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DETERMINATION OF THE BIOCHEMICAL PROPERTIES OF LIVER OIL FROM SELECTED CARTILAGINOUS FISH LIVING IN THE NORTHEASTERN MEDITERRANEAN

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ABSTRACT

The objective of this research was to investigate the biochemical properties of liver oil from cartilaginous fish.The levels of carotene, α-tocopherol, trace elements, and fatty acid components were measured. The average carotene level of stingray liver oil was found to be thirteen times greater than that from the bignose shark. The levels of α-tocopherol in liver oil from three rays were found to be higher than those from all other cartilaginous fish. Levels of trace elements were additionally found to be lower.Total unsaturated fatty acids were found to be 63.70%, 64.85%, 63.04%, 63.29%, 59.04%, and 58.38% for the smooth-hound, bignose shark, guitarfish, stingray, eagle ray, and cownose ray, respectively. Surprisingly, the highest C18:1n9 and C18:1n7 levels and the lowest EPA-DHA levels were observed to be in the liver oil from the bignose shark. The findings also indicate that the levels of EPA in the liver oil of all the fish species studied were lower than those of DHA.This study concluded that the lipid levels and the amount of EPA and DHA in the liver oil of all the cartilaginous fish are sufficient to be used as commercial raw materials for the manufacturing of pharmaceutical and cosmetic products.

Keywords: Carotene, α-tocopherol, element, fatty acids, hepatosomatic index, cartilaginous fish

INTRODUCTION

Elasmobranchs (e.g., sharks, rays) form a group of fish that are also described as cartilaginous because these fish have a skeleton made of cartilage instead of bone. There are approximately 1200 species of cartilaginous fish world-wide, and more than 80 species are found in the Mediterranean Sea alone (Golani *et al*., 2006). The smooth-hound, bignose shark, guitarfish, stingray, eagle ray, and cownose ray are some of the cartilaginous fish that live in the northeastern Mediterranean and are occasionally found in the nets of fishermen.

Some cartilaginous fish (e.g., sharks) are intentionally caught for their fins. The fins are used to make shark fin soup in Far East Asia or are air dried, salted, and exported to the Southwest African countries (El Kebir *et al*., 1995). Along the coastlines of the Mediterranean and Aegean region, cartilaginous fish are not generally targeted for fishing, but instead, these fish are unintentionally caught in abundance and are well known in the region. Specifically, the muscles of the guitarfish are consumed in traditional dishes (e.g., shish kebab, doner kebab) in these regions (Karalar, 2005).

Although the muscles of cartilaginous fish are consumed in traditional dishes, their livers, and all of the other internal organs, are generally either thrown back into the sea or discarded as slaughter waste. This is a waste of a highly beneficial resource because the livers of cartilaginous fish could be used as raw material in the production of many industrial products (e.g., pharmacy, cosmetics) due to their high fatty acid content (Ould El Kebir *et al*., 2003; Navarro-Garcia *et al*., 2004; Ould El Kebir *et al*., 2007). Therefore, to better understand how these by-products could be used as raw materials in the pharmaceutical and cosmetic industries, it is necessary to investigate the liver oil content of cartilaginous fish.

Because cartilaginous fish are an important raw material, previous studies have investigated the liver oils from cartilaginous fish in order to guide the industrial production efforts of many different types of products in different parts of the world (e.g., Caribbean and Gulf of California (Navarro-Garcia *et al*., 2000); East Tropical Atlantic Ocean (Ould El Kebir *et al*., 2003); the Gulf of California (Mexico) (Navarro Garcia *et al*., 2004); Mauritania/West Africa (Ould El Kebir *et al*., 2007); and East Coast of India (Nechet *et al*., 2007)). However, the cartilaginous fish from the northeastern Mediterranean, especially the Gulf of Iskenderun, have not previously been studied. We know that the biochemical properties of the liver oil of cartilaginous fish differ by region, and the differences are of high interest to industrial manufacturers of pharmaceutical and cosmetic products.

Fish lipids have received great attention over the last two decades because they are rich in fatty acids. The fatty acid content of fish liver oil is an excellent source of polyunsaturated fatty acids (PUFA) including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These two acids have been previously reported to be very important for the prevention or reduction of many health-related problems such as cardiovascular disease (Mayneris-Perxachs*et al*., 2010) and depressive disorders (Mischoulon*et al*., 2009). These fatty acids also are essential for normal human brain development (Innis, 2007). In addition, tocopherols contribute to the stabilization of membrane structures and other active agents (e.g., vitamin A, hormones, and enzymes) against oxidation (Belitz*et al*., 2009). We know that high levels of unsaturated omega-3 PUFA, α-tocopherols, and carotenes are indicators of high-quality liver oil and that low levels of trace elements are indicators of the safety of the liver oil. Therefore, it is essential to investigate the vitamin content, element levels, and fatty acid composition of cartilaginous fish in order to develop products, control industrial manufacturing processes, and determine conditions for oil storage.

