



## Fatty Acid Profiles of Fish Oil Derived by Different Techniques from By-products of Cultured Black Sea Salmon, *Oncorhynchus mykiss*

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### ABSTRACT

This paper investigates the fatty acid profiles of fish oil extracted from by-products of cultured Black Sea salmon, *Oncorhynchus mykiss*, using conventional [conventional fish oil (CFO)] and dry freezing oil [dry freezer oil (DFO)] techniques. In the CFO and DFO groups, monounsaturated fatty acid + polyunsaturated fatty acid (PUFA) comprised 74.00% and 72.68% of the total fatty acids, respectively. The highest PUFA was linoleic acid (CFO=14.22%, DFO=13.15%) with Docosahexaenoic acid (DHA, C22:6n3) being the second most concentrated fatty acid for PUFA in the CFO (8.12%) and DFO (8.02%)

groups, followed by eicosapentaenoic acid (EPA, C20:5n3) (CFO=4.39%, DFO=2.87%). Similarly, the difference between groups in omega-3 was statistically significant ( $p<0.05$ ) and the CFO ratio was higher in the DFO. The PI, AI, TI, h/H, and UI percentages in the CFO group were 0.99, 0.37, 0.26, 2.98, and 1.73%, respectively, while in the DFO group they were 0.80, 0.35, 0.31, 2.83, and 1.61%, respectively. The findings of this study conclude that the oils obtained from Black Sea salmon by-products are rich in omega-3 fatty acids and have good lipid quality indexes.

Keywords: By-product, Conventional fish oil, Dry freezing, Fish oil quality, *Oncorhynchus mykiss*

## 1. Introduction

The global fish production from fishing is constrained by factors such as overfishing, climate change, and habitat destruction, which limit the amount of fish that can be sustainably caught. The increasing world population and the search for quality seafood support the development of aquaculture (Filogh et al. 2023; Bayrakli & Duyar 2021a,b; Sönmez et al. 2022). The use of fish meal and oil in feed rations is important for quality fish farming (Bayrakli & Duyar 2019a). Fish meal and oil production made from whole fish is also limited (Bayrakli et al. 2019).

The use of fish oil in the aquaculture sector to feed farmed fish is increasing, as well as in the food industry to supplement foods with omega-3 fatty acids and in the pharmaceutical industry to produce omega-3 concentrates.

Fish oil is mainly derived from fish caught specifically for this purpose, such as anchovies, sardines, mackerel, and menhaden (Bayrakli et al. 2019). There are 85.4 million tons of aquaculture production in the world, with great potential for oil extraction from fish waste and by-products from the fish processing industry (TUIK 2021). According to TUIK (2021) data, Black Sea salmon production on land and in the sea in Turkey saw an increase from 123,089 tons in 2019 to 144,283 tons in 2020. Every year, the production of Black Sea salmon in Turkey continues to increase, and as a result, the amount of by-products generated also rises accordingly. By-products are fish pieces that have been removed before the fish reaches consumers to maintain fish quality, reduce shipping weight, or increase the value of the main fish product. Fish by-products contain significant amounts of fat and protein that can be used for human nutrition and animal feed, from a nutritional or food safety point of view (Ramirez 2007). The main components of aquaculture discards are the head, viscera, gills, bones, scales, fins, and sometimes skin. A significant amount of waste (20-80%) is generated depending on the fish

processing technique and fish species (Ghaly et al. 2013). Fish waste typically contains 58% crude protein, 22% ash, 19-25% ether extract and 1% crude fibre (Esteban et al. 2007).

A characterization of the lipid profile of oils found in fish is crucial to explore its potential application to new fish sources for omega-3 production. Recently, the potential use of these ingredients has attracted a great deal of attention. Fish oil is the main natural source of the healthiest omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish oil suitable for dietary supplementation is the relative amount of omega-3 and omega-6 fatty acids. To maintain the correct omega-3/omega-6 ratio in the diet, regular consumption of omega-3-rich and omega-6-poor fish is recommended, and the optimal omega-3/omega-6 dose or ratio ranges from 1/4 to 1/1. The demand for fish oil for direct human use as fish oil capsules and additives in the food industry continues to increase (Simopoulos 2002). The main fatty acid quality assessment indexes include the polyene index (PI), a ratio of polyunsaturated fatty acids to other fatty acids, as well as the atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic/hypercholesterolemic ratio (h/H), fish lipid quality (FLQ), and unsaturation index (UI).

