LWT - Food Science and Technology 48 (2012) 89–95

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Functional food products made from fish protein isolate recovered with isoelectric solubilization/precipitation

Reza Tahergorabi, Sarah K. Beamer, Kristen E. Matak, Jacek Jaczynski*

Animal and Nutritional Sciences, PO Box 6108, West Virginia University, Morgantown, WV, USA

ARTICLE INFO

Article history:
Received 31 August 2011
Received in revised form
17 February 2012
Accepted 20 February 2012

Keywords:
Protein isolates
Isoelectric solubilization/precipitation
Functional food
Value-added food
Product development
Omega-3 polyunsaturated oil
Physicochemical properties

ABSTRACT

Isoelectric solubilization/precipitation (ISP) allows efficient recovery of fish protein isolate (FPI) that could be used in functional foods. There is an increasing interest in incorporating ω-3 polyunsaturated fatty acids (PUFAs) oils in food with a simultaneous sodium reduction. FPI was recovered from whole gutted trout using ISP. FPI was used as a main ingredient in heat-set gels made with ω-3 PUFAs oils (flaxseed, algae, fish, krill, and blend) and KCl-based salt substitute. The objectives were to determine (1) protein gelation, (2) color and texture, and (3) sodium and potassium content of the developed functional food (i.e., heat-set gels). Color properties were improved except when krill or algae oil was added. Texture profile analysis showed that ω-3 PUFAs generally did not affect texture of trout protein gels. The addition of ω-3 PUFAs oil improved heat-induced protein gelation as demonstrated by dynamic rheology. Elastic modulus increased when ω-3 PUFAs oil was added except krill oil. Salt substitute resulted in reduced sodium and increased potassium content in the heat-set gels. The functional food products developed from FRI were nutritionally enhanced with ω-3 PUFAs, had reduced sodium and increased potassium; while the color and texture properties were good and gelation properties were improved.

1. Introduction

Isoelectric solubilization/precipitation (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. (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011). Muscle proteins from fish have thus far been recovered at the laboratory- and pilot-scale ISP using a batch mode (Choi & Park, 2002; Kim, Park, & Choi, 2003; Kristinsson & Hultin, 2003; Mireles DeWitt, Nabors, & Kleinholz, 2007; Undeland, Kelleher, & Hultin, 2002). The ISP processing has been applied to beef and fish processing by-products (Chen & Jaczynski, 2007a, b; Mireles DeWitt, Gomez, & James, 2002). Most recently, ISP processing has been used to recover muscle proteins from chicken meat processing by-products (Tahergorabi, Beamer, Matak, & Jaczynski, 2011a; Tahergorabi, Sivanandan, & Jaczynski, 2012). The ISP processing allows high protein recovery yields from such difficult to process sources as fish/chicken/beef processing by-products (Chen & Jaczynski, 2007b; Taskaya, Chen, Beamer, & Jaczynski, 2009). Recovered proteins retain functional properties and nutritional value (Chen, Tou, & Jaczynski, 2007, 2009; Gigliotti, Jaczynski, & Tou, 2008; Nolsoe & Undeland, 2009; Taskaya, Chen, Beamer, Tou, & Jaczynski, 2009; Taskaya, Chen, & Jaczynski, 2009). Due to extreme pH shifts during ISP, this processing technology also results in mild, non-thermal pasteurization (Lansdowne, Beamer, Jaczynski, & Matak, 2009a, b). Dioxin and polychlorinated biphenyls (PCBs) are significantly reduced in the ISP-recovered fish proteins (Marmon, Liljelind, & Undeland, 2009). In summary, ISP processing offers several advantages over mechanical processing and may be a useful technology to recover functional and nutritious proteins from whole gutted fish (i.e., without filleting) or fish processing by-products (i.e., frames, heads, etc.) for subsequent development of food products. Although ISP processing offers efficient recovery of high quality fish protein, there have been no successful food products developed from the ISP-recovered protein.

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), particularly eicosapentaenoic (EPA, 20:5 ω-3) and docosahexaenoic (DHA, 22:6 ω-3) FAs have been correlated with improved cardiovascular health in humans. However, they are highly susceptible to lipid oxidation leading to rancidity (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006, 2007; Lee, Faustman, Djordjevic, Faraji, & Decker, 2006; Nicholas, Petrie, & Singh, 2010). Seafood is naturally rich in these essential nutrients. It has been estimated that the Western diet is deficient in ω-3 PUFAs with the ω-6/ω-3 ratio at 15–20/1, instead of the suggested 1/1. Elevated blood pressure caused by excessive

* Corresponding author. Tel.: +1 304 293 1893.
E-mail address: Jacek.Jaczynski@mail.wvu.edu (J. Jaczynski).

0023-6438/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2012.02.018

dietary sodium intake (i.e., with food) also contributes to poor cardiovascular health. Therefore, in order to improve cardiovascular health, health and professional organizations recommend increased consumption of food rich in ω-3 PUFAs (Simopoulos, 2002); while the American Heart Association, Centres for Disease Control and Prevention, and Food and Drug Administration have been working on sodium reduction in processed food products by 50%. There is an increasing interest in the fortification of food products with ω-3 PUFAs and concurrent sodium reduction.

Functional foods are food products that contain added, technologically developed ingredients with specific health benefits. The food products nutrified with ω-3 PUFAs provide a means to achieve desired biochemical effects of these nutrients without the ingestion of dietary supplements, medications or a major change in dietary habits. Since the protein isolate in the present study is recovered from fish and used in a formulated food product, it is a logical vehicle for increasing the consumption of ω-3 PUFAs and concurrent reduction of sodium without the need for dietary supplements in a pill or capsule form.

The overall objective of this study was to determine physicochemical properties of a functional food products developed with ω-3 PUFAs and salt substitute using muscle protein isolate recovered by ISP processing from whole gutted rainbow trout (bone-in, skin- and scale-on). The specific objectives were to determine: (1) protein gelation properties, (2) color and texture properties, and (3) sodium and potassium content of the developed functional food products.

2. Materials and methods

2.1. Sample preparation and protein recovery

Whole gutted rainbow trout (bone-in, skin- and scale-on) were purchased from a local aquaculture farm. The fish were the input material for the isoelectric solubilization/precipitation (ISP) processing to recover muscle protein isolate.

