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#### **Scientific Research**

**Effect of storage of rainbow trout (***Oncorhynchus mykiss***) by -products on fatty acids composition, oxidation, and nutritional properties of oil obtained using autolysis** 

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# **ARTICLE INFO ABSTRACT**

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The effects of storage of rainbow trout processing by-products at 4 °C on fatty acid composition and nutritional values of hydrolyzed -derived oils were determined. Polyunsaturated fatty acids (PUFA, 39.97 -43.70%) were the major fatty acids followed by monounsaturated (MUFA, 31.90 -34.05%) and saturated (SFA, 19.27 -22.59%) fatty acids. Among fatty acids, linoleic, cis vaccinic, and palmitic were the main fatty acids. N -6 fatty acids represented 34 -38% while  $n-3$ fatty acids were  $4.5 - 6.6\%$ of all fatty acids  $(P<0.05)$ . Hypocholesterolemic/hypercholesterolemic ratio (h/H) index in oils was between 4.23 and 5.15. The highest value was found at day 2 while showed no changes among other storage times (P>0.05). Thrombogenic index (TI) at days 0 and 2 were the lowest among storage times while at days 5 it represented the highest value. However, the change was not significant (P>0.05). N-6/n-3 ratio ranged from 5.01 to 7.6. PUFA/SFA was between 1.77 and 2.29 and showed the highest value at day 2 while after 5 days significantly decreased (P<0.05). The highest fish lipid quality (FLQ) index was for fresh by -products (day 0)  $(P<0.05)$  and showed no differences from day 1 to 5  $(P>0.05)$ . Also, nutritive value index (NVI) ranged from 2.25 to 2.55 with no significant differences during storage (P>0.05). The sum of EPA and DHA was between 1.73 and 4.05% and decreased with increasing storage days (P<0.05). Polyene index (PI) decreased to the end of storage time up to 4 days compared with the fresh by products and significantly decreased thereafter after 5 days (P<0.05). Results of the present study showed that fatty acid composition and nutritional quality of oils from hydrolysis -derived oils were influenced by the storage days to some degree.

# **1. Introduction**

Today, with the development of the concept of sustainable, economic and diverse aquaculture with less adverse environmental effects, better use of aquaculture waste can be beneficial.[1,2]. The use of marine waste for compounds with biological properties shows a special strategy in the production of added value. Aquaculture wastes or marine resources can be a useful source for producing valuable compounds such as hydrolyzed protein for use in food or feed if they are collected, stored and processed with proper management to minimize adverse qualitative reactions.[3,4]. If the wastes are to be used as raw materials in the production of various compounds, health guidelines related to the collection, storage and transportation of specific types of wastes should be established. Aquatic wastes have different amounts of protein (between 10 and  $20\%$ ). Waste including pieces of meat<sup>1</sup>, fins, meat attached to the bone 2 , head, skin, intestines and viscera, as well as non -macular species[5]. Various studies have shown that food proteins are a rich source of peptides that have a positive effect on the health of consumers by reducing the risk of chronic diseases.[6,7]. Muscle, bone, skin and intestines and viscera of fish, remaining muscles after preparation of fillet and waste of shrimp are sources of hydrolyzed protein and bioactive peptides production.[4] .

Different studies investigated the fatty acid composition of fish waste in fresh and preserved form. Hydrolysis is a method for the effective use of aquatic waste, especially rainbow trout processing waste. Or this existence, the fatty acid composition of the waste oil and the nutritional value of the oil are affected by the conditions of hydrolysis or pretreatment on the raw material. Storing the waste before starting the hydrolysis process, either at its production site (frozen fillet processing companies) or during transportation, has a significant effect on the onset of adverse

qualitative reactions in the waste. The presence of hemoglobin as a pro -oxidant as well as fats in the abdominal area of fish can accelerate spoilage and therefore increase the amount of oxidation inside the reactor. The occurrence of such adverse chemical reactions during hydrolysis will ultimately affect organoleptic properties, aquatic performance (such as growth reduction), and shelf life of products including oil. In general, marine oils are rich in long -chain unsaturated fatty acids such asEPA AndDH A are vulnerable to oxidation. The increase in reactive compounds with thiobarbituric acid indicates the decomposition of hydroperoxides and the progress of oil oxidation.[8] .

