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Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnura marmorata* rays

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Abstract

The omega-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (EPA) and docosahexaenoic (DHA), have been recognized for their important role in health. EPA has several beneficial effects regarding coronary heart disease, while DHA has been found to be important for the development of the brain and retina. The increase in the consumption of fish oil, a commercial source of PUFA, has made necessary the search for new fish species rich in PUFA that could be used as raw material for the fish oil production. The lipid, fatty acid composition and natural antioxidant contents (carotene, tocopherol) were analyzed for the liver oil of *Dasyatis brevis* (arenera) and *Gymnura marmorata* (mariposa), two ray species commercially captured in the Gulf of California. The liver oil yield 25–50% (w/w) for *D. brevis* and 38–56% for *G. marmorata*. The triglyceride fraction was the major lipid class (577–758 mg/g) for both species, with smaller proportions of sterol esters, free sterols, polar lipids and diacyl glyceryl ethers. *D. brevis* showed a greater carotene and tocopherols concentration (6.9 mg/100 g, 25.3 mg/100 g, respectively) than *G. marmorata* (1.8 mg/100 g, 2.8 mg/100 g, respectively). The content of saturated and monoenoic fatty acid was similar for both species, however, the liver oil of *G. marmorata* had twice as much DHA than *D. brevis*. The composite percentage composition of DHA plus EPA with respect to the total of fatty acids in liver oil was 18% for *G. marmorata* and 16% for *D. brevis*. The liver oil of *G. marmorata* and *D. brevis* represent a new source of omega-3 PUFA that can be used for human and animal nutrition.

Keywords: Pufa; Lipids; Ray; Antioxidant

1. Introduction

The fish oils, constitute an important source of omega-3 polyunsaturated fatty acids (PUFA), mainly the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA). The omega-3 PUFA provides several benefits to the human health; they are essential for the development and function of certain organs and for several biochemical and physiological responses of the organism (Meyer et al., 1999).

The brain is one of the organs where the omega-3 PUFA's are essential. The tissue of this organ is particularly rich in DHA, showing a close correlation between the consumption of this acid and its deposition in the cellular membrane (Rapoport, Chang, & Spector, 2001). DHA take part in the brain development and retina formation of the child during pregnancy (Hoffman & Uauy, 1992), so it is recommended that the future mother incorporates fish to her diet as a source of omega-3 PUFA's (Koletzko et al., 2001). Premature children fed with enriched DHA formulas reach visual sharpness faster than those with deficiencies (Ksiazyk, 2000). The omega-3 PUFA's have shown to be useful in the treatment of mental disorders like schizophrenia (Peet, Brind, Ramchand, Shah, & Vankar, 2001) and in the combat against cancer (Yam, Peled, & Shinitzky, 2001). Additionally, the consumption of omega-3 PUFA prevents the appearance of cardiovascular diseases, due to the hypolipidemic (Harper & Jacobson, 2001; Weber, 2000) and antithrombotic (Knapp, 1997) property of these fatty acids.

The highly unsaturated character of the omega-3 PUFA makes them target of the oxidation, therefore,

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tocopherols and carotenes that occur naturally in fish oil, help to maintain oil quality by terminating free radicals.