A processing flowchart for the recovery of muscle protein isolates from whole gutted rainbow trout and subsequent development of heat-set protein gels is shown in Fig. 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, g:mL) using a laboratory homogenizer (PowerGen 700, Fisher Scientific, Fairlawn, NJ) set at speed five for five minutes. 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 aliquot of the trout homogenate was transferred to a beaker and the pH was adjusted to 11.50 ± 0.05 with 5 and 0.5 mol L^{-1} NaOH (Chen & Jaczynski, 2007a, b). The 5 and 0.5 mol L^{-1} 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, and bottom – insolubles (bones, skin, scale, insoluble proteins, membrane lipids, etc.).

The middle layer was collected and the pH was adjusted to 5.50 ± 0.05 by 5 and 0.5 mol L^{-1} HCl to isoelectrically precipitate trout muscle proteins. Once the desired pH was obtained, the precipitation reaction was allowed for 10 min. The solution with precipitated proteins was de-watered by centrifugation as above. The centrifugation resulted in two layers: top – process water, and bottom – precipitated and de-watered trout muscle protein isolate. The precipitated and de-watered protein isolate was collected and used in the development of heat-set gels.

2.2. Preparation of fish protein paste

Fish protein pastes were made using the procedure described by Jaczynski and Park (2004). The recovered protein isolate was chopped in a universal food processor (Model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. A fish protein paste was obtained by extracting myofibrillar proteins with 0.34 mol L^{-1} of KCl using a KCl-based salt substitute (AlsoSalt® sodium-free salt substitute, AlsoSalt, Maple Valley, WA) (hereafter called salt substitute) 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 (Tahergorabi, Beamer, Matak, & Jaczynski, in press). The salt substitute contained 68 g/100 g of KCl and L-lysine mono-hydrochloride and calcium stearate. The concentration of 0.34 mol L^{-1} of KCl added to the recovered fish protein isolate paste is equivalent to 20 g of NaCl per 1000 g batch. According to the manufacturer, the patented L-lysine derivative masks the metallic-bitter aftertaste of KCl.

Final moisture content of the fish protein paste was adjusted to 68 g/100 g by adding functional additives at the following final concentrations: 10 g/100 g of a ω-3 PUFAs-rich oil (see below), 3.7 g/100 g of crab flavor (F-11019, Activ International, Mitry-Mory Cedex, France), 2 g/100 g of potato starch (PS) (Penbind 1000 modified potato starch, Penford Food Ingredients Corp., Centennial, CO), 0.5 g/100 g of microbial transglutaminase (MTGase) (Activa RM, Ajinomoto USA Inc., Teaneck, NJ), and 0.3 g/100 g of polyphosphate (PP) (Kena FP-28, Innophos, Cranbury, NJ). The above levels of functional additives were found in previous studies as optimal for gelation of fish muscle protein isolate and consequently texture development as well as closely resembling commercial surimi-based seafood products (Park, 2005; Perez-Mateos, Boyd, & Lanier, 2004; Taskaya, Chen, & Jaczynski, 2010). A 0.5 g/100 g of titanium dioxide (TiO_2) [Titanium (IV) oxide, Sigma–Aldrich, Inc., St. Louis, MO] was also added to the paste (Tahergorabi et al., 2011a, 2012). Up to 1 g/100 g of TiO_2 is commonly added to food products as a whitening agent. The PS, MTGase, TiO_2, and PP were in a dry powder form. The crab flavor was a water-soluble liquid. The ω-3 PUFAs-rich oils (see below) were added at 10 g/100 g by replacing ice/water (1:1) that is normally added to a fish protein-based paste such as surimi paste. One treatment without added oil was used as a control. The final moisture content of the control paste was adjusted to 78 g/100 g by adding ice/water to the paste (Park, 2005; Perez-Mateos et al., 2004). Chopping at low speed for 1 min was applied to mix all of the ingredients with the fish protein paste. Addition of TiO_2 to a protein paste results in poor quality of heat-set gel due to the pH lowering effect of TiO_2 (Park, 2005). Therefore, the final pH of the paste in the present study was adjusted to 7.20 ± 0.05 (Tahergorabi et al., 2011a; Taskaya et al., 2010). Additional chopping was performed at high speed under vacuum (50 kPa) for the last 3 min. The paste temperature was controlled between 1 and 4 °C during chopping. Fish protein pastes were prepared in 1 kg batches.

The following ω-3 PUFAs-rich oils were added to the fish protein paste:

1) Flaxseed oil was obtained from Jedwards International, Inc. (Quincy, MA).

