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**THE EFFECT OF VACUUM PACKAGING ON FAT PEROXIDE VARIATIONS IN  
THE FILLET OF *HYPOPHTHALMICHTHYS MOLITRIX* STORED AT -18 °C**

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**ABSTRACT**

All food products need to be packaged due to health, appropriate distribution, and quality. The present study was conducted to investigate the effects of vacuum packaging on fat peroxide in fillets of *Hypophthalmichthys molitrix* and determine shelf time at -18 °C for 50 days. For this purpose, 20 fillets in 33 packages of 100 gr were packed and stored in a vacuum by EBOR. The fillets were stored in days 0, 10, 20, 30, 40, and 50 at -18°C in three replications so that fat and peroxide were measured. The peroxide value at -18°C was significantly increased by time in comparison with the control ( $p \leq 0.5$ ). Storing the fillets of *Hypophthalmichthys molitrix* in vacuum caused to alleviate peroxide variations in different days and reduce the spoiling speed of *Hypophthalmichthys molitrix*.

**Keywords: Vacuum packaging, *Hypophthalmichthys molitrix*, Peroxide, Fat, Shelf time**

**INTRODUCTION**

The need to protein materials and providing it is the major issue which nowadays attracts the human attention and has an eminent role on various aspects of human beings such as health, economy, technology and researches. Fish proteins regarding sensitive to proteolytic degradation have equivalent values or even higher than red meat so that facilitate their digestion. Fat digestion of fish is fast and also it is rich in terms of the unsaturated fatty

acids (mainly EPA and DHA) as well as fat-soluble vitamins (A-D) microelements such as iodine, iron and calcium (Perez-Alonso et al., 2003; Razavi Shirazi, 2006), is an important and valuable resource. Therefore, nutrition experts recommend eating fish in the diet. Fish are very sensitive to corruption and are spoiled faster than other meat dishes (Fan et al., 2009). Fishery products, although having a large amount of nutritional value, have a lot of corruptions in relation to environmental factors, since a lot of changes are occurred in fish muscle tissue after catching. These variations usually have enzymatic and chemical reactions which cause an initial drop in new fish, while microbial activity is responsible for secondary corruption and determining the shelf life of fish (Sallam et al., 2006, Huss, 1994). In this respect, more attention is required to develop and store it (Canel, 2004). The common time of fish storage is 1 to 2 days but fish can be stored in fridge condition for a long time to maintain its quality (Johnston et al., 1995). Freezing is the most important method of storing seafood (Vidya et al., 1999). The shelf life and quality of frozen fish at the end of the storing period are different based on biological differences, how to catch and how to prepare them for the period before freezing. If the quality of the fish is not desirable, certainly shelf life under freezing condition, as well as the quality of the final product will not be as optimal. Undoubtedly, preventing the total spoil is very important and difficult, however shingling non-ice cover and packaging are the ways which can be effective here (Razavi Shirazi, 2007). Freezing fish via forming ice crystals increases salt concentration and organic compounds in the liquid phase which finally may dehydrate and denature muscle proteins or cell membranes will be destroyed (Aubourge et al., 1999). One of the methods of processing and storage of fish products is packaging and vacuuming in a vacuum. This process protects the health and safety of fish and ease of its use, and well as to introduce the product. Packaging and vacuuming are applied to fresh and frozen fish and can maintain the quality of products for more time (Davies, 1997). When the fresh fish is packaged in the vicinity of the air, its shelf life is limited, but outing the air of the package and keeping it in a vacuum increases the shelf life of fresh fish. Lipids and fats are the most important biochemical compounds of fish. Lipids of fish are stored in various organs, especially the liver and muscles (Kandomir et al., 2007). Fish fat with increasing shelf life due to the production of peroxide compounds (pv), its rate gradually decreases. Peroxide index can be considered as the main factor of the shelf time (Moeini, 1989). *Hypophthalmichthys molitrix* (silver carp) is one of the most eminent fish species in Iran which is grown due to its low-cost diet. In terms of high annual production and accessibility for consumers and good distribution is more significant among

farmers and often can be purchased as fresh fillet or whole fish by consumers from retail shops (Siddaiah et al., 2001). Hence, this species was used as one of the important fish species in the present study. So the study was aimed to assess vacuum packaging on fat peroxide variations in the fillet of *Hypophthalmichthys molitrix* stored at -18°C.

