

Effect of vacuum packaging on body chemical composition, peroxide, and TVB-N of phytophagous fish during frozen storage

Abbas Abdolmaleki ^a, Mehdi Mohammadalikhani ^{*b}, Zeinab Rahimi Afzal ^c

^a Department of Fisheries, Faculty of Natural Resource, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran

^b Ph.D graduated, Young Researchers and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, Iran

^c Dvsc Aquatic Animal Health & disease, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:

Received: 28 May 2022

Accepted: 3 January 2023

Available online: 10 January 2023

Keywords:

Ash, Protein

Fat

Moisture

Peroxide (PV)

TVB-N

ABSTRACT

This study aimed to investigate the effects of vacuum packaging on body chemical composition and changes in peroxide and TVB-N of phytophagous fish and determine the optimal storage time in freezing conditions (-18 °C). In this study, 36 samples of 100 g fillets were prepared for both vacuum and non-vacuum conditions in completely hygienic conditions. Then half of the samples were vacuum-packed using an EBOR vacuum packing machine, and the other half were packaged under conventional conditions. These fillets were stored for five months at freezing temperature (-18 °C). After packing, all factors were analyzed in 6 replications by standard methods. Peroxide content increased significantly from 1.96 meq/kg to 44.44 meq/kg in vacuum conditions and to 47.77 meq/kg in non-vacuum conditions. Also, the amount of TVB-N increased significantly from 6.53 mg/100g to 18.66 mg/100g in vacuum conditions and from 6.53 mg/100g to 38.26 mg/100g in non-vacuum conditions. The result of the analysis shows the positive effect of packaging in vacuum conditions on the process of changes in the body's chemical composition. The samples in these conditions had less change range than in conventional conditions, which has reduced the spoilage rate of these samples.

Highlights

- This study aimed to determine the optimal freezing storage time and the effects of vacuum packaging on the chemical composition of phytophagous fish.
- Half of the samples were then vacuum-packed with an EBOR vacuum packing machine.
- Peroxide concentration rose from 1.96 meq/kg in vacuum to 44.44 meq/kg in non-vacuum.
- TVB-N concentration rose from 6.53 mg/100g to 18.66 mg/100g in vacuum and to 38.26 mg/100g in non-vacuum.

1. Introduction

Undoubtedly, food and nutrition are the most crucial subjects on a global scale at this time. The imperative to address the nutritional requirements of future generations and the expansion of the human population highlight the criticality of research in numerous domains, including agriculture, animal husbandry, technology, and related sciences. In this situation, it is crucial to consider not only the matter of food preparation, but also the provision of nutritious food that is chemically and health-friendly (Safi Yari et al., 2005). Fisheries and their byproducts are vital to human nutrition because they can supply the desired

quantity of nutrients that are essential to human health (Piri et al., 1998). Fish and seafood consumption has increased in recent years. The preference for fish and aquatic products over alternative foods, rising income, and population expansion are all contributing factors to the escalating demand for aquatic products (Alasalvar, 2002). Aquatic fish are regarded as a nutritious food source due to their abundance of unsaturated fats, vitamins, minerals, and high-quality proteins (Venugopal I, 2006). Aquatic animals constitute an essential and substantial proportion (16%) of the protein consumed by humans (FAO, 2004).

* Corresponding author.

E-mail address: m.mohammadalikhani1982@gmail.com
<https://doi.org/10.22034/aes.2023.340914.1036>

Environmental factors render fishery products exceedingly perishable, notwithstanding their considerable nutritional value, because the muscle tissue of fish undergoes numerous changes subsequent to the fishing process. These alterations manifest in a biochemical manner, causing numerous microbial, chemical, and enzymatic decay processes to commence simultaneously (Huss, 1994). Therefore, greater care must be taken in its preparation and maintenance. The approximate nutritional components of fish meat, namely protein, fat, and ash, are the most significant in terms of nutritional value (Ali et al., 2005). Presently, a critical component of the technological, scientific, and economic capabilities of human society is devoted to the study, evaluation, and implementation of projects that offer superior quality and sustainability. Notwithstanding the advancements achieved in the manufacturing and distribution of aquatic products and their byproducts within our nation, the assortment of novel seafood products produced and supplied by processing machines and plants has fallen short of expectations. As a consequence of this circumstance, a multitude of products that can be manufactured and prepared from aquatic organisms are currently unavailable. Presently, scientific and nutritional experts concur that consuming fish as a nutritious food item serves as a preventative measure against numerous illnesses and a viable treatment for certain conditions; thus, those responsible for nutrition and production are concerned with ensuring that it is accessible and included in household food baskets (Safi Yari et al., 2005). The silver carp is referred to as Phytophagous or Tolstolobic, and the market is erroneously acquainted with the brand name for farmed salmon. This fish has a spherical head adorned with minute scales (Adeli, 2005). These are lengthy gill blades. Its diet consists of 1.5 centimeters of algae (Vosoughi and Mostajir, 2002). It initially consumes zooplankton and phytoplankton, but eventually transitions to phytoplankton. It possesses a long, silver body and a lower mouth. Asgari (2009) reports that within our nation, one-row pharyngeal teeth with the formula 4-4 are observed in animals weighing up to 14 kg. Its maximum feeding capacity is 17% of its total weight, which is 20 kg. Spawning occurs in water that is stagnant. The mean quantity of eggs falls within the range of 467 to 542 thousand. Their optimal laying temperature is between 21 and 23 degrees. The incubation period for the 0.7-1 mm in diameter eggs is 1.5 days. Approximately 50 to 85 percent of co-cultivation consists of this fish. Differentiating it from the stubborn carp are its small head, full keel, and lack of connection between the pectoral fin and ventral fin. The public considers this fish to be the finest-farmed due to its delectable, fatty, and skeletal flesh (Adeli, 2005).