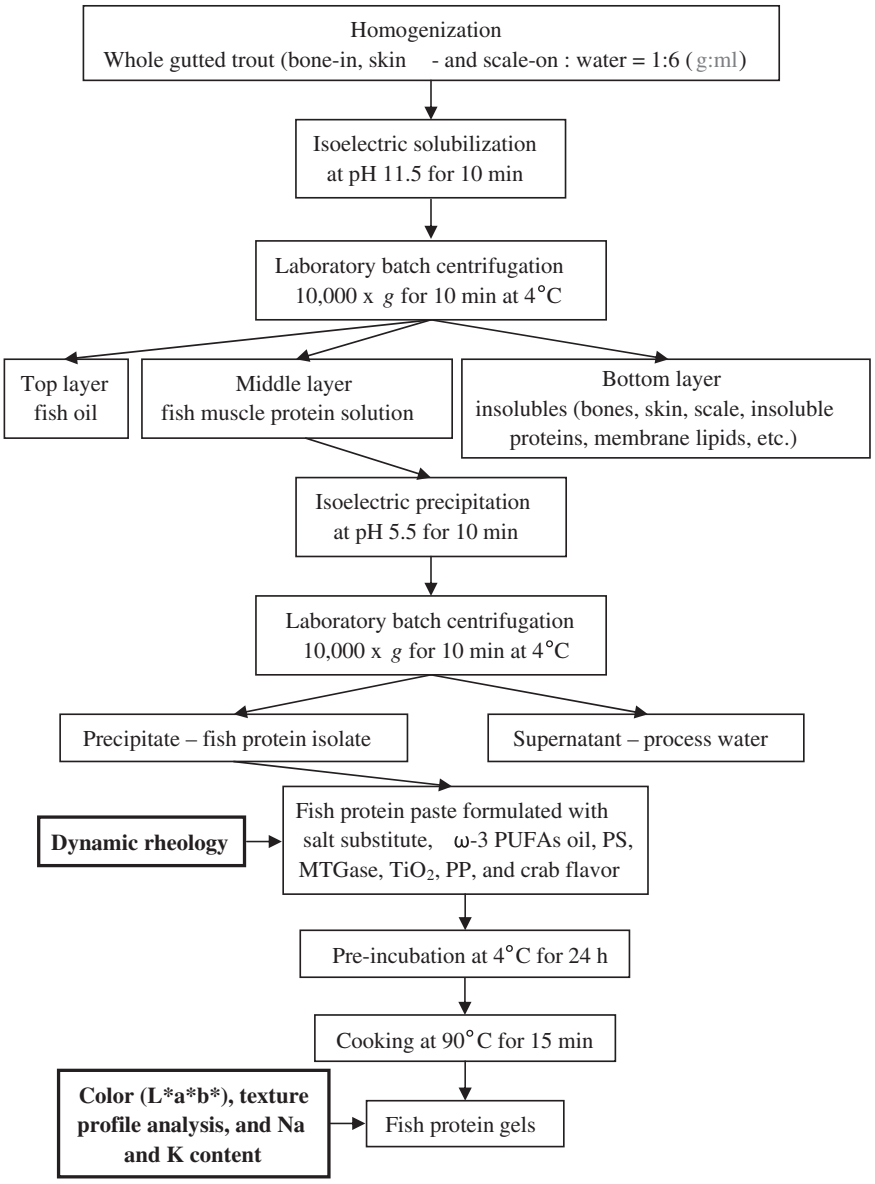

Fig. 1. A flowchart for recovery of fish protein isolates using isoelectric solubilization/precipitation (ISP) and subsequent development of fish protein gels. The gels were formulated to contain 68 g/100 g moisture, 0.34 M salt substitute, 10 g/100 g ω-3 PUFAs oil (flaxseed, algae, fish, krill, or blend), 2 g/100 g potato starch (PS), 0.5 g/100 g microbial transglutaminase (MTGase), 0.5 g/100 g titanium dioxide (TiO_2) 0.3 g/100 g polyphosphate (PP), and 3.7 g/100 g crab flavor. The paste pH was adjusted to 7.2.

2) Algae oil (DHAS) was obtained from Martek Biosciences (Columbia, MD).
3) Fish oil (Omega Pure 8042TE) was obtained from Omega Pure (Reedsville, VA).
4) Krill oil (4225F) was obtained from Enzymotec USA, Inc. (Springfield, NJ).
5) Blend (Flaxseed:Algae:Fish, 8:1:1).

When oil is added to a comminuted protein-based paste, it results in light scattering, and therefore, improves whiteness of cooked gels (Park, 2005). This is why 10 g/100 g of ω-3 PUFAs-rich oil was added to the paste.

The trout protein paste prepared in this manner was immediately used for dynamic rheology tests to determine elastic modulus (G'). Pastes prepared in this manner were also used to develop heat-set fish protein gels for evaluation of color (tristimulus color values, $L^*a^*b^*$) and texture (texture profile analysis, TPA) as well as sodium and potassium content.

2.3. Oscillatory dynamic rheology

Non-destructive gelation properties (elastic modulus, G') of the trout protein paste were determined with a dynamic rheometer (Bohlin CVOR 200, 7 Malvern Instruments Ltd., Worcestershire, UK) in oscillation mode using a cone and plate geometry (4 and 4 cm diameter). The gap between cone and plate was 150 μm. A plastic cover supplied by the manufacturer was used to prevent moisture loss during measurement. Tests were conducted at 1% strain and 0.1 Hz frequency. The temperature ramp was programmed to increase from 25 to 95 °C at the rate of 1 °C/min (Chen & Jaczynski, 2007a, b). The rheogram is reported as a mean value of three measurements per treatment.

2.4. Preparation of heat-set fish protein gels

The trout protein paste was stuffed into stainless steel cylindrical tubes (length = 17.5 cm, internal diameter = 1.9 cm) with

screw end caps. The tubes were heated in a water bath at 90 °C for 15 min. Following heating, the tubes were chilled in ice slush and the fish protein gels were removed for analyses.

2.5. Color properties of heat-set fish protein gels

The gel samples were equilibrated to room temperature for 2 h prior to the color measurement. The color properties of heat-set fish gels 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 ten 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 L^* (lightness), a^* (redness), and b^* (yellowness) tristimulus color values were determined. A D_{65} illuminant was used and reflected light was viewed at 10°. Whiteness of gels was calculated by the following equation (Kristinsson, Theodore, Demir, & Ingadottir, 2005; National Fisheries Institute, 1991):

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

2.6. Texture properties of heat-set fish protein gels

Texture profile analysis (TPA) was conducted using a Texture Analyzer (Model TA-HDi, Texture Technologies Corp., Scarsdale, NY). The trout gel samples were equilibrated to room temperature for 2 h prior to the texture measurement. TPA of the gels was performed according to Cheret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, and Lamballerie (2005). At least eight cylindrical gels (height = 2.54 cm, diameter = 1.90 cm) per treatment were used for the TPA measurement. Gel samples were subjected to two-cycle compression at 50% compression using the texture analyzer with a 70-mm TPA compression plate attachment moving at a speed of 127 mm/min. From the resulting force-time curves, hardness, cohesiveness, gumminess, springiness, chewiness, and resilience were determined. Hardness was determined as the maximum force (N) detected during first compression. Cohesiveness was measured as the ratio of the positive force during the second compression to the positive force during the first compression. Gumminess was determined as the product of hardness × cohesiveness. Springiness was determined as the ratio of the distance from the second area to the second probe reversal over the distance. Chewiness was defined as the energy required to chew a solid food to the point required for swallowing it and was calculated as gumminess × springiness. Resilience was determined as the degree of how well a sample recovers from deformation in relation to speed and force applied (Alvarez, Canet, & Lopez, 2002). Cohesiveness, gumminess, springiness, chewiness, and resilience do not have units.

