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ORIGINAL ARTICLE

The Qualitative characteristics of Silver Carp Fish burger fortified with isolate protein of fish

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ABSTRACT
The aim of this research was the use of isolate protein of tuna fish in the burger carp to improve the nutritional value and the examining of qualitative characteristics and shelf life of the fish burger. For this purpose silver carp after early preparations, was head and tail grinded and raw fillet was washed and then to prepare for a burger stuffed and were mixed with other ingredients (treatment 1 or control) and in the enriched samples, the isolate protein of tuna fish was added to burgers mixture at a rate of 25% (treatment 2). Then the samples for evaluation of chemical, corruption and oxidation and sensory evaluation for 5 months in 4 Phase for testing were evaluated. The results showed that moisture, fat and pH percentage in treatment 1 was significantly higher than treatment 2 (p<0.05). But the percentage of ash, protein and TBARS in treatment 2 was significantly higher than treatment 1 (p<0.05). With the passage of time during storage of numeric values of fat, ash and TBARS values increased but protein, moisture and pH values decreased during storage. The results of sensory evaluation showed that sensory data during storage was reduced in treatments over time but the results were similar and the reduction was not statistically significant. According to the results, we can conclude that burger contains isolated tone has had a higher nutritional value and quality than the control sample and shelf-life in both samples has been 5 months.
Keywords *Fish Burger, Fish Silver Carp, Fish Protein Isolate, Tuna, Shelf Life, Nutritional Value*

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INTRODUCTION

The seafood due to the high quality of protein is very important. Seafood contains all essential amino acids are required to value ratio [1]. In recent decades people are more concerned about their health and therefore prefer fish to meat because the fish protein is easier digested than red meat and is more useful for health. So, large amounts of high quality protein can be available for mankind through the marine products. These proteins have appropriate functional features that increase the nutritional value of food products [2]. Several studies have been done to optimize the use of fish processing waste that has been led to produce valuable compounds, especially proteins [3]. The increasing world's population has increased need for protein, so that over the past decade the need for securing new sources of protein concentrate in world has been increased. Thus, much attention has been paid to Supplements of fish processing industry and small and low-value fish species. Every year there is a large volume of fish processing waste that without considering biological hazards are discarded. These materials are protein-rich and typically are used as fish meal as animal feed [4]. If the optimal use of these compounds, in addition to reducing environmental pollution, can be produced value-added products. With turning low-cost and low- consumption to value-added products such as surimi or fish protein isolate, not only can minimize fish processing waste, but also the loss of protein sources can be prevented [5]. The fish protein isolate by change in pH is a suitable intermediate product with nutritional value and a high strength of gel

production. The protein isolated and surimi, both are intermediate products that are used in other manufacturing processes, their texture brightness and whiteness is very important and affects the color of secondary products made from them. The brightness and whiteness of these products, make it is possible that the color of made product can be changed to the desired color [6, 7].
The isolated protein is highly digestible and can play a major role in formulated products. Generally the isolated protein is not used directly, but can be used as a raw material to produce other valuable products. The primary sources in the isolated protein are usually processed fish waste (dark muscle - fatty fish – the muscle and skin of fish and the remaining raw materials from the production of fish fillet) which is an economic process and develops the application of protein [8]. For protein recovery from mentioned resources, various methods such as chemical methods are used [9].
The products with FPI added value by mixing it with various additives such as vegetable proteins, starch, wheat flour, spices and turning into forms of intended products are made [10, 9]. To enter (injection) recycled fish protein from left-over of raw materials to fillet in order to improve efficiency and water-holding capacity and also use them to reduce oil uptake in fried products, many studies have been done and satisfactory results have been reported . In recent years, with the development of urban and machine life, the proliferation of restaurants and cafeterias and more jobs for women in the community, in fact the preparation and cooking of food at home has declined and more people are considering the consumption of prepared and semi-prepared foods [11]. Obviously, meat products, especially products derived from minced meat such as burgers are of particular importance in this regard.
The fish tuna is considered as one of the most valuable industrial fish in the world tuna that allocated a significant account of the annual fishing to itself. In our country remains of raw material derived from tuna cannery is estimated about 60-50 percent from which 10-20 percent is related to dark muscle, which in canning is isolated from clear meat and along with other isolated parts of fish (including head and viscus) as the waste is left at disposal of fishmeal processing units. In a study recently conducted in the country using this technology the dark muscle protein of *Acanthopagrus latus* (yellow fin fish) tuna was extracted and its qualitative characteristics were evaluated. The results showed that more than 80 percent of proteins were extracted through this method and more than 70% of fats in the muscle dark were isolated. Although the functional characteristics of isolated of tuna is weaker than isolate produced from fish fillet, but it may be used in the construction and enrichment of food products due to high protein. Fish protein isolate can be used to enrich fish fillets, increase the consistency of fried products glaze and help to reduce oil uptake in fried products as well as manufacturing formulated products ready to use. Thus, protein recovery from these raw materials and utilization of it in the manufacture of food products of important issues which be considered the country's fish processing industry.
Given the importance of these products processing and use of them in food processing, this study aimed to evaluate the qualitative characteristics of silver carp fish burger enriched with fish protein isolate.

