Fat content and EPA and DHA levels of selected marine, freshwater fish and shellfish species from the east coast of Peninsular Malaysia

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Abstract: The total lipid contents and the concentration of eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acid of fourteen selected marine, three freshwater fish species, four shellfish species and two selected canned fish species of East Coast of Peninsular Malaysia were determined. The fat content of all samples ranged from 1.01 % to 15.83 % with silver catfish reported to have the highest value. Most of the fish had fat amounts lower than 10% of their total weight. In general, DHA concentrations (50.50-165.21 μ g/g) were significantly higher than EPA (11.12-55.38 μ g/g) in all of the fish species analyzed. Among all marine fish species, the sixbar grouper recorded the highest concentration of DHA (165.21 μ g/g) while barramundi had the highest concentration of EPA (55.38 μ g/g). In conclusion, all fish and shellfish species are considered a good source of EPA and DHA, representing a very valuable essential nutrient for maintenance of human health.

Keywords: Fat content, marine fish, shellfish, eicosapentaenoic (EPA), docosahexaenoic (DHA).

Introduction

Eicosapentaenoic (EPA) and docosahexaenoic (DHA) are the two main omega-3 fatty acids typically found in marine fish and originate from the phytoplankton and seaweed that are part of their food chain. Previous research indicates that omega-3 fatty acids are associated with lowering the occurrence hypertrigliceridaemia (Cavington, 2004), inflammation (Calder, 2001), rheumatoid arthritisand other diseases. Besides these functions, EPA and DHA are highly concentrated in the brain and appear to be particularly vital for early development of cognitive function and visual sharpness (Birch et al., 2000) and also can act as major components of cell membranes and precursors of the eicosanoids hormones (Ng, 2006). In addition, these fatty acids have been effectively proven to be useful in the prevention and treatment of wide variety of disorders including hypertension, eczema, psoriasis, osteoporosis, breast cancer, asthma and allergy (Guil-Guerrero et al., 2011). Hence, DHA and EPA provide a wide range of health benefits, both by means of fish ingestion, or through the consumption of dietary supplement.

Previous epidemiological studies have shown that minimal intake of the long chain omega-3

polyunsaturated fatty acids, EPA and DHA are linked to having an increased risk of coronary heart disease (Albert *et al.*, 2002; Hu *et al.*, 2002; Abu and Oluwatowoju, 2009). The importance of omega-3 fatty acids in human nutrition is widely recognized (Simopoulos, 2004), especially HUFA with 20–22 carbons and 5 or 6 double bonds and particularly DHA and EPA. The potential of these compounds in the prevention of certain cardiovascular diseases (Connor, 2000) and other chronic diseases have also been documented (Innis, 2000; Moyad, 2005). The benefits of n-3 PUFA (polyunsaturated fatty acids) are associated with its role in the synthesis of prostaglandins, thromboxanes, and leukotrienes (Kapoor and Patil, 2011).

In the perspective of functional food, EPA and DHA fatty acids provide the taste and texture of many foods. Variations in fatty acid composition may occur due to fluctuations in the quality and amount of food available, especially for phytoplankton (Ackman, 1967). The nutritional recommendations for daily intakes of ω -3 from DHA and EPA ranged between 0.5 and 1.6 g for healthy adults, infants, pregnant and lactating women published by several international scientific authorities (Loukas *et al.*, 2010).

The fatty acid composition of the edible portion of fish is affected by many factors, such as species,

*Corresponding author. Email: wrosli@kck.usm.my sex, sexual maturity, size, place of capture, water temperature, feeding and season (Armstrong *et al.*, 1991). Presently, there is little information about the EPA and DHA levels of various marine fish species from East Coast region of Malaysia. This research primarily focuses on fish fillets consumed as food. In addition, this study investigates the composition of EPA and DHA and the amount of total lipids of twenty three marine, freshwater fish and shellfish species from East Coast of Peninsular Malaysia.

