Optimum conditions for protein extraction from tuna processing by-products using isoelectric solubilization and precipitation processes

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Received: April 2016 Accepted: September 2016

Abstract

The by-product from tuna processing is a potential source of edible protein. Therefore, it is very important to extract protein from such raw materials for human food. In this study the optimum pH for protein extraction from tuna by-products was optimized by using isoelectric solubilization and precipitation processes. The Response Surface Methodology (RSM) and the single factor model were used for optimization of the protein extraction process. From ANOVA (one-factor design) tests, significant effects were detected for process variables, functional properties and stability between tuna protein isolate prototypes extracted at acidic and alkaline pH, the latter having the least Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric Acid Reactive Substances (TBARS), but the highest Water Holding Capacity (WHC), hardness, cohesiveness, springiness and viscosity values. The highest yield percentage was found for the alkaline aided process, too. The alkali-aided process recovered proteins of higher whiteness than the acid-aided process. Accordingly the optimum pH of protein extraction was obtained. The model was then validated and maximized based on the functional properties, stability and recovery yield data. Under the optimized pH, the experimental values were in good agreement with those predicted by the software. Then the properties of the optimum prototypes were compared to the fish protein isolated from different by-products. The results suggest that the proteins recovered from tuna processing by-products could be a valuable source of protein ingredient for fortification/ developing formulated ready-to-eat products.

Keywords: Tuna protein isolate, Yellow fin tuna, Dark muscle, Texture profile analysis, Response surface methodology (RSM)

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Introduction

Tuna, among the most important species in the world fisheries industry, is caught in commercial quantities and widely distributed globally (Herpandi *et al.,* 2011). Worldwide catch of tuna *"i.e."* albacore, big eye, skipjack and yellowfin was estimated at about 4,780,000 MT in 2014 (Western and Central Pacific Fisheries Commission, 2015). Tuna flesh generally is processed in raw form and marketed as either loins/steaks or canned products. In the tuna canning process, only the white meat of about one-third of whole fish is used (Herpandi *et al.,* 2011). Tuna processing by-products containing the head, gills, viscera, dark/red meat, bone, and skin, can constitute up to 70% of its original material (Guerard *et al.,* 2002; Hernández *et al.,* 2013) wherein about 12% is blood/ dark/ red meat (BCTFA, 2001), which are commonly considered as low-value resources with negligible market value (Herpandi *et al.*, 2011).

In Iran, whilst tuna catch constitutes 20% of the total amount of fishery production (Shilat, 2015), an estimate of 50% of 250,000 metric tons reported as processed outputs (Shaviklo, 2016) is by-products that go as waste, which may well be channeled toward other resourceful products like fish meal and fertilizers. Therefore, due to limited biological resources and increasing environmental pollution the need for improved optimization/ utilization of fish by-products is of increasing emphasizes by globally recognized authorities/ organizations. However, among key challenges facing the tuna processing industry is the establishment of improved approaches that optimize the conversion of underutilized by-products into valuable food resources (Choa *et al.,* 2005). Accordingly, the utilization of such by-products to develop value-added and better quality products is crucial. One of the knowledge-based alternatives is to extract the proteins from these underused tuna by-product resources (Kim and Mendis, 2006; Shaviklo, 2008).

Various protein extraction processes have been reported (Shaviklo, 2015; Shabanpour and Etemadian, 2016a), but to our knowledge, it appears that not much emphasis has been given to isoelectric solubilization and precipitation, which may well be less tasking yet apractical method to achieve a relatively pure protein with a high yield and functionality (Hultin *et al.,* 2005).

Isoelectric solubilization/ precipitation method by the pH shift method is widely applied for extraction of proteins from fish by-products (Kristinsson and Hultin, 2003; Shabanpour and Etemadian, 2016b). Additionally, high recovery yield, economical feasibility, and improved functionalities of the recovered proteins compared with surimi seems to be the promising advantages of this process (Undeland *et al.,* 2002; Kristinsson *et al.,* 2005; Shaviklo, 2006).

The use of isoelectric solubilization and precipitation methods to recover fish proteins involves the solubilisation of homogenized fish flesh either in aqueous acidic (pH≤3.5) or alkaline (pH≥10.5) solutions. The protein rich solution is separated from solids (insoluble proteins, skin, bones, and scales) and neutral lipids by centrifugation. The soluble proteins are then recovered by isoelectric precipitation by adjusting the pH to 5.5 and the precipitated proteins are removed again by centrifugation (Hultin *et al.*, 2005; Kristinsson and Ingadottir, 2006). The isolated protein is free from fishy odor and flavors and can be a useful candidate for the formulation of fishery products (Shaviklo, 2006). Fish protein isolate (FPI), which is obtained after a pH shift process of tuna by-products, can serve as a food ingredient in food industries to provide functional effects such as, gelling, and texturing properties (Shaviklo *et al.*, 2010a; 2016).