MATERIALS AND METHODS

Three silver carp fish with a total weight of 3 kg and average size of 50 cm was purchased from Rasht fish market and with ice (ice - fish ratio 1: 2) was transferred to the National Center for aquatic product. 50 kg frozen yellow fin tuna (6) with an average size of 60 cm from Cannery homes purchased in the province and in good condition was transferred to the National Center for aquatic product. Fifty kg of frozen Acanthopagrus latus (yellow fin fish) tuna (6 numbers) with an average size of 60 cm was purchased from canning factory of Guilan state and was transported in good condition to National Center for processing of aquatic product. Additives to produce fish burger such as onions, garlic, salt, spices, dried parsley, vegetable oil, wiped soybean and bread powder were bought from the local market of Anzali city.
After thawing frozen tuna, its dark muscle was separated by hand. Then the dark muscle of fish tuna using a meat grinder (saya, Promeat, W1800, 5mm) was minced and then minced meat of fish with 9 parts of cooled distilled water was mixed [6] and using a mechanical stirrer (min1, speed 50) was homogenized. Then, using a soda solution (NaOH) 1 M, pH mixture was brought to 11 [6]. In the next step, to separate the insoluble materials from the dissolved ones, the laboratory (8000× g, 25 min) was used [9]. After centrifugation of the samples, 3 phases were formed. A bottom layer contains impurities, a gel layer on the top which includes a series of lipids and a layer in the middle which was soluble proteins. In the next stage, the pH of isolated solution using hydrochloric acid (HCL) 1 M to pH isoelectric was reached (5.5) whereby the proteins of Sarcoplasm and myofibrils precipitated. In the final stage, the precipitated proteins by centrifuge (4000 × g, min 20) were recycled / isolated and the protein isolate was reduced by pressing handle in the filter cloth (70-72%). The extracted proteins by alkaline method were refrigerated until processing of product .

According to the tests and initial prototyping, the levels of 20 to 50 percent of isolate tuna and minced meat of Silver carp was used to produce product. The total of isolation and minced meat was 70 percent of formulation and other 30 percent included vegetable oil and dried herbs, spices, salt, bread powder, wiped soya, onion and garlic which evenly applied in all treatments.
So using specialized software design expert (Design @ Expert, 7.0, USA) 13 treatments were designed as following and after sensory evaluation the best example in terms of having the highest sensory scores was selected.

Table 1 Examples of treatments designed to produce Burger samples

SN.	Component 1: Tuna Isolate	Component 2: Silver carp mince	Response 1: Odour	Response 2: Flavour	Response 3: Texture	Response 4: Overal 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 production of fish burger

