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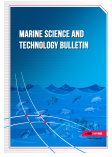

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RESEARCH ARTICLE

A study on fatty acid composition and quality indicators of anchovy (*Engraulis encrasicolus*) oils from different factories

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ABSTRACT

This study aims to investigate the impact of anchovy (*Engraulis encrasicolus*) oil produced in different factories on the fatty acid composition and quality indicators. The study utilizes anchovy oils obtained from three different factories. Fatty acid analysis was conducted using gas chromatography, and the results were expressed as percentages. Additionally, fatty acid quality indices such as atherogenic index (AI), thrombogenic index (TI), Polyene index (PI), and hypocholesterolemic/hypercholesterolemic ratio (h/H) were calculated. The findings of the study indicate that different processing technologies may influence the fatty acid composition of anchovy oil. Anchovy oils were observed to be rich in polyunsaturated fatty acids (PUFA) and notably contain omega-3 fatty acids such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). This study provides valuable insights into anchovy oil production and quality, offering an in-depth understanding of sustainable nutrition. In conclusion, this study sheds light on a significant issue in the anchovy oil industry and may guide researchers and industry experts interested in improving the quality of fish oil products and supporting human health with potential opportunities.

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Introduction

Fish oil plays a pivotal role in both human health and various industrial applications (Bayrakli, 2021a; Yildiz et al., 2023). In recent times, the significance of omega-3 fatty acids, particularly DHA and EPA, in human nutrition has gained widespread recognition (Bayrakli et al., 2019; Bayrakli & Duyar, 2019a, 2019b, 2021a, 2021b; Bayrakli, 2021b; Duyar & Bayrakli, 2023). Polyunsaturated fatty acids (PUFAs) are well known to have specific pharmacological and physiological effects on humans (Singer & Calder, 2023).

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Fish oil is a highly valuable product produced in fish meal and oil factories, with approximately 18 million tons of fish being processed in these facilities worldwide annually (FAO, 2020). However, the sustainability of this sector is being questioned due to environmental factors and overfishing. Additionally, climate change is increasing drought conditions globally, impacting food resources. Therefore, to sustain civilizations, it is imperative to implement proper food policies (Bayrakli & Duyar, 2019b).

Fish oil is used as a crucial raw material in pharmaceuticals and dietary supplements, primarily owing to its preservative and healing properties. It is also a key component in the production of fish feeds, containing high levels of omega-3 fatty acids such as EPA and DHA. The fatty acid composition found in fish meat obtained through aquaculture has been linked to the composition of fatty acids in their feed (Truzzi et al., 2023). Consequently, it is desirable for fish raised in farms to have high levels of fatty acids, especially EPA and DHA (Santigosa et al., 2023). In the machines designed for fish meal and oil, firstly it is cooked and then pressed and the watery and solid parts are separated. the watery part is separated from the oil in the separator and refined and stored.

This study aims to investigate the impact of different processing technologies used in various factories on the quality and composition of products derived from anchovy (*Engraulis encrasicolus*). Furthermore, it seeks to evaluate the contributions of anchovy oils fatty acid values and calculated fatty acid quality indices to the literature. This research will deepen the understanding of fish oil production and quality, providing valuable insights for sustainable nutrition.

Material and Method

Material

This study was conducted in three different fish meal and oil factories located between the cities of Sinop and Samsun in the Black Sea region of Türkiye. Each of these factories has a daily average capacity of 2000 ton/day, forming the core material of the study. Fish oil samples were obtained from these factories in November 2022 during the processing of raw materials. The fish oil samples were filled into two 60-gauge lids for each group and stored in cold conditions to preserve their quality.

For laboratory analysis, the samples were sent to the Sinop University Scientific and Technological Research Application and Research Center. This center is equipped with a laboratory infrastructure that ensures reliable analysis procedures in compliance with standardized protocols.

