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ARAŞTIRMA MAKALESİ ***RESEARCH PAPER***

The Effect of Raw Material Freshness on Fish Oil Quality Produced in Fish Meal and Oil Plant [*]

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Abstract: The effect of raw material freshness on fish oil quality produced in fish meal and oil factories in the Black Sea during the 2007-2008 fishing season was investigated. The fish oil was produced from anchovy (*Engraulis encrasicolus*) which were processed on catch day (D0), Day 1(D1), Day 2 (D2), and Day 3 (D3) after catching and thiobarbituric acid (TBA) values of the fish oils that having different freshness degree of raw material were determined as D0, D1, D2, and D3. Thirty-one fatty acids in total were identified and the total ratio of identified fatty acids ranged between 87.35% and 88.22% among the groups. In the freshest group (D0), the average saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids contents were 34.21%, 21.51%, and 32.04%, respectively. In the 1 day old material (D1), the average SFA, MUFA, and PUFA contents were 34.17%, 21.73%, and 32.32%, respectively. In the 2 days old material (D2), the average SFA, MUFA, and PUFA contents were 34.06%, 21.29%, and 32.18%, respectively. In the 3 days old material (D3), the average SFA, MUFA, and PUFA contents were 34.48%, 21.66%, and 31.21%. In addition, atherogenicity (AI) and thrombogenicity index (TI), which is an evaluation index for fatty acids, and polyene (PI) index indicating the degradation of PUFA were calculated, and the obtained data were compored with the recommended limit values for human health. Based on the findings, it was determined that if the raw material was processed earlier, the amount of PUFA in fish oil would be higher. In light of these results, it can be recommended that the factories that produce according to the feed law should produce the fish oil that is rich in ω3 and ω6 according to the food law instead of feed law.

Keywords: Anchovy, Black Sea, DHA, *Engraulis encrasicolus*, EPA, fatty acids.

Hammadde Tazeliğinin Balık Unu ve Yağı Fabrikasında Üretilen Balık Yağı Kalitesine Etkisi

Öz: Bu araştırmada, Karadeniz'de 2007-2008 av sezonunda balık unu ve yağı fabrikalarında üretilen balık yağı kalitesine kullanılan hammadde tazeliğinin etkisi araştırılmıştır. Avlandıktan sonra 0, 1, 2 ve 3 üncü gün işlenen ve tiyobarbitürik asit (TBA) analizleri yapılan hamsi (*Engraulis encrasicolus*) balığından üretilen balık yağı grupları D0, D1, D2 ve D3 olarak belirlenmiştir. Gruplar arasında tespit edilebilen 31 çeşit yağ asitlerinin toplam oranı %87.35 ile %88.22 arasında değiştiği belirlenmiştir. Tazelik gruplarından D0 için ortalama doymuş yağ asidi (SFA) %34.13, tekli doymamış yağ asidi (MUFA) %21.36 ve çoklu doymamış yağ asidi (PUFA) miktarı %32.45 olarak tespit edilmiştir. Birinci gün örneğinde (D1), SFA oranı %34.17, MUFA oranı %21.73 ve PUFA oranı %32.32 olarak tespit edilmiştir. İkinci gün örneğinde (D2), SFA oranı %34.06, MUFA oranı %21.29 ve PUFA oranı %32.18 olarak bulunmuştur. Üçüncü gün örneğinde (D3) ise, SFA oranının %34.48, MUFA oranının %21.66 ve PUFA oranının %31.21 olduğu belirlenmiştir. Tazelik grupları arasında PUFA miktarının zamana bağlı olarak azaldığı ve gruplar arasında ki farkın istatistiki olarak önemli olduğu bulunmuştur ($P<0.05$). Ayrıca, çalışmada insan sağlığı açısından yağ asitleri için bir değerlendirme indeksi olan atherogenicity (AI) ve thrombogenicity indeksi (TI) ile PUFA'nın bozulma değerini gösteren polyene (PI) indeksi hesaplanmış ve elde dilen veriler tavsiye edilen limit değerleri ile kıyaslanmıştır. Elde edilen sonuçlara göre hammadde ne kadar kısa sürede işlenirse balık yağında ki PUFA miktarının daha yüksek olacağı belirlenmiştir. Bu sonuçlar ışığında, yem kanununa göre üretim yapan balık unu ve yağı fabrikalarının ω3 ve ω6 bakımından zengin olan balık yağını gıda kanununa göre üretmeleri önerilmektedir.

