

Effects of cooking mahi-mahi (*Coryphaena hippurus*) fish fillets with different techniques on their phospholipid and triacylglycerol fatty acid composition

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SUMMARY: In this study, the fatty acid (FA) composition in phospholipid (PL) and triacylglycerol (TAG) fractions of mahi-mahi fish fillets cooked with different methods was investigated. Compared to the triacylglycerol fraction, 16:0, 18:0, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3, 22:6 ω 3, Σ SFA and $\Sigma\omega$ -3 PUFA were found to be higher in the phospholipid fraction. However, 18:1 ω 9, 18:2 ω 6 and $\Sigma\omega$ -6 PUFA were found to be low. The ω -3/ ω -6 ratio in the phospholipid fraction of mahi-mahi fillets, both raw and cooked using different methods (frying with sunflower oil, frying with olive oil, frying with corn oil, frying with hazelnut oil, oven, grill, microwave and steamed), was found to be significantly higher than triacylglycerol. 22:6 ω 3, Σ PUFA and $\Sigma\omega$ -3PUFA levels were higher in the phospholipid fraction of oven-cooked fillets compared to the control fillets. In the triacylglycerol fraction, the ω -3/ ω -6 value, one of the important nutritional parameters, was found to be higher in the microwave-cooked fillets than in oven-cooked, grilled or steamed fillets.

KEY WORDS: Cooking methods; Fatty acid; Mahi-mahi; Phospholipid; Triacylglycerol; Vegetable oils; ω -3/ ω -6.

Resumen: Efectos del cocinado de filetes de pescado dorado (*Coryphaena hippurus*) con diferentes técnicas sobre su composición en ácidos grasos de fosfolípidos y triacilglicéricos. En este estudio, se investigó la composición de ácidos grasos (AG) en las fracciones de fosfolípidos (PL) y triacilglicérol (TAG) de filetes de pescado dorado cocinados con diferentes métodos de cocción. En comparación con la fracción de triacilglicérol, se encontró que los ácidos grasos 16:0, 18:0, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3, 22:6 ω 3, así como Σ SFA y $\Sigma\omega$ -3 PUFA eran más altos en la fracción de fosfolípidos. Sin embargo, se encontró que los ácidos 18:1 ω 9, 18:2 ω 6 y $\Sigma\omega$ -6 PUFA eran bajos. La relación ω -3/ ω -6 en la fracción de fosfolípidos de los filetes de pescado, tanto crudos como cocinados con diferentes métodos de cocción (fritos con aceite de girasol, con aceite de oliva, con aceite de maíz, con aceite de avellana, al horno, parrilla, microondas y al vapor), resultó ser significativamente mayor que en la fracción de triacilglicérol. Los niveles de 22:6 ω 3, Σ PUFA y $\Sigma\omega$ -3PUFA fueron mayores en la fracción de fosfolípidos de los filetes cocinados al horno en comparación con los filetes de control. En la fracción de triacilglicérol, la relación ω -3/ ω -6, uno de los parámetros nutricionales importantes, resultó ser mayor en los filetes cocinados al microondas que en los filetes cocinados al horno, a la parrilla o al vapor.

PALABRAS CLAVE: Aceites vegetales; Ácidos grasos; Fosfolípidos; Mahi-mahi; Métodos de cocción; Triacilglicérol; ω -3/ ω -6.

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1. INTRODUCTION

Fish is a food source that is consumed all over the world because of its high protein, PUFA (polyunsaturated fatty acid), and vitamin and mineral contents (Abraha *et al.*, 2018). Mahi-mahi fish (*Coryphaena hippurus*), consumed in the Mediterranean region, is a marine product that has economic and commercial value along with high nutritional quality. Therefore, it is important to know the fatty acid profiles of fish with economic and commercial value in order to reveal the nutritional value of fish. The fat content and FA composition of fish lipids vary depending on different biotic and abiotic factors such as season, feed type and amount, fish size, age, water temperature, pH, salinity and reproductive cycle (Kaushik *et al.*, 2006; Cengiz *et al.*, 2012; Kaçar *et al.*, 2023a). Fatty acids play an important role in energy storage and transport, as primary metabolic fuels in metabolism, as membrane components, and as gene regulators. In addition to providing polyunsaturated fatty acids, which are precursors of eicosanoids, dietary lipids are also important for thermal insulation and mechanical protection (Rustan *et al.*, 2005). The lipids in fish meat mainly consist of PL, TAG, sterols, and small amounts of their metabolic products, which are glycolipids and sulfolipids. Triacylglycerol is the storage lipid in almost all fish species. As the lipid content in the muscles increases, the TAG content also increases (Drazen, 2007). The benefits of fish consumption are mainly attributed to the effects of ω -3 PUFA, which have been reported to have a number of benefits for human health. The fatty acid composition of marine fish makes it a delicious and high-quality food product because it provides high protein and fat content and contains fatty acids such as EPA and DHA (Suganthi *et al.*, 2015). Fish oil contains ω -3 fatty acids, which have been shown to protect against hypertension, cardiovascular disease, depression, cancer, and other disorders (Kaçar *et al.*, 2023b). Therefore, PUFAs are necessary to maintain optimal health. For example, DHA (docosahexaenoic acid) and arachidonic acid (AA) are the major long-chain PUFAs in the brain. Since they are important components of neuron membranes, they help nerve cells communicate with each other, which is important for maintaining mental health. DHA is essential for the structure and function of all membranes, especially nerves

and muscles. Both EPA (eicosapentaenoic acid) and DHA are important for the cardiovascular system. In particular, EPA is a component of eicosanoids that contribute to the anti-inflammatory response. These messengers affect blood pressure, blood clotting, immune function, and allergic response. There is also suggestive evidence that an early intake of ω -3 fatty acids has a beneficial effect on children's cognitive development (IFFO, 2017; Osendarp, 2011). Different cooking methods also affect the FA composition of fish (oven-baking, microwaving, frying, poaching, boiling, and grilling etc.). Cooking is the process of applying heat to foods for a certain period of time to improve properties such as flavor and taste. Oven-baking, a dry heat cooking technique, is one of the most commonly used. Oven cooking is the best way to preserve the nutritional value and PUFA contents in cooked fish. Steaming fish meat results in nutritional benefits which are similar to consuming raw fish meat.