2.7. Sodium and potassium content of fish protein gels

All glassware was washed overnight in a solution of 0.3 mol L^{-1} HCl in dd H_2O prior to use. Ashed samples were dissolved in 2 mL of 7.7 mol L^{-1} nitric acid. The acidified samples were neutralized and filtered through Whatman #1 paper (Whatman International Ltd., Maidstone, United Kingdom). Samples were diluted to volume with dd H_2O in a 50 mL volumetric flask. Sodium and potassium content were determined using inductively coupled plasma optical emission spectrometry (model P400, Perkin Elmer, Shelton, CN). The measurements were performed in triplicate and mean values ± standard deviation are reported.

2.8. Statistics

The experiments were independently triplicated ($n = 3$). In each triplicate at least ten measurements were performed for color ($L^*a^*b^*$), eight for TPA, three for sodium and potassium, and three for G'. 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 the Fisher's Least Significant Difference (LSD) test (Freud & Wilson, 1997, p. 464). All statistical analyses of data were performed using SAS (2002). The data are reported as mean values ± standard deviation (SD).

3. Results and discussion

3.1. Color properties of heat-set fish protein gels

Several published studies have shown that solubilization of fish muscle protein at basic pH during isoelectric solubilization/precipitation (ISP) allows recovery of proteins with better gelation properties as well as texture and color properties compared to acidic solubilization (Chen & Jaczynski, 2007a; Kristinsson & Hultin, 2003; Kristinsson & Liang, 2006; Tahergorabi et al., 2011a). Fish lipids are also removed to a greater degree from the recovered muscle proteins when solubilization is conducted at basic pH (Chen & Jaczynski, 2007a, b). Since dioxin and polychlorinated biphenyls (PCBs) are lipophilic, they can accumulate in fish lipids. However, they are efficiently reduced by ISP processing (Marmon et al., 2009). Considering the above advantages of basic solubilization, pH 11.50 ± 0.05 was used in the present study to solubilize trout muscle proteins with subsequent precipitation at pH 5.50 ± 0.05.

Proper color and texture are two critical quality attributes of restructured fish food products. In the present study whole gutted rainbow trout was used as an input material for the ISP processing. Some of the dark pigment components were probably removed from various fish parts during ISP processing and retained with the recovered trout proteins; therefore, contributing to high yellowness (b^*) of the gels ("No oil" in Table 1). Although ISP processing allows efficient recovery of functional muscle proteins from difficult sources for processing such as fish by-products or whole fish, the proteins recovered from these sources result in poor color properties of the resultant gels. TiO_2 is often added to various food products and cosmetics to improve whiteness by blocking/scattering light and giving white appearance (Feng et al., 2007; Ferin, Oberdorster, & Peney, 1992; Meacock, Taylor, Knowles, & Himonides, 1997; Rajh, Nedeljkovic, Chen, Poluektov, & Thurnauer, 1999). Vegetable oils are also commonly used in surimi-based products (Park, 2005). It has been shown that oil addition results in proportional increase of lightness (L^*) due to

Table 1
Color properties[a] of fish protein gels formulated with different ω-3 PUFAs oils and salt substitute.

	No oil	Flaxseed	Fish	Algae	Krill	Blend
L^*	86.3 ± 0.2 b	87.2 ± 0.2 a	86.9 ± 0.2 a	84.0 ± 0.5 c	60.5 ± 0.8 d	86.1 ± 0.2 b
a^*	−0.7 ± 0.1 bc	−0.9 ± 0.1 c	−0.5 ± 0.0 b	−0.6 ± 0.1 b	29.3 ± 0.8 a	−0.8 ± 0.0 bc
b^*	8.1 ± 0.2 f	11.7 ± 0.1 d	9.1 ± 0.1 e	29.0 ± 0.7 b	39.2 ± 0.7 a	14.2 ± 0.1 c

[a] Data are given as mean ± SD ($n = 3$). Mean values in rows with different letters indicate significant differences (Least Squared Difference test; $P < 0.05$).

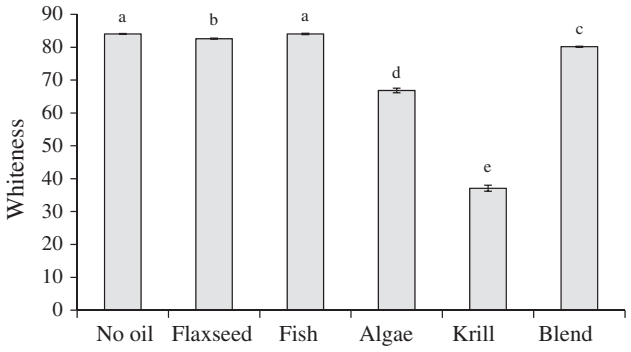

Fig. 2. Whiteness* of fish protein gels formulated with different ω-3 PUFAs oils and salt substitute. *Data are given as mean ± SD ($n = 3$). The small bars of the top of data bars indicate SD. Different letters on the top of SD bars indicate significant differences between mean values (Least Squared Difference test; $P < 0.05$).

light scattering; thereby, improving whiteness of surimi gels (Park, 2005). In a previous study, addition of 10% of canola oil to the ISP protein isolate recovered from chicken processing by-products resulted in the best color and textural properties (Tahergorabi et al., 2011a). Therefore, in the present study different types of ω-3 PUFAs oils were added at 10 g/100 g in order to improve the color and texture of fish protein gels with concurrent enhancement of their nutritional value.

