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COMPARATIVE STUDIES OF THE EFFECT OF FREEZING ON THE PHYSICOCHEMICAL PROPERTIES OF THE FILLETS OF TWO FISH SPECIES IN IRAN

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Abstract. The *Sparidentex hasta* and *Pampus argenteus* species of fish consumed in the south of Iran are abundant in a particular season, so they should be frozen for consumption throughout the year. That is why this research was carried out to investigate the effects of freezing on some of the physicochemical properties of the fillets of the fishes. Such parameters were determined as fat (by the chloroform-methanol method), the amount of TBA (thiobarbituric acid) in fish muscle (by Pearson's method), pH (with a pH meter), and FFA (Free fatty acids) – by the titration method, in the presence of phenolphthalein. They were determined basing on the percentage of oleic acid. The peroxide value, according to AOAC (Association of Official Analytical Chemists), was tested in fresh samples at time zero and after different periods of freezing. The results showed that the TBA content in fish fillet for *Pampus argenteus* and *Sparidentex hasta* was 0.65 and 0.53, respectively. The highest percentage of fat was found for *Pampus argenteus* after 95 days (24.22%), and for *Sparidentex hasta* after 35 days (25.19%). The highest free fatty acids contents (0.9% and 0.97%) was found for *Sparidentex hasta* and *Pampus argenteus* after 95 days. It can be concluded that the TBA and FFA contents and pH of both fish species increased during storage in a freezer. The peroxide value in *Pampus argenteus* was reduced, but in *Sparidentex hasta* showed no significant differentce. The best time of storage of *Pampus argenteus* and *Sparidentex hasta* at –18°C was 35 days of freezing, but the nutritional value of the fillets and the fatty acids reduced greatly.

Key words: freezing, oil, physicochemical properties, *Pampus argenteus*, *Sparidentex hasta*, Iranian fish species

ПОРІВНЯЛЬНА ХАРАКТЕРИСТИКА ФІЗИКО-ХІМІЧНИХ ВЛАСТИВОСТЕЙ ФІЛЕ СПЕЦИФІЧНИХ РИБ ІРАНУ ПІСЛЯ ЗАМОРОЖУВАННЯ

Анотація. Дослідження проведено з метою вивчення впливу заморожування на деякі фізико-хімічні gпоказники філе специфічних видів риб Ірану. Визначено такі фізико-хімічні параметри заморожених філе при зберіганні як вміст ліпідів, тіобарбітурової кислоти, pH, вільних жирних кислот. Вміст перекису протестовано на свіжому зразку та після різних періодів заморожування. Результати показали, що вміст тіобарбітурової кислоти у рибному філе *Pampus argenteus* і *Sparidentex hasta* становив 0,65 і 0,53%, відповідно. Найбільший відсоток жиру було визначено для *Pampus argenteus* через 95 днів (24,22%), а для *Sparidentex* – через 35 днів зберігання (25,19%). Найбільш високий вміст вільних жирних кислот (0,9% та 0,97%) було виявлено для *Sparidentex hasta* та *Pampus argenteus* через 95 днів. Можна зробити висновок, що вміст вміст тіобарбітурової кислоти та вільних жирних кислот обох видів риби під час зберігання в морозильній камері збільшувався. Вміст пероксиду в *Pampus argenteus* було зменшено, а у *Sparidentex hasta* суттєвих змін не відбулось. Визначено, що найкращий час зберігання заморожених зразків *Pampus argenteus* і *Sparidentex* складає 35 днів при –18°C.

Ключові слова: заморожування, ліпіди, фізико-хімічні властивості, *Pampus argenteus, Sparidentex hasta*, іранські види риби.

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Introduction. Formulation of the problem

Because the *Sparidentex hasta* and *Pampus argenteus* species of fish consumed in some parts of southern Iran are abundant in a particular season, they should be frozen for storage and consumption throughout the year. As the fish species are considered delicious among the people of southern Iran, this research has been carried out to investigate the effects of freezing on some of the physicochemical properties of the fillets of the fishes.