Understanding the chemical makeup of fish flesh is currently critical. Because, based on the information at hand (including the proportions of water, protein, fat, starch, minerals, and vitamins in fish meat), the optimal processing method can be determined. Without a doubt, the primary distinction in the chemical composition of fish ought to be associated with the nutrition or food sources they consume. As a result, once the fish have adequate access to food, the relative increase in muscle protein is

followed by a rapid increase in fat. On the contrary, when fish encounter food scarcity during spawning or migration, protein, and fat reductions result in gradual alterations to the chemical composition of muscles (Moeini, 2001).

Although the typical shelf life of fish is one to two days, long-term preservation of the product's quality is possible in the refrigerator. According to Vidya et al. (1996), freezing is the most critical method for storing seafood. The quality and shelf life of frozen fish will vary significantly by the end of the storage period due to biological differences, methods of capture, and pre-freezing preparation. Both the final quality and the shelf life of the product in cold storage will undoubtedly fall short of expectations if the initial quality of the fish is unsaturated. Furthermore, the level of attention and precision that is maintained while the product is stored in the refrigerator will significantly influence its ultimate quality. By forming ice crystals, for instance, the concentration of salt and organic compounds in the liquid phase is increased when fish is frozen. Consequently, the destruction of denatured and dehydrated muscle proteins or cell membranes may occur (Aubourge and Medina, 1999).

Nonetheless, the ongoing oxidation and hydrolysis of fish fat result in undesired alterations during the freezing phase, consequently diminishing the product's quality. The best shelf life was determined and the qualitative and chemical index, as well as the measurement and comparison of body chemical compounds, in fresh and vacuumed tissue of phytophagous fish (*Hypophthalmichthys molitrix*), were assessed in this study.

2. Materials and Methods

2.1. Preparation and processing of phytophagous fish

Under vacuum conditions, fish were frozen at -18 °C for five months using the vacuum packaging method in this study. A total of eighteen phytophagous fish samples, each weighing 1.5 kg, were acquired. When selecting, every effort is made to ensure that the fish have a consistent length and weight range and lack any abnormal appearance signs. The heads and fins were removed from the fish subsequent to their weighing, while their stomachs were emptied. From the captured samples, 100 g fillets were prepared under ultra-hygienic conditions; 33 packages of 100 g fish fillets were produced.

Subsequently, under vacuum conditions and in the presence of ice, these fillets were transferred to the packing center of Kaleh Amol Company for packaging. These fillets were assessed at three different time points throughout the day and at -18 °C for 1, 2, 3, and 4.5 months in three replicates. Initially, the chemical composition of fresh phytophagous fish was assessed on day zero. Subsequently, fillet packages were examined on day zero and throughout months 1, 2, 3, 4, and 5 in order to determine the peroxide and TVB-N spoilage indices.

2.2. Vacuum packaging (VP)

A vacuum pump-equipped EBOR vacuum packing machine was utilized for this objective. Following the establishment of a steady-state condition in the apparatus

using the control panel and the provided program, the pressure of the adjusting gas within the package was evacuated for a duration of 1.5 seconds, resulting in the creation of a vacuum within the package. The plastic bag lid was subsequently sealed using heat stitching at a stitching temperature of 250 °C and coated with polyamide.

2.3. Protein measurement

Protein extraction is done in three steps.

2.3.1. Digestion stage

Pour 1 gram of the weighed sample into the digestion tube to begin. Subsequently, incorporate 18 ccs of 95% sulfuric acid, followed by 10 g of potassium sulfate, 0.3 g of titanium dioxide, and 0.3 g of copper sulfate. The digestion step was then initiated by placing the tube in the digester and setting its temperature to between 300 and 100 degrees Celsius for three to five hours.

2.3.2. Distillation step

Subsequently, the digested sample is combined with 20 cc of distilled water and positioned on the left side of the Kajeldal distillation apparatus. On the right side of the Kajeldal distiller, combine 3-5 drops of methylene red as a reagent with 40 cc of 2% boric acid containing a human subject. Activate the device and configure the settings to set the gain to 80 cc; the amount is then appended automatically to the pipe on the left. After a five-minute period of operation, this device will shut down automatically. As the distillation process nears its culmination, nitrogen is introduced into the beaker via the tube.

2.3.3. Titration stage

As a titrant at this stage, 0.1 N sulfuric acid is utilized. The aforementioned substance is introduced into the distillation material to induce a color transformation from yellow to purple. For the percentage of protein in the carcass, multiply the quantity of sulfuric acid consumed by 0.875.

2.4. Measurement of fat and peroxide

- A. Determination and measurement of fat by Kinsella et al. (1971), which is expressed in grams per hundred grams of muscle.
- B. Determination and measurement of peroxide number (PV) by Malaysian palm oil method (Johnson et al., 1995).

2.5. Chemical experiments

Following the meat grinder's grinding of the sample, 50 g of it was simultaneously mixed with 50 cc chloroform and 100 cc methanol at high speed for three minutes using an electric mixer. Following the addition of an additional 50 cc of chloroform, the mixture was stirred for 30 to 60 seconds. Following this, 50 cc of distilled water was added and the mixture was stirred at high speed for an additional 30 to 60 seconds.

After transferring the mixture to a decanter and observing the oil separate from the meat, the water was

positioned at the top of the pan, the meat was positioned in the middle, and the oil containing the solvent was located at the bottom. By agitating a metal or glass rod subtly within the mixture, one can accelerate the oil's accumulation at the container's base. The oil and solvent can be introduced into the glass balloon for the Rotavipur device using a funnel lined with filter paper, albeit with a degree of perseverance. Notably, the glass balloon ought to have undergone prior weighing.

Following this, the balloon is linked to the Rotavipur device. The temperature of the water is adjusted between 50 and 60 °C. Once the vacuum pump is activated, the solvent and oil are separated. The amount of oil that remains in the balloon is determined by reweighing it and calculating the difference in weight from its empty weight. The quantity of fat that was extracted was measured in grams per gram of moist muscle.

