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RESEARCH PAPER

Product Optimization of Fish Burger Containing Tuna Protein Isolates for Better Sensory Quality and Frozen Storage Stability

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Abstract

The effect of applying tuna protein isolated from red/dark meat on product design and development and storage stability of the silver carp mince burger was studied using Design-Expert® Statistical Software. D-Optimal Mixture Design model was used to investigate the effect of different mixtures of tuna protein isolates and silver carp mince and their interactions on sensory and stability of the prototypes. The results indicated that the fish burgers containing 20% tuna protein isolate and 50% silver carp mince, as selected product, had better sensory scores and acceptability than the other prototypes. The results also indicated that added tuna protein isolate to silver carp mince and storage time did not affect the sensory attribute of the prototype. Soapy taste and grainy texture, which were reported as quality defects in the previous work, were not detected in the present study. The prototypes were also stable within 6 months frozen storage.

Keywords: Tuna red meat, silver carp mince, D-Optimal Mixture Design.

Introduction

Seafood is an important source of animal protein for human food. Fishery products play an important role in the diet of people in many countries (Min *et al.,* 1988). Along with a growing interest for animal proteins to be used as food ingredients, the large stocks of fishery by-products (rest raw materials) have received increased attention as potential protein resources (Shaviklo, 2008; Arason *et al.,* 2009). At least 40% of the estimated 300,000 metric tons of tuna species that are processed in Iran (Shilat, 2014) is red meat, which is wasted and converted to non-human products as fish meal or fertilizers. Therefore, a major challenge for tuna canning industry is to find the new processing methods for applying tuna by-products (mainly red meat) in food formulations (Razavi-Shirazi, 2002).

The red/dark meat is a band of red/dark tissue that lies beneath the skin throughout the fish's body (Sánchez-Zapata and Pérez-Alvarez, 2007). There are many variations in the chemical characteristics of the two types of fish meats *"i.e."* light and red, but the most important are the high fat and hemopigment contents in red meat. Therefore, the higher lipid contents, less stable proteins, and higher concentrations of sarcoplasmic proteins of red meat are the main obstacles in its application and developing food products (Hultin and Kelleher, 2000; Sánchez-Zapata *et al.,* 2011).

Annually, a large quantity of yellow fin tuna (*Thunnus albacares*) is commercially used in the canning industry (Razavi Shirazi, 2002). Yellow fin tuna is a fatty fish and contains more than 40% of red meat (Sánchez-Zapata and Pérez-Alvarez, 2007). The tuna red meat is not usually used by the tuna canneries, and removed from the processing line as a by-product (Razavi Shirazi 2002). However, the characteristics of tuna red meat make it not acceptable for food product development. Strong red color and highly susceptible to lipid oxidation accelerate its deterioration (Razavi Shirazi, 2002; Hultin *et al.,* 2005; Nishioka *et al.,* 2007). However, the isolation of proteins for food application could be a practical approach for utilization of such raw materials.

Fish protein isolate is produced from different types of raw materials by the pH-shift technology (Hultin *et al.,* 2005). It is a kind of protein ingredient, without retaining the original shape of the muscle and can be used as raw material for production of value

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added and ready-to-eat products based on mince or surimi (Shaviklo *et al.,* 2012).

Designing and developing new seafood products including fish protein isolate requires a comprehensive study on raw material stability and sensory properties of the final product (Shaviklo *et al.*, 2010). The level of incorporation of fish protein isolates into food formulation and storage time can influence the sensory quality and stability of the product (Shaviklo *et al.,* 2010). The presence of hemoglobin and myoglobin during pH-shift process and in the final product could lead to a serious lipid oxidation (Gutteridge, 1988; Richards and Hultin, 2002). Protein oxidation is another important issue in frozen stored fish and fishery products, which can affect protein functionality and sensory quality (Davies and Dean, 1997; Hawkins and Davies, 2001). There are few published studies on characteristics of fish protein isolate in ready-to-eat products. Development of fish sausages (Piers *et al.*, 2009), fish balls (Shaviklo *et al.*, 2010) and beef balls (Hayam, 2015) incorporated with fish protein isolate has been already reported. But there is not any published work regarding to ingredient optimization of fish burger formulated with fish protein isolated from tuna by-product and its storage stability study.

Therefore, the overall objective of this study was (1) to determine the optimum level of protein isolated from tuna red meat in silver carp burger formula and (2) to investigate the influence of protein isolates and frozen storage time on sensory quality and stability of the optimized product. The results help industry for commercial use of tuna protein isolate.