After buying silver carp, alongside the ice with a ratio of one by one in the refrigerator were transported to National Research Center for aquatic processing and after weighing were kept at low temperature (4 ° C) to begin operation. After receiving and weighing the fish, to remove dirt and mud and mucus on the fish, were washed with clean water.
The silver carp after being washed and head grinding and discharged from gut. The fillet fish was washed with clean water and fish guts, blood and debris were removed by brushing. Due to the lack of appropriate systems to fillet of this kind of fish, fillet operation was carried out by hand. At the end of fillet practice, the fillet fish to remove plasma and debris were rewashed. The silver carp fillets directly after being washed by meat catcher were meat isolated.
Firstly, the intended supplementary materials based on the composition percentage were weighed (Table 1). After weighing the material dumped in a mixer with minced meat fish and was uniformed and through this way control sample was provided. In the next step to prepare isolate sample, in addition to previous material, protein isolate was used which was prepared from fish tuna. So, at this stage, two types of mixing was prepared, the first from single silver carp fish meat tuna and second by adding protein isolate of tuna fish were produced.
The prepared mix by forming machine was formed as a round shape. At this stage, the granular flour was used, in order to glaze making be easily done. The simple glaze which was making according to formula of Bandar Anzali aquatic research institute was used. Fried powder was used to cover Burgers. Frying was done using teflon machine made in France. Frying action was done at 180 ° and for 120 seconds. After frying, the burgers were transported to freezer at -18° for maintaining for 5 months to be frozen.

Chemical and sensory testing

The protein measuring was performed using Macro-Kjeldahl method. To measure fat, ash, pH and moisture, the Soxhlet method was used. To calculate, sulfuric acid amount was multiplied by a factor of 14 to amount of volatile nitrogen base in milligrams per 1 hundred grams of meat is calculated [12].
The amount of TBA Malondialdehyde mg per kg of fish tissue was calculated using the following formula

$$\mathrm{TBA} = \frac{(\mathrm{As} - \mathrm{Ab}) \times 50}{200}$$

The amount of peroxide in terms of ml Equivalent per kg fatty substance was calculated according to equation -

N= Normality sodium thiosulfate
S = titration of development (growth)
W= oil sample weight

$$PV = \frac{S \times N \times 1000}{W}$$

Fife trained experts for evaluating the burger samples were used. The samples with 3-digit code were randomly selected were provided in the plastic dish with evaluation sheets to experts. In this study, 2 tests were used for sensory evaluation of production samples. For evaluating the produced burgers and selection of the most acceptable burger, the Hedonic method and 9-point scale Yi (9-point hedonic scale) and to detect sensory changes of burger, the QDA method was used . In both tests, 7 experts were used.

The statistical analysis

After averaging, the data were entered into the Software Design Expert (Version 7.0.0, state-Ease, Minneapolis, MN, USA) to the software data analysis be applied, which included analysis of variance (ANOVA), the calculation of degrees of freedom of graphs resulted by identifying early indicators of response. The analysis of trial data relating to the samples was performed using SPSS version 17. To compare the means between the burger containing isolated tuna and control the Paired Sample T test and Mann-Whithney U test were used. The Panel Check statistical software version V1.3.2, Norway) was used for the analysis of sensory data.

RESULTS AND DISCUSSION

Chemical test

According to the statistical results of Table 2, the protein percentage in the both treatments during maintaining time has been decreased that due to release of free amines and water dripping (Drip) (resulted by thawing and exiting of the amount of protein but in terms of percentage of protein, enriched treatment has been much better than the control treatment and the difference between the 2 treatments was significant ($p < 0.05$).

The higher levels of protein in treatment 2 could be due to the addition of protein isolate of fish to fish burgers which is composed of minced meat and has increased the amount of protein compared to treatment 1. On the other hand oxidation of fat is a major problem in maintenance, particularly maintenance of the frozen fishery products [13, 14] which can have a negative impact on the functional properties of protein [15]. This due to enzyme hydrolysis exiting from context can be cause of the decrease in protein content during maintenance at treatment 1 and 2. The protein oxidation at maintenance of frozen fish and fishery products is another important issue that can affect the functionality of protein and sensory qualities [16, 17]. That's probably another reason to reduce the protein content in non-FPI burger and burger containing isolated tuna with time during maintenance.

Fat percentage has been among effective indicators of the shelf life of treatments which in control treatment is more than the treatment enriched with protein and the its data difference in the 2 treatments was significant over time and fat percentage was significantly increased with the passage of time during maintenance ($P < 0.05$) (Table 2).

