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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of different thawing methods on fatty acid composition of cultured sea bass (*Dicentrarchus labrax* Linnaeus, 1758)

Farklı çözündürme yöntemlerinin kültür levreği (*Dicentrarchus labrax* Linnaeus, 1758)'nin yağ asidi kompozisyonu üzerine etkisi

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Abstract: This study aimed to determine the potential changes in the fatty acid composition of frozen cultured sea bass (*Dicentrarchus labrax*) thawed at different environmental conditions. Sea bass fillets were thawed using four different methodologies: refrigerator (+4°C), water (+15°C), microwave (defrost mode) and ambient conditions (22±2°C). Some part of the fish was thawed once (on the 7th and 30th days), and the other part was thawed twice (on the 30th day). Thus, crude lipid analysis and fatty acid composition by gas chromatography were carried out in the thawed sea bass fillets. The results showed negative effects on the fatty acid composition caused by the different thawing methods. The most suitable thawing method was determined as refrigerator thawing, especially on the 30th day 1st thawing according to least loss of the lipid values (9.19±0.18%) and unsaturated fatty acids (C18:2 25.92±0.13%, C20:5 5.56±0.02%, C22:6n-3 8.90±0.09%, ∑PUFA 44.70±0.04%). Samples thawed in water and ambient conditions follow the refrigerator thawing method in terms of lipid and fatty acids. The highest lipid and fatty acid loss was observed in microwave thawing. Although the samples thawed in the refrigerator were better than the other groups in terms of lipid content and fatty acid composition, it is recommended to the consumers that frozen foods should be thawed only once and consumed immediately, and that fish should be frozen according to their needs and thaw as much as they can consume.

Keywords: Sea bass, *Dicentrarchus labrax*, freezing, thawing, fatty acid composition

Öz: Bu çalışmada, dondurma ve farklı ortam şartlarında çözündürme işlemi uygulanan kültür levrek balığının (*Dicentrarchus labrax* Linnaeus, 1758) yağ asidi kompozisyonunda meydana gelen değişimlerin tespiti amaçlanmıştır. Levrek balığı filetoları, buzdolabı (+4°C), su (+15°C), mikrodalga (buz çözme modu) ve ortam şartları (22±2°C)'nda olmak üzere dört farklı ortamda çözündürülmüştür. Balıkların bir kısmı bir (7. ve 30. günde), diğer kısmı ise iki kez (30. günde) çözündürülmüştür. Çözündürülen levrek balığı filetolarında ham yağ ve yağ asitleri kompozisyonu analizleri gerçekleştirilmiştir. Tüm grupların yağ asidi kompozisyonu gaz kromatografi cihazı kullanılarak belirlenmiştir. Elde edilen bulgular farklı ortamlarda birden fazla çözündürmenin yağ asitleri kompozisyonu üzerinde olumsuz etkilere sebep olduğunu ortaya koymuştur. Uygulanan çözündürme tekniklerinden en uygun çözündürme ortamının özellikle 30. gün ilk çözündürme günü ele alındığında yağ değerleri (%9,19±0,18) ve doymamış yağ asitleri sonuçlarında (C18:2 %25,92±0,13, C20:5 %5,56±0,02, C22:6n-3 %8,90±0,09, ∑PUFA %44,70±0,04) en az kayıp görülmesinden dolayı buzdolabında çözündürme yöntemi olduğu sonucuna varılmıştır. Suda ve ortam koşullarında çözündürülen örnekler yağ ve yağ asitleri sonuçları bakımından buzdolabında çözündürme yöntemini takip etmektedir. Yağ ve yağ asidi miktarında en fazla kayıp mikrodalga çözündürme yönteminde gözlenmiştir. Buzdolabında çözündürülen örnekler her ne kadar yağ miktarı ve yağ asitleri kompozisyonu açısından diğer gruplara göre daha iyi olsalar bile tüketicilere dondurulmuş gıdaların sadece bir kere çözündürülerek hemen tüketilmeleri gerektiği ve ihtiyaçları oranında balıkları dondurup, yiyebilecekleri kadar miktarları çözündürmeleri önerilmektedir.

Anahtar kelimeler: Levrek, *Dicentrarchus labrax*, dondurma, çözündürme, yağ asidi kompozisyonu

INTRODUCTION

Seafood is an increasingly important food both in the world and in Turkey. One of the most important factors that determine the quality of food is its digestibility and utilization of its proteins, lipids, carbohydrates, vitamins and minerals by our body. Fish are important food sources with these characteristics. Fish meat is a recommended meal by all health and nutrition experts (Metli, 2006; Murray and Burt, 2011; Chen et al., 2022). Fish have functional effects due to their richness in polyunsaturated fatty acids. As a result of several researches, it has been concluded that dietary supplements containing fish are beneficial in the treatment of many diseases such as cardiovascular diseases, ulcerative colitis and hyperlipidemia

(Chen et al., 2022). The consumption amount of fresh and processed seafood in Turkey is quite low, especially compared to European countries. However, animal protein intake is insufficient in our country. Fish meat and other seafood are important for human nutrition due to their contents of both high-quality protein and especially long-chain unsaturated fatty acids (Gülyavuz and Ünlüsayın, 1999; Anonymous, 2019). In recent years, the Republic of Turkey Ministry of Agriculture and Forestry has been taking steps to increase aquaculture production and export. The main products of our aquaculture exports are sea bass, seabream, rainbow trout and tuna. A large part of our export consists of fresh and chilled fish (Anonymous, 2014).