Materials and Methods

Fish Protein Isolates

The frozen Tuna (*Thunnus albacares*) with total weight 100 kg (15 individuals) was obtained from a local tuna cannery (Gil Chika Co., Anzali, Guilan, Iran). The fishes were 30 days old when they brought to the processing laboratory (National Fish Processing Research Center Anzali, Guilan, Iran) in frozen conditions (-18°C). The fish transportation and handling from fish cannery to the laboratory took less than 1 h. The frozen tuna (each piece 5000-8000g) was thawed in chill storage (4°C) over night. The red meats were removed from the fish flesh manually. A localized filtration based pH-shift method based on Nolsøe *et al.*, (2007) work was used for isolating protein from tuna red meat. No cryoprotectants were added to tuna protein isolate (TPI) and it was used immediately for product development.

Fish Mince

Fresh silver carp (*Hypophthalmichthys molitrix*) with total weight 20 kg (10 individuals) were obtained from a local fish market (Rasht, Guilan, Iran) and transported by ice (1:1) to National Fish Processing Research Center (Anzali, Guilan, Iran). Individual fishes (weight range: 600-900 g) were gutted, dressed and filleted manually and minced by using a mechanical deboner (Baader model 694, Lubeck Germany).

Food Ingredients

Breadcrumbs, textured soy protein were obtained from the Amoon Shirin Part Company, (Karaj, Iran), and Zardaneh Soy Protein (Esfehan, Iran), respectively. Fresh onion, fresh garlic, salt, spices, vegetable oil, dried vegetable, and wheat flour was purchased from a local market (Anzali, Guilan, Iran).

Product Design and Development

D-Optimal Mixture Design (Shaviklo and Fahim, 2014) was used to optimize the fish burger containing TPI and SCM. Statistical software package Design-Expert (Version 6.0.2, State-Ease, Minneapolis, MN) was applied to construct as well as to analyze the design. The suggestion of low level and high level of TPI and SCM in fish burger formula (20-50%) was based on the pre-test results. The attributes like odor, flavor, texture and overall acceptance were also determined as the responses. Therefore, 13 representative formulations (1–13) were suggested by the software (Table 1). Initially, different proportions of silver carp mince (SCM), TPI and other ingredients (as mentioned in the footnote under the table 1) were mixed with a kitchen blender (Panasonic, MJ. W176P, Japan). The fish burgers were molded manually using a plastic former, followed by battering (30% wheat flour, 10% corn flour and 60% cold water) and breading (conventional breadcrumbs, Amoon Shirin Part Company) and deep frying (for 30 s at 180ºC in sunflower oil) using a pilot processing line (Convenience Food Systems, Bakel, The Netherlands) based on a previous work (Shaviklo and Fahim, 2014). The fried prototypes were used for sensory evaluation tests to find the optimum mixture.

Then, the optimized prototype was developed using the same aforementioned process. The fried and chilled prototypes, except samples which were measured freshly the following day, were individually quick frozen at -40°C for 20 min and packed in polyethylene bags, sealed and stored at -18°C. During the storage time, the frozen prototypes were removed randomly from the freezer and were put in a refrigerator for thawing overnight before the physico-chemical and sensory measurements.

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 the sample in an air oven at 105°C until it reached a constant weight (AOAC, 1995). Ash content was determined by charring samples at 550°C for around 18 h (AOAC, 1995). Carbohydrate content was calculated by difference. 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. Malondialdehyde 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 pH of the 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.

Microbiological Analyses

National standard methods were used for preparation of the samples (ISIRI, 2007). Aerobic plate counts were determined by the spread plate method on Plate Count Agar (30°C for 24-48 h). Most Probable Number method was carried out for coliform bacteria count. Lauryl Tryptose Broth was used as a medium and confirmation test was made in Brilliant Green Bile 2% (37°C for 24-48 h). Most Probable Number method and Lauryl Sulfate broth as a medium were used for *E. coli* counts. Confirmation test was made in Peptone Water and EC broth accordingly. Yeast and molds were enumerated with Potato Dextrose Agar (25°C for 5 days). *Salmonella* spp. was determined using *Salmonella* and *Shigella* Agar (37°C, 72 h) (ISIRI, 2007). All media were provided from Merck KGaA (Darmstadt, Germany).