The elasmobranch (shark and ray) fishery for Sonora State in the Gulf of California is important, however, underdeveloped. Particularly for the ray fishery only the fins are utilized; head and viscera including the liver are thrown back to the sea. The annual ray captures from 1995 to 2000 were within 2000 ton; these volumes accounted for the 25% of the total nationwide rays catch (Anon, 2000). In order to propose the utilization of the ray liver as a source of high quality oil to the domestic fishermen and plant processors, it is necessary to calculate the oil recovery from these underutilized species and characterize its composition and stability. At the present time, scientific information on composition of elasmobranch liver oil is mainly on shark species (Bakes & Nichols, 1995; Bordier, Sellier, Foucault, & Le Goffic, 1994; Deprez, Volkman, & Davenport, 1990; Hayashi & Kishimura, 2000; Kang, Timmins, & Ackman, 1998; Navarro-García, Pacheco-Aguilar, Vallejo-Córdova, Ramírez-Suarez, & Bolaños, 2000). Little information has been reported for ray liver oil. Pal, Banerjee, Patra, Patra, and Ghosh (1998), analyzed the liver lipid composition of the ray *Dasyatis bleekeri* from India while Ould El Kebir, Barnathan, Siau, Miralles, and Gaydou (2003), the fatty acid composition in muscle, liver and gonads from three ray species from the Mauritanian coast. The ray species caught in Sonora, have not been study before. Therefore, the objective of this work was to characterize the lipid composition of liver oil of two commercial ray species from the Gulf of California, their natural antioxidant content and physicochemical properties. Results are intent to be used for triggering the development of this fishery.

2. Materials and methods

2.1. Ray samples

Six specimens of *Dasyatis brevis* and four of *Gymnura marmorata*, were collected off the coast of Kino Bay, Sonora, in the Gulf of California (Mexico), during May of 1999. The rays were measured and weighed soon after collection. Rays were eviscerated and livers were weighed and placed in polyethylene bags, rapidly frozen and stored at −70 °C until used. Frozen period was no longer than 2 weeks. The hepatosomatic index [(liver weigh/total body weigh) × 100] was determined for each individual ray specimen.

2.2. Lipid extraction

Prior to analysis, each liver was defrosted and homogenized for 2 min in a Waring blender homogenizer (Fisher Scientific, Pittsburgh, PA). Temperature during homogenization did not exceed 20 °C. The liver homogenates were immediately subjected to lipid extraction.

Lipid extraction was carried out following the extraction procedure outlined by McGill and Moffat (1992). Anhydrous sodium sulfate (30%, w/w) was added to the liver homogenate, mixed for 3 min and centrifuged at 1150*g* for 20 min at 25 °C. The resulting oil was divided in 15 ml aliquots and stored at −70 °C in small glass vials under nitrogen atmosphere. This oil was used for the determination of lipid classes, carotenes, tocopherols and physicochemical properties. Separately, the lipid content of a 10 g aliquot of each liver homogenate was immediately obtained by the sohxlet extraction procedure (AOAC, 1995 method 948.16) using petroleum ether as solvent.

2.3. Lipid classes

Qualitative and quantitative analyses of the lipid constituents in liver oil were carried out by thin-layer chromatography (TLC) (Navarro-García et al., 2000). A 20×20 cm pre-coated TLC plates without fluorescent indicator containing silica gel 60 (0.25 mm thick) (Merck-México, SA) were used for fractionation of steryl esters, triacylglycerols, sterols and polar lipids. Ten μl of oil hexane solution (40 mg/ml), was spotted over a width of 10 cm at 1 cm beneath the top of the plate. TLC was carried out at 25 °C in a glass tank ($15 \times 25 \times 25$ cm) using as the carrier agent a 90:10:0.5 mixture of hexane, diethyl-ether and acetic acid (v/v/v). Plates were developed by spraying a solution of 10% phosphomolybdic acid in ethanol (w/v). Lipid constituents were identified by comparison of their Rf values with those from the corresponding component of the neutral lipid mixture (cholesterol, cholesteryl oleate, oleic acid, oleic acid methyl ester, triolein), phosphatidyl choline (Matreya, Inc., Pleasant Gap, PA) and diacyl glyceryl ethers (DAGE) concentrated (Iceland Health, New York, NY), used as standard. The TLC plates were densitometrically scanned in a Bio-Rad scanner model GS-700 (Bio-Rad Inc. Lab., Hercules, CA). The areas were integrated with the software Multi-Analyst v1.1 (Bio-Rad Inc. Lab. Hercules, CA), and calibrated with standards at the 10–300 μg range.