Anahtar sözcükler: DHA, Hamsi, *Engraulis encrasicolus*, EPA, Karadeniz, Yağ asitleri.

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INTRODUCTION

The anchovy (*Engraulis encrasicolus*), the most important pelagic specie of the Black Sea, and are caught plenty in fishing season and excess fish are utilized as fish meal and oil (Özdemir et al., 2007; Erdem et al., 2008; Özdemir, 2010). According to TÜİK (2019) data, minimum 96452 tons (2018) and maximum 229023 tons (2010) anchovies in the last decade were caught in Turkey. Approximately 46% of the hunted anchovies are processed in fish meal and oil plants, while the rest of anchovies are consumed as fresh, freeze and marinade. Anchovy has a high quality fatty acid composition (Öksüz and Özyılmaz, 2010). Fresh fish and fishery products play an important role in the healthy nutrition of developed and developing countries. Developed countries promote omega-3 fatty acid-supplemented diets that are abundant in aquaculture to combat increasing obesity and heart disease (Jahns, 2016). Fish oil is a very valuable product produced in fish flour and oil factories. Approximately 900.000 tons of fish oil are produced annually in the world (IFFO, 2016). Fish oil is primarily used in industry as raw material for pharmaceutical and food supplements due to its protective and healing properties. It is also considered as an additive in the feed industry.

The importance of DHA and EPA fatty acids in human nutrition is a widely accepted fact today (Tocher et al., 2019). Polyunsaturated fatty acids (PUFA) have been recognized to have specific pharmacological and physiological effects on humans (Siscovick et al., 1995). The changing climate conditions of the world bring drought. Therefore, to keep their civilization sustainable, food resources must be presented to consumption with the right policies. It is possible to add omega-3 rich fish oils to foods without any fish taste by using high refining or microencapsulated fish oils (Karabulut & Yandı, 2006). In parallel with the technological developments in this field, it is necessary to increase the quality criteria for obtaining fish oil.

Fish oil is one of the raw materials used in fish feeds and contains the high omega 3 fatty acids such as EPA and DHA. Fatty acid composition determined in fish meat obtained by aquaculture is reported to be related to the fatty acid composition in the feed (Farkas, 1984). In fish farming, it is desirable to have a high proportion of EPA and DHA in the fatty acid composition of fish meat. (Torstensen et al., 2005).

The amount of EPA and DHA decreases in fish oil and cooked fish during storage, the freshness of raw materials was important in fish fatty acid composition, PUFA and ω3 ratio decreased during storage of fish products, and polyunsaturated fatty acids were higher than saturated fatty acids. Polyunsaturated fatty acids were determined to be rapidly oxidized (Bayraklı, 2015). Because of this feature, fish meal and oil sector are developed in the Black Sea Region in Turkey. The study was carried out in a fish meal and oil plant in Sinop province during the 2007-2008 fishing season. In this study, the effect of freshness degree of raw material on fatty acid composition of fish oil that processed in fish meal and oil plant was investigated

MATERIAL and METHODS

The average capacity of each fish meal oil factory established in the Black Sea region is 2000 tons/day. For this reason, the factories cannot process the fish coming for the production of fish meal and oil immediately if the continuity of the fish is not possible and the raw material is kept in spare ships, truck containers or collection ponds. Collecting ponds have a capacity of approximately 250 tons. Thus, fish oil samples were taken from the raw material that processed soon at 0, 24, 48, and 72 hours, and then TBA analyses were performed. Fish oil samples were taken from the centrifuge outlet during the processing of raw materials representing each group. The fish oil samples were filled into two 60-gauge lids for each group. The samples kept under chill storage until analyses, then were sent to TUBITAK Marmara Research Center in a styrofoam box together with -80 ºC shocked ice capsules. TBA analysis samples were done for representing each process based on their storage before oil extraction. Samples were homogenized in the laboratory present in the fish meal plant without losing time and the samples were independently analyzed in duplicate on each sampling hour.

