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## Evaluating the nutritional value and shelf life of fish patty enriched with protein concentrate from *Abramis brama* at ambient temperature

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### Abstract

In recent decades, because of the increased population and the importance of animal protein, the production of high value added products from marine resources has attracted much attention. On the other hand, protein deficiency-induced nutrition is one of the most important problems in most developing countries. Therefore, the addition of controlled amounts of FPC to daily food intake can solve this problem. The purpose of present study is evaluating the nutritional value and shelf life of fish patty enriched with protein concentrate from *Abramis brama* (FPC) at ambient temperature. In this study, FPC (type A) was prepared from bream fish by chemical method. Then the fish patty produced in 4 groups of control, 5%, 10% and 20% FPC were packed and kept at ambient temperature. Chemical parameters such as pH, peroxide value and total volatile base nitrogen and sensory evaluation (taste, smell, color and texture) of fish patty were studied by trained members for a period of 30 days. Chemical results of the samples showed that the pH value was low in all treatments during storage, but TVB-N and PV values increased significantly in all treatments during storage ( $P < 0.05$ ). Nonetheless, fish patty unenriched (control) and enriched with 5% and 10% FPC in terms of TBA content and fish patty contain 5% FPC in terms of PV content were in an acceptable range until the end of storage period. Reduction of sensory factors (flavor and odor) was also observed in all treatments, but no significant difference was observed between control and other treatments ( $P > 0.05$ ). Decrease in color factor was not significant. The color factor was not significantly decreased ( $P > 0.05$ ), and 10% FPC and control were better than other treatments at the end of storage period. Regarding the tissue parameter, fish patty containing 10% FPC in 10 and 30 days of storage compared to the control treatment showed a significant difference ( $P < 0.05$ ) and also had a better rating than other treatments. In general, 5 and 10% FPC treatments were considered as superior formulations and equal to control treatments. Therefore, 5% FPC treatment was proposed for final production.

**Keywords:** Nutritional value, shelf life, protein concentrate, bream fish

### Introduction

The importance of fish as a very useful food source and a valuable animal protein is no worn on one; so that about 20% of the animal protein required by humans is provided in this way and has a close competition with other sources of animal protein [1]. In this regard, advanced countries, with a better exploitation of water resources, breeding and cultivating a variety of aquatic species and investment in the processing sector, can step up the per capita consumption of fish and the elimination of protein poverty in their societies. Today, more than 150 kinds of food and seafood such as sausages, sausages, surimi, salami, etc. are produced in these countries using various types of fish.

The global standard of aquaculture per capita consumption is 19.2 kg [2]. Unfortunately, in Iran, this is estimated at 8.5 kg [3], which is much low than the global standard. It is no comparable to the per capita consumption of countries such as Japan with an annual consumption of about 70 kg [1]. Based on the average age and weight of the population, the average person's need for protein content is about 15.50 kg per year. So, in total, millions of tons of pure protein will be needed annually. The World Food and Agricultural Organization (FAO) also estimate the amount of protein required for each person recommends an average of 29 grams per day. On the other hand, nutritionists believe that each person's daily diet should contain at least 15 grams of animal protein. The rest of the body protein needs can be provided with the help of vegetable proteins, and if the consumption of animal protein be less than 15 g per day can cause protein poverty [4]. Based on the above, a large proportion of people are suffering from protein poverty,

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because studies have shown that 19.5% of people in different societies use more than 30 g and 19.8% of them use 30-50 g per day of animal protein. While about 60-70% of humans suffer from protein deficiency when they eat less than 15 grams of protein per day [1].

Bream fish (*Abramis brama*) is one of the species that has an economic value and has been commonly used for its good taste [5]. Fish Protein Concentrate (FPC) is also a white powder, odorless and flavorless from fish that contains 80 to 90% of the protein with a good quality [6, 7]. For the first time in the late 60s, it was recognized as the most fruitful way to overcome global malnutrition [8]. This type of protein is a human consumable product that can usually be used in combination of various cooked materials. Fat and undesirable substances remove from the final product by using the solvents such as alcohol, ethanol or isopropanol [9]. In fact, FPC is used as cheap food supplements [10, 11] and because FPC type A does not smell and taste, its consumption alone is not pleasant [12]. Therefore, finding ways to increase consumption is a major concern, as well as the amount of FPC added to food, it should be some extent that it does not affect the characteristics and characteristics of the food.

So far, good results from the combination of FPC with all kinds of macaroni, milk-based beverages, sauce, baby food, dietary foods and baby's breakfast is obtained [4]. On the other hand, the experimental production of surimi-based fish products such as fish cakes, fish patty and etc began in China in 1984 to 1985, but did not continue and was restarted in 1993 [13]. Fish patty is a ready food that is well cooked and has a good demand [14].

The production of fish patty has not been done so far in Iran and is also limited in other countries. Therefore, the purpose of this research was to produce fish protein concentrate by using a solvent (isopropanol) of bream fish (*A. brama*) meat, and then producing patty with different percentages from it. The nutritional value and shelf-life of them compared to common patty (unenriched) were studied for 30 days at an ambient temperature.