Freezing is one of the most preferred methods to store fish and other seafood. Although low temperatures protect the food shelf life and quality, incorrect applications can cause negative effects on the quality. If the necessary conditions are not followed, quality losses may occur in fish tissue due to physical, chemical and enzymatic changes, which adversely affect the consumer's preferences (Abraha et al., 2018). The factors mostly causing these quality losses can be storage temperature, freezing and thawing times, temperature changes, incorrect freezing and thawing processes. Various studies demonstrated that multiple freezing and thawing processes lead to physiological and biochemical damage in muscle systems, such as nutrient loss, lipid oxidation, protein denaturation, and hydrolysis (Wu et al., 2021). Food preservation methods aiming to offer fish to consumers under healthier and hygienic conditions can protect the products. Although this, quality losses especially in terms of physical and sensory characteristics may occur in these products due to incorrect applications of the consumers. Bozkır et al. (2014) stated that thawing foods using traditional methods may have some negative effects such as microorganisms development or oxidation (and accordingly color changes). Although the negative effects of traditional methods, consumers use these thawing methods at home in order to save time and consume the products quickly (Konak et al., 2009).

The fact that unconscious storage processes in small businesses and fish shops have a negative effect on the quality of fish is one of the most important reasons why consumers avoid to buy fish. This study aimed to determine the effects of incorrect/unconscious freezing and different thawing processes on the fatty acid composition of sea bass, a species highly consumed in our country and in the world and of very high economic value. Thus, the study evaluated the changes in the amount of lipid and fatty acid composition occurring during the thawing of the fish in different conditions (refrigerator, ambient conditions, water and microwave) after the storage process in the -18°C freezer, which is frequently used in homes and plants.

MATERIAL AND METHODS

Study area and stations

Twenty kg of sea bass (50 fish) with an average weight of 350±20 g and lenght of 30±3 cm, farmed in offshore cage systems in Milas, Bodrum (Turkey), were used for the experiments. Samples were taken from the fish farms in Milas, Bodrum, and brought to Muğla Sıtkı Koçman University, Faculty of Fisheries, Quality Control Laboratory within 1 hour in cold chain conditions with styrofoam boxes in ice.

Methods

The sea bass samples were divided into groups of 5 and stored in refrigerator bags at -18°C. Lipid of fish was extracted before the fresh fish were taken into storage, and methylation was performed to determine the initial fatty acid composition. After 7 days of storage, fish were thawed (7th day 1st thaw) in four different conditions (refrigerator (4±1°C), ambient conditions (22±2°C), water (15±2°C) and microwave (defrost mode). Lipid and fatty acid analyses were applied to some of the thawed fish, and the remaining sea bass were re-frozen. After 30 days of storage, this re-frozen fish group (30th day 2nd thaw) and the fish group that stored for a month (30th day 1st thaw) were thawed with these four different thawing methods. After the 30th day thawing processes, lipid was extracted and the fatty acid composition was determined using gas chromatography for the groups of 30th day thawing.

Crude lipid analysis

The samples were analyzed in triplicate for lipid content following the Bligh and Dyer (1959)'s method. A mixture of methanol and chloroform (1:2, v/v, 100 ml) was added to 5 g of the fish sample (fish/solvent, 1:20, w/v) and homogenized. Then, 20 ml of 0.4% $CaCl_2$ solution was added onto the samples. The samples were filtered on a filter paper into the tared balloons kept in the oven at 105ºC for 2 h. These balloons were kept in a dark environment overnight and the upper layer consisting of methanol+water was separated with the help of a separation funnel. Chloroform from the chloroform+lipid part in the solution remaining in the flask was evaporated using a rotary evaporator (Heidolph) with the help of a water bath set at 60ºC. At the end of the method, balloons were kept in the oven at 60ºC in order to remove the remaining solvent, then they were kept in a desiccator for 30 min and their final weighings were taken after cooling as following:

Yield of crude lipid = [(Final weight - Initial weight) / Sample weight] x 100

Fatty acids analysis

The methyl esters of lipid from the samples were prepared by transmethylation using gas chromatography-flame ionizing detector (GC-FID) according to the method described by Ichihara et al. (1996). 25 mg of extracted oil was dissolved in 2 ml isooctane, followed by addition of 4 ml of 2 M KOH (in methanol). Then, the tube was vortexed for 2 min at room temperature. Separation into methyl esters was performed in

triplicate for each sample. After centrifugation at 4000 rpm for 10 min, the isooctane layer was taken for gas chromatography (GC) analysis.

Gas chromatography (GC) conditions

The fatty acid methyl esters were analyzed using gas chromatography of Agilent Technologies model 7820 equipped with a flame ionization detector (FID) and fitted with an HP-88 capillary column (60 m × 0.25 mm × 0.25 µm thickness). Helium was used as the carrier gas at a constant pressure of 16 psi. Injection port was maintained at 220ºC, and the sample was injected in split mode with a split ratio of 50:1. During the analysis, detector temperature was 280ºC. Column temperature was started at 175ºC, and then programmed at 3ºC/min to 220ºC, ramped at 1°C/min to 220ºC, and held for 10 min. The total running time was 26 min. Helium was used as the make up gas at a constant flow of 40 ml/min, and hydrogen and dry air were used as detector gases. Identification of fatty acids was carried out by comparing sample fatty acids methyl esters (FAME) peak relative retention times with those obtained for Supelco standards (Supelco 37 Compounds FAME mix 10 mg/l in CH_2 Cl_2–47,885 U, Supelco 1819–1 Ampule FAME mix C4-C24). The results were expressed as percentage of total fatty acid methyl esters (ISO, 1990).

Statistical analysis

All experiments were carried out in triplicate and the results were reported as mean and standard deviation of measurements. Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) using SPSS (Version 21, SPSS Inc., Chicago, IL, USA) software. Tukey's multiple range test ($p<0.05$) was used to detect differences among mean values of all test intervals.