Thiobarbituric Acid Value (TBA): The degree of oxidation in fish oils was determined performing thiobarbituric acid (TBA) analysis according to the method described by Tarladgis et al. (1960).

TBA (mg malondialdehyde (MA)/kg) = (Sample - Blank) x 7.8

The amount of TBA used in the determination of the degree of oxidation in seafood should be less than 3 mg MA/kg in very good material and not more than 5 in a good material and the consumable limit value should be maximum 7-8 mg MA/kg (Schormüller, 1969).

Fatty Acid Composition: Fatty acid methyl esters were prepared according to the esterification and extraction principle (IUPAC, 1994). According to this method; The fatty acid methyl esters were analyzed by 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 µl of Supelco GP% OV-275 on 100/120 PAW-PMCS.

Lipid Quality İndices: The polyene index (PI) was used as a measure of PUFAs damage (Lubis & Buckle, 1990) and calculated according to the formula below.

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

Lipid quality indices as atherogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic / hypercholesterolemic ratio (h/H) were calculated with the formulas below (Ulbricht & Southgate, 1991; Abrami et al. 1992; Fernández et al., 2007), taking into account the different effects of different fatty acids on human health:

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

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

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

Statistical analysis: The data obtained from three different times were analyzed by one-way analysis of variance (ANOVA) using the SPSS statistical package program (Version 10, SPSS Inc., Chicago, IL, USA), and differences among the means were compared using Duncan's multiple range test. A significance level of 0.05 was chosen and the results were shown as mean values ± SD.

RESULTS and DISCUSSION

The raw materials of the product processed in the fish meal-oil plant in Turkey are anchovy and sprat (Duyar and Bayraklı, 2005). According to previous studies, the quality of the fatty acid and chemical composition of anchovy has reported better than sprat. Among the quality parameters, especially TBA and TVB-N are very essential oxidation parameters for processed seafood (Bayraklı, 2009; Bayraklı, 2015).

In the study, TBA value of fish samples taken from raw material collection ponds were determined as 5.55±1.125 (D0), 8.85±0.324 (D1), 15.76±0.486 (D2), and 21.01±1.347 (D3) mg MA/kg. The difference among the freshness groups was statistically significant ($p < 0.05$). The TBA value of D0 was found within the consumable limit value of 7-8 mg MA/kg reported by Schormüller (1969) for good quality fish, however, the TBA values of other groups exceed the limit value.

Palmitic acid (19.80%) has the highest amount among SFA in all groups, followed by myristic acid (6.52%) and stearic acid (3.60%). The highest SFA ratio among freshness groups was determined in the D3 group, and it was determined that SFA ratios in fish oil increased with aging of the raw material. The difference among the groups was statistically significant ($p < 0.05$) (Table 1).

Among MUFA, oleic acid was the highest rate (13.59%) in all groups followed by palmitoleic acid (5.43%). Although the value increased from 21.36% in the D0 group to 21.66% in the D3 group, the differences among the groups were statistically insignificant ($p > 0.05$) (Table 1).

PUFA values decreased significantly depending on the aging of the raw material ($p<0.05$) and the values of D0, D1, D2 and D3 were found as 32.45%, 32.32%, 32.18% and 31.21%, respectively. Docosahexaenoic acid (DHA, C22: 6n3) was the most concentrated (18.64%) fatty acid among PUFA in all groups, followed by eicosapentaenoic acid (EPA, C20: 5n3) (8.68%). Similarly, omega-3 ratios decreased depending on the aging of raw material and the difference among the groups was statistically significant ($p<0.05$). However, the highest omega-6 ratio was determined in the D2 group. Also, the decrease in the omega-3/omega-6 ratios was found parallel to the decrease in the amount of omega-3 depending on the aging of raw material, and the significant differences were found among the freshness groups ($p < 0.05$). The highest EPA/DHA ratio was calculated in the D0 group (0.48). The PI value (the coefficient of deterioration of PUFA) of D3, was found the lowest among the freshness groups, and there were statistically significant differences among the groups ($p < 0.05$). The mean AI used in the determination of fatty acid quality for human health was found to be 0.86, and the differences observed among freshness groups were not statistically significant ($p > 0.05$). The highest IT value, was determined in the D3 group (0.28) and the value was found to be statistically significant ($p < 0.05$) than those of other freshness groups.