The development of the aquaculture industry and the lack of fish powder and oil from surface fish is one of the most important problems facing aquaculture. Due to the decrease in the production of fishmeal and its excessive price in recent years, it is predicted that in 2025, about 38% of the production of fishmeal on a global scale will be provided from fish waste. For example, in 2015, 13.9 million tons of fish meal was produced from low -value fish in a complete form, while in the same year 5.69 million tons of fish meal was produced from waste.[9]. The products obtained from the waste can be used again in the fish feed production cycle, and due to the lack of aquatic feed production institutions, the price increase and of course the increase in production costs, the production of inputs, supplements and additives of aquatic feed, from aquatic waste and promotion The quality of fish feed is one of the priorities of the country's aquaculture industry. Although the hydrolyzed protein is produced during the processing of waste by hydrolysis method, at the same time the oil is separated from the waste and can be used. Therefore, the storage conditions of waste as raw materials and hydrolysis variables will also

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affect the amount and nutritional characteristics of the resulting oil.

Iran is one of the largest producers of rainbow trout.*Oncorhynchus mykiss*) in the world, according to the latest production statistics, in 1998, it produced more than 182 thousand tons of trout. If boneless fillet is prepared, salmon has 30% waste, which mainly includes head, intestines, viscera and bones.[10,11] which is mostly thrown away without use. With the expansion of aquaculture in the country and the lack of feed ingredients including fish powder and oil, which has caused the price of fish to increase, the effective use of salmon waste in the production of some feed ingredients, including oil, can supply part of the factories' needs. To produce fish feed[12,13]. Therefore, this study aims to investigate The effect of keeping rainbow trout waste at 4°C on the composition of fatty acids, oxidation, and the nutritional value of the hydrolyzed oil has been made to take an effective step in the production of some fish feed inputs, including oil.

## **2- Materials and methods**

## **2-1- Preparation of trout waste**

In this project, fresh scraps of rainbow trout were used. Salmon was bought live from a fish store in Urmia and transported to the fishery processing laboratory of Artemia and Aquaculture Research Institute by Unilite and ice. In the laboratory, the wastes (including head, intestines and viscera, fins, bones) were separated and made into a paste by a meat grinder (Pars Khazar, Tehran, Iran) with a hole diameter of 3 mm. In order to prevent the temperature from rising while grinding the waste, the meat grinder attachments were cooled for 15 minutes at -18°C. This process was repeated for a week.

# **2-2- Hydrolysis process**

Experimental treatments were the days of waste storage, so sampling was done at times 1, 2, 3, 4, and 5. In order to perform hydrolysis,

after grinding, the waste was mixed with distilled water at a ratio of 1:1 (w/w) and homogenized by a homogenizer for 2 minutes. Hydrolysis was done at 40°C for 1 hour by autolysis method[12]. During the reaction time, the mixture was continuously stirred by a mechanical stirrer. In order to stop the enzymatic reaction, the solution was heated for 10 minutes at a temperature of 95°C and after cooling and initial filtering through a mesh cloth, it was centrifuged for 15 minutes at 4000 x g for 15 minutes at a temperature of 4°C. . The amount of Lord and other hydrolysis components (peptide phase, oil, resulting water) was measured by measuring the weight of each component.

# **2-3- Measurement of fatty acids**

The amount of fatty acids by the Miquel method[14] Was determined. A gas chromatograph (Agilent Technologies 7890A, Santa Clara, CA, USA) and a DB225MS column were used to measure fatty acids. One gram of sample was mixed with one milliliter of extraction solution (2.5% sulfuric acid + 98% methanol, at a volume/volume ratio of 1:40) and kept at 80°C for one hour. Then, 500 microliters of hexane was added with 1.5 ml of 0.5% sodium chloride solution to extract fatty acid methyl esters. Then the sample was separated for 10 minutes with a 4000 rpm centrifuge and the upper part of the solution (including hexane -methyl ester). The amount of fatty acids was calculated as a percentage of the total peaks, assuming that 100 were considered.