2.6. Measurement of peroxide (PV) number

Utilizing 3 grams of extracted oil in a 250 ml laboratory container, this procedure was carried out. In this container, 10 ml of a chloroform and acetic acid solution was added while stirring. Following the addition of one milliliter of saturated potassium iodide solution, the mixture was left in the dark for five minutes. Following this, 20 ml of distilled water was added while stirring the mixture. 1% normal sodium thiosulfate was then used to titrate the mixture until the yellow hue disappeared. Following the addition of 1 ml of a 1.5% starch solution to the mixture, titration was maintained until the absence of the dark blue hue was observed. The control sample was conducted in a comparable manner, with the exception that the mixture did not contain any oil. Using the following formula, the quantity of peroxide in mill equivalents per 1000 g of fat (fat kg / meqO₂) was determined:

$$100 (V_1 - V_2) N / W$$

V₁ represents the quantity of sodium thiosulfate, while N denotes the standard deviation in milliliters. The volume of sodium thiosulfate utilized for the control test is denoted by V₂. The sample's weight (g) is denoted by W. The normality of sodium thiosulfate intake is denoted by N.

2.7. TVB-N Assessment

In order to quantify TVB-N, 5 g of the sample must be separated, followed by the addition of 1.5 g of copper oxide. We subjected it to five minutes in the distillation machine. The quantity of Caustic soda is currently zero. Along with a few drops of Methyl Red (MR) Reagent, incorporate 40 cc. Titration is conducted using a 0.1 N sodium thiosulfate solution at this juncture. The quantity of TVB-N present in 5 g of sample meat is determined by multiplying the volume of acid consumed by 28 by the color change of the aforementioned solution.

2.8. Humidity measurement

After preparing a watch glass that is both clean and dry, 5 grams of minced fish meat are inserted into the watch glass after being separated with a scalpel. As soon as the watch glass containing the meat is weighed and placed in the oven, preheat it to 100 °C for three to five hours. Once

the fish meat has dried for the designated duration, each container containing the samples is assessed for weight. Subsequently, the secondary weight is subtracted from the initial weight. By performing this operation, the moisture content of 5 grams of fish meat is determined. The moisture content of the fish meat can be determined by dividing the weight of the moisture obtained by the weight of the raw sample, which is 5 grams.

2.9. Ash Assessment

The crucible is initially weighed, and the resultant value is documented. Following this, 2 grams of fish mince are weighed and added to the crucible. Following the completion of the initial smoking or burning process, the specimen is subsequently heated in an electric oven. The sample is heated to 550 degrees Celsius for two to five hours, or until it turns gray. Following the completion of the crucible's cooling process and the completion of the machine time, the sample was re-weighed. The value derived from the empty crucible's weight was subtracted, thereby yielding the quantity of ash. The quantity of ash present in the fish meat can be determined by dividing the weight of the resulting ash by the weight of the raw sample, which is 2 grams.

2.10. Statistical analysis

SPSS16 and Excel 2007 were utilized for the generation of graphs and statistical analyses, respectively. Furthermore, the acquired data are subjected to statistical analysis using factorial method 2.6 (GLM) and Duncan's mean comparison test at the 0.05 significance level.

3. Results

3.1. Fat tests

Table 1 presents the fat content of phytophagous fish fillets that were stored at -18 °C under standard packing conditions on various days of the experiment. The mean fat contents of phytophagous fish fillets, which were stored at -18 °C for various days of the experiment, were as follows: 3.90 g, 3.54 g, 3.15 g, 2.80 g, 2.26 g, and 2.00 g. Table 2 presents the fat content of phytophagous fish fillets that were vacuum-packed and stored at -18 °C on various days throughout the experiment. On various days of the experiment, the mean fat content of rainbow trout fillets stored at -18 °C was as follows: 3.91 grams, 3.71 grams, 3.47 grams, 3.12 grams, 2.88 grams, and 2.79 grams, respectively.

Table 1. Measurement of fat content in phytophage fish fillets kept under usual packaging at -18 °C on different days of the experiment

Day	0	30	60	90	120	150
Average	3.903	3.545	3.151	2.802	2.260	2.008
Standard deviation	0.044	0.131	0.088	0.181	0.235	0.097
Minimum	3.869	3.415	3.051	2.615	2.219	1.899
Maximum	3.954	3.677	3.218	2.978	2.514	2.040
Number of repetitions	3	3	3	3	3	3

Table 2. The amount of fat measured in phytophage fish fillets stored in vacuum packaging at -18 °C on different days of the experiment

Day	0	30	60	90	120	150
Average (g)	3.918	3.713	3.474	3.120	2.888	2.79
Standard deviation	0.047	0.062	0.109	0.145	0.121	0.229
Minimum (g)	3.864	3.641	3.389	2.999	2.745	2.541
Maximum (g)	3.951	3.750	3.598	3.282	2.969	2.986
Number of repetitions	3	3	3	3	3	3

Table 3. Comparison of the average fat content of phytophagous fish fillets in different treatments

Package type	Day	Number	Average cStandard daviation
Vacuum	0	3	3.9183±0.04737 ^a
	30	3	3.7130±0.06236 ^{ab}
	60	3	3.4743±0.010965 ^b
	90	3	3.1207±0.14561 ^{cd}
	120	3	2.8880±0.12421 ^{de}
	150	3	2.8880±0.12421 ^{de}
No Vacuum	0	3	3.9030±0.04498 ^a
	30	3	3.5457±0.13100 ^b
	60	3	3.1510±0.08826 ^c
	90	3	2.8023±0.18178 ^e
	120	3	2.2603±0.23573 ^f
	150	3	2.0083±0.09744 ^g

* Similar letters do not differ significantly (P = 0.05)

Using Duncan's multiple amplitude method, it was possible to ascertain which day of the experiment exhibited a significantly different fat content compared to the others. A statistical analysis of means revealed that the treatments assigned to distinct groups differed significantly from one another, whereas the treatments assigned to the same group did not differ significantly. Table 4 presents the outcomes of the average fat comparison test conducted at -18 °C. The

data presented in Tables 3 and 4 indicates that there are notable variations in fat quantities across different days. Furthermore, as shown in the tables, the positive impact of packaging on these values is quite apparent

Figure 1 illustrates a comparison of the mean fat contents of phytophagous fish fillets stored at -18 °C. The process of reducing the fat content of vacuum-packed fillets and conventionally packaged fillets is illustrated in

this diagram. Furthermore, it was observed that the fat content of phytophagous fish fillets diminishes with time in both packaging types. However, with the exception of the initial day of the experiment, the vacuum-packed samples exhibited a higher fat content than the control samples on all other days. As time progresses, the rate of fat reduction in samples packaged conventionally is greater than that of samples packed vacuum-style.