2.4. Fatty acids

Fatty acids were derivatized to their correspondent methyl-esters using 7% BF_3MEOH following the method EC 2-66 of the AOCS (1993). About 200 mg of ray liver oil was hydrolyzed with 0.5 N NAOH in methanol (50 ml) for 1 h at reflux temperature. Afterward, BF_3-methanol reagent (5 ml) was added and the mixture was boiled for 2 min, then 2 ml of hexane was added and boiled for one more minute. After cooling, 15

ml of a saturated sodium chloride solution was added. The hexane solution of methyl-esters at the top, was extracted and transferred into a test tube with anhydrous sodium sulfate. The dry hexane solution (1 μl) was injected directly in gas chromatograph. Identification and quantification of fatty acid methyl-esters (FAME) was obtained by capillary gas chromatography (GC) in a Varian 3400 gas chromatograph (Walnut Creek, CA) equipped with a flame ionization detector and fitted with a 30 m × 0.25 mm i.d. fused silica capillary column coated with a 0.25 mm thick film of Omega Wax 250 (Supelco-Sigma, Mexico). Initial oven temperature was 180 °C. After 2 min the temperature was raised to 205 °C at 6 °C/min. Individual components were identified by comparing retention times with those obtained from a FAME mixture standard (Supelco-Sigma, cat. no. 4-7885, Aldrich Quimica, Mexico). Quantitative composition was calculated using an internal standard (17:0). Quantitative data were corrected for differences in detector responses through analysis of authentic standards of each reported fatty acid. All solvents used were analytical reagent grade from Merck (Merck, SA Mexico).

2.5. Carotenes and tocopherols

The total carotene analysis in the oil was carried out according to Simpson and Haard (1985). The weighed oil samples were dissolved in hexane and the absorbance at 468 nm recorded using a UV–Vis spectrophotometer Perkin Elmer Lambda 2S. The tocopherol determination was carried out according to Medina-Juarez et al. (2000) in a Varian 9050 HPLC chromatograph (Walnut Creek, CA), equipped with a ultraviolet light detector Varian model 3400 and a Lychrosorb column Si 60 (25 cm × 4 mm, 5 μm) (Supelco-Sigma, Mexico). The mobile phase was a mixture of hexane/isopropanol, 99.5:0.5 (v/v), with a flow of 1.6 ml/min. Before use, the mobile phase was filtered through a 0.45 μm filter. Two grams of oil were dissolved in 25 ml of hexane and filtrated to low pressure through a 0.45 μm filter. The samples (10 μl) were injected to the HPLC. The chromatographic peaks were identified and quantified by the comparison of the retention time and the areas of standards of α-tocopherol, β-tocopherol and γ-tocopherol (Supelco-Sigma, Aldrich Química, Mexico). Tocopherols were measured at 292 nm.

2.6. Physicochemical properties of the oil

The refractive index (RI), specific gravity (SG), iodine index (II) and saponification index (SI), were determined following the methodology outlined in the AOAC (1995) (methods 921.08, 920.213, 993.20 and 921.160, respectively). The oil color was measured according to AOCS (1993) (Method Cc 1e-92).

2.7. Statistical analysis

Descriptive statistics (mean, standard deviation and coefficient of variation) were applied. A linear regression analysis was carried out to establish the correlation of the carotene concentration in oil with the oil color. For data analysis the JMP Statistical Computer Package "JMP 4.04" for Windows 98 was used. For all determinations, six replicates were carried out for *D. brevis* and four for *Gymnura marmorata*. All analyses were carried out in duplicate.

3. Results and discussion

Sampled specimens of *D. brevis* presented a bigger size and total and liver weight than those of *G. marmorata.* Even though the hepasomatic index (HI) was also bigger for *D. brevis*, the parameter showed a wide variation within this species (Table 1). Literature reports that the HI present a wide variation among fish species. Pal et al. (1998), reported a specimen of ray (*D. bleekeri*) with a HI of 14.8%, while Navarro-García et al. (2000) a HI of 5.1% for the pelagic shark *Carcharhinus falciformis*, and Deprez et al. (1990) a HI in the range 17–26% for depth sharks.