Table 1 and Fig. 2 show tristimulus color values ($L^*a^*b^*$) and whiteness of trout protein gels, respectively. Flaxseed and fish oils resulted in the highest ($P < 0.05$) L^* of the protein gels due to the minimal pigmentation in the oils, which also gave greatest ($P < 0.05$) whiteness. The L^* value for protein gels made with the blend oil was slightly lower ($P < 0.05$) than the gels with flaxseed and fish oils due to the yellow pigmentation of algae oil used in the blend. In contrast, krill had markedly lower ($P < 0.05$) L^* than any other sample due to the rich astaxanthin pigmentation (Bustos, Romo, Yanex, Diaz, & Romo, 2003; Tou, Jaczynski, & Chen, 2007). These results are in accordance with Pietrowski et al. (2011) who reported the effect of ω-3 PUFAs oils on Alaska pollock surimi gels. The two other color values, a^* and b^* indicate redness and yellowness of food products, respectively. All of the trout protein gels except those with krill oil had slightly negative a^* indicating the lack of redness and very little greenish hue (Table 1). The gels made with krill oil had by far the highest ($P < 0.05$) redness resulting from the bright red antioxidant, astaxanthin (Bustos et al., 2003; Tou et al., 2007). The b^* value for trout protein gels with added oils were higher ($P < 0.05$) than the gels without oil (Table 1). Similar to a^*, gels with krill oil had the highest ($P < 0.05$) b^* followed by gels with algae, blend, flaxseed, and fish oil. Algae oil contains yellow-orange carotenoids, which contributed to the high b^* of gels made with this oil in the present study. Park, Kelleher, McClements, and Decker (2004) reported very similar results for gels made of cod surimi with algae oil homogenized into aqueous emulsion and stabilized with whey protein isolate. The whiteness values were the highest ($P < 0.05$) for trout protein gels made with fish oil and when oil was not added, followed by only slightly lower ($P < 0.05$) whiteness for gels with flaxseed and blend oils; while gels with algae and krill oils had markedly lower ($P < 0.05$) whiteness. These results are in agreement with Perez-Mateos et al. (2004) and Pietrowski et al. (2011).

3.2. Texture properties of heat-set fish protein gels

Texture is a complex sensory attribute involving such parameters as hardness, springiness, cohesiveness, gumminess, chewiness, and resilience that cumulatively contribute to the mouth feel/texture experienced by humans (Haard, 1992; Szczesniak, 2002). Szczesniak (2002) defined cohesiveness as "the strength of the internal bonds making up the body of the product" and springiness as "the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed". Texture profile analysis (TPA) of the trout protein gels made with and without added oil revealed some differences in the texture parameters (Table 2). In general, gels with krill and blend oils tended to have slightly lower TPA parameters. This may be due to the oil inherent properties such as a high degree of unsaturation and its propensity for oxidation that may interfere with protein thermal gelation resulting in slightly poorer texture. However, when flaxseed, fish, and particularly algae oil was added, the gel texture was improved. The results in the present study suggest that functional food products with good texture properties can be developed using ω-3 PUFAs oils in combination with ISP-recovered proteins.

3.3. Oscillatory dynamic rheology

Dynamic rheology has been used extensively to study the heat-induced gelation of myofibrillar proteins (Hamann, 1992; Visessanguan, Ogawa, Nakai, & An, 2000). 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' are typically used to monitor heat-induced protein gelation (Venugopal et al., 2002). Elastic moduli (G') of trout protein pastes with and without the ω-3 PUFAs oils are shown in Fig. 3. Trout protein paste without oil showed a typical G' pattern for the ISP-recovered fish muscle proteins. This pattern was also similar to the pattern for trout protein paste with krill oil. However, the gelation of ISP-recovered trout proteins was greatly improved by adding algae, fish, blend, and flaxseed oil. For these pastes, there was an initial increase of G' at approximately 45 °C followed by a more pronounced increase. It is important to note that this increase occurred at a lower temperature and to a greater extent than the trout paste without oil or with krill oil. These results are similar to the G' of Alaska pollock surimi paste with added ω-3 PUFAs oils previously reported (Pietrowski, Tahergorabi, & Jaczynski, 2012; Taskaya, Chen, Beamer, & Jaczynski, 2009). The increase at 45 °C indicates the initial stages of gel network formation due to partial un-folding of myosin (Sano, Noguchi, Tsuchiya, & Matsumoto, 1988). The myosin subfragment

Table 2
Texture profile analysis[a] (TPA) of fish protein gels formulated with different ω-3 PUFAs oils and salt substitute.

	No oil	Flaxseed	Fish	Algae	Krill	Blend
Hardness (N)	3884 ± 116 bc	3738 ± 336 b	3166 ± 337 d	4354 ± 357 a	3278 ± 82 cd	2774 ± 221 e
Springiness	1.93 ± 0.00 ab	1.93 ± 0.01 ab	1.94 ± 0.00 a	1.93 ± 0.02 b	1.94 ± 0.01 a	1.93 ± 0.00 ab
Cohesiveness	0.64 ± 0.02 c	0.70 ± 0.01 a	0.71 ± 0.01 a	0.69 ± 0.01 b	0.71 ± 0.01 a	0.71 ± 0.01 a
Gumminess	2192 ± 109 c	2623 ± 201 b	2228 ± 223 c	3024 ± 243 a	2303 ± 61 c	1952 ± 127 d
Chewiness	4245 ± 212 c	4722 ± 316 b	4319 ± 443 c	5638 ± 532 a	4423 ± 129 bc	3770 ± 252 d
Resilience	0.33 ± 0.01 d	0.38 ± 0.01 b	0.39 ± 0.01 a	0.36 ± 0.01 c	0.38 ± 0.01 b	0.39 ± 0.01 a

[a] Data are given as mean ± SD ($n = 3$). Mean values in rows with different letters indicate significant differences (Least Squared Difference test; $P < 0.05$).

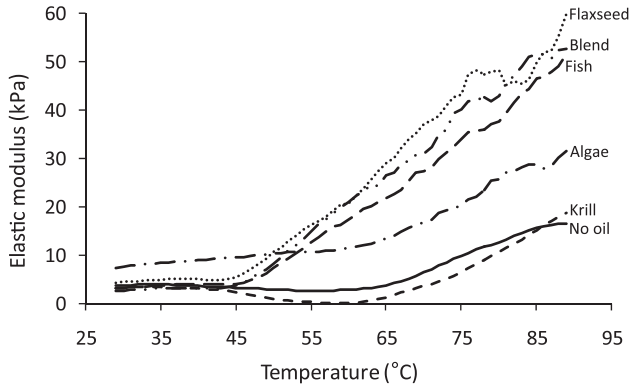

Fig. 3. Average elastic moduli (G') of fish protein pastes formulated with different ω-3 PUFAs oils and salt substitute.