## MATERIAL AND METHODS

The treatment of samples: 20 samples of silver carp fish with an average weight of  $20 \pm 1100$  gr were lively purchased from a hydrothermal fish farm in Kermanshah, west of Iran. For this purpose, it was tried to select fish without any abnormal symptoms and as far as possible have the same length and weight. Then the fish were transferred to the Lab of Agriculture and Natural Resources college in impervious packs along with ice in order to preliminary tests. Sample preparation included washing with water, removing scales, abdominal drain, cut off heads and tails and with full respect of health, fish were filleted weighing about 100 gr and after washing, they were transferred to the laboratory vacuum packaging of Razi University in Kermanshah. The fillets at days 10, 20, 30, 40, and 50 were evaluated in triplicate at -18 °C. For this purpose, 33 100g-packs were used. First, fresh silver carp was analyzed at first day, subsequently fillets on days 10, 20, 30, 40, and 50 were tested to obtain the amount of fat and determine the peroxide value.

### Measurement of fat and peroxide

1. Determining and measuring fat was done based on Kinsella et al. (1971), which are expressed as gram per hundred grams of muscle.
2. Determining and measuring peroxide value (pv) based on the method of Malaysian palm oil (1995).

### Chemical tests Fat extracted by cold or Kinsella method

At first samples were minced and then 50 gr of minced meat with 50 cc chloroform and 100 cc methanol were simultaneously stirred by electric mixer on high speed for 3 minutes. After that 50 cc chloroform was added to the mixture and was stirred for 30 to 60 seconds. Next, 50 cc distilled water was added and stirred again at high speed for 30 to 60 seconds. The mixture was transferred to decanter to separate oil from the meat, so the water was concentrated at the top, the meat in the middle and the oil at the bottom of the container. *Rotary* evaporator was used to separate the oil. Hence the oil can be weighted in the balloon of *rotary* evaporator. The extracted fat was expressed as gram per hundred grams of wet muscle.

### Measuring peroxide value (PV)

In this method, 3 gr of extracted oil was transferred into a 250 ml beaker. 10 ml of chloroform and acetic acid were added and stirred to this container. Then one ml of saturated solution of potassium iodide was added to the mixture for 5 minutes and was kept in the dark. After that, 20 ml of distilled water was added and stirred. This mixture was titrated by 1% normalized Thiosulfate to disappear the yellow color. Then one ml of 1.5% starch solution was added to the mixture and also titration continued until dark blue was disappeared. Control sample was done as this method with the exception that the mixture had no oil. The amount of peroxide based on mEq /1000 gr was calculated as follows:

$$(V1-V2)N/W1000$$

Where V1: the amount of sodium thiosulfate with normality N per ml; V2: the amount of sodium thiosulfate used to test control; W:Weight (gr) of the sample; N: normality of sodium thiosulfate.

The data was analyzed with SPSS16 and were submitted to one way analysis of variance.

## RESULTS

The results of fat in fillets of silver carp stored at -18 °C have been presented in **Table 1**.

According to the findings, the mean fat in fillets at -18 °C in days 0, 10, 20, 30, 40, and 50 is 4.10, 3.95, 3.37, 3.00, 2.53, and 2.11 gr, respectively (**Table 1**).

The results of peroxidase in fillets of silver carp stored at -18 °C have been presented in **Table 2**.

According to table 2, the mean peroxide of silver carp fillet in day 0 is 2.73 meqO<sub>2</sub>/kg fat. This value was found 3.04, 4.28, 4.75, 4.80, and 5.72 meqO<sub>2</sub>/kg fat, respectively, for days 10, 20, 30, 40, and 50.

The results of one way analysis of variance at -18 °C has been presented in table 3.

According to table 3, the mean of peroxide in different days are not similar (F=422.83, P<0.05). Here, Duncan multiple test was used for mean comparison, which its results has been shown in table 4.

According to table 4, the peroxide of fillets in different days has been divided to five groups. The mean peroxide in days 0, 10 and 20 is in three different classes. It shows that the increase of peroxide in fillets was significant until day 20 (P<0.05). However, the mean peroxide in days 40 and 50 is in a class, which shows the increase after day 30 is not statistically significant (P<0.05). Based on Duncan results, the peroxide changes in some days had no significant difference with before days. In other words, storing fillets of silver carp at -18°C caused to alleviate changes in peroxide. Hence, the hypothesis of the study is accepted.

