

Lipid content and fatty acid composition of the liver from the rajiforms *Urotrygon chilensis*, *Urobatis halleri*, *Rhinobatos glaucostigma*, *Rhinoptera steindachneri* and *Dasyatis dipeteura* captured in Sinaloa, México

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Abstract

The lipid content and fatty acid composition of the liver oil from the ray fish species *Urotrygon chilensis*, *Urobatis halleri*, *Rhinobatos glaucostigma*, *Rhinoptera steindachneri* and *Dasyatis dipeteura*, rajiforms captured in the State of Sinaloa, México, was examined, and the variation in the fatty acid composition determined by multivariate analysis. Total lipid content in their liver was relatively high, ranging between 30.67 - 46.41%, the highest observed in *D. dipeteura*. These species showed important concentrations of n-3 long chain polyunsaturated fatty acids, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (EPA+DHA= 4.79 - 14.47 g/100 g of liver oil), both provide health and medical benefits, including the reduction in the risk of death from coronary heart disease. The atherogenicity (0.93 - 1.53) and thrombogenicity (0.31 - 0.53) indexes were noticeably low, supporting the evidence of their high quality for cardiovascular disease prevention. Variation in the fatty acid composition, determined by multivariate analysis, showed the separation of the five species group means is fully explained in the two-dimensional discriminant space accounting for 83.34% of the dispersion. In conclusion, ray fish liver oil can be considered an important and interesting alternative to fish oil as a source of EPA and DHA.

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Introduction

Nowadays consumption of nutraceuticals is significant and it has increased steadily over the past few years. Nutraceuticals are natural food products that provide health and medical benefits, including the prevention and treatment of disease. Among these food products essential fatty acids stand out, particularly the n-3 long-chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6n-3). Among their health benefits, a reduction in the risk of death from coronary heart disease with a daily consumption of 400 - 500 mg of EPA+DHA in healthy patients has been reported (Harris *et al.*, 2008). In patients with schizophrenia and attention-deficit/hyperactivity disorder, low plasma DHA levels have been observed (Riediger *et al.*, 2009). On the other hand, DHA consumption during pregnancy and later in infant formulas results in improved brain and eye (retina) development in babies (Dobs *et al.*, 2008).

Up until today fish oil is the most important source of EPA and DHA. Among the fish species with the highest content of these essential fatty acids are wild salmon, cod and herrings. However, the sustained increment in fish oil demand over the last years, and the projected increment in the near future, requires the search for alternative sources of these essential fatty acids (González *et al.*, 2010).

Ray fish liver oil has presented itself as an important and interesting alternative (Ould El Kebir *et al.*, 2007) found that the liver of three species of ray fish captured in the Republic of Mauritania have concentrations of EPA and DHA ranging from 1.88-5.01% and 10.00 - 13.04%, respectively. In Mexico some of the first studies on lipid content of ray fish liver oil were presented by Navarro *et al.* (2004a) and Navarro *et al.* (2004b), they found that the liver of some ray fish species captured in the Gulf of California represents between 5 and 11% of the animals' wet weight, and the lipid content of the liver corresponds to 50% of that weight. The liver oil of the studied species had 16 - 18% of EPA+DHA,

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a similar concentration to that reported for cod oil characterized for its high n-3 LC-PUFA content. Three more species from the state of Campeche, in the Gulf of Mexico, *Rhinoptera bonasus*, *Aetobatus narinari* and *Dasyatis americana*, showed an oil yield of 43.0, 41.2 and 38.2% of the liver wet weight, respectively. The highest sum of eicosapentaenoic, docosahexaenoic and docosapentaenoic n-3 LC-PUFA were found in *Rhinoptera bonasus* (22.4%) and *Dasyatis americana* (21.6%) (Navarro et al., 2009).