Table 5 presents the protein content of phytophagous fish fillets that were stored at -18 °C under standard

packaging conditions on various days throughout the experiment. On various days of the experiment, the mean protein content of phytophage fish fillets stored at -18 °C was as follows: 20.1, 18.4, 16.27, 15.14, 14.14, and 13.87%, respectively. Additionally, the protein content of phytophagous fish fillets preserved in vacuum packaging is illustrated in Figure 2. On various days of the experiment, the mean protein content of phytophage fish fillets stored at -18 °C was calculated to be 20.76, 20.03, 18.59, 17.69, 16.84, and 15.47%, respectively.

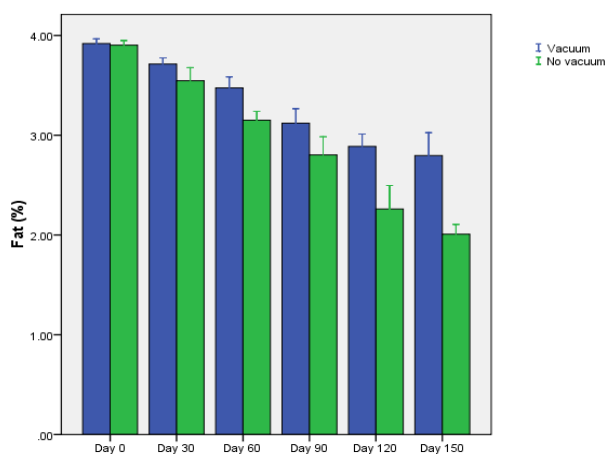


Figure 1. Comparison of the amount of fat in phytophagous fish fillets kept at -18 degrees on different days

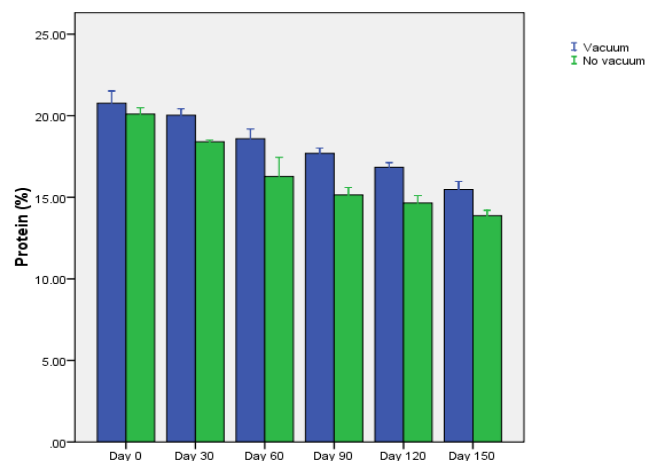


Figure 2. Amount of protein measured in phytophagous fish fillets stored in vacuum packaging

Table 4. The result of the comparison test of the average fat content of phytophagous fish fillets kept at -18 °C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
150 Normal	3	2.008						
120 Normal	3		2.260					
150 Vacum	3			2.796				
90 Normal	3			2.802				
120 Vacum	3			2.888	2.888			
90 Vacum	3				3.120	3.120		
60 Normal	3					3.151		
60 Vacum	3						3.474	
30 Normal	3						3.545	
30 Vacum	3						3.713	3.713
0 Normal	3							3.903
0 Vacum	3							3.918

The results of a substantial analysis of protein content across various days and packaging conditions revealed that the treatment means of the various groups differed significantly. There is minimal variation among the means comprising a group with respect to the degree of significance. (0.05 P-value) (Table 6). Significant variations in protein concentrations across different days are evident in Tables 6 and 7, suggesting that vacuum packing has contributed positively to these fluctuations.

Figure 2 further illustrates the trend of this disparity. As time has passed, the protein content of phytophagous fish fillets has decreased, as indicated by data on both types of packaging. Additionally, with the exception of the initial day of the experiment, the vacuum-packed samples contain a greater quantity of protein than the control samples on all other days. The rate of protein reduction in conventionally packaged samples is greater than that in vacuum-packed samples over time.

Table 5. The amount of protein measured in phytophagous fish fillets kept under conventional packaging conditions at -18 °C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	20.103	18.408	16.274	15.146	14.646	13.877
Standard deviation	0.381	0.104	1.77	0.452	0.460	0.319
Minimum (%)	19.718	18.475	13.312	14.814	14.125	13.540
Maximum (%)	20.481	18.175	17.587	15.622	15.001	14.175
Number of repetitions	3	3	3	3	3	3

Table 6. The amount of protein measured in fish fillets stored under vacuum packaging at -18 °C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	20.764	20.033	18.592	17.692	16.842	15.475
Standard deviation	0.753	0.395	0.596	0.333	0.284	0.499
Minimum (%)	19.955	19.712	17.914	17.325	16.514	15.008
Maximum (%)	21.446	20.475	19.037	17.937	17.011	16.001
Number of repetitions	3	3	3	3	3	3

Table 7. Comparison of mean values of phytophagous fish fillet protein in different treatments

Package type	Day	Number	Average ±Standard deviation
Vacuum	0	3	20.7643 ±0.75365 ^a
	30	3	20.0330 ± 0.39563 ^a
	60	3	18.5920 ± 0.59666 ^b
	90	3	17.6920 ± 0.32372 ^{bc}
	120	3	16.8420 ± 0.28410 ^{cd}
	150	3	15.4757 ± 0.49901 ^{ef}
No Vacuum	0	3	20.1037 ± 0.38157 ^a
	30	3	18.4080 ± 0.10499 ^b
	60	3	16.27476 ± 1.17712 ^{de}
	90	3	15.460 ± 0.45295 ^f
	120	3	14.6460 ± 0.46099 ^{fg}
	150	3	13.8773 ± 0.31329 ^g