Response surface methodology (RSM) among notable mathematical modeling and statistical techniques employed in studying the relationships between one or more responses (dependent variables) and corresponding factors (independent variables) in the view to optimize different processes (Cho *et al.*, 2004). Some variations of design models of RSM have been provided by the Design Expert software. Selection of the design model depends on the number of design factors (Jeirani *et al.*, 2013). Considering that pH is the most important factor affecting both quality and functionality of fish protein that has been isolated by pH-shift method (Nolsøe and Undeland, 2009), one factor design- which is one of the various design types for RSM in the software successfully applied to optimize food processing operations, can therefore be used for such studies (Diniz and Martin, 1996; Chenyan *et al.*, 2011).

There is some relevant literature concerning the extraction of protein from different kinds of raw material using Isoelectric solubilization/precipitation technology (Nolsøe and Undeland, 2009; Matak *et al.*, 2015), but seemingly not so for the isolating protein from tuna by-products. Specially therefore, the objectives of this study was (1) to investigate, through RSM, the influence of different pH, on stability, yield and functional properties of protein extracted from tuna by-products for optimizing pH for protein extracting and (2) to compare physical-chemical properties and sensory attributes of selected tuna proteins isolate to the attributes of conventional surimi or FPI made from different fish raw materials.

Material and methods

In this study, investigations were divided into 2 parts. Initially, the protein extraction from the red meat of yellowfin tuna (*Thunnus albacares*) was optimized by using response surface methodology (Jeirani *et al.*, 2013). Accordingly, 12 runs based on a computer-aided statistical programme (Design-Expert software) were developed. Functional properties and stability of FPIs were analyzed. Then the physical properties of the optimized yellowfin tuna protein isolate was compared to the fish protein isolates extracted from fish by-products reported by other works.

Tuna protein isolate (TPI)

Frozen yellow tail tuna (50 kg) was obtained and transferred from a tuna canning company in Bandar Anzali (Guilan, Iran) to the processing lab at National Fish Processing Center in Bandar Anzali (Guilan, Iran) under frozen conditions (-18˚C). Thawed tuna fish was filleted manually and red meat was removed from light meat. The pH-shift method (Hultin *et al.,* 2005) was used to extract proteins from tuna red meat. The ground tuna red meat (TRM) was homogenized for 1 min (speed 50) with 9 volumes of ice-cold distilled water. The proteins in the homogenate were solubilized by dropwise addition of 1 N HCl or 1 N NaOH until the intended (2.5, 3.0 and 3.5or10.5, 11.0 and 11.5) was reached (Hultin *et al.,* 2005). The protein suspension was centrifuged within 25 min at 8000 *g* (Thawornchinsombut and Park, 2006). The supernatant was separated from the emulsion layer by filtering these two phases through double cheese cloth. The soluble proteins were precipitated by adjusting the pH to 5.5 using 1 N NaOH or 1 N HCl. Precipitated proteins were collected via a second centrifugation at 8000 *g* (25 min). The moisture content of the tuna protein isolate (TPI) was adjusted by manual pressing.

Physico-chemical Analysis

Crude lipid content was determined by the Soxhlet method (Soxtec System-Texator, Sweden) (AOAC, 1995). Crude protein content was determined using the Kjeldahl method (Kjeltex System-Texator, Hagonas, Sweden). The moisture content was determined by drying samples for 4 h at 105°C until constant weight was achieved. Ash content was determined by charring samples overnight at 550°C (AOAC, 1995). Thiobarbituric Acid Reactive Substances (TBARS) were determined by a slightly modified steam distillation method (Tarladgis *et al.,* 1960), where the sample size was reduced to 5 g and antioxidants (5 mL of 0.5% propyl gallate and 0.5% ethylene diamine tetra acetic acid in water) were added to the sample during blending. Malondialdehyd-bis- (diethyl acetate) was used as a standard. Total Volatile Basic Nitrogen (TVB-N) was determined using steam distillation followed by titration method (Malle and Poumeyrol, 1989). The peroxide value (PV) was determined by the modified AOAC method (1995) and expressed as milliequivalent of oxygen per kilogram of lipid. The pH of samples was measured using a digital pH meter (Knick-Portamess 913 pH, Berlin, Germany). All samples were measured at room temperature. The pH value was the average value of two readings.

Gel preparation

The TPI gel was prepared by comminuting 500 g of thawed TPI with 3% salt in a vertical cutter mixer

(Stephan Machinery, Columbus, Ohio, U.S.A.) at <5°C for 10 min (FAO/WHO, 2005). Homogenized fish paste was stuffed by a manual stainless steel sausage stuffer (7KVSSL, Omcan, OMCAN INC. Mississauga, ON) into a plastic casing (3 cm diam) with the minimum incorporation of air bubbles and sealed at both ends. Test materials were then kept in the fridge for 1 h, subsequently followed by being subjected to hot water (85-90°C) for 30 min. Immediately after finishing the heating treatment the test materials were cooled in a mixture of ice and water for 10 min. Then, they were kept at 4 °C for overnight until testing.

