



## Changes in Fatty Acid Profiles of Guitarfish' (*Rhinobatos rhinobatos*; Linnaeus 1758) Liver Oil, a Cartilaginous Fish Species, in Different Storage Conditions

Ayşe ÖZYILMAZ<sup>1\*</sup> Abdullah ÖKSÜZ<sup>2</sup>

<sup>1</sup>Iskenderun Technical University, Faculty of Marine Sciences and Technology, Department of Marine Technologies, Hatay, Turkey.

<sup>2</sup>Necmettin Erbakan University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Konya, Turkey.

Geliş/Received: 11.10.2021

Kabul/Accepted: 13.12.2021

Yayın/Published: 31.12.2021

How to cite: Özyılmaz, A. & Öksüz, A. (2021). Changes in Fatty Acid Profiles of Guitarfish' (*Rhinobatos rhinobatos*; Linnaeus 1758) Liver Oil, a Cartilaginous Fish Species, in Different Storage Conditions. *J. Anatolian Env. and Anim. Sciences*, 6(4), 662-667.

Atıf yapmak için: Özyılmaz, A. & Öksüz, A. (2021). Bir Kıkırdaklı Balık Türü Olan Kemane Balığı (*Rhinobatos rhinobatos*; Linnaeus 1758) Karaciğer Yağlarının Farklı Saklama Koşullarında Yağ Asitlerindeki Değişimler. *Anadolu Çev. ve Hay. Dergisi*, 6(4), 662-667.

\*ID: <https://orcid.org/0000-0002-4376-0873>  
ID: <https://orcid.org/0000-0001-8778-9320>

**\*Corresponding author:**

Ayşe ÖZYILMAZ  
Iskenderun Technical University, Faculty of  
Marine Sciences and Technology, Department  
of Marine Technologies, Hatay,  
Turkey.  
✉: [ayse.ozyilmaz@iste.edu.tr](mailto:ayse.ozyilmaz@iste.edu.tr)

**Abstract:** Guitarfish (*Rhinobatos rhinobatos*; Linnaeus 1758) is one of the commonly found cartilaginous fish species in the North-eastern Mediterranean. The objective of the current study is to explore the changes of fatty acid profiles in liver oil of guitarfish stored in two different storage places; one stored in refrigerator (+4°C) and the other stored in room temperature in a period of 180 days. Changes in fatty acid profiles in liver oil of the guitarfish were analyzed and observed in monthly during storage period. The results of the analysis for storing in refrigerator (+4°C) and room temperature showed some certain differences in terms of the fatty acid components of the cartilaginous fish liver oils. In particular, increasing in saturated fatty acids (SFA) and degreasing in monounsaturated fatty acids (MUFA) was observed during the storage period for the liver oils. The average levels of polyunsaturated fatty acids (PUFA) also showed some changes, in decreasing patterns, during the trial for both storage conditions.

**Keywords:** Fatty acid, guitarfish, liver oil, *Rhinobatos rhinobatos*, storage condition.

## Bir Kıkırdaklı Balık Türü Olan Kemane Balığı (*Rhinobatos rhinobatos*; Linnaeus 1758) Karaciğer Yağlarının Farklı Saklama Koşullarında Yağ Asitlerindeki Değişimler

**\*Sorumlu yazar:**

Ayşe ÖZYILMAZ  
Iskenderun Teknik Üniversitesi, Deniz  
Bilimleri ve Teknolojisi Fakültesi, Deniz  
Teknolojileri Bölümü, Iskenderun, Hatay,  
Türkiye..  
✉: [ayse.ozyilmaz@iste.edu.tr](mailto:ayse.ozyilmaz@iste.edu.tr)

**Öz:** Kemane balığı (*Rhinobatos rhinobatos*; Linnaeus 1758), Kuzeydoğu Akdeniz'de yaygın olarak bulunan kıkırdaklı balık türlerinden biridir. Bu çalışmanın amacı, iki farklı depolama koşulunda depolanan kemane balıklarının karaciğer yağındaki yağ asidi profillerindeki değişiklikleri araştırmaktır. Depolama koşullarından biri buzdolabında (+4°C) diğeri ise oda sıcaklığıdır. Depolama süresi 180 gündür. Kemane balıklarının karaciğer yağındaki yağ asidi profillerindeki değişimler, depolama süresince aylık olarak analiz edilmiş ve izlenmiştir. Buzdolabında (+4°C) ve oda sıcaklığında depolanan yağlarda için yapılan analiz sonuçları, kıkırdaklı balık karaciğeri yağlarının yağ asidi bileşenleri açısından bazı farklılıklar olduğunu ortaya koymuştur. Özellikle depolama süresince karaciğer yağlarında doymuş yağ asitlerinde (SFA) artış ve tekli doymamış yağ asitlerinde (MUFA) azalma şeklinde gözlenmiştir. Çoklu doymamış yağ asitlerinin (PUFA) ortalama seviyelerinde azalmıştır.