* Similar letters do not differ significantly (P < 0.05)

Table 8. The result of the comparison test of the average phytophagous fish fillet protein stored at -18 °C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
150 Normal	3	13.877						
120 Normal	3	14.646	14.646					
150 Vacuum	3		15.146					
90 Normal	3		15.475	15.475				
120 Vacuum	3			16.276	16.274			
90 Vacuum	3				16.842	16.842		
60 Normal	3					17.692	17.692	
60 Vacuum	3						18.408	
30 Normal	3						18.592	
30 Vacuum	3							20.033
0 Normal	3							20.103
0 Vacuum	3							20.764

Tables 9 and 10 detail the quantities of ash that were quantified in phytophagous fish fillets that were vacuum-sealed and stored at -18 °C under standard conditions on various days of the experiment. An examination of the mean ash values across various treatments (Table 11)

revealed that there is a statistically significant difference in the means of the treatments in the different groups. A further observation was that there was no significant difference in the means of the groups within a given group (P = 0.05).

Table 9. The amount of ash measured in fish fillets kept under normal packaging conditions at -18 °C on different days of the experiment

Day	0	30	60	90	120	150
Average	2.366	1.683	1.433	1.150	0.816	0.041
Standard deviation	0.057	0.028	0.028	0.132	0.104	0.076
Minimum	2.21	1.65	1.40	1	0.7	0.45
Maximum	2.4	1.7	1.45	1.25	0.9	0.5
Number of repetitions	3	3	3	3	3	3

Table 10. Measure ash content in phytophage fish fillets stored in vacuum packing conditions at -18 °C on different experiment days

Day	0	30	60	90	120	150
Average (%)	2.266	1.966	1.733	1.300	0.950	0.583
Standard deviation	0.189	0.076	0.246	0.132	1.00	0.104
Minimum (%)	2.05	2.05	1.45	1.2	0.85	0.5
Maximum (%)	2.4	1.9	1.9	1.45	1.05	0.7
Number of repetitions	3	3	3	3	3	3

As indicated by the data presented in Tables 11 and 12, as well as the explanations provided regarding the method for determining whether a particular factor's influence on the number of factor changes (ash) is significant and evident, the quantity of ash has decreased considerably. As

indicated by data from both packaging types, the ash content of fillets of phytophagous fish diminishes with time. With the exception of the initial day of the experiment, the vacuum-packed specimens exhibit a greater concentration of ash on all other days compared to

the conventional specimens. Ash reduction in vacuum-packed samples is slower than the rate of ash reduction in conventional packing ash over time.

3.3. Humidity

Tables 13 and 14 detail the moisture content of phytophagous fish fillets that were vacuum-sealed and routinely packaged at -18 °C on various days of the experiment. An examination of the mean humidity values across various treatments (Tables 15 and 16) reveals a statistically significant difference in the means of

treatments within distinct groups. A further observation was that there was no significant difference in the means of the groups within a given group (P = 0.05). The moisture content of phytophagous fish fillets diminishes with time in both packaging types. With the exception of the initial day of the experiment, the humidity level of the vacuum-packed specimens is consistently higher than that of the conventional specimens on all other days. The rate of moisture depletion is greater for samples packaged vacuum-packed than for those packaged in conventional packaging over time.

Table 11. Comparison of mean values of phytophagous fish fillet ash in different treatments

Package type	Day	Number	Average ±Standard deviation
Vacuum	0	3	2.2667 ± 0.18930 ^a
	30	3	1.9667 ± 0.07638 ^b
	60	3	1.7333 ± 0.24664 ^c
	90	3	1.3000 ± 0.13229 ^{de}
	120	3	0.9500 ± 0.1000 ^{fg}
	150	3	0.5833 ± 0.10408 ^h
No Vacuum	0	3	1.3000 ± 0.13229 ^{de}
	30	3	1.6833 ± 0.02887 ^c
	60	3	1.4333 ± 0.02887 ^d
	90	3	1.1500 ± 0.13229 ^{ef}
	120	3	0.8167 ± 0.10407 ^g
	150	3	0.4167 ± 0.07638 ^h

* Similar letters do not differ significantly (P <0.05).

Table 12. The result of the comparison test of the average phytophagous fish fillet ash kept at -18° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
150 Normal	3	0.416							
120 Normal	3	0.583							
150Vacum	3		0.816						
90 Normal	3		0.950						
120Vacum	3			1.150	1.150				
90 Vacum	3			1.300		1.300			
60Normal	3					1.433			
60Vacum	3						1.683		
30Normal	3						1.733		
30Vacum	3							1.966	
0Normal	3								2.266
0Vacum	3								2.366

Table 13. The amount of moisture measured in phytophagous fish fillets kept under normal packaging conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	77.686	66.333	58.640	54.993	52.306	46.233
Standard deviation	2.200	1.990	2.053	2.518	6.984	2.366
Minimum (%)	75.48	46.36	56.28	52.72	45.08	43.72
Maximum (%)	79.88	68.34	60.02	57.7	52.82	48.42
Number of repetitions	3	3	3	3	3	3

Table 14- The amount of moisture measured in phytophagous fish fillets stored in vacuum packing conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	77.593	69.460	62.993	58.833	56.753	51.353
Standard deviation	0.650	2.862	1.678	2.198	2.078	2.839
Minimum (%)	76.94	66.88	61.12	56.30	54.90	48.26
Maximum (%)	78.24	72.54	64.36	60.24	59.00	51.96
Number of repetitions	3	3	3	3	3	3

3.4. TVB-N Assessment Results

The quantities of TVB-N ascertained in phytophagous fish fillets that were preserved under standard vacuum

conditions at -18 °C on various days of the investigation are detailed in Tables 17 and 18.