LMM (light meromyosin) and subfragment-1 (S-1) are involved in the initiation of denaturation at 40–45 °C resulting in protein–protein interactions (Smythe, Smith, Vega-Warner, & O'Neill, 1996). Park et al. (2004) suggested that the increased elasticity of fish proteins in the presence of oil is a result of the emulsified oil droplets being surrounded by myofibrillar proteins and occupying proteins' hydrophobic regions; thus, not interfering with gelation and consequently allowing efficient formation of protein–protein covalent bonds. Furthermore, Ziegler and Foegeding (1990) suggested that the emulsified oil acts as a protein copolymer filling the voids in the network of the protein gel matrix; therefore, enhancing its elasticity.

3.4. Sodium and potassium content of fish protein gels

Sodium and potassium content of trout protein gels made with salt substitute and different ω-3 PUFAs oils are presented in Fig. 4. Sodium content was 200–300 mg/100 g of trout gel, while potassium content ranged between 1150 and 1350 mg/100 g. It has been reported that surimi gels made without salt or salt substitute have sodium content under 100 mg/100 g of surimi gels, while the gels made with 1–3% salt contain 300–1000 mg/100 g of gel (Tahergorabi et al., in press). Therefore, using a salt substitute in restructured food products developed from the ISP-recovered proteins allows reduction of sodium content with simultaneous increase of potassium. NaOH and HCl were used in the present study to induce protein solubilization and precipitation, respectively during ISP processing. This inevitably resulted in accumulation of NaCl during ISP processing. Some NaCl was likely retained

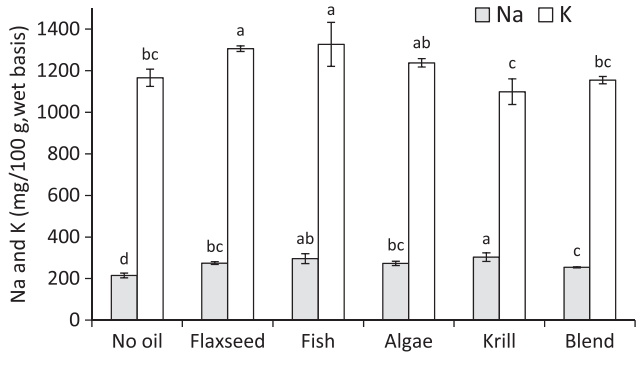

Fig. 4. Sodium* and potassium* content of fish protein gels formulated with different ω-3 PUFAs oils and salt substitute. *Data are given as mean ± SD ($n = 3$). The small bars of the top of data bars indicate SD. Different letters on the top of SD bars indicate significant differences between mean values within sodium or potassium content (Least Squared Difference test; $P < 0.05$).

with the protein isolate, resulting in Na content at 200–300 mg/100 of gel even though NaCl was not added during paste formulation. However, sodium content could be further reduced in final gels by using different reagents for protein solubilization/precipitation. For example, using KOH could further reduce sodium content, while increasing potassium content in fish protein gels.

Current average dietary sodium intake in the United States exceeds 3400 mg/day, which is considerably higher than the recommended maximum intake of 2300 mg/day established in the 2005 Dietary Guidelines for Americans. Unlike sodium, which increases hypertension, potassium has antihypertensive properties and much higher recommended maximum intake level than sodium (sodium – 2300 mg/day, potassium – 4700 mg/day) (McGuire & Beerman, 2007, chap. 13). Elevated blood pressure caused by excessive intake of sodium with diet accounts for 62% of strokes and 49% of coronary heart disease (WHO, 2002). Increasing current potassium intake to the recommended level may reduce the risk of stroke mortality by 8–15% and the risk of heart disease mortality by 6–11% (ICRG, 1988). This is of similar magnitude to what can be achieved by lowering dietary sodium intake; highlighting, therefore, the importance of dietary strategies focusing on a reduction of sodium intake and simultaneous increase of potassium intake.

3.5. ω-3 polyunsaturated fatty acids in fish protein gels

Anderson and Ma (2009) provided an up-to-date and comprehensive review of health benefits specific for α-linolenic (ALA, 18:3 ω3), eicosapentaenoic (EPA, 20:5 ω-3), and docosahexaenoic (DHA, 22:6 ω3) FAs. Flaxseed oil contains over 40 g/100 g of ALA and algae oil nearly 40 g/100 g of DHA, but both of these oils contain negligible amount of EPA. While fish and krill oils contain trace levels of ALA; fish oil has approximately 11 and 10 g/100 g of EPA and DHA, respectively; and krill oil 24 and 15 g/100 g, respectively (Kassis, Gigliotti, Beamer, Tou, & Jaczynski, 2012; Pietrowski et al., 2011). In addition, DHA and EPA in krill are esterified in phospholipids (Kassis et al., 2012), which may have significant implications for health benefits in humans. Thus, the concentrations of particular FAs and their ratios per serving size can be readily calculated. For example, 10 g/100 g of flaxseed oil incorporated in the protein paste would result in over 4000 mg of ALA per 100 g (approximate size of 1 serving) of trout protein gels developed in the present study.

4. Conclusion

This study demonstrated a feasibility to develop functional food products made of muscle protein isolate recovered with isoelectric solubilization/precipitation from whole gutted fish (bone-in, skin- and scale-on). The functional foods were nutritionally enhanced with ω-3 PUFAs, had reduced sodium and increased potassium; while the color and texture properties were good and gelation properties were improved. Although the results of this study point toward the potential for novel, marketable functional food products developed from inexpensive sources, sensory tests and storage stability study are recommended.

References

Alvarez, M. D., Canet, W., & Lopez, M. E. (2002). Influence of determination rate and degree of compression on textural parameters of potato and apple tissue in texture profile analysis. *European Food Research and Technology, 215*, 13–20.

Anderson, B. M., & Ma, D. W. L. (2009). Are all n-3 polyunsaturated fatty acids created equal? *Lipids in Health and Disease, 8*, 33.

Bustos, R., Romo, L., Yanex, K., Diaz, G., & Romo, C. (2003). Oxidative stability of carotenoid pigments and polyunsaturated fatty acids in microparticulate diets containing krill oil for nutrition of marine fish larvae. *Journal of Food Engineering, 56*, 289–293.