Shavikloo and colleagues [23] in studying haddock fish protein isolate extraction and adding protein isolate of haddock fish (FPI) to mince haddock fish and ultimately fish ball reported that the percentage of fat in mince haddock was 0.6±0.06 and in haddock fish protein isolate was 0.1±0.05 that revealed the reduction of protein in fish protein isolate. Zakipour Rahim Abadi and colleagues [18] in the study of quality assessment of minced meat and silver carp surimi to produce fish fingers reported the amount of fat content in minced fish meat and surimi 2.27±0.33 and 1.98±0.34, respectively, that washing mincemeat during the surimi production has been somewhat reduced fat content and Tukur and colleagues [19] for fish fingers receipt of washed meat of mirror carp achieved similar results.

According to the statistical results shown in table 2, the moisture in treatment has been significantly reduced over time due to moisture loss caused by evaporation of springhouse, but the moisture in the enriched treatment was lower and its data difference with control treatment has been significant ($P < 0.05$). Since 80% of the weight of tissue is water and fat, the difference in water percentage per muscle is effective on fat and its content varies. With increasing fat content in the fish burger context during maintenance, the moisture amount has been reduced which is in line with the results of the present research. The obtained results are matched with the results of Taskaya and colleagues [20] about the fish burgers resulted by rainbow trout.

The ash amount in the enriched treatment has been more than control that was due to the high enriched protein content and its data has been significantly different with control treatment. Over time during the maintenance, the ash percentage in treatments significantly increased ($p<0.05$). Shaviklo and colleagues [23] examined the haddock fish protein isolate extraction and adding haddock fish protein isolate ((FPI) to mince prepared from haddock fish and ultimately fish ball and reported ash percentage in haddock fish was 1.3 ± 0.05 and the haddock fish protein isolate was 0.1 ± 0.05, respectively.

Table 2 The measuring of percentage of protein, fat and moisture in control treatments (silver carp burger) and fortified burger with isolated protein of tuna dark meat after 5 months of maintenance at refrigeration temperature (-18^{0c})

	Protein		fat		moisture		ash	
Time	Control	Burger contained isolated protein	Control	Burger contained isolated protein	Control	Burger contained isolated protein	Control	Burger contained isolated protein
Zero phase	19 ± 0.14^{a}	25.14 ± 0.42^{a}	8.00 ± 0.14^{b}	5.7 ± 0.02^{b}	61.03 ± 0.09^{b}	57.00 ± 0.11^{b}	2.40 ± 0.14^{b}	2.82 ± 0.03^{b}
After 5 months	17 ± 0.04^{b}	21.20 ± 0.13^{b}	8.95 ± 0.21^{a}	6.86 ± 0.04^{a}	56.65 ± 0.41^{b}	51.16 ± 0.41^{b}	2.65 ± 0.07^{a}	3.17 ± 0.05^{a}

The numbers in the table represent mean and standard deviation (Mean ± SD) the three replications. Different small letters in each column shows the significant differences during maintenance in 5% level in springhouse ($P<0.05$).

According to the statistical results of measuring pH in both treatments have been decreasing, but the decrease has been preserved after 5 months in the standard range (pH: 5.5). But shelf life of control treatment has been better and data difference of 2 treatments was significant ($P<0.05$) (Table 3). In the above mentioned research the higher pH of surimi and protein isolated compared to mince meat ($p<0.05$) may be due to the loss of free fatty acids, free amino acids, lactic acid or other acid dissolved in water [25].

According to the statistical results shown in table 3, TBArs has been significantly increased over time, but after 5 months in has been preserved at standard level (2 TBArs: mg MDA / 1000 g), but the differences in 2 treatments data after phase zero to the end of 5 months have been significant and significantly has have been increased ($P<0.05$). The increase in oxidation in treatment 2 could be due to the effects of freezing and protein denaturation and also aw reduction and its effect on fat oxidation could be another reason in the increased oxidation.

Fat oxidation is a major problem in maintenance, especially maintenance of fishery products as frozen [13, 14]. The minced fish meat and isolated fish protein are susceptible to lipid oxidation.

According to the results of Richards and Hultin [9] the existence of hemoglobin and myoglobin pigments during the process as well as of changing the pH in the final product (FPI) and products containing FPI can lead to fat oxidation and increase the amount of TBA. However, with the addition of antioxidants to fish isolated protein (FPI) and products containing FPI the unwanted changes can be prevented [27].