Because of the scarcity of research about the cartilaginous fish living in the northeastern Mediterranean and the importance of the fish to industrial manufacturing of pharmaceutical and cosmetics products, the objective of this study was to determine the biochemical composition of some native cartilaginous fish living in the northeastern Mediterranean Sea. The cartilaginous fish included in this study were smooth-hound, bignose shark, guitarfish, stingray, eagle ray, and cownose ray. The levels of the liver oil, trace elements, total carotene (vitamin A), and total α-tocopherol (vitamin E) were measured, and the ratio of hepatosomatic index (HSI) and fatty acid profiles of the fish liver oil were determined.

MATERIALS AND METHODS

Materials: Smooth-hound (*Mustelusmustelus*, Linnaeus 1758), common guitarfish (*Rhinobatosrhinobatos*, Linnaeus 1758), common stingray (*Dasyatispastinaca*, Linnaeus 1758), eagle ray (*Myliobatisaquila*, Linnaeus 1758), Lusitaniancownose ray (*Rhinopteramarginata*, Geoffroy Saint-Hilaire, 1817), andbignose shark (*Carcharhinusaltimus*, Springer, 1950) were caught in the Gulf of Iskenderun (northeastern Mediterranean Sea, Turkey) by professional fishermen with trawl nets.Amap of the study area is shown in Figure 1.

The average body weights of the cartilaginous fish were found to be 12296 g, 9304 g, 8633 g, 6735 g, and 5186 g for the eagle ray, cownose ray, guitarfish, stingray, and smooth-hound, respectively. Additionally, the average lengths of the smooth-hound, guitarfish, stingray, eagle ray, and cownose ray were measured to be 107.67 cm, 135.67 cm, 126.25 cm, 110.67 cm, and 104.00 cm, respectively.

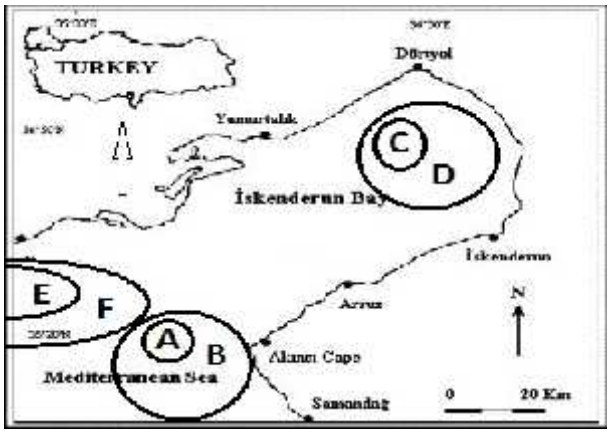

Figure 1. Map of the study area (A:smooth-hound, B:bignose, C: guitarfish, D: stingray, E: eagle ray, and F: cownose ray).

Sample Preparation: First, the lengths and the weights of the whole fish were measured. Second, the fish livers were removed, weighed, and stored at -20°C for further analysis.

Hepatosomatic Index (HSI): The HSI is calculated as the ratio of liver weight to body weight.

Crude Lipid Analysis: Amodified Bligh and Dyer method (Hanson and Olley, 1963) was used to determine the crude lipid composition of all cartilaginous fish species. Briefly, 10 g of chopped liver was weighed and placed into a homogenisation flask. Pure water (6 mL of) was then added. After the addition of 20 mL of chloroform (Merck, Darmstadt, Germany) and 40 mL of methanol (Merck, Darmstadt, Germany), the mixture was homogenised for 1 minute. An additional 20 mL of chloroform was added, and the mixture was homogenised for 30 seconds. Finally, 20 mL of water was added, and the mixture was homogenised for a further 30 seconds. The homogenate was centrifuged for 10 minutes at 3000 rpm. Following centrifugation, the aqueous layer was removed by aspiration. A total of 10 mL of the chloroform layer was transferred into a dry pre-weighed round-bottom flask. The chloroform was evaporated using a rotary vacuum evaporator at 35 °C. Final drying was performed in an oven at 105 °C for 30 minutes. The flask was allowed to cool to room temperature in desiccators for approximately 30 minutes and weighed. The following equationwas used to calculate the lipid content in the sample.