This study was carried out to characterize the fish oil profiles obtained from the non-consumed waste parts of Black Sea salmon reared in net cages in the Black Sea, by the conventional method and the freeze-drying method. Data obtained from this study can be the basis for analyzing the possibility of using Black Sea salmon waste as a source of omega-3 rich oil.

## 2. Material and Methods

### 2.1. Fish by-products

Fish by-products were obtained from an industry-leading aquaculture processing facility headquartered in Samsun, Turkey. After Black Sea salmon is processed at the factory, about 40% is considered unsuitable for human consumption and subsequently leftover from industrial processing. Most of this leftover excess is the internal organs, as well as the head and gills. In order to conduct our study, we obtained 5 kg of Black Sea salmon by-products (Trim B waste obtained after fillet trimming in an aquaculture processing plant) and transported them to a fish meal oil factory in a Styrofoam box.

### 2.2. Extraction techniques and procedures

In this study, the fatty acid profiles of fish oil extracted from Black Sea salmon by-products through two different techniques were investigated.

1. Conventional fish oil (CFO): CFO was obtained from the company from which we provided our research material. This company applies the industrial conventional method. This wet reduction process involves fish cooking, pressing, solid and liquid separation (decanter), and centrifugation for extraction.

2. Dry freezer oil (DFO): Black Sea salmon wastes obtained from the same company were brought to the research laboratory and homogenized using a Mateka brand (GPS 300) food shredder (15 L and 5000 rpm). Then, the Black Sea salmon structure was obtained by drying in a lyophilizer. For this purpose, a Lyoquest-85 Plus Eco laboratory lyophilizer, made by Telstar, was used. The operating procedure of the device is provided in the Table 1.

**Table 1- Method used in the lyophilizer device**

<i>Step no</i>	<i>Process</i>	<i>Parameters</i>		
		<i>Time (hh:mm)</i>	<i>Pressure (mBar)</i>	<i>Temperature (°C)</i>
1	Freezing	01:00	0.2	-
2	Cool + vacuum	00:30	0.2	-
3	Heat shelves	24:00	0.2	20
4	Heat shelves	06:30	0.001	35
5	Stop	-	-	-

### 2.3. Fatty acid composition

Fatty acid methyl esters were prepared with hot alkali esterification according to IUPAC (1994) method. Basically, 150 mg of fish oil was weighed into an Erlenmeyer flask. Five mL of methanolic 0.5 N NaOH added, then boiled for 15 minutes by using a condenser. The mixture was cooled down to room the temperature. Further 5 mL of methanolic  $\text{BF}_3$  (14%), was added into the mixture and boiled for 5 minutes. Methylated sample was cooled and a 5 mL of saturated NaCl solution was added into the solution. The solution was transferred into a screw capped tube and Fames were extracted with 2 mL of n heptane into an amber vial. Some anhydrous sodium sulphate (full spoon of microspatula) was added into a vial in order to hold moisture. The vials were kept at  $-20^\circ\text{C}$  till GC analysis.

The GC system consists of an FID detector (Flame Ionization Detector), gas chromatography (Shimadzu, GC2014, Japan), and autoinjector (AOC-20i, Shimadzu, Japan). The instrument is controlled by GC solution software (Version 2.41.00 su\_1). FAME WAX (polyethylene glycol, 30 meter\*0.25 mm I.D\*0.2  $\mu\text{m}$ , GC Columns Restek) was used as the chromatographic colon. The GC operation conditions were as follows; injection mode: split ratio, split: 1/10, injection and detector temperature: 260 and 280  $^\circ\text{C}$ , carrier gas and column flow: helium and 1.4 mL  $\text{min}^{-1}$ , temperature program: initial temperature 5 m 100  $^\circ\text{C}$ , 5  $^\circ\text{C}$  increase per minute from 100  $^\circ\text{C}$  to 150  $^\circ\text{C}$ , 15 m at 150  $^\circ\text{C}$ , 10  $^\circ\text{C}$  increase per minute to 210  $^\circ\text{C}$ , and 20 m at 210  $^\circ\text{C}$ .