Analysis of recent research and publications

A review of literature has shown that the fish species are known, among other seafood, for their delicious meat, lack of scale (because of shedding in the catch), and high commercial and nutritive value [1], and it is the most expensive and valuable fish in the Persian Gulf countries, especially Iran, Kuwait, and Iraq [2]. Freezing is one of the best food storage methods. Freezing ensures that nutrients will be preserved in food with minimal changes for a relatively long time and prevents the growth of microorganisms and the activity of enzymes. Different methods of freezing have been created to maintain the better quality of food [3].

The TBA index is used in measuring malondialdehyde, a byproduct of the oxidation of fatty acids, such as polyunsaturated fatty acids [4]. The pH alone is not a good scale for fish quality control and can only be used as a guide and a tool to determine the quality of fish and seafood [5]. The findings of other researchers have shown that there is a significant correlation between pH and fresh fish. The physical property can be used to assess fish freshness [6]. The increase in pH

could be due to the fundamental analysis of compounds such as ammonia and trimethylamine. These compounds are produced by bacterial enzymes and body enzymes in fish, a process followed by spoilage [7]. Omidvar and Karimzadeh [8] found no significant difference in the moisture, protein, ash, fat and pH contents in fish of the *Rutilus firsi* species during three months of storage at –20°C. Fishery products in the freezing process cause the lowering of chemical compounds such as denaturation of protein, fat oxidation, discoloration and decrease in taste quality, textural changes and weight and moisture loss [9–11]. Fat and fatty acids oxidation, in addition to developing rancidity in fish products during freezing, cause denaturation of proteins and, as a result, cause degradation and intolerance of the products tissue [12]. These changes are the result of the freezing, storage temperature, temperature fluctuations, etc. [10,11,13].

The purpose of this study was determining the pH, free fatty acids, fat content, peroxide value, and thiobarbituric acid in the freezing period, and assessing the nutritional value of the fillets of the fishes *Sparidentex hasta* and *Pampus argenteus* in the freezing time.

Research Materials and Methods

Fish samples preparing. This project was carried out in the spring of 2014, in the biology laboratory, at Behbahan Khatam Alanbia University of Technology. 10 kg of each fish species, *Pampus argenteus* and *Sparidentex hasta*, were purchased at the Behbahan city market in Khuzestan province, with an average weight 350±20.2 g and 750±15.38 g, respectively, in May 2015. All fish samples, after washing and removing wastes and bones, were put in 200 g packages in the freezers, at –18°C. First, all the factors, including moisture, ash, fat, protein, TBA (thiobarbituric acid), pH, FFA, peroxide value, and fatty acid of the fresh samples were tested at time zero. To evaluate the effects of freezing on the samples at – 18°C, they were transferred to the freezer and were stored for zero, 35, 65, and 95 days. Then, after defrosting, they were put to tests.

Determining the chemical composition of the fish fillets. At the end of each period of freezing, to analyze the proximate compounds, the fish fillets were ground so that homogeneous mixtures were obtained. Then the sample were placed in an oven, at 60 °C for 24 h, to remove moisture between body tissues, so that the samples became dried. The samples were powdered by an electric mixer and then transferred to the laboratory for an analysis of their nutrient composition. Chemical analyses of the samples were carried out in the central veterinary laboratory in Ahwaz, Iran.

To study the oxidation, first, lipids were extracted from a g per 100 g sample by the chloroform-methanol method [17]. To measure the peroxide, 20 ml of the low phase of the decanter were carefully transferred to a 250 ml flask. About 25 ml of acetic acid – chloroform, in the proportion 3:2, was added to the flask content. 0.5 ml of the saturated potassium iodide solution, 30 ml of distilled water, and 0.5 ml of 1% soluble starch were added, too. The amount of free iodine was titrated with 0.01 normal thiosulfate solutions [14]. To measure free fatty acid, 25 ml of neutralized ethanol with normal NaoH was added to the oil sample (due to evaporation of the solvent remaining in the bottom phase decanter). The amount of free fatty acid based on the amount of normal NaoH was used during the titration in the presence of phenolphthalein and was determined basing on the percentage of oleic acid per oil [15].