Table 3 Mean ± Sd measuring pH, TBArs in the control treatments (silver carp burger) and burger fortified with isolated protein of dark meat after 5 months of maintenance at refrigeration temperature (-180c)

	pH		TBArs	
Time	Treatment 1 (Control)	Treatment 2 (Burger contained isolated protein)	Treatment 1 (Control)	Treatment 2 (Burger contained isolated protein)
Zero phase	6.40 ± 0.01^{a}	6.20 ± 0.01^{a}	6.10 ± 0.01^{c}	0.19 ± 0.02^{d}
After 1 month	6.33 ± 0.01^{a}	6.13 ± 0.01^{b}	0.41 ± 0.2^{b}	0.69 ± 0.02^{c}
After 3 months	6.03 ± 0.05^{a}	5.79 ± 0.02^{b}	1.03 ± 0.09^{a}	1.48 ± 0.03^{b}
After 5 months	5.97 ± 0.01^{a}	5.58 ± 0.02^{b}	1.03 ± 0.04^{a}	1.71 ± 0.02^{a}

The numbers in the table represent mean and standard deviation (Mean ± SD) the three replications. Different small letters in each column shows the significant differences during maintenance in 5% level in springhouse ($P<0.05$).

The results of sensory analysis

As shown in Figure 1, the highest rating of smell and taste, texture and overall acceptability are relating to samples containing 20% isolated fish meat and 50% minced silver carp. So, 20% of isolated fish tuna with utility-grade of 0.83 was considered as recommended and selected level (Figure 2). The selected production samples were packed in plastic bags and were kept in the freezer until test time.