Equation: Percentage of lipid in the fish (Bligh & Dyer Method).

$$\% \text{ Lipid (B\&D)} = \frac{W_L}{Ws} \times \frac{V_1}{V_2} \times 100$$

Where:

W_L= Weight of the lipid extracted (g)
W_S= Weight of the sample (g)
V_1 = Total volume of chloroform that used for lipid extraction (mL)
V_2= Volume of chloroform used for evaporation (mL)

Determination of Total Levels of Carotene and α-Tocopherol: Spectrophotometric techniqueswere used to measure total carotene and α-tocopherol. The α-tocopherol and total carotene levels of the liver oils from cartilaginous fish were measured using protocols according to TS (Turkish Standard) 5036 (1987) and Morello *et al*. (2004), respectively.

Fatty Acid Methyl Esters (FAMEs) Preparation: The conversion and separation of FAMEs were performed as described in Oksuz *et al*. (2009).

Instrument and Column: Fatty acids were analysed 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 HP-INNOWAX Polyethylene Glycol Capillary Column, Model Number: HP 19091N-133.

The oven programme and the identification of individual fatty acids were performed according to Öksüz and Özyılmaz (2010).

Extraction and Determination of Trace Elements: The wet digestion method was used to digest the organic matter. This procedure was carried out according to the AOAC Method 975.03 with a minor modification. The sample was weighed, and a total of 2 mL of 60% perchloric acid (Merck, Darmstadt, Germany) was added to samples in fume hood. The sample was incubated until the dense gas output decreased. Then, 8 mL of 65% HNO_3 (Merck, Darmstadt, Germany) was added to complete digestion. Next, the sample was heated on a hot plate for at least 6 hours. Heated samples were removed from the hot plate and allowed to cool. The digests were filtered into a 25 mL volumetric flask, using ash-free filter paper, and filled to 25 mL with ultra-pure water. The determination and quantification of trace elements were performed by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry, Varian Model-Liberty series II). Calibration curves for each of the individual elements were prepared from ICP Multi element stocks (Merck, Darmstadt, Germany).

Statistical Analysis: The data were subjected to one-way ANOVA, and a mean comparison was carried out using Duncan's Multiple Range test at $P \leq 0.05$ (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Liver weight, HSI, total body weight, and average length of the cartilaginous fish are shown in Table 1. The bignose shark had the heaviest liver (2000 g) among the six cartilaginous fish investigated in this study. The other five cartilaginous fish ranked by weight (g) in descending order were eagle ray, stingray, cownose ray, guitarfish, and smooth-hound. The liver weights of the eagle ray and stingray were found to be 667 and 562 g, and their HSIs were calculated to be 5.36% and 8.25%, respectively. The smooth-hound had the lowest liver weight of 481.80 g, and the HSI of the smooth-hound was calculated to be 5.15%. Moreover, the average lengths of smooth-hound, guitarfish, stingray, eagle ray, and cownose ray were measured to be 107.67, 135.67, 126.25, 110.67, and 104.00 cm, respectively. The ratio of HSI varied from one species to another (4.24-8.25, excluding the bignose shark).

Table 1. The average length (cm), total weight (g), liver weight (g), and hepatosomatic index (HSI) of the cartilaginous fish.

Fish species	Length (cm)	Total weight (g)	Liver weight (g)	HSI (%)
Smooth-hound	107.67	5186.67	353.00	6.34
Bignose shark	-	-	2000.00	-
Guitarfish	135.67	8633.33	368.33	4.24
Stingray	126.25	6735.00	562.50	8.25
Eagle ray	110.67	12296.67	667.67	5.36
Cownose ray	104.00	9304.00	481.80	5.15

Mean±SD values were presented (n = 3), (except bignose shark).

According to Golani *et al*. (2006), the length of the smooth-hound was generally in the range of 50-100 cm (max. 120 cm). Measurements of smooth-hound length in this study were higher than those previously reported by Golani *et al*. (2006). The data generated from the body measurements of the smooth-hound are an indication that this cartilaginous fish lives and breeds very well in the northeastern Mediterranean.