The purpose of the frying process is to increase digestibility by changing the colors, shapes and structures of food, in order to make it desirable and edible, to inactivate enzymes and pathogenic microorganisms, to reduce water activity and to extend the shelf-life of food (Garcia-Arias *et al.*, 2003). Because of their nutritional value and durability, vegetable oils are frequently used in frying (Bansal *et al.*, 2010). In the food industry and at home, frying is a common technique for enhancing the flavor and shelf-life of fish, giving it a distinctive sensory quality, and enhancing the quality of the food product. Frying produces aldehydes, furans, ketones, alcohols, and acids, which contribute to the aroma of oils and products (Zarulakmam *et al.*, 2021). As the number of frying cycles increases, color, taste, odor, juiciness, appearance, and overall acceptability are reduced (Tadesse *et al.*, 2020). Flavor acceptability decreases due to the synthesis of free FA compounds in the oxidation process of lipids and proteins; whereas odor acceptability decreases due to the formation of peroxides and free radical compounds (Ketaona *et al.*, 2013). Discoloration may be caused by polymerization reactions at high temperatures (Idun-Acquah *et al.*, 2016). Triacylglycerol dimers and polymers, as well as non-volatile polar molecules, are the main degradation products of frying oil during polymerization. Increased levels of hydroperoxides and polymer molecules in

food may provide health risks to consumers (Jurid *et al.*, 2020). For this reason, frying parameters such as frying cycle, frying time, frying temperature, frying oil and frying technique should be controlled as they can change the oil content, lipid fractions and FA profiles of fish lipids. Fish frying techniques, raw fish lipid content, and frying oil composition all have a major impact on these variations (Moradi *et al.*, 2011). Possible mechanisms for changes occurring during the cooking process include moisture loss in the food, the leakage of fat-soluble molecules from the food, and oxidation reactions with free radicals produced in hot cooking oil (Little *et al.*, 2000). In addition, digestibility increases due to protein denaturation during cooking, but the contents of thermolabile compounds and polyunsaturated fatty acids generally decrease (Finot 1997). PUFAs such as EPA and DHA are considered to be particularly susceptible to oxidation during heating and other cooking processes (Loughrill *et al.*, 2016). Up to now, studies have generally been conducted on the effect of different cooking methods on the total fatty acid composition of freshwater or marine fish. However there is no study on the fatty acid composition of triacylglycerol, and phospholipids in fish. The aim of this study is to investigate the effects of different cooking methods on the fatty acid composition in the PL and TAG fractions of *C. hippurus*.

2. MATERIALS AND METHODS

In this research, mahi-mahi (*Coryphaena hippurus*) sea fish caught with a fishing line in the Karatepe Bay of the Aydıncık district of the Mersin province in September 2021 was used. Three fish weighing 300-400 grams were brought to the laboratory on a cold chain, and each fish was divided into four fillet pieces. The fillets were kept in the laboratory at -25 °C until analysis (day 1). The fish fillets were cooked using eight different cooking techniques (Sunflower oil, Olive oil, Corn oil, Hazelnut oil, Oven, Grill, Microwave and Steamed) and uncooked (raw) fillets were determined as the standard. In the frying process, olive oil, corn oil, sunflower oil and hazelnut oil were used. The fillets were cooked in hot oil for three minutes on each side once the oil in the pan had risen to a high temperature. The other cooking processes (Oven, Grill, Microwave and Steamed) were carried out three times. The fish fillets cooked by

cooking methods other than frying were evaluated according to their ability to maintain a constant internal temperature of 75 °C during the cooking process (USDA, 2023). For oven baking, heating in a pre-heated conventional oven at 180 °C for 30 min was used (center temperature was 73.6 ± 0.5 °C). Cooking was carried out in the microwave oven ((2450 MHz 500 W) for 6 minutes (the average temperature after cooking was 88.3 ± 1 °C). During steaming, the fillet did not come into contact with water, only the steam of the water was used. Steaming occurred at 100 °C for 45 minutes (center temperature was 92.5 ± 2 °C). In the grill cooking method, both sides of the fillet were cooked over a coal fire for 8 minutes.

2.1. Lipid extraction and transmethylation of fatty acids

To determine the FA composition of the samples, after the fillets were disintegrated and homogenized in 2:1 chloroform-methanol (Folch *et al.*, 1957), the lipid part was removed using 0.88% KCl to wash and facilitate the lipid fraction separation, and then the solvent was evaporated in the evaporator. After this process, the remaining lipid was weighed and the total lipid amount was determined. Then, 4 ml of methanol and 4-5 drops of sulfuric acid were added to the lipid and heated at 85 °C for 2 hours (Stanley-Samuelson and Dadd, 1983), thus converting the fatty acids into methyl esters. The mixtures were extracted three times with 5 mL hexane.