Although the fat depot is located in most of the fish mainly underneath the skin, usually throughout the ventral line and around the abdomen, some species, like the codfish and the sharks, accumulate a considerable amount of oil in the liver (Kinsella, 1988), which is related to the function of energy storage of this organ. The total liver oil content of the two ray species also showed a wide variation within a given species. For *D. brevis*, it varied from 25% to 50%, while for *G. marmorata* the variation was between 38% and 56% (Table 1). Liver oil data was lower than the value of 63.4% reported by Pal et al. (1998) for the ray *D. bleekeri*. The variations found for this indicator can be due to age and differences in the physiological state of the animals among some other factors.

The physicochemical properties of a given oil are parameters, that allow its general characterization and are used in the quality control of technological processes. In the present work, all oil parameters (density, refraction index, saponification and iodine indices) except color, were similar for both species (Table 2). The saponification index for the two species ranged between 184 and 193, similar to the value of 188 reported by Hamm (1950), for the ray *Dasyatis uarnak*. The liver oil of *D. brevis* showed an orange-intense tone that corresponded to its high value for the red component (6 R to >9 R), whereas, the oil tone for *G. marmorata* was pale yellow to orange with values for the red component between 2 R and 5 R (Table 2).

Table 1
Rays body data and liver lipid content

Scientific name	Specimen no.	Body disk length[c] (m)	Body weight (kg)	Liver weight (kg)	Liver lipid (%)	Hepasomatic index[d] (%)
Dasyatis brevis	1	0.69	8.58	0.96	50.80	11.14
	2	0.69	9.58	0.94	42.30	9.85
	3	0.65	8.48	0.78	40.80	9.22
	4	0.69	9.58	0.63	29.80	6.57
	5	0.68	9.30	0.80	25.60	8.64
	6	0.62	7.70	0.38	34.50	4.91
Mean[a]		0.67	8.87	0.75	37.30	8.39
Standard deviation[a]		0.03	0.75	0.22	9.17	2.27
Gymnura marmorata	1	0.48	4.57	0.28	51.50	6.11
	2	0.59	6.96	0.30	56.20	4.33
	3	0.45	4.00	0.30	38.80	7.42
	4	0.50	4.96	0.35	56.20	6.95
Mean[b]		0.51	5.12	0.31	50.68	6.20
Standard deviation[b]		0.06	1.29	0.03	8.22	1.36

Figures are the mean of duplicate analyses.
[a] $n = 6$.
[b] $n = 4$.
[c] Ray body diameter.
[d] (Liver weight/body weight) $\times 100$.

Table 2
Physicochemical characteristics of rays liver oil

Scientific name	Specimen no.	Color[c]	Density	Refraction index	Saponification index	Iodine index
Dasyiatis brevis	1	50Y, 6.6R	0.9170	1.4770	186.7	150.8
	2	50Y, 6.5R	0.9193	1.4770	190.2	153.2
	3	50Y, 7.4R	0.9207	1.4775	193.1	148.6
	4	50Y, 8.0R	0.9145	1.4765	193.8	135.9
	5	50Y, 7.3R	0.9143	1.4745	190.8	128.2
	6	50Y, 9.9R	0.9115	1.4755	192.7	128.9
Mean[a]			0.9162	1.4763	191.2	140.9
Standard deviation[a]			0.0034	0.0011	2.6	11.3
Gymnura marmorata	1	50Y, 2.0R	0.9208	1.4790	184.5	149.1
	2	50Y, 5.0R	0.9235	1.4800	190.3	146.2
	3	50Y, 3.0R	0.9229	1.4780	192.2	161.3
	4	50Y, 3.0R	0.9256	1.4780	185.7	146.0
Mean[b]			0.9232	1.4788	188.2	150.7
Standard deviation[b]			0.0020	0.0010	3.7	7.2

Figures are the mean of duplicate analyses.
[a] $n = 6$.
[b] $n = 4$.
[c] Y, value of the yellow component; R, value of the red component.