Fatty Acid Composition

Fatty acid methyl esters were prepared according to the esterification and extraction principle (IUPAC, 1994). To esterify, 0.150 g of fish oil was weighed in a volumetric flask and 5 ml of methanolic 0.5 N NaOH was added on top of it. Then, accompanied by a cooler, it was saponified by boiling in a water bath for 15 m with boiling chip addition. After flowing 5 ml of BF3 reagent over the cooler, it was boiled for 5 more minutes and 2 ml of heptane was added. Then, after another 1 minute of boiling, the cooler was removed and the sample was gently taken into a 25 ml volumetric flask. The flask was rinsed with saturated NaCl, and the resultant solution was also added. 1-2 ml of liquid was taken via micropipette from the upper heptane phase and transferred to a glass bottle with a test tube. A few crystalline anhydrous Na_2SO_4 was thrown into the bottle, 1 µl of this solution was injected into Shimadzu gas chromatography (GC) and the fatty acid composition was determined (Erickson, 1993).

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 µm, GC Columns Restek) was used as the chromatographic colon. GC operation conditions were as follows; injection mode: split ratio, split: 1/10, injection and detector temperature: 260 and 280°C, carrier gas and column flow: helium and 1.4 ml min-1, temperature program: initial temperature 5 m 100°C, 5°C increase per minute from 100°C to 150°C, 15 m at 150°C, 10°C increase per minute to 210°C, and 20 m at 210°C.

Peaks in gas chromatography by using the "Supelco 37 Component FAMEs Mix" standard. In obtaining data, methyl esters of fatty acids were calculated as the percentage of total fatty acids. The spectrum includes all commonly known fatty acid methyl esters.

According to this method, the fatty acid methyl esters were analysed 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 Indices

Polyene index (PI) was used as a measure of PUFAs damage (Lubis & Buckle, 1990) and calculated according to the Equation 1:

$$PI = \frac{[EPA(C20{:}5n3)+DHA(22{:}6n3)]}{Palmitic\ acid(C16{:}0)} \quad (1)$$

AI, TI and h/H were calculated using the following equations (Ulbricht & Southgate, 1991; Abrami et al., 1992; Bayrakli & Duyar, 2019b; Bayrakli, 2021b; Duyar & Bayrakli, 2023) taking into account the different effects of different fatty acids on human health (Equations 2-4):

$$AI = \frac{(12{:}0+4(14{:}0)+16{:}0)}{\sum MUFA+\sum PUFA} \quad (2)$$

$$TI = \frac{[(14{:}0+16{:}0+18{:}0)]}{0.5(\sum MUFA)+0.5(n6PUFA)+3(n3PUFA)+\left(\frac{n3PUFA}{n6PUFA}\right)} \quad (3)$$

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

Statistical Analysis

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

Results and Discussion

In this study, it was aimed to investigate the ΣSFA, ΣMUFA, ΣPUFA fatty acid content and AI, TI, PI, h/H quality indices of fish oils obtained from anchovy. The findings revealed the ΣSFA content of samples collected from three different factories. The average ΣSFA values were calculated as follows: Factory A, 34.25%±0.561; Factory B, 35.22%±0.188; and Factory C, 34.87%±0.374 (Table 1). There was no statistically significant difference among these values ($p>0.05$).

Atherogenic index (AI), thrombogenic index (TI), polyene index (PI), and hypocholesterolemic/hypercholesterolemic ratio (h/H) were calculated. The findings of the study indicate that different processing technologies may influence the fatty acid composition of anchovy oil. Anchovy oils were observed to be rich in polyunsaturated fatty acids (PUFA) and notably contain omega-3 fatty acids such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid)

However, a noteworthy outcome observed in the present study is that specific SFAs such as palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0) were more dominant in certain factories. Palmitic acid was found to be the highest in Factory A at 15.54%±0.296, while stearic acid was most prominent in Factory C at 6.93%±0.157. Similarly, myristic acid significantly varied, with Factory B recording 5.78%±0.035. Statistically significant differences were observed among groups for these SFAs ($p<0.05$). The results of this study demonstrate that the SFA content of fish oils obtained from anchovies can vary depending on different factors such as the processing temperature and duration in the factories, as well as the freshness of the raw materials used in the process. These findings appear to be consistent with previous research. For instance, a study by Bayrakli & Duyar (2019b) examined the ΣSFA content of anchovy oils and similarly found that palmitic acid (19.80 %) was the most abundant in all groups, although this study (reported higher levels of palmitic acid. Furthermore, a study by Duyar & Bayrakli (2023) investigated the ΣSFA content of fish oils obtained from salmon internal organs and found similarities with the ΣSFA content of anchovy oils. Particularly, the conclusion that palmitic acid is the highest in ΣSFA aligns with this study (Öksuz et al., 2009; Oksuz & Ozyilmaz, 2010; Gencbay & Turhan, 2016; Durmuş, 2019; Yuneva et al., 2019).