Texture Profile Analyses (TPA)

The TPA was carried out using a texture profile analyzer (TA-XT Express, Stable Micro Systems Ltd.). Samples (weighing 6 g) cylindrical in shape (1.0 cm height and 3.0 cm width) were prepared from the recovered protein samples. The analysis was carried out in TPA mode, using the TA-XT Express software included with the equipment. A double compression cycle test was performed up to 50% compression of the original portion height with an aluminum cylinder probe of 5-cm in diameter. A time interval of 1 s was allowed to elapse between the 2 compression cycles. The trigger force used for the test was 5 g, with a test speed of 5 mm/s. When the test was finished, the software calculated the values for hardness (maximum force required to compress the sample), cohesiveness (extent to which the sample could be deformed before rupture), adhesiveness, springiness (ability of the sample to recover its original form after deforming force was removed), and resilience. The analysis was carried out for 6 replicates (Omana *et al.*, 2010).

Viscosity measurement

The TPI was diluted with distilled water (1:5) and homogenized for 1 min and 10,000 rpm using a high speed homogenizer (Wiggen Hauser, D-500, Kreuzbergstr, Berlin, Germany). The solution was subjected to viscosity measurements using a Brookfield viscometer (model DV II+, Brookfield Engineering Labs Inc., Stoughton, MA, USA.) with spindle No.31 and a speed of 100 rpm, heated using a rotary water bath from 5 to 50°C with a heating rate of 5°C/min. 600 ml of the sample solution was incubated for 30 min at each temperature before the measurements. The relative viscosity was calculated in comparison to that obtained at 5°C, which was adjusted using ice flake. The viscosity was read and reported in terms of centipoises (cP). This procedure was performed in triplicate for each sample.

Determination of Water Holding Capacity (WHC)

The WHC was determined by centrifugation (Shahidi *et al.*, 1995; Shaviklo *et al.*, 2010b). Two grams of TPI was placed in a plastic cylinder in a plastic holder cup. The cylinder had a fine mesh at the other end (diameter of sample cylinder 2.5 cm). This mesh had the purpose of holding the sample and also to allow liquid to pass through it since it was porous. The sample cylinder was then placed in a Biofuge Stratos; Heraeus Instruments (Hanau, Germany). Temperature interval was set at 5°C, speed 1350 rpm and the time was 5 min. After the centrifugation was completed, the sample cylinder was weighed and the difference in weight of the sample before and after was noted. The WHC was expressed as the amount of water retained after centrifugation per gram of dry weight of the product. The WHC was calculated according to the following formula (Shaviklo *et al.*, 2012):

$$WHC (\%)=((A\times B)-C)/(A\times B)\times 100 \quad (1)$$

Where:

A= Moisture content of sample before centrifugation (g),

B= Sample weight (g),

C= Sample weight after centrifugation (g)

Color evaluation

The TPI gel was cut into flat and smooth slices 15 mm in thickness or more. The samples were evaluated immediately with a color-difference meter instrument by measuring the values of L^* (lightness), a^* (red-green colors) and b^* (yellow-blue colors) to the first decimal place. Three or more sliced pieces were tested. The whiteness was calculated using the following equation as referred by the Codex Alimentarius (Park *et al.*, 2005):

$$\text{Whiteness} = 100-[(100-L^*)^2 + (a^*)^2 + (b^*)^2]^{½} \quad (2)$$

Sensory attributes of TPI and TRM

Test samples (TPIs and TRM) were mixed separately with 3% salt for 2 min in a food homogenizer (model 1094, Tecator, Co., Paris, France). The mixtures were then used to make fish cake each weighing about 40 g and 1 cm thick (about 5 cm in width and 1 cm in thickness).Thermal processing (setting) was boiling in hot water (95-100°C) for 5 min (Shaviklo *et al.*, 2010a). Sensory analyses of test samples were done by 8 panelists (5 females) who were selected consistent with general guidance of International Organization for Standardization (ISO, 2012). The average age of the panelists was 28 years and they were familiar with the Quantitative Descriptive Analysis (QDA) method (Meilgaard *et al.*, 2007). Panelists had experiences in sensory assessment of seafood products and they were trained during two sessions to evaluate fish cakes samples using the QDA method. Informed consent was sought from each panelist prior to their participation. They were asked to generate terms concerning the color, flavor, odor and texture. They were asked to smell and taste the samples and to record their comments about all the odor, flavor, color and texture attributes. Individual score sheets were prepared and during the session the assessors evaluated the intensity of each sensory attribute of 2 fish cake samples, placing a mark on unstructured 15 cm line scales anchored at the ends with the term low and much. A list of sensory lexicon (Table 1) to describe the intensity of each attribute for the given samples using an unstructured scale (from 0 to 100%) was adapted from Shaviklo *et al.* 2010a).