Sensory Analysis

Step 1: Sensory evaluation of the prototype to select an optimized mixture was made by an expert panel consisting of 5 (2 females) trained and experienced experts in seafood processing (Shaviklo and Fahim, 2014). The evaluation and selection of the prototypes were based upon the highest scores of sensory liking (odor, flavor, texture and overall acceptance). A 15-cm unstructured line scale anchored with little to much was used to quantitate each sensory attribute (Meilgaard *et al.,* 2007). The experts were requested to first evaluate each sample by sniffing alone and then by tasting. They rinsed their mouths with water after tasting each sample.

Step 2: During 6 months storage, sensory analysis of the selected fish burger containing TPI and control was done by 9 panelists (4 females) at the sensory lab of the National Fish Processing Research Center (Anzali, Guilan, Iran) and had been selected according to the general guidance of the International Organization for Standardization (ISO, 2012). The average age of the panelists was 30 years and they were familiar with the Quantitative Descriptive Analysis (QDA) method. Panelists had experiences in sensory assessment of the fishery products and they were trained during two sessions to evaluate fish burger prototypes using the QDA method (Meilgaard *et al.,* 2007). The panel observed differences in appearance, texture, odor, and flavor of the prototypes (P<0.05). A list of sensory lexicon (Table 2) to describe the intensity of each attribute for the given samples using an unstructured scale (from 0 to 100%)

Table 1. Design of experiment for optimizing 2 main components of fish burgers containing tuna protein isolates and silver carp mince and related responses

Run	Component 1: Tuna protein solate (%)	Component 2: Silver carp mince (%)	Response 1: Odor	Response 2: Flavor	Response 3: Texture	Response 4: Overall acceptance
1	42	28	73	73	80	75
2	50	20	73	73	73	72
3	31	39	81	82	82	79
4	35	35	80	82	84	87
5	24	46	73	73	73	74
6	20	50	83	84	82	85
7	35	35	80	82	84	87
8	50	20	73	73	73	72
9	50	20	73	73	73	72
10	46	24	70	63	74	66
11	28	42	74	77	80	77
12	20	50	83	84	82	85
13	20	50	83	84	82	85

The 2 mixture components, tuna protein isolates and silver carp mince, made up a total of 70% of the actual formulation, with the complement being, fresh onion (4.5%), fresh garlic (1.4%), salt (1.3%), spices (0.3%), dried parsley (1%), vegetable oil (2.5%), breadcrumbs (12%) and textured soy protein (7%) used to make up 100% of the formulation.

was adapted from Shaviklo *et al.*, (2010). All sample observations were done based on ISO guidelines (ISO, 2007). All prototypes were coded with three-digit random numbers and presented to the panelists on a plastic tray in individual booths. Orders of serving were completely randomized in duplicate. Water was provided between evaluations to cleanse the palate (Shaviklo *et al.*, 2010). The panel evaluated the fish burgers without information about the ingredients, using a list of sensory vocabulary. 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

PanelCheck software (version V1.3.2, Matforsk, Ås, Norway) was applied to monitor panelists' performance and to analyze sensory data. Multivariate comparison of sensory attributes of the isolate mince burger and control samples was also carried out with Principal Component Analysis (PCA) using the statistical program Unscrambler (V 9.7 (CAMO Software AS, OSLO, Norway).

Analysis of variance (ANOVA) was performed using the statistical program NCSS 2007 (NCSS, Statistical Software, Kaysville, UT) for the statistical analysis of physico-chemical and microbial results. Student's t-test was used to determine whether there was a difference in proximate composition of control and isolate mince burgers. The results were given as a mean ± standard deviation. Significance of difference was defined at the 5% level.

Results and Discussion

Prototype Selection

The experimental design with independent variables and the related observed responses for the fish burger prototypes are given in Table 1. The changes in selected responses for both control and isolate mince burger as is given by the two component design are shown in Figure 1. The responses of these models can be plotted as a function of two components in the mixture keeping the total as 70%. Mixture response surface contour plots (Figure 1) display that the mixture of SCM and TPI, affect sensory properties significantly. It reveals that TPI plays a major role in fish burger odor, flavor, texture and the overall acceptance. As the level of TPI increased, the acceptance of the product decreased. Using such data for product design and development and prototype selection depends on the specifications of the product and expert panel comments (Shaviklo *et al.*, 2013).

