Iranian Journal of Fisheries Sciences 18(4) 1083-1092 2019
DOI: 10.22092/ijfs.2019.118283

Influence of vacuum packaging and frozen storage time on fatty acids, amino acids and ω -3/ ω-6 ratio of rainbow trout (*Oncorhynchus mykiss*)

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Received: May 2016 Accepted: August 2017

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Keywords: Rainbow trout, Fatty acid, Amino acid, Freezing, Vacuum packaging.

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a native species of North America and Russia which has been widely farmed as a recreational and food fish around the world (Salem *et al*., 2010). Rapid growth rate, high nutritional value and tolerant to the wide range of environment and handling caused rainbow trout to be cultured for human consumption in many temperature regions like US, Europe, Chili, Japan, Australia and Iran (Celik *et al*., 2008). Fishery products are high in protein, essential minerals, polyunsaturated fatty acids (PUFA) and low in cholesterol content (Fallah *et al*., 2011).The n-3 and n-6 polyunsaturated fatty acids (PUFAs) have been shown positive effects on cardiovascular diseases and cancers (Ozden, 2005). Also, fish meat plays an important role in supplement of human protein necessity (Osibon *et al*., 2009). On the other hand, fish is an extremely perishable food and deterioration of fish begins immediately upon harvest, and continues to various degrees depending on storage conditions. Several methods are used to extend the shelf life of fish products such as salting, roasting, drying and freezing (Beklevik *et al*., 2005). Freezing is one of the common methods largely used to lessen many enzymatic and non-enzymatic processes leading to putrefaction of aquatic production (Erkan and Bilen, 2000). However, the fish component such as amino acids and unsaturated fatty acids changes during period of frozen storage, and influence of freezing on fish muscle proteins mainly concerns the changes in solubility of protein fraction, their water- holding capacity and the activity of proteolytic enzymes. Also the use of packaging technologies are important preservation of fish products against deteriorative effects which many include microbial, biochemical and physical activities from environmental

influences (Restuccia *et al*., 2010). One of the appropriate methods to access this goal is using Vacuum Packaging (VP) in order to control rancidity in oil and lipid containing food (Rostamzad *et al*., 2010). VP is a way for delaying lipid oxidation (Auto oxidation) because of limiting oxygen molecule and has managed to keep the product hygienic with a highly extended shelf life (Rajesh *et al*., 2002). In recent years, rainbow trout is a species with high commercial value and much appreciated by consumers and it is sold as either whole fresh fish or in fillet form. Additionally, frozen vacuum packaged rainbow trout are being consumed (Etemadi *et al*., 2013). Therefore, this present study was aimed to the study of effect of vacuum packaging on proximate composition, fatty acids and amino acids profile of rainbow trout fillet and its changes during 9 months storage at -18°C.

Materials and methods

Sample preparation: In total 30 fresh rainbow trout sample (350±4.3 g weight and 28.5cm length) were obtained from a local farm in Babol, Mazandaran province, Iran and were kept in ice inside a Styrofoam box. Then they sent to the laboratory and were immediately headed, eviscerated and filleted. Then fillets were divided into 2 groups: samples of the first group were left untreated (control) and directly packed in polyamide/polyethylene bags without using vacuum. The second group was packed under vacuum conditions in individual polyamide/polyethylene bags. Then all samples were immediately frozen at -30°C and kept in this temperature for 24 hours. Later all fish fillets were placed in -18°C freezer. For all fillets, analysis was carried out after the freezing process at zero time and after 1, 3, 6 and 9 months of storage at -18°C. For each kind of fillets, three different batches (n=3) were considered that were taken out randomly from frozen storage, thawed at 4°C overnight, then homogenized and subjected to analytical determination with triplication (n=3). Finally the results were subjected to statistical analysis.

Proximate composition

The moisture content in rainbow trout fillet was determined by drying to constant weight at 105°C for 24 hours according to AOAC standard method (2005). Crude ash was determined after heating the sample overnight at 550°C (AOAC, 2005). The crude protein content was determined as kjeldal-protein using a factor 6.26 following the AOAC standard method (2005). Fillets fat content was determined by the AOAC (2005). In all cases, results are expressed g 100g of muscle.