## Material and methods

### Preparation of fish

Bream fish after catch of the Aras dam (west Azarbaijan province, Iran) along with ice and water in the CSW (cilled sea water) tank, was transferred to the National Fish Processing Research Center, Bandar Anzali (Guilan province, Iran) and was placed in a chill room at 5 to 8 °C. After weighing the fish (35 kg), they were placed in baskets for washing with tap water. Then the head, tail, and viscera were discarded. After this stage, the fish were transferred to the debone machine and the bone, skin, and fin were removed from the meat.

### Production of FPC type A from bream fish

For production of FPC, fish meat and solvent (99% isopropanol) with the ratio of 1 to 1 for 50 minutes at ambient temperature (24 °C) were placed. After this time, the first press was performed and again isopropanol was added to the pressed sample with a ratio of 1 to 1. Samples were placed in a water bath for 90 minutes at 75 °C. After this time, the sample was re-pressed and placed in a water bath at a temperature of 75 °C for 70 minutes (1 meat: 1 isopropanol solution). The sample was again pressed and transferred to a dryer for 8 hours at 125 °C. At this point, the

final moisture content should be less than 8%. The sample was milled and passed through a sieve (the mesh was 100 micron) [4].

### Production of fish patty with FPC

In order to produce the fish patty, 1 kg of sugar was added to the lukewarm water. Then the leaven (it was suitable for puffing the patties and forming the tissue) was added and stirred. 15 eggs and 2 kg of flour added to the mixture and well mixed. Then the cryoprotectant was added to the mixture and the resulting paste was kneaded to allow adhesion. At the end, a little oil was added to the paste. The paste was divided into four equal parts for preparing the treatments. FPC was added in concentrations of 0%, 5%, 10% and 20%. The pastes in a container containing some flour on the floor were placed in an oven for half an hour to prepare for cooking. Then, the temperature of the oven was increase to 180 °C so that it can be cooked for half an hour. After cooling, samples were packed in polyethylene bags under normal air and stored at ambient temperature for one month. Measurement intervals were 1, 10, 20 and 30 days after the production of patties.

### Measuring the amount of crude protein

The crude protein content of samples was calculated on the basis of nitrogen content; which was determined by the Macro-Kjeldahl system (Techno Service Co, Behr, K 24, Germany) and finally by multiplying the nitrogen number by a factor of 6.25 [15]. The formula is as follows:

$$\text{Protein (\%)} = [\text{consumed H}_2\text{So}_4 \text{ content (ml)} \times 0.014 \times 0.1 \times 6.25 \times 100] / \text{sample (g)}$$

### Measuring the amount of ash

The ash content of samples was estimated by heating the samples overnight in a furnace (Muffle Furnace, SEF-202, Korea) at 525°C for 12h [15]. The formula is as follows:

$$\text{Ash (\%)} = [\text{ash (g)} / \text{sample (g)}] \times 100$$

### Measuring the amount of moisture

The moisture content of samples was determined by drying to a constant weight at 105°C in an oven [15]. The formula is as follows:

$$\text{Moisture (\%)} = [(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100$$

### Measuring the amount of fat

Fat was extracted from minced fish and fish patty in a Soxhlet extractor (Behr, Labor-Technik, Germany) using petroleum ether. The crude lipid content was determined gravimetrically following oven-drying of the extract at 105°C overnight [15].

$$\text{Fat (\%)} = [\text{fat (g)} / \text{sample (g)}] \times 100$$

### pH measurement

20 g of fish patty was added to 100 ml of distilled water and mixed. The resulting mixture was then straightened after a few minutes. Then, pH of the samples was measured using a pH meter (Digital-Microprocessor pH Meter, LT-50, India), calibrated at pH 4 and 7 [16].

### Determination of total volatile base nitrogen (TVB-N)

Total volatile nitrogen of samples was determined according to the protocol of Parvaneh [17]. Briefly, 10 g fish patty and 300 ml distilled water was added to a round bottom

distillation flask. After a little shaking the balloons by hand, 2 g magnesium oxide and anti-bumping granules were added to them. 25 ml of 2 % boric acid and a few drops of indicator (dissolve 0.1 g phenolphthalein in 100 ml ethanol 95%) was added to 250 ml Erlenmeyer flask. The distilling flask was heated so that the liquid was boiled in exactly 10 min. Using the same rate of heating was distilled for exactly 25 min. After the distillation, the solution collected in a receiver flask was titrated to purple endpoint 0.1 N H<sub>2</sub>SO<sub>4</sub>. Total volatile base nitrogen expressed as mg nitrogen per 100 g sample:

TVB-N (mg nitrogen/100 g sample) = consumed H<sub>2</sub>SO<sub>4</sub> content (ml) × 14