RESULTS

Crude Lipid Analysis Results

The % lipid analysis results of the fresh material, multiple freezed/thawed and only once freezed/thawed sea bass were reported in Table 1. While the lipid content was 10.29% in fresh sea bass (day 0), a decrease was observed in the lipid content of fish thawed more than once in different mediums ($p<0.05$). In the refrigerator, a decrease was observed on the 7th day 1st thawing and on the 30th day 2nd thawing with 9.19% and 8.67%, respectively. Also in other thawing mediums, decreases were observed on the 30th day 2nd thawing compared to the 7th day 1st thawing. The highest lipid loss was observed in the microwave thawing method on the 30th day 1st thawing.

Table 1. Lipid analysis results of sea bass thawed in different conditions (%)

	Thawing Medium			
Thawing Time	**Refrigerator**	**Microwave**	**Water**	**Ambient Conditions**
Day 0	10.29±0.85^{aA}	10.29±0.85^{aA}	10.29±0.85^{aA}	10.29±0.85^{aA}
Day 7 (1st Thawing)	9.19±0.08^{aB}	8.18±0.05^{bBC}	9.53±0.44^{aB}	9.48±0.36^{aB}
Day 30 (2nd Thawing)	8.67±0.07^{aC}	8.87±0.48^{aB}	8.38±0.01^{bC}	8.23±0.05^{bC}
Day 30 (1st Thawing)	9.19±0.18^{bB}	7.46±0.57^{cC}	8.87±0.34^{bC}	8.33±0.09^{bC}

Data are expressed as the mean±SD. Lowercase letters indicate the statistical difference between the thawing methods, and uppercase letters indicate the statistical difference between the thawing times of the groups ($p<0.05$).

According to the results of fatty acid composition, the total saturated fatty acid (∑SFA) of fresh samples was 22.38%, hovewer it decreased to 12.96% in the water thawing method on the 7th day 1st thawing ($p<0.05$). With regard to the total monounsaturated fatty acid (∑MUFA), the value was 30.07% in fresh sea bass, however the highest increase (33.28%) occurred in water thawing samples. Total polyunsaturated (∑PUFA) was 40.34% in fresh sea bass, but it increased to 44.67% in water thawing. Eicosapentaenoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3) were detected in the range of 4.64±0.03-5.39±0.04% and 7.53±0.23-8.71±0.07%, respectively.

No statistically significant difference emerged between ambient conditions and water thawed groups with fresh material in terms of EPA and DHA ($p>0.05$). The n-3/n-6 ratio was 0.65 in fresh sea bass, 0.60 in refrigerator, 0.59 in microwave, 0.62 in ambient conditions and 0.61 in water thawed samples ($p>0.05$) (Table 2.)

Table 2. Fatty acid analysis results of sea bass thawed for the 1st time on the 7th day in different conditions (%)