Fatty acids analysis

The total lipids were extracted by chloroform-methanol (1:1, v/v) and estimated gravimetrically (Bligh and Dyer, 1959). The fatty acids in the total lipids were esterifies into methyl esters by saponification with 0.5 N-methanolic NaOH and trances teriried with 14% BF3 in methanol. The fatty acid methyl esters were analysis on a Hetwlett Packard 6890 Gas

Chromatograph (GC) Equipped with a Flame Ionization Detector (FID). The esters were separated on a BPX-70 column (120 m×0.25mm). Column injector and detector temperatures were 285 and 320°C, respectively.

Amino acid analysis

Total amino acid composition was determined after acid hydrolysis in 6N HCl for 22h at 110°C. Chromatography analysis was carried out using an Agilent chromatograph L 1100 High Performance Liquid Chromatography (HPLC). Tryptophan was not determined in this study.

Statistical analysis

Nonparametric statistics used to analyze the data. Repeated measures analysis of variance used to compare between the groups and times. Repeated measures was made with General Line Models (GLM) with significant level of $p<0.05$. The Duncan´s multiple range tests with significant difference at $p<0.05$ used to compare sample means by using SPSS 16 software.

Results and discussion

Proximate composition

The results of proximate analysis and its changes of control and vacuum packed samples during 9 months storage at -18°C in rainbow trout fillet are giving in Tables 1 and 2. The results shown that moisture content of the fillets gradually reduced from 72.1±0.04 g $100g^{-1}$ to 71.7±0.021 g $100g^{-1}$ of muscle and 72.05±0.042 g $100g^{-1}$ to 71.0.063 g $100g^{-1}$ of muscle in control and VP samples, respectively after 9 months storage at -18 °C. There were significant difference in moisture content of control and VP during 9 months of frozen storage ($p<0.05$). Sabetian *et al.* (2012) and Popelka *et al.* (2014) were also have been recorded similar results for moisture contents of rainbow trout fillets after 10 and 6 months of frozen storage at -18 °C, respectively. The reduction of moisture contents during the frozen storage maybe explained by temperature uniformity, resulting slight difference between the vapor pressure of frozen product and air (Sabetian *et al*., 2012). In this study protein content was decreased significantly from 20.78±0.03 g $100g^{-1}$ to 18.12±0.13 g $100g^{-1}$ of muscle and 20.73±0.1 g $100g^{-1}$ to 18.59±0.02 g $100g^{-1}$ of muscle in control and VP samples after 9 months storage, respectively ($p<0.05$). Decrease in protein contents could be related to the proteins breakdown by enzymatic activities and production of volatile basic substances (Erkan and Belin, 2000). On the other hand, lipid content in rainbow trout fillet samples were steadily increased from 7.1±0.042 g $100g^{-1}$ to 7.18±0.134 g $100g^{-1}$ of muscle and 7.14±0.04 g $100g^{-1}$ to 7.25±0.049 g $100g^{-1}$ of muscle in control and VP samples at the end of experiment time at -18 °C, respectively ($p<0.05$). Theses finding are in accordance with those reported previously illustrated a converse correlation between the moisture and lipid contents of fish meat (Beklevik *et al*., 2005). According, the raise of lipid contents during the experimental time could be elucidated by decline in the

moisture contents causing an increase in lipid amount per 100 g^{-1} of rainbow trout fillet (Sabetian *et al*., 2012). Also, ash content was slowly increased from 2.27±0.12 g $100g^{-1}$ to 2.87±0.502 g $100g^{-1}$ of muscle and 2.3±0.12 g $100g^{-1}$ to 2.8±0.08 g $100g^{-1}$ of muscle during frozen storage in 9 months at -18°C, respectively (p<0.05). Increase of ash content could be explain by decreased of moisture content of the fillets as reported previously by Ozden (2005).

In general, one can see that the results for moisture, protein, lipid and ash content of rainbow trout fillet in this research were similar to the earlier finding of other researcher such as Rostamzad *et al*. (2010), Sabetian *et al*. (2012), Taheri and Motalebi (2012), Etemadi *et al*. (2013) and popelka *et al*. (2014). The slight differences could be related to the seasonal factor, geographical location, size and physiological conditions (Tzikas *et al*., 2007).