2.2. PL and TAG separation

Thin-layer chromatography (TLC) was used to fractionate the total lipids in the tissue samples. For the PL and TAG fractions, the plates were activated by being kept in an oven at 100 °C for an hour after being dried in the air. PL and TG were separated using thin-layer chromatography (TLC) using a mobile phase composed of petroleum ether, diethyl ether, and acetic acid (80:20:1 by volume). In order to make the lipid fractions visible under the UV lamp, 2',7'-dichlorofluorescein was sprayed onto the plates, 4 ml of methanol and 4-5 drops of sulfuric acid were added and heated at 85 °C under reflux for two hours. Then, the cooled solution was extracted with five ml hexane three times and the methyl esters were extracted. A gas chromatography device with an FID detector was used to analyze the methyl esters.

The fatty acid methyl ester analysis was conducted using a GC 2010 Plus gas chromatograph with a flame ionization detector and a DB-23 capillary column. The flow rates of compressed air and hydrogen were 400 mL/min and 30 mL/min, respectively. The carrier gas was helium at a flow rate of 0.5 mL/min. The injection port and detector temperatures were 250 °C. Split ratio was 1:20. The oven temperature was programmed at an initial temperature of 170 °C, kept constant for 2 min, and then to risen from 170 °C to 210 °C at a rate of 2 °C/min.

2.3. Statistical analysis

The percentages of fatty acids were compared using the SPSS 22 computer program. The analysis was carried out three times. The mean value and standard deviation (mean±SD) of the results are provided. Analysis of variance (ANOVA) was used to examine fatty acids, and Tukey's test was used to compare means. The 't' test was used to compare the fatty acid percentages, moisture, and total lipids of the cooked fillets to the raw fillets, and Duncan's (1955) "Multiple Range" test was used to evaluate the differences between the averages. Significant differences between means were defined as $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. FA composition in PL fractions of mahi-mahi fried with different vegetable oils

The major SFA; 16:0, was found to be 15.98% in the fish fried in corn oil, 16.41% in sunflower oil, 19.77% in olive oil and 21.52% in hazelnut oil. In the raw fish, it was 22.03%. The SFA percentage was found to be at its highest value in the raw fish, 37.08%. It was found to be 37.01% in the fish fried with olive oil, 30.78% with hazelnut oil, 28.70% with sunflower oil, and 26.70% with corn oil. 18:1 ω -9, which is the major component among MUFAs, was determined as 32.67% in hazelnut oil, 23.22% in sunflower oil, 21.60% in corn oil and 17.30% in olive oil. 18:2 ω -6, one of the ω -6 PUFAs, was found in high amounts in sunflower (19.98%) and corn oil (13.61%). DHA was highest in the raw fish (37.59%). It was determined as 32.76% in the fish fried with olive oil, 29.19% in corn oil, 23.0% in hazelnut oil and 21.34% in sunflower oil. It was observed that the phospholipid fatty acid contents in the fillets fried

in different vegetable oils changed. For example, 18:1 ω 9 and Σ MUFA was determined in the fillets fried in hazelnut oil compared to both the control and other oils; percentages of 18:2 ω 6, Σ PUFA and $\Sigma\omega$ -6 PUFA increased in those fillets fried in sunflower and corn oils. However, 16:0 and Σ SFA levels decreased significantly in the samples fried in corn oil and sunflower oils (Table 1). When the control fillets were compared to the deep-fried fillets, 20:5 ω 3, 22:5 ω 3, 22:6 ω 3, and the ω 3/ ω 6 ratio were significantly higher. 18:1 ω 9, Σ MUFA and 18:2 ω 6 levels were found to be lower. As stated in previous studies, this is because vegetable oils do not contain such fatty acids and some vegetable oils are rich in 18:2 ω 6 and some are rich in 18:1 ω 9 (Akgül and Başhan, 2023). The ω 3/ ω 6 ratio, which was found to be 7.96 in the raw samples, was 1.03 in sunflower oil, 4.99 in olive oil, 1.87 in corn oil and 1.15 in hazelnut oil (Table 1). According to these data, among the dietary fats, the ω 3/ ω 6 ratio was found to be highest in olive oil and lowest in the fillets fried in sunflower oil. (Table 1).

Phospholipids, which are polar lipids, are important structural components of cell membranes and eicosanoids (De Leonardis and Macciola, 2004). Few data were available on the effects of the frying process on the PL composition in fish and shellfish tissues (Boselli *et al.*, 2012; Liu *et al.*, 2019). SFA and PUFA levels, as well as their important components, increased in corn oil-fried tissues, but MUFA varied similarly in Tunisian clams in PL (Bejaoui *et al.*, 2019). Such SFA increases could be attributed to the oil composition, which was identified as high in SFA, particularly C16:0 and C18:0. The rise in PUFA was associated with higher levels of ω 6 PUFA, AA, and C18:2 ω -6 (Bejaoui *et al.*, 2019). In our study, PUFA also increased.

In comparison to the other conditions, clams fried in olive oil had a minimal impact. While there were slight variations in the SFA and PUFA compounds, there were no substantial differences compared to the fresh tissues. There was a small increase in MUFA in the tissues fried with olive oil (Bejaoui *et al.*, 2019). Similarly, in our study, SFA did not change, while MUFA increased slightly. All of this information showed that the fatty acid contents in the samples fried in various oils varied, and the FA composition in the PL fractions of the fillets fried in oils differed from that of the raw fish.