Determining thiobarbituric acid. The amount of TBA (thiobarbituric acid in malondialdehyde, mg, per sample, kg) in fish muscle was measured by Pearson's method [15]. To determine the amount of TBA (thiobarbituric acid) in fish muscle, the amount of 10 g of minced fish muscle was weighted (each sample was obtained from the homogenized mixture of three fishes), and transferred to the distillation balloon (digestion). 50 ml of distilled water was added and stirred for 2 minutes. Again, 47.5 ml of distilled water with 2.5 ml 4 of normal hydrochloric acid was added. Digestion continued until obtaining 50 ml of distilled solution. Then 5 ml of the distilled solution was transferred into a test tube, and 5 ml of thiobarbituric reagent was added (288.3 mg of thiobarbituric dissolved in 100 ml of glacial acetic acid, 90% achieved). The test tubes were placed for 35 minutes in the water bath at 100°C, and then for 10 minutes were cooled in cold water. Then, with the help of spectrophotometer in the 538 nm, absorption was determined.

Determining pH. 10 g of fish fillet were ground completely and homogenized in 10 ml of distilled water, then, using a pH meter, pH samples were measured [16].

Determining the peroxide value. To measure the peroxide value, 50 g of the sample was transferred into the 500 ml flask, and 200 ml of chloroform was added to it. For extraction, the flasks were shaken on a shaker for 2 h. Then the solution was transferred to the flask. The samples were transferred to the rotary evaporator to evaporate the solvent. After the evaporation of the solvent, the weight of the oil remaining in the flask was determined. To measure the peroxide value according to AOAC, Association of Official Analytical Chemists [17], the oil extracted in 30 ml of the mixture of acetic acid and chloroform was added to the obtained mixture, 5.0 ml of saturated potassium iodide was added, and the mixture was shaken for one minute (AOAC, Association of Official Analytical Chemists, [17]). 30 ml of distilled water was added to the mixture. After stirring thoroughly, the solution mixed with a sodium thiosulphate solution (0.01 normal) was titrated until it became bright yellow. 0.5 ml of starch solution was added to the mixture so that the mixture color became dark blue. The titration continued to remove the blue and give a bright color.

Statistical analysis. The analysis was carried out by CRD – Completely Randomized Design. Using the analysis of variance (One Way ANOVA), the differences between the means of treatment were determined. By means of Duncan's Multiple Range Test, significant differences between treatments were evaluated basing on a 95% level. For a statistical analysis, SPSS version 22 was used.

Results of the research and their discussion

The effect of freezing on the thiobarbituric acid. The results showed that the TBA (thiobarbituric acid) content in fish fillet of the *Pampus argenteus* and *Sparidentex hasta* species, being 0.65 and 0.53 respectively (Table 1), after 95 days of freezer storage at a temperature of -18°C, changed to 1.29 and 1.14 mg of malondialdehyde per kg (MDA) in the muscle tissue of the fish, which was a little high. Cadun *et al.* [18] noted that levels lower than 3 for TBA (thiobarbituric acid) are acceptable conditions for seafood that is kept frozen, which agrees with the results of the present study.

Table 1 – Thiobarbituric acid changes (mg/g) in the fillets of fishes *Pampus argenteus* and *Sparidentex hasta* in different periods of storage at –18°C (n=2, P≤0.05)

Time (days)	0	35 days	65 days	95 days
Sparidentex hasta	0.53 ± 0.02^{a}	0.72 ± 0.01^{a}	0.65 ± 0.02^{a}	1.14 ± 0.02^{a}
Pampus argenteus	0.65 ± 0.02^{a}	0.77 ± 0.02^{b}	0.96 ± 0.01^{c}	1.29 ± 0.04^{d}

The effect of freezing on the free fatty acids composition of the fillet of fishes Pampus argenteus and Sparidentex hasta. Khorramgah and Rezai's results [21] showed that, because of the effects of freezing on chemical changes in the fish *Rutilus frisii Kutum*, the amounts of TBA (thiobarbituric acid) and FFA were increased during storage in the frozen state (P<0.05). In our study, the amount of free fatty acids in frozen fish *Sparidentex hasta* increased during the period while the amount of it in *Pampus argenteus* increased after 35 days (Table 2) (P>0.05). But a significant decrease was registered in the next periods as compared to the fresh fish. FFA alone do not reduce the nutritional value, but its assessment in the process of spoilage in fish is important [19–21].