**Anahtar kelimeler:** Depolama koşulu, karaciğer yağı, kemane balığı, *Rhinobatos rhinobatos*, yağ asidi.

## INTRODUCTION

The cartilaginous fish species are abundant and generally are not targeted fish species, excluding few of them in the Northeastern Mediterranean (Ragheb & Hasan, 2021). The guitarfish is one of the few regarding its consumption due to its consumption. Apart from the other cartilaginous fish, this one has an economically valuable raw material. Even though guitarfish is a cartilaginous fish species, it has different meanings for the people around the regions. This fish is in consuming list for a lot of people. People around the coast of the in the Northeastern Mediterranean have different dish relating guitarfish which is pointed out in many previously published studies that the fish is consumed not only people around the Turkish coastal waters but also other parts of the Mediterranean (Lteif et al., 2016)

Some previously reported studies showed that liver oil of the cartilaginous can be considered as raw materials and turned out something useful and beneficial (Ayas et al., 2019; Yığın et al., 2019; Ozyilmaz 2016; Özyılmaz & Öksüz 2015; Ould El Kebir et al., 2007; Nechet et al., 2007). These attributes may make them as important issue to the study.

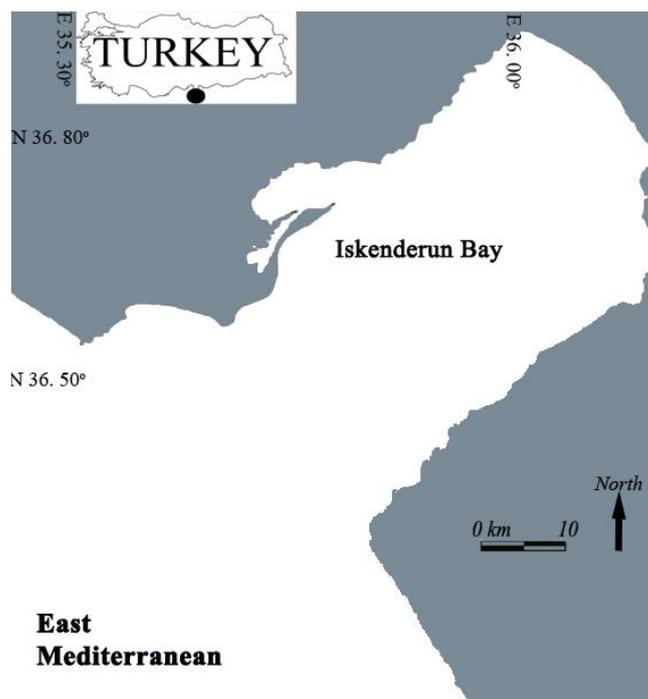
Fish lipids are known to be rich components due to their health benefit effects substances e.g., polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). This health promoted fatty acids have many different positive health effects on human health (Bagge et al., 2017; Simat et al., 2015). That's why it might be useful to know the effects of two different storage conditions on the quality of oil obtained from cartilaginous fish livers.

Some studies also showed that some certain differences might be obtain with storage conditions by itself as well as different storage conditions in fish and liver oils (Ozogul et al., 2017; Özyılmaz & Öksüz, 2018a; Özyılmaz & Öksüz, 2018b; Wang et al., 2011; Boran et al., 2006; Özden, 2005). The aim of this present study is to search the changes of fatty acid profiles in liver oil of guitarfish in two different storage conditions; one storage in refrigerator (+4°C) and the other storage at room temperature for 180 days each. The results may guide the very next studies and food industry applications in this field.

## MATERIAL AND METHOD

The cartilaginous fish species was chosen as the guitarfish (*Rhinobatos rhinobatos*; Linnaeus 1758) for this current study. All guitarfish used in this research were obtained from Mediterranean Sea in 2010 by professional fishermen. The map of the study area for guitarfish in Northeastern Mediterranean was given in Figure 1.

The liver parts of the all guitarfish used for the study were separated from their bodies. The livers were gathered in containers which were placed in cool chains. Fish livers were homogenized thoroughly then lipid extraction was carried out as mentioned below. Extracted lipids were divided into two batches and filled into an air tight 250 ml volume brown bottles. First batch was stored at 4°C in a fridge and the second batch was stored at room temperature in order to observe chemical changes at different storage conditions.



**Figure 1.** The map of the study area for guitarfish (modified from Simsek and Demirci, 2018)

**Lipid analysis:** It has been performed the Modified Bligh & Dyer Modified by Hanson & Olley (1963) method for determining the crude lipid levels of the cartilaginous fish' livers. This method methanol and chloroform ratio of kept 1:1 level, a total of 40 mL of chloroform and methanol, 20 mL of distilled water. Roughly, 10 g of liver samples were weighed into a 250 mL homogenizing flasks. A total of 8mL of pure water (a total volume of 16 mL), 20 mL of chloroform, and 40 mL of methanol were added the homogenate. The mixtures were homogenized at least 1 min in icy water during the whole steps of the analysis. After that, 20 mL chloroform was added the and then the mixtures were homogenized for further 30 second. Finally, 20 mL of distilled water was added the homogenate and the mixtures were homogenized another 30 s. The homogenates were transferred into two 50-mL centrifuge tubes and centrifuged for at 3000 rpm for 10 min. Aqueous layers were removed from sucking pipet. From chloroform layer, of 20 mL of mix (chloroform and lipid) transferred into a pre dried and weight

pear-shaped flask and evaporated using a rotary evaporator (Heidolph-Germany) under the vacuum.