TABLE 1. Fatty acid composition in the phospholipid fractions of mahi-mahi fried in different vegetable oils and raw mahi-mahi*

Fatty acid	Raw	Sunflower oil	Olive oil	Corn oil	Hazelnut oil
12:0**	0.29±0.02b	0.00±0.00d	0.13±0.01c	0.35±0.02b	0.43±0.03a
14:0	1.05±0.08a	0.45±0.03c	0.47±0.03c	0.29±0.02d	0.88±0.06b
15:0	0.66±0.05a	0.25±0.02c	0.38±0.03b	0.18±0.01d	0.37±0.03b
16:0	22.03±1.75a	16.41±1.30b	19.77±1.57b	15.98±1.27b	21.52±1.71a
17:0	1.32±0.10a	0.63±0.05b	1.58±0.12a	1.02±0.08a	0.64±0.05b
18:0	12.45±0.98a	10.96±0.87a	14.68±1.16a	8.88±0.70b	6.94±0.55c
∑SFA***	37.80±3.00a	28.70±2.28c	37.01±2.94a	26.70±2.12c	30.78±2.44b
16:1 ω7	0.56±0.04c	0.86±0.06b	1.34±0.10a	1.40±0.11a	0.66±0.05c
18:1 ω9	13.49±1.07c	23.22±1.84b	17.30±1.37c	21.60±1.71b	32.67±2.59a
20:1 ω9	0.11±0.01b	0.11±0.01b	0.23±0.02a	0.22±0.02a	0.13±0.01b
∑MUFA	14.16±1.12c	24.19±1.92b	18.87±1.50c	23.22±1.84b	33.46±2.65a
18:2 ω6	1.07±0.08e	19.98±1.59a	2.75±0.21d	13.61±1.08b	7.00±0.55c
18:3 ω6	0.07±0.01b	0.17±0.02a	0.15±0.02a	0.13±0.02a	0.20±0.02a
18:3 ω3	0.05±0.01e	0.19±0.02c	0.25±0.02b	0.42±0.03a	0.12±0.01d
20:2 ω6	0.26±0.02b	0.10±0.01c	0.33±0.03a	0.31±0.03a	0.06±0.01d
20:3 ω6	0.05±0.01b	0.12±0.01a	0.14±0.01a	0.10±0.01a	0.05±0.01b
20:4 ω6	3.97±0.31a	2.70±0.21b	3.98±0.31a	3.24±0.25a	2.50±0.18b
20:5 ω3	3.16±0.24a	1.92±0.15c	2.95±0.22a	2.29±0.18b	1.96±0.16c
22:5 ω3	1.74±0.15a	0.53±0.04c	0.74±0.06b	0.77±0.05b	0.78±0.06b
22:6 ω3	37.59±2.98a	21.34±1.69c	32.76±2.60b	29.19±2.32b	23.00±1.79c
∑PUFA	47.96±3.62b	47.05±3.58b	44.05±3.42c	50.06±3.91a	35.67±2.81d
∑ω-3 PUFA	42.61±3.28a	23.98±1.81d	36.70±2.72b	32.67±2.34c	25.86±1.76d
∑ω-6 PUFA	5.35±0.41d	23.07±1.79a	7.35±0.72d	17.39±1.26b	9.81±0.89c
ω3/ω6	7.96±0.61a	1.03±0.07e	4.99±0.34b	1.87±0.14d	2.63±0.19c
∑PUFA/∑SFA	1.26±0.10c	1.63±0.12b	1.19±0.10c	1.87±0.12a	1.15±0.10c

*Means are the average of 3 replicates. ** Values reported are means±standard deviation; means followed by different letters in the same line are significantly different ($p < 0.05$) by Tukey's test. ***SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

3.2. FA composition in the PL fractions of mahi-mahi cooked by methods other than frying

The FA 16:0 and 18:0, the major components, did not differ much according to different cooking methods. It was determined that 18:1 ω -9, which is the most abundant MUFA, was found at its lowest level in oven cooking (9.95%) and at its highest level in grill cooking (13.65%). DHA, one of the ω -3 PUFAs, was found at the highest level in oven cooking (44.47%), followed by steaming (42.49%), and microwave cooking (42.12%). In the raw samples and in mahi-mahi fillets cooked by methods other than frying, SFAs, 16:0 and 18:0 MUFAs, 18:1 ω 9 among PUFAs, 22:6 ω 3 fatty acids were found to be dominant. However, cooking methods other than frying changed the FA composition in the PL fractions of mahi-mahi fish. In oven-cooked samples, microwave and steaming methods, it was observed that the percentages of 16:0, Σ SFA, 18:1 ω 9, Σ MUFA, 22:6 ω 3, Σ PUFA, Σ ω -3 PUFA, Σ ω -6 PUFA, the ω 3/ ω 6 ratio and PUFA/SFA ratios were quite similar (Table 2). The 16:0, Σ SFA, 18:1 ω 9 and Σ MUFA levels in the grilled fillets were higher than those cooked using the oven, microwave or steaming methods. However, 22:6 ω 3, Σ PUFA, Σ ω -3 PUFA percentages and PUFA/SFA ratios were determined to be lower (Table 2). In samples cooked with the control and grill methods, it was observed that the 16:0, Σ SFA, 22:6 ω 3, Σ PUFA, Σ ω -3 PUFA percentages and the PUFA/SFA ratio were close to each other. In fish cooked using the oven, microwave and steam methods, 16:0 and Σ SFA levels were lower than in the raw and grilled fish. It was determined that the percentages of 22:6 ω 3, Σ PUFA, Σ ω -3 PUFA and the PUFA/SFA ratio were significantly higher (Table 2). The ω 3/ ω 6 ratio was found to be 8.00 in the raw fillets; while the values were found to be close to each other in the grill (7.43), microwave (7.44) and steaming (7.73) methods. However, in the oven cooking method (9.73), this ratio increased slightly (Table 2). Similar results were obtained in previous studies. DHA, especially among ω 3 PUFAs, exhibited the highest heat resistance in oven-baked fish. Cooked with this method, the phospholipid fatty acid profile was not affected in anchovy, sardine or sprat fish (Farabegoli *et al.*, 2019). Because phospholipids show better resistance to oxidation compared to triacylglycerols from the same source (Adkins and