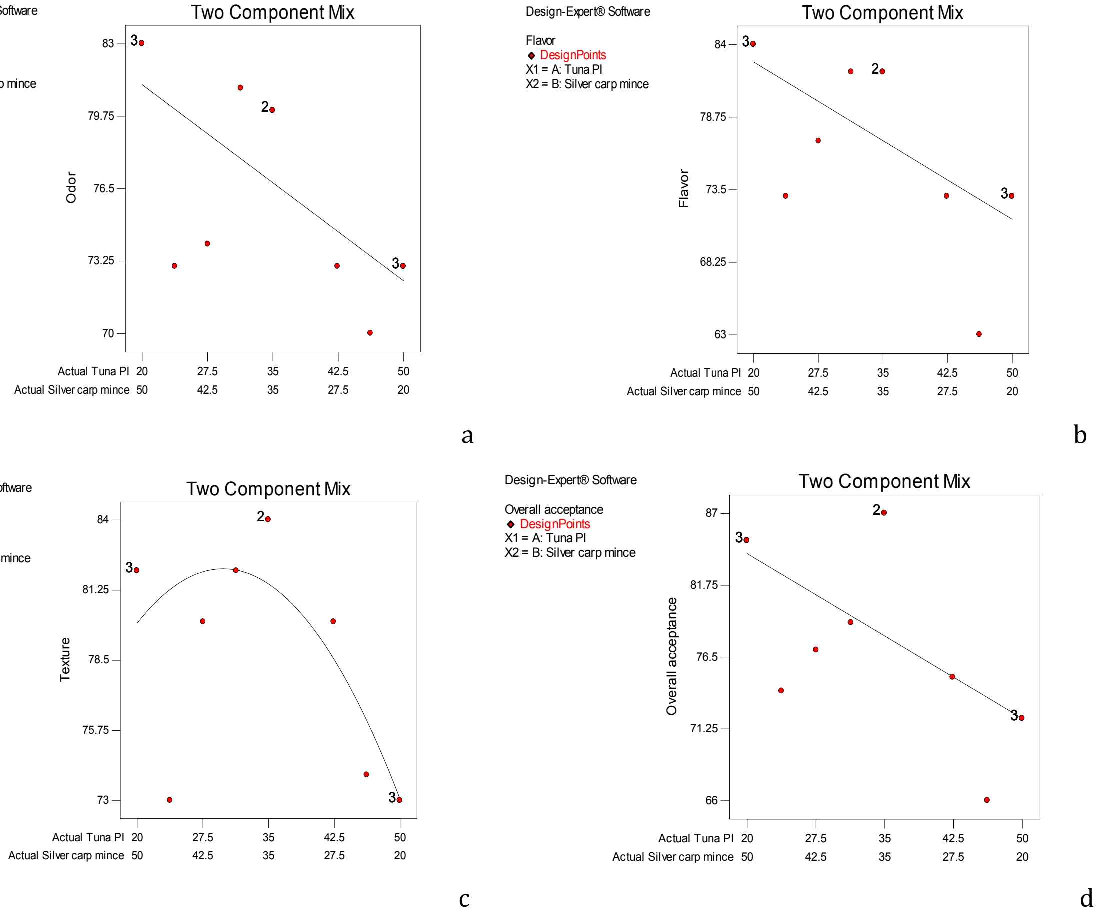

Figure 1 Effect of different levels of isolated tuna and silver carp minced meat on a) smell, b) flavor, c) tissue, and d) general acceptance of produced burger

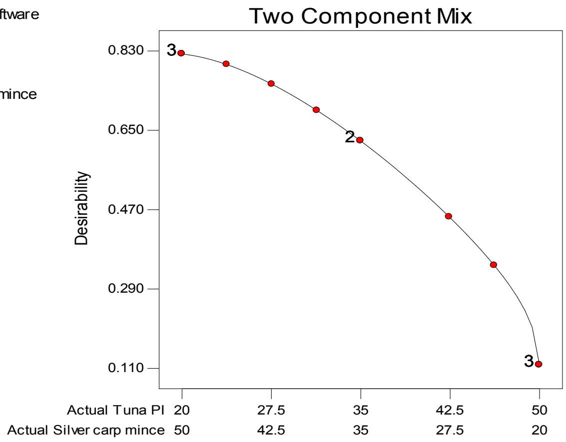

Figure 2 The optimum and recommended level amount of insulated in the formulation

The sensory characteristics of burger containing isolated tuna and control from production time by the end of maintenance time has been shown in table 4.
Average Sensory Scores (0-100) SCM

Table 4 Average Sensory Scores (0-100) Scm Burger (C) And Isolate Mince Burger Containing 20% Tpi And 50% Scm (F)

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
F5	29^{b}	37	33	62^{a}	29^{a}	64	13	17^{b}	35	36	8^{b}	35	32	17	26	15	6
C5	32^{b}	34	22	72^{a}	30^{a}	65	10	14^{b}	40	35	7^{b}	38	27	24	20	17	5
Sig.	<0.05	NS	NS	<0.05	<0.05	NS	NS	<0.05	NS	NS	<0.05	NS	NS	NS	NS	NS	NS

Sig: significant. Different letters show a significant difference between samples within a row. 0 to 6 indicate storage months. NS: not significant.

During maintenance due to inevitable physical and chemical changes, the samples in terms of scents of spices, the smell of fish, the smell of springhouse and softness and elasticity has significant differences with each other. But these increases or decreases were very small and can't have an impact on the acceptance of samples. Burger containing isolated tuna in terms of other sensory characteristics (fried flavor and smell, smell and taste of rancidity, odor and taste of cold (springhouse) maintenance, color, shrinkage, spices smell, etc, had no significant differences with control samples. Differences and similarities of sensory characteristics between the two groups of samples are shown in figure 3. Multivariate analysis of sensory data shows that more than 80% of changes of data between the 2 main components have been described. The identified areas in the diagram (PCA) are related to two groups of samples that are clearly separated. All samples by the end of the maintenance (shelf life) have similar sensory characteristics. However, as shown in the diagram (PCA), the sample containing isolated tuna in the third and fifth month of maintenance are different from control sample and are placed in a separate category, but these changes have not been significant.