The Gulf of California is an important ray fish fishing area in Mexico. Recent surveys indicated a high diversity of elasmobranchs (shark and batoid) in the artisanal fisheries of this region. Assessments of Mexican elasmobranchs fisheries indicate that sharks dominate landings (Bonfil, 1994, 1997; Castillo et al., 1998), but rays are important fishery targets by the artisanal ray fishery (Márquez et al., 2002) and are commonly caught as bycatch by shrimp trawlers in the area (Flores et al., 1995; García et al., 2000). The ray fish species incidentally captured in shrimp trawlers include the families Urotrygonidae, Myliobatidae, Narcinidae, Rhinobatidae, and Rhinopteridae, which include demersal species that distribute along the Mexican Pacific. Abundance of a particular species in the catches depends on the season, depth and whether conditions. In spite of the progress of their artisanal fishery, still very little is known on their biology and biochemistry (Márquez, 2002; Navarro et al., 2004a, b). Most of the ray fish species are underutilized; only the meat is used and its price depends on its color.

Sinaloa is one of the states with an important ray fisheries activity in the Gulf of California (CONAPESCA, 2010). However, no information is available on the liver quality and oil yield of the captured species that would encourage the utilization and commercialization of this resource. Thus, the objective of the present study was to analyze the lipid content and fatty acid composition of the liver oil from *Urotrygon chilensis*, *Urobatis halleri*, *Rhinobatos glaucostigma*, *Rhinoptera steindachneri* and *Dasyatis dipterura*, rajiforms captured in the State of Sinaloa, México, and evaluate the variation in the fatty acid composition determined by multivariate analysis.

Materials and methods

Sample acquisition

Samples of *U. chilensis* (20), *U. halleri* (20), *R. glaucostigma* (5), *R. steindachneri* (3) and *D. dipterura* (7) were obtained during 2009 from experimental shrimp trawl surveys conducted in Teacapan, Sinaloa by the Instituto Nacional de Pesca, and Facultad de

Ciencias del Mar from the Universidad Autónoma de Sinaloa. Sets were performed at different depths including 2, 5, 8, 10 and 12 fathoms (Fig. 1). The livers were dissected, placed in polyethylene bags and frozen at -20°C for their transportation in coolers to the Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora (DICTUS) (Hermosillo, Sonora, Mexico). Livers were stored at -80°C, for no more than 2 weeks, until lipid extraction.

Lipid content and fatty acid analysis

Total lipid was extracted from the liver by homogenizing in chloroform/methanol (2:1, v/v) in an Ultra-Turrax T-18 tissue disrupter (IKA Work, Inc, Wilmington, NC, USA.) and measured gravimetrically according to the method of Folch et al. (1957). Fatty acids were derivatized to their corresponding methyl-esters using 7% BF₃-MeOH, according to method Ce 2-66, A.O.C.S. (1992). Identification and quantification of fatty acids methyl-esters (FAME) was done by capillary gas chromatography with a Varian 3800 gas chromatograph (Varian Inc., Walnut Creek, CA) fitted with a 50 m, 0.25 mm i.d. CP-Cil 88 52CB capillary column (Varian, Walnut Creek, CA, USA) and equipped with a flame ionization detector. The initial oven temperature was set at 195°C, raised to 240°C at 5°C/min and maintained at this temperature for 3 min. Individual components were identified by comparing retention times with those obtained from the FAME mixture standard (Supelco-Sigma cat. No. 4-7885, Aldrich, Química, México). Tricosanoic acid (C23:0) was used as an internal standard. The fatty acid content was expressed as g of FAME per 100 g of liver oil. Once the fatty acid content and composition were determined, dietary scores such as the atherogenicity index (AI) and index of thrombogenicity (IT) (Ulbricht and Southgate, 1991) were determined to obtain information on the oils' benefits as nutraceuticals for cardiovascular disease prevention.

Statistical analysis

Sample analysis was performed in triplicate and data presented are means ± standard deviation. Analysis of variance of total lipid content of the liver was performed, and Duncan's multiple range test was used as the mean separation procedure (P < 0.05). Fisher discriminant analysis (FDA) was performed by using centred and standardised ray fish species data. The data set was composed by sixteen fatty acids and five ray fish species. Subsequently, the Lachenbruch's holdout procedure [C, D] (sometimes referred to

as jackknifing or cross-validation) was used in conjunction with the Fisher's classification analysis based on the first two sample discriminants in order to estimate the total misclassification probability or actual error rate (AER). The last process was repeated for each variables subset possible. The variables subset with dimension minimum associated to the estimated AER minimum was selected in order to see how well they classify compared to the discriminant function, which uses all the variables. Data were processed with MATLAB program version 7.0.