Kelley 2010), it has often been observed that cooking produces significant changes in the percentages of fatty acids depending on season and species. In anchovy fish, while 16:0 and Σ SFA increased, EPA, DHA and Σ ω -3 PUFA decreased by baking the fish in the oven in autumn. In sardine fish, 16:0 and Σ SFA increased with oven cooking in spring and autumn. Σ MUFA decreased significantly with oven cooking in autumn; while EPA, DHA, Σ ω -3 PUFA and Σ PUFA decreased in sardine fillets caught in both spring and autumn seasons. There was no significant difference in the PL fraction of sprat fish in The major and other components of fish caught in winter and spring by oven cooking. The PL fraction of horse mackerel increased 16:0 and Σ SFA, EPA, DHA, Σ PUFA and Σ ω -3 PUFA slightly due to oven cooking, while 18:1 ω -9 and Σ MUFA slightly increased in autumn-caught fish (Farabegoli *et al.*, 2019). As reported by other researchers, the response to baking is species-specific (García-Arias *et al.*, 2003; Schneedorferová *et al.*, 2015). In baked sardines, there were significant increases in the percentages of MUFA and Σ n-6 PUFA in the autumn (Farabegoli *et al.*, 2019).

The levels of 22:6 ω -3, Σ PUFA, Σ ω -3 PUFA were found to be higher in the oven-baked fillets than in the control fillets. These data show that baking and grilling, which are thermal processes, do not adversely change the phospholipid FA composition in the cells or the organelle membranes of fish fillets. Cell membranes in fish are resistant to heat treatment. Similar results were determined in the previous study. Among the ω -3 PUFAs, DHA exhibited the highest heat resistance in oven-baked fish. The phospholipid FA profile was not affected in anchovies, sardines or sprats cooked by this method (Farabegoli *et al.*, 2019). As noted by Adkins and Kelley (2010), phospholipids also have better resistance to oxidation than triacylglycerols from the same source. These data, especially those other than grilling, where thermal processes are applied, showed that oven and microwave cooking and steaming methods changed the phospholipid composition of mahi-mahi.

3.3. FA composition in the TG fractions of mahi-mahi fried with different vegetable oils

The 16:0 ratio was determined as 31.42% in raw fish, 14.51% in olive oil, 12.91% in corn oil, 12.13%

TABLE 2. Fatty acid composition in the phospholipid fractions of mahi-mahi fillets and raw fillets cooked with different methods*

Fatty acid	Raw	Oven	Grill	Microwave	Steamed
12:0**	0.23±0.01a	0.00±0.00b	0.00±0.00b	0.26±0.01a	0.00±0.00b
14:0	0.44±0.02a	0.40±0.02a	0.36±0.01a	0.52±0.03a	0.43±0.02a
15:0	0.39±0.02a	0.24±0.01b	0.34±0.02a	0.34±0.02a	0.23±0.01b
16:0	22.87±1.81a	19.45±1.67a	20.95±1.74a	19.53±1.68a	17.40±1.38b
17:0	1.93±0.15a	1.55±0.12a	1.38±0.09a	1.62±0.13a	0.31±0.02b
18:0	13.20±1.04b	12.41±0.86b	15.13±1.15a	12.77±0.92b	15.13±1.15a
∑SFA***	39.06±3.10a	34.05±2.77b	38.16±3.03a	35.04±2.86b	33.50±2.79b
16:1 ω7	0.68±0.05b	0.99±0.07b	0.85±0.06b	1.18±0.12a	1.55±0.15a
18:1 ω9	10.92±0.93b	9.95±0.73b	13.65±1.07a	10.95±0.93b	11.40±0.90b
20:1 ω9	0.26±0.02a	0.10±0.01b	0.14±0.01b	0.13±0.01b	0.05±0.00c
∑MUFA	11.86±0.87a	11.04±0.79a	14.64±1.14a	12.26±0.97a	13.00±1.03a
18:2 ω6	1.22±0.09b	1.08±0.08b	1.67±0.14a	1.44±0.11a	1.58±0.12a
18:3 ω6	0.09±0.01b	0.10±0.01b	0.14±0.01a	0.16±0.01a	0.06±0.01c
18:3 ω3	0.14±0.01a	0.15±0.01a	0.10±0.01b	0.12±0.01b	0.10±0.01b
20:2 ω6	0.26±0.01a	0.25±0.01a	0.21±0.01a	0.24±0.01a	0.05±0.00b
20:3 ω6	0.03±0.00c	0.10±0.01b	0.12±0.01b	0.22±0.01a	0.28±0.01a
20:4 ω6	3.86±0.30a	3.58±0.26a	3.53±0.25a	4.18±0.33a	4.15±0.32a
20:5 ω3	2.91±0.23a	3.02±0.23a	2.70±0.19a	3.07±0.21a	2.90±0.21a
22:5 ω3	1.09±0.13c	2.09±0.16a	0.83±0.06b	1.13±0.11b	1.82±0.14a
22:6 ω3	39.54±3.12b	44.47±3.53a	38.51±3.05b	42.12±3.28a	42.49±3.29a
∑PUFA	49.14±3.76b	54.84±3.85a	47.81±3.46b	52.68±3.21a	53.43±3.36a
∑ω-3 PUFA	43.68±3.29b	49.73±3.60a	42.14±3.14b	46.44±3.62a	47.32±3.98a
∑ω-6 PUFA	5.46±0.41a	5.11±0.39a	5.67±0.43a	6.24±0.52a	6.12±0.47a
ω3/ω6	8.00±0.63a	9.73±0.73a	7.43±0.52a	7.44±0.52a	7.73±0.53a
∑PUFA/∑SFA	1.25±0.09b	1.61±0.10a	1.25±0.10b	1.50±0.08a	1.59±0.05a