In this study, we focused on the ΣMUFA (monounsaturated fatty acid) content, which was found to be 23.23%±0.949, 23.77%±0.066, and 23.50%±0.055 in the A, B and B factories, respectively. There was no statistically significant difference among these values ($p>0.05$). Oleic acid (C18:1 n9) was identified as the predominant ΣMUFA in all groups, with percentages of 14.30%, 14.75%, and 14.77%, respectively. These results indicate similarities in ΣMUFA content among the factories.

This finding is in accordance with previous research, such as the study of Bayrakli & Duyar (2019b) study, which examined the ΣMUFA content in anchovy oil and similarly identified oleic acid (C18:1 n9) as the predominant fatty acid in all groups. Previous studies have also reported similar ΣMUFA values and identified oleic acid as the dominant component (Durmuş, 2019; Gencbay & Turhan, 2016; Öksuz et al., 2009; Oksuz & Ozyilmaz, 2010; Yuneva et al., 2019). These consistent findings suggest that the ΣMUFA content of anchovy oil remains relatively stable across different factories and that oleic acid is a characteristic component of this oil. In line with these results, a study by Duyar and Bayraklı (2023) also reported a

Table 1. Fatty acid values (%) of fish oils obtained from 3 different factories

Fatty acid	A Factory	B Factory	C Factory
C10:0	0.04±0.000	0.04±0.006	0.05±0.012
C11:0	0.02±0.006	0.02±0.000	0.02±0.006
C12:0	0.15±0.006	0.18±0.000	0.17±0.006
C13:0	0.12±0.006	0.15±0.000	0.14±0.006
C14:0	5.35±0.084^{a}	5.78±0.035^{b}	5.65±0.046^{b}
C15:0	1.76±0.031	2.06±0.025	2.02±0.026
C16:0	15.54±0.296^{b}	14.85±0.115^{ab}	14.56±0.357^{a}
C17:0	2.03±0.036	2.17±0.021	2.10±0.006
C18:0	6.03±0.110^{c}	6.60±0.032^{b}	6.93±0.157^{a}
c20:0	1.97±0.026	2.10±0.021	1.90±0.032
C21:0	0.17±0.010	0.15±0.000	0.18±0.012
C22:0	0.06±0.000	0.07±0.01	0.09±0.015
C23:0	0.04±0.036	0.06±0.017	0.08±0.026
C24:0	0.97±0.015	0.99±0.020	0.97±0.057
C14:1	0.68±0.015	0.82±0.015	0.81±0.006
C15:1	0.39±0.010	0.48±0.021	0.47±0.015
C16:1	1.84±0.035	0.79±0.006	0.73±0.015
C17:1	1.40±0.012	1.54±0.017	1.49±0.015
C18:1n9c	14.30±0.183^{a}	14.75±0.070^{b}	14.77±0.044^{b}
C18:1n9t	1.26±1.218	2.58±0.021	2.32±0.061
C20:1	1.05±0.540	0.70±0.015	0.75±0.021
C22:1n9	0.52±0.168	0.38±0.006	0.39±0.006
C24:1	1.79±0.026	1.74±0.010	1.78±0.047
C18:2n6c	4.48±0.060^{a}	4.66±0.015^{b}	4.52±0.047^{a}
C18:2n6t	1.19±0.020	1.29±0.006	1.22±0.025
C18:3n3	3.68±0.061	3.86±0.006	3.82±0.023
C18:3n6	1.10±0.021	1.20±0.010	1.09±0.020
C20:2	1.14±0.017	1.17±0.020	1.16±0.020
C20:3n3	0.18±0.006	0.17±0.006	0.99±0.676
C20:3n6	2.80±0.081	2.62±0.102	3.13±0.256
C20:4n6	2.43±0.026	2.59±0.021	2.50±0.020
C20:5n3	9.29±0.095^{a}	9.61±0.140^{b}	9.36±0.040^{a}
C22:2	0.34±0.006	0.34±0.015	0.32±0.020
C22:6n3	15.89±0.106^{b}	13.47±0.042^{a}	13.49±0.388^{a}
ΣSFA	34.25±0.561^{a}	35.22±0.188^{a}	34.87±0.374^{a}
ΣMUFA	23.23±0.949^{a}	23.77±0.066^{a}	23.50±0.055^{a}
ΣPUFA	42.52±0.420^{b}	41.00±0.152^{a}	41.61±0.406^{a}
EFA	25.18±0.200^{b}	23.08±0.159^{a}	22.85±0.359^{a}
UNSFA/SFA	1.92±0.048^{a}	1.84±0.015^{a}	1.87±0.030^{a}
PUFA/SFA	1.24±0.011^{b}	1.16±0.010^{a}	1.19±0.024^{a}
DHA/EPA	1.71±0.007^{b}	1.40±0.020^{a}	1.44±0.046^{a}
omega-3	29.04±0.256^{b}	27.11±0.166^{a}	27.67±0.804^{a}
omega-6	12.00±0.150^{a}	12.37±0.067^{a}	12.46±0.359^{a}
omega-3 / omega-6	2.42±0.013^{b}	2.19±0.021^{a}	2.22±0.128^{a}
omega-6 / omega-3	0.41±0.002^{a}	0.46±0.004^{b}	0.45±0.026^{ab}
omega-9	16.08±1.269^{a}	17.70±0.072^{a}	17.47±0.025^{a}