Navarro-Garcia *et al*. (2000) studied the shark species *Carcharhinusfalciformus* and reported that the liver weight of this cartilaginous fish was between 1300 and 2300 g. In this study, the weight of the liver of the big nose shark (*Carcharhinusaltimus*) was found to be

2000 g, which is in the range reported by Navarro-Garcia *et al*. (2000). For the guitarfish, previous reports showed that these fish become mature and can bear offspring after reaching 86 cm in length (Karalar, 2005). In this study, each guitarfish was considered to be mature according to the Karalar (2005) classification. Additionally, the average length of the guitarfish in this study was in agreement with the measurement of the guitarfish length reported in previous studies (e.g., Yılmaz and Akpinar, 2003; Karalar, 2005; Golani *et al*., 2006).

The average liver lipid levels, total carotenes, and α-tocopherol of the cartilaginous fish liver oils are shown in Table 2. All of the cartilaginous fish had a large amount of lipids in their liver. However, the levels of liver lipid varied greatly from one species to another. The highest (73.10%) and lowest (57.19%) lipid levels were found in the livers of the bignose shark and smooth-hound, respectively.

Table 2. Liver lipid levels (%), total carotenes (µg/100 g), and α-tocopherol (mg/kg) of cartilaginous fish liver oil

Fish species	Liver lipid levels	Total carotenes	α-Tocopherol
Smooth-hound	57.19±0.00^{a}	83.78±3.53^{a}	82.84±0.63^{a}
Bignose shark	73.10±0.75^{b}	29.26±2.83^{b}	64.15±0.75^{b}
Guitarfish	69.20±0.52^{c}	249.72±69.6^{c}	60.55±0.57^{b}
Stingray	68.04±1.17^{c}	401.49±4.06^{d}	101.91±5.17^{c}
Eagle ray	65.71±0.68^{d}	104.53±2.10^{a}	77.36±12.55^{a}
Cownose ray	58.01±0.8^{a}	73.22±0.35^{ab}	114.69±6.47^{d}

Mean±SD values were presented (n = 3).
Mean±SD followed by the different letters within a column refers to significant differences ($P \leq 0.05$).

The amounts of total carotenes in the livers of the smooth-hound, bignose shark, guitarfish, stingray, eagle ray, and cownose ray were found to be 83.78±3.53, 29.26±2.83, 249.72±69.6, 401.49±4.06, 104.53±2.10, and 73.22±0.35µg/100 g, respectively. The differences between the amounts of carotene in the liver lipids of the various fish were statistically significant ($P \leq 0.05$). The highest carotene level was in the stingray liver lipid, followed by the guitarfish and eagle ray.

The α-tocopherol quantity in liver oils of cartilaginous fish was calculated to be 82.84±0.63 mg/kg for the smooth-hound, 64.15±0.75 mg/kg for the bignose shark, 60.55±0.57 mg/kg for the guitarfish, 101.91±5.17 mg/kg for the stingray, 77.36±12.55 mg/kg for the eagle ray, and 114.69±6.47 mg/kg for the cownose ray.

The liver lipid levels in all the cartilaginous fish livers in this study differed from each other, and these differences were statistically significant ($P \leq 0.05$). Fats were found in at least half of the livers, which clearly show that cartilaginous fish store a large amount of fat in their livers. Some studies on different cartilaginous fish species are in agreement with the finding that cartilaginous fish have a high amount of lipids in their livers (Navarro-Garcia *et al*., 2000; Ould El Kebir *et al*., 2003; Navarro-Garcia *et al*., 2004; Ould El Kebir *et al*., 2007; Nechet *et al*., 2007). The variations in the liver lipid levels in the cartilaginous fish livers could be the result of differences in the fish species, stage of fish life, seasons, location, or gender.

Navarro-Garcia *et al*., (2010) found that the levels of carotenes in the livers of two cartilaginous fish species, *Rhinopterabonasus* and *Aetobatusnarinari,* from the Gulf of Mexico were 0.90±0.0 and 0.60±0.0 mg/100 mg, respectively. In the same study, the authors also found that the levels of tocopherol for the same two species were 48.70±0.0 and 69.10±0.0 mg/100 mg. The highest tocopherol levels were found in the liver of the cownose rays, followed by the stingray, smooth-hound, eagle ray, bignose shark, and guitarfish.

Table 3. Contents of trace elements of cartilaginous fish (mg/kg).