Peaks were identified by comparing the retention time of the “Supelco 37 Component FAMES Mix” standard. Results were expressed as percentage area of the identified individual fatty acids. The spectrum includes all commonly known fatty acid methyl esters.

According to this method, the fatty acid methyl esters were analysed using a PUE UNICAM 204 Gas Chromatography equipped with a flame ionization detector using a Degs capillary column (2 MX 1-8 inc) coated with 0.25  $\mu\text{l}$  of Supelco GP% OV-275 on 100/120 PAW-PMCS.

### 2.4. Lipid quality indices

PI was used as a measure of PUFAs damage (Lubis & Buckle 2007) and calculated according to the formula below.

$$\text{PI} = \frac{[\text{EPA (C20: 5n3)} + \text{DHA(22: 6n3)}]}{\text{Palmitic acid (C16: 0)}}$$

AI, TI and h/H were calculated using the following equations (Abrami et al. 1992; Bayraklı 2021; Ulbricht & Southgate 1991), taking into account the different effects of different fatty acids on human health:

$$\text{AI} = \frac{[12:0 + 4(14:0) + 16:0]}{\text{MUFA} + \text{PUFA}}$$

$$\text{TI} = \frac{(14:0 + 16:0 + 18:0)}{[0.5(\text{MUFA}) + 0.5(\text{n6PUFA}) + 3(\text{n3PUFA}) + (\text{n3PUFA}/\text{n6PUFA})]}$$

$$\text{h/H} = \frac{(\text{C18: 1} + \text{C18: 2} + \text{C18: 3} + \text{C20: 3} + \text{C20: 4} + \text{C20: 5} + \text{C22: 4} + \text{C22: 5} + \text{C22: 6})}{(\text{C14: 0} + \text{C16: 0})}$$

$$\text{UI} = [1 \times (\% \text{ monoenoics}) + 2 \times (\% \text{ dienoics}) + 3 \times (\% \text{ trienoics}) + 4 \times (\% \text{ tetraenoics}) + 5 \times (\% \text{ pentaenoics}) + 6 \times (\% \text{ hexaenoics})]/100$$

### 2.5. Statistical analysis

The data obtained from three different time periods were analysed by Student's t-test analysis using the SPSS statistical package program (Version 10, SPSS Inc., Chicago, IL, USA), and the differences among the means were compared using Duncan's multiple range test. A significance level of 0.05 was used and the results were shown as mean values  $\pm$  standard deviation.

### 3. Results and Discussion

Although the aim of the research was not to optimize both techniques, the characterizations of the fish oil profiles obtained between these two techniques were compared and the analyses were performed with three replications. The fatty acid profiles of fish oil obtained from freeze-dried Black Sea salmon by-product are given in Table 2.