#### Determination of peroxide value (PV)

The peroxide value (PV) of samples was determined by AOAC (15) and expressed as milli-equivalents of oxygen per 1000 g oil. Accurately 50 g fish patty was weighed into 250 ml ground glass. 100 ml chloroform was added. The specimens were kept in a dark place for 2 hours and then filtered. 25 ml of the filtered solution was transferred into a glass jar and placed under the hood to evaporate the solvent. To the rest of the filtered solution, 37 ml acetic acid, 30 ml distilled water, 1 ml potassium iodide and approximately 1 ml starch solution were added. The solution was gently swirled. When it was completely evaporated, the amount of residual fat in the container was weighed. The residual solution was titrated with 0.01 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The PV of samples was calculated by using the following formula:

PV (milli-equivalents of oxygen per 1000g fat) = 0.01 × 1000 × ml of sodium thiosulfate for titration/ residual fat in the container (g).

#### Sensory analysis

For sensory analysis, samples were fried in a fryer with sunflower oil at 180 ° C for 4 minutes [18]. Sensory testing was performed using a semi-trained panel of 10 people. These people evaluated the flavor, odor, color and texture of samples on the hedonic scale [19]. After evaluating each sample, the panel members were rinsed thoroughly with some water and the next test was performed.

#### Statistical analysis

Statistical analysis was performed using SPSS-17 and Minitab-16 softwares. After the normal distribution of data using the Kolmogorov-Smirnov test, Duncan's Multiple Range and Tukey tests ( $p = 0.05$ ) were used to determine any significance of differences between means. The nonparametric method of Mann-Whitney was used to analyze the sensory test. All tests were performed in triplicate, and the data are expressed in terms of mean ± standard deviation (SD).

#### Results

The proximate composition of FPC and minced bream fish is shown in Table 1. The results of this study showed that there was a significant difference between the FPC and minced bream fish in terms of protein content ( $p < 0.05$ ), so that the amount of protein in FPC and minced bream fish was  $20.11 \pm 0.02$  and  $91.63 \pm 0.09$ , respectively. The high protein in the FPC is due to the protein purity compare with minced bream fish. The results of fat content were converse of protein content and the fat of FPC was significantly lower than minced meat of bream fish. This difference was statistically significant ( $p < 0.05$ ). The moisture and ash values were also lower in the FPC compared to the minced meat of bream fish, and there was statistically a significant difference between them ( $p < 0.05$ ).

**Table 1:** Comparison of proximate compositions of FPC and minced bream fish

| Test (%)          | Protein                   | Fat                      | Ash                      | Moisture                  |
|-------------------|---------------------------|--------------------------|--------------------------|---------------------------|
| Minced bream fish | 20.11 ± 0.02 <sup>b</sup> | 7.00 ± 0.14 <sup>a</sup> | 0.99 ± 0.01 <sup>a</sup> | 72.30 ± 0.47 <sup>a</sup> |
| FPC               | 91.63 ± 0.09 <sup>a</sup> | 0.57 ± 0.02 <sup>b</sup> | 0.26 ± 0.01 <sup>b</sup> | 6.12 ± 0.03 <sup>b</sup>  |

The lowercase letters in each column indicate significant differences ( $p < 0.05$ ) between the treatments. Results are expressed as Mean ± SD (n=3).

The amount of protein during the storage period at ambient temperature is shown in Table 2. Based on the results, protein reduction in different treatments was not significant during the whole maintenance period ( $p > 0.05$ ). However, at the end of the maintenance period (day 30), the highest and lowest protein contents was related to fish patty enriched with 20% FPC (17.44%) and control treatment (4.78%), respectively. Meanwhile, there was a significant difference between treatments ( $p < 0.05$ ). In the measurement of fat percentage (Table 2), due to the addition of oil in the formulation of fish patties, it is not possible to accurately analyze the effects of different proportions of FPC on the increase of final absorption in comparison with control treatment. Based on this study, there was no significant difference between different treatments during storage period ( $p > 0.05$ ). In each treatment, fat content increased with increasing time, which was statistically significant. At the end of the maintenance period, the highest fat content

was associated with 20% FPC-enriched fish patty with 23.67% of fat and the lowest was for control treatment with 23.12% of fat. There was no significant difference in moisture content between treatments ( $p > 0.05$ ). And the amount of moisture in all treatments gradually decreased with increasing time. The highest moisture content was observed on 30 days in fish patty enriched with 20% FPC, but moisture changes were not significant ( $p > 0.05$ ) (Table 2). Also in this study, ash increased during the storage period in all treatments, except on the first day, as compared to control treatment ( $p < 0.05$ ) (Table 2). Between enriched treatments, fish patty enriched with 20% FPC had the highest amount of ash. However, no significant difference was observed between two treatments with 5 and 10% FPC ( $p > 0.05$ ). Also, this difference was not significant between two treatments with 10% and 20% FPC ( $p > 0.05$ ). On the other hand, there was significant difference between treatments enriched with 5% and 20% FPC ( $p < 0.05$ ).