	Day 7 (1st Thawing)				
	Fresh	**Refrigerator**	**Microwave**	**Ambient Conditions**	**Water**
C12:0	0.03±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}
C13:0	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}
C14:0	2.28±0.03^{a}	2.23±0.05^{a}	2.14±0.01^{a}	2.41±0.04^{a}	2.37±0.02^{a}
C15:0	0.28±0.00^{a}	0.26±0.00^{a}	0.28±0.00^{a}	0.28±0.00^{a}	0.27±0.00^{a}
C16:0	15.14±0.09^{a}	12.60±0.16^{b}	14.98±0.01^{a}	10.15±0.05^{c}	7.88±0.88^{d}
C17:0	0.25±0.01^{a}	0.21±0.05^{a}	0.29±0.00^{a}	0.20±0.00^{a}	0.15±0.01^{a}
C18:0	4.10±0.03^{a}	3.01±0.60^{b}	4.19±0.02^{a}	2.72±0.03^{b}	1.89±0.26^{c}
C22:0	0.12±0.00^{a}	0.13±0.00^{a}	0.12±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}
C24:0	0.18±0.02^{a}	0.26±0.03^{a}	0.22±0.00^{a}	0.22±0.01^{a}	0.23±0.00^{a}
∑SFA	**22.38±0.03^{a}**	**18.73±0.19^{b}**	**22.25±0.01^{a}**	**16.17±0.02^{c}**	**12.96±0.29^{d}**
C14:1	0.09±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.04±0.00^{a}
C16:1	3.26±0.03^{a}	3.36±0.11^{a}	3.13±0.02^{a}	3.52±0.02^{a}	3.61±0.07^{a}
C17:1	0.44±0.01^{a}	0.45±0.01^{a}	0.43±0.00^{a}	0.48±0.01^{a}	0.49±0.01^{a}
C18:1n-9c	22.54±0.12^{c}	24.11±0.08^{b}	22.70±0.16^{c}	23.97±0.11^{b}	25.21±0.35^{a}
C18:1n-9t	0.17±0.00^{a}	0.18±0.00^{a}	0.17±0.01^{a}	0.18±0.00^{a}	0.19±0.00^{a}
C20:1n-9	2.76±0.00^{a}	2.94±0.10^{a}	2.76±0.00^{a}	3.08±0.00^{a}	3.14±0.06^{a}
C22:1n-9	0.81±0.01^{a}	0.55±0.01^{a}	0.52±0.01^{a}	0.54±0.01^{a}	0.60±0.00^{a}
∑MUFA	**30.07±0.04^{c}**	**31.62±0.05^{b}**	**29.73±0.06^{c}**	**31.81±0.04^{b}**	**33.28±0.13^{a}**
C18:2n-6t	0.82±0.00^{a}	0.07±0.01^{a}	0.09±0.00^{a}	0.07±0.00^{a}	0.06±0.00^{a}
C18:2n-6c	22.43±0.12^{e}	24.68±0.19^{c}	23.68±0.14^{d}	25.65±0.12^{b}	26.44±0.37^{a}
C18:3n-6	0.27±0.02^{a}	0.41±0.07^{a}	0.34±0.01^{a}	0.20±0.02^{a}	0.19±0.00^{a}
C18:3n-3	1.42±0.01^{a}	1.61±0.06 ^{a}	1.52±0.01^{a}	1.56±0.01^{a}	1.64±0.02^{a}
C20:2	0.95±0.02^{a}	1.05±0.04^{a}	0.99±0.00^{a}	1.00±0.01^{a}	1.03±0.01^{a}
C20:3n-6	0.12±0.01^{a}	0.10±0.03^{a}	0.15±0.00^{a}	0.09±0.01^{a}	0.07±0.01^{a}
C20:3n-3	0.47±0.01^{a}	0.86±0.02^{a}	0.84±0.01^{a}	0.89±0.02^{a}	0.90±0.00^{a}
C20:4n-6	0.19±0.00^{a}	0.21±0.01^{a}	0.37±0.22 ^{a}	0.22±0.01^{a}	0.24±0.00^{a}
C22:2	0.07±0.01^{a}	0.08±0.01^{a}	0.03±0.01^{a}	0.07±0.01^{a}	0.06±0.01^{a}
C20:5n-3	5.01±0.05^{ab}	4.91±0.17^{b}	4.64±0.03^{b}	5.39±0.04^{a}	5.32±0.05^{a}
C22:6n-3	8.60±0.21^{a}	7.94±0.28^{b}	7.53±0.23^{b}	8.42±0.09^{a}	8.71±0.07^{a}
∑PUFA	**40.34±0.07^{d}**	**41.94±0.09^{c}**	**40.18±0.09^{d}**	**43.56±0.04^{b}**	**44.67±0.11^{a}**
Total n-3	**15.50±0.10^{b}**	**15.32±0.12^{b}**	**14.52±0.11^{c}**	**16.26±0.04^{a}**	**16.57±0.03^{a}**
Total n-6	**23.83±0.05^{d}**	**25.48±0.08^{bc}**	**24.63±0.10^{cd}**	**26.23±0.05^{ab}**	**27.00±0.17^{a}**
n-3/n-6	**0.65±0.01^{a}**	**0.60±0.05^{a}**	**0.59±0.06^{a}**	**0.62±0.01^{a}**	**0.61±0.01^{a}**
EPA/DHA	**0.58±0.01^{a}**	**0.62±0.00^{a}**	**0.62±0.02^{a}**	**0.64±0.01^{a}**	**0.61±0.00^{a}**
Undefined	**7.20**	**7.70**	**7.84**	**8.47**	**9.09**

Lowercase letters indicate statistical differences between groups ($p<0.05$).
SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, n - omega.

Table 3 reports the results of the fatty acid composition of the 30th day 2nd thawing. Although the ∑SFA value was 22.38% in fresh sea bass, it decreased to 15.53% in thawing at ambient conditions. With regard to the ∑MUFA, the value was 30.07% in fresh sea bass, however it increased to 33.54% in thawing under ambient conditions and was determined as the group with the highest increase. The ∑PUFA was 40.34% in fresh sea bass and it increased to 42.22% in thawing under ambient conditions.

In addition, DHA was determined as 8.60±0.21% in fresh fish, but significant losses occurred in all thawing methods ($p<0.05$). The n-3/n-6 ratio was 0.65 in fresh sea bass, 0.63 in the refrigerator, 0.64 in the microwave, 0.60 in ambient conditions, and 0.57 in water thawed samples.

Table 3. Fatty acid analysis results of sea bass thawed for the 2nd time on the 30th day in different conditions (%)