The analysis of lipids by class is shown in Table 3. The triglycerides are the main component of lipid depots as much in animal as in vegetal cells. In the fish, the triglycerides are mobilized from lipid depots toward the liver with dependency of the energy needs of the organism (Sheridan, 1988). Considering both species, the triglycerides content varied from 577 to 758 mg/g. Similar levels of triglycerides had been reported in liver oil of ray *D. blekeeri* (Pal et al., 1998), shark *Carcharhinus falciformis* (Navarro-García et al., 2000) and fish globe (*Chelonodon patoca*, *Sphaeroides oblongus*, *Lagocephalus lunaris*, *Lagocephalus inermis*) (Hazra, Ghosh, Banerjee, & Mukherjee, 1998). Pal et al. (1998) reported that the polar lipids (PL) in the liver oil of the rays, are made up of glycolipids and phospholipids. For the species under study the content of PL ranged from 25 to 44 mg/g for *D. brevis* and from 36 to 60 mg/g for *G. marmorata*. These values were inferior to the PL content reported by Pal et al. (1998) in the liver oil of *D. bleekeri* (82 mg/g).

The sterols participate in the synthesis of vitamin D, sexual hormones, bile acid and in the absorption and transport of fatty acids. The content of free sterols (FS) and steryl esters (SE) were similar for both species under study (Table 3). The content for SE was higher than the one for FS. The SE ranged from 63 to 88 mg/g, while the

Table 3
Lipid by class, α-tocopherol and carotens contents in ray liver oil

Scientific name	Lipids by class (mg/g oil)						α-Tocoferols (mg/100 g oil)	Carotenes (mg/100 g oil)
	No.	TG	PL	SE	FS	DAGE		
Dasyatis brevis	1	759	44.8	72.4	33.3	–	15.6	2.7
	2	577	30.9	85.7	38.0	90.2	17.5	4.3
	3	749	33.4	–	60.6	–	16.6	5.9
	4	711	30.3	–	71.8	–	29.0	6.1
	5	617	26.7	84.2	46.8	96.2	8.4	6.1
	6	606	25.6	87.3	44.2	84.0	64.9	16.5
Mean[a]		670	32.0	82.4	49.1	90.1	25.3	6.9
Standard deviation[a]		79	6.9	6.7	14.5	6.1	20.5	4.9
Gymnura marmorata	1	744	44.5	79.4	38.4	–	2.5	0.2
	2	658	60.8	63.8	38.9	–	1.6	1.6
	3	696	36.4	77.8	62.3	–	4.3	0.2
	4	695	54.9	88.4	50.7	–	2.8	5.2
Mean[b]		698	49.2	77.4	47.6		2.8	1.8
Standard deviation[b]		35	10.8	10.2	11.3		1.1	2.4

TG, triglycerides; PL, polar lipid; SE, steryl esters; FS, free sterols; DAGE, diacyl glyceryl ethers.
Figures are the mean of duplicate analyses. (–) Not detected.
[a] $n = 6$.
[b] $n = 4$.

FS from 33 to 62 mg/g; however, in two specimens of *D. brevis* almost no SE were detected. Similar sterols content in liver oil for elasmobranches has been reported. Kayama, Tsuchiya, and Nevenzel (1969), reported that the FS contents in the liver oil of six species of shark was in the range from 30 to 68 mg/g of oil.

The DAGE were detected only in three specimens of *D. brevis* (Table 3) in concentrations that varied from 84 to 96 mg/g. Pal et al. (1998) reported DAGE (11.9 mg/g) in ray species of the family *Dasiatidae*. The DAGE have been found in shark liver oil (Kang et al., 1998; Bordier, Sellier, Foucault, & Le Goffic, 1996; Hayashi & Kishimura, 2000) in concentrations up to 76.6% of the lipid classes (Bakes & Nichols, 1995). It is considered that these compounds contribute to the buoyancy of the fish, in addition to serve as energy reserve similar to triglycerides. Hydrocarbons and wax esters have been found in elasmobranch oils among others lipid classes. Sargent, Gatten, and McIntosh, (1973) reported levels of hydrocarbons and wax esters (39% and 2%, respectively), in the liver neutral lipids of surface living shark, *Prionace glauca*. Those lipids classes were not analyzed in the present study and could be the remaining components of the liver oil for both ray species.