	Fish species						*P*-
Elements	Smooth-hound	Bignose shark	Guitarfish	Stingray	Eagle ray	Cownose ray	value
Cd	0.02±0.02^{a}	0.05±0.02^{ab}	0.03±0.02^{ab}	0.03±0.0^{ab}	0.07±0.04^{b}	0.07±0.04^{b}	0.086
Cr	0.09±0.02^{a}	0.02–0.8^{a}	0.09–0.73^{a}	0.07–0.44^{a}	0.44±0.11^{a}	0.00–0.25^{a}	0.405
Cu	0.15±0.08^{a}	0.30±0.16^{a}	0.08–0.68^{a}	0.09–0.27^{a}	0.14±0.05^{a}	0.01–1.37^{a}	0.470
Fe	0.07±0.04^{a}	0.05±0.05^{a}	0.32–0.32^{ab}	0.14±0.08^{abc}	1.02–1.07^{bc}	1.18±0.32^{c}	0.039
Mn	0.57±0.03^{a}	0.68±0.14^{a}	0.63±0.01^{a}	0.59±0.05^{a}	0.55±0.09^{a}	0.08±0.06^{b}	0.000
Pb	0.17–1.26^{a}	0.52±0.14^{a}	0.31±0.14^{a}	0.79±0.15^{a}	0.90±0.22^{a}	0.89±0.40^{a}	0.213
Zn	0.33±0.03^{a}	0.52±0.01^{b}	0.39±0.12^{ab}	0.42±0.08^{ab}	0.36±0.15^{a}	0.27±0.02^{a}	0.045

Mean±SD values were presented (n = 3).
Mean±SD followed by the different letters within a row refers to significant differences ($P \leq 0.05$).

A total of seven trace elements in the livers of the six cartilaginous fish were analysed, and the results are shown in Table 3. The average trace element contents in fish liver oils varied greatly. The variability was statistically insignificant for some of the trace elements (Cd, Cr, Cu, Pb) (P> 0.05), whereas the variability was statistically significant for some of the other trace elements (Fe, Mn, Zn) ($P\leq$ 0.05).

The levels of Cd and Pb in the liver oil of the cartilaginous fish were found to be 0.02-0.07 mg/kg and 0.15-0.90 mg/kg, respectively. The amount of Cd in the liver oil of the guitarfish (0.03 mg/kg, on average) was found to be very close to the amount found in stingrays. Cd is an unwanted trace element that can be found in some species of seafood. Cd levels are controlled by legislation enacted by the European Commission (EC, 2006). The EC enforces the maximum permitted levels of 0.05-3.0 mg/kg wet weight for different types of seafood, excluding cartilaginous fish liver. The Cd measured in the selected cartilaginous fish liver was found to be in the range enforced by the EC. Therefore, fish liver can be considered safe to consume with regard to Cd levels. According toFood and Agriculture Organization (FAO, 1983), the tolerable daily intake of Cd and Pb for a 70-kg person was recommended to be 0.24 and 0.07 mg/day, respectively.

The average levels of Cr in the liver oil of the bignose shark, guitarfish, stingrays, and cownose rays were found to be 0.02-0.81 mg/kg, 0.09-0.73 mg/kg,0.07-0.44 mg/kg, and 0.00-0.25 mg/kg, respectively. Cr is an essential trace element for the human body; however, higher levels of Cr may harm people (Agency for Toxic Substances and Disease Registry, 2004). Although an upper level for the safe consumption of this trace element has not been established, the U.S. Institute of Medicine (2002) recommends a daily intake of 20-30 µg for men 51-70 years old.

The average levels of Cu in the liver oil of the smooth-hound, bignose shark, guitarfish, stringray, eagle ray, and cownose ray were 0.15, 0.30, 0.80, 0.09, 0.14, and 0.01 mg/kg, respectively. Additionally, the levels of Mn and Zn in cartilaginous fish liver oils varied from 0.08 to 0.68 mg/kg for Mn and from 0.27 to 0.52 mg/kg for Zn. These differences in the Mn level in the liver oils of the fish were statistically significant ($P\leq$ 0.05). Further investigation revealed that the main reason for the variation in Mn was the low level of liver oils in the cownose ray, which also indicates that the rest of the fish had similar levels of liver oil. Moreover, the amount of Fe in all of the liver oils of the cartilaginous fish was found to be lower than 1.51 mg/kg. Fe is an undesirable element in food processing due to its catalytic effect on the oxidation of fats and oils (Belitz *et al*., 2009).