Fish oil is a very important nutrient for human health. Particularly polyunsaturated fatty acids such as EPA and DHA are abundant in seafood. In cases where the fish is not utilized as human consumption during the fishing period, it should be processed with different processing technologies and alternative products such as fish meal-oil.

The TBA value is the secondary lipid oxidation index which measures malondialdehyde (MDA) content. MDA is a secondary oxidation product resulting from the degradation of lipid hydroperoxides formed during the oxidation process of polyunsaturated fatty acids (Fernandez et al., 1997). Bensid et al. (2014) reported that, TBA value of anchovy, stored by freezing in foam boxes, reached to 14.38 mg MA/kg on the 9th day of the storage. In this study, the TBA value of fish oil produced from samples stored 48 h (D2) in fish meal and oil plant fish collection ponds was reached 15.761 mg MA/kg. To stop the rapid deterioration of the anchovy, it is essential to store the product at chill storage after fishing. Both the protection of the cold chain after fishing and the cooling of the collection ponds are essential for the production of quality fish oil. Again, in addition to antimicrobial activity, a process involving the addition of EDTA/BHQ combination and ascorbic acid to delay the destructive oxidation of lipids and fats can be applied (Ghaly et al., 2010).

Table 1. Fatty acid composition (%) of anchovy oil having different raw material freshness.