A statistically significant distinction was observed in the

mean values of TVB-N across various treatments, as indicated by Tables 19 and 20. These treatments were categorized into distinct groups. Over time, the quantity of TVB-N phytophagous fish fillets increased in both packaging types. On all other days, with the exception of

the initial day of testing, vacuum-packed samples contain a reduced quantity of TVB-N compared to standard TVB-N. The rate of increase in TVB-N for samples packaged conventionally is greater than that for samples packed vacuum-packed

Table 15. Comparison of average moisture values of phytophagous fish fillets in different treatments

Package type	Day	Number	Average ±Standard deviation
vacuum	0	3	77.5933± 0.65003 ^a
	30	3	69.4600± 2.8694 ^b
	60	3	62.933± 1.67837 ^{cd}
	90	3	58.8333± 2.19839 ^{de}
	120	3	56.7533± 2.07811 ^{ef}
	150	3	51.3533± 2.83904 ^g
No vacuum	0	3	77.6867± 2.2003 ^a
	30	3	66.3333± 1.99021 ^{bc}
	60	3	58.6400± 2.05358 ^{de}
	90	3	54.9933± 2.5181 ^{efg}
	120	3	52.3067± 6.98416 ^{fg}
	150	3	46.2333± 2.36697 ^h

* Similar letters do not differ significantly (P<0.05)

Table 16. The result of the comparison test of the average moisture content of phytophagous fish fillets kept at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
150 Normal	3	46.233							
120 Normal	3		51.353						
150Vacum	3		52.306	52.306					
90 Normal	3		54.993	54.993	54.993				
120Vacum	3			56.753	56.753				
90 Vacum	3				58.640	58.640			
60Normal	3				58.833	58.833			
60Vacum	3					62.993	62.993		
30Normal	3						66.333	66.333	
30Vacum	3							69.460	
0Normal	3								77.593
0Vacum	3								77.686

Table 17. The amount of TVB-N measured in phytophagous fish fillets kept under normal packaging conditions at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	6.533	26.133	27.066	30.800	33.600	38.266
Standard deviation	1.616	1.616	4.277	2.800	2.800	1.616
Minimum (%)	5.60	25.20	22.40	28.00	30.80	39.20
Maximum (%)	8.40	28.00	30.80	33.60	36.40	36.40
Number of repetitions	3	3	3	3	3	3

Table 18- The amount of TVB-N measured in phytophagous fish fillets kept under normal packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	6.533	13.066	15.866	14.00	15.866	18.666
Standard deviation	1.616	1.616	1.616	28.00	1.616	1.616
Minimum (%)	5.60	11.20	14.00	11.20	14.00	16.8
Maximum (%)	19.60	16.80	16.80	16.80	14.00	8.40
Number of repetitions	3	3	3	3	3	3

Table 19. Comparison of average TVB-N values of phytophagous fish fillets

Package type	Day	Number	Average ±Standard deviation
vacuum	0	3	6.5333±1.61658 ^g
	30	3	13.0667± 1.61658 ^f
	60	3	15.8667± 1.61658 ^{ef}
	90	3	14.000± 2.8000 ^f
	120	3	15.8667± 1.61658 ^{ef}
	150	3	18.6667± 1.61658 ^e
No vacuum	0	3	6.5333±1.61658 ^g
	30	3	26.1333± 1.61658 ^d
	60	3	27.0667± 4.27707 ^{cd}
	90	3	30.800± 2.8000 ^{bc}
	120	3	33.6000± 2.8000 ^b
	150	3	38.2667±1.61658 ^a

* Similar letters do not differ significantly (P <0.05).

Table 20. The result of the comparison test of the average TVB-N of phytophagous fish fillets kept at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
150 Normal	3	6.533						
120 Normal	3	6.533						
150Vacum	3		13.066					
90 Normal	3		14.00					
120Vacum	3		15.566	15.566				
90 Vacum	3		15.866	15.866				
60Normal	3			18.666				
60Vacum	3				26.133			
30Normal	3				27.066	27.066		
30Vacum	3					30.800	30.800	
0Normal	3						33.600	
0Vacum	3							38.266

3.5. Peroxide assay results

The quantities of peroxide ascertained in phytophagous fish fillets that were vacuum-sealed and packaged as usual at -18 °C on various days of the experiment are detailed in Tables 21 and 22. An examination of the average peroxide levels across various treatments revealed a statistically significant difference in the means of treatments between groups (Table 23). The peroxide content of phytophagous

fish fillets increases with time in both packaging types. With the exception of the initial day of testing, the peroxide concentration in the vacuum-packed samples is comparatively lower than that of the standard samples on all other days. The rate of increase in peroxide concentration in conventional packaging is found to be greater than that in vacuum packaging over time (Table 24).

Table 21. Measurement of peroxide measured in phytophagous fish fillets kept under usual packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	1.966	33.330	28.886	39.996	38.883	47.773
Standard deviation	0.611	3.330	1.928	3.335	3.850	1.928
Minimum (%)	1.66	30.00	26.66	36.66	36.66	46.66
Maximum (%)	2.06	36.66	30.00	40.00	43.33	50.00
Number of repetitions	3	3	3	3	3	3

Table 22. Measurement of peroxide measured in phytophagous fish fillets stored in vacuum packaging at -18 ° C on different days of the experiment

Day	0	30	60	90	120	150
Average (%)	1.693	33.220	24.440	34.440	32.220	44.440
Standard deviation	0.542	3.845	1.922	1.922	3.845	1.922
Minimum (%)	1.45	30.00	23.33	33.33	30.00	43.33
Maximum (%)	1.86	36.66	26.66	36.66	36.66	46.66
Number of repetitions	3	3	3	3	3	3

Table 23. Comparison of average values of phytophage fish fillet peroxide in different treatments

Package type	Day	Number	Average ±Standard deviation
Vacuum	0	3	1.6933± 0.54271 ^h
	30	3	32.2200± 3.84515 ^{ef}
	60	3	24.4400± 1.92258 ^g
	90	3	34.4400± 1.92258 ^{de}
	120	3	32.2200± 3.84515 ^{ef}
	150	3	44.4400± 1.92258 ^{ab}
No Vacuum	0	3	1.9667± 0.61199 ^h
	30	3	33.3300± 3.3300 ^{ef}
	60	3	28.8867± 3.33000 ^{fg}
	90	3	39.3367± 3.33500 ^c
	120	3	38.8333± 3.85093 ^{cd}
	150	3	47.7733± 1.92835 ^a

* Similar letters do not differ significantly (P <0.05).