The desirable maximization of the isolate mince burger was performed by numerical techniques using mathematical optimization procedure of the Design Expert Software Package. Optimization criterion was based on the highest level of sensory scores, including acceptance, which is thought to be the most important

Table 2. Lexicon for sensory attributes of fish burgers (adapted from Shaviklo *et al*., 2010)

Sensory attribute	Scale (0-100)	Definitions
Odor		
Spicy	none | much	Inside burger: odor of spices, onion, etc.
Frying oil	none | much	Inside burger: odor of fat from frying oil.
Rancid	none | much	Inside burger: rancid odor can remind of cardboard, paints, nuts, etc.
Fish	none | much	Inside burger: fish odor.
Frozen storage	none | much	Inside burger: odor from frozen storage, old fish, etc.
Appearance		
Wrinkle	none | much	Wrinkle on the surface of the burgers.
Color (inside)	light | dark	Inside burger: Is the color dark or light?
Texture		
Softness	firm | soft	Outside and inside: Softness in the first bite.
Cohesiveness	little | much	Inside burger: Little (easy to take apart with a fork), Much (the inside of the burger is firm).
Juiciness	dry | juicy	Outside and inside when chewing: Dry (sample draws liquid from mouth), Juicy (Samples give away liquid)
Graininess	none | much	Inside burger: when rubbed against palate with tongue, grainy reminds of couscous or sand.
Rubbery	none | much	Outside and inside: when chewing rubbery, springy.
Flavor		
Spicy	none | much	Outside and inside: flavor of spices or onion.
Frying oil	none | much	Outside and inside: flavor from frying oil.
Rancid	none | much	Outside and inside: sign of decay.
Fish	none | much	Outside and inside: fish flavor.
Frozen storage	none | much	Outside and inside: flavor from long frozen storage.
Soapy	none | much	Soapy, chemical flavor.

parameter in product development studies (Varghese *et al.*, 2015). The solution was obtained using the software, which sought to maximize the desirability function by being at random starting points and proceeding on a path of the steepest slope to a maximum. The best among them was taken as optimum. The desirability model (Figure 2) was obtained from the software (Design-Expert) calculation. It recommended a mixture containing 20% TPI, 50% SCM and 30% other ingredients (Table 1). The reconstitution of the mixtures was done. No significant differences were found between the predicted responses and actual obtained response values (Table 3). These results revealed the capability of 2-mixture design in food formulation.Therefore, fish burger containing TPI and silver carp burger (as control sample) were developed, packed and stored 6 months in cold storage (-18 ℃) to study quality changes and shelf life studies.

Physico-Chemical Characterization

Raw SCM and TPI

Significant differences ($P<0.05$) were found between SCM and TPI for protein, moisture, fat content, pH (Table 4) and TBARS levels (Figure 3). The same level of ash, carbohydrate (Table 4) and