#### **Fatty Acid Methyl Esters (FAMES) Preparation:**

The methylation process of fatty acids was started as taking approximately 45-60 mg of liver oil which was weighed into a 10 mL teflon screw cap test tube. A total of 1.5 mL 0.5 M NaOH was added on it and tightly sealed. It was heated in a heating block at 115°C for 7 minutes and then let to be cooled. Additionally, 2 mL of Boron trifluoride-methanol solution 14% in methanol (called methanolic 14% BF<sub>3</sub>) was added to the cooled flask and heated at 115°C for 5 minutes this time. Tubes left to be cooled again and 2 mL of iso-octane was added. The tube has been shaken very well and waited until the phase separation takes place. It was taken approximately 1.5 mL from the upper phase after the separation and placed in 2 ml brown vial tubes which was stored in the freezer at -20 °C until injection.

Lipid fatty acid profile of guitarfish at the beginning of both storages (on day 0) was published in somewhere (Özyılmaz & Öksüz, 2015) and tabulated with the results of remaining storage periods. Instrument and Column: Fatty acids were analyzed by GC-MS (Gas Chromatography-Mass Spectrometry (GC-MS) using a Hewlett Packard GC (model 6890) coupled with a Hewlett Packard model 5972A HP 6890 system MS detector. Fatty acids were separated using an HPINNOWAX Polyethylene Glycol Capillary Column, Model Number: HP 19091N-133. The temperature program was kept at 120 °C for 3 minutes and with an increase of 10 °C/min, it reached 250 °C and was kept at this temperature for a while. Furthermore, Injector, detector temperatures, and identifying the individual fatty acids were described in Öksüz and Özyılmaz (2010).

**Statistic:** “Windows SPSS 13.00 Software” package program was used for statistical analysis and the data were analyzed at 95% confidence interval. One-way analysis of variance was used for comparison the difference between storage types and periods. To examine the effect of the duration and storage types on lipid of the fish' liver and their interaction with each other was evaluated with repeated measurements. Significance level of the values were determined at 95% level where  $p < 0.05$  was considered to be significantly different.

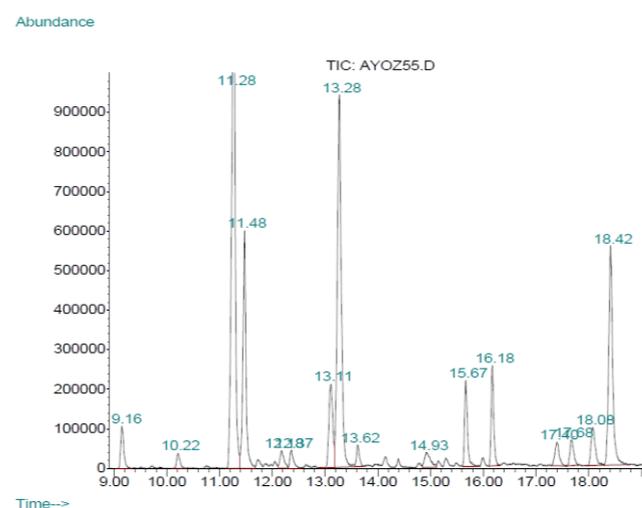
## **RESULTS AND DISCUSSION**

The liver oil of the guitarfish used in this study stored in two different storage conditions in a period of 180 days. The SFA, MUFA, and PUFA and their individual components of the guitarfish liver oil stored in room temperature and refrigerator (+4°C) were shown in Table 1 and Table 2. The first column in the both Tables was published in Özyılmaz and Öksüz (2015) because it was a part of a doctoral thesis study and this present one is another

major part of the same study which was too extensive to fit into one study.

Apart from that, their average fatty acid values of the oil stored in room temperature and refrigerator (+4°C) were analyzed on the days of the 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup>, 120<sup>th</sup>, 150<sup>th</sup>, 180<sup>th</sup>. The all fatty acid components determined for this study did not given in this current study. Only the majority parts were given for this paper. Because it is considered that to show the results in this way in order to point out the main parts of the results.

A chromatogram of fatty acids from the wild brown guitarfish was represented in Figure 2. The mean levels of the liver oil of the guitarfish's SFA, MUFA, and PUFA were changed during the storage. The SFA in liver oil of guitarfish tend to be higher while MUFA and PUFA in liver oil of guitarfish showed a lowering pattern in refrigerator and room temperature during storage. The main components of SFA were measured as C14:0, C16:0, and C18:0 which were increased in general but the increment was not constant however at the end of the sampling data were higher than sampling in the first place for both storing conditions.



**Figure 2.** A typical fatty acid chromatogram of guitarfish used in this current study

A study related changes in fatty acid contents of sea bass fillet in an 18 day of storage period, it was reported that the total SFA amount was 17.64% at the beginning of the storage, increased during the storage period, and reached 19.25% at the end of the storage Ozogul et al. (2017). The SFA content in fish were reported to be increased during the storage in this previously reported study. It was observed that the SFA values obtained from the present study were higher than the results reported by other researchers. However, SFA values decreased in first 30 days of the experiment for room temperature, it generally had an increasing pattern rest of the trial during the storage period. The interaction between storage types and periods regarding

SFA values during storage had significant effects on the liver oil ( $P < 0.05$ ).