Note: ΣSFA: Saturated fatty acid, ΣMUFA: Monounsaturated fatty acid, ΣPUFA: Polyunsaturated fatty acid, EFA: EPA+DHA. Different letters (a,b,c) in the same row shows significant differences ($p<0.05$) among the freshness groups.

high concentration of oleic acid with no statistically significant differences among groups. The stability and consistency of ΣMUFA content in anchovy oil, with oleic acid as the dominant fatty acid, underscore the potential for this oil to serve as a valuable and consistent source of monounsaturated fats for various industrial and nutritional applications.

In this study, the polyunsaturated fatty acid (PUFA) values exhibited variation among the different factories. The ΣPUFA content was calculated as follows: Factory A, 42.52%; Factory B, 41.00%; and Factory C, 41.61%. Statistical analysis did not reveal any significant differences between these values ($p>0.05$). Dominant PUFAs were identified as Docosahexaenoic acid (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3). DHA was found to be present at levels of 15.89%, 13.47%, and 13.49%, respectively, while EPA was determined at 9.29%, 9.61%, and 9.36%. Omega-3 fatty acids exhibited similarity among factories, while omega-6 fatty acids were recorded at 12.00%, 12.37%, and 12.46%, respectively. The omega-3/omega-6 ratio was calculated as 2.42, 2.19, and 2.22 for the three factories, respectively.

The importance of polyunsaturated fatty acids, particularly long-chain omega-3 fatty acids such as EPA and DHA, in human and animal nutrition has been increasing over time. Various studies have indicated their role in the treatment of coronary heart disease, COVID-19, and organ damage when taken through diets (Singer & Calder, 2023). Additionally, there is evidence suggesting that the consumption of these fatty acids can reduce the risk of cancer (Gheorghe et al., 2022).

The PUFA/SFA ratio in the fish oils obtained from factories ranged from 1.19 to 1.64 (Table 2). This value significantly exceeds the recommended minimum value of 0.45 for human health (HMSO, 1994). It demonstrates the nutritive quality of fish oil obtained from anchovies in terms of human health. These findings highlight the nutritional value of fish oils obtained from anchovies, particularly their high PUFA/SFA ratio. This is of great importance in the context of dietary recommendations and public health, as these fatty acids are associated with various health benefits, including cardiovascular health and the potential prevention of chronic diseases. Further research in this area can provide valuable insights into optimizing fish oil production for human consumption.

Previous studies have highlighted the variability in ΣPUFA content of Black Sea anchovy oil, ranging from 23.00% to 38.01%, with valuable proportions of EPA (4.9%–11.55%) and DHA (14.03%–20.05%) (Öksuz et al., 2009; Oksuz & Ozyilmaz, 2010; Gencbay & Turhan, 2016; Durmuş, 2019; Yuneva et al., 2019). The work of Bayrakli & Duyar (2019b) supports these findings.