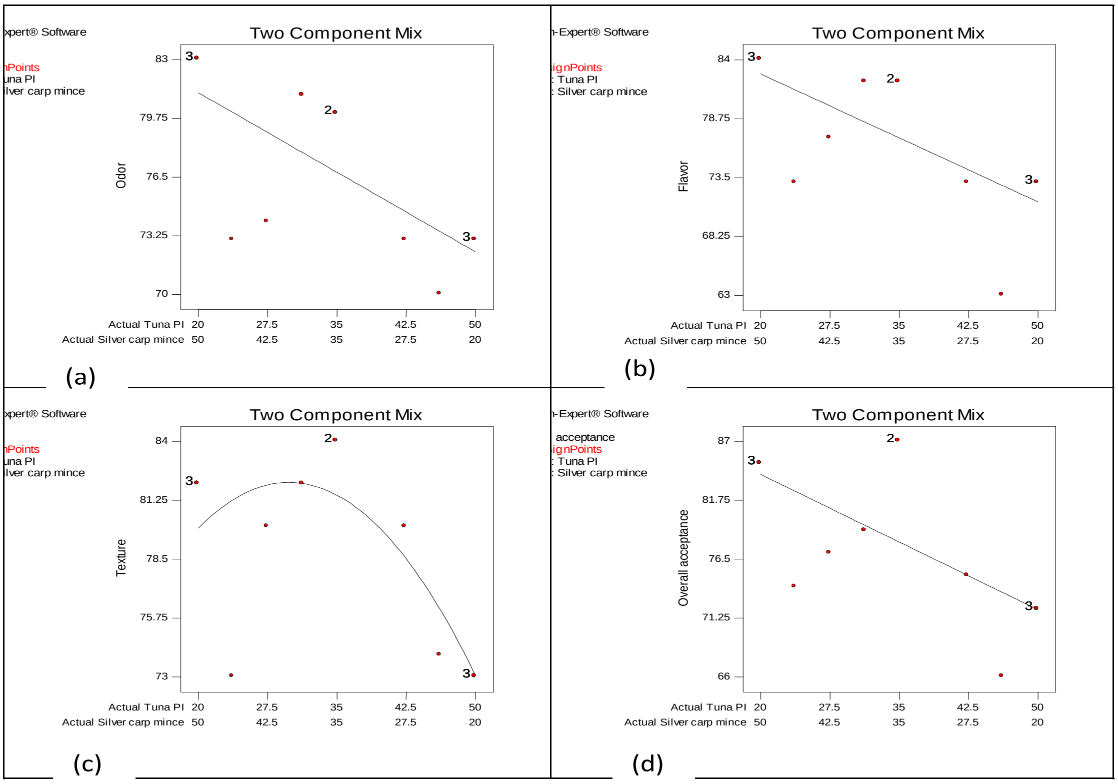

Figure 1. Variations in odor (a), flavor (b), texture (c) and overall acceptance (d) of fish burger with varying proportions of TPI and SCM in the mixture.

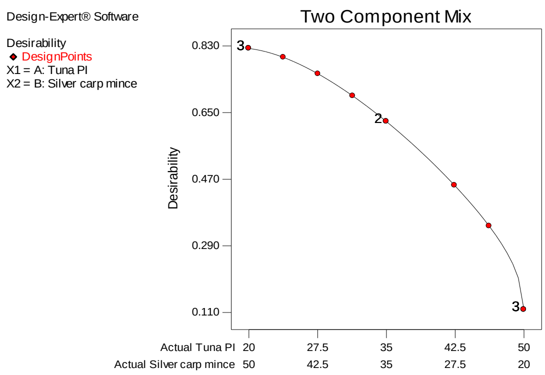

Figure 2. Desirability of fish burger with varying proportions of TPI and SCM in the mixture.

TVB-N (Figure 4) was observed for SCM and TPI samples. Proximate analysis of fish protein isolate can vary with the physiological characteristics of fish and also depends on the processing methods. Fat content of fish protein isolate is usually less than 1%, if a high speed centrifuge is used (Shaviklo, 2007). The higher amount of fat will be found, if filtration is applied to separate soluble and insoluble phases in the pH-shift process (Nolsøe *et al.*, 2007). The moisture content of fish protein isolate depends on dewatering methods, too. The average pH value of SCM and TPI were within the limits considered acceptable for fresh fish (Huss, 1995).

The TVB-N and the TBARS are important indicators for fish quality and freshness. The TVB-N and TBARS value for raw SCM and TPI were 5.6 mg/100 g and 0.11 mg malondialdehyde/kg and 15.6 mg/100 g and 0.25 mg malondialdehyde/kg, respectively, before processing, which were lower than permitted levels (Huss, 1995; ISIRI, 2003).

Fish Burger Prototypes

Protein and moisture contents of SCM burgers were significantly different from isolate mince burger (containing 20% TPI and 50% SCM). Isolate mince burger had higher values of protein and lower value of moisture than that of the control sample (Table 4).

The TVB-N and the TBARS for SCM burger and isolate mince burger were 13.6 mg/ 100 g and 0.10 mg malondialdehyde/kg and 13.9 mg/100 g and 0.19 mg malondialdehyde/ kg, respectively just after processing. No significant difference was found among samples within 6 months storage for The

Table 3. Prediction values (suggested by the software) and actual values obtained from the panelists for sensory responses of fish burger containing 20% TPI and 50% SCM

Response	Prediction values	Actual obtained values	*P value*
Odor	81.16	83.43	NS
Flavor	82.74	81.22	NS
Texture	79.95	77.45	NS
Overall acceptance	84.06	82.78	NS

NS: not statistically significant (p>0.05)

Table 4. Proximate analysis (%) of SCM and TPI and burgers

Sample	Protein	Moisture	Fat	Ash	Carbohydrate	pH
Raw samples						
Silver carp mince	17.26 ± 1.21^{b}	78.81 ± 0.91^{a}	2.82 ± 0.32^{b}	1.11 ± 0.10	0.0	6.7 ± 0.01^{a}
Tuna protein isolate	23.61 ± 0.85^{a}	69.74 ± 0.69^{b}	5.75 ± 0.54^{a}	0.90 ± 0.09	0.0	5.8 ± 0.04^{b}
Cooked samples						
Silver carp burger (control)	19.01 ± 0.51^{b}	61.32 ± 1.01^{b}	7.02 ± 0.21	2.63 ± 0.17^{a}	10.02±0.87	6.4±0.03
Isolate mince burger	28.62 ± 0.33^{a}	52.12 ± 0.04^{b}	6.71 ± 0.21	3.21 ± 0.07^{a}	9.34±0.74	6,2±0.01

Values are means of 3 analyses. Different Superscripts denote significant differences within a column (P<0.05). NS: not significant. * containing 20% TPI and 50% SCM.