Additionally, the MUFA components especially, the average values of C18:1n9+n7 in liver oil of guitarfish at room temperature and refrigerator ( $+4^{\circ}\text{C}$ ) changed in the range of 19.74% to 24.22%. Even though some changes were observed during the storage for both storage conditions, surprisingly the mean levels of C18:1n9+n7 in liver oil of guitarfish at the end of the trial were so close to the values at

the beginning ones. The second major fatty acid were determined as C16:1n9 which averagely was 10.85% at the beginning of the storage. It decreased 9.25% and 8.72% on the days of the 150<sup>th</sup> for liver oil stored in refrigerator and room temperature respectively. The interaction between storage types and periods regarding the average values of C18:1n9+n7 in liver oil of guitarfish in room temperature and refrigerator showed no significant effects on the liver oil ( $P > 0.05$ ).

**Table 1.** Changes in the fatty acid composition of guitarfish liver oil stored in refrigerator ( $+4^{\circ}\text{C}$ )

Fatty acids	Days						
	Day 1*	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
C14:0	1.95±0.07 <sup>a</sup>	2.94±0.06 <sup>bd</sup>	2.74±0.27 <sup>bc</sup>	2.62±0.26 <sup>c</sup>	2.92±0.09 <sup>bd</sup>	2.09±0.07 <sup>a</sup>	3.07±0.03 <sup>d</sup>
C15:0	0.74±0.01 <sup>a</sup>	1.12±0.02 <sup>bd</sup>	1.03±0.13 <sup>bc</sup>	0.96±0.11 <sup>c</sup>	1.08±0.03 <sup>bcd</sup>	0.76±0.02 <sup>a</sup>	1.19±0.02 <sup>d</sup>
C16:0	28.63±1.65 <sup>ab</sup>	29.87±0.76 <sup>bc</sup>	30.48±1.11 <sup>c</sup>	30.09±2.08 <sup>c</sup>	26.31±0.36 <sup>a</sup>	31.13±1.52 <sup>c</sup>	27.53±0.95 <sup>ab</sup>
C17:0	1.07±0.01 <sup>ac</sup>	1.30±0.16 <sup>b</sup>	1.26±0.16 <sup>b</sup>	1.07±0.04 <sup>a</sup>	1.30±0.12 <sup>b</sup>	0.90±0.02 <sup>a</sup>	1.52±0.03 <sup>c</sup>
C18:0	4.44±0.67 <sup>a</sup>	7.06±0.31 <sup>b</sup>	6.54±0.47 <sup>bc</sup>	5.97±0.33 <sup>c</sup>	6.22±0.58 <sup>c</sup>	6.27±0.15 <sup>c</sup>	7.09±0.14 <sup>b</sup>
<b>ΣSFA</b>	<b>36.84</b>	<b>42.29</b>	<b>42.05</b>	<b>40.71</b>	<b>37.83</b>	<b>41.14</b>	<b>40.40</b>
C16:1n9	10.85±0.51 <sup>ab</sup>	10.31±0.48 <sup>a</sup>	10.86±0.29 <sup>a</sup>	10.86±0.23 <sup>ab</sup>	11.03±0.26 <sup>ab</sup>	9.25±0.40 <sup>c</sup>	11.59±0.56 <sup>b</sup>
C17:1	0.96±0.01 <sup>a</sup>	1.16±0.22 <sup>ab</sup>	1.04±0.09 <sup>ab</sup>	1.09±0.21 <sup>ab</sup>	1.19±0.03 <sup>ab</sup>	0.97±0.04 <sup>a</sup>	1.26±0.02 <sup>b</sup>
C18:1n9+n7	22.33±0.43 <sup>a</sup>	19.23±0.93 <sup>b</sup>	21.14±0.90 <sup>ac</sup>	21.18±0.93 <sup>ac</sup>	20.29±1.20 <sup>bc</sup>	20.59±0.50 <sup>bc</sup>	21.59±0.68 <sup>ac</sup>
C20:1n9	1.56±0.72 <sup>a</sup>	1.16±0.20 <sup>a</sup>	1.24±0.14 <sup>a</sup>	1.11±0.07 <sup>a</sup>	1.41±0.10 <sup>a</sup>	1.68±0.08 <sup>a</sup>	1.30±0.00 <sup>a</sup>
<b>ΣMUFA</b>	<b>35.7</b>	<b>31.86</b>	<b>34.28</b>	<b>34.24</b>	<b>33.92</b>	<b>32.49</b>	<b>35.75</b>
C18:2n6	1.45±0.11 <sup>a</sup>	1.06±0.05 <sup>b</sup>	1.07±0.09 <sup>b</sup>	1.06±0.10 <sup>b</sup>	1.24±0.09 <sup>b</sup>	1.07±0.04 <sup>b</sup>	1.17±0.17 <sup>b</sup>
C20:4n6	3.14±0.97 <sup>a</sup>	3.12±0.20 <sup>a</sup>	3.24±0.08 <sup>a</sup>	3.22±0.07 <sup>a</sup>	3.43±0.01 <sup>a</sup>	3.07±0.16 <sup>a</sup>	3.21±0.11 <sup>a</sup>
C22:4n6	1.62±0.20 <sup>a</sup>	1.19±0.10 <sup>bc</sup>	1.24±0.03 <sup>bc</sup>	1.19±0.05 <sup>bc</sup>	1.38±0.13 <sup>c</sup>	1.13±0.01 <sup>b</sup>	1.24±0.14 <sup>bc</sup>
C22:5n6	1.48±0.11 <sup>a</sup>	1.30±0.12 <sup>b</sup>	1.39±0.04 <sup>ab</sup>	1.29±0.09 <sup>b</sup>	1.48±0.05 <sup>a</sup>	1.25±0.02 <sup>b</sup>	1.37±0.01 <sup>ab</sup>
<b>Σn6</b>	<b>7.69</b>	<b>6.7</b>	<b>6.94</b>	<b>6.77</b>	<b>7.54</b>	<b>6.52</b>	<b>6.99</b>
C20:5n3	4.21±0.66 <sup>a</sup>	3.43±0.12 <sup>b</sup>	3.65±0.08 <sup>b</sup>	3.63±0.12 <sup>b</sup>	3.81±0.01 <sup>b</sup>	3.38±0.12 <sup>ab</sup>	3.34±0.11 <sup>b</sup>
C22:5n3	2.16±0.51 <sup>a</sup>	1.93±0.08 <sup>ab</sup>	2.03±0.05 <sup>ab</sup>	1.98±0.13 <sup>ab</sup>	2.13±0.07 <sup>ab</sup>	1.73±0.02 <sup>b</sup>	1.97±0.09 <sup>ab</sup>
C22:6n3	13.28±0.41 <sup>a</sup>	9.53±0.41 <sup>bc</sup>	10.31±0.59 <sup>c</sup>	10.17±0.20 <sup>c</sup>	10.40±0.66 <sup>c</sup>	9.23±0.26 <sup>b</sup>	8.76±0.52 <sup>b</sup>
<b>Σn3</b>	<b>19.64</b>	<b>14.89</b>	<b>15.99</b>	<b>15.78</b>	<b>16.34</b>	<b>14.34</b>	<b>14.07</b>
<b>ΣPUFA</b>	<b>27.34</b>	<b>21.56</b>	<b>22.93</b>	<b>22.54</b>	<b>23.88</b>	<b>20.86</b>	<b>21.06</b>
<b>Identified ΣFA</b>	<b>99.87</b>	<b>95.71</b>	<b>99.26</b>	<b>97.49</b>	<b>95.62</b>	<b>94.49</b>	<b>97.21</b>
<b>n3/n6</b>	<b>2.55</b>	<b>2.23</b>	<b>2.30</b>	<b>2.33</b>	<b>2.17</b>	<b>2.20</b>	<b>2.01</b>
<b>DHA/EPA</b>	<b>3.16</b>	<b>2.78</b>	<b>2.82</b>	<b>2.80</b>	<b>2.73</b>	<b>2.73</b>	<b>2.62</b>