	Day 30 (2nd Thawing)				
	Fresh	**Refrigerator**	**Microwave**	**Ambient Conditions**	**Water**
C12:0	0.03±0.00^{a}	0.02±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}
C13:0	0.01±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.07±0.09^{a}
C14:0	2.28±0.03^{a}	2.44±0.25^{a}	2.44±0.14^{a}	2.36±0.02^{a}	2.38±0.07^{a}
C15:0	0.28±0.00^{a}	0.29±0.01^{a}	0.29±0.00^{a}	0.29±0.00^{a}	0.29±0.00^{a}
C16:0	15.14±0.09^{a}	13.92±0.04^{b}	13.09±1.10^{b}	9.81±0.96^{c}	9.81±0.21^{c}
C17:0	0.25±0.01^{a}	0.27±0.03^{a}	0.26±0.07^{a}	0.20±0.01^{a}	0.21±0.01^{a}
C18:0	4.10±0.03^{a}	4.02±0.64^{a}	3.45±1.47^{b}	2.50±0.24^{c}	3.13±0.76^{b}
C22:0	0.12±0.00^{a}	0.12±0.01^{a}	0.12±0.01^{a}	0.14±0.01^{a}	0.13±0.00^{a}
C24:0	0.18±0.02^{a}	0.20±0.00^{a}	0.22±0.02^{a}	0.19±0.00^{a}	0.22±0.02^{a}
∑SFA	**22.38±0.03^{a}**	**21.30±0.22^{b}**	**19.91±0.56^{c}**	**15.53±0.32^{d}**	**16.27±0.25^{d}**
C14:1	0.09±0.00^{a}	0.03±0.01^{a}	0.03±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}
C16:1	3.26±0.03^{a}	3.17±0.14^{a}	3.38±0.26^{a}	3.45±0.04^{a}	3.42±0.11^{a}
C17:1	0.44±0.01^{a}	0.43±0.01^{a}	0.47±0.03^{a}	0.47±0.00^{a}	0.47±0.01^{a}
C18:1n-9c	22.54±0.12^{b}	23.09±0.11^{b}	22.71±0.27^{b}	25.76±0.43^{a}	25.20±0.03^{a}
C18:1n-9t	0.17±0.00^{a}	0.17±0.01^{a}	0.18±0.02^{a}	0.19±0.01^{a}	0.18±0.01^{a}
C20:1n-9	2.76±0.00^{a}	2.80±0.15^{a}	2.96±0.19^{a}	3.16±0.04^{a}	3.04±0.06^{a}
C22:1n-9	0.81±0.01^{a}	0.47±0.03^{a}	0.54±0.03^{a}	0.48±0.02^{a}	0.52±0.01^{a}
∑MUFA	**30.07±0.04^{b}**	**30.16±0.07^{b}**	**30.28±0.12^{b}**	**33.54±0.15^{a}**	**32.86±0.04^{a}**
C18:2n-6t	0.82±0.00^{a}	0.09±0.00^{a}	0.08±0.01^{a}	0.07±0.00^{a}	0.08±0.01^{a}
C18:2n-6c	22.43±0.12^{c}	23.45±0.38^{b}	23.69±0.36^{b}	24.88±0.34^{a}	25.24±0.24^{a}
C18:3n-6	0.27±0.02^{a}	0.37±0.16^{a}	0.46±0.05^{a}	0.42±0.01^{a}	0.37±0.11^{a}
C18:3n-3	1.42±0.01^{a}	1.54±0.09^{a}	1.52±0.10^{a}	1.64±0.03^{a}	1.58±0.03^{a}
C20:2	0.95±0.02^{a}	0.96±0.02^{a}	1.03±0.07^{a}	1.08±0.03^{a}	1.01±0.02^{a}
C20:3n-6	0.12±0.01^{a}	0.13±0.01^{a}	0.13±0.03^{a}	0.10±0.01^{a}	0.12±0.02^{a}
C20:3n-3	0.47±0.01^{a}	0.81±0.03^{a}	0.84±0.07^{a}	0.84±0.03^{a}	0.81±0.08^{a}
C20:4n-6	0.19±0.00^{a}	0.20±0.00^{a}	0.21±0.02^{a}	0.22±0.02^{a}	0.21±0.00^{a}
C22:2	0.07±0.01^{a}	0.08±0.00^{a}	0.09±0.02^{a}	0.07±0.00^{a}	0.07±0.00^{a}
C20:5n-3	5.01±0.05^{b}	4.82±0.15^{b}	5.56±0.32^{a}	4.76±0.04^{b}	4.75±0.28^{b}
C22:6n-3	8.60±0.21^{a}	8.04±0.17^{bc}	7.87±0.76^{c}	8.14±0.00^{b}	7.71±0.03^{c}
∑PUFA	**40.34±0.07^{c}**	**40.47±0.12^{c}**	**41.48±0.23^{b}**	**42.22±0.10^{a}**	**41.94±0.10^{ab}**
Total n-3	**15.50±0.10^{a}**	**15.20±0.06^{a}**	**15.78±0.32^{a}**	**15.37±0.02^{a}**	**14.85±0.12^{b}**
Total n-6	**23.83±0.05^{c}**	**24.23±0.16^{bc}**	**24.58±0.15^{b}**	**25.70±0.15^{a}**	**26.01±0.10^{a}**
n-3/n-6	**0.65±0.01^{a}**	**0.63±0.02^{a}**	**0.64±0.04^{a}**	**0.60±0.01^{b}**	**0.57±0.03^{c}**
EPA/DHA	**0.58±0.01^{c}**	**0.60±0.03^{b}**	**0.71±0.11^{a}**	**0.58±0.00^{c}**	**0.62±0.03^{b}**
Undefined	**7.20**	**8.08**	**8.32**	**8.70**	**8.93**

Lowercase letters indicate statistical differences between groups (p<0.05).
SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, EPA - eicosapentaenoic acid, DHA – docosahexaenoic acid, n - omega.

Table 4 shows the results of the fatty acid composition of the 30th day 1st thawing. The ∑SFA was 22.38% in fresh sea bass, but it significantly decreased to 14.38% in thawing in the refrigerator (p<0.05). The highest value (22.44%) was obtained in the microwave thawing method, and its difference with the control group was found to be statistically insignificant (p<0.05). It was determined that while ∑MUFA was 30.07% in fresh sea bass, it significantly decreased to 28.77% in microwave thawing and increased to 33.18% in ambient conditions (p<0.05). The ∑PUFA was 40.34% in fresh sea bass and it increased to 44.70% in refrigerator thawing with the highest value among the groups (p<0.05). The n-3/n-6 ratio was 0.65 in fresh sea bass, 0.63 in refrigerator and ambient conditions, and 0.64 in microwave and water thawing (p>0.05).