Each assessor evaluated 2 samples per session in duplicate. All sample observations were done based on ISO guidelines (ISO, 2007). Boiled fish cakes were coded with three-digit random numbers and presented to the panelists on a tray in individual booths. Water was provided for mouth-rinsing between samples to cleanse the palate (Shaviklo *et al.,* 2010a). Orders of serving were completely randomized in duplicate. Average scores of the judges were calculated for each sample and the reported values were the average of the two analyses.

Experimental design and statistical analysis

Statistical software package Design-Expert (Version 6.0.2, State-Ease, Minneapolis, MN) was applied in data analysis and design of this study. Accordingly, RSM was adopted to determine the functional relationships between the process variable (pH) and stability of prototypes. The range of the design factor was divided into two sub-ranges (acidic: 2.5, 3.0, 3.5 and alkaline: 10.5, 11.0, 11.5 pH) in such a way that each range has only one maximum.

Table 1: Lexicon for sensory attributes of fish cake made from TPI (adapted from Shaviklo *et al.,* 2010a).

Sensory attribute	Scale (0-100)	Definitions
Odor		
Rancid	None/much	Inside fish cake: rancid odor can remind of cardboard, paints,
Fishy	None/much	Inside fish cake: fish odor.
Appearance		
Wrinkle	None/much	Wrinkle on the surface of the burgers.
Color (inside)	Light/dark	Inside fish cake: Is the color dark or light?
Texture		
Softness	Firm/soft	Outside and inside: Softness in the first bite.
Cohesiveness	Little/much	Inside fish cake: Little (easy to take apart with a fork), Much (the inside of the burger is firm).
Graininess	None/much	Inside fish cake: when rubbed against palate with tongue, grainy reminds of couscous or sand.
Rubbery	None/much	Outside and inside: when chewing rubbery, springy.
Flavor		
Rancid	None/much	Outside and inside: sign of decay.
Fishy	None/much	Outside and inside: fish flavor.
Soapy	None/much	Soapy, chemical flavor.

The selected minimum and maximum values for the first design sub-range were 2.5 and 3.5 respectively. The selected minimum and maximum for the second design sub-range were 10.5 and 11.5, respectively. In performing one-factor design and applying the design sub ranges, the seven design points were automatically determined by the software for both design sub-ranges individually. The corresponding experimental response values were entered into the model after the seven experiments were carried out at the predetermined conditions. Multiple regression analysis technique included in the one-factor design was used to estimate the coefficients of models as per responses.

The statistical adequacy of one-factor models for physic-chemical data were performed through analysis of variance (ANOVA) using the statistical program NCSS 2007 (NCSS, Statistical Software, Kaysville, UT). Student's t-test was used to test the prediction and actual values obtained for TPI. The results are presented in terms of means of repeated measurements and standard deviations. Significance of difference was defined at the 5% level. PanelCheck software (version V1.3.2, Matforsk, Ås, Norway) was applied to monitor panelists' performance and to analyze sensory data.

Results

From ANOVA (one-factor design) tests, significant effects occurred at process variables, functional properties and stability between TPIs prototypes extracted in the acidic and alkaline pH, the latter having the least TVB-N and TBARS, but the highest WHC, hardness, cohesiveness, springiness and viscosity values (Figs.1 and 2). The highest yield percentage was found for the alkaline aided process, too. The alkali-aided process recovered proteins of higher whiteness than the acid-aided process (Fig. 1).

To optimize pH for protein extraction, the maximum TPI prototypes desirable was sought by numerical techniques using mathematical optimization procedure of Design Expert Software Package. Optimization criterion considered the highest level of functionality and lowest level of oxidation. The TVB-N have been considered the most important parameters in protein isolation studies (Nolsøe and Undeland, 2009; Matak *et al.,* 2015). Accordingly, to maximize the desirability function via random starting points that identify the path of the steepest slope up to a maximum was deemed a way out, that is, desirability model (Fig. 2, plot f) obtained from Design-Expert software. It recommended pH 11.0 as an optimum pH that allowed for the reconstitution of the mixtures.

No significant differences between the predicted responses and actual obtained response values (Table 2) indicated the capability of one factor design to help achieve process optimization.