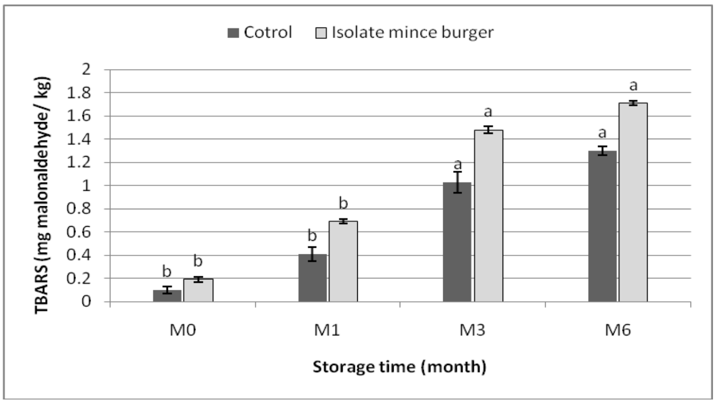

Figure 3. TBARS values of SCM burger (control) and isolate mince burger (containing 20% TPI and 50% SCM) during 6 months storage at -18◦C. M: month of storage.

TVB-N values (Figure 4). The TBARS values between the sample groups changed significantly after 1 month of storage (Figure 3), but they were lower than permitted levels (Huss, 1995; ISIRI, 2003). The TVB-N, which should not be over 30-35 mg/100 g in the fish meat. And the TBARS value of 3-4 mg malondialdehyde/kg leads to souring and quality loss in fish meat (Huss, 1995).

Fish and fishery products are susceptible to chemical and physical changes during frozen storage. These changes adversely affect product's quality and storage stability (Al-Bulushi *et al.*, 2013). Serious lipid oxidation in fish protein isolate is caused by hemoglobin and myoglobin during pH-shift process and final product storage (Rechards and Hultin, 2002; Nolsøe and Undeland, 2009). Therefore, it was expected to observe intense lipid oxidation in fish burgers containing TPI during frozen storage. But the results indicated low levels of TBARS values in isolate mince burger possibly due to using ingredients such as garlic and spices. It is well documented that spices are effective in delaying lipid oxidation, however, they are not able to stop lipid oxidation due to low antioxidant concentration (Yanishlieva *et al.*, 2006).

Microbial Analysis

No significant difference was observed between control and isolate mince burgers for all microbial analyses within 6 months frozen storage. Neither type of fish burger nor frozen storage time influenced the number of microorganisms. The total plate count was less than 2×10^5 cfu/g within the 6 months of storage and no *E. coli* (cfu/g) and *Salmonella* (cfu/g) were detected. Counts (cfu/g) of coliforms, mold and yeast were less than 10 throughout the study (data not shown) indicating that the products were microbiologically safe and stable (ISIRI, 2007).

Sensory Evaluation

Results from the analysis of variance (ANOVA) of sensory attributes of fish burger samples during 6 months storage are presented in Table 5. All sample groups had the same sensory attributes after the production and within 6 months frozen storage at -18°C. Storage time influenced just spicy, fish and

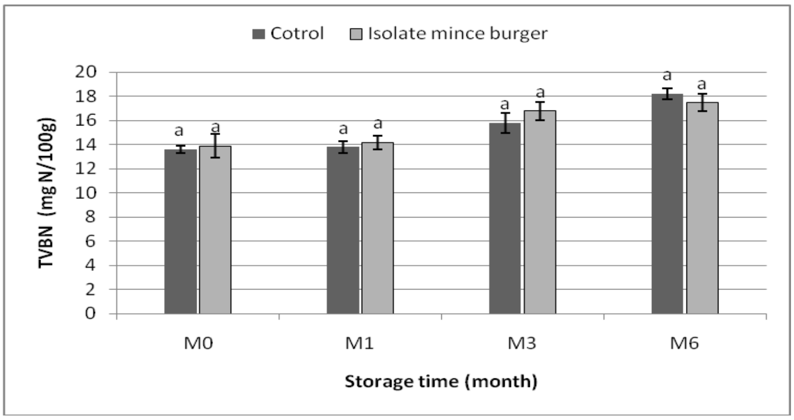

Figure 4. TVBN values of SCM burger (control) and isolate mince burger (containing 20% TPI and 50% SCM) during 6 months storage at -18◦C. M: month of storage.