Chen, Y. C., & Jaczynski, J. (2007a). Gelation of protein recovered from Antarctic krill (*Euphausia superba*) by isoelectric solubilization/precipitation as affected by function additives. *Journal of Agricultural and Food Chemistry, 55*, 1814–1822.
Chen, Y. C., & Jaczynski, J. (2007b). Protein recovery from rainbow trout (*Oncorhynchus mykiss*) processing by-products via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. *Journal of Agricultural and Food Chemistry, 55*, 9079–9088.
Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S., & Jaczynski, J. (2006). Enhancement of omega-3 fatty acid content in rainbow trout (*Oncorhynchus mykiss*) fillets. *Journal of Food Science, 71*, C383–C389.
Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S., & Jaczynski, J. (2007). Physicochemical changes in omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) muscle during refrigerated storage. *Food Chemistry, 104*(3), 1143–1152.
Chen, Y. C., Tou, J. C., & Jaczynski, J. (2007). Amino acid, fatty acid, and mineral profiles of materials recovered from rainbow trout (*Oncorhynchus mykiss*) processing by-products using isoelectric solubilization/precipitation. *Journal of Food Science, 72*(9), C527–C535.
Chen, Y. C., Tou, J. C., & Jaczynski, J. (2009). Amino acid and mineral composition of protein and other components and their recovery yields from whole Antarctic krill (*Euphausia superba*) using isoelectric solubilization/precipitation. *Journal of Food Science, 74*(2), H31–H39.
Cheret, R., Chapleau, N., Delbarre-Ladrat, C., Verrez-Bagnis, V., & Lamballerie, M. D. (2005). Effects of high pressure on texture and microstructure of sea bass (*Dicentrarchus labrax* L.) fillets. *Journal of Food Science, 70*, E477–E483.
Choi, Y. J., & Park, J. W. (2002). Acid-aided protein recovery from enzyme-rich Pacific whiting. *Journal of Food Science, 67*, 2962–2967.
Feng, X. X., Zhang, L. L., Chen, J. Y., Guo, Y. H., Zhang, H. P., & Jia, C. I. (2007). Preparation and characterization of novel nanocomposite films formed from silk fibroin and nano-TiO_2. *International Journal of Biological Macromolecules, 40*(2), 105–111.
Ferin, J., Oberdorster, G., & Peney, D. P. (1992). Pulmonary retention of ultrafine and fine particle in rats. *American Journal of Respiratory Cell and Molecular Biology, 6*(5), 532–542.
Freud, R. J., & Wilson, W. J. (1997). *Design of experiments*. San Diego (CA): Academic Press.
Gehring, C. K., Gigliotti, J. C., Moritz, J. S., Tou, J. C., & Jaczynski, J. (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish – a review. *Food Chemistry, 124*(2), 422–431.
Gigliotti, J. C., Jaczynski, J., & Tou, J. C. (2008). Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique. *Food Chemistry, 111*(1), 209–214.
Haard, N. F. (1992). Control of chemical composition and food quality attributes of cultured fish. *Food Research International, 25*, 289–307.
Hamann, D. D. (1992). Rheological studies of fish proteins. In *International conference and industrial exhibition on food hydrocolloids* (pp. 4–49). Tokyo, Japan: Japanese Society of Food Hydrocolloids.
Intersalt Cooperative Research Group. (1988). Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *British Medical Journal, 297*, 319–328.
Jaczynski, J., & Park, J. W. (2004). Physicochemical changes in Alaska pollock surimi and surimi gel as affected by electron beam. *Journal of Food Science, 69*, 53–57.
Kassis, N., Gigliotti, J. C., Beamer, S. K., Tou, J. C., & Jaczynski, J. (2012). Characterization of lipids and antioxidant capacity of novel nutraceutical egg products developed with omega-3-rich oils. *Journal of the Science of Food and Agriculture, 92*(1), 66–73.
Kim, Y. S., Park, J. W., & Choi, Y. J. (2003). New approaches for the effective recovery of fish proteins and their physicochemical characteristics. *Fisheries Science, 69*(6), 1231–1239.
Kristinsson, H. G., & Hultin, H. O. (2003). Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. *Journal of Agricultural and Food Chemistry, 51*(24), 7187–7196.
Kristinsson, H. G., & Liang, Y. (2006). Effect of pH-shift processing and surimi processing on Atlantic croaker (*Micropogonias undulates*) muscle proteins. *Journal of Food Science, 71*, 304–312.
Kristinsson, H. G., Theodore, A. E., Demir, N., & Ingadottir, B. (2005). A comparative study between acid- and alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. *Journal of Food Science, 70*, C298–C306.
Lansdowne, L., Beamer, S., Jaczynski, J., & Matak, K. E. (2009a). Survival of *Escherichia coli* after isoelectric solubilization/precipitation of fish. *Journal of Food Protection, 72*(7), 1398–1403.
Lansdowne, L., Beamer, S., Jaczynski, J., & Matak, K. E. (2009b). Survival of *Listeria innocua* after isoelectric solubilization and precipitation of fish protein. *Journal of Food Science, 74*(4), M201–M205.
Lee, S., Faustman, C., Djordjevic, D., Faraji, H., & Decker, E. (2006). Effect of antioxidants on stabilization of meat products fortified with n-3 fatty acids. *Meat Science, 72*(1), 18–24.
Marmon, S. K., Liljelind, P., & Undeland, I. (2009). Removal of lipids, dioxins and polychlorinated biphenyls during production of protein isolates from baltic herring (*Clupea harengus*) using pH-shift process. *Journal of Agricultural and Food Chemistry, 57*, 7819–7825.
McGuire, M., & Beerman, K. A. (2007). *Nutritional sciences, from fundamentals to food*. Belmont (CA): Thomson Learning.
Meacock, G., Taylor, K. D. A., Knowles, M. J., & Himonides, A. (1997). The improved whitening of minced cod flesh using dispersed titanium dioxide. *Journal of the Science of Food and Agriculture, 73*, 221–225.