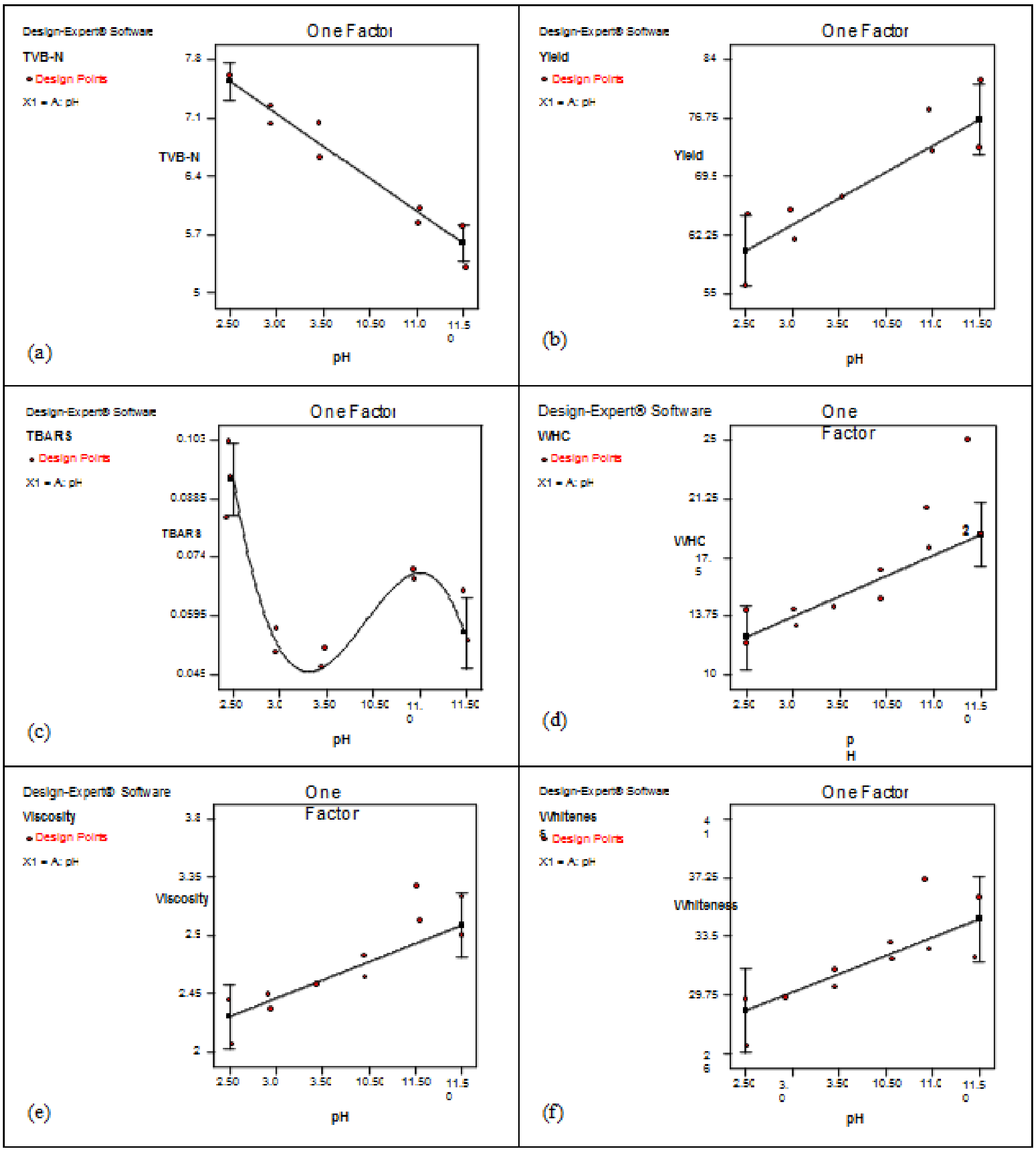

Figure1: Response surface design plots: the effect of pH on (a) TVBN (mg N/100 g sample) (b) Yield (%) (c) TBARS (mg malonaldehide/kg sample) (d) WHC (%) (e) Viscosity (cP) (f) whiteness of tuna protein isolates.

From the desirability model using optimization procedure, the protein extracted at pH 11 appeared more stable with superior functional properties compared with the other samples.

Physico-chemical analysis of the selected TPI (extracted at pH:11) and TRM are shown in Table 3. Significant differences were found between TRM and the selected TPI for protein, moisture, and fat contents, PV, and TVB-N.