Table 4. Fatty acid analysis results of sea bass thawed for the 1st time on the 30th day in different conditions (%)

	Day 30 (1st Thawing)				
	Fresh	**Refrigerator**	**Microwave**	**Ambient Conditions**	**Water**
C12:0	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}
C13:0	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
C14:0	2.28±0.03^{a}	2.51±0.01^{a}	2.27±0.02^{a}	2.38±0.01^{a}	2.37±0.00^{a}
C15:0	0.28±0.00^{a}	0.29±0.00^{a}	0.29±0.00^{a}	0.28±0.00^{a}	0.29±0.00^{a}
C16:0	15.14±0.09^{a}	8.74±0.44^{c}	15.11±0.02^{a}	9.28±1.26^{bc}	10.40±0.36^{b}
C17:0	0.25±0.01^{a}	0.17±0.01^{a}	0.26±0.00^{a}	0.18±0.01^{a}	0.19±0.00^{a}
C18:0	4.10±0.03^{a}	2.28±0.09^{b}	4.16±0.02^{a}	2.24±0.38^{b}	2.50±0.13^{b}
C22:0	0.12±0.00^{a}	0.13±0.00^{a}	0.12±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}
C24:0	0.18±0.02^{a}	0.21±0.01^{a}	0.19±0.00^{a}	0.21±0.03^{a}	0.18±0.00^{a}
∑SFA	**22.38±0.03^{a}**	**14.38±0.15^{c}**	**22.44±0.01^{a}**	**14.74±0.42^{c}**	**16.10±0.12^{b}**
C14:1	0.09±0.00^{a}	0.04±0.00^{a}	0.03±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}
C16:1	3.26±0.03^{a}	3.60±0.02^{a}	3.20±0.02^{a}	3.52±0.03^{a}	3.46±0.01^{a}
C17:1	0.44±0.01^{a}	0.51±0.00^{a}	0.43±0.01^{a}	0.49±0.00^{a}	0.49±0.00^{a}
C18:1n-9c	22.54±0.12^{c}	23.88±0.18^{b}	21.67±0.10^{d}	25.35±0.68^{a}	24.28±0.15^{ab}
C18:1n-9t	0.17±0.00^{a}	0.19±0.00^{a}	0.17±0.00^{a}	0.19±0.00^{a}	0.18±0.00^{a}
C20:1n-9	2.76±0.00^{a}	3.18±0.02^{a}	2.80±0.01^{a}	3.11±0.04^{a}	3.06±0.01^{a}
C22:1n-9	0.81±0.01^{a}	0.55±0.02^{a}	0.47±0.00^{a}	0.48±0.02^{a}	0.46±0.00^{a}
∑MUFA	**30.07±0.04^{c}**	**31.93±0.06^{b}**	**28.77±0.04^{d}**	**33.18±0.25^{a}**	**31.97±0.06^{b}**
C18:2n-6t	0.82±0.00^{a}	0.07±0.00^{a}	0.09±0.00^{a}	0.07±0.00^{a}	0.07±0.00^{a}
C18:2n-6c	22.43±0.12^{d}	25.92±0.13^{a}	23.30±0.04^{c}	25.22±0.41^{ab}	24.88±0.11^{b}
C18:3n-6	0.27±0.02^{a}	0.40±0.01^{a}	0.47±0.00^{a}	0.41±0.00^{a}	0.40±0.01^{a}
C18:3n-3	1.42±0.01^{a}	1.56±0.01^{a}	1.41±0.01^{a}	1.54±0.06^{a}	1.55±0.01^{a}
C20:2	0.95±0.02^{a}	1.04±0.01^{a}	0.96±0.00^{a}	0.97±0.03^{a}	1.05±0.01^{a}
C20:3n-6	0.12±0.01^{a}	0.10±0.01^{a}	0.13±0.00^{a}	0.08±0.00^{a}	0.09±0.00^{a}
C20:3n-3	0.47±0.01^{a}	0.88±0.01^{a}	0.85±0.01^{a}	0.88±0.02^{a}	0.87±0.01^{a}
C20:4n-6	0.19±0.00^{a}	0.22±0.01^{a}	0.20±0.00^{a}	0.21±0.01^{a}	0.21±0.00^{a}
C22:2	0.07±0.01^{a}	0.07±0.01^{a}	0.09±0.01^{a}	0.07±0.00^{a}	0.07±0.00^{a}
C20:5n-3	5.01±0.05^{a}	5.56±0.02^{a}	5.00±0.04^{a}	5.00±0.03^{a}	5.15±0.01^{a}
C22:6n-3	8.60±0.21^{a}	8.90±0.09^{a}	8.28±0.11^{a}	8.97±0.09^{a}	8.86±0.13^{a}
∑PUFA	**40.34±0.07^{c}**	**44.70±0.04^{a}**	**40.79±0.03^{c}**	**43.41±0.12^{b}**	**43.21±0.05^{b}**
Total n-3	**15.50±0.10^{b}**	**16.89±0.04^{c}**	**15.55±0.05^{b}**	**16.39±0.03^{c}**	**16.43±0.06^{c}**
Total n-6	**23.83±0.05^{c}**	**26.70±0.06^{a}**	**24.19±0.02^{c}**	**25.99±0.18^{ab}**	**25.66±0.05^{b}**
n-3/n-6	**0.65±0.01^{a}**	**0.63±0.01^{a}**	**0.64±0.02^{a}**	**0.63±0.01^{a}**	**0.64±0.01^{a}**
EPA/DHA	**0.58±0.01^{c}**	**0.63±0.00^{a}**	**0.60±0.00^{b}**	**0.56±0.00^{d}**	**0.58±0.01^{c}**
Undefined	**7.20**	**8.98**	**8.00**	**8.67**	**8.71**

Lowercase letters indicate statistical differences between groups ($p<0.05$).
SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, n - omega.