Table 1: The measured fat in fillets of silver carp stored at -18 °C in different days

day						
50	40	30	20	10	0	
2.11	2.53	3.00	3.37	3.95	4.10	Mean
0.10	0.10	0.10	0.10	0.10	0.10	Standard deviation
2.01	2.43	2.90	3.27	3.85	4.00	Minimum
2.21	2.63	3.10	3.47	4.05	4.20	Maximum
3	3	3	3	3	3	Replication

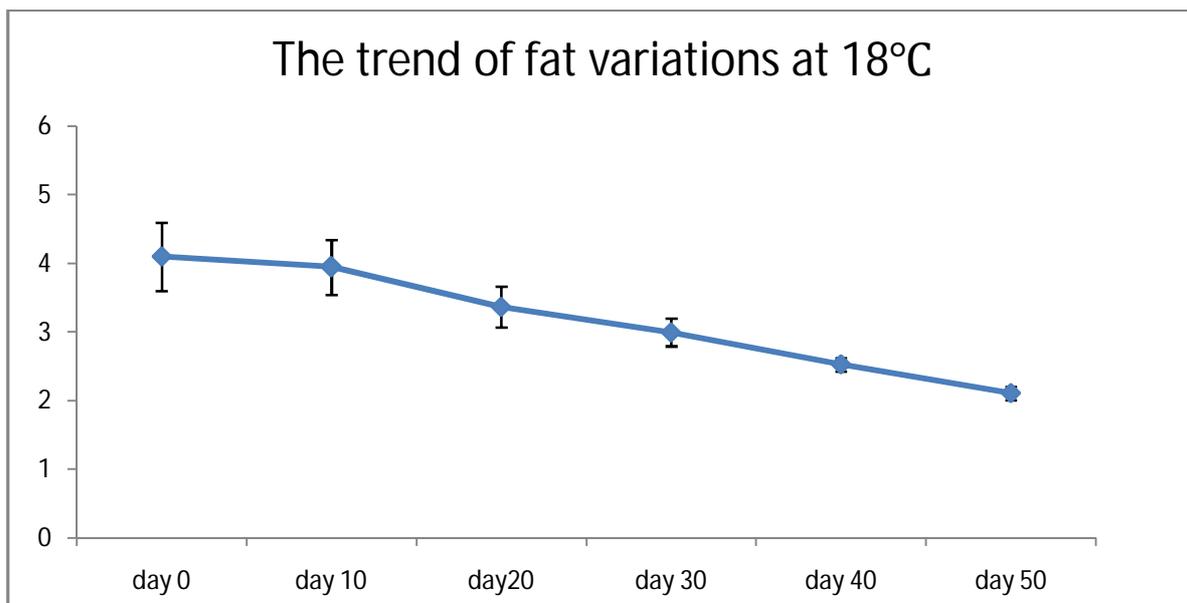


Figure 1: The trend of fat variations at -18 °C in days of 0, 10, 20, 30, 40, and 50

Table 2: descriptive factors of peroxide in fillets of silver carp stored at -18 °C in different days

day						
50	40	30	20	10	0	
5.72	4.80	4.75	4.28	3.04	2.73	Mean
0.10	0.10	0.10	0.10	0.07	0.10	Standard deviation
5.62	4.70	4.65	4.18	2.98	2.63	Minimum
5.82	4.90	4.85	4.38	3.12	2.83	Maximum
3	3	3	3	3	3	Replication

Table 3: The analysis of variance for peroxide changes in fillets of fish stored at -18 °C

Sig.	Test	MS	df	SS	Source variable
0.00	422.83	3.89	5	19.45	Between group
		0.01	12	0.110	Inter group
			17	19.56	Total

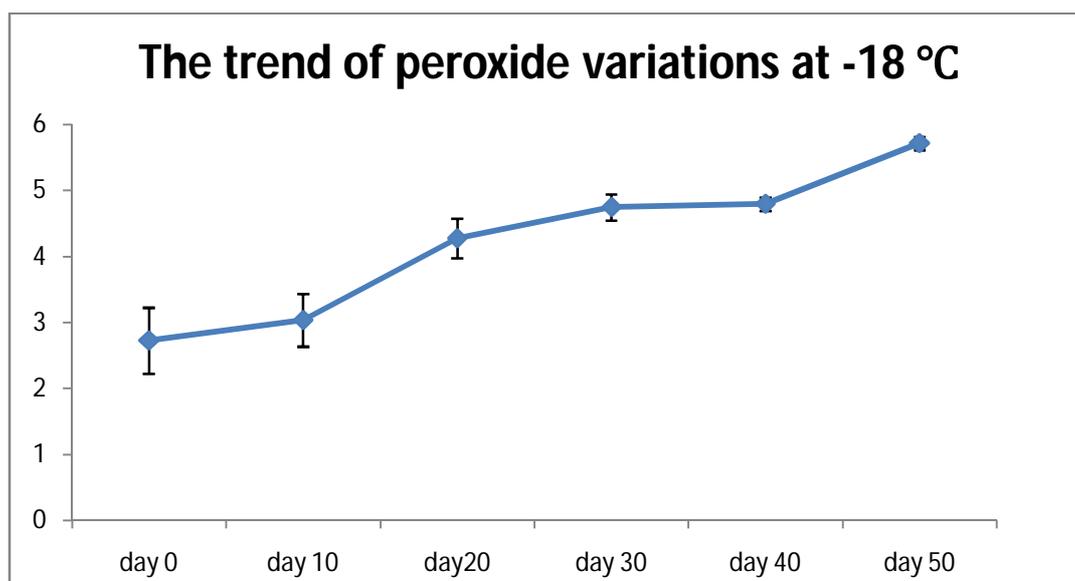


Figure 2: The trend of peroxide changes at -18 °C in days of 0, 10, 20, 30, 40, and 50