The Cu levels in all of the cartilaginous fish liver oils were lower than the limits reported by Oehlenschlager (2002), which provides further evidence that fish liver oil can be considered safe to consume. Like Fe, Cu is not a desirable trace element during food processing and storage because this element can catalyse many unwanted reactions (Belitz *et al*., 2009). According to the Turkish Food Codex (TFC, 2002), the acceptable Cu content determined for animal fats (not specified for fish lipid) is a maximum of 4 mg/kg and for fish is a maximum of 20 mg/kg. The Cu levels for all of the fish liver oils were much lower than these maximum levels enforced by the TFC (2002).

The fatty acid compositions of the cartilaginous fish are shown in Table 4. Fatty acids that were less than 1% were omitted and are not shown in Table 4. A maximum of 19 fatty acids are shown in the table because some fatty acids were neither calculated (less than 1%) nor detected in some species. The determination of the fatty acid composition of the cartilaginous fish was performed by GC-MS. The levels of monounsaturated fatty acid (MUFA) were found to be higher than the levels of both saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) in liver oils of all of the cartilaginous fish except for the eagle ray.

The total SFA percentages of the smooth-hound, stingray, eagle ray, and cownose ray were similar to each other (P >0.05) with values of 34.79%, 34.97%, 35.10%, and 34.95%, respectively. In contrast, the levels of C14:0 (myristic acid), C16:0 (palmitic acid), and C18:0 (stearic acid) in the four cartilaginous fish liver oils differed from each other. The differences inC16:0 were statistically significant ($P\leq$ 0.05) for all six fish liver oils. The levels of C16:0 were 30.88% for the smooth-hound, 16.59% for the bignose shark, 28.63% for the guitarfish, the 26.86% for stingray, 21.16% for the eagle ray, and 18.63% for the cownose ray. Interestingly, the levels of C16:0 in all of the cartilaginous fish liver oils were higher than 21%, except for the bignose shark and the cownose ray. Moreover, C16:0 levels in all of the cartilaginous fish liver oils differed from each other. These differences were statistically significant ($P\leq$ 0.05). Furthermore, the highest C14:0 levels in SFA were found in the liver oil of the stingray ($P\leq$ 0.05).

As shown in Table 4, the predominant fatty acid in the smooth-hound, bignose shark, guitarfish, stingray, eagle ray, and cownose ray MUFA was C18:1n9+n7 (oleic+vaccenic acid), which had a value of 28.72%, 40.70%, 22.33%, 21.11%, 16.02%, and 12.65%, respectively. Clearly, the average level of C18:1n9+n7 in the bignose shark liver oil were significantly higher than the level in all of the other cartilaginous fish liver oils. The level is almost twice the level found in the guitarfish and stingray.

The total levels of PUFA in all the fish liver oils differed significantly ($P \leq$ 0.05). The major fatty acids identified in all fish liver oils were C22:6n3 (docosahexaenoic acid, DHA), C20:5n3 (eicosapentaenoic acid, EPA), and C20:4n6 (arachidonic

acid, ARA or AA). One exception was the liver oil from the bignose shark, which had C18:2n6 as the second highest fatty acid in its PUFA content instead of EPA. Additionally, the total n3, n6, and PUFA levels in cownose ray liver oil were significantly higher than those found in all other fish liver oils ($P \leq 0.05$).

Table 4. Fatty acid composition (percent of total fatty acids) of cartilaginous fish liver oil