Results and discussion

Total lipid content in the liver of the analyzed species was relatively high, ranging between 30.67 and 46.41% (Table 1); *D. dipterura* showed the highest lipid content and *R. steindachneri* the lowest. Navarro *et al.* (2004 a, b) reported higher lipid content for *R. steindachneri* and lower for *D. dipterura*, this makes evident the role of the liver as an energy storage organ, which determines that its total lipid content varies according to the animals' feeding status, age or size, gender, geographical distribution, or season, even for the same species (Wetherbee and Nichols, 2000). Similar total liver lipid contents have also been reported for other ray species, such as *D. bleekeri* (63.4%) (Pal *et al.*, 1998), *D. marmorata*, *R. cemiculus*, *R. marginata* (47.4, 40.5, and 22.6% respectively) (Ould El Kebir *et al.*, 2003), *Himantura bleekeri* (54.0%) (Le Nèchet *et al.*, 2007), *R. bonasus*, *D. americana* and *A. narinari* (43.0, 38.2 and 41.2%, respectively) (Navarro *et al.*, 2009), as well as shark liver (40.3%) (Navarro *et al.*, 2000).

The fatty acid analyses of the lipid extracted from ray fish livers showed eighteen fatty acids that were considered abundant (Table 2). In spite of the complexity of fatty acid profiles from fish oils, their composition can be described based on 8 to 10 key fatty acids (Lambertsen, 1978). The present study concurs, since fatty acid analysis of ray fish liver oils demonstrated that C16:0 (4.74 - 23.44 g/100 g of liver oil) and C18:0 (3.10 - 10.71 g/100 g of liver oil) were the main saturated fatty acids (SFA), coinciding with has already been reported in liver lipids of *D. dipterura* and *R. steindachneri* captured off the Sonora state coast (Navarro *et al.*, 2004 a, b), for *R. bonasus*, *D. americana* and *A. narinari* (Navarro *et al.*, 2009) and for *R. marginata*, *D. marmorata* and *R. cemiculus* (Ould El Kebir *et al.*, 2003), but also comparable to fatty acids in muscle of *Rachycentron canadum* (Liu *et al.*, 2009) and *Raja clavata* (Turan *et al.*, 2007). Both, C16:0 and C18:0 can be used as energy sources. Additionally, they may serve as

Table 1. Total lipid content in the liver of *U. chillensis*, *U. halleri*, *R. glaucostigma*, *R. steindachneri* and *D. dipterura*

Species	Lipid (%)
<i>U. chillensis</i>	36.18 ^{ab} ± 10.18
<i>U. halleri</i>	36.27 ^{ab} ± 7.51
<i>R. glaucostigma</i>	31.83 ^b ± 10.27
<i>R. steindachneri</i>	30.67 ^b ± 4.84
<i>D. dipterura</i>	46.41 ^a ± 4.98

Table 2. Fatty acid composition of liver oil from the rajiforms *U. chillensis*, *U. halleri*, *R. glaucostigma*, *R. steindachneri* and *D. dipterura* (g/100 g of liver oil)

Fatty acid	<i>U. chillensis</i>		<i>U. halleri</i>		<i>R. glaucostigma</i>		<i>R. steindachneri</i>		<i>D. dipterura</i>	
	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.
14:0	1.54	0.53	2.49	1.09	2.22	0.38	0.58	0.36	2.65	1.28
14:1	0.96	0.32	1.40	0.52	1.12	0.37	0.41	0.22	1.53	0.74
16:0	15.28	4.17	16.70	4.94	15.20	1.76	4.74	1.11	23.44	10.85
16:1	5.02	1.53	7.96	2.36	4.39	0.47	1.74	0.55	11.62	4.62
18:0	10.71	2.93	7.21	1.95	6.48	0.66	3.10	0.87	8.41	3.24
18:1	6.24	1.78	12.37	3.38	8.15	1.30	2.86	1.13	17.61	6.96
18:2n-6	1.73	0.40	1.79	0.63	1.44	0.31	0.61	0.27	2.12	1.04
18:3n-3	0.39	0.11	0.39	0.32	0.32	0.09	0.09	0.06	0.31	0.18
20:0	0.22	0.12	0.10	0.10	0.00	0.00	0.12	0.10	0.17	0.08
20:1	2.27	0.53	2.05	0.58	0.96	0.17	1.02	0.24	2.50	1.02
20:4n-6	3.24	0.85	3.10	0.87	2.86	1.21	1.30	0.24	2.88	0.95
20:5n-3	5.41	1.54	5.09	1.75	3.28	1.20	0.99	0.18	4.33	1.42
22:0	1.59	0.60	1.35	0.38	0.81	0.13	0.51	0.16	1.03	0.46
22:1	0.64	0.13	0.52	0.17	0.32	0.02	0.30	0.09	0.55	0.17
22:5n-3	2.27	0.41	2.21	0.62	1.21	0.23	1.10	0.41	1.85	0.55
22:6n-3	8.55	1.66	8.37	3.22	11.19	1.82	5.70	2.84	4.73	1.44
24:1	3.02	0.58	2.29	0.59	0.73	0.09	1.52	0.40	2.44	0.73
24:2	2.02	0.38	1.69	0.66	1.43	0.32	2.29	0.41	1.07	0.35