Table 1: Changes in proximate composition of control rainbow trout fillet (g $100g^{-1}$) stored at -18 °C for 9 months. Data are express as mean±SE.

Proximate composition	Initial	30 days	90 days	180 days	270 days
Moisture	72.1±0.04	72.06±0.04	72±0.03	71.99±0.007	71.7±0.02
Ash	2.27±0.1	2.37±0.05	2.47±0.04	2.48±0.08	2.87±0.5
Protein	20.78±0.03	20.48±0.03	18.78±0.19	18.46±0.12	18.12±0.134
Lipid	7.1±0.04	7.1±0.01	7.15±0.01	7.16±0.07	7.18±0.02

Table 2: Changes in proximate composition of vacuum packed rainbow trout fillet (g $100g^{-1}$) stored at -18°C for 9 months. Data are express as mean ±SE.

Proximate composition	Initial	30 days	90 days	180 days	270 days
Moisture	72.05±0.04	72±0.04	71.98±0	71.91±0.02	71.53±0.06
Ash	2.3±0.1	2.47±0.05	2.53±0.2	2.57±0.08	2.8±0.08
Protein	20.73±0.1	20.56±0.2	18.94±0.2	18.59±0.02	18.32±0.13
Lipid	7.14±0.04	7.18±0.02	7.2±0.03	7.21±0.04	7.25±0.04

Fatty acids analysis

Fatty acids composition of control and vacuum packed rainbow trout fillets and their changes during 9 months frozen storage are given in Tables 3 and 4. The finding indicated that value of saturated and unsaturated fatty acids have been changed over the experimental period. The results revealed that Monounsaturated fatty acids (MUFAs) were the more predominant in the total fatty acids followed by Saturated fatty acids (SFAs) and Polyunsaturated fatty acids (PUFAs) at the beginning of the experiment (MUFA>SFA>PUFA).

Stearic acid (C18:0), palmitic acid (C16:0), and arachidic acid (C20:0) were the most abundant SFAs in control and vacuum packed samples at the end of experiment time (270 days). Except for initial time and 30 days, significant differences were observed among the SFAs during frozen storage in control samples (p<0.05). In additional, all samples showed an increased SFAs value with increased storage time. So that, the ΣSFA increased from

29.51±0.2% to31.78±0.2% and 29.39±0.3% to 32.03±0.1% in control and vacuum packed samples of total fatty acids composition at the end of experimental period, respectively.

A decrease was observed in MUFAs value with increased storage period in both treatment (p<0.05). Oleic acid content in control (27.8%) and vacuum packed samples (27.9%) was higher than that of other monounsaturated fatty acids. Conversely, ΣMUFA reduced during freezing storage from 32.54±0.23% and 32.63±0.1% to 31.31±0.75% and 31.6±0.83% in control and vacuum packed samples after 9 months storage, respectively. Among n-6 PUFAs, the linoleic acid (C18:2) was also shown the final dominance. DHA (C22:6 n-3), linolenic acid (C18:3 n-3) and EPA (C20:5 n-3) were also dominated among n-3 PUFAs, respectively. There were significant differences among PUFAs contents during 270 days (p<0.05). All samples showed a decreased PUFAs value with increased storage time. Also, ΣPUFA decrease from 24.27±0.22 to 22.82±0.4 and 24.6±0.2 to 22.88±0.1 in control and vacuum packed samples after 9 months of storage, respectively. The decrease in the PUFAs concentrations is commonly attributable to oxidation and it is normally accepted that the oxidation rate increases dramatically with degree of unsaturation (Erdem *et al*., 2009). The PUFA/SFA ratio in rainbow trout fillets was less than 1 and the decrease of PUFAs, in contrast to SFAs, led to a significant decrease in this ratio (p<0.05). The PUFA/SFA ratio was steadily decreased from 0.82% and 0.83% to 71% and 71% in control and vacuum packed samples throughout the experiment, indicating the susceptibility of rainbow trout fillet to significant changes during the frozen storage.