**Table 2:** Evaluation of proximate compositions of fish patty enriched with FPC from bream fish during one month storage

| Fish patty   | Time (days) | Control (without FPC)     | 5% FPC                    | 10% FPC                   | 20% FPC                   |
|--------------|-------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Protein (%)  | 1           | 5.65±0.04 <sup>dA</sup>   | 11.78±0.03 <sup>cA</sup>  | 16.44±0.02 <sup>bA</sup>  | 21.10±0.04 <sup>aA</sup>  |
|              | 10          | 5.56±0.04 <sup>dA</sup>   | 11.21±0.02 <sup>cA</sup>  | 16.15±0.02 <sup>bA</sup>  | 20.93±0.03 <sup>aA</sup>  |
|              | 20          | 5.24±0.04 <sup>dA</sup>   | 10.90±0.04 <sup>cA</sup>  | 15.63±0.05 <sup>bA</sup>  | 19.79±0.04 <sup>aA</sup>  |
|              | 30          | 4.78±0.03 <sup>dA</sup>   | 10.14±0.04 <sup>cA</sup>  | 14.69±0.04 <sup>bA</sup>  | 17.84±0.03 <sup>aA</sup>  |
| Fat (%)      | 1           | 19.39±0.02 <sup>aB</sup>  | 19.45±0.007 <sup>aB</sup> | 19.48±0.01 <sup>aB</sup>  | 19.51±0.02 <sup>aB</sup>  |
|              | 10          | 19.58±0.03 <sup>bB</sup>  | 19.70±0.04 <sup>bB</sup>  | 19.88±0.01 <sup>bB</sup>  | 20.04±0.09 <sup>bB</sup>  |
|              | 20          | 22.39±0.02 <sup>cA</sup>  | 23.18±0.01 <sup>cA</sup>  | 23.29±0.02 <sup>cA</sup>  | 23.49±0.02 <sup>cA</sup>  |
|              | 30          | 23.12±0.02 <sup>dA</sup>  | 23.39±0.03 <sup>dA</sup>  | 23.55±0.02 <sup>dA</sup>  | 23.67±0.05 <sup>dA</sup>  |
| Moisture (%) | 1           | 21.01±0.08 <sup>dA</sup>  | 21.17±0.07 <sup>dA</sup>  | 21.56±0.19 <sup>dA</sup>  | 21.88±0.07 <sup>dA</sup>  |
|              | 10          | 20.39±0.02 <sup>cA</sup>  | 20.71±0.09 <sup>cA</sup>  | 21.06±0.09 <sup>cA</sup>  | 21.29±0.02 <sup>cA</sup>  |
|              | 20          | 19.60±0.09 <sup>bAB</sup> | 19.72±0.02 <sup>bAB</sup> | 19.79±0.02 <sup>bAB</sup> | 19.86±0.02 <sup>bAB</sup> |
|              | 30          | 17.58±0.02 <sup>aB</sup>  | 17.85±0.02 <sup>aB</sup>  | 17.91±0.01 <sup>aB</sup>  | 18.11±0.02 <sup>aB</sup>  |
| Ash (%)      | 1           | 0.99±0.12 <sup>aB</sup>   | 0.99±0.11 <sup>aB</sup>   | 0.99±0.03 <sup>aB</sup>   | 0.99±0.04 <sup>aB</sup>   |
|              | 10          | 0.99±0.007 <sup>bB</sup>  | 1.06±0.01 <sup>aAB</sup>  | 1.12±0.01 <sup>aA</sup>   | 1.18±0.007 <sup>aA</sup>  |
|              | 20          | 1.04±0.01 <sup>cA</sup>   | 1.10±0.007 <sup>bA</sup>  | 1.17±0.02 <sup>abA</sup>  | 1.20±0.007 <sup>aA</sup>  |
|              | 30          | 1.13±0.04 <sup>cA</sup>   | 1.19±0.007 <sup>bA</sup>  | 1.25±0.02 <sup>abA</sup>  | 1.32±0.01 <sup>aA</sup>   |

Lowercase letters in each row indicate significant differences ( $p<0.05$ ) between the different treatments.

Capital letters in each column indicate significant differences ( $p<0.05$ ) between the different times.

Results are expressed as Mean±SD (n=3).

Changes in the pH of various treatments during the 30 days of storage at ambient temperature are shown in Table 3. The pH of all treatments decreased during storage ( $p<0.05$ ). Significant differences were observed between control and fish patty enriched with 10% and 20% FPC ( $p<0.05$ ). The highest pH was observed on the 30th day in control treatment and the lowest pH was observed in 20% FPC-enriched fish patties. The value of volatile bases nitrogen in different treatments increased significantly during the maintenance period ( $p<0.05$ ). In the control sample, it was 7 mg N per 100 g sample on the first day and then reached to 21 mg N per 100 g sample on the 30th day of storage. TVB-N in 5%, 10% and 20% FPC-enriched fish patties respectively increased from 8.40%, 9.80% and 11.22% on the first day to 23.10%, 23.80% and 25.90% on 30th day of

storage. In fact, on this day, the lowest increase was observed in control treatment and the highest increase was observed in 20% FPC-enriched fish patty, but there was no significant difference between them and other enriched samples (Table 3). The results also showed that the amount of peroxide (PV) in all treatments except the first day was significantly higher than that of control ( $p<0.05$ ) sample (Table 3). With increasing time, the amount of PV increased. At the end of the maintenance period, the lowest levels of peroxide were belonged to the control treatment (4.19 mg peroxide per kg fat) and the highest amount was belonged to the fish patty enriched with 20% FPC (5.44 mg peroxide per kg fat). In general, control treatments and 5% FPC-enriched fish patty were at an acceptable level (5 mg peroxide per kg fat) until the end of the maintenance period.