The fish is not able to synthesize carotenes, so they need to get them from the diet (Nègre-Sadargues, Castillo, & Segonzac, 2000). The rays incorporate carotenes through the shrimp and small crustaceans fundamental part of their feeding (Shahidi, Synowiwcki, & Penney, 1993). The carotenes are stored in the liver (Metusalach, Synowiecki, Brown, & Shahidi, 1996) and due to their liposoluble nature are extracted with the oil. In certain species of cultured fish, such as salmon, its quality is very related to the pink coloration of the meat, which in turn is due to its carotene content. The carotene content in the liver oil of *D. brevis* (2–16 mg/100 g oil), was superior to the one of *G. marmorata* (0.2 to 5 mg/100 g oil) (Table 3). The correlation equation of the red component (R) of the oil color (Table 2) with the carotene content (Table 3) considering the whole set of data from the two species was: R component $= 0.49$ [carotenes] $+ 3.77$ ($p < 0.01$, $r^2 = 0.6140$ and $p < 0.01$). However, using only the set of values from *D. brevis*, the corresponding equation was: R component $= 0.02$ [carotenes] $+ 5.89$ ($p < 0.01$, $r^2 = 0.924$ and $p < 0.001$). The correlation for the oil of *G. marmorata* oil was not statistically significant ($p \geqslant 0.05$). The result indicate that, the red color of the liver oil is closely related to its carotene content.

Similarly, the concentration of tocopherols was higher for the oil of *D. brevis* (8–64 mg/100 g oil vs. 1–4 mg/100 g oil) (Table 3). Only α-tocopherol was present, other tocopherols were not detected. Parazo, Lall, Castell, and Ackman (1998) reported that, fish liver presents a high selectivity to the deposition of the α form over the rest of tocopherols. The presence of α-tocopherol has been reported in liver oil of other species of elasmobranches (shark) in concentrations that varies from 8.3 to 25.0 mg/100 g of oil (Nichols, Bakes, & Elliott, 1998; Sunarya, Hole, & Taylor, 1996), and in menhaden oil (4.7 mg/100 g oil) (Kulås & Ackman, 2001). The antioxidant capacity of carotenes (Clark, Faustman, Chan, Furr, & Riesen, 1996), and tocopherols (Wanasundara, Shahidi, & Amarowicz, 1998), on the oxidative stability of fish lipids is well documented. Moreover, Takeuchi, Hara, Totani, Hibino, and Tanaka (1997), mentioned a synergistic effect of phospholipids on the antioxidant activity of carotenes and tocopherols.

Table 4
Quantitative amounts of fatty acids in ray liver oil (g/100 g oil)