The ω-3/ω-6 ratio is a better index in comparing relative nutritional value of fish oils of different species (Pirestani *et al*., 2010). The ω-3/ω-6 ratio of rainbow trout was shown in Table 3 and 4. A decrease in this ratio from 0.4 to 0.35 in control and 0.41 to 0.35 in vacuum packed samples showed that the nutritional value of this fish had declined during frozen storage.

Table 3: Changes in fatty acids profiles of control rainbow trout fillets during frozen storage up to 9 months in -18˚C (Means±SD (n = 3); p<0.05).

Fatty acids	Time of storage (Months)				
	0	1	3	6	9
C14:0	0.75±0.04	0.85±0	0.858±0.004	0.862±0.02	0.87±0.02
C16:0	11.6±0.18	11.8±0.007	11.85±0.03	11.93±0.02	11.96±0.01
C18:0	14.15±0.01	14.61±0.01	14.63±0.03	14.79±0.02	14.8±0.02
C20:0	2.3±0	2.68±0.09	2.74±0.04	2.83±0.03	2.86±0.03
C22:0	0.33±0.02	0.41±0.042	0.59±0.03	0.68±0.01	0.79±0.02
C24:0	0.4±0.02	0.41±0.02	0.47±0.04	0.58±0.04	0.6±0.04
SFAΣ	29.51±0.29	30.74±0.18	31.05±0.22	31.62±0.18	31.78±0.12
C14:1	0.17±0.02	0.17±0.03	0.15±0.01	0.12±0.02	0.13±0.01
C16:1	2.9±0.07	2.88±0.03	2.79±0.07	2.73±0.1	2.59±0.5
C18:1	27.850.04	27.79±0.01	27.78±0.03	27.66±0.04	27.2±0.2
C20:1	1.3±0.03	1.32±0.03	1.29±0.04	1.25±0.03	1.2±0.02
C22:1	0.17±0.05	0.16±0.02	0.15±0.01	0.1±0.01	0.09±0.01
C24:1	0.15±0.02	0.13±0.02	0.165±0.01	0.15±0.01	0.1±0.01

Table 3 continued:					
ΣMUFA	32.54±0.23	32.29±0.14	32.33±0.18	32.01±0.22	31.31±0.75
C18:2 n-6	16.47±0.04	16.44±0.04	16.43±0.03	16.41±0.03	16.3±0.07
C18:3 n-6	0.38±0.01	0.35±0.01	0.34±0.02	0.31±0	0.24±0.12
C20:4 n-6	0.37±0.05	0.34±0.04	0.315±0.21	0.3±0.03	0.28±0.04
C18:3 n-3	0.74±0.01	0.58±0.24	0.46±0.02	0.42±0.028	0.32±0.007
C20:5 n-3	0.59±0.03	0.52±0.02	0.48±0.05	0.42±0.021	0.4±0.01
C22:6 n-3	5.72±0.06	5.64±0.02	5.61±0.02	5.56±0.02	5.27±0.05
PUFAΣ	24.27±0.22	23.87±0.42	23.63±0.38	23.42±0.14	22.82±0.4
PUFA/SFA	0.82	0.77	0.76	0.74	0.71
Σ ω-3	7.05±0.1	6.74±0.4	6.55±0.1	6.4±0.07	5.99±0.07
Σ ω-6	17.22±0.11	17.13±0.13	17.08±0.2	17.02±0.07	16.82±0.32
ω-3/ω-6	0.4	0.39	0.38	0.37	0.35
EPA+DHA/C16	0.5	0.52	0.51	0.5	0.47
Σ SFA+PUFA	87.32±0.54	86.9±0.71	87±0.78	87.05±0.54	85.91±0.1

It is known that EPA and DHA have an essential role in the human diet to prevent diseases. A decrease were observed from 0.59 to 0.4 and 0.63 to 0.43 for EPA and 5.72 to 5.27 and 5.87 to 5.2 for DHA, in control and vacuum packed samples at the end of storage time, respectively. The losses of these products were probably related to autoxidation of lipids. These results are in agreement with other research which reported a decrease in the EPA and DHA levels during frozen storage in fish samples (Erdem *et al*., 2009). The EPA+DHA/C16:0 ratio is a good index to determine lipid oxidation (Ozden,2005). Although this ratio in control and vacuum packed samples were 0.5 and 0.51, it has been decreased to 0.47 in both experimental samples toward the end of frozen storage. The same result has been found for Japanese oyster (*Crassostera gigas*) and mackerel (*Scomberomorus commerson*) flesh (Ozden, 2005). The negative correlation between EPA+DHA/C16:0 ratio and storage time showed that oxidation mechanism and enzymatic breakdown have active process during frozen storage.