Table 24. The result of the comparison test of the average phytophage fish fillet peroxide stored at -18 ° C

Day	Repetitions	Group 1	Group 2	Group 3	Group 4	group5	Group 6	Group 7	Repetitions
150 Normal	3	1.693							
120 Normal	3	1.966							
150Vacum	3		24.440						
90 Normal	3		28.886	28.886					
120Vacum	3			32.220	32.220				
90 Vacum	3			32.220	32.220				
60Normal	3			33.330	33.330				
60Vacum	3				34.440	34.440			
30Normal	3					38.883	38.883		
30Vacum	3						39.996	39.996	
0Normal	3							44.440	44.440
0Vacum	3								47.773

4. Discussion

Different results were obtained when body chemical compounds, peroxide, and TVB-N in phytophage fish fillets were subjected to vacuum packaging for five months or 150 days at freezing temperature (-18 °C). We discuss the results of various studies on other compounds and species in order to facilitate comparisons with the present study's findings.

All the variables assessed in this investigation exhibited substantial variations, with a discernible contrast in the rate of change between the two packaging types (refer to the results). Significantly slower trend changes were observed in the packing factors of samples packed under vacuum conditions compared to the packing factors under standard operating conditions. Furthermore, the majority of the assessed factors were determined for samples that were vacuum-packed during the higher months, whereas the initial month's values were derived from samples that were packed according to standard procedure.

The aforementioned data indicates that there was a decline in the outcomes of chemical compound measurements, including those for fat, protein, moisture, and ash, throughout the specified time period. It is worth mentioning that vacuum packaging played a substantial and beneficial role in decelerating the progression of these alterations, while also ensuring the samples remained intact in terms of quality for an extended duration.

Storage time is dependent on variables including freezing status, type of fish, and storage conditions, as demonstrated by Ben et al. (2009). Additionally, these variables have a substantial impact on the chemical composition of the body and spoilage samples. The aforementioned findings align with the results reported in this study. They indicate that the quality of the product degrades as its shelf life increases, and that all other factors undergo substantial changes. Additionally, it is worth noting that under vacuum conditions, the trend of these changes will be diminished. This article alludes to the identical freezing storage method utilized in the aforementioned research; consequently, the outcomes of the two investigations are congruent and aligned.

According to a study conducted by Jorkesh in 2004 regarding the shelf life of Caspian white fish (*Rutilus frisii kutum*) packaged under vacuum conditions at 4 °C, all indicators exhibited viability for a maximum of nine days; thereafter, they progressively deviated from their intended standards. Furthermore, during the course of this study's

analyses, all the measured indicators deviated from their standard ranges gradually. However, this deviation was more pronounced in vacuum packages and corresponded to the outcome reported by Jorkesh (2004).

The effects of temperature, duration, and freezing storage on fat changes in *Clupeonella engrauliformis* were investigated by Rezaei (2003). The findings of his investigation revealed that the values of spoilage and peroxide characteristics are significantly influenced by temperature and storage time in the frozen state. Vacuum packaging and freezing significantly slowed the ascent of these characteristics in the current investigation; thus, the quantity of peroxide determined in the second month of vacuum-packed samples was equivalent to the quantity of peroxide determined in the first month of closed samples classified under standard operating conditions. These results indicate that vacuum packaging is more effective, and the sample exhibits a slight oscillation pattern that persists for a longer period of time.

The vacuum packaging technique was also applicable to peroxide and TVB-N. As indicated by the results, there was an increase in the values of these two factors. Once more, vacuum packaging played a significant role in retarding the rate of this increase, allowing the samples that were sealed using this method to reach their unauthorized limit for a longer duration. Consequently, they can be utilized for a longer period of time.

In his study, Falaki Moghadam (2012) examined the impact of vacuum packaging on fat peroxide alterations in fillets of Caspian white fish stored at temperatures of -4 and -18 degrees Celsius. The findings of this research demonstrated that the quantity of peroxide, as measured at temperatures of -4 and -18 degrees Celsius, exhibited a significant decrease over time when compared to the fresh fillet of Caspian whitefish. On day zero, the peroxide content was found to be zero. Undoubtedly, what was noteworthy was the favorable impact that vacuum packaging had on the augmentation of said factors. As a result, at -18 degrees Celsius, the samples that were vacuum-packed increased at a slower rate than the control samples and required a more extended period of time to reach the critical level of these attributes; this allowed for an extended storage period. Consistent with the findings of the present study are these results. The current investigation revealed a marginal increase in the quantity of this index in the vacuum, resulting in a rise in its value from 6.53 to 18.66. Consequently, the current study reveals a discernible upward trajectory in this quantity; under typical packaging

and conditions, this growth has escalated from 6.53 to 38.26.

The current investigation revealed that the range of changes in vacuum packaging during the 150-day duration was significantly reduced compared to the range of changes observed under typical circumstances. This amounted to \$26.13 within a single month, which would normally transpire. Additionally, the current investigation demonstrates the impact of vacuum packaging on said value.

Stamatis et al. (2007) conducted a quality assessment of *Scombercolias japonicas* fish using two methods: packaging in a vacuum at temperatures of 6 and 3 °C and exposure to a modified atmosphere (MAP). The findings indicated that samples subjected to the altered atmosphere exhibited a more rapid deterioration in quality when compared to samples maintained under vacuum conditions. The aforementioned study yielded findings pertaining to the impact of vacuum packaging on chemical compounds. They showed that the rate of change in samples packed in vacuum conditions was less than that of packaged samples under normal conditions, that the samples with longer shelf life have better quality, and that vacuum packaging has a positive role in the shelf life of samples. The findings presented here align with those documented in the study conducted by Stamatis et al. (2007).