Materials and Methods

Prior to the laboratory analysis, the dietary pattern of fish and shellfish intake were implemented to identify the type of fish and shellfish species that frequently consumed among the local community in the East Coast region of Peninsular Malaysia. In order to identify the most preferable species consumed by the community in this region, two steps of dietary intake survey have been implemented thoroughly to 100 selected respondents (n=100) from all part of vicinities in Kelantan state of Malaysia. All respondents were asked to recall their intake for breakfast, lunch and dinner by two nutritionists who have familiar with the name and type of local foods in Kelantan. Subjects were asked to report portion sizes of their intake based on the standard household measures which included a glass, bowl and teaspoon. Nutritionists were also probe in details particulars in term of fish and shellfish consumption and checked the food list for face validity if any local foods were missed from the 24 hour dietary recalls particularly on fish and shellfish consumed. After application of this method and restricted only to the fish and shellfish item, the food frequency questionnaire was constructed for interview process. For each item of fish and shellfish lists, the frequency of consumption either 'day', 'week', 'month' or 'year' were documented.

Eventually, nineteen fishes (fourteen marine, three freshwater and two commercial canned products) and four shellfishes based on the frequency of consumption were identified and purchased from local wet market in Kota Bharu, a capital city of market. Kota Bharu wet market was being chosen to select the sample of fish and shellfish due to the marketing activities of varieties of fish which on demand by local community. The samples were immediately stored on ice until they reached the laboratory and then were kept frozen at -20°C until chemical analysis. Sampling was performed irrespective of organism sex and size.

The following marine (fourteen) and freshwater

(three) fishes and shellfishes (four) species of East Coast of Peninsular Malaysian were investigated: Thunnus tonggol (small tuna, local name aya kecil), Euthynnus affinis (large tuna, aya besar), Magalapsis cordyla (hardtail pomfret, cencaru), Selarides leptolejus (small yellow striped scad, selar kuning kecil), Selarides spp. (yellow striped scad, selar besar), Parastromateus niger (black pomfret, bawal hitam), Pampus argenteus (silver pomfret, bawal putih), Nemipterus japonicas (delagoa threadfish bream kerisi), Stolephorus commersonii (anchovy, bilis), Scomberomorus guttatus (spotted mackerel, tenggiri), Decapterus russelli (selayang, Indian scad), Rastrelliger kanagurta (Indian mackerel, kembung), Epinephelus sexfasciatus (sixbar grouper, kerapu), Lates calcarifer (barramundi, siakap), Clarias batrachu (catfish, keli), Synbranchus bengalensis (eel, belut), *Pangasius pangasius* (silver catfish, patin), Liocarcinus vernalis (crab, ketam), Sepia officinalis, (cuttlefish, sotong), Arca granosa (cockles, kerang) and Metapenaeus affinis (shrimp, udang). In addition, two commercial canned fish products also included in this study. They are Clupeoides spp. [sardine, sardin (Ayam brandTM, Ayam SARL, Shah Alam, Malaysia)], and Scomberomorus spp [mackerel, makeral (Ayam brandTM, Ayam SARL, Shah Alam, Malaysia)].

Preparation of fish, shellfish and selected commercial canned fish samples

Fresh fish fillet samples were prepared by removing 3 g pieces of meat from the middle portion of the fish with skin on. The tissue was cut into small pieces and homogenized (Ultra turrax T25D Ika, Germany). The homogenized fish tissue samples were then investigated for their fat content and fatty acid composition profiles. However, the whole fish body was used during homogenization for smaller fish (anchovy). Meanwhile, in preparation of shellfish samples, only flesh was used for homogenization of crab, cockles and shrimp samples where the shells were discarded. On the other hand, fish fillets with skin on but without sauces were used in preparation of canned fish samples.

Total fat content

The fat content was measured in triplicates by gravimetric technique with chloroform/ methanol/ water according to the method described by Kinsella *et al.* (1977). The extracted fats were stored at -18°C before further analysis. The yields of the extracted fish samples were calculated based on the difference in the mass before and after lipid extraction.

Fatty acid determination

Fatty acid methyl esters (FAME) were prepared by esterification with sodium methoxide (analytical grade) in methanol (Abu and Oluwatowoju, 2009). The samples were then analyzed using an Agilent CP9001 model gas chromatograph equipped with a flame ionization detector (FID) and a fused silica capillary column CP Sil-88 (50 x 0.25 mm id., 0.20 Im film thickness, Chrompack) and hydrogen as carrier gas with a flow of 1.0 mL/min, split ratio of 1/100. The Injection and detection temperatures were set at 250°C and 280°C, respectively. The column temperature was maintained at 80°C for 7 min and programmed from 80 to 180°C at 10°C/min and from 180 to 210°C at 3°C/min. The retention times and peak areas were computed automatically by a computing integrator. Fatty acids were identified and quantified by comparison with the retention times and peak areas of known standards purchased from Sigma Chemicals. Data were calculated using the normalized peak area percentages of total fatty acid content and converted into percentage lipids with 0.9 as a conversion factor for dark meat fishes (tuna, hardtail pomfret, black pomfret, anchovy, spotted mackerel, Indian mackerel, sixbar grouper, barramundi, catfish and eel) and 0.7 for light meat fishes (small yellow striped scad, yellow striped scad, silver pomfret, delagoa threadfish bream and silver catfish) respectively as described by Holland et al. (1994).