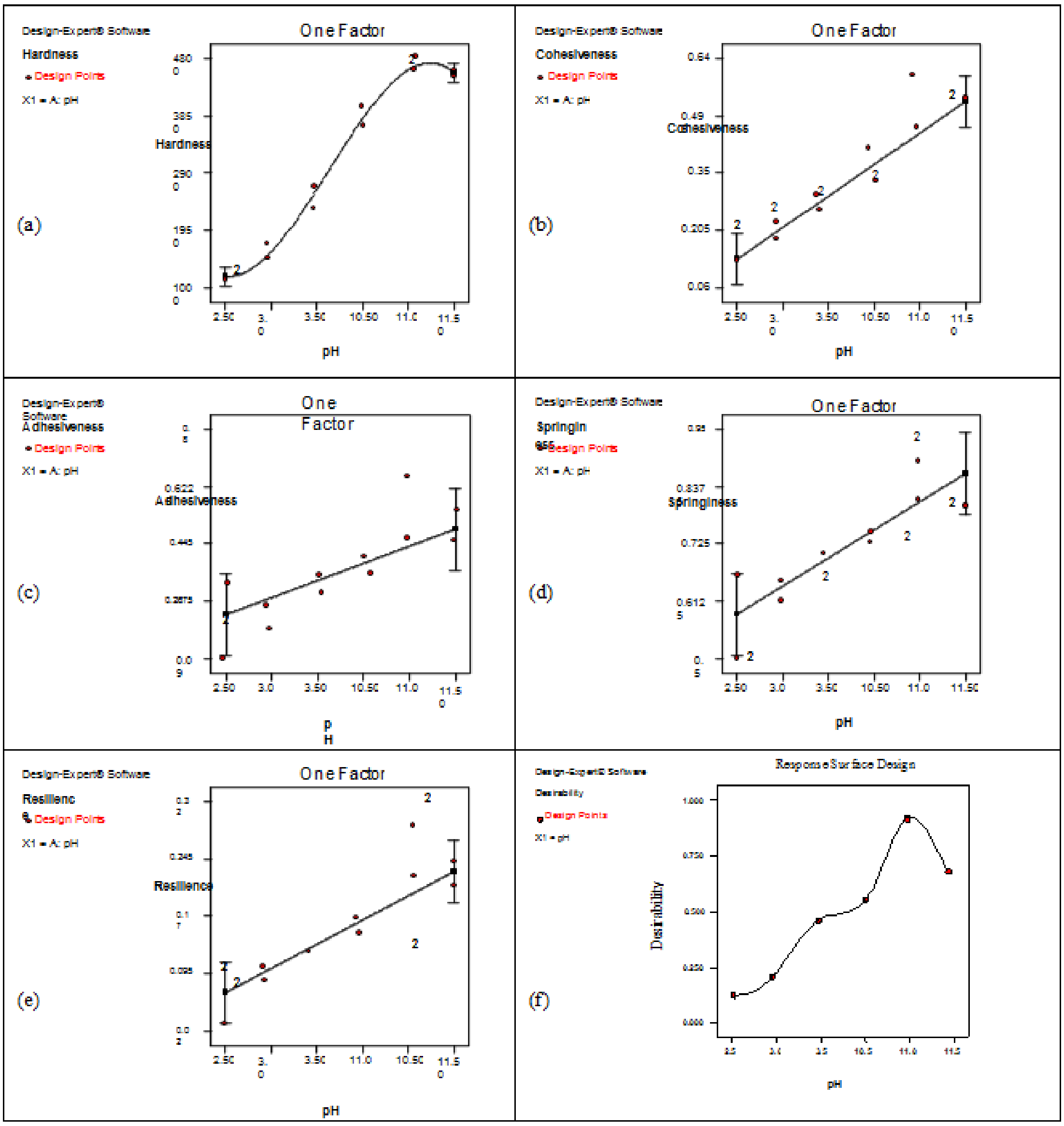

Figure 2: Response surface design plots: the effect of pH on (a) hardness (g) (b) cohesiveness (c) adhesiveness (d) springiness and (e) resilience of tuna protein isolates. (f) Desirability of pH for protein extraction.

The TVB-N and TBARS were 6.81 mg N/100 g sample and 0.33 mg malondialdehyde/ kg for TPI and 19.6 mg N/100 g sample and 0.56 mg malondialdehyde/ kg for TRM, respectively.

Average sensory scores (0-100) for TDM and TPI fish cake prototypes are presented in Table 4. The panel observed differences in appearance, texture, odor, and flavor. Fish cake from TRM had more rancid and fishy odor and flavor than the prototype prepared from TPI. Fish cake made from TPI was whiter and had better texture properties than the TRM cake.

Table 2: Obtained experiment data considered for validation.

Response	Prediction values	Actual obtained values	*P value*
Protein content (%)	30.67	28.94	NS
Moisture (%)	72.21	74.06	NS
Lipid (%)	3.94	4.21	NS
TBARS (mg malondialdehyde	0.067	0.074	NS
WHC (%)	16.51	14.61	NS
TVBN(mg N/100 g sample)	6.91	6.82	NS
Yield (%)	87.21	88.12	NS
Viscosity (cP)	2.78	3.02	NS
whiteness	29.10	51.41	NS
Hardness (g)	4655.47	4791.24	NS
Cohesiveness	0.85	0.91	NS
Springiness	0.22	0.30	NS

NS: not statistically significant (p>0.05)

Table 3: Physicochemical analysis of TRM and TPI.