Mireles DeWitt, C. A., Gomez, G., & James, J. M. (2002). Protein extraction from beef heart using acid solubilization. *Journal of Food Science, 67*, 3335–3341.
Mireles DeWitt, C. A., Nabors, R. L., & Kleinholz, C. W. (2007). Pilot plant scale production of protein from catfish treated by acid solubilization/isoelectric precipitation. *Journal of Food Science, 72*, E351–E355.
National Fisheries Institute. (1991). *A manual of standard methods for measuring and specifying the properties of surimi*. Washington (D.C.): National Fisheries Institute.
Nicholas, P. D., Petrie, J., & Singh, S. (2010). Long-chain omega-3 oils – an update on sustainable sources. *Nutrients, 2*, 572–585.
Nolsoe, H., & Undeland, I. (2009). The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. *Food Bioprocess Technology, 2*, 1–27.
Park, J. W. (2005). Ingredient technology for surimi and surimi seafood. In J. W. Park (Ed.), *Surimi and surimi seafood* (2nd ed.). (pp. 649–707) Boca Raton (FL): Taylor and Francis Group.
Park, Y., Kelleher, S. D., McClements, D. J., & Decker, E. A. (2004). Incorporation and stabilization of omega-3 fatty acids in surimi made from cod, *Gadus morhua*. *Journal of Agricultural and Food Chemistry, 52*, 597–601.
Perez-Mateos, M., Boyd, L., & Lanier, T. (2004). Stability of omega-3 fatty acids in fortified surimi seafoods during chilled storage. *Journal of Agricultural and Food Chemistry, 52*, 7944–7949.
Pietrowski, B., Tahergorabi, R., Beamer, S., Matak, K., Tou, J., & Jaczynski, J. (2011). Chemical properties of surimi seafood nutrified with ω-3 rich oils. *Food Chemistry, 129*, 912–919.
Pietrowski, B., Tahergorabi, R., & Jaczynski, J. (2012). Dynamic rheology and thermal transitions of surimi seafood enhanced with ω-3-rich oils. *Food Hydrocolloids, 27*(2), 384–389.
Rajh, T., Nedeljkovic, J. M., Chen, L. X., Poluektov, O., & Thurnauer, M. C. (1999). Improving optical and charge separation properties of nanocrystaline TiO_2 by surface modification with vitamin C. *Journal of Physical Chemistry B, 103*(18), 3515–3519.
Sano, T., Noguchi, S. F., Tsuchiya, T., & Matsumoto, J. J. (1988). Dynamic viscoelastic behavior of natural actomyosin and myosin during thermal gelation. *Journal of Food Science, 53*, 924–928.
SAS Institute. (2002). *SAS/STAT guide for personal computers, version 8.1*. Cary (NC): SAS Institute.
Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*, 365–379.
Smythe, A. B., Smith, D. M., Vega-Warner, V., & O'Neill, E. (1996). Thermal denaturation and aggregation of chicken breast muscle myosin and subfragments. *Journal of Agricultural and Food Chemistry, 44*, 1005–1010.
Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference, 13*, 215–225.
Tahergorabi, R., Beamer, S., Matak, K. E., & Jaczynski, J. (2011a). Effect of isoelectric solubilisation/precipitation and titanium dioxide on whitening and texture of proteins recovered from dark chicken-meat processing by-products. *LWT – Food Science and Technology, 44*(4), 896–903.
Tahergorabi, R., Beamer, S. K., Matak, K. E., & Jaczynski, J. Physicochemical properties of surimi gels with salt substitute. *LWT – Food Science and Technology*, in press.
Tahergorabi, R., Sivanandan, L., & Jaczynski, J. (2012). Dynamic rheology and endothermic transitions of proteins recovered from chicken-meat processing by-products using isoelectric solubilization/precipitation. *LWT – Food Science and Technology, 46*(1), 148–155.
Taskaya, L., Chen, Y. C., Beamer, S., & Jaczynski, J. (2009). Texture and color properties of proteins recovered from whole gutted silver carp (*Hypophthalmichthys molitrix*) using isoelectric solubilisation/precipitation. *Journal of the Science of Food and Agriculture, 89*(2), 349–358.
Taskaya, L., Chen, Y. C., Beamer, S., Tou, J. C., & Jaczynski, J. (2009). Compositional characteristics of materials recovered from whole gutted silver carp (*Hypophthalmichthys molitrix*) using isoelectric solubilization/precipitation. *Journal of Agricultural and Food Chemistry, 57*(10), 4259–4266.
Taskaya, L., Chen, Y. C., & Jaczynski, J. (2009). Functional properties of proteins recovered from whole gutted silver carp (*Hypophthalmichthys molitrix*) by isoelectric solubilization/precipitation. *LWT – Food Science and Technology, 42*(6), 1082–1089.
Taskaya, L., Chen, Y. C., & Jaczynski, J. (2010). Color improvement by titanium dioxide and its effect on gelation and texture of proteins recovered from whole fish using isoelectric solubilization/precipitation. *LWT – Food Science and Technology, 43*(3), 401–408.
Tou, J. C., Jaczynski, J., & Chen, Y. C. (2007). Krill for human consumption: nutritional value and potential health benefits. *Nutrition Reviews, 65*(2), 63–77.
Undeland, I., Kelleher, S. D., & Hultin, H. O. (2002). Recovery of functional proteins from herring light muscle by an acid or alkaline solubilization process. *Journal of Agricultural and Food Chemistry, 50*, 7371–7379.
Venugopal, V., Kakatkar, A., Bongirwar, D. R., Karthikeyan, M., Mathew, S., & Shamasundar, B. A. (2002). Gelation of shark meat under mild acidic conditions: physicochemical and rheological characterization of the gel. *Journal of Food Science, 67*, 2681–2686.
Visessanguan, W., Ogawa, M., Nakai, S., & An, H. (2000). Physiological changes and mechanism of heat induced gelation of arrowtooth flounder. *Journal of Agricultural and Food Chemistry, 48*, 1016–1023.
World Health Organization. (2002). *World health report 2002. Reducing risks, promoting healthy life*. Geneva, Switzerland. Available at http://www.who.int/whr/2002 Accessed 14.08.11.
Ziegler, G. R., & Foegeding, E. A. (1990). The gelation of proteins. In J. E. Kinsella (Ed.), *Advances in food and nutrition research* (pp. 203–298). New York: Academic Press.