Table 4: Changes in fatty acids profiles of vacuum pack rainbow trout fillets during frozen storage up to 9 months in -18˚C(Means±SD (n=3); *p*<0.05).

Fatty acid	**Time of storage (Months)**				
	0	**1**	**3**	**6**	**9**
C14:0	0.753±0.04	0.862±0.001	0.865±0.002	0.867±0.03	0.868±0.002
C16:0	11.61±0.1	11.88±0.01	11.94±0.09	11.96±0.01	12±0
C18:0	14.05±0.01	14.46±0.2	14.63±0.2	14.74±0.01	14.8±0.07
C20:0	2.28±0	2.71±0.1	2.8±0.04	2.83±0.02	2.86±0.01
C22:0	0.33±0.02	0.41±0.042	0.59±0.03	0.68±0.01	0.79±0.02
C24:0	0.37±0.02	0.43±0.02	0.51±0.05	0.57±0.007	0.715±0.06
ΣSFA	29.39±0.3	30.75±0.46	31.33±0.43	31.65±0.1	32.03±0.17
C14:1	0.16±0.02	0.15±0.03	0.18±0.01	0.17±0.02	0.14±0.01
C16:1	2.95±0.02	2.94±0.02	2.8±0.11	2.78±0.12	2.5±0.05
C18:1	27.91±0.02	27.81±0.04	27.79±0.02	27.76±0.01	27.6±0.2
C20:1	1.32±0.03	1.31±0.06	1.3±0.04	1.27±0.03	1.19±0.02
C22:1	0.16±0.05	0.15±0.02	0.15±0.01	0.08±0.01	0.05±0.01
C24:1	0.13±0.02	0.14±0.02	0.16±0.01	0.15±0.01	0.12±0.01
ΣMUFA	32.63±0.17	32.5±0.19	32.38±0.21	32.21±0.21	31.6±0.32
C18:2 n-6	16.58±0.06	16.47±0.02	16.4±0.02	16.39±0.03	16.28±0.07
C18:3 n-6	0.435±0.02	0.355±0.04	0.33±0.02	0.31±0.04	0.3±0.04

Table 4 continued:

C20:4 n-6	0.360.01	0.32±0.03	0.31±0.02	0.3±0.02	0.27±0.01
C18:3 n-3	0.72±0.02	0.53±0.02	0.45±0.01	0.43±0.001	**0.32±0.007**
C20:5 n-3	0.63±0.77	0.52±0.02	0.52±0.04	0.46±0.02	**0.43±0.01**
C22:6 n-3	5.87±0.07	5.67±0.02	5.62±0.02	5.6±0.03	**5.2±0.05**
PUFAΣ	24.6±0.27	23.87±0.18	23.64±0.14	23.49±0.17	22.88±0.18
PUFA/SFA	0.83	0.77	0.75	0.74	0.71
Σ ω-3	7.22±0.3	6.72±0.07	6.59±0.07	6.49±0.06	6.02±0.07
Σ ω-6	17.37±0.09	17.15±0.1	17.05±0.07	17±0.11	16.85±0.1
ω-3/ω-6	0.41	0.39	0.38	0.38	0.35
EPA+DHA/C16	0.51	0.52	0.51	0.5	0.47
Σ SFA+PUFA	86.62±0.74	87.12±0.83	87.350.78	87.35±0.48	86.51±0.67