Fatty acids	Groups				
	D0	D1	D2	D3	Average
C10:0 Capric	0.07±0.002^{ab}	0.07±0.001^{a}	0.07±0.003^{ab}	0.08±0.001^{b}	0.07±0.002
C12:0 Lauric	0.08±0.000^{a}	0.08±0.002^{a}	0.09±0.008^{a}	0.08±0.002^{a}	0.08±0.002
C13:0 Tridecanoic	0.07±0.003^{a}	0.07±0.001^{a}	0.09±0.013^{a}	0.07±0.001^{a}	0.07±0.004
C14:0 Myristic	6.50±0.074^{a}	6.56±0.029^{a}	6.47±0.016^{a}	6.57±0.015^{a}	6.52±0.022
C15:0 Pentadecanoic	1.04±0.002^{a}	1.09±0.029^{a}	1.06±0.002^{a}	1.06±0.002^{a}	1.06±0.008
C16:0 Palmitic	19.73±0.018^{a}	19.77±0.016^{a}	19.75±0.030^{a}	19.92±0.030^{b}	19.80±0.030
C17:0 Heptadecanoic	1.65±0.007^{a}	1.69±0.003^{b}	1.71±0.001^{b}	1.69±0.002^{b}	1.69±0.009
C18:0 Stearic	3.61±0.002^{a}	3.56±0.018^{ab}	3.54±0.006^{b}	3.69±0.004^{c}	3.60±0.022
C20:0 Arachidic	0.66±0.001^{a}	0.64±0.045^{a}	0.61±0.010^{a}	0.72±0.004^{a}	0.66±0.018
C21:0 Henicosanoic	0.21±0.001^{a}	0.13±0.018^{b}	0.11±0.008^{b}	0.12±0.001^{b}	0.14±0.016
C22:0 Behenic	0.20±0.000^{a}	0.21±0.011^{a}	0.21±0.010^{a}	0.21±0.000^{a}	0.21±0.003
C23:0 Tricosanoic	0.10±0.002^{a}	0.10±0.004^{a}	0.13±0.001^{b}	0.10±0.002^{a}	0.11±0.005
C24:0 Lignoceric	0.21±0.001^{a}	0.21±0.009^{a}	0.22±0.004^{a}	0.17±0.000^{b}	0.20±0.007
∑ SFA	34.13±0.084^{a}	34.17±0.127^{ab}	34.06±0.029^{a}	34.48±0.057^{b}	34.21±0.069
C14:1 Myristolec	0.20±0.003^{a}	0.29±0.006^{b}	0.21±0.002^{a}	0.20±0.001^{a}	0.22±0.015
C16:1 Palmiteloic	5.44±0.002^{a}	5.43±0.006^{a}	5.26±0.008^{b}	5.60±0.013^{c}	5.43±0.045
C17:1 c Heptadecenoic	0.59±0.001^{a}	0.59±0.003^{a}	0.59±0.008^{a}	0.62±0.001^{b}	0.60±0.005
C18:1n9t Elaidic	0.13±0.001^{a}	0.15±0.019^{a}	0.11±0.004^{a}	0.14±0.001^{a}	0.13±0.007
C18:1n9c Oleic	13.67±0.008^{a}	13.56±0.282^{a}	13.55±0.035^{a}	13.59±0.012^{a}	13.59±0.057
C20:1n9 cEicossenoic	0.72±0.001^{a}	1.05±0.020^{b}	0.96±0.003^{c}	0.90±0.003^{c}	0.91±0.046
C22:1n9 Erucic	0.33±0.001^{a}	0.37±0.002^{b}	0.36±0.009^{b}	0.32±0.003^{a}	0.35±0.007
C24:1n9 Nervonic	0.95±0.004^{a}	0.99±0.025^{a}	0.97±0.004^{a}	0.96±0.000^{a}	0.97±0.008
∑ MUFA	21.36±0.012^{a}	21.73±0.22^{a}	21.29±0.026^{a}	21.66±0.033^{a}	21.51±0.083
C18:2n6t Linoleaidic	0.28±0.003^{a}	0.29±0.006^{a}	0.26±0.009^{a}	0.28±0.006^{a}	0.28±0.005
C18:2n6c Linoleic	0.29±0.001^{a}	0.27±0.013^{b}	0.26±0.001^{b}	0.28±0.002^{a}	0.28±0.005
C18:3n6 γ -Linolenic	1.92±0.005^{a}	2.03±0.009^{a}	2.06±0.002^{a}	1.95±0.011^{a}	1.99±0.021
C18:3n3a-Linolenic	0.15±0.001^{a}	0.14±0.005^{a}	0.16±0.007^{a}	0.16±0.005^{a}	0.15±0.003
C20:2 c Eicosadienoic	0.23±0.001^{a}	0.27±0.026^{a}	0.27±0.002^{a}	0.24±0.001^{a}	0.25±0.008
C20:3n3 α Eicosatrienoic	0.09±0.002^{a}	0.09±0.002^{a}	0.09±0.005^{a}	0.10±0.007^{a}	0.09±0.002
C20:4n6 Arachidonic	0.14±0.001^{a}	0.13±0.012^{a}	0.14±0.007^{a}	0.11±0.004^{a}	0.13±0.006
C22:2 Docosadienoic	0.87±0.004^{a}	0.84±0.003^{a}	0.85±0.013^{a}	0.85±0.003^{a}	0.85±0.005
C20:5n3 EPA	9.06±0.008^{a}	8.57±0.004^{b}	8.59±0.019^{b}	8.53±0.002^{b}	8.68±0.082
C22:6n3 DHA	18.75±0.006^{a}	18.99±0.081^{a}	18.80±0.038^{a}	18.03±0.055^{b}	18.64±0.139
∑ PUFA	32.45±0.012^{a}	32.32±0.043^{ab}	32.18±0.071^{b}	31.21±0.040^{c}	32.04±0.185

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. D0: Fresh material, D1: 1 day old material, D2: 2 days old material, and D3: 3 days old material.

Table 2. Fatty acid ratios and lipid quality indexes.