n=3±std; \* (Özyılmaz & Öksüz 2015)

**Table 2.** Changes in the fatty acid composition of guitarfish liver oil stored in room temperature

Fatty acids	Days						
	Day 1*	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
C14:0	1.95±0.07 <sup>a</sup>	2.50±0.02 <sup>b</sup>	2.77±0.14 <sup>c</sup>	2.63±0.25 <sup>bc</sup>	2.97±0.06 <sup>a</sup>	1.97±0.05 <sup>d</sup>	3.16±0.12 <sup>a</sup>
C15:0	0.74±0.01 <sup>a</sup>	0.76±0.02 <sup>b</sup>	1.21±0.10 <sup>a</sup>	0.94±0.11 <sup>c</sup>	1.11±0.02 <sup>a</sup>	0.65±0.00 <sup>d</sup>	1.21±0.04 <sup>a</sup>
C16:0	28.63±1.65 <sup>a</sup>	26.74±0.21 <sup>a</sup>	29.93±0.68 <sup>b</sup>	29.88±1.91 <sup>c</sup>	27.85±0.40 <sup>ac</sup>	28.47±0.58 <sup>a</sup>	28.55±0.35 <sup>ac</sup>
C17:0	1.07±0.01 <sup>a</sup>	0.82±0.01 <sup>b</sup>	0.84±0.03 <sup>b</sup>	1.13±0.20 <sup>c</sup>	1.32±0.02 <sup>c</sup>	0.82±0.03 <sup>b</sup>	1.53±0.06 <sup>d</sup>
C18:0	4.44±0.67 <sup>a</sup>	3.86±0.26 <sup>b</sup>	6.88±0.32 <sup>c</sup>	5.68±0.58 <sup>d</sup>	6.21±0.09 <sup>d</sup>	6.76±0.12 <sup>c</sup>	7.13±0.14 <sup>c</sup>
<b>ΣSFA</b>	<b>36.84</b>	<b>34.67</b>	<b>41.62</b>	<b>40.26</b>	<b>39.46</b>	<b>38.66</b>	<b>41.58</b>
C16:1n9	10.85±0.51 <sup>a</sup>	14.55±0.02 <sup>b</sup>	11.65±0.47 <sup>c</sup>	11.13±0.17 <sup>d</sup>	11.10±0.17 <sup>d</sup>	8.72±0.21 <sup>e</sup>	11.99±0.21 <sup>c</sup>
C17:1	0.96±0.01 <sup>a</sup>	0.98±0.04 <sup>b</sup>	1.26±0.13 <sup>c</sup>	1.03±0.09 <sup>b</sup>	1.24±0.03 <sup>c</sup>	0.92±0.01 <sup>b</sup>	1.24±0.02 <sup>c</sup>
C18:1n9+n7	22.33±0.43 <sup>a</sup>	24.22±0.11 <sup>b</sup>	21.19±0.28 <sup>b</sup>	20.87±0.59 <sup>ac</sup>	19.74±0.27 <sup>c</sup>	20.58±0.36 <sup>ac</sup>	22.16±0.38 <sup>a</sup>
C20:1n9	1.56±0.72 <sup>a</sup>	2.10±0.13 <sup>b</sup>	1.54±0.04 <sup>cb</sup>	1.30±0.40 <sup>cd</sup>	1.36±0.24 <sup>cd</sup>	1.70±0.07 <sup>c</sup>	0.73±0.01 <sup>e</sup>
<b>ΣMUFA</b>	<b>35.7</b>	<b>41.83</b>	<b>35.63</b>	<b>34.33</b>	<b>33.44</b>	<b>31.92</b>	<b>36.11</b>
C18:2n6	1.45±0.11 <sup>a</sup>	0.80±0.02 <sup>a</sup>	1.11±0.09 <sup>bc</sup>	1.06±0.10 <sup>bc</sup>	1.16±0.01 <sup>b</sup>	1.01±0.02 <sup>c</sup>	1.14±0.08 <sup>c</sup>
C20:4n6	3.14±0.97 <sup>a</sup>	2.05±0.01 <sup>b</sup>	3.30±0.28 <sup>a</sup>	3.18±0.11 <sup>a</sup>	3.10±0.10 <sup>a</sup>	3.20±0.03 <sup>a</sup>	3.32±0.04 <sup>a</sup>
C22:4n6	1.62±0.20 <sup>a</sup>	1.24±0.13 <sup>bc</sup>	1.24±0.01 <sup>bc</sup>	1.20±0.03 <sup>bc</sup>	1.16±0.02 <sup>b</sup>	1.23±0.14 <sup>bc</sup>	1.34±0.14 <sup>c</sup>
