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The effect of freezing at the temperature of -18°C on chemical compositions of the body of *Lutjanus johnii*

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Abstract. In order to investigate the effect of freezing at the temperature of -18°C on chemical compositions (crude protein, crude fat, ash and moisture) of the body of *Lutjanus johnii* during 180 days of the preservation in freezer, sampling the mentioned fish was done from fish seller market of Bushehr Province in January 2014. After removing scales, cutting fins and evacuation of viscera, 3 fishes were randomly separated in order to do zero day experiments (fresh sample). The rest of the fish was separately placed in plastic freezer bags and they were placed in freezer at the temperature of -18°C. At the next samplings (the 30th, 60th, 90th, 120th, 150th, and 180th days) the number of replication for each treatment was 3 and the meat sample from different parts of homogeneous fish were used. The results showed that the levels of crude protein, crude fat, ash and moisture (%) in fresh sample were 15.87±0.15, 4.50±0.14, 2.37±0.21, 77.72±0.24 respectively that reached 15.57±0.33, 4.02±0.05, 3.00±0.15, 77.20±0.22 respectively after 180 days of the preservation in freezer. The statistical analysis results showed that the preservation duration in freezer at the temperature of -18°C had significant effect on the levels of crude fat and ash ($p < 0.05$), but it had no significant effect on the levels of crude protein and moisture ($p > 0.05$). Investigation of chemical changes of the fish body at the end of the research showed that the nutritional value of the fish did not fall into tangible decline though some of these rates increased and decreased. Thus the preservation of the product at this temperature during 180 days keeps the quality desirably and acceptably for the consumer.
Key Words: freezing, chemical changes, quality, *Lutjanus johnii*.

Introduction. According to the fact that two-thirds of the Earth's surface is covered with water and these waters have important and valuable biological fishery resources, thus human's knowledge is oriented towards the sea and the oceans so that he can encounter land food resource restriction by replacing them with the present resources in the sea. *Lutjanus johnii* belonging to Perciformes order and Lutjanidae family is considered as the most important and valuable edible fishes of the tropical region waters (Sadeghi 2001). This fish is benthic and is found in rocky regions and coral reefs and disperse widely in warm and semi-warm areas of the Atlantic Ocean, the Pacific Ocean, the Indian Ocean, and the Persian Gulf and Oman sea (Allen 1985).

Regardless of vital importance of proteins and having the other mineral and organic matters, the delicious flesh of this fish has caused to have a good chance for human nutrition and consumption. Freshness is the most important issue in determination of the fish quality and its rate is variable from fishing to consumption. According to the limits of fishing season and dispersal of the fish consumption place, different techniques are used in order to maintain the quality of the fish flesh among which freezing is one of the most common ways of preservation (Lugasia et al 2007; Badii & Howell 2002). Freezing has more advantages in comparison with the traditional methods such as salting, smoking and drying since in this method the least changes are made to the product (Razavi-Shirazi 2002). Freezing is one of important methods of the preservation of marine foods that causes an increase in shelf-life of the product by preventing from internal dehydration or water immobilization, decreasing the temperature (Ersoy et al 2008) and preventing from microbial growth (Morkore &

Lilleholt 2007). However the fish flesh may be accompanied by the quality index decline such as protein denaturation, fat oxidation of the fish muscle, color and smell degradation, tissue change and losing weight during freezing process (Alizadeh et al 2007; Zhu et al 2004; Srinivasan et al 1997). These changes affect the characteristics of the fish body cause injuries to the physicochemical and tissue qualities (Srinivasan et al 1997; Nilsson & Ekstrand 1995). Thus according to the fact that *L. johnii* is among the valuable fishery fishes of the Persian Gulf sea including a large population of fishing in this area and also according to people taste in order for the consumption of this fish during the year, the process of freezing effects at the temperature of -18^{o}C on chemical compositions (crude protein, crude fat, ash and moisture) of the body of this fish was investigated in this research.

Material and Method

Sampling and samples preparation. In this study we prepared 30 samples of *L. johnii* from local markets in Bushehr Province in January 2014. The fishes were placed in special unolits containing ice and were transported to the veterinary laboratory of Bushehr province in the shortest time. After transporting, the fish samples were washed using potable water. After removing scales, cutting fins and evacuation of viscera, 3 fishes were randomly separated in order to do zero day experiments (fresh sample). The rest of the fish was separately placed in plastic freezer bags and they were placed in freezer at the temperature of -18^{o}C. At the next samplings (the 30th, 60th, 90th, 120th, 150th, and 180th days) the number of replication for each treatment was 3 and the meat sample from different parts of homogeneous fish were used.