# **2-4- Determining the nutritional value of the oil**

The nutritional value of the oil was measured by measuring the amount of omega -3 fatty acids and the ratios between different fatty acids after measuring the fatty acid profile. Thermogenicity index <sup>3</sup> which indicates platelet aggregation induction index was calculated according to the following formula[15] :

# IT =  $[(C14: 0) + (C16: 0) + (C18: 0)]/[(0.5 \times MUFA) + (0.5 \times PUFAn - 6) + (3 \times PUFAn - 3) + (PUFAn - 3)PUFAn - 6]$

In terms of nutritional value, smaller TI is beneficial for health.

The hypo/hypercholesterolemia (h/H) index, which expresses the unbalanced level of blood cholesterol, was calculated according to the following formula[16] :

$$
h/H = (PUFAn - 3 + PUFAn - 6 + C18: 1)/(C14: 0 + C16: 0)
$$

The nutritional value index (NVI) was presented based on the following formula[17] :

Nutritive Value Index (NVI) [39]

$$
NVI = \frac{(C18:0 + C18:1)}{C16:0}
$$

Fish oil quality index (FLQ) was presented based on the following formula[17] :

Fish Lipid Quality (FLQ) [40]

$$
FLQ = 100 \times \ \frac{[{\text{C22:6 (n-3) + C20:5 (n-3)} } }{\text{EFA}}
$$

# **5 - 2 -Statistical analysis**

 SPSS software (SPSS Inc., Chicago, IL, USA) number 16 was used for statistical analysis. The statistical analysis of the data was done using two -way analysis of variance (ANOVA) and the comparison between the means was done by Duncan's multi -range test. The significance of the data was checked at the confidence level of 95% (P≤0.05). Data are averaged ± The standard deviation (SD) of three replicates was presented.

# **3. Results and Discussion**

Chromatogram of fatty acids of rainbow trout waste oil obtained from hydrolysis on different days of storage at 4°C is shown in Figure 1. Polyunsaturated fatty acids (39.43 -97.70%) are the most abundant fatty acids in salmon waste oil, followed by monounsaturated fatty acids (31.90 -34.05%) and saturated fatty acids. (19.27 -2.59%) had the highest amount of fatty acids (Table 1). The amount of SFA showed a slight difference during different storage times, so that it increased at the end of the storage time and the lowest percentage of these fatty acids was observed on days 1 and 2. However, this difference was not significant (<0.05). *p*). MUFA fatty acids showed changes during the storage period, so that in fresh waste and on the 5th day, it showed the highest percentage, but on the second day, it was lower than other times  $(<0.05)$ .*p*). The percentage of PUFA fatty acids on days 2 to 4 was higher than other times and similar to each other  $( $0.05p$ )$  but showed a significant decrease on the fifth day (>0.05).*p*). Among the fatty acids, linoleic acid (C18:2n6Cis), vaccinic (C18:1n9) and palmitic (C16:0) were the most abundant acids. Nikoo et al[12] Regarding the change of the composition of fatty acids in relation to the time and temperature of the hydrolysis of salmon waste, they showed that the composition of the fatty acids of the oil after centrifugation in different treatments, the above 3 fatty acids had the highest percentage.