**Table 2- Fatty acid composition (%) of Black Sea salmon fish oil processing by-product (CFO-DFO)**

<i>Fatty acid</i>	<i>CFO</i>	<i>DFO</i>
C10:0 Capric	0.01±0.000	0.00±0.000
C12:0 Lauric	0.11±0.006	0.07±0.000
C13:0 Tridecanoic	0.03±0.000	0.02±0.000
C14:0 Myristic	3.69±0.101 <sup>a</sup>	2.96±0.052 <sup>b</sup>
C15:0 Pentadecanoic	0.61±0.026 <sup>a</sup>	0.44±0.012 <sup>b</sup>
C16:0 Palmitic	12.66±0.062 <sup>b</sup>	13.65±0.344 <sup>a</sup>
C17:0 Heptadecanoic	0.64±0.015 <sup>a</sup>	0.56±0.006 <sup>b</sup>
C18:0 Stearic	5.75±0.576 <sup>b</sup>	8.13±0.165 <sup>a</sup>
C20:0 Arachidic	0.95±0.006 <sup>a</sup>	0.83±0.046 <sup>b</sup>
C21:0 Henicosanoic	0.04±0.006	0.03±0.006
C22:0 Behenic	0.63±0.087	0.54±0.015
C23:0 Tricosanoic	0.09±0.010	0.07±0.006
C24:0 Lignoceric	0.79±0.026 <sup>a</sup>	0.02±0.006 <sup>b</sup>
Σ SFA	26.01±0.860 <sup>a</sup>	27.32±0.642 <sup>a</sup>
C14:1 Myristolec	0.25±0.006	0.15±0.006
C15:1	0.09±0.006	0.06±0.006
C16:1 Palmitoleic	0.55±0.010	0.41±0.012
C17:1 c Heptadecenoic	0.86±0.021 <sup>a</sup>	0.51±0.015 <sup>b</sup>
C18:1n9t Elaidic	1.58±0.667 <sup>b</sup>	2.82±0.044 <sup>a</sup>
C18:1n9c Oleic	23.28±1.020 <sup>a</sup>	23.66±0.814 <sup>a</sup>
C20:1n9 cEicossenoic	1.30±0.010 <sup>b</sup>	3.51±2.246 <sup>a</sup>
C22:1n9 Erucic	3.49±0.036 <sup>a</sup>	3.44±0.058 <sup>a</sup>
C24:1n9 Nervonic	1.33±0.035 <sup>a</sup>	1.20±0.025 <sup>b</sup>
Σ MUFA	32.74±1.236 <sup>b</sup>	35.76±1.285 <sup>a</sup>
C18:2n6t Linoleaidic	0.75±0.015 <sup>a</sup>	0.49±0.015 <sup>b</sup>
C18:2n6c Linoleic	14.22±0.119 <sup>a</sup>	13.15±0.179 <sup>b</sup>
C18:3n6 γ -Linolenic	0.87±0.006 <sup>a</sup>	0.60±0.092 <sup>b</sup>
C18:3n3a-Linolenic	5.72±0.070 <sup>a</sup>	5.15±0.078 <sup>b</sup>
C20:2 c Eicosadienoic	3.18±0.036 <sup>a</sup>	3.20±0.069 <sup>a</sup>
C20:3n3 α Eicosatrienoic	1.76±0.010 <sup>a</sup>	1.63±0.038 <sup>b</sup>
C20:3n6	0.57±0.026 <sup>a</sup>	0.30±0.006 <sup>b</sup>
C20:4n6 Arachidonic	1.60±0.065 <sup>a</sup>	1.45±0.040 <sup>b</sup>
C22:2 Docosadienoic	0.08±0.000	0.06±0.035
C20:5n3 EPA	4.39±0.165 <sup>a</sup>	2.87±0.046 <sup>b</sup>
C22:6n3 DHA	8.12±0.255 <sup>a</sup>	8.02±0.167 <sup>a</sup>
Σ PUFA	41.26±0.385 <sup>a</sup>	36.92±0.662 <sup>b</sup>

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, Different letters (a,b,c) in the same row shows significant differences ( $p<0.05$ ) among the freshness groups.

The SFA, MUFA, PUFA and MUFA+PUFA values were determined in fish oil obtained from Black Sea salmon by-products with 2 different techniques (CFO and DFO groups). SFA in CFO and DFO groups were calculated as 26.01% and 27.32%, respectively. MUFA was higher in the DFO group than the CFO group. PUFA values of CFO and DFO groups were found as 41.26% and 36.92%, respectively (Table 2). Compared to the DFO group, PUFA was higher in the CFO group. In the CFO and DFO groups, MUFA+PUFA constituted 74.00% and 72.68% of total fatty acids, respectively. The difference in SFA between CFO and DFO groups was insignificant ( $p>0.05$ ), but the difference between the MUFA and PUFA groups were statistically significant ( $p<0.05$ ). It was observed that the SFA results obtained in this study and the SFA values in fish oil obtained from Black Sea salmon, tilapia and carp processing by-products in other studies were similar (Table 3). The MUFA content of fish oil obtained from various fish by-products has been reported in several studies. Zhong et al. (2007), Crexi et al. (2010), Korkmaz & Tokur (2020), and Pateiro et al. (2020) reported lower MUFA content compared to our study. On the other hand, Bayraklı & Duyar (2019b), Khoddami et al. (2012), and Brelaz et al. (2019) reported higher MUFA content. The MUFA content reported by Nascimento et al. (2015) and Abiona et al. (2021) was found to be similar to our results. The PUFA obtained in this study was found to be higher than all the studies reported in Table 3.