Amino acids analysis

Amino acids composition of rainbow trout fillets and their changes during 9 months frozen storage is given in Table 5 and 6. In this research Aspartic acid (2.9 mg g^{-1}) and Glutamic acid (2.86 mg g^{-1}) were the main amino acids of rainbow trout fillet in control and vacuum packed samples. These amino acids changes at the storage time. So that, they reduction to 1.8 mg g^{-1} and 2.6 mg g^{-1} in control and 1.7 mg g^{-1}. and 2.69 mg g^{-1} in vacuum packed samples, respectively. Iwasaki and Harada (1985) similarly reported that the main amino acids in fish muscles were aspartic acid, glutamic acid, and lysine. The most changes between amino acids were found in in Aspartic acid from 2.9 mg g^{-1} to 1.8 mg g^{-1} and 2.8 mg g^{-1} to 1.7 mg g^{-1} in control and vacuum packed samples at the storage time, respectively. The content of amino acids which contain Sulphur such as Methionine and Cysteine at the initial time were found 1.32 mg g^{-1} and 0.34 mg g^{-1} in control and 1.32 and 0.44 mg g^{-1} in vacuum packed samples, respectively, but they changes at the storage time. So that, they reduced to 0.9 mg g^{-1} and 0.15 mg g^{-1} in control and 0.9 mg g^{-1} and 0.14 mg g^{-1} in vacuum packed samples at the end of experimental time (270 days). In this study seventeen different amino acids were obtained in rainbow trout fillets, 10 essential amino acids and 7 nonessential amino acids were identified. The changes in amino acids contents in fish muscle were affected by spawning period and feeding psychology of fish and some parts of protein was used by fish for spawning (Iwasaki and Harada,1985). The ~~A~~mount of essential amino acids in rainbow trout fillets was observed higher than nonessential amino acids. Therefore, the essential amino acids / nonessential amino acids ratio (EAA/Non-EAA) of rainbow trout fillets was 1.06 and 1.12 in initial time, in control and vacuum packed, respectively. At the end of the experiment, EAA/Non-EAA ratio was increased from 1.06 to 1.24 and 1.12 to 1.22, in control and vacuum packed, respectively. In present study, the proportion of EAA/Non-EAA has shown a greater amount than other works: It was 0.78 for *Huso huso* ,0.77 for sea bream (*Pagrus major*), 0.77 for mackerel (*Scomber japonicas*), 0.71 for mullet (*Mugil cephalus*), 0.69 for sardine (*Sardine melonostica*), 0.74 for

herring (*Clupea pallasi*), 0.75 for chum salmon (*Oncorhynchus keta*) and 0.77 for pacific flounder (*Paralichths olivaceus*) (Iwasaki and Harada,1985).

Table 5: Changes in amino acids profiles of control rainbow trout fillets (mg g^{-1}) during frozen storage up to 9 months in -18°C (Means±SD (n = 3); $p<0.05$).

Amino acid	initial	30 days	90 days	180 days	270 days
Histidine	0.81±0.007	0.76±0.01	0.7±0.02	0.56±0.04	0.5±0.02
Isoleucine	1.84±0.01	1.82±0.01	1.8±0.02	1.54±0.07	1.52±0.07
Leucine	2.41±0.04	2.35±0.00	2.31±0.02	2.19±0.02	2.15±0.01
Lysine	2.31±0.02	2.17±0	2.13±0.02	1.48±0.03	1.41±0.01
Methionine	1.32±0.01	1.31±0.02	1.21±0.05	0.95±0.04	0.9±0.01
Phenylalanine	1.72±0.01	1.68±0.03	1.64±0.007	1.53±0.06	1.5±0.02
Threonine	0.93±0.02	0.92±0.04	0.87±0.02	0.81±0.2	0.71±0.04
Arginine	2.49±0.01	0.02±2.44	2.28±0.03	2.15±0.02	2.1±0
Valine	1.43±0.02	1.39±0.02	1.38±0.007	1.31±0.02	1.31±0.04
Tyrosine	1.43±0.03	1.35±0.02	1.22±0.01	1.14±0.03	1±0.02
Aspartic acid	2.9±0.03	2.83±0.03	1.93±0.02	1.91±0.04	1.82±0.04
Glycine	1.37±0.02	1.32±0.02	1.2±0.02	1.1±0.04	1±0.01
Proline	1.27±0.02	1.21±0.02	0.98±0.02	0.9±0.01	0.8±0.02
Serine	1.25±0.02	1.2±0.028	1.15±0.02	0.96±0.056	0.95±0.01
Cysteine	0.34±0.02	0.3±0.04	0.23±0.01	0.17±0.02	0.15±0.03
Glutamic acid	2.86±0.02	2.74±0.02	2.73±0.07	2.72±0.02	2.66±0.02
Alanine	1.86±0.01	1.8±0.04	1.66±0.03	1.57±0.02	1.55±0.01
EAAs*Σ	14.43	14.08	14.5	13.21	12.82
Non-EAAs**Σ	13.52	11.96	11.26	11.43	9.97
EAAs/Non-EAAs	1.06	1.17	1.28	1.15	1.24