**Table 3:** Evaluation of corruption factors of fish patty enriched with FPC from bream fish during one month storage.

| Fish patty        | Time (days) | Control (without FPC)    | 5% FPC                    | 10% FPC                   | 20% FPC                  |
|-------------------|-------------|--------------------------|---------------------------|---------------------------|--------------------------|
| pH                | 1           | 6.08±0.02 <sup>bA</sup>  | 6.10±0.02 <sup>aA</sup>   | 6.10±0.02 <sup>aA</sup>   | 6.10±0.11 <sup>aA</sup>  |
|                   | 10          | 6.07±0.01 <sup>aA</sup>  | 6.02±0.01 <sup>aB</sup>   | 5.93±0.03 <sup>aB</sup>   | 5.85±0.02 <sup>bB</sup>  |
|                   | 20          | 5.91±0.02 <sup>aB</sup>  | 5.84±0.04 <sup>aC</sup>   | 5.76±0.03 <sup>bC</sup>   | 5.71±0.02 <sup>bC</sup>  |
|                   | 30          | 5.81±0.02 <sup>aC</sup>  | 5.73±0.02 <sup>aD</sup>   | 5.65±0.02 <sup>bD</sup>   | 5.51±0.02 <sup>cD</sup>  |
| TVB-N (mg N/100g) | 1           | 7.00±0.97 <sup>bC</sup>  | 8.40±0.88 <sup>bD</sup>   | 9.80±0.85 <sup>bC</sup>   | 11.20±0.98 <sup>aC</sup> |
|                   | 10          | 9.10±0.98 <sup>bC</sup>  | 11.90±0.98 <sup>aC</sup>  | 15.40±0.76 <sup>aB</sup>  | 17.50±0.98 <sup>aB</sup> |
|                   | 20          | 16.10±0.98 <sup>cB</sup> | 19.60±0.98 <sup>bcB</sup> | 21.70±0.98 <sup>abA</sup> | 23.80±0.88 <sup>aA</sup> |
|                   | 30          | 21.00±0.86 <sup>bA</sup> | 23.10±0.98 <sup>abA</sup> | 23.80±0.89 <sup>aA</sup>  | 25.90±0.98 <sup>aA</sup> |
| PV (meq/kg)       | 1           | 0.00±0.00 <sup>aD</sup>  | 0.00±0.00 <sup>aD</sup>   | 0.00±0.00 <sup>aD</sup>   | 0.00±0.00 <sup>aD</sup>  |
|                   | 10          | 0.70±0.03 <sup>cC</sup>  | 0.96±0.01 <sup>bC</sup>   | 1.05±0.02 <sup>aC</sup>   | 1.13±0.02 <sup>aC</sup>  |
|                   | 20          | 2.37±0.04 <sup>bB</sup>  | 2.89±0.04 <sup>bcB</sup>  | 3.14±0.06 <sup>abB</sup>  | 3.34±0.02 <sup>aB</sup>  |
|                   | 30          | 4.19±0.007 <sup>bA</sup> | 4.35±0.02 <sup>bA</sup>   | 5.24±0.02 <sup>aA</sup>   | 5.44±0.04 <sup>aA</sup>  |

Lowercase letters in each row indicate significant differences ( $p<0.05$ ) between the different treatments.

Capital letters in each column indicate significant differences ( $p<0.05$ ) between the different times.

Results are expressed as Mean±SD (n=3).

The results of flavor evaluation of fish patty during 30 days of storage at ambient temperature are given in Table 4. The results showed that the flavor decreased during storage, but at the end of the maintenance period (30th day), control treatment and other treatments containing FPH were at a qualitative level ( $p>0.05$ ). This difference was not significant. The results showed that the odor factor decreased during storage, but there was no significant difference between the control treatment and other treatments ( $p>0.05$ ). Also, the results of color attributes showed that this index had the highest rating at the

beginning of the maintenance period, but its rating decreased during storage time ( $p>0.05$ ). There was no significant difference between treatments. The texture of the samples had a high score at the beginning of the maintenance period, but during the maintenance period their score decreased and this decrease was statistically significant ( $p<0.05$ ). At the end of the maintenance period, the highest score was for the fish patty enriched with 10% FPC (2.5), but there was no significant difference between other treatments (Table 4).