Carbon Chain	Fish species					
	Smooth-hound	Bignose shark	Guitarfish	Stingray	Eagle ray	Cownose ray
C14:0	1.71±0.25^{a}	1.93±0.07^{a}	1.95±0.07^{a}	2.51±0.25^{b}	1.89±0.01^{a}	1.63±0.34^{a}
C15:0	≤1	≤1	0.74±0.01^{a}	≤1	0.99±0.01^{b}	1.02±0.15^{b}
C16:0	30.88±0.48^{a}	16.59±0.48^{b}	28.63±1.65^{c}	26.86±0.76^{d}	21.16±0.00^{e}	18.63±0.18^{f}
C17:0	≤1	1.13±0.14^{a}	1.07±0.01^{a}	1.05±0.07^{a}	1.77±0.02^{b}	1.49±0.23^{c}
C18:0	2.20±0.28^{a}	6.06±2.32^{b}	4.44±0.67^{b}	4.56±0.45^{b}	7.47±0.18^{c}	10.13±0.66^{d}
C20:0	≤1	≤1	≤1	≤1	1.84±0.01	2.06±0.28
ΣSFA	**34.79^{a}**	**24.28^{b}**	**36.84^{a}**	**34.97^{a}**	**35.10^{a}**	**34.95^{a}**
C16:1	12.94±0.35^{a}	10.28±0.33^{b}	10.85±0.51^{b}	16.63±0.60^{c}	9.15±0.06^{d}	6.74±0.74^{e}
C17:1	≤1	1.11±0.04^{a}	0.96±0.01^{a}	1.09±0.13^{a}	0.98±0.01^{a}	1.96±0.26^{b}
C18:1n9+n7	28.72±1.24^{a}	40.70±1.60^{b}	22.33±0.43^{c}	21.11±0.04^{c}	16.02±0.41^{d}	12.65±0.69^{e}
C20:1n9	1.64±0.14^{a}	3.90±0.09^{b}	1.56±0.72^{a}	2.40±0.46^{c}	1.14±0.15^{a}	1.47±0.59^{a}
C22:1n9	ND	ND	ND	ND	1.32±0.14	1.39±0.26
ΣMUFA	**43.30^{a}**	**55.98^{b}**	**35.7^{c}**	**41.22^{a}**	**28.61**	**24.19^{e}**
C16:2n4	≤1	≤1	≤1	0.98±0.03	1.43±0.04	ND
C18:2n6	0.75±0.18^{a}	1.77±0.02^{b}	1.45±0.11^{c}	≤1	1.41±0.01^{c}	1.18±0.12^{d}
C20:4n6	1.88±0.10^{a}	0.75±0.06^{b}	3.14±0.97^{c}	2.18±0.21^{ad}	2.38±0.06^{ad}	2.82±0.06^{cd}
C22:4n6	0.89±0.05^{a}	≤1	1.62±0.20^{b}	1.56±0.15^{b}	2.29±0.01^{c}	2.63±0.01^{d}
C22:5n6	1.03±0.05^{a}	≤1	1.48±0.11^{b}	1.68±0.15^{c}	2.43±0.05^{d}	2.23±0.04^{e}
Σn6	**4.55 ^{a}**	**2.64^{b}**	**7.69^{c}**	**5.41^{d}**	**8.49^{e}**	**8.85^{e}**
C20:5n3	2.31±0.09^{a}	0.79±0.05^{b}	4.21±0.66^{c}	4.04±0.65^{c}	3.05±0.06^{d}	4.12±0.3^{c}
C22:5n3	1.37±0.08^{a}	0.63±0.02^{b}	2.16±0.51^{c}	1.47±0.18^{a}	2.52±0.04^{cd}	2.78±0.21^{d}
C22:6n3	11.81±0.60^{ac}	4.93±0.46^{b}	13.28±0.41^{cd}	10.17±0.59^{a}	14.27±0.40^{d}	17.57±3.55^{e}
Σn3	**15.49^{a}**	**6.35^{b}**	**19.64^{c}**	**15.68^{a}**	**20.52^{c}**	**24.47^{d}**
ΣPUFA	**20.04^{a}**	**8.87^{b}**	**27.34^{c}**	**22.07^{d}**	**30.43^{e}**	**34.19^{f}**
n3/n6	**3.41^{a}**	**2.52^{b}**	**2.55^{b}**	**2.90^{b}**	**2.42^{b}**	**2.77^{b}**
DHA/EPA	**5.10^{a}**	**6.27^{b}**	**3.16^{c}**	**2.52^{d}**	**4.69^{ae}**	**4.26^{e}**

Mean±SD values were presented (n = 3). ND: Not Detected, ≤ 1: Less than 1%
Mean±SD followed by the different letters within a column refers to significant differences ($P \leq 0.05$).

The total SFA percentages of all the cartilaginous fish in this study were in the range of 24.28-36.84%. The total SFA percentages of two cartilaginous fish liver oils from *R. bonasus* and *A. narinari* that were previously reported were 34.60 and 35.30, respectively (Navarro-Garcia *et al*., 2010). The percentages of total SFA in both fish (*R. bonasus* and *A. narinari*) (Navarro-Garcia *et al*., 2010) were in agreement with the finding from the current study.

Among the SFA components, the levels of C14:0 in the livers of stingrays (*Dasyatispastinaca*) in this study were lower than that of female *Dasyatismarmorata* (Ould El Kebir *et al*., 2007) and both*Dasyatis brevis* and *Gymnuramarmorata* (Navarro-Garcia *et al*., 2004) but were higher than the levels of C14:0 in male Dasyatismarmorata (Ould El Kebir *et al*., 2007). Additionally, the levels of C14:0 in the liver oil of guitarfish (*Rhinopteramarginata*) in this study were lower than the levels in both male and female *Rhinobatoscemiculus*(Ould El Kebir *et al*., 2003).