structural components of phospholipids (Perez *et al.*, 1999). As for monounsaturated fatty acids (MUFA), the most abundant were C16:1 (1.74 - 11.62%) and C18:1 (2.86 - 17.61%) (Table 2), showing similar values to those reported for other Mexican ray fish species captured in different locations (Navarro-García *et al.*, 2009; Navarro-García *et al.*, 2004a, b). These fatty acids can be biosynthesized or obtained from the diet.

The n-3 LC-PUFA, particularly EPA and DHA have a significant therapeutic and nutritional value, and among other applications, they have been used for cardiovascular disease treatment (Kryzhanovskii and Vititnova, 2009), for babies brain development (Schuchardt *et al.*, 2010) and as hypotensors (Chen *et al.*, 2009). In general, the species in this study showed important concentrations of EPA and DHA (Table 2), although *D. dipterura* showed a DHA level (4.73%) lower than 5%. DHA values were higher than EPA values for the studied species, similarly to what has been reported for other ray fish (Navarro *et al.*, 2009; Navarro *et al.*, 2004a, b) and some warm water species (Ackman *et al.*, 1980).

Unsaturated fatty acids (\sum MUFA+ \sum PUFA+ \sum LC-PUFA) were a majority (58-69%), whereas the ratio \sum PUFA+ \sum LC-PUFA/ \sum SFA was higher for *R. steindachneri* (1.16). This ratio was over 0.50 in all remaining species (Table 3), which is the minimum

Table 3. Fatty acid sums (g/100 g of liver oil) and nutritional indexes of liver oil from the rajiforms *U. chilensis*, *U. halleri*, *R. glaucostigma*, *R. steindachneri* and *D. dipterura*

Fatty acid	<i>U. chilensis</i>		<i>U. halleri</i>		<i>R. glaucostigma</i>		<i>R. steindachneri</i>		<i>D. dipterura</i>	
	Mean	Std.Dev	Mean	Std.Dev	Mean	Std.Dev	Mean	Std.Dev	Mean	Std.Dev
ΣSFA	29.30	7.57	27.79	7.82	24.72	2.11	9.01	2.55	35.62	15.48
ΣMUFA	18.15	4.30	26.47	6.53	15.67	2.06	7.84	2.49	36.25	13.71
ΣPUFA+LC-PUFA	23.61	4.64	22.62	6.14	21.73	2.40	10.16	3.80	17.28	5.48
PUFA/ΣSFA	0.82	0.11	0.83	0.14	0.88	0.12	1.16	0.41	0.51	0.10
Σn-3	16.62	3.38	16.05	4.35	16.00	1.97	5.95	3.82	11.21	3.33
Σn-6	4.97	1.17	4.88	1.42	4.30	1.15	1.91	0.05	5.00	1.93
Σn-3/Σn-6	3.39	0.38	3.32	0.47	4.03	1.60	3.08	1.90	2.32	0.36
EPA+DHA	13.95	2.95	13.45	3.73	14.47	1.87	4.79	3.83	9.06	2.71
AI	1.40		1.25		1.53		0.93		1.47	
IT	0.41		0.36		0.36		0.31		0.53	

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; LC-PUFA: Long Chain Polyunsaturated Fatty Acids; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid; AI: Atherogenicity index; IT: Index of Thrombogenicity.