**Table 4:** Sensory evolution of fish patty enriched with FPC from bream fish during one month storage

| Fish patty | Time (days) | Control (without FPC)   | 5% FPC                   | 10% FPC                  | 20% FPC                  |
|------------|-------------|-------------------------|--------------------------|--------------------------|--------------------------|
| Flavor     | 1           | 4.30±0.82 <sup>aA</sup> | 4.20±0.63 <sup>aA</sup>  | 4.20±0.78 <sup>aA</sup>  | 4.40±0.51 <sup>aA</sup>  |
|            | 10          | 4.11±0.78 <sup>aA</sup> | 3.77±0.66 <sup>bB</sup>  | 3.66±0.5 <sup>bB</sup>   | 3.55±0.72 <sup>bB</sup>  |
|            | 20          | 2.50±0.53 <sup>aB</sup> | 2.25±0.46 <sup>aC</sup>  | 2.12±0.35 <sup>aC</sup>  | 2.12±0.35 <sup>aC</sup>  |
|            | 30          | 2.50±0.54 <sup>aB</sup> | 2.33±0.5 <sup>aC</sup>   | 2.33±0.50 <sup>aC</sup>  | 2.16±0.40 <sup>aC</sup>  |
| Odor       | 1           | 4.60±0.69 <sup>aA</sup> | 4.50±0.70 <sup>aA</sup>  | 4.30±0.82 <sup>aA</sup>  | 4.50±0.52 <sup>aA</sup>  |
|            | 10          | 4.11±0.78 <sup>aA</sup> | 3.88±0.60 <sup>bB</sup>  | 3.77±0.44 <sup>bB</sup>  | 3.88±0.78 <sup>bB</sup>  |
|            | 20          | 2.62±0.51 <sup>aB</sup> | 2.37±0.51 <sup>aC</sup>  | 2.37±0.51 <sup>aC</sup>  | 2.50±0.53 <sup>aC</sup>  |
|            | 30          | 2.66±0.51 <sup>aB</sup> | 2.33±0.51 <sup>aC</sup>  | 2.33±0.51 <sup>aC</sup>  | 2.50±0.54 <sup>aC</sup>  |
| Color      | 1           | 4.60±0.69 <sup>bA</sup> | 4.50±0.84 <sup>bA</sup>  | 4.20±0.91 <sup>bA</sup>  | 4.80±0.63 <sup>aA</sup>  |
|            | 10          | 4.55±0.52 <sup>aA</sup> | 4.00±0.86 <sup>aA</sup>  | 4.00±0.70 <sup>aA</sup>  | 3.88±0.74 <sup>aB</sup>  |
|            | 20          | 3.37±0.51 <sup>aB</sup> | 2.75±0.46 <sup>aB</sup>  | 2.37±0.51 <sup>aB</sup>  | 2.87±0.64 <sup>aC</sup>  |
|            | 30          | 2.83±0.40 <sup>aC</sup> | 2.33±0.51 <sup>aB</sup>  | 2.66±0.51 <sup>aB</sup>  | 2.33±0.51 <sup>aC</sup>  |
| Texture    | 1           | 4.40±0.84 <sup>bA</sup> | 4.30±0.67 <sup>bA</sup>  | 4.10±0.56 <sup>bA</sup>  | 4.30±0.48 <sup>bA</sup>  |
|            | 10          | 3.88±0.78 <sup>aB</sup> | 3.22±0.83 <sup>abB</sup> | 3.11±1.16 <sup>bB</sup>  | 2.88±0.92 <sup>bB</sup>  |
|            | 20          | 2.50±0.53 <sup>aC</sup> | 2.12±0.35 <sup>bC</sup>  | 2.12±0.35 <sup>bC</sup>  | 2.00±0.88 <sup>bcC</sup> |
|            | 30          | 2.33±0.51 <sup>bc</sup> | 2.16±0.40 <sup>bc</sup>  | 2.50±0.54 <sup>aBD</sup> | 2.00±0.96 <sup>bc</sup>  |

Lowercase letters in each row indicate significant differences ( $p < 0.05$ ) between the different treatments.

Capital letters in each column indicate significant differences ( $p < 0.05$ ) between the different times.

Results are expressed as Mean±SD (n=3).