Table 5. Average sensory scores (0-100) SCM burger and isolate mince burger containing 20% TPI and 50% SCM

Sample	O. Spicy	O. Frying oil	O. Rancid	O. Fish	O. Frozen storage	Internal color	Wrinkle	Softness	Cohesiveness	Juiciness	Rubbery	F. Spicy	F. Frying oil	F. Rancid	F. Fish	F. frozen storage	F. Soapy
F0	51^{a}	48	27	33^{c}	17^{b}	75	18	45^{a}	52	47	23^{a}	44	37	8	33	10	12
C0	52^{a}	46	14	26^{c}	15^{b}	75	20	36^{a}	60	46	28^{a}	42	38	10	26	9	9
F1	42^{a}	49	14	44^{b}	18^{b}	66	23	37^{a}	54	46	14^{a}	36	33	14	30	11	11
C1	38^{a}	42	20	49^{b}	21^{b}	76	21	27^{ab}	63	48	18^{a}	43	39	18	20	17	10
F3	30^{b}	38	34	44^{b}	17^{b}	74	7	22^{b}	42	38	7^{b}	33	24	12	20	12	9
C3	25^{b}	36	18	46^{b}	16^{b}	64	9	24^{b}	35	40	10^{b}	34	25	10	16	9	8
F6	29^{b}	37	33	62^{a}	29^{a}	64	13	17^{b}	35	36	8^{b}	35	32	17	26	15	6
C6	32^{b}	34	22	72^{a}	30^{a}	65	10	14^{b}	40	35	7^{b}	38	27	24	20	17	5

Sig: significant. Different letters show a significant difference between samples within a row. C: SCM burger; F: isolate mince burger containing 20% TPI and 50% SCM. 0 to 6 indicate storage months. NS: not significant.

frozen storage odors, softness and rubbery texture after 6 months of storage. Other attributes *"i.e."* frying oil and rancid odor and flavor, internal color, wrinkle, cohesiveness, juiciness, grainy and spicy, fish, frozen storage and soapy flavors unaffected by the storage time.

A multivariate analysis of the data (Figure 5) showed that 87% of the variation between the samples was explained in the first two principal components. The PCA indicated the effect of storage and product on sensory attributes. The control (C0, C2) and isolate mince burger (F0, F2) groups are located on the left sides of the plot. Attributes located in the upper left sides of the plot indicate that both sample groups have sensory similarities during storage. The changes in the attributes describing oxidation can be seen by the sample groups located in the upper right part of the plot along with the products after 6 months storage. Two sample groups (C4, C6 and F4, F6) are quite separate from each other and are located in the lower and higher right parts of the plot. Control samples were more characterized by softness and cohesiveness, while the mince isolate samples were more characterized by rubbery texture and spicy odor and flavor. The intensity of fish odor and frozen storage odor were increased in mince isolate samples (F4, F6) at the end of the study due to hint lipid oxidation. Frozen storage time affected texture attributes of fish burgers significantly ($P<0.05$). All sample groups were softer and less rubbery after 6 months of frozen storage possibly due lipid oxidation and protein degradation during storage (Undeland and Lingnert, 1999; Ninan *et al.*, 2008). Similar results were reported for fish finger (Tokur *et al.*, 2006), fish burger (Al Bulushi *et al.*, 2005), fish sausage (Al-Bulushi *et al.*, 2013), fish cutlets (Talab, 2014) and fish ball (Shaviklo *et al.*, 2010).

It is well documented that post-harvest activities and also storage time can influence the sensory quality of the fish and fishery products (Huss, 1995). The most important problem for preservation of fresh/frozen fish is lipid oxidation (Ackman, 1980; Belitz *et al.*, 2008) and it relates to high content of polyunsaturated fatty acids in the fish flesh. Comminuted fish flesh is susceptible to lipid oxidation due to the presence of enzymes and oxidation catalysts in the product (Taylor *et al.*, 2006). Lipid and protein oxidation in frozen stored fish and fishery products is also a critical problem, which can affect protein functionality and sensory quality (Davies and Dean, 1997; Hawkins and Davies, 2001; Belitz *et al.*, 2008). However, it could be possible to eliminate such quality deterioration by using natural antioxidants such as different herbs and spices (Yanishlieva *et al.*, 2006).

Soapy/ chemical taste which was reported in our previous work (Shaviklo *et al.*, 2010) was not detected in fish burgers incorporated with tuna isolate due to applying spices. Applying aromatic spices for masking fishy taste originated from fishery derived ingredient has already been reported (Kolanowski *et al.*, 2007; Shaviklo *et al.*, 2011; 2013). Several methods can be probably used for masking the soapy/ chemical taste of fish protein isolates. But, the addition of spices and herbs to the isolate based products would be the most practical and economical approaches to solving this problem.