**Table 3- Literature information on fatty acids contents and Lipid Quality Indices determined for different fish oil products**

Fatty Acids	<i>Abiona et al. (2021)</i>	<i>Inguglia et al. (2020)</i>	<i>Brelaz et al. (2019)</i>	<i>Khoddami et al. (2012)</i>	<i>Nascimento et al. (2015)</i>	<i>Pateiro et al. (2020)</i>	<i>Korkmaz and Tokur (2020)</i>	<i>Fiori et al. (2012)</i>	<i>Zhong et al. (2010)</i>	<i>Crexi et al. (2010)</i>	<i>Bayraklı &amp; Duyar (2019b)</i>	<i>This Study</i>
	<i>Scomber scombrus</i>	<i>Salmo Salar</i>	<i>Fresh water fish</i>	<i>Euthynnus affinis</i>	<i>Fish of various species</i>	<i>Sparus aurata</i>	<i>Rainbow trout</i>	<i>Rainbow trout</i>	<i>Rainbow trout</i>	<i>Cyprinus carpio</i>	<i>Anchovy*</i>	<i>Rainbow trout</i>
C14:0 Myristic	2.24-3.48	2.56		3.76-7.49	6.33	1.73-2.84	2.03-2.28	4.75-6.04	3.43	3.80	6.52	2.96-3.69
C16:0 Palmitic	27.49-34.16	9.57	29.01	27.63-32.74	19.67	13.27-14.13	14.27-15.47	15.7-17.8	15,7	16,20	19.80	12.66-13.65
C18:0 Stearic	8.17-10.20	0.54	9.62	8.82-13.62	4.91	2.70-4.51	4.44-4.88	3.59-3.88	4,50	3.13	3.60	5.75-8.13
C18:1n9c Oleic	24.31-31.99	39.47	18.48	9.16-11.95	12.65	32.99-35.91	37.14-39.47	17.9-19.0	28,6	25,84	13.59	23.28-23.66
C18:2n6c Linoleic	1.95-4.38	14.56	4.39	1.0-2.49	0.2	16.57-19.58	17.94-19.23	13.8-17.9	9,0	9.17	0.28	13.15-14.22
C18:3n3a-Linolenic		4.46	4.51		2	3.86-4.73	3.05-3.27	0.80	0,11	7.17	1.99	5.15-5.72
C22:6n3 DHA	1.62-9.31	2.78	4.20	14.18-15.70	9.12	3.51-5.20	4.37-5.11	6.02-7.30	7,98	1.20	18.64	8.02-8.12
C20:5n3 EPA					10.36	1.83-2.78	1.62-1.90	5.98-8.75	3,35	3.83	8.68	2.87-4.39
SFA	40-45.36		48.23	47.02-55.20	31.63	19.72-20.85	21.94-23.74	24.3-27.9	25	26.85	34.21	26.01-27.32
MUFA	33.85-42.32		28.92	20.82-24.20	30.59	44.05-46.08	44.05-46.46	72.1-75.7	40	41.90	21.51	32.74-35.76
PUFA	12.31-25.36		21.91	23.98-28.77	32.78	31.17-34	23.15-24.41		26	25.54	32.04	36.92-41.26
Omega 3		10.64	12.79	15.88-17.18		11.91-14.18	6.37-7.38	17.4-22.2		13.61	28.39	17.67-19.99
Omega 6		19.06	8.57	8.10-11.59		18.66-21.04	22.51-23.71	17.5-20.4		11.90	3.65	15.99-18.01
Omega 3/ Omega 6							0.27-0.33	0.85-1.18		1.14	7.78	1.10-1.11
UI								1.76-2.01				1.61-1.73

In terms of MUFA + PUFA values, the results of this study were similar to the literature on fish oil obtained from fish processing by-products of Black Sea salmon, *Cyprinus carpio*, *Spratus aurata*, *Oreochromis niloticus*, *Scomber scombrus* (Table 3). Fish oil made from whole anchovy fish and fish oil obtained from *Euthynus affinis* and some freshwater fish by-products were found to be higher. Fatty acid values differ according to the type of fish, whether it is wild or cultured fish, the processed part of the fish, and the processing method.

Palmitic acid has the highest amount among SFA in CFO and DFO groups (12.66% and 13.65%), followed by stearic acid (5.75% and 8.13%) and myristic acid (3.69% and 2.96%). The values of fish oil studies with Black Sea salmon by-product were lower than those made with similar different kinds of fish by-product. In addition, as seen in Table 3, the dominant SFA in all studies was palmitic acid, followed by stearic acid and myristic acid, as in this study.