Table 2 – Free fatty acids contents (%) in the fillets of the fishes *Pampus argenteus* and *Sparidentex hasta* in different periods of storage at –18°C (n=2, P≤0.05)

Time (day)	0	35days	65days	95days
Sparidentex hasta	0.37 ± 0.006^{a}	0.4 ± 0.01^{a}	0.68 ± 0.001^{b}	0.9 ± 0.002^{c}
Pampus argenteus	0.34 ± 0.01^{a}	0.45 ± 0.02^{a}	0.68 ± 0.009^{b}	0.97 ± 0.01^{c}

The oil present in the fish fillet undergoes hydrolysis, resulting in the release of free fatty acids [20–23]. Due to the fat hydrolysis, FFA accumulates in the tissue during frozen storage, in low freezing, especially at high temperatures –10 to -20 °C [24–26]. Slow freezing rates or fluctuating storage temperatures may result in increased activity of some endogenous lipases enzymes resulting in increased rates of FFA (free fatty acids) accumulation [26]. The obtained results were lower than those recorded by Gandotra *et al.* [27], that, at –12°C, the FFA (free fatty acids) content in Mustus seengala fillet was 0.57% on the day zero and 5.61% on the 21st day of storage. Rodriguez *et al.* [25] showed increasing FFA (free fatty acids) during frozen storage in farmed Coho salmon (*Oncorhynchus kisutch*), while Seifzadeh *et al.* [28] made a study to evaluate the fat quality in packaged sprats (*Clupeonella cultriventris caspia*) soaked in whey protein compared with sodium alginate. The FFA (free fatty acids) they recorded on the first day was 4.10 and increased to 12.38 in the sixth month.

Effects of freezing on fish fillets pH. The results of this study showed that pH of *Pampus argenteus* and *Sparidentex hasta* at the start of freezing was 6.32 and 6.36, and after 95 days, it was 7.53 and 7.43, respectively (Table 3).

Table 3 – pH changes in the fillets of the fishes *Pampus argenteus* and *Sparidentex hasta* in different periods of storage at –18°C (n=2, P≤0.05)

Time (day)	0	35days	65days	95days
Sparidentex hasta	6.32 ± 0.04^{a}	6.60 ± 0.04^{b}	6.96 ± 0.01^{c}	7.53 ± 0.02^{c}
Pampus argenteus	6.36 ± 0.01^{a}	6.47 ± 0.06^{a}	6.91 ± 0.08^{b}	7.43 ± 0.01^{b}

Therefore, pH higher than 7.9 is known as spoilage, and a pH level higher than this value shows that the fillet is unusable. The temperature in this case is very important because if the temperature becomes higher, the analysis of protein will be increased, more ammonia and ammonium will be produced, and pH will be increased. Kilinc and Cakli [29] showed in their study of fresh Sardine fishes that the pH was 6.72, Gandotra, *et al.* [27] found the effect of freezing on fish muscles. They used the fillet of *Mystus seenghala*, and their results showed that when it was frozen at –12ºC for 21 days, the pH of the fillet increased slightly from 6.8 to 7.1. However, Aubourg *et al.* [24] found that frozen storage did not have significant effect on pH changes during the storage period.

The effect of freezing on the peroxide values of fish fillets. The results showed a significant decrease in the peroxide value in the fish *Pampus argenteus* within 3 months of storage at –18°C, compared to fresh fish. But the amount of it in the fish *Sparidentex hasta* in all periods was expressed by a negative number which showed no significant difference (Table 4).