Table 4: the findings of Duncan test for fillets stored at -18 °C

Day	Rep.	Group 1	Group 2	Group 3	Group 4	Group 5
0	3	2.73 <sup>a</sup>				
10	3		3.04 <sup>b</sup>			
20	3			4.28 <sup>c</sup>		
50	3				4.75 <sup>d</sup>	
40	3				4.80 <sup>d</sup>	
30	3					5.72 <sup>c</sup>
Sig.		1.00	1.00	1.00	.54	1.00

The similar letters show no significant difference at 0.05 % level.

**DISCUSSION**

The present study was aimed to investigate the packaging and vacuuming impacts on fat peroxide of fillet of silver carp. For this purpose, fat and peroxide tests were conducted for 50 days in freeze condition (-18 °C). There were found different results which would be discussed in comparison with other findings obtained by different authors. According to the results of this study, mean fat in fillets of silver carp stored at -18 °C in different days of experiment including 0, 10, 20, 30, 40, and 50, respectively, was 4.10, 3.95, 3.37, 3.00, 2.53, 2.11 gr. However, the results obtained by Basharati (2004) on rainbow trout

showed that the fat value has a descend trend. A study was done on silver carp indicated that fat is decreased by time in a 60-day period. Mean peroxide in fillets of fish stored -18°C in days 0, 10, 20, 30, 40, and 50 was, respectively, 2.73, 3.04, 4.28, 4.75, 4.80, and 5.72 meq<sub>2</sub>/kg fat. Parvaneh (1998) showed the peroxide value in oil should be less than 5 meq<sub>2</sub>/kg fat or even less than international limit. Karim (1991) concluded that if the peroxide was more than 10, the fish would not be fresh in terms of fat, but the adverse smell will be obtained when it is more than 20 meq<sub>2</sub>/kg fat. Smitt et al. (1980) on the storage of

herring showed that the peroxide value is 0.8-1.20 meqO<sub>2</sub>/kg fat. Pacheco- Aguilar et al. (2000) found the peroxide value as 2.9-8.9 meqO<sub>2</sub>/kg fat for monterey sardine muscle stored at 0 °C. Different authors have been found various results on this issue (Rezaee, 2003; Dragoev et al., 1993; Ben et al., 1999; Kaivanfar et al., 2003; Nazemroaya, 2006). Lyhs et al. (2001) on a study titled microbiological quality and Shelf-life of Vacuum Packaged, rainbow trout stored at 3 and 8 °C showed the fillets can be stored till 21 days at 3 °C and 18 days at 8 °C. Hedaiatifard et al. (2010) obtained the increase of peroxide in pike at 4 °C in a 60-day period. Zaree (1994) in a study on Sturgeon concluded that vacuum Sturgeon at -18 °C was stable without any change in the taste of its fillet for 5 months, and also chemical features were able to stable for 6 months at -18 °C. Jourkesh (2004) concluded that the peroxide value was normal till 18 days. Esmaili et al. (2007) showed the peroxide variations and organoleptic features have direct relation with storing time in refrigerator. Developing the inappropriate smell can be due to oxidation of fat (Fagan, 2003). Besides, fat oxidation results in color and feed changes (Dragoev et al., 1993). Hence, to alleviate the adverse impacts of oxidation, the natural anti-oxidative compounds can be used. During lipid

peroxidation, free radicals are created and oxidation happened for some vitamins. The oxidation outputs don't change the sensory characteristics of fish but are dangerous to human health. Hydro peroxides have no toxic effects but carcinogenic. The results are similar to those obtained by Hultin et al. (1992). This reduction can be expressed by one and two molecule mechanism, i.e. when the hydroperoxide of fish muscles decrease, the formation speed of these compounds is higher than their corruption. Therefore, according to this research, shelf time of silver carp based on peroxide stored in a vacuum at -18°C in day 50 would be reached to unallowable value of 10 meqO<sub>2</sub>/kg fat. So the increase of peroxide at -18 °C is low. In addition, packaging in vacuum can increase the shelf time of fish. This value reduces the economic dangers of fish spoil and maintains the nutritional value of fish.

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