Among MUFA, oleic acid had the highest percentage in CFO and DFO groups (23.28% and 23.66%, respectively) followed by erucic acid (3.49% and 3.44%, respectively). There was no statistically significant difference between the groups ( $p>0.05$ ). Oleic acid was the highest MUFA as reported in other studies (Table 3). In the studies conducted by Korkmaz & Tokur (2020) and Zhong et al. (2007) with Black Sea salmon by-products, oleic acid was found to be lower compared to our findings, while it was higher than the value reported by Fiori et al. (2012). It was observed that the oleic acid values in fish oils made with the by-products of other fish species were lower than some studies and higher than others.

The PUFA results in this study were higher than the fish oil value obtained from all anchovy investigated by Bayraklı and Duyar (2019b). The highest concentration of PUFA in the CFO and DFO groups was found to be linoleic acid, similar to other fish by-products. In contrast, fish oil obtained from whole anchovy contained only 2% of this fatty acid (Bayraklı & Duyar 2019b). Among the PUFA, DHA (C22: 6n3) was the most abundant in both groups, with concentrations of 8.12% and 8.02%, followed by EPA (C20: 5n3) at 4.39% and 2.87%, respectively (Table 2). The difference between the two groups was statistically significant for omega-3 ( $p<0.05$ ), with the CFO group having a higher concentration. The highest DHA/EPA ratio was observed in the DFO group (2.80) (Table 4). The omega-3 fatty acid value in this study was higher than in similar research by Fiori et al. (2012), while omega-6 fatty acid values were similar to those reported by the same study. It was also observed that the omega-3 and omega-6 values were lower or higher than some research results. According to the results, fish oil obtained from anchovy investigated by Bayraklı and Duyar (2019b) had a lower omega-3 value and a higher omega-6 value compared to CFO and DFO groups. The omega-3/omega-6 ratios were 1.11:1 and 1.10:1 in the CFO and DFO groups, respectively, which were higher than Korkmaz and Tokur's (2020) values, but similar to the results obtained from the Black Sea salmon fish processing by-products studied by Fiori et al. (2012) and Zhong et al. (2007). According to the results of this study, the fish oil value obtained from the whole anchovy was considerably lower (Bayraklı & Duyar 2019b). It was reported that the omega-3/omega-6 ratio in the fatty acid ratio of wild Black Sea salmon was 3.08, while it was 0.46 in culture (Oz & Dikel 2015). Plant-based raw materials are commonly used in feed rations for Black Sea salmon fish grown in aquaculture farms. For this reason, it was evaluated that omega-6 levels increased and omega-3 levels decreased, so this ratio may be lower than oils obtained from wild fish. The minimum recommended PUFA/SFA ratio value is 0.45 (HMSO 1994). This value is lower than the values obtained in both fish oil groups in this study.

**Table 4- Fatty acid ratios and lipid quality indexes**

<i>Indexes</i>	<i>CFO</i>	<i>DFO</i>
UNSFA/SFA	2.85±0.125	2.66±0.085
PUFA/SFA	1.58±0.144	1.35±0.103
n3	19.99±0.175	17.67±0.326
n6	18.01±0.187	15.99±0.294
n3/n6	1.11±0.003	1.10±0.008
n9	28.35±1.271	29.92±0.915
DHA/EPA	1.85±0.130	2.80±0.018
A <sub>1</sub>	0.37±0.010	0.35±0.011
T <sub>1</sub>	0.26±0.008	0.31±0.005
P <sub>1</sub>	0.99±0.008	0.80±0.005
h/H	2.98±0.088	2.83±0.015
UI	1.73±0.004	1.61±0.011

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, PI: Index of polyene, IA: Atherogenicity index, IT: Thrombogenicity index, FLQ: Fish lipid quality

Several studies have reported that individuals who consume omega-3 rich products are less likely to develop hypertension or other cardiovascular diseases. It has been emphasized that drugs used in the treatment of obesity and cardiovascular diseases can be reduced by decreasing the omega 6 ratio in diets and increasing the omega-3 ratios (Simopoulos et al. 2000). In the early studies on fatty acids, the omega-3/omega-6 ratio was initially reported to be 1:1, but over time, due to changing dietary habits in industrial societies, this ratio has increased to 30:1 to 50:1. The World Health Organization reported that the omega-3/omega-6 ratio should be between 5:1 and 10:1 (FAO/WHO 1994). However, this ratio should be between 1:1 and 1:4 in a healthy diet (Simopoulos et al. 2000). In the present study, omega-3/omega-6 ratio of 1.10:1 indicates that by-products of cultured Black Sea salmon appears to be a healthy food and can provide source of omega-3 fatty acids (Table 4).