Table 4 – Peroxides index changes in the fillets of the fishes *Pampus argenteus* and *Sparidentex hasta* in different periods of storage at –18°C (n=2, P≤0.05)

Time (day)	0	35days	65days	95days
Sparidentex hasta	3.9 ± 0.17	Negative	Negative	Negative
Pampus argenteus	Negative	Negative	Negative	Negative

In Khorramgah and Rezai's study [21], it was found that the amount of peroxide in the fish *Rutilus frisii Kutum* was increasing until the third month of storage and then reduced. According to Connell [30], when the peroxide number exceeded 10 meq of oxygen per kg of oil of fish fillet, the fish fillet is then considered unfit for human consumption; all the marks inspected were within the acceptable limits. Egan *et al.* [15] suggests that the rancidity flavor appears when peroxide numbers reach 20–40 meq of oxygen per kg of fat. So, in the present investigation, it has been found that both fish species tested do not show an increased rancidity flavor. The current result was lower than the results of Rostamzad *et al.* [31] who recorded an increase in the PV (peroxide value) content in control fillets of the Persian sturgeon during frozen storage from 0.2 on the first day of storage to 10 in the sixth month. Seifzadeh *et al.* [28], though, recorded the PV (peroxide value) on the first day being 0.2 and increasing to 5.10 in the sixth month.

The results of changes in crude oil of the fish *Pampus argenteus* and *Sparidentex hasta* after freezing for different periods are shown in Table 5.

Table 5 – Fat contents changes in the fillets of the fishes *Pampus argenteus* and *Sparidentex hasta* in different periods of storage at –18°C
(n=2, P≤0.05)

Time (day)	0	35days	65days	95days
Sparidentex hasta	17.78 ± 0.01^{b}	25.19 ± 1.09^{c}	11.04 ± 0.04^{a}	10.5 ± 0.02^{a}
Pampus argenteus	17.42 ± 1.07^{a}	17.99 ± 1.02^{a}	17.66 ± 1.04^{a}	24.22 ± 2.05^{b}

The *Sparidentex hasta* fillet fat amount after 95 days of freezing was of the lowest percentage (10.5±0.02a), but no significant difference was observed in the fresh sample (P<0.05). The results showed that the percentage of fat fillet of *Pampus argenteus* in the freezer after 65 days and 35 days were not significantly different from the fresh samples (P<0.05). On the other hand, with other treatments, after 95 days, there was a significant difference in the frozen fillets (P<0.05). Nazemroaya *et al.* [22] reported the effects of freezing storage on the fatty acids of the mackerel (*Scomberomorus commersoni*) and sharks (*Carcharhinus dussumieri*), and showed that for both fish species, PUFA (polyunsaturated fatty acids) and HUFA (highly unsaturated fatty acids) in fresh fish were higher than all saturated fatty acids, although after the freezing period the ratio of unsaturated fatty acids to saturated fatty acids reduced. As can be seen in the present study, after 95 days of freezing, due to the changes in the fatty acid chains, the amounts of saturated and unsaturated fatty acids changed.The increase in the lipid content is related to the lipid oxidation resulting from the action of lipolytic enzymes (Lipases and phospholipases). Fish phospholipids undergo degradation to produce hydroperoxides, aldehydes, and ketones which are responsible for the development of oxidative rancidity (Raharjo *et al.* [32]). The recorded results were less than the results of Rostamzad *et al.* [31], who revealed that the TBA (thiobarbituric acid) value was 0.2 on the first day of storage and increased to 2.7 in the sixth month. And as Seifzadeh *et al.* [28] recorded, the TBA (thiobarbituric acid) on the first day was 0.03 and increased to 0.32 in the sixth month.

Conclusions

Freezing and frozen storage affected the free fatty acids, thiobarbituric acid, and pH. The increase in the contents of free fatty acids, thiobarbituric acid, and pH in the fish fillets was observed as compared to the control. A statistical analysis revealed that there was no significant difference between thiobarbituric acid changes in the fish *Sparidentex hasta* in freezing periods, but a significant difference was found in the thiobarbituric acid content in the fish *Pampus argenteus.* The peroxide value in *Pampus argenteus* was reduced, but in *Sparidentex hasta*, revealed itself. Between free fatty acids and pH contents in the fillets of *Pampus argenteus* and *Sparidentex hasta*, significant differences were observed. This study has shown low fat oxidation and rancidity in the fillet of the fish *Pampus argenteus* in a freezing period as compared to the control.

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