Measuring the rates of chemical compositions. Measuring the crude protein rate was done using Kjeldahl method that its calculation method is mentioned in equation (1) (AOAC 2005).

$$C = \frac{A \times 100 \times 1.4 \times 6.25}{1000 \times B} \quad (1)$$

In this equation (C) is the protein percent in meat products, (A) is the used rate of acid for the sample titration, (B) is the sample rate and 6.25 is the conversion coefficient of nitrogen into protein. Crude fat was extracted using petroleum ether and Soxhlet device. Measuring the ash rate was done by heating the sample within the electric furnace at a temperature of 550^{o}C by the time it reached the stable weight (AOAC 1990). Measuring the moisture rate was determined using oven at a temperature of 100^{o}C by the time it reached the stable weight (AOAC 2005).

Statistical analysis. Statistical analysis of the obtained data was carried out using SPSS 18 software. The accuracy of the normality of the data was specified using one sample Kolmogorov-Smirnov test. One–Way ANOVA was used in order to compare the data and Duncan test was used to test the significance of the differences at a 95% certainty level. Excel software was used to draw diagrams (Zar 1999).

Results. The results of this research indicated that the most rates of the muscle crude protein 15.87±0.15 (%) were observed in fresh sample that they had no significant differences in comparison with their rates during all perseveration months ($p > 0.05$). The least rates of the muscle crude protein 15.57±0.33 (%) were observed in 180th day of preservation in freezer that they had no significant differences in comparison with the crude protein rates in the other months of preservation in freezer ($p > 0.05$) (Figure 1).

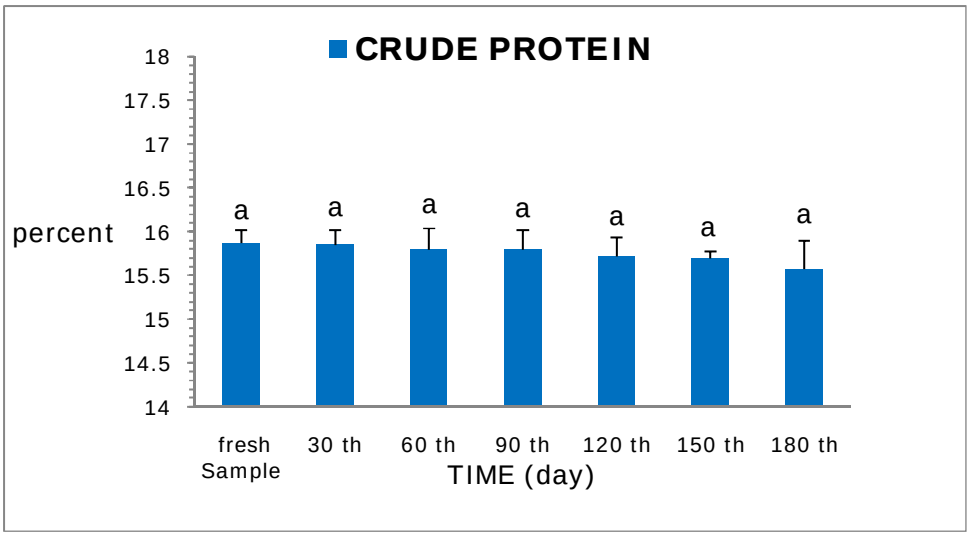

Figure 1. The change process of muscle crude protein rates of *Lutjanus johnii* during 180 days of preservation in freezer.

The results of this research indicated that the most rates of the muscle crude fat 4.50±0.14 (%) were observed in fresh sample that they had significant differences in comparison with their rates during all perseveration months ($p < 0.05$). The least rates of the muscle crude fat 4.02±0.05 (%) were observed in 180th day of preservation in freezer that they had no significant differences in comparison with the crude fat rates in the other months of preservation in freezer ($p > 0.05$) (Figure 2).