The PI value (the coefficient of deterioration of PUFA) may be a useful tool for measuring the oxidative stability of fish oil. In general, a higher PI value is preferred. However, in our study, we found that the PI value was the lowest in the DFO group (0.80), and there were statistically significant differences when compared to the CFO group (0.96) (Table 4). Additionally, we found that fish oil obtained from whole anchovy (Bayraklı & Duyar 2019b) had a relatively low PI value (1.73). Based on this result, it can be concluded that the oxidation of PUFA was higher in oil obtained from fish processing by-products.

It is reported that atherogenic (AI) and thrombogenic (TI) indices that are higher than (>1.0) are harmful to human health (Ouraji et al. 2009). If this value gets lower, the risk of coronary heart disease decreases (Cuttrignelli et al. 2008). The average AI values for the CFO and DFO groups were determined as 0.37, and 0.35, respectively. The average TI values for the CFO and DFO groups were determined as 0.26, and 0.31, respectively. The AI and TI values of fish oil made from whole anchovy were reported to be 0.86, and 0.28, respectively. Karsli (2021) reported AI values between 0.11-0.70 and TI between 0.01-0.36 fish oil supplement products. In conclusion, our study demonstrated that fish oil obtained from fish processing by-products had lower AI and TI compared to fish oil obtained from whole anchovy, and the consumption of fish oil supplements made from fish processing by-products does not pose significant risks to human health in terms of AI and TI.

The h/H ratio of fatty acids is the indicator of whether the fat in the product is nutritionally adequate (Caglak & Karsli 2017). The fact that this ratio is high indicates that the oil contained in the product is suitable for nutrition. In this study, the h/H ratio was determined as 2.98 for the CFO group and 2.83 for the DFO group, and these values were found to be higher than the data of the study (1.73) conducted by Bayraklı & Duyar (2019b). The high rate indicates that the oil in the product is suitable for nutrition. The fatty acid composition can be variable depending on fish species (Ozogul et al. 2013).

The UI shows the degree of unsaturation in lipids and is calculated as the sum of the percentage of each unsaturated fatty acids multiplied by the number of double bonds in that fatty acids (Logue et al. 2000). That this ratio is high indicates that the oil contained in the product is suitable for nutrition. In this study, the UI was found to be 1.73 and 1.61 in the CFO and DFO groups, respectively. The difference between the groups were found to be statistically significant. Fiori et al. (2012) reported that the UI value in Black Sea salmon processing by-product ranged from 1.76 to 2.01, similar to the results of this research.

#### 4. Conclusions

The study suggests that the use of vegetable oil in farmed fish feed may lead to an increase in the amount of omega-6 and a decrease in the amount of omega-3/omega-6 ratio as a cost-saving measure. In the Black Sea, aquaculture Black Sea salmon can be considered as a resource with good lipid quality indices, which can be used for the production of fish oil rich in omega-3.

An evaluation of Black Sea salmon by-products is especially important today when considering the ongoing global food shortages and it would be particularly beneficial to use this waste for human and animal consumption.

Fish by-products are organs where microbial degradation rapidly takes place. Considering the environmental factors, these fish by-products must be processed quickly. There are various difficulties in the traditional fishmeal and oil production processing of fish by-products. Future studies should consider economical, simple and feasible extraction of fish oil from fish residues processed by using different processing techniques or new technologies in small-capacity aquaculture processing plants.

**Data availability:** Data are available on request due to privacy or other restrictions.

**Authorship Contributions:** Concept: H.A.D., B.B., Design: H.A.D., B.B., Data Collection or Processing: H.A.D., B.B., Analysis or Interpretation: B.B., Literature Search: H.A.D., B.B., Writing: H.A.D., B.B.

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