Fatty acid	1	2	3	4	5	6	Mean[a,b]	Standard deviation[a,b]
Dasyatis brevis								
14:0	4.4	4.9	4.4	4.9	4.7	4.4	4.6	0.2
16:0	11.4	12.7	11.3	12.7	12.2	11.3	11.9	0.7
16:1	9.3	10.3	9.2	10.4	9.9	9.2	9.7	0.6
18:0	1.6	1.8	1.6	1.8	1.7	1.6	1.7	0.1
18:1	12.5	13.9	12.4	14.0	13.4	12.4	13.1	0.8
18:2	1.1	1.2	1.1	1.3	1.2	1.1	1.2	0.1
18:3	1.2	1.3	1.2	1.3	1.3	1.2	1.3	0.1
18:4	1.2	1.3	1.2	1.3	1.2	1.2	1.2	0.1
20:1	3.3	3.7	3.3	3.7	3.5	3.3	3.5	0.2
20:4	3.0	3.4	3.0	3.4	3.2	3.0	3.2	0.1
20:5	5.1	5.6	5.0	5.7	5.4	5.0	5.3	0.3
22:6	4.6	5.1	4.6	5.1	4.9	4.6	4.8	0.2
$n-3$	12.1	13.4	12.0	13.5	12.9	12.0	12.6	0.7
$n-6$	4.2	4.6	4.1	4.6	4.4	4.1	4.3	0.2
Gymnura marmorata								
14:0	3.6	4.6	4.5	3.7			4.1	0.5
16:0	13.1	17.0	16.3	13.6			15.0	1.9
16:1	8.0	10.4	10.0	8.7			9.3	1.1
18:0	0.8	1.0	1.0	0.8			0.9	0.1
18:1	10.9	13.2	13.6	11.9			12.4	1.2
18:2	5.7	7.4	7.2	6.2			6.6	0.8
18:3	3.7	4.8	4.8	4.0			4.3	0.5
18:4	6.7	8.8	8.6	7.4			7.9	0.9
20:1	1.7	2.3	2.1	1.9			2.0	0.2
20:4	2.1	2.8	2.7	2.4			2.5	0.3
20:5	5.2	6.7	6.5	5.4			5.9	0.7
22:6	8.7	11.4	10.7	9.4			10.0	1.2
$n-3$	24.4	31.8	30.7	26.4			28.3	3.5
$n-6$	7.9	10.3	9.9	8.6			9.2	1.1

Figures are the means of duplicate analyses.
[a] $n = 6$.
[b] $n = 4$.

Further studies (Pacheco-Aguilar et al., in preparation) are under development to evaluate the impact of the carotenes, tocopherols and phospholipids content in the oxidative stability of the liver oil extracted from both species.

The unsaturated fatty acids (UFA) were the major fraction in the liver oil of the two ray species studied (Table 4). The monoenoic fatty acids content was similar for both species; however, the liver oil of *G. marmorata* has twice as much polyenoic acids as *D. brevis*. Within the monoenoic fatty acids, the oleic (18:1) presented the higher concentration in both species (13.1 ± 0.8 *D. brevis*; 12.4 ± 1.2 *G. marmorata*). The palmitoleic acid (16:1) was also present in substantial amounts. These results agreed with the studies on fatty acid composition made by Nichols et al. (1998) and Bordier et al. (1994) for shark liver oil and those of Méndez, González, Inocente, Giudice, and Grompone (1996), and Andrade, Rubira, Matsushita, and Souza. (1995) for fillets of *Pogonias cromis*, *Menticirrhus americanus* and *Micropogonias furnieri*. With regard to the omega-3 PUFA's, both species have similar content of EPA (20:5) (5.3 ± 0.3 and 5.9 ± 0.7 g/100 g oil for *D. brevis* and *G. marmorata*, respectively); in the other hand, the concentration of the DHA (22:6) in *G. marmorata* was two times higher in *D. brevis* (10.0 ± 1.2 vs. 4.8 ± 0.2).

The percentage composition of DHA plus EPA with respect to the total of fatty acids in liver oil was 18% for *G. marmorata* and 16% for *D. brevis*. Méndez et al. (1996) reported a composite content of EPA and DHA of 16.1% for codfish liver oil, considered the traditional omega-3 PUFA's source. Result of this study indicated that, ray liver oil could easily replace the codfish liver oil in its different nutritional applications (human and aquaculture). With respect to saturated fatty acids, the higher proportion was for palmitic acid (16:0) followed by myristic (14:0) in both species.

In conclusion, liver oils from *D. brevis* and *G. marmorata* is an excellent sources of omega-3 fatty acids, especially EPA and DHA. Also, the presence of α-tocopherols and carotenes prove their exceptional nutritional characteristic for human food as well as for feed. Nutritional-wise, the ray fishery in Mexico could be

developed into an important source of several value-added biologic active compounds.

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