EPA and DHA play a fundamental role in the prevention and treatment of numerous diseases, and since fish oil contains high levels of EPA and DHA, the sum of EPA + DHA is used to assess nutritional quality. In this study, EPA + DHA levels were determined to be 25.18%, 23.08%, and 22.85% in the respective factories. Considering the well-established potential health benefits of EPA and DHA in human metabolism, governmental and medical institutions worldwide recommend the regular consumption of approximately 500 mg/day of EPA + DHA to reduce the risk of cardiovascular diseases (Biandolino et al., 2023). Based on the results obtained from this study, fish oils obtained from the factories would meet the recommended amount for reducing the risk of cardiovascular diseases when added at 2 g to 100 g of food.

Atherogenic (AI) and thrombogenic (TI) indices, when exceeding 1.0, have been reported to be detrimental to human health (Ouraji et al., 2009). Lowering these values reduces the risk of coronary heart disease (Cutrignelli et al., 2008). According to the results of this study, AI values were similar among factories, calculated as 0.56 for Factory A, 0.59 for Factory B, and 0.57 for Factory C. These values were within the recommended limits for human health. Notably, in the study of Bayrakli & Duyar (2019b) study found an AI value of 0.86, and in the study of Duyar & Bayrakli (2023) study, this value decreased to 0.36. Similarly, TI values were also similar among factories, calculated as 0.25 for Factory A, 0.27 for Factory B, and 0.26 for Factory C. These values were acceptable for human health. in the study of Bayrakli & Duyar (2019b) study, the TI value was 0.28, and in the study of Duyar & Bayrakli (2023) study, it was also determined as 0.28. Additionally, in the study of Karsli (2021), AI values ranged from 0.11 to 0.70, and TI values ranged from 0.01 to 0.36, indicating no significant risk to human health.

Table 2. Calculated fatty acid quality indices

Index	A Factory	B Factory	C Factory
AI	0.56	0.59	0.57
TI	0.25	0.27	0.26
PI	1.62	1.55	1.57
h/H	2.50	2.53	2.61

Note: AI: atherogenic index, TI: thrombogenic index, PI: Polyene index, h/H: hypocholesterolemic/ hypercholesterolemic ratio

Polyene index (PI) can provide a meaningful tool to measure the oxidative stability of fish oils. A high PI value is

preferred. PI values varied among factories, measuring 1.62 for Factory A, 1.55 for Factory B, and 1.57 for Factory C in this study. In the study of Bayrakli & Duyar (2019b) study, the PI value was 0.95, and in the study of Duyar & Bayrakli (2023) study, it was calculated as 1.38.

The hypocholesterolemic/hypercholesterolemic ratio (h/H) of fatty acids is an indicator of whether the oil in the product is nutritious (Caglak & Karsli, 2017). A high h/H ratio signifies that the oil in the product is suitable for nutrition. The composition of fatty acids can vary depending on the fish species (Ozogul et al., 2013). h/H values also varied among factories, calculated as 2.50 for Factory A, 2.53 for Factory B, and 2.61 for Factory C. These values indicate that the products are suitable for nutrition. In the study of Bayrakli & Duyar (2019b) study, the h/H value was 1.73, and in the study of Duyar & Bayrakli (2023) study, it was recorded as 2.90.

Conclusion

This study has demonstrated some variations in the quality and composition of anchovy fish oils obtained from different factories. However, it is important to note that these differences are generally not statistically significant. This study focused on evaluating the quality of products obtained from factories that carry out anchovy fish oil production using different processing technologies.

While the saturated fatty acid content was similar between factories, specific SFAs (palmitic acid, stearic acid, and myristic acid) were observed to be more dominant in the factories. Monounsaturated fatty acid content also exhibited similarity among factories, with oleic acid being identified as the dominant MUFA. Polyunsaturated fatty acid content varied among factories, but essential PUFAs like EPA and DHA were found at similar levels. The EPA + DHA levels were indicative of these fish oils being a valuable source of nutrition for human health.

Atherogenic, thrombogenic indices and hypocholesterolemic/hypercholesterolemic ratio were within acceptable limits for human health, indicating the high quality of these fish oils. Polyene index (PI) values reflected good oxidative stability.

This study provides significant insights into anchovy fish oil production and quality. The results underscore the value of this product as a valuable resource for human nutrition and industrial applications. In the future, further research and development efforts will be needed to enhance the sustainability and quality of anchovy fish oil production.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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