Analysis	TDM	TPI
Fat	12.6±0.65[a]	5.7±0.54[b]
Moisture	75.1± 0.42[a]	70.80±0.69[b]
Ash	0.90±0.11[a]	0.88±0.09[a]
Protein	11.4± 0.87[b]	23.61±0.85[a]
TVBN(mg N/100 g sample)	19.6±0.75[a]	6.8±0.35[b]
TBARS (mg malonaldehide/kg sample)	0.56±0.05[a]	0.33±0.03[b]
Peroxide value (meq/ kg)	6.51±0.13[a]	2.51±0.97[b]

TRM: tuna red meat; TPI: tuna protein isolate. Dissimilar superscripts in the same row denote significant difference (p<0.05). [1]Results are presented as means values (n = 6).

Table 4: Average sensory scores (0–100) for 2 groups of fish cakes.

Attribute	*P* value	Fish cake from TRM	Fish cake from TPI
Odor			
Rancid	**	80.32[a]	5.23[b]
Fishy	***	90.26[a]	10.45[b]
Appearance			
Wrinkle	*	56.32[a]	35.21[b]
Color (inside)	**	79.38[a]	45.17[b]
Texture			
Softness	*	26.54[b]	32.89[a]
Cohesiveness	**	58.69[b]	66.25[a]
Graininess	NS	23.21	26.32
Rubbery	*	77.69[b]	83.21[a]
Flavor			
Rancid	***	70.25[a]	3.23[b]
Fishy	***	79.25[a]	5.69[b]
Soapy	**	12.25[b]	20.56[a]

TRM: tuna red meat; TPI: tuna protein isolate. Values are means of two evaluations. Different small letters show significant difference within a row (∗p<0.05; ∗∗ p<0.01; ∗∗∗ p<0.001). TRM= Tuna red meat; TPI= Tuna protein isolate; NS = Not significant.

Discussion

In this work the pH of protein solubilization was the only factor that influences the responses; therefore one-factor design was used for the modeling as the suitable design for the experiment (Diniz and Martin, 1996; Choa *et al.,* 2005; Jeirani *et al.,* 2013).

Proximate analysis of fish products can vary with the physiological characteristics and dependent on processing methods. Fat content of fish protein isolate can be as low as 1% in high speed centrifuge situations (Shaviklo, 2006). Differences in methods for separating soluble and insoluble phases may bring about increases in fat with pH-shifts (Nolsøe *et al.* 2007). Moisture content of fish protein isolate may well depend on dewatering methods, too.

The TVB-N and TBARS are important indicators for fish quality and freshness (Huss, 1988). In the present study, tuna may have started to spoil during handling and frozen storage, which shows the essence of proper fish handling and frozen storage before processing. However, the protein isolation process could decrease TVB-N, and TBARS levels significantly in the TPI samples, due to considerable removal of amounts of fat as well as already formed oxidation products owing to the nitrogen containing compounds and water soluble proteins during the dewatering steps (Shaviklo *et al.,* 2010c; Shaviklo and Rafipour, 2013). Amid the abovementioned results, the TVB-N levels and TBARS values specific to TPIs were still lower than permitted levels (Huss, 1988). The TVB-N, should not be over 30-35 mg/100 g in fish meat. The TBARS value of 3-4 mg malondialdehyde points to reduced quality of fish meat (Huss, 1988). A similar removal efficiency of chemical and microbial hazards has been recently reported in protein isolation processes using pH-shift technology (Kokkaew *et al.,* 2015; Panpipat and Chaijan, 2016).

The TRM mainly consists of unsaturated fatty acids, which are prone to oxidation. Accordingly, the oxidation of unsaturated fatty acids is adequately analyzed by using the TBARS method. Lipid oxidation in fish protein isolates has been reported during the pH-shift process (Kristinsson and Hultin, 2003; Kristinsson *et al.,* 2005; Kristinsson and Liang, 2006; Shaviklo *et al.,* 2012). The acid-aided pH-shift process can lead to increased oxidation of the lipid fraction of fish flesh compared to the alkaline assisted process due to activation of heme proteins as prooxidants at low pH (Hultin *et al*., 2005; Kristinsson and Liang, 2006; Shabanpour and Etemadian, 2016a).

The average viscosity of TPI at pH 11.0 was 3.3 cP (Centipoise). This result was lower than that for hake fillets (*Merluccius capensis*) reported by Moreno *et al.* (2013). However, it was similar to the values reported by Zhou and Regenstein (2004) for skin gelatin extracted from Alaska Pollock, which were between 1.5 and 6.6 cP yet lower than values reported by Boran and Regenstein (2009) for the skin gelatin extracted from silver carp, which was between 2.5 to 13.5 cP. Low viscosity might be due to low cross linking degree of protein molecules. The low viscosity of prototypes may possibly be explained by decreasing interaction between proteins and surrounding medium (Belitz *et al.,* 2009). Therefore, denaturation and modification of protein conformation in tuna protein samples may have affected the viscosity.