DISCUSSION

The lipid content of fish varies widely and not only according to the fish species. Changes in the lipid amount of fish meat can also occur due to seasonal conditions, nutritional characteristics, salt content of the water and various other factors (Çilingir Yeltekin, 2012). For this reason, although it is difficult to specify a general amount of lipid content of fish (Baysal, 2002), it is reported that the total amount of lipid varies between 0.5% and 20% (Koubaa et al., 2012). Fish are generally classified as lean, medium oily and oily on the basis of their lipid content. Less than 5% fat content is considered lean, 5-10% medium oily, and more than 10% oily fish (Ackman, 1989). The quality of fish stems from 15-20% saturated and 80-85% unsaturated fatty acids in its composition. Polyunsaturated fatty acids constitute the majority of these unsaturated fatty acids (Yalçın and Yalçın, 2016). Periago et al. (2005) in their study examining the meat quality of cultured and wild sea bass, reported an initial lipid content for fresh cultured sea bass of 6.66%. Orban et al. (2003) also determined a value of 9.36% in their similar studies. In our study, the lipid content of fresh sea bass was determined as 10.29%. According to the results of the present study, sea bass are generally in the class of medium oily fish when compared with other studies.

Lipid content decreased in the thawing processes performed on the 7th and 30th days after the freezing process compared to the fresh fish on day 0 in our study. In general, there was a decrease in lipid content depending on time. On the 7th day and 30th day 1st thawing, the lowest lipid value was determined at microwave thawed samples. The highest lipid

content was in the samples thawed in the refrigerator. Samantaray et al. (2021), applied thawing several times in samples of different carps species. As a result, while the lipid content was 2.23±0.03% and 2.02±0.03%, respectively in the fresh sample of Catla and Rohu species, the value decreased to 1.04±0.06% and 1.49±0.03%, respectively in the 5th thawing. Pourshamsian et al. (2012) applied the frying of cultured sturgeon with different oils, followed by freezing and thawing in the refrigerator and microwave three days later. They reported that the lipid ratios in the samples decreased after the thawing processes performed on the 3rd day, but no significant difference was observed between the two thawing methods.

Thawing is the final stage of the freezing process. In the stage of processing frozen fish or consuming it as fresh fish, thawing under inappropriate conditions causes quality loss in the product (Binici and Kurtkaya, 2014). Air thawing, water thawing, vacuum thawing, electrical thawing and high pressure thawing methods are all methods used for food (Binici and Kurtkaya, 2014; Genç et al., 2015). Multiple freezing and thawing processes are generally used in restaurants, hypermarkets and homes. According to researches in the literature, it is not possible to give valid information for the best thawing method (Baygar et al., 2004). For instance, thawing at room temperature is widely practiced in small businesses, but it is not recommended due to the risk of microorganisms development. Excessive water loss, bad odor and taste may occur in the refrigerator thawing due to the slow thawing and bacterial growth over time. In principle, seafood should be thawed as quickly as possible. Faster and more advantageous thawing methods are possible for large facilities, and microwave thawing is one of them (Turan et al., 2006).

Mol et al. (2004) found that different thawing conditions (water, ambient conditions and refrigerator) did not cause a significant change on the quality of imported mackerel according to the sensory, chemical and microbiological analyzes and mackerels can be consumed by thawing at refrigerator, ambient conditions and refrigerator in a healthy way. Tokur and Kandemir (2008) examined the effects of different thawing methods (microwave, flowing water and ambient conditions) on protein quality of frozen fish, and reported that different thawing processes caused a significant decrease in protein solubility. Turan et al. (2006) evaluated the effects of different thawing methods (microwave, water, ambient temperature, refrigerator) on the quality of frozen trout by sensory, chemical and microbiological methods, and their results showed that the best quality was in microwave and water thawing in terms of both chemical and sensory characteristics. It was concluded that the water thawing method would be more appropriate in large facilities, since microwave thawing method is an expensive and requiring control. For consumers who thaw small quantities of fish, the microwave thawing method is recommended because of taking less time. Ersoy et al. (2008) applied refrigerator, water, ambient conditions and microwave thawing to frozen eel, and they concluded that the water thawing was the most appropriate method by a microbiological point of view. Karami et al. (2022) in their study that invastigated the effect of different (microwave, ambient and air refrigerator) thawing processes on quality of mullet (*Liza aurata*) fillets during the 60 day storage, while on the first day protein content were 18.25%, 19.13% and 19.18% in the groups of microwave, ambient air and refrigerator thawing, after 60 days it decreased to 17.37%, 18.89% and 18.95%, respectively. They found the lipid content on the first day as 3.76%, 4% and 4% in the groups of microwave, ambient air and refrigerator thawing, after 60 days it decreased to 3.57%, 3.77%, 3.78%, respectively. The highest decrease was observed in the microwave thawing group in terms of protein and lipid content. Also in the same study maximum decrease in moisture content was in the microwave thawing (77.27% on the first day, 66% on the 60th day). Wang et al. (2022) studied the effects of different thawing methods (water immersion thawing, air thawing and refrigerator thawing) on the biochemical properties of tilapia (*Oreochromis niloticus*) fillets during storage at -18°C for 6 months. According to the thawing and cooking losses refrigerator thawing is suggested to be an effective thawing method similar with our study to minimise quality change during frozen storage. Han and Gökoğlu (2022) studied the effects of different freezing and thawing (on air, in refrigerator and in microwave oven) methods on the quality of giant red shrimp (*Aristaeomorpha foliacea*). Results showed that microwave thawing conditions are not suitable similar with our study for thawing of frozen shrimp as it negatively affects texture and colour and increases cooking loss. Benjakul and Bauer (2001) studied thawing of catfish using different techniques and they determined that the fast thawing process caused a better change in the sensory and textural quality of the meat compared to the slow thawing process. They also reported that deterioration in the structure of meat, lipid oxidation and protein denaturation occured due to the osmotic pressure of water during thawing in water. In thawing methods, temperature fluctuations significantly affect the quality. Frozen foods are mostly thawed before processing or consumption and it can cause chemical, physical and microbiological damage to food (Pourshamsian et al., 2012). Polyunsaturated fatty acids, which are abundant in the structure of fish, are also oxidatively damaged during storage (Nazemroaya et al., 2009).