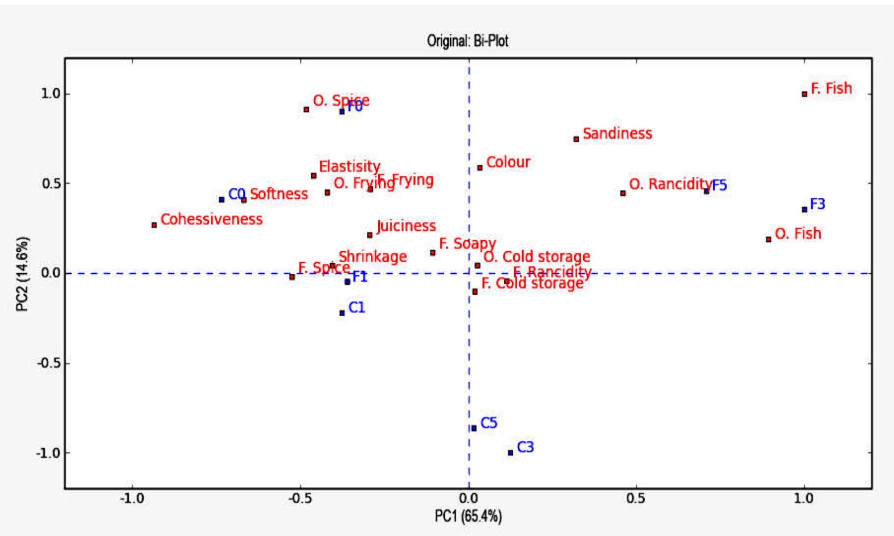

Figure 3 Diagram of principal components analysis represents the sensory evaluation scores of isolated tuna burger (F) and control (C) during 5 months of maintenance at refrigeration temperature (-180c)

The functional properties of fish protein isolate (FPI) can be effective as an element affecting the sensory characteristics of food products (flavor, smell, texture, appearance, etc.) or on properties affecting food products processing (pumping, the extrude ability , tear or wastes resistance, etc.). This definition suggests that the functional properties of protein isolate are effective on food ingredients and its manufacturing process. All the measuring physical or sensory characteristics should be done on a baked product and the evaluation of appearance, flavor, smell and texture of products containing FPI is necessary to improve the quality and effectiveness [23].
Oxidation or rancidity of fat can lead to bad breath out, creating an unpleasant taste and overall undesirable acceptability color, texture and appearance [14, 22] and finally the slight bad odor, taste, color and texture as well as undesirable appearance and overall acceptability of fish burger can be caused by fat oxidation. These changes resulted by a high content of polyunsaturated fatty acids in fish [13, 21, 22].
The sensory index of tissue in the burger devoid of fish protein isolate (FPI) and burger containing fish protein isolate (FPI) during maintenance at frozen mode retains its softness which can be due to products reaction resulted by fat oxidation with protein and the formation of links between them [21]. But after frying in oil and baking, the tissue may have grainy and roughness states that due to water evaporation from the surface layer and denaturation of protein in muscles during cooking process [24]. According to the results of Reppond and Babbitt [24], the mince has usually rough and grain tissue. Using high temperature and pressure during processing and production of burger in treatment 1 (devoid of fish protein isolate) and treatment 2 (burger containing fish protein isolate) and chemical processing during the production is of one of the other main reasons in fish burger tissue roughness [23]. Belitz and colleagues [21] reported on a study that raw samples of fish ball containing fish protein isolate (FPI) are softer and during maintenance time because of the products reaction caused by protein and fat oxidation and the formation of links between them after 4 weeks the tissue is juicy. The results listed correspond with the results of present study.

Total conclusion

Chemical and sensory test results of the study showed that the percentage of moisture and fat content and pH levels in control treatment is significantly higher than treatment 2 (containing isolated tuna) ($p<0.05$), but the ash, protein, and TBA in treatment 2 was significantly higher than treatment 1 ($p<0.05$). With the passage of time during maintenance, numeric values of fat, ash and TBA increased but numeric values of protein, moisture and pH during maintenance decreased. The results of sensory evaluation showed that: sensory characteristics in treatments during maintenance were reduced over time, but the results were similar and the reduction was not statistically significant ($p<0.05$). In the comparative study between treatments it was found in samples containing high protein isolate in the third and fifth maintenance was different from control samples and achieved better sensory scores, but the difference was not significant ($p<0.05$). According to the obtained results we can conclude that burger with tuna isolate has a higher nutritional value compared to the control (no protein isolate tuna) and shelf-life in both samples has been 5 months.

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