Table 4. Matrix of holdout procedure calculated using all fatty acids

Actual membership		Predicted membership				
		E1	E2	E3	E4	E5
	E1	17	1	0	2	0
	E2	1	17	0	1	1
	E3	0	0	5	0	0
	E4	1	0	0	3	0
	E5	0	2	0	0	5

E1: *U. chilensis*; E2: *U. halleri*; E3: *R. glaucostigma*; E4: *R. steindachneri*; E5: *D. dipterura*

recommended ratio for cardiovascular disease prevention (Hunty, 1995) and comparable to the ratio reported for *R. clavata*'s oil (0.50) (Turan *et al.*, 2007).

The n-3 and n-6 polyunsaturated fatty acids have antagonistic effects in the human body. While n-3 fatty acids are precursors of vasodilators, platelet anti-aggregation and anti-inflammatory compounds, the n-6 are precursors of compounds with opposite effects. Thus, the n-3/n-6 ratio is an important indicator to determine the nutritional value of oils. Simopoulos (2000) proposed an optimal range of 0.5 - 1.0 for this ratio. The n-3/n-6 ratios determined for the five studied ray fish species ranged between 2.32 and 4.03, the highest corresponding to *R. glaucostigma*. Similar ratios have been determined for liver oils of *Sardinella lemuru* (2.26) (Khoddami *et al.*, 2009) and tuna (6.5) (Jaturasitha *et al.*, 2009). Slightly lower n-3/n-6 ratios have been reported for some of these ray fish species and others, such as *R. steindachneri* (1.13), *A. narinari* (0.3), *R. bonasus* (1.33) and *D. dipterura* (1.21) (Perez *et al.*, 2008) that are probably a result of a dietary influence in the tissue's fatty acid profile.

Predicting the capacity to avoid cardiovascular accidents by consuming a particular kind of oil may be done through the atherogenicity (AI) and thrombogenicity (IT) indexes (Ulbricht and Southgate, 1991) determined from the fatty acid profile of the oil. The AI evaluates the effect of various fatty acids on the serum cholesterol. The

Table 5. Matrix of holdout procedure calculated using thirteen fatty acids*

Actual membership		Predicted membership				
		E1	E2	E3	E4	E5
	E1	18	1	0	1	0
	E2	0	20	0	0	0
	E3	0	0	5	0	0
	E4	0	0	0	3	0
	E5	0	1	0	0	6

*Fatty acids: 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2n-6, 20:1, 22:0, 22:5n-3, 22:6n-3, 24:1, 24:2.

E1: *U. chilensis*; E2: *U. halleri*; E3: *R. glaucostigma*; E4: *R. steindachneri*; E5: *D. dipterura*

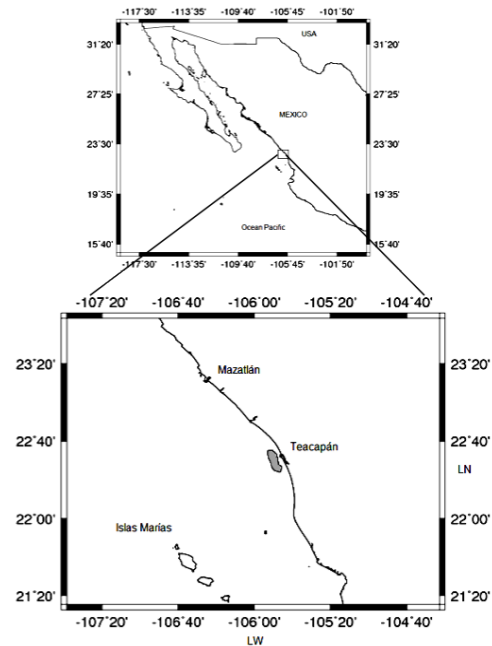


Figure 1. Study area and location of sampling stations in the coasts of the Gulf of California

saturated fatty acids C14:0 and C16:0 are four times more atherogenic, while unsaturated fatty acids do not contribute to the increment of the index. The IT allows us to know the degree of contribution of fatty acids to platelet aggregation raising the levels of esterified fatty acids, cholesterol, and phospholipids, which are factors that favor the development of thrombosis (Turan *et al.*, 2007). The determined IA (0.93 - 1.53) and IT (0.31 - 0.53) values for the liver oils of the evaluated ray fish were noticeably low in both cases (Table 3), which supports the evidence of the high quality of ray fish liver oil for cardiovascular disease prevention. Values for AI and IT for *R. clavata* (2.37; 0.63) (Turan *et al.*, 2007), anchovy (0.45; 1.35), eel (0.37; 0.57), and rainbow trout (0.25; 0.45) (Valfré *et al.*, 2003) have been reported.

