 c	88.38 ± 0.52 b
12.5	94.89 ± 0.32 cd	0.91 ± 0.14 a	4.43 ± 0.25 a	81.61 ± 1.02c
13.0	94.20 ± 0.58 d	0.51 ± 0.32 ab	3.47 ± 0.43 b	83.79 ± 1.51 c

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

than the gels from acidic treatments, suggesting better gel quality. Kristinsson and Liang (*29*) speculated that higher solid and protein contents partially contribute higher shear stress and strain values of Atlantic croaker surimi. Although the average protein and ash contents in the recovered krill protein (dry basis) in our study were not different between acidic and basic treatments (**Table 1**), the results of torsion test were in agreement with the rheogram (**Figure 3B** vs **5**). **Figure 4** also shows that despite addition of BPP, the proteins solubilized at acidic pH were degraded to a higher degree likely by krill endogenous proteases than those at basic pH. This higher degradation likely contributed to poorer texture of gels developed from krill proteins solubilized at acidic pH (**Figure 5**). In addition, Kristinsson and co-workers (*48*) suggested that the alkali-aided processing led to less denaturation than the acid-aided process. The lower denaturation of krill proteins solubilized at basic pH might also have contributed to better texture.

A similar trend was also observed for Kramer shear force determined for krill protein-based gels shown in **Figure 6**. Overall, the gels developed from krill proteins that were solubilized at basic pH resulted in stronger gel than those solubilized at acidic pH. A TPA of the krill protein-based gels is summarized in **Table 2**, and the TPA parameters (hardness, springiness, cohesiveness, gumminess, chewiness, and resilience) have been defined according to Cheret and co-workers (*33*). The higher ($P < 0.05$) hardness of krill protein-based gels developed from krill protein that was solubilized at basic pH was in agreement with the rheogram (**Figure 3B**), torsion test (**Figure 5**), and Kramer shear test (**Figure 6**). In summary, of the six TPA parameters (**Table 2**), krill protein-based gels developed from krill protein that was solubilized at basic pH exhibited better texture properties than the gels made from protein solubilized at acidic pH. Within the basic pH treatment (**Table 2**), solubilization at pH 12.0 resulted in krill proteins that yielded the highest gel quality. This highest gel quality at pH 12.0 was likely due to the least protein denaturation (*48*) and enzymatic proteolysis (**Figure 4**) as compared to other pH treatments.

The brighter (L^*) ($P < 0.05$) and whiter as well as less red and yellow gels were obtained from acid than basic treatments (**Table 3**). It has been reported that addition of a vegetable oil to surimi resulted in brighter (L^*) and whiter ($L^* - 3b^*$) surimi seafood (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 (*57*). It is likely, therefore, that the krill protein-based gels developed from krill proteins solubilized at acidic pH exhibited whiter and brighter color due to higher krill oil content (**Table 1**).

In conclusion, functional protein can be recovered from whole Antarctic krill by isoelectric precipitation/solubilization in a continuous mode. However, protein degradation, likely due to endogenous krill proteases, necessitates addition of protease inhibitors during heat-induced gelation. In addition, functional additives such as exogenous TGase, starch, and phosphates enhance textural properties of krill protein-based gels. Protein denaturation during solubilization at acidic pH values occurs to a higher extent than at basic pH; therefore, the gels developed from krill protein solubilized at basic pH have a firmer texture than the acidic counterparts. The protein recovered from krill by isoelectric precipitation/solubilization retains a relatively high amount of krill lipid; therefore, the krill protein-based gels have high whiteness ($L^* - 3b^*$) values.

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