The WHC is one of the most important quality parameters of muscle and fish products, because of reduced weight loss during cutting and storage, and improved ability of muscle to retain water during processing (Belitz *et al.*, 2009). The WHC of TPIs at pH 11.0 was 26%, which resembled saithe protein isolate yet it was greater than Arctic Charr protein isolate and cod protein isolate from by-products (5.3%) (Shaviklo, 2006). WHC of tilapia protein isolates made from fish fillet was 2-4% as reported by Ingadottir (2004). The degree of WHC in fish species may well be associated with quantities of salt, different processing method together with the interaction of these factors (Hultin *et al.,* 2005; Park *et al.*, 2005). However, fish protein isolate can serve as a potential candidate in different food systems.

In the present work, hardness and cohesiveness obtained peaks for samples prepared at alkaline pH. The muscle proteins have been considered particularly responsible for gelation are myosin and actomyosin (Park *et al.*, 2000). Alkali-aided protein extraction can bring about less denaturation compared to the acid-aided process (Kristinsson *et al.,* 2005). The exposure of more hydrophobic groups due to protein extraction at higher pH values leads to more protein-protein interactions (Omana *et al.*, 2010). These interactions may lead to a gel network to form with increased hardness. Such a gel network is likely to differ from a typical gel network of myofibrillar proteins (Tadpitchayangkoon and Yongsawatdigul, 2009). Elastic gels can be got from numerous cross links between the myofibrillar proteins. When this gel network is unevenly distributed due to local aggregation of myofibrillar proteins, it brings about poor gel formation (Omana *et al.*, 2010). And such gels would appear harder due to the presence of this local protein aggregation to break easily when some force is applied (Feng and Hultin, 2001). Increased hardness may also be accounted for by a stronger gel network formed when myofibrillar proteins concentrated in the protein isolate. The low values for cohesiveness, adhesiveness, springiness and resilience were mainly due to the fat content (Caine *et al.*, 2003).

The whiteness value of TPIs at pH 11.0 was 31.21, which was considerably whiter than that of TRM (26.09). Color of fish protein isolates can depend on factors such as kind of raw material, and muscle color and freshness of fish (Shaviklo, 2006; Nolsøe and Undeland, 2009). Color characteristics of fish protein isolates can depend on; a) connective tissue which can increase L* value of fish protein isolates; b) retention of lipids in the products that can influence yellowness value; co-precipitation of heme proteins which can affect redness value; c) denaturation and oxidation of hemoglobin which cause yellowish-brown color in products; and d) concentration of haem proteins in the final product that contribute to a higher a* value (Kristinsson *et al.,* 2005; Shabanpour and Etemadian, 2016b).

No objectionable matters (impurities) were detected in the raw TPI prototypes of this study, given that this attribute are usually about 5-10 in conventional surimi (Lanier Tyre, 2000). The results equally suggest that all impurities from the raw materials can be separated and removed by the use of the pH - shift method. Previous authors showed 13 objectionable matters for conventional surimi and 15 impurities for recovered surimi (Park *et al.,* 2005).

Poor sensory rating for attributes of fish cake made from TRM is associated with the accumulation of TVB-N compounds in fish flesh and lipid oxidation (Huss, 1988; Belitz *et al.,* 2009; Shaviklo *et al.*, 2016). Serious lipid oxidation in tuna meat can be activated by hemoglobin and myoglobin during improper handling and storage (The Codex Alimentarius Commission, 2009). The pH-shift process can be contributing to the fishy odor and flavor from TRM, which may also be linked to removal of TVB-N compounds and lipid oxidation products of tuna isolates (Table 3). The potential elimination of unwanted chemicals/ components associated with fishy odor by using acid/alkaline aided processes has also been evidenced previously (Shaviklo 2006; Kokkaew *et al.,* 2015; Panpipat and Chaijan, 2016).

The Tuna canning operations generate large amounts of by-products annually that could be utilized to produce value-added products. To develop protein isolates from TRM is therefore plausible. The TPI could be a potential/ promising raw material for the food industry, despite its poor functionality demonstrated in this study, which could be avoided by proper handling of tuna, preserving the by-product under conditions to prevent the lipid oxidation/ spoilage or by adding antioxidants during processing. The TPI can be incorporated in the product development of formulated fishery products. However, more studies on storage stability, product development and consumer preferences are necessary towards the commercialized TPI products.

Acknowledgments

The authors are grateful for the support provided by the Iran National Science Foundation (INSF).

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