Grainy/ gritty texture is also another properties of the fish protein isolate that is sensed by touch in the mouth and even with the hands (Shaviklo *et al.*, 2010). It is one of the most important texture attributes reported for fish ball (Shaviklo *et al.*, 2010) and beef ball incorporated with fish protein isolate (Hayam, 2015). In this study, grainy texture was not

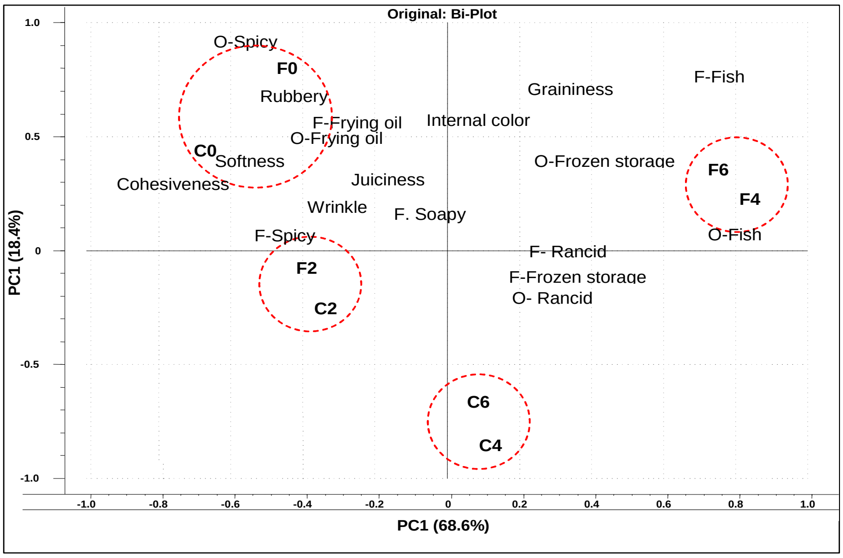

Figure 5. Principal Component Analysis (PCA) describing sensory quality scores of of scmburger (c) and isolate mince burger containing 20% TPI, and 50% SCM (f) stored 6 months at -18˚c as evaluated by a trained sensory panel. O: odor, F: flavor. Numbers 0 to 6 indicate the storage months.

detected in the isolate mince burger due to comminuting tuna protein isolate along with silver carp mince for texture modification. Size reduction/ comminution is used to improve food texture and therefore, eating quality. Accordingly, different methods of size reduction are used in food unit operations based on the size range of particles produced (Fellows, 2000). However, the shape and size of these particles as well as their distribution affect flavor, texture, and appearance of the intended food (Rosenthal, 1999; Suman and Sharma, 2003). In our observations, when FPI is ground or comminuted, the appearance and the taste of the product is changed. It becomes whiter and tastes more soapy than before grinding. However, it can be covered as mentioned before.

Sensory quality of ready-to-eat fish products during frozen storage is dramatically influenced by chemical and microbial deterioration (Tokur *et al.*, 2004; Calci *et al.*, 2005; Al-Bulushi *et al.*, 2005; Boran and Kose, 2007). Several techniques are used for preserving fishery formulated products. Among these methods using spices and herbs, which are natural antioxiants and antibacterial, and covring the produt with batter/and breading are common techniques in the seafood industry (Al-Bulushi *et al.*, 2013; Shaviklo *et al.*, 2010; 2013). Battering and breading of fish burger can act as oxygen barrier, which provide better protection against lipid oxidation and microbial deterioration (Joseph *et al.*, 1992; Vanitha *et al.*, 2013). Accordingly, in this study, both control and mince isolate samples were stable and had the same sensory attributes after production and during frozen storage. This is a positive point for applying TPI in formulated muscle foods.

Conclusions

The results of the product design and development showed that the prototype quality was influenced by the different proportions of TPI and SCM. The optimum components were determined as a 20% TPI and 50% SCM. Both groups of fish burgers had the same odor, flavor and texture attributes and overall acceptance after production and during frozen storage. Soapy taste and grainy texture, which were reported as quality defects in the previous work, were not detected in the present study due to applying spices and size reduction of the fish protein isolate, respectively. The prototypes were also stable within 6 months frozen storage. This was a positive point for commercial application of TPI products. Mixture design was found to be a beneficial tool in product design and development with a high degree of predictive accuracy of the responses.

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