*Means are the average of 3 replicates. ** Values reported are means±standard deviation; means followed by different letters in the same line are significantly different ($p < 0.05$) by Tukey's test. ***SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

in hazelnut oil and 8.63% in sunflower oil. The 18:1 ω -9 ratio was found to be highest in olive oil (67.61%), followed by hazelnut oil (56.57%), sunflower oil (34.38%) and corn oil (26.11%). Therefore, the highest MUFA rate was determined in frying with olive oil (68.72%). 18:2 ω -6 was 48.93% in sunflower oil, 48.32% in corn oil, 18.01% in hazelnut oil, 10.76% in olive oil and 3.04% in the raw fish. DHA was found in the highest amount (19.80%) in the raw fish. It decreased to 4.47% in hazelnut oil. It was determined as 2.47% in corn oil, 1.94% in sunflower oil and 1.29% in olive oil. Since 18:2 ω -6 is high in sunflower and corn oils, PUFAs were found to be high in fish fried in these oils. In the triacylglycerol fraction of raw samples, which was compared to fillets fried separately with different vegetable oils, the percentages of SFAs such as 16:0, 18:0, Σ SFA, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3, 22:6 ω 3 and $\Sigma\omega$ 3 PUFA were higher. The levels of 18:1 ω 9, Σ MUFA and 18:2 ω 6 were determined to be lower (Table 3). The ω 3/ ω 6 ratio, which was 0.05 in the samples fried with sunflower oil, 0.16 in olive oil, 0.07 in corn oil, and 0.32 in hazelnut oil, showed a significant increase in the control and was found to be 3.32 (Table 3). The fish fried in sunflower oil had lower 16:0 and Σ SFA percentages, while fillets fried in corn oil had lower 18:1 ω 9 and Σ MUFA percentages. 18:2 ω 6, Σ PUFA, $\Sigma\omega$ -6 PUFA and the PUFA/SFA ratio were higher in the samples cooked in olive oil and hazelnut oil. The fish fried with olive oil was found to have significantly higher percentages of 18:1 ω 9 and Σ MUFA compared to the other frying methods. (Table 3). Neutral lipids showed a different trend due to cooking processes compared to polar lipids. Neutral lipids; SFA, MUFA and $\Sigma\omega$ -6 PUFA contents were quite stable or could even be found to be concentrated in the cooked fillets because of water loss. However, the tendency of $\Sigma\omega$ -3 PUFA was similar to polar lipids (Farabegoli *et al.*, 2019).

3.4. FA composition in the TG fractions of mahi-mahi cooked by methods other than frying

The 16:0 was found to be 23.62% in the microwave cooking process, 23.86% in steaming, 31.94% in grilling, and 34.37% in the oven. The amount of 18:0 did not differ much among the cooking techniques. SFA was determined to be highest in the oven (54.97%) and the lowest in steaming (37.46%).

The amount of 18:1 ω -9 did not differ in oven, grill, or microwave cooking (9.74-11.86%). It was found to be high in steaming (21.87%). DHA was found to be (35.85%) in microwave cooking, (26.12%) in raw fish, (24.27%) in grilled, (20.98%) in steamed, and (19.41%) in baked fish. In the fillets cooked by methods other than frying, 16:0 and 18:0 among SFAs, 18:1 ω 9 among monounsaturated fatty acids, and 22:6 ω 3 among PUFAs were determined as dominant components. 16:0 and Σ SFA levels in the fillets cooked with microwave and steaming methods were lower than in the samples cooked with the control and other cooking methods; the percentage of 18:1 ω 9, Σ MUFA and 18:2 ω 6 was higher in those cooked by steaming. It was determined that 20:5 ω 3, 22:6 ω 3, Σ PUFA and $\Sigma\omega$ -3 PUFA were significantly higher in the fillets cooked in the microwave (Table 4). The value of ω 3/ ω 6, an important nutritional parameter, was higher in the raw samples than the oven-cooked fillets, grilled or steamed fillets. However, it was found to be lower than those cooked in the microwave. Hence, it can be said that cooking methods other than frying change the fatty acid composition in the triacylglycerol fractions of mahi-mahi fillets. It was observed that the FA composition in the PL and TG fractions of fillets fried in different vegetable oils was different from that of raw fish, and the fatty acid composition of the samples fried in these oils changed. For example, the phospholipid fraction compared to the triacylglycerol fraction is richer in fatty acids such as 16:0, 18:0, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3, 22:6 ω 3, and fatty acid groups such as Σ SFA, $\Sigma\omega$ -3 PUFA. It has been determined that the triacylglycerol fraction is poorer in terms of fatty acids such as oleic acid, linoleic acid and $\Sigma\omega$ -6 PUFA. Because some of the vegetable oils contain very high levels of linoleic acid (in sunflower and corn oil, 18:2 ω 6) and in some of them, oleic acid (in hazelnut oil and olive oil, 18:1 ω 9). Therefore, these fatty acids that pass into the fish fillets during the frying process are collected into the triacylglycerol fraction rather than the phospholipids. The ω -3/ ω -6 ratio in the phospholipid fraction of fish fillets, both raw and cooked with different cooking methods, was found to be significantly higher than the triacylglycerol fraction. These data show that the fatty acid composition of the triacylglycerol fractions in fish is related to dietary fatty acids and that the dominant fatty acids taken from the diet are mostly introduced into the triacylglycerol