C22:5n6	1.48±0.11 <sup>a</sup>	1.28±0.08 <sup>a</sup>	1.24±0.01 <sup>a</sup>	1.29±0.06 <sup>a</sup>	1.34±0.03 <sup>a</sup>	1.31±0.21 <sup>a</sup>	1.38±0.05 <sup>b</sup>
<b>Σn6</b>	<b>7.69</b>	<b>5.36</b>	<b>6.90</b>	<b>6.72</b>	<b>6.75</b>	<b>6.75</b>	<b>7.18</b>
C20:5n3	4.21±0.66 <sup>a</sup>	2.61±0.01 <sup>b</sup>	3.39±0.17 <sup>c</sup>	3.51±0.16 <sup>c</sup>	3.41±0.04 <sup>c</sup>	3.95±0.08 <sup>d</sup>	3.47±0.03 <sup>c</sup>
C22:5n3	2.16±0.51 <sup>a</sup>	1.53±0.01 <sup>b</sup>	1.87±0.02 <sup>c</sup>	1.90±0.06 <sup>c</sup>	2.02±0.01 <sup>a</sup>	1.87±0.13 <sup>c</sup>	2.10±0.04 <sup>a</sup>
C22:6n3	13.28±0.41 <sup>a</sup>	9.90±0.15 <sup>ab</sup>	8.53±0.50 <sup>b</sup>	9.46±0.48 <sup>b</sup>	9.48±0.19 <sup>b</sup>	10.31±0.09 <sup>b</sup>	9.56±0.24 <sup>b</sup>
<b>Σn3</b>	<b>19.64</b>	<b>14.04</b>	<b>13.79</b>	<b>14.88</b>	<b>14.91</b>	<b>16.13</b>	<b>15.13</b>
<b>ΣPUFA</b>	<b>27.34</b>	<b>19.39</b>	<b>20.69</b>	<b>21.60</b>	<b>21.66</b>	<b>22.88</b>	<b>22.31</b>
<b>Identified ΣFA</b>	<b>99.87</b>	<b>95.89</b>	<b>97.95</b>	<b>96.20</b>	<b>94.56</b>	<b>93.45</b>	<b>100.00</b>
<b>n3/n6</b>	<b>2.55</b>	<b>2.62</b>	<b>2.00</b>	<b>2.21</b>	<b>2.21</b>	<b>2.39</b>	<b>2.11</b>
<b>DHA/EPA</b>	<b>3.16</b>	<b>3.79</b>	<b>2.52</b>	<b>2.69</b>	<b>2.78</b>	<b>2.61</b>	<b>2.75</b>

n=3±std; \* (Özyılmaz & Öksüz 2015)

The average amounts of PUFA on the days of the 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup>, 120<sup>th</sup>, 150<sup>th</sup>, and 180<sup>th</sup> were calculated to be 27.34%, 21.56%, 22.93%, 22.54%, 23.88%, 20.86%, and 21.06 in liver oil of guitarfish stored in refrigerator and 27.34%, 19.39%, 20.69%, 21.60%, 21.66%, 22.88%, and 22.31% in liver oil of guitarfish stored room temperature respectively. These results give us information about how storage effected fatty acid compositions of the liver oil guitarfish. The mean levels of PUFA had a quick effect on the days of the 30<sup>th</sup> for both storage conditions. Additionally, storage in refrigerator for liver oil of guitarfish was better than that of room temperature regarding their average PUFA contents on the days of the 30<sup>th</sup>. This could be the beginning of the lipid oxidations which was vulnerable in unsaturated fatty acids. Based on our data, the oxidation rate showed a sharp decreased in the first month and a very smooth changes in the following months during the storage period.