Table 6: Changes in amino acids profiles of vacuum pack rainbow trout fillets (mg g^{-1}) during frozen storage up to 9 months in -18°C(Means±SD (n = 3); $p<0.05$).

Amino acid	initial	30 days	90 days	180 days	270 days
Histidine	0.875±0.02	0.77±0	0.75±0.01	0.59±0.04	0.49±0.02
Isoleucine	1.9±0.04	1.84±0.01	1.81±0.02	1.68±0.05	1.5±0.03
Leucine	2.47±0.02	2.37±0.01	2.36±0.02	2.21±0.06	2.12±0.01
Lysine	2.46±0.03	2.38±0.03	2.17±0	2.16±0.04	1.53±0.02
Methionine	1.32±0.04	1.31±0.02	1.21±0.056	0.95±0.04	0.9±0.04
Phenylalanine	1.81±0.04	1.67±0.01	1.66±0.02	1.64±0.02	1.5±0.02
Threonine	0.95±0.01	0.92±0.01	0.81±0.2	0.79±0.04	0.75±0.02
Arginine	2.4±0.05	0.03±2.38	2.34±0.01	2.135±0.03	2.13±0
Valine	1.55±0.01	1.39±0.02	1.35±0.02	1.34±0.03	1.31±0.04
Tyrosine	1.53±0.01	1.37±0.02	1.3±0.06	1.2±0.01	1.05±0.02
Aspartic acid	2.89±0.03	2.01±0.02	1.94±0.02	1.89±0.02	1.74±0.04
Glycine	1.48±0.04	1.31±0.04	1.25±0.02	1.17±0.01	1.06±0.01
Proline	1.3±0.09	1.18±0.04	1.08±0.05	0.86±0.01	0.85±0.02
Serine	1.25±0.04	1.23±0.01	1.22±0.02	1.07±0.05	0.97±0.01
Cysteine	0.44±0.04	0.28±0.01	0.28±0.1	0.25±0.04	0.145±0.03
Glutamic acid	2.94±0.02	2.77±0.02	2.72±0.01	2.72±0.02	2.69±0.02
Alanine	1.87±0.01	1.77±0.02	1.72±0.01	1.63±0	1.56±0.01
EAAs*Σ	15.82±0.23	15.08±0.16	14.55±0.35	13.58±0.39	12.23±0.2
Non-EAAs**Σ	14.01±0.36	12.12±0.23	11.69±0.25	10.87±0.18	10.10±0.21
EAAs/Non-EAAs	1.12	1.24	1.244	1.249	1.22

In this study the effects of packaging and storage time on the fatty acids and amino acids of rainbow trout fillets were examined. As a result of a frozen storage period up to 9 months, changes in SFAs, MUFAs, PUFAs, ω-3/ω-6 ratio and EAA/Non-EAA ratio were observed. So that, in fatty acids, SFAs was increased but MUFAs, PUFAs and, ω-3/ω-6 ratio were decreased.

Although, a protection effect on such fatty acids could be observed due to the vacuum packed samples. Therefore, the results showed that usage of vacuum packaging had a positive influence on delaying lipid oxidation and increasing shelf-life of rainbow trout fillets.

Accordingly, a vacuum packaging method can be considered as most suitable, especially if such a method includes properties, like easy availability, and low commercial cost.

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