## Discussion

The amount of protein in the muscle of fish is reported to be about 21 to 16% [20]. But research on various aspects of the production of FPC from the different fish especially bream fish, and its use in human diet as a valuable protein supplement plays an important role in providing nutritional needs and eliminating protein deficiencies in the community. Dust *et al.* [21] considered FPC as a very valuable protein source, and noted that its absorption rate was more than 8.2%. The Food and Drug Administration of the United States (FDA) also controlled use of FPC as a protein supplement in the human diet has been approved, while that the FPC is used in terms of factors such as protein content (more than 75%), fat (Maximum of 0.75%), moisture, microbial, etc. are in good condition [4, 22]. In the present study, protein concentrate produced from bream fish with a protein content of 91.63%, is FPC type A, which complies with FDA and FAO rules. In another study by Khoshkhoo *et al.* [23] on the production of FPC from kilka fish (*Clupeonella cultriventris*), the protein content was reported about 0.92%. Differences in the concentrations of proteins and other chemical and physical factors may depend on the season, the species used, the production method, and other parameters [24, 25]. Mohamed *et al.* [26] reported that FPC produced from carp and shark fish contained 88.70% and 89.12% of protein, respectively. Azhdari [24] also reported the FPC from silver carp was 81% and Dan Syahurul, [27] and Dewita *et al.* [28] reported the amount of FPC from catfish was 75.31%. Also, the amount of protein in FPC derived from bream fish in this study was more than the protein content derived from FPC of other fish such as common carp (72%), menhaden (78%) and hake (85%) [29, 30, 31]. This can be due to the non-use of pre-extraction cooking method, because heat reduces the amount of protein in the product, changing the structure and reducing access to essential amino acids. For example, the reduction of access to amino acids of cysteine, lysine, arginine, threonine and serine in various protein sources has been reported as a result of thermal treatments [32]. It should be noted that amino acids, such as lysine, at high temperatures produce form complexes with carbohydrates such as glucose, which cause the reaction of Millard [33]. Therefore, high temperatures can reduce the quality of food

and the amount of proteins [34]. On the other hand, the results of fish patty maintenance with 5%, 10%, and 20% of FPC during one month showed that at the beginning of the maintenance period, the protein content was respectively 11.78%, 16.44% and 21.10%. It was more than the amount of protein in the control fish patty (5.65%). Their amount at the end of the maintenance period was 10/14, 14/69 and 17/84 percent, respectively. This decrease is due to the denaturation of the protein during storage. Nagaral *et al.* [35] reported that the protein content of fish patty from *Catla catla* was significantly reduced during the 16 days of storage at the refrigerator temperature, which is consistent with the results of the present study. A study by Bavitha *et al.* [36] also found that the protein content of fish cake from *Cyprinus carpio* was significantly reduced during the 15 days of storage ( $P < 0.05$ ), so that in The end of the maintenance period reached the protein content of 4.92%. Total fat is a qualitative indicator of fish corruption [37]. The results of fat content of protein concentrate obtained from bream fish was 58%. The reason for this compared with minced meat of bream fish, the effect of solvent during the process of preparation of FPC. In a study by Mohamed *et al.* [26], the fat content of FPC produced from carp and shark fish contained 0.04 and 0.01%, respectively. With a study on extraction of FPC from phytophagus fish, Azhdari [24] reported fat content of 0.37%, which was less than the amount of fat obtained from the results of this study. But the results of FPC fat content from catfish [10, 28] and common carp [30] were more than the results of this study. The difference in fat content can be due to the type of solvent used or its purity. On the other hand, the fat content of fish varies as well. To prove this, studies by Cordova [31] on the effects of various solvents such as isopropanol, hexane, ether, benzene, toluene, cyclohexane, carbon tetrachloride and ethanol on the extraction of fat and water from fish for the production of FPC showed that isopropanol is more suitable solvent. And while extracting fats, the flavor, smell and coloring material also well separates from the fish tissue and is safer than other solvents such as hexane and toluene. Regarding the measurement of fat during storage, the lowest amount of fat on the first day was observed in the control treatment with 19.39% and the highest on day 30 in fish patty enriched with 20% FPC. The reason for the high fat in

the fish patty was the use of vegetable oil in the formulation of the patty. Romero *et al.* [38] also reported that, among different treatments of fish patty from *Pseudoplatystoma corruscans*, the highest fat content was found in fish patty treated with 20.28 of corn flour and the lowest was 10.55% in control treatment. Moisture is an important factor in determining the quality and shelf-life of foodstuffs [39]. The difference in moisture content can also be due to the effect of solvent and the use of a dryer during the process as well as the difference in the duration of use of the dryer. FAO (The Food and Agriculture Organization of the United Nations) has set 8% the maximum acceptable moisture content of FPC type A [4]. Khoshkhoo *et al.* [23] with producing FPC from kilka fish reported the moisture content of 3.2%, and Mohamed *et al.* [26] with producing FPC of carp and shark reported the moisture contents of 9.10 and 8.55%, respectively. Also, in the present study, by measuring the moisture content of fish patty containing FPC during 30 days of storage at ambient temperature, observed that moisture changes were not significant between control sample and treatments containing FPC in different days, but the moisture content of the samples was significant from 0 to 30 days of storage. Bello and Pigott [40] reported that the moisture content of patty produced from various fish such as rockfish, pacific cod, codling and pacific herring was 3.4% on zero day and 8.3% in the fourth month, which this increase in moisture was due to increased moisture absorption from the environment. Sehgal *et al.* [41] reported the moisture content of fish patty produced by the Indian carp (*Labeo rohita*) about 52%. Romero *et al.* [38] reported the moisture content of fish patty from *Pseudoplatystoma corruscans* containing rice flour was about 67.95% that there was a significant difference between different treatments except control treatment and fish patty containing Amaranth flour. The amount of ash of FPC in accordance with FAO standards is 18% [4], which is much higher than the amount of protein derived from bream FPC. During the storage period, the ash content of all treatments increased as compared to control treatment, so that at the end of the maintenance period, the highest amount of ash was associated with the fish patty enriched with 20% FPC. These results were not consistent with the study of Romero *et al.* [38] on the amount of ash from fish patty containing corn flour compared to control treatment. Regarding the factors of corruption, the decrease in pH of treatments and the absence of increase during the maintenance period at ambient temperature could be due to the proper packaging, the lower release of volatile compounds and the absence of microbial growth due to the dry matter of the foodstuff. But Turhan *et al.* [42] reported that after 10 days of storage, the pH of fish patty from anchovy increased from 6.33 to 6.56. Because the growth of microorganisms and enzymes produced by them, by liberating oxygen and hydrogen, increased the concentration of hydroxyl ions and cause the increase in pH. Also, the results of this study did not match the results of Desturan and Haard [43], Yerlikaya *et al.* [44] and Kilinc *et al.* [45]; but was consistent with the results of Raut *et al.* [46] Ilhak *et al.* [47] also found that there was no significant increase in the pH of different treatments from fish patty during the whole period of storage. The TVB-N factor is one of the indicators widely used to assess the quality of fish and fish products, which includes measurement of trimethylamine, dimethylamine, ammonia and other volatile nitrogen compounds associated with food