The levels of DHA + EPA are issued and detailed in reports of the studies because these two fatty acids are the precious omega 3 fatty acids for a healthy life and their presences and levels are very important in order to maintain a healthy diet e.g., cell membrane, hormones (Schlotz, et al., 2012). The eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid contents (DHA; 22:6n-3) are two precious fatty acids in polyunsaturated fatty acids (PUFA). Their sums (levels of DHA + EPA) in fatty acid components especially were given in studies in order to point out their importance.

In this study, the average levels of DHA + EPA in guitarfish liver oil were calculated as 17.49%. The liver oil of two cartilaginous fish, *R. bonasus* and *A. narinari*, EPA+DHA values were reported as 13.2% and 8.0%, respectively (Navarro-Garcia et al., 2010). These values for liver oil of these two cartilaginous fish are lower than that of the liver oil of the average EPA+DHA levels obtained in this study. The mean EPA+DHA values in liver oil of males and females' *R. clavata* were calculated to be 28.8% and 31.1% from Black sea and 28.2% to 30.6% from Mediterranean, respectively (Ozyilmaz 2016). These given values for liver oil of this cartilaginous fish for *R. clavata* are higher than that of the liver oil of the average EPA+DHA levels obtained in this study. The liver oils of the guitarfish used in this current are in the range of the previously given information related the average EPA+DHA levels in different kinds of cartilaginous fish.

The average amount of C14:0, C16:0, and C18:0 were increased in general but fluctuated during the storage. The level of saturated fatty acids were much higher at the end of storage than initial one for both storage conditions. On the other hand MUFA components especially, the average value of C18:1n9+n7 decreased for stored oil in room temperature and refrigerator (+4°C).

Fish oil are generally known to have higher amount of eicosapentaenoic acid (EPA, C20:5n3) and (DHA, C22:6n3). However, this fish liver oil surprisingly has got greatest amount of the C18:1n9+n7 comparing other cartilaginous fish species (Ozyilmaz 2016; Özyilmaz & Öksüz 2015; Ould El Kebir et al., 2007; Nechet et al., 2007). The C18:1n9+n7 is vulnerable to oxidize.

Oxidation is one of the major problem in oil. In particular fish oil are susceptible to oxidation because it has highly unsaturated fatty acids in its structure. Oils with unsaturated fatty acids (multiple double bonds between their carbon atoms) makes them unstable. The data regarding monthly analysis of this study indicated that the average level of the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and their individual components e.g., EPA, DHA in muscle lipid of the fish did not have a constantly degreasing or increasing pattern during storage period in 180 days.

## CONCLUSION

In conclusion, increase in all SFA components were observed and as a result, total SFA increased during storage for both storage conditions. In contrast to SFA, MUFA and PUFA decreased and this decrease may be explained by the progress of oxidation in unsaturated fatty acids. Stability of unsaturated fatty acids are vulnerable to oxidation than that of saturated fatty acids. This could be considered as an important issue for quality of the product. Unsaturated fatty acids are generally known to be less stable than saturated ones. This attributes make them vulnerable to rancidity. Based on the data of this present study, the data regarding unsaturated fatty acids oxidation is in parallel with the previously given fact. It was also supported these changes by monthly analysis. It is crucial to take the all necessary precaution to delay or prevent the fish lipid deterioration during the storage, such as proper packaging, low temperature storage and use of antioxidant.

## ACKNOWLEDGEMENTS

This work was a part of doctoral thesis of Ayse Ozyilmaz and financially supported by MKUBAP (Project Number: 04 Y 0126). Authors would like to thank to Dr. Aydın Demirci and Sevil Demirci for helping out the providing the guitarfish and MKUBAP for the financial the support.

## REFERENCES

Ayas, D., Eliuz, E., Peri, F. & Bakan, M. (2019). Determination of usage potential of some