In this study, the level of ΣMUFA (41.22%) in the liver oil of the stingray was higher than the levels found in both male and female *Dasyatismarmorata* (Ould El Kebir *et al*., 2003). Interestingly, one MUFA component, C18:ln9+n7, was found at an exceptionally high level in the liver oil of the bignose shark, and this finding has drawn attention to this fatty acid. This high amount of C18:1n9+n7 were not observed in any other cartilaginous fish (Ould El Kebir *et al*., 2003; Navarro Garcia *et al*., 2004; Ould El Kebir *et al*., 2007; Navarro-Garcia *et al*., 2010). Surprisingly, in contrast to containing high levels of C18:ln9+n7, the liver oil of the bignose shark has low amounts of EPA, DHA, and ARA, which are generally dominant in marine fish. Having a high level of C18:1n9+n7 could be a distinctive feature of the Carcharhinidae family. According to Osborn and

Akoh (2002), n-9 fatty acids, found as oleic acids (C18:1 n-9), play a moderately important role in the human body. Thus, the fatty acid profile of the bignose shark fish liver oil was very surprising in terms of the levels of C18:1n9+n7, DHA, EPA, and AA.

Erucic acid (C22:1n9) is a MUFA omega-9 fatty acid that is considered monounsaturated because it has only one double-bonded carbon atom in its fatty acid chain. Erucic acid was not detected in the liver oil of the smooth-hound, bignose shark, guitarfish, or stingray and was found to be less than 2% in the liver oil of the eagle ray and cownose ray. Oils with high levels of erucic fatty acids are not permitted in U.S. foods according to the U.S. Food and Drug Administration (FDA) regulations. For example, the FDA guidelines state that oils must not exceed a maximum erucic fatty acid content of 2% (O'Brian, 2004).

In this study, the levels of DHA, EPA, and ARA in fish liver oil differed greatly, and the differences were statistically significant ($P \leq 0.05$). The levels of DHA, EPA, and ARA in stingray (*Dasyatispastinaca*) liver oil were 10.17%, 4.04%, and 2.18%, respectively. Ould El Kebir *et al*. (2007) reported that the levels of DHA, EPA, and ARA were 11.6%, 1.88%, and 5.07% for female and 13.4%, 3.41%, and 5.66% for male *Dasyatis marmorata*. Navarro-Garcia *et al*. (2004) previously reported that the levels of DHA, EPA, and ARA were 4.80%, 5.30%, and 3.2% for liver oil from *Dasyatis brevis* and 10%, 5.90%, and 2.50%for liver oil from *Gymnuramarmorata*.

Although the DHA levels varied greatly, all fish liver oils have a high amount of DHA except the oil from the bignose shark. The average level of EPA in the guitarfish liver oil was in agreement with the findings from the liver oil of both male and female guitarfish species (*Rhinobatoscemiculus*) (Ould El Kebir *et al*., 2007). Moreover, the average level of ARA in guitarfish liver oil was the highest of all the cartilaginous fish liver oils. The levels of ARA varied greatly, and these variations were statistically significant ($P \leq 0.05$). Furthermore, the levels of ΣPUFA (22.07%), Σn3 (15.49%), and Σn6 (4.55%) in the liver oil of the stingray in this study were lower than those of both male and female *Dasyatismarmorata* (Ould El Kebir *et al*., 2003).

Conclusions: Based on the results of this study, all fish livers, with the exception of the bignose shark, are a good source of n-3 fatty acids, especially DHA. On the other hand, compared to other PUFA components, some of the n-6 fatty acids were either not detected or found in low levels in all fish species (e.g., C16:2n4, C22:4n6).

The lipid levels, total carotene (vitamin A), total tocopherol (vitamin E), trace elements, and fatty acid profiles of liver oil of the smooth-hound, bignose shark, guitarfish, stingray, eagle ray, and cownose ray were investigated. Although some features of the cartilaginous fish oils varied between the fish, all of the fish liver oils are qualitatively and quantitatively suitable for consumption with regard to the level of trace elements and fatty acid profiles. With the exception of the bignose shark, the livers from all of the other cartilaginous fish are rich in DHA (an important fatty acid). Therefore, the livers of these cartilaginous fish should be utilised and considered to be an alternative source of fish oil for the industrial production of useful products, such as pharmaceuticals and cosmetics.

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