corruption [20]. In the present study, the number of total volatile base nitrogen from fish patty in all treatments increased at the end of the maintenance period. The increase in the amount of TVB-N can be attributed to the decomposition of nitrogen compounds, such as proteins and free amino acids [48]. The results of this study were complied with Yerlikaya *et al.* [44] studies on the patty produced from Anchovy (*Engraulis encrasicolus*), Kilinc *et al.* [45] studies on the patty produced from *Sardina pilchardus*, Kaba *et al.* [49] studies on the patty produced from *Engraulis engrasicholus* and Guran *et al.* [50] studies on the patty produced from Bonito fish (*Sarda sarda*). The legal limit for this index is 35 mg N per 100 g sample during the maintenance period [45]. Peroxide is used as the primary product of fat oxidation in fish [51]. Corrupt products are rapidly degraded and aldehydes are formed. These compounds have a severe and unpleasant odor and smell, which can lead to consumer hazards [52]. The results of this study showed that the amount of peroxide in all treatments increased significantly during the maintenance period except on the first day compared to the control treatment. This increase was due to the decomposition of fatty acids and the breakdown of triglycerides and free radicals. However, Yerlikaya *et al.* [44] studies on the proxide value of fish patty from *Engraulis encrasicolus* during the six days of storage reported that the amount of peroxide increased until the fifth day of storage, and then decreased, and the reduction of peroxide value at the end of the maintenance period can be attributed to the degradation of hydroperoxide to secondary oxidation products. The results of this study were consistent with the results of Guran *et al.* [50] and Bou *et al.* [53]. In terms of sensory evaluation, the odor quality, taste, color and texture of the product will change during the maintenance period and will lead to consumer dissatisfaction [54]. The present study showed that flavor loss was observed in each treatment during storage, but no significant difference was observed between treatments. Rockower *et al.* [55] investigated the taste of the patty produced from trout and pollack protein concentrate, and found that the best taste was found in the patties containing the protein concentrates of the both fishes. Sehgal *et al.* [14] also examined the flavor of fish patty produced from common carp (*Cyprinus carpio*) and reported that there was no significant difference in their flavor compared to the control sample. Also, the results of tissue measurements showed that with increasing time, the quality of the tissue was reduced and the highest tissue score at the end of the maintenance period belonged to 10% FPC-enriched patty and then the control treatment. Zhu *et al.* [56] observed that with increasing storage period, the texture hardness and chewing ability of fish patty increased and its springiness ability decreased. Therefore, the results of the evaluation of sensory indices during the maintenance period showed that although at the beginning of the experiment, the taste was desirable, the texture was flexible and the color was acceptable, but lipid oxidative degradation, microbial activity, the presence of free fatty acids [57], the formation of high molecular weight compounds such as phospholipids and triglycerides [58], the increase of peroxide value, PUFA reduction and protein degradation [59] resulted in loss of flavor, odor, texture and color of the product [60].

## Conclusion

The results of dietary value tests (protein, fat, moisture and

ash), and evaluation of corruption factors (pH, peroxide value and total volatile base nitrogen) during 30 days of storage at ambient temperature showed that protein was the most important indicator in fish patty enriched with FPC, but there was no significant difference in the factors of corruption between control sample and other treatments (5% and 10% FPC-enriched patties), especially with 5% FPC treatment. However, in the duration of storage, fish patty enriched with 20% FPC had a lower shelf life. So, 5% and 10% treatments were considered as treatments with superior formulation and equal treatment with control. But due to the economic cost, 5% FPC treatment was proposed for final production.

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