- Mediterranean rays in fish oil production. *Marine Science and Technology Bulletin*, 8(1), 13-22. <https://doi.org/10.33714/masteb.480957>
- Bagge, C. N., Strandhave, C., Skov, C. M., Svensson, M., Schmidt, E. B., & Christensen, J. H. (2017).** Marine n-3 polyunsaturated fatty acids affect the blood pressure control in patients with newly diagnosed hypertension a 1- year follow-up study. *Nutrition Research*, 38, 71-78. <https://doi.org/10.1016/j.nutres.2017.02.009>
- Boran, G., Karaçam, H., & Boran, M. (2006).** Changes in the quality of fish oils due to storage temperature and time. *Food chemistry*, 98(4), 693-698.
- Hanson, S.W.F. & Olley, J. (1963).** Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochemical Journal*, 89, 101-102.
- Lteif, M., Mouawad, R., Khalaf, G., Lenfant, P., & Verdoit-Jarraya, M. (2016).** Population biology of an endangered species: the common guitarfish *Rhinobatos rhinobatos* in L ebanese marine waters of the eastern Mediterranean Sea. *Journal of Fish Biology*, 88(4), 1441-1459.
- Navarro-García, G., Ramírez-Suárez, J. C., Cota-Quñones, E., Márquez-Farías, F., & Bringas-Alvarado, L. (2010).** Storage stability of liver oil from two ray (Rhinoptera bonasus and Aetobatus narinari) species from the Gulf of Mexico. *Food Chemistry*, 119(4), 1578-1583.
- Nechet, S.L., Dubois, N., Gouyguou, J.P. & Berge J.P. (2007).** Lipid composition of the liver oil of the ray, Himantura bleekeri. *Food Chemistry*, 104, 559-564.
- Ould El Kebir, M.V., Barnathan, G. Gaydau, E.M. Siau, Y. & Miralle's J. (2007).** Fatty acid in liver, muscle, and gonad of three tropical rays including Non-Methylene-Interrupted Dienoic fatty acids. *Lipids*, 42, 525-535.
- Ragheb, E. & Hasan, M.W.A. (2021).** First record of Pteroplatytrygon violacea (Bonaparte, 1832) with annotation of cartilaginous fishes by-catch by gill nets (Egyptian Mediterranean). *The Egyptian Journal of Aquatic Research*, 47(4), 387-392.
- Simsek, E. & Demirci, A. (2018).** Barotrauma treatment effects on survival rates for some discarded fish by trawl fishery. *Fresenius Environmental Bulletin*, 27(7), 4867-4873.
- Ozyilmaz, A. (2016).** Tocopherol, heavy metals (Cd, Pb, Cu, Fe, Mn, Zn), and fatty acid contents of thornback ray (*Raja clavata* Linnaeus, 1758) liver oil in relation to gender and origin in the Mediterranean and Black seas. *Journal of Applied Ichthyology*, 32(3), 564-568. DOI: [10.1111/jai.13041](https://doi.org/10.1111/jai.13041)
- Özden, Ö. (2005).** Changes in amino acid and fatty acid composition during shelf-life of marinated fish. *Journal of the Science of Food and Agriculture*, 85(12), 2015-2020.
- Özogul, Y., Durmus, M., Uçar, Y., Köşker, A.R. & Ozogul, F. (2017).** The combined impact of nanoemulsion based on commercial oils and vacuum packing on the fatty acid profiles of sea bass fillets. *Journal of Food Processing and Preservation*, 41(6), e13222. DOI: [10.1111/jfpp.13222](https://doi.org/10.1111/jfpp.13222)
- Özyilmaz A. & Öksüz A. (2018 a).** The Effects of Different Storage Conditions on Fatty Acid Profile of Shark, Smooth-hound (*Mustelus mustelus*, Linnaeus 1758), Liver Oil; II. *International Fisheries Symposium*, 4-8 November 2018. Kyrenia, Cyprus, 54-55.
- Özyilmaz A. & Öksüz A. (2018 b).** The effects of storage conditions on fatty acid profile of shark, bignose shark (*Carcharhinus altimus*, Springer, 1950), liver oil; Çukurova I. *International Multidisciplinary Studies Congress*, 13-16 December 2018, Adana, Turkey.
- Özyilmaz, A. & Öksüz, A. (2015).** Determination of the biochemical properties of liver oil from selected cartilaginous fish living in the northeastern Mediterranean. *Journal of Animal and Plant Science*, 25, 160-167.
- Öksüz, A. & Özyilmaz, A. (2010).** Changes in fatty acid compositions of Black Sea anchovy (*Engraulis encrasicolus* L. 1758) during catching season. *Turkish Journal of Fisheries and Aquatic Sciences*, 10(3), 381-385.
- Schlotz, N., Sørensen, J.G. & Martin-Creuzburg, D. (2012).** The potential of dietary polyunsaturated fatty acids to modulate eicosanoid synthesis and reproduction in *Daphnia magna*: a gene expression approach. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 162(4), 449-454.
- Simat, V., Bogdanovic, T., Poljac, V. & Petricevic, S. (2015).** Changes in fatty acid composition, atherogenic and thrombogenic health lipid indices and lipid stability of bogue (*Boops boops* Linnaeus, 1758) during storage on ice: effect of fish farming activities. *Journal of Food Composition Analysis*, 40, 120-125. DOI: [10.1016/j.jfca.2014.12.026](https://doi.org/10.1016/j.jfca.2014.12.026)
- Wang, H., Liu, F., Yang, L., Zu, Y., Wang, H., Qu, S. & Zhang, Y. (2011).** Oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during long-term storage. *Food Chemistry*, 128(1), 93-99.
- Yığın, C., Çakır, Ç., Cabbar, F., Kızılkaya, K., Ormancı, B., Öztekin, H. B. & Özüdoğru, Y. (2019).** The liver lipid fatty acid composition consisting of two cartilaginous fish, thornback ray (*Raja clavata*) and common smooth-hound (*Mustelus mustelus*). *Aquatic Research*, 2(3), 143-153.