TABLE 3. Fatty acid composition in the triacylglycerol fractions of mahi-mahi fried in different vegetable oils and raw mahi-mahi*

Fatty acid	Raw	Sunflower oil	Olive oil	Corn oil	Hazelnut oil
12:0**	1.39±0.11a	0.08±0.00c	0.10±0.01c	0.45±0.03b	0.40±0.03b
14:0	2.09±0.15a	0.24±0.02b	0.18±0.01b	0.30±0.02c	0.32±0.02c
15:0	0.84±0.05a	0.11±0.00c	0.10±0.00c	0.28±0.02b	0.11±0.00c
16:0	31.42±2.49a	8.63±0.61c	14.51±1.20b	12.91±0.91b	12.13±0.83b
17:0	2.44±0.19a	0.15±0.01b	0.24±0.02b	0.23±0.02b	0.10±0.01c
18:0	13.26±1.05a	3.56±0.28c	3.45±0.25c	6.96±0.54b	4.09±0.32c
∑SFA***	51.44±3.90a	12.77±1.01d	18.58±1.50c	21.13±1.62b	17.15±1.12c
16:1 ω7	2.13±0.14a	0.37±0.02c	1.10±0.09b	0.41±0.03c	0.83±0.04b
18:1 ω9	14.94±1.15e	34.38±2.71c	67.61±5.28a	26.11±2.03d	56.57±4.42b
20:1 ω9	0.28±0.02a	0.17±0.01b	0.01±0.00c	0.18±0.01b	0.25±0.02a
∑MUFA	17.35±1.31e	34.92±2.77c	68.72±5.38a	26.70±2.12d	57.65±4.69b
18:2 ω6	3.04±0.23d	48.93±3.81a	10.76±0.90c	48.32±3.76a	18.01±1.48b
18:3 ω6	0.33±0.02a	0.19±0.01b	0.34±0.02a	0.11±0.00c	0.05±0.00d
18:3 ω3	0.57±0.04b	0.26±0.02c	0.26±0.02c	0.76±0.06a	0.33±0.02c
20:2 ω6	0.34±0.02a	0.02±0.00c	0.03±0.00c	0.23±0.02a	0.15±0.01b
20:3 ω6	0.11±0.00a	0.02±0.00d	0.02±0.00d	0.05±0.00c	0.07±0.00b
20:4 ω6	3.39±0.25a	0.30±0.02c	0.21±0.01d	0.39±0.02c	0.68±0.04b
20:5 ω3	2.58±0.19a	0.43±0.03c	0.28±0.02d	0.30±0.02d	1.14±0.09b
22:5 ω3	0.99±0.07a	0.16±0.01b	0.04±0.00c	0.12±0.01b	0.22±0.02b
22:6 ω3	19.80±1.57a	1.94±0.15c	1.29±0.09c	2.47±0.19c	4.47±0.33b
∑PUFA	31.15±2.47b	52.25±4.09a	13.23±1.10d	52.75±4.11a	25.12±2.03c
∑ω-3 PUFA	23.94±1.98a	2.79±0.22c	1.87±0.13d	3.65±0.29c	6.16±0.46b
∑ω-6 PUFA	7.21±0.56d	49.46±4.33a	11.36±0.93c	49.10±4.82a	18.96±1.48b
ω3/ω6	3.32±0.24a	0.05±0.00d	0.16±0.01c	0.07±0.00d	0.32±0.02b
∑PUFA/∑SFA	0.60±0.04d	4.09±0.32a	0.71±0.05d	2.49±0.20b	1.46±0.13c

*Means are the average of 3 replicates. ** Values reported are means±standard deviation; means followed by different letters in the same line are significantly different ($p < 0.05$) by Tukey's test. ***SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

TABLE 4. Fatty acid composition in the triacylglycerol fractions of mahi-mahi fillets cooked with different methods and raw fillets*