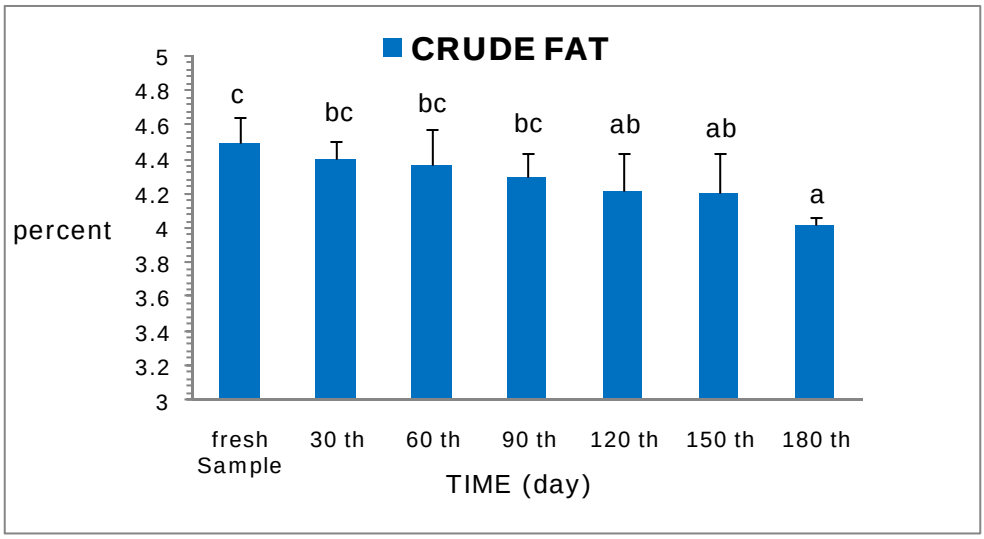

Figure 2. The change process of muscle crude fat rates of *Lutjanus johnii* during 180 days of preservation in freezer.

The results of this research indicated that the most rates of the muscle ash 3.00±0.15 (%) were observed in 180th day of preservation in freezer that they had significant differences in comparison with the fresh sample and the other months of preservation in freezer ($p < 0.05$). The least rates of the muscle ash 2.37±0.21 (%) were observed in fresh sample that they had significant differences in comparison with the other months of preservation in freezer ($p < 0.05$) (Figure 3).

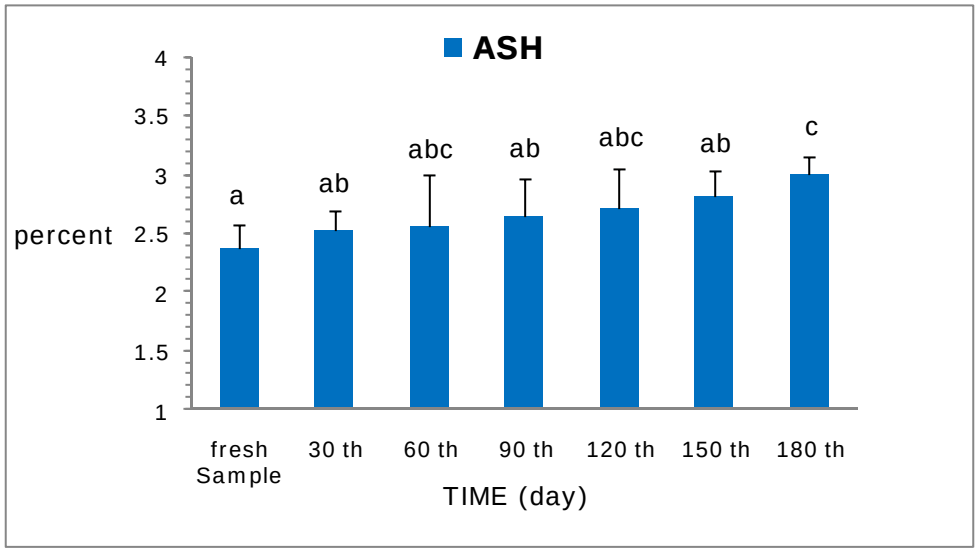

Figure 3. The change process of muscle ash rates of *Lutjanus johnii* during 180 days of preservation in freezer.

The results of this research indicated that the most rates of the muscle moisture 77.72±0.24 (%) were observed in fresh sample that they had no significant differences in comparison with moisture rates during the other months of perseveration in freezer ($p > 0.05$). The least rates of the muscle moisture 77.12±0.43 (%) were observed in 150th day of preservation in freezer that they had no significant differences in comparison with the other months of preservation in freezer ($p > 0.05$) (Figure 4).