In our study, according to the fatty acid composition of sea bass thawed at different times with different methods, it was determined that the dominant saturated fatty acids were palmitic acid (C16:0), myristic acid (C14:0) and stearic acid (C18:0) in all groups. The highest palmitic acid was determined in fresh samples with a value of 15.14%. A decrease in palmitic acid was observed with the storage period. In general, the lowest total SFA occurred in the water and ambient conditions thawing on all thawing days. Durmuş and Özoğul (2018) studied the effect of nanoemulsions on the fatty acids of sea bass fillets in cold storage and they reported the total SFA of the control group as 19.21% at the beginning of storage, and an increase in the SFA during the storage period. In addition,

the total SFA increased to 24.49% on the 8th day of storage for the control group, and this value was 24.05% at the end of the storage (12th day). In our study, the total SFA was 22.38% at the beginning of the storage, and the total SFA of all thawing methods during the storage were lower than the results of the researcher.

In our study, linoleic acid, EPA and DHA were determined as 22.43%, 5.01% and 8.60%, respectively, in the fresh sample. The total PUFA was determined as 40.34% in the fresh sample and between 40.47-44.70% in the samples thawed in the refrigerator. Durmuş and Özoğul (2018) stated that the highest polyunsaturated fatty acids were linoleic acid, EPA and DHA with 14.52%, 4.21% and 8.09%, respectively, for the control group at the beginning of storage. Kocatepe and Turan (2012) determined that the total polyunsaturated fatty acids (PUFA) in the fatty acid composition of cultured sea bass were 33.2%. Durmuş and Özoğul (2018) reported that the total polyunsaturated fatty acids (PUFA) was 29.25% at the beginning of storage, while there was a decrease in PUFA during the storage period and this value was 20.94% at the end of the storage (12th day). The PUFA values in our study were higher than those reported by the other researchers. Comparing the thawing methods, the highest PUFA and n-3 values were found in the samples thawed in the water (41.94±0.10%-44.67±0.11% and 14.85±0.12%-16.57±0.03%) and ambient conditions (42.22±0.10%-43.56±0.0% and 15.37±0.02%-16.39±0.03%). Lower values were obtained on the 30th day 2nd thawing. Javadian et al. (2013) in a study applying different thawing methods (refrigerator, water, microwave, ambient conditions) to frozen rainbow trout (*Oncorhynchus mykiss*), reported that after freezing/thawing, the contents of polyunsaturated fatty acids (PUFA) and n-3 were significantly reduced compared to the fresh sample. They concluded that thawing in water after freezing is the best method for all rainbow trout. Wu et al. (2021) examined the effects of freezing and thawing processes applied to raw pork meat on protein and lipid oxidation, applying thawing 7 times. As a result, while in the control group, saturated and polyunsaturated fatty acids were 36.27±0.96% and 15.85±0.90%, respectively, the values decreased significantly to 31.77±0.68% and 8.91±1.04% at the 7th thawing. Pourshamsian et al. (2012) evaluated the fatty acid profile of the cultured sturgeon that was fried, freezed and then thawed in refrigerator and microwave after 3 days. They reported that fatty acid profiles was similar to the control group in the refrigerator thawing method than microwave thawing method.

Refrigerator and microwave thawing methods are the most effective methods for thawing animal tissues in large portions. The microwave thawing method is faster and less damaging the tissues (Boonsumrej et al., 2007). The thawing method should preserve the original quality of the food as much as possible. When the studies of various researchers were investigated, results of different thawing methods were obtained. Researchers' results may differ due to fish species, region, season, gender or ovulation period besides thawing method. Thus, it is not possible to give a clear conclusion on the most efficient thawing method.

CONCLUSION

In our study, according to the the lipids analysis, the lowest fat loss was observed in the refrigerator thawing method (9.19±0.18%) and the highest lipid loss (7.46±0.57%) was in the microwave thawed samples at Day 30 (1st thawing). According to the fatty acid profile, due to the lowest loss of unsaturated fatty acids (C18:2 25.92±0.13%, C20:5 5.56±0.02%, C22:6n-3 8.90±0.09%, ∑PUFA 44.70±0.04%) at Day 30 (1st thawing) the most convenient method was determied as refrigerator thawing. As a result, all seafood, including fish, are the products that are highly susceptible to quality loss and deterioration in terms of meat structure and content. Incorrect practices during the storage and thawing of seafood accelerate the losses in all other quality parameters, especially meat quality. Even if the freezing conditions are appropriate, incorrect applications during the thawing of the products can cause undesired quality changes. Thus, the cold chain conditions should not be broken in such processes, the ambient and water temperature should not be high during thawing, the products should not be kept in ambient conditions for a long time. In the case of water thawed samples, the water pressure should not be high to avoid to damage the meat. In addition it should be prevented water penetration inside the meat and excessive freezing and thawing, together with avoid to damage the meat with the effect of heat in applications such as microwaves. As a result of the study, it is recommended to the consumers that frozen foods should be thawed only once, to consume immediately and to freeze and thaw the fish according to their needs.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Cansu Metin, Yunus Alparslan and Zerrin Ekşi carried out the laboratory studies; material preparation, lipid and fatty acid analyses. Taçnur Baygar, Yunus Alparslan and Cansu Metin interpreted the results. The writing/editing was carried out by Cansu Metin, Yunus Alparslan and Zerrin Ekşi, Taçnur Baygar and all authors have read and approved the article.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

The data supporting the conclusions of this paper are available in the main paper.

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