Fatty acids	Groups				
	D0		D0		D0
Total fatty acids	87.95±0.084^{ab}	88.22±0.137^{a}	87.53±0.126^{b}	87.35±0.050^{b}	87.76±0.135
Unidentified	12.05±0.084^{ab}	11.78±0.136^{a}	12.47±0.126^{b}	12.65±0.050^{b}	12.24±0.135
∑MUFA/∑SFA	0.63±0.001^{a}	0.64±0.012^{a}	0.62±0.001^{a}	0.63±0.001^{a}	0.63±0.006
∑PUFA/∑SFA	0.95±0.004^{a}	0.95±0.007^{a}	0.94±0.002^{a}	0.90±0.004^{b}	0.94±0.020
∑ω3	28.85±0.018^{a}	28.64±0.083^{ab}	28.45±0.083^{b}	27.62±0.070^{c}	28.39±0.499
∑ω6	3.60±0.001^{a}	3.68±0.022^{b}	3.73±0.018^{b}	3.59±0.013^{a}	3.65±0.062
ω3/ω6	8.00±0.008^{a}	7.78±0.069^{b}	7.63±0.014^{b}	7.70±0.048^{b}	7.78±0.154
ω6/ω3	0.12±0.001^{a}	0.13±0.001^{a}	0.12±0.001^{a}	0.13±0.001^{a}	0.13±0.002
EPA/DHA	0.48±0.001^{a}	0.45±0.002^{b}	0.46±0.001^{b}	0.47±0.002^{c}	0.47±0.014
AI	0.85±0.008^{a}	0.85±0.008^{a}	0.85±0.001^{a}	0.88±0.003^{a}	0.86±0.011
TI	0.28±0.001^{a}	0.28±0.001^{a}	0.28±0.001^{a}	0.29±0.001^{b}	0.28±0.006
PI	1.41±0.003^{a}	1.39±0.004^{ab}	1.39±0.001^{b}	1.33±0.007^{c}	1.38±0.031
h/H	1.76±0.008^{a}	1.74±0.019^{a}	1.74±0.001^{a}	1.69±0.006^{b}	1.73±0.028

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, PI: Index of Polyene,AI: Atherogenicity index and TI: Thrombogenicity index. Different letters (a,b,c) in the same fraction shows significant differences ($p < 0.05$). D0: Fresh material, D1: 1 day old material, D2: 2 days old material, and D3: 3 days old material.

Öksüz et al., (2009) reported that SFA value in the Black Sea anchovy was 35.41%, and palmitic acid (19.19%), myristic acid (7.21%) and stearic acid (4.82%) among SFA were dominant. Durmus (2018) has investigated fatty acids compositions of 13 different fish species, and he found that SFAs ranged between 27.68% and 36.59%. Also he stated

that the predominant SFA was palmitic acid in all species examined. Öksüz & Özyılmaz (2010) determined that the SFA ratios of monthly caught Black Sea anchovy during the 2008-2009 fishing season were between 33.40% and 37.91%, respectively. Yuneva et al. (2019), investigated fatty acid concentrations of anchovy between 2006 and 2013, and they found that palmitic acid, which changed between 33.1% (2009) and 39% (2012) values, was dominant in all fatty acids. Gencbay and Turhan (2016) reported that the amount of SFA in the Black Sea anchovy was 38.67%. In the present study, it was found that palmitic acid constitutes 57.88% of the SFA (34.21%). Among the freshness groups, the highest SFA was found in the D3 group and a statistically significant difference was found among the groups ($p < 0.05$). These evaluations and results are similar to our study.

Öksüz et al. (2009) reported that the value of MUFA in the Black Sea anchovy is 29.46%, and oleic acid is the most dominant fatty acid among MUFA. Öksüz and Özyılmaz (2010) determined that the ratio of MUFA in the Black Sea anchovy is between 25.91% and 31.51%, and oleic acid is the most characteristic fatty acid among MUFA. Yuneva et al. (2019) reported that the MUFA value in the Azov sea anchovy ranged from 31.2% to 39.7%, and that oleic acid was dominant. Gencbay and Turhan (2016) reported that the amount of MUFA in the Black Sea anchovy was 23.32%. In the research conducted, MUFA value was determined as 21.51%, and the predominant MUFA in all samples was oleic acid (63.17%). It was determined that the MUFA ratio increased and decreased in fish freshness groups according to ageing time of samples. Some researchers have reported that the time-dependent change of the total amount of MUFA is irregular and may change according to fish species, feeding area, season, processing temperature and storage time (Castro et al., 2007; Öksüz et al., 2009.; Öksüz & Özyılmaz, 2010; Durmus, 2018).