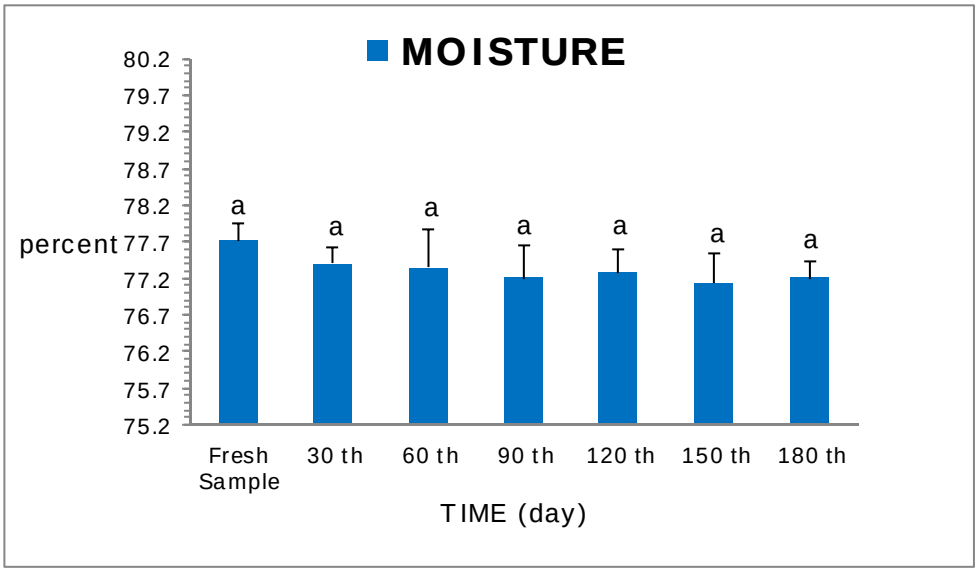

Figure 4. The change process of muscle moisture rates of *Lutjanus johnii* during 180 days of preservation in freezer.

Discussion. Freshness is the most important issue in determination of the quality of the fish meat. The loss of freshness is accompanied by decomposition. One of the ways of increasing shelf-life of the fish is to preserve it at a temperature below 0°C (freezing). Freezing process delays the procedure of the chemical and microbial reactions but some of these reactions are carried out at the freezing temperature, though (Ranken & Kill 1993). Chemical composition of the fish's body is different from one species to another one. This difference may be seen even among the fishes of the same species due to the differences in age, sex, environmental conditions and season. Doing all chemical reactions and biological activities requires two main factors, that is, heat and water. Thus

both a fall in temperature by below zero and lower than that or lack of access to open water due to freezing (formation of ice crystals) are among the factors that can affect the speed and intensity of chemical reactions and biological activities and stop them in some circumstances. Even if the fish is frozen immediately immediately after fishing and was preserved in freezer under desirable conditions in terms of coldness and moisture, we cannot be sure that the product keeps its qualitative characteristics for unlimited time. The rate of the quality decline of the product depends on many factors such as preparing product before freezing, freezing method, temperature fluctuations and thawing method (Ersoy et al 2008; Boonsomrej et al 2007).

The present study showed that the rates of crude protein, crude fat, ash and moisture in *Lutjanus johnii* during 180 days of preservation in freezer are not the same. The results indicated that the rate of crude protein in fresh sample decreased from 15.87±0.15 (%) to 15.57±0.33 (%) at the end of the preservation period. The study of the effects of freezing on protein states that denaturation of protein due to freezing is the main factor of tissue changes such as toughness of the product (Mackie 1993). Making drip after thawing process, relative change of chemical compositions of the muscle and protein denaturation are among the reasons of protein decline (Castrillon et al 1996). Arannilewa et al (2005) examined the effect of freezing on the rate of crude protein of the fish *Sarotherodon galilaeus* during 60 days. The results showed that the most rate of crude protein was observed in fresh sample that reached the least rate with a decline procedure at the end of the period. Aberoumand (2013) studied the effect of shelf-life duration freezing on the crude protein rates of the body of four fishes (*Liza dussumieri*, Sparidae, Sciaenidae, Platycephalidae). The results showed that shelf-life in freezer during 60 days had significant effect on crude protein rate of the fishes and its rate decreased in all species. Decline procedure of crude protein rate was similar to the results of the present research.