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**Fig1. Chromatograms of fatty acids of oils obtained from hydrolysis of rainbow trout processing by -products at different storage times (0 -5 days) at 4 °C.**

Table 1. The major groups of fatty acids in oils derived from the hydrolysis of rainbow trout byproducts at different times of storage at 4 °C . **Fatty Days of storage**

$\blacksquare$ utti acids	<b>Duty of Storage</b>								
$\Sigma$ SFA	$20.96 \pm 2.47$ <sup>a</sup>	$19.35 \pm 1.15^a$ $19.27 \pm 1.24^a$ $20.51 \pm 0.36^a$			$20.23 + 0.38$ <sup>a</sup>	$22.59 + 2.43^{\circ}$			
$\sum MUFA$	$33.80 \pm 0.07^{\rm a}$			$32.33\pm0.39^{bc}$ $31.90\pm0.07^{c}$ $32.35\pm0.14^{bc}$ $32.71\pm0.02^{b}$		$34.05 \pm 0.14^a$			



### **2-4- Nutritional value of fatty acids of salmon waste**

The nutritional value of fish oil fatty acids was evaluated by measuring TI, h/H, NVI, FLQ, n - 6/n -3 and PUFA/SFA indices (Table 2). Hypercholesterolemia (high cholesterol) is a form of hyperlipidemia and refers to the high level of cholesterol in the blood. The cause of this disease can be environmental (such as morbid obesity and diet) or genetics. People with hypercholesterolemia are at risk of developing heart diseases and heart attacks. A higher h/h ratio is better in terms of nutritional value between different oils[16] Although the recommended limit for aquatic products was not reported, but for meat products, the number 2 is recommended for this index.[18]. The amount higher than 2 indicates the product's composition of fatty acids favorable for health. In the European eel <sup>4</sup> This index was 1.94 and then showed changes after salting, cooking, sterilization, and storage during 12 months and it varied between 2.01 and 4.03.[16]. The value of this index in salmon waste oil varied from 4.23 to 5.15, which indicates the usefulness of this oil in terms of blood cholesterol. The highest value of this index was related to the second day of storage. But there was no significant difference between samples between different days of storage (<0.05). *p*). The amount of h/H in mackerel waste oil<sup>5</sup> and carp 6 It was reported between 2/21 and 2/77[17].

The TI index indicates the induction of platelet aggregation. A lower value of this index  $(>1)$  is reported to be beneficial for human health, and foods with lower TI levels play a more important role in protecting against vascular diseases.[18]. The value of this index for European eel is 0.86%.[16] And 0.69% was reported for common carp[19]. The lower level of this index for white needle fish<sup>7</sup>  $(0.44)$  and sardines 8 (0.20) was reported[20]. The value of TI in the waste oil of salmon is between 0.34 and 0.45, which corresponds to its value of 0.5

in the oil of different types of marine fish in Brazilian waters.[20]. The lower level of this index (0.23 to 0.33) was reported in mackerel and carp waste oil[17]. In this study, the amount of TI on days 0 and 2 was lower than other times and the highest ratio was related to the fifth day of waste storage, but this difference was not significant (Table 2) (<0.05*p*).

The n -6/n -3 ratio is used as an index in evaluating the nutritional value of edible oils. In this study, the ratio was between 5.01 and 7.61 (Table 2). On the sixth day, the oil showed the highest ratio and the oil on day 0 (fresh waste) showed the lowest n -6/n -3 ratio. This ratio did not show a significant difference in the 2 -4 days  $(<0.05)$ .*p*). In raw eel meat, this ratio was 0.41, but after canning and storage for 1 year, it reached 10.23.[16]. Also, in mackerel waste oil, it varied between 0.18 and 0.22 [17]. Nutritionally, n -6/n -3 ratio is reported between 1:1 and 3:1[5]. The high level of this ratio in salmon waste oil indicates a higher level of omega -6 fatty acids compared to omega -3. As shown in Table 1, the amount of n -6 fatty acids was between 34 and 38.7% and n -3 fatty acids between 7.47 -47.51%. Matos et al[19] They reported n -6/n -3 ratio for common carp, grass carp, silver carp and silver carp as 5.40, 5.27, 0.16 and 0.40 respectively. In tuna and sardine waste oil, the amount of n -6 fatty acids was lower compared to n -3, and on the contrary, the amount of n -6 fatty acids in the waste oil of sea bass and sea bass was higher than n -3 fatty acids.[21]. The results of various studies show that the high amount of omega -6 and omega -3 fatty acids in fish oil does not necessarily indicate the nutritional value of the oil, but the balance in the n -6/n -3 ratio is important for health.[22] .