The importance of PUFA in human and animal nutrition is increasing day by day. Various studies have shown that consumption of foods containing long-chain ω3 fatty acids such as EPA and DHA is associated with a reduced risk of coronary heart disease (Harper & Jakobson, 2005) and cancer (Roynette et al., 2004). The recommended minimum PUFA/SFA ratio was 0.45 (HMSO, 1994), while the mean ratio was 0.94 in all groups. This value is above the recommended value for human health. The fish oil obtained from the anchovy of the Black Sea was found to have a nutritious structure for human health. The PUFA ratio in the Black Sea anchovy was found between 23.00% and 38.01% in various studies (Öksüz et al., 2009; Öksüz & Özyılmaz, 2010; Gencbay & Turhan, 2016; Yuneva et al., 2019). In the same studies, it was emphasized that Black Sea anchovy is a valuable product in terms of EPA (4.9-11.55%) and DHA (14.03-20.05%). In this study, the PUFA, EPA, and DHA values were 32.04%, 8.68%, and 18.64%, respectively. Although the obtained values were generally determined in the ranges of result obtained by other researchers, some of them were found to be low and high. This difference is thought to be due to fish species (Durmus, 2018), feeding area (Öksüz et al., 2009), season (Öksüz & Özyılmaz 2010), processing temperature and storage time (Castro et al., 2007). In addition, fatty acid composition of fish can be variable depending on factors such as gender, location and environmental conditions (Özoğul et al., 2007).

Besides, it was found that the amount of total PUFA decreased with storage time, and the difference among the groups was statistically significant ($P \leq 0,05$). This can be attributed to the rapid oxidation of PUFA compared to other fatty acid groups (Castro et al., 2007). Considering the health benefits of the fatty acids, it has been reported that those fed with omega-3 rich products are less likely to develop hypertension or other cardiovascular diseases (Su et al., 1996). It has been emphasized that drugs used in the treatment of obesity and cardiovascular diseases can be reduced by decreasing the ω6 ratio in diets and increasing the 3 ratios (Simopoulos et al., 2000). In the first studies on fatty acids, the ratio of ω6 / ω3 was 1: 1, but with the development of industrial society, this ratio increased to 1:30 to 1:50 with changing feeding habits. The World Health Organization reported that the ω6/ω3 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, ω6/ω3 ratio of 0.13 indicates that anchovy caught in the Black Sea seems to be healthy food and can provide a good balanced omega-3 fatty acids (Table 2).

In our study, anchovy caught in the Black Sea were found to have a fatty acid composition rich in omega-3 and omega-6. To obtain high-quality fish oil, it was determined that the raw material should be processed fresh. It is necessary to investigate the fatty acid change until the product in fish oil stores is sold. It is very important for human health that fish oil produced in fish flour and oil plant according to feed law is produced according to food law and used in human consumption.

It is reported that the atherogenic (AI) and thrombogenic (TI) indices that are higher than (>1.0) is harmful to human health (Ouraji et al., 2009). If this value gets lower, the risk of coronary heart disease decreases (Cutrignelli et al., 2008). Çağlak and Karslı (2017), found TI value between 0.22-0.31 and AI value between 0.38-0.49 in zander fish (*Sander lucioperca*). In the present study, the average TI and AI values were determined as 0.28, and 0.86, respectively. In light of this data, it was determined that there were no risks for human health.

The h/H ratio of fatty acids is the indicator of whether the fat in the product is nutritionally adequate (Çağlak and Karslı, 2017). In this study, the h/H ratio was determined 1.73 and this value was found lower compored to the data of study conducted (2.17-2.77) by Çağlak and Karslı (2017). The fatty acid composition can be variable depending on fish species (Özoğul et al., 2007).

As a result, anchovy, which was catched in the Black Sea, is quite rich in polyunsaturated fatty acids.

According to the results of TBA analysis in raw material freshness groups, it is considered that fish oil obtained by the immediate processing of anchovy coming to fish meal and oil plants is suitable for human consumption, however, fish oil obtained from anchovy processed after 24 hours is not suitable for this purpose. In addition, it was found that the amount of polyunsaturated fatty acids obtained in the fish oil were seen to be positively correlated with the freshness of the processed raw anchovy material.

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