Fatty acid	Raw	Oven	Grill	Microwave	Steamed
12:0**	0.67±0.05b	1.86±0.14a	0.50±0.04b	0.46±0.04b	0.00±0.00c
14:0	1.27±0.09a	1.28±0.09a	0.80±0.06b	1.14±0.08a	1.34±0.10a
15:0	0.64±0.05a	0.73±0.06a	0.56±0.04a	0.43±0.04b	0.49±0.05b
16:0	30.01±2.38b	34.37±2.73a	31.94±2.53b	23.62±1.97c	23.86±1.98c
17:0	2.71±0.20a	2.58±0.17a	2.07±0.15a	1.44±0.11b	1.37±0.10b
18:0	12.27±0.96b	14.15±1.12a	12.57±0.99b	11.53±0.91b	10.40±0.82c
Σ SFA***	47.57±3.78b	54.97±4.33a	48.44±3.85b	38.62±3.06c	37.46±2.97c
16: ω 7	1.96±0.15a	1.91±0.13a	1.81±0.11a	0.58±0.04c	0.93±0.07b
18:1 ω 9	11.11±0.88b	11.86±0.91b	9.74±0.77b	10.34±0.89b	21.87±1.73a
20:1 ω 9	0.17±0.01c	0.23±0.02b	0.14±0.01c	0.34±0.03a	0.19±0.01b
Σ MUFA	13.24±1.05b	14.00±1.11b	11.69±0.92b	11.26±0.86b	22.99±1.81a
18:2 ω 6	4.06±0.32c	4.61±0.36c	8.09±0.64b	3.71±0.28c	12.00±0.95a
18:3 ω 6	0.39±0.03a	0.36±0.03a	0.23±0.02b	0.44±0.04a	0.24±0.02b
18:3 ω 3	0.28±0.02b	0.26±0.02b	0.26±0.02b	0.46±0.04a	0.31±0.03a
20:2 ω 6	0.20±0.02a	0.23±0.02a	0.15±0.01a	0.24±0.02a	0.26±0.02a
20:3 ω 6	0.03±0.00c	0.10±0.01b	0.11±0.01b	0.19±0.02a	0.15±0.01b
20:4 ω 6	3.32±0.24a	2.75±0.16a	2.97±0.18a	3.68±0.31a	2.28±0.09b
20:5 ω 3	3.89±0.30b	2.62±0.18b	2.95±0.28b	5.12±0.40a	2.84±0.19b
22:5 ω 3	0.83±0.07b	0.86±0.07b	0.76±0.06b	1.17±0.08a	0.93±0.08b
22:6 ω 3	26.12±2.07b	19.41±1.56c	24.27±1.92b	35.85±2.84a	20.98±1.66c
Σ PUFA	39.12±3.10b	31.20±2.48c	39.79±3.16b	50.86±4.04a	39.99±3.17b
Σ ω -3 PUFA	31.12±2.47b	23.15±1.84d	28.24±2.24c	42.60±3.38a	25.06±1.99d
Σ ω -6 PUFA	8.00±0.63c	8.05±0.64c	11.55±0.91b	8.26±0.67c	14.93±1.18a
ω 3/ ω 6	3.89±0.30b	2.87±0.22c	2.33±0.18c	5.15±0.40a	1.67±0.13d
Σ PUFA/ Σ SFA	0.82±0.06c	0.56±0.040	2.44±0.19a	1.31±0.10b	1.06±0.09b

*Means are the average of 3 replicates. ** Values reported are means±standard deviation; means followed by different letters in the same line are significantly different ($p < 0.05$) by Tukey's test. ***SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

fraction. In the phospholipid fractions of methods other than frying, 22:6 ω 3, Σ PUFA, $\Sigma\omega$ -3 PUFA levels were found to be higher in oven-baked fillets than in the control fillets. This is because the cell membranes in fish resist the heat treatments applied. This determination shows that oven cooking and grilling, which are thermal processes, do not adversely change the phospholipid fatty acid composition in the cell or organelle membranes of mahi-mahi fillets. In triacylglycerol fractions of methods other than frying, 16:0 and Σ SFA levels in fillets cooked with microwave and steaming methods were lower than in samples cooked with the control and other cooking methods; the percentage of 18:1 ω 9, Σ MUFA and 18:2 ω 6 iwas higher in the fillets cooked by steaming. It was determined that 20:5 ω 3, 22:6 ω 3, Σ PUFA and $\Sigma\omega$ -3 PUFA were significantly higher in the microwave-cooked fillets. The value of ω -3/ ω -6, an important nutritional parameters, was found to be higher than fillets cooked by oven, grill or steaming methods. It was found to be significantly higher in the fillets which were cooked in the microwave.

Neutral lipids consist mainly of triacylglycerol storage lipids, used for energy for the maturation of gametes during the breeding season and as a temporary store of polyunsaturated fatty acids that can be delivered to structural lipids or directed to specific metabolic pathways (Varljen *et al.*, 2004). Neutral lipids showed a different behavior as a result of the cooking processes compared to polar lipids. The SFA, MUFA and $\Sigma\omega$ -6 PUFA contents of neutral lipids are quite stable. However, the tendency of $\Sigma\omega$ -3 PUFA is similar to polar lipids (Farabegoli *et al.*, 2019). In a previous study, the neutral lipid Σ SFA increased, EPA, DHA and $\Sigma\omega$ -3 PUFA decreased due to the autumn baking of anchovy fish (Farabegoli *et al.*, 2019). The same findings were detected in the fatty acid composition of the total lipids in lambuca baked in the oven in our study. In our study, as in sardine fish (Farabegoli *et al.*, 2019), 16:0 and Σ SFA increased, EPA, DHA and $\Sigma\omega$ -3 PUFA decreased.

4. CONCLUSIONS

It has been observed that some FAs and FA groups are more dominant in the triacylglycerol fraction of mahi fish fillets when they are fried in different nutritional oils, depending on the type of oil used in the frying process. However, the oils used significantly

changed the FA composition in the triacylglycerol fractions of the fish. It is known that some frying oils contain very high levels of linoleic acid and some of them contain oleic acid. Therefore, these FAs and the FA groups attached to the fatty acids, which pass into the fish fillets during the frying process, are collected into the triacylglycerol fraction rather than phospholipids. It was observed that the FA composition in the PL fractions of fillets fried in corn oil and hazelnut oil was different from that of raw fish, and the FA composition of the samples fried in these oils changed significantly.

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No potential conflict of interest was reported by the author(s).

Ethical approval

All applicable national guidelines for the care and use of animals were followed.

Authorship contribution statement

S. Kaçar: Writing – original draft, Writing – review & editing

M. Başhan: Investigation, Methodology, Project administration

N. Akgül: Formal analysis, Funding acquisition, Writing, Investigation, Methodology

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