PUFA/SFA index to evaluate the effect of nutrition on cardiovascular health<sup>9</sup> is. In this index, it is assumed that all fatty acids of the PUFAs group can reduce bad cholesterol (low density lipoprotein).<sup>10</sup> and reduce the level of serum cholesterol, while SFAs cause an increase in serum cholesterol. Therefore, the

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<sup>4-</sup> *Anguilla anguilla*

<sup>5-</sup> *Scomberomorus sinensis*

<sup>6</sup> -*Gilded Carassius*

<sup>7</sup> -*Hyporhamphus unifasciatus*

<sup>8</sup> -*Opisthonema oglinum*

<sup>9-</sup> CVH

higher the ratio, the better[22]. However, not all saturated fatty acids increase cholesterol, such as C18:0 fatty acid, which is biologically neutral and does not play a role in bad cholesterol levels. However, fatty acids such as C12:0, C14:0 and C16:0 play an important role in raising blood cholesterol.[23]. The recommended amount of PUFA/SFA in the human diet should be more than 0.45 and the nutritional optimum should be around 1[22]. This ratio varied between 1.22 and 1.33 in mackerel waste oil and between 0.85 and 1.06 in carp waste oil.[17]. Also, in 4 species of freshwater fish in Brazilian waters, this ratio was between 1.09 and 1.47.[20]. In salmon waste oil, the ratio of PUFA/SFA was higher and between 1.77 and 2.29 due to the higher polyunsaturated fatty acids compared to the saturated ones (Table 2). On the second day of storage, the ratio of PUFA/SFA was significantly higher than at times 0, 1, 3, and 4, and there was no significant difference  $(<0.05)$ .*p*). The lowest ratio was on the 5th day of storage, which was significantly lower than other samples  $(>0.05)$ .*p*).

The FLQ index is calculated from the sum of the two fatty acids, EPA + DHA, as a percentage of the total fatty acids. This index is more useful in marine food products due to more EPA and DHA. So far, the FLQ index has been mostly used to evaluate the quality of fish oil. In different fish species, this index varied between 13.01 and 36.37[22]. It was reported between 26.88 and 28.88 in mackerel fish waste oil[17]. In the waste oil of salmon in this study, this ratio varied between 1.79 and 4.22 (Table 2) and was lower than the value reported in previous studies. This change could be due to the cultured nature of salmon and the lack of these fatty acids in the feed compared to marine fish oil or their waste. The highest rate was related to fresh lesions (day 0)  $(>0.05)$ .*p*). The amount of this index after 1 day of storage until the end of the fifth day had no significant difference (<0.05).*p*).

The NVI index, which shows the effect of fatty acids on cholesterol metabolism, and its higher value is more beneficial for health. The higher ratio indicates the higher amount of stearic (C18:0) and oleic (C18:1) fatty acids compared to palmitic fatty acid. In mackerel and carp waste oil, this index was reported as 0.87 and 1.68[17]. In this study, NVI between 2.25 and 2.55 and did not show any difference between different storage times (Table 2) (<0.05*p*).

The total amount of omega -3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was between 1.73 and 4.05% of total fatty acids and showed a decrease with the passage of storage time at 4 degrees Celsius. Gad (>0.05*p*). In seabass waste oil and cultured sea wire, the sum of EPA + DHA is about 12% of the total fatty acids, which is more than that in salmon waste oil. Also, a higher amount of EPA + DHA was reported in the waste oil of marine fish such as bluefin tuna (31%) and sardines (30%).[21] .