Studies on the fatty acid profiles of oils from marine species not only provide information on their nutritional value, but also allow their classification through the application of multivariate statistical analysis. Some studies have reported that it is possible to differentiate different salmon species (Mjaavatten *et al.*, 1998) or species from the genus *Sebastes* (Joensen and Grahl-Nielsen, 2000) and

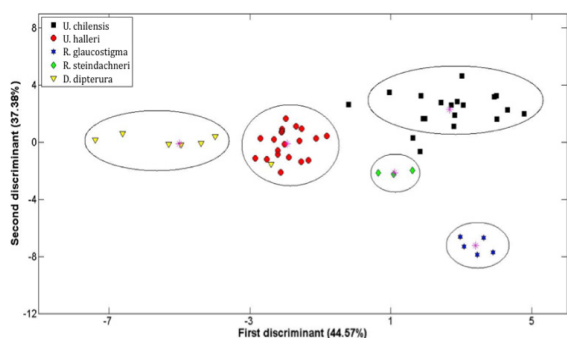


Figure 2. Ray fish species samples in discriminant space based on all fatty acids. The percentage of the dispersion of the group means along each of the discriminants may be observed as asterisks in the graph.

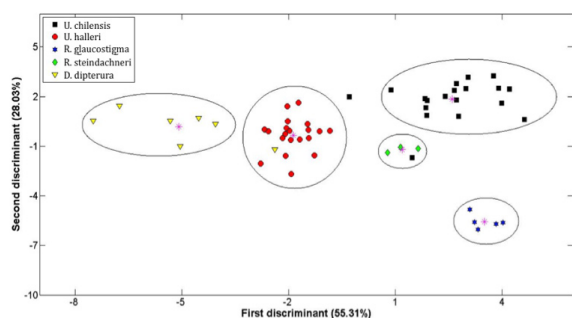


Figure 3. Ray fish Ray fish species samples in discriminant space based on thirteen fatty acids. The percentage of the dispersion of the group means along each of the discriminants may be observed as asterisks in the graph.

even tell apart a cultured or wild organisms (Tritt *et al.*, 2005). In the present paper, result from the multivariate statistical analysis showed the separation of five group means fully explained in the two-dimensional “discriminant space”. The group means and the scatter of the individual observations in the discriminant coordinate system are shown in Figure 2. The separation is good, and the first and second discriminant functions, which taken together account for 81.95% of the dispersion of the group means, shows that they have the strongest power of discrimination of the four functions. The elements in Table 4 were generated using the holdout procedure, so the overall error rate (8/55 or 14.55%) is low. On the other hand, adopting the holdout estimate of the expected AER as our criterion in order to explore effective classification with fewer variables, it was observed that thirteen fatty acids (14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2n-6, 20:1, 22:0, 22:5n-3, 22:6n-3, 24:1, 24:2) produced an error rate of 5.45%, quite low (Table 5). A plot of the group means and individual observations using the first two discriminant scores is shown in Figure 3, where the separation is as good as in the previous figure. The first and second discriminant functions account for

83.34% of the dispersion of the group means. Thus the mean vectors lie largely in two dimension, and two discriminant functions suffice to describe most of the separation among the five ray fish species.

Conclusion

In conclusion, the value of multivariate analysis to determine the variation in the fatty acid composition of the oils was demonstrated. Total lipid content in the liver of all five species was relatively high, *D. dipterura* showed the highest total lipid content. All five species also showed important concentrations of n-3 LC-PUFA, particularly EPA and DHA, and *R. glaucostigma* showed the highest content of EPA+DHA. The AI and TI were noticeably low, supporting the evidence of the high quality of ray fish liver oil for cardiovascular disease prevention. Therefore, ray fish liver oil can be considered an important and interesting alternative to fish oil as a source of EPA and DHA.

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