5. Conclusion and final remarks

As indicated by the findings of this study and comparisons with other researchers, the storage duration of aquatic products is significantly impacted by the implementation of non-oxygen or vacuum conditions. In contrast, samples preserved under vacuum conditions exhibit reduced fluctuations across all factors when compared to samples preserved under standard conditions with oxygen present. Consequently, the quality of the products remains consistent for a more prolonged duration.

Given the findings of the research, the following recommendations may be put forth:

1. Given that augmenting the population, diversifying consumption patterns, and increasing the proportion of aquatic protein will effectively increase demand and consumption, it is imperative to prioritize the processing of various farmed and marine fish species.

2. Following production, various products derived from different fish (particularly phytophagous fish) should be stored at a low temperature and in suitable packaging (vacuum, vacuum packaging) to preserve their quality, value, and marketability. It is possible to produce a product of superior quality and durability by employing these procedures.

3. An investigation should be conducted into the impact of various packaging materials on alterations in body chemical compounds, peroxide, and TVB-N.

4. Prolonged exposure to environmental conditions for the storage of aquatic products should be avoided, and special provisions should be implemented to ensure the safekeeping of fish for extended periods of time.

Additionally, it is recommended that investigations concerning fluctuations in the chemical composition of the body, peroxide levels, and TVB-N be conducted at more frequent time intervals.

Reference

- Adeli, A. (2005). The role of aquatic packaging on the consumption behavior of households in Tehran. Unpublished doctoral seminar. Gorgan University of Agricultural Sciences and Natural Resources. [In Persian]
- Alasalvar, C. (2002). Seafoods: quality, technology and nutraceutical application an overview. In C. Alasalvar & T. Taylor (Eds.), *Seafoods-quality, technology and nutraceutical application* (pp. 1–5). Springer-Verlag Berlin Heidelberg.
- Ali, M., Iqbal, F., Salam, A., Iram, S., & Athar, M. (2005). Comparative study of body composition of different fish species from brackish water pond. *International Journal of Environmental Science and Technology*, 2, 229-232. doi: 10.1007/bf03325880
- Asgari, R. (2009). A review of systematic fisheries. Sarva Publications. [In Persian]
- Aubourge, S. P., & Medina, I. (1999). Influence of storage time and temperature on lipid deterioration during cod (*Gadus morhua*) and haddock (*Melanogrammus asglifimus*) frozen storage. *Journal of the Science of Food and Agriculture*, 79, 1943–1948.
- Ben-Smida, M. A., Marzouk, B., & El-Cafsi, M. (2009). The composition of fatty acids in the tissues of Tunisian swordfish (*Xiphias gladius*). *Food Chemistry*, 115, 522–528. doi:10.1016/j.foodchem.2008.12.084
- Etedali, M. (2009). Application of packaging in the country's fisheries industry (1st ed.). Naghsh Mehr Publications. [In Persian]
- Falaki-Moghaddam, H. (2012). Estimation of variance components for composite reproduction traits in moghani sheep using repeatability and random regression models. Master's thesis, Bu-Ali Sina University. [In Persian]
- FAO. (2004). FAO yearbook of fishery statistics 2004 (Vol. 1/2). Food and Agriculture Organization of the United Nations.
- Friedrich, M., & Stepanowska, K. (1999). Effect of diet composition the levels of glucose lipid lipoproteins of the blood on the chemical composition of two year-old carp (*Cyprinus carpio* L.) reared on cooling waters. *Acta Ichthyologica et Piscatorial*, 24, 1–24.
- Huss, H. H. (1994). Quality and quality changes in fresh fish (FAO Fisheries Technical Paper No. 348). Food and Agriculture Organization of the United Nations.
- Johnson, W. A., Nicholson, F. J., Roger, A., & Stroud, G. D. (1995). Freezing and refrigerated storage in fisheries (FAO Fisheries Technical Paper No. 340). Food and Agriculture Organization of the United Nations.
- Jorkesh, M. (2004). Investigation of increasing the shelf life of fresh fish using modified atmospheric packaging technique Master's thesis, Amirkabir University of Technology. [In Persian]

- Moeini, S., & Danesh Nooran, B. (2001). Produce cold marinade from word fish. *Iranian Journal of Natural Resources*, 54(1), 63–73. [In Persian]
- Piri, M., Nezami, S., & Ordag, V. (1998). The effects of diazinon, malathion, macheti and saturn toxins on juvenile mortality of whitefish. *Iranian Journal of Fisheries*, 7(4), 18–19. [In Persian]
- Rezaei, M. (2003). Effects of temperature and storage time on freezing in fat changes in Kilka Anchovy Doctoral dissertation, Tarbiat Modares University. [In Persian]
- Rouhani Moghaddam, B. (2004). Eating seafood is good for everyone. Fisheries information bases. Retrieved from www.shilat.com/persian/https://www.scribbr.com/category/apa-style/archive/page [In Persian]
- Safi Yari, S., & Moradi, G. (2005). Guide to producing value-added products from aquaculture. Safavis. [In Persian]
- Stamatis, N., & Arkoudelos, J. S. (2007). Effect of modified atmosphere and vacuum packaging on microbial, chemical and sensory quality indicators of fresh filleted *Sardina pilchardus* at 3 °C. *Journal of the Science of Food and Agriculture*, 87, 1164–1171. **doi:10.1002/jsfa.2858**
- Venugopal, V. (2006). Seafood processing: adding value through quick freezing, retortable packaging and cook-chilling. Taylor & Francis Group.
- Vidya Sagar Reddy, G., & Sriker, L. N. (1996). Effect of preprocess ice storage on the lipid change of Japanese threadfin bream mince during frozen storage. *Asian Fisheries Science*, 9, 109–114.
- Vosoughi, G., & Mostajir, B. (2002). Freshwater fish (5th ed.). University of Tehran Press. [In Persian]