**Table 2.** Nutritional and health value of the fatty acids of oils derived from the hydrolysis of rainbow trout by products at different storage times at 4 °C . **Nutritional and Days of storage**

health indices						
					4	
TI	$0.37 + 0.06^a$	$0.42 + 0.11^a$	$0.34 + 0.02^a$	$0.41 + 0.01^a$	$0.39 + 0.2^a$	$0.45 + 0.06^a$
h/H	$4.80 + 0.26^{\circ}$	$5.08 + 0.32$ <sup>a</sup>	$5.15 + 0.12^a$	$4.62 + 0.40^a$	$4.78 + 0.10^a$	$4.23 + 0.17^b$
<b>FLO</b>	$4.22 + 1.00^a$	$2.92 + 0.30^b$	$2.39 + 0.58^b$	$2.50 + 0.18^b$	$2.41 + 0.11^b$	$1.79 + 0.30^b$
<b>NVI</b>	$2.55 + 0.47^{\circ}$	$2.55 + 0.28$ <sup>a</sup>	$2.49 + 0.29$ <sup>a</sup>	$2.32 + 0.07^{\circ}$	$2.45 + 0.04^a$	$2.28 + 0.36^a$
$N-6/n-3$	$5.01 + 0.64^{\circ}$	$5.92 + 0.57$ <sup>bc</sup>	$6.89 + 0.67$ <sup>ab</sup>	$6.57 + 0.15^{ab}$	$6.68 + 0.13^{ab}$	$7.61 + 0.33^a$
$EPA+DHA$	$4.06 + 0.89$ <sup>a</sup>	$2.77+0.30b$	$2.29+0.55^{\rm b}$	$2.40+0.17b$	$2.29+0.10b$	$1.73 + 0.26^b$
PUFA/SFA	$2.00 \pm 0.26^{ab}$	$2.23 + 0.19^{ab}$	$2.29 + 0.19^a$	$2.08 \pm 0.06^{ab}$	$2.07 \pm 0.05^{ab}$	$1.77 \pm 0.21^{\circ}$

\* Different small superscript letters in the same row indicate significant difference (P≤0.05).

# **4-3- Oxidation**

Poly Yen index $11$  It indicates the oxidation of polyunsaturated fatty acids and the formation of secondary fat oxidation compounds[21]. Storing the waste for 5 days at 4°C caused this

index to change due to the oxidation of essential fatty acids. On day zero, this index was significantly higher than other samples  $(0.05)$ .*p*). This was due to the higher amounts

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of EPA and DHA in waste oil. With the increase in the storage time of wastes, PI decreased and until the 4th day of storage, it was less than fresh wastes, but no significant difference was observed between different times (<0.05).*p*). With the increase of storage time until the fifth day, a significant decrease in this ratio was again observed  $(<0.05)$ .*p*) (figure 2). The waste oil of Atlas salmon, especially the oil obtained from intestines and viscera, which was obtained

during hydrolysis with alkalase, was oxidized.[24]. In seabass waste oil and cultured sea wire with less amount of EPA + DHA, this index is lower compared to sardine waste oil, tuna and cod liver oil containing more EPA + DHA.[21]. Because this index is calculated from the amount of essential fatty acids, any oxidation and loss of oil quality during hydrolysis will decrease this ratio.



Figure 2. PI index diagram of fatty acids of oils obtained from hydrolysis of rainbow trout by-products at different times of storage at 4 °C.

## **4 - Conclusion**

Storing the waste before starting the hydrolysis process can have a significant effect on the adverse qualitative reactions in the waste. The main groups of fatty acids including saturated, monounsaturated and polyunsaturated fatty acids showed changes during different storage days. Also, the nutritional indicators of the oil differed depending on the storage time. The findings of this research showed that it is possible to store salmon waste for up to 5 days at a temperature of 4°C without significant change in waste quality. Therefore, the possibility of short -term storage of salmon waste at 4°C temperature before processing by hydrolysis method is possible. However, the presence of hemoglobin as a pro -oxidant as well as fat in wastes can accelerate oxidative reactions during hydrolysis. Oxidation control by antioxidant compounds is suggested to increase the shelf life of the oil. Conducting a study on the interaction between waste freshness and hydrolysis variables will provide useful information regarding oil extraction efficiency and its characteristics.

## **5- Destiny and gratitude**

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مجله علوم و صنایع غذایی ایران





**پژوهشی \_ مقاله علمی**

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**ارزش تغذیه ای روغن** 

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