Investigations showed that in the present study the crude fat rate in fresh sample decreased from 4.50±0.14 (%) to 4.02±0.05 (%) at the end of the shelf-life. Although the fat rate is variable in various species, all fishes have almost unsaturated fatty acids in their fat structure oxidized when it is exposed to the air and creates undesirable changes in fats and cause the quality of the product to be deceased (Jalili 2008). Arannilewa et al (2005) studied the effect of 60 day of preservation freezing on the crude fat rate of the muscle of the (*Sarotherodun galiaenus*). The results showed that the highest rate of crude fat with 9.72% in fresh samples and the lowest crude fat rate with 7.20% were observed in fishes which were preserved freezing during 60 days. This decline procedure was similar to the results of the present study.

In the present study ash rate in fresh sample increased from 2.37±0.21 (%) to 3.00±0.15 (%) at the end of preservation period. Freezing with a decrease in moisture of nutrition increases mineral concentration of the product tissue and as a result ash reaches a higher percent (Jalili 2008). Moini et al (2012) in a study investigated the ash rate of the meat of *Scomberomorus guttatus*. The results showed 3.76% ash in -18°C after 240 days that was similar to the results of the present research. Aberoumand (2013) studied the ash rate in the body of four fishes (*Liza dussumieri*, Sparidae, Sciaenidae, Platycephalidae) during 60 days of preservation in freezer at a temperature of -18^{o}C. The results indicated that the ash rate was variable from 1.36% to 0.81%, 0.66% to 0.81%, 0.97% to 0.47% and 0.68% to 0.53% respectively during 60 days of preservation in freezer. Ogundiran et al (2014) measured the ash rate in the body of four fishes (*Scomber scombrus*, *Sardina pilchardus*, *Gadus macrocephalus*, *Trachurus symmetricus*) chosen from two markets located in the southwest of Nigeria 1.11%, 1.30%, 1.48% and 1.41% respectively that the ash rate was not similar to the results of the present study.

In the present study the moisture rate in fresh sample decreased from 77.72±0.24 (%) to 77.20±0.22 (%) at the end of the preservation period. The moisture rate almost includes about 80-85% of the fish muscle weight. Loss of moisture is done during freezing and thawing period that this issue may result in the toughness of the product tissue. It appears that a decrease in the capacity of the water preservation after thawing process is in relation with denaturation and aggregation of proteins especially myosin

(Morkore & Lilleholt 2007). Nazemroaya et al (2011) stated that moisture in the *Scomberomorus commerson* and *Carcharhinus dussumieri* was variable between 73.32% to 75.05% and 74.68% to 76.33% respectively in both species (*S. commerson* and *C. dussumieri*) during 6 months of preservation in freezer at a temperature of -18^{o}C. Based on approximate analysis the moisture rate in *C. dussumieri* was significantly higher than *S. commerson*). In both fish no significant differences were observed in moisture rate as a result of preservation time and temperature between fresh fillets and preserved fillets during 6 months in freezer. The process of moisture changes in both species was not the same during 6 months of preservation in freezer. Also the moisture rate in both species was lower than the measured moisture rate in the present study. Mackie (1993) reported that freezing can decrease the capacity of water preservation in the fish muscle and increases its toughness state that the incidence of this state is due to protein degradation and loss of flexibility property of myofibril protein. Cheng et al (1979) reported that the capacity of preservation of the water of tissues during freezing period is related with a decrease in solubility of myofibril protein and little changes in capacity rate of water preservation of thawing samples in water is due to little denaturation of myofibril proteins (soluble in salt) in such a manner that the decline procedure of moisture in this research approve this issue.

Conclusions. Finally we can conclude that we may consume the fish after freezing. But we should try to consume the fish in fresh condition as early as possible as quality remain better in earlier stage. Since fish is not normally consumed raw, freezing processing method is employed in preparation them for consumption which could have varying effect on their nutrients, texture and falvour. Freezing processing made fish less susceptible to spoilage. Consequently according the results of the analysis of chemical compositions of the body of the *L. johnii* it can be stated that using this method keep the quality of the meat of the *L. johnii* desirably and acceptably for the consumer.

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