Results and Discussion

Generally, the fat contents of the 14 marine, 3 freshwater fishes, 4 shellfishes and 2 selected commercial canned fishes from the Northern East Coast of Peninsular Malaysia studied ranged from 1.01% (delagoa threadfish bream, kerisi) to 15.83% (canned sardine). Figure 1 presents the data for fat content obtained for 14 different marine fish species. Six local fish fillet species [small tuna (aya kecil), large tuna (aya besar), hardtail pomfret (cencaru), delagoa threadfish bream (kerisi), sixbar grouper (kerapu) and barramundi (siakap)] contained fat at less than 2.00%. On the other hand, six marine fish species [small yellow striped scad (selar kuning kecil), yellow striped scad (selar besar), black pomfret (bawal hitam), silver pomfret (bawal putih), Indian scad (selayang) and Indian Mackeral (kembung)] recorded fat content less than 4.00%. However, on the other data, the anchovy (ikan bilis) and spotted mackerel (tengggiri) contained fats at 5.00 and 6.50%, respectively.

The present study indicate that the total fat content

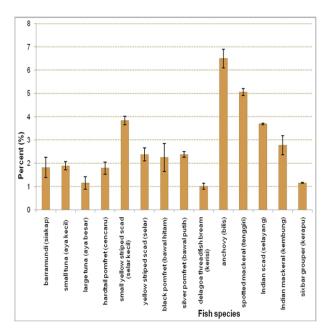


Figure 1. Total fat content of selected marine fish species from East Coast of Peninsular Malaysia

of black pomfret and silver pomfret (Figure 1) was slightly lower (2.25 and 2.38%) than those reported by Osman et al. (2001) at 2.79 and 2.91 g/100g of crude fats, respectively. However, Tee et al. (1997) reported that whole medium black pomfret and silver pomfret recorded fat content at 2.8 g in 100% and 3.6 g in 90.1 % edible portion, respectively. These variable values may possibly due to the different techniques of sample preparation practiced by previous studies and present study. Previously, Tee et al. (1997) utilized whole fish sample while our study only used fillet of fish during preparation of sample. The other differences may also possibly be due to the fact that the fishes were obtained from different geographical area compared to this study where samples from the West Coast of Malaysia have been used.

Among three of the freshwater fishes analyzed, silver catfish (*Patin*) contained the highest level of fat (14.07%) (Table 1). Catfish (*Keli*) had 10.60% while eel (Belut) recorded 0.71% of fat content. Our study indicated that the fat content of these fresh water fishes are generally higher than that previously reported (Suriah et al., 1995) where patin had 5.67 g/100g while catfish had 4.25 g/100g. The difference may be due to geographical variation from which catfish the fishes were sourced (West Coast of Peninsular Malaysia) compared to our study (East Coast region of Peninsular Malaysia). Other variables may be due to the different types of diet and feeding systems practiced in both areas. Another possible reason why different fat content reported in this study for Silver catfish and Catfish compared to previous studies may possibly due to the different method of sample

preparation used to quantify fat content. Meanwhile, Tee *et al.* (1997) reported that whole medium size of keli and patin recorded 3.6g in 115.2 g and 13.4g in 243.6g edible portion, respectively. The difference between our data as compared to Tee *et al.* (1997) in fat content may possibly due the different sampling technique and extraction method used.

The fat content values obtained for all the shellfish species were lower than 2.50%, particularly for cockles (kerang), which presented a very low content of 1.08% (Table 2). The lipid concentration of two species, such as crab (*ketam*) and cuttlefish (*sotong*), were much higher (2.49% and 2.06%, respectively) than those of all the other species analyzed. The present data was comparatively higher than what Tee et al. (1997) have reported. They recorded that ketam and sotong had fat content at 1.2 and 1.3g in 100g edible portion, respectively. The variable was may due to the different fat extraction and detection methods used in previous and present studies. Besides that, both canned sardine and mackeral had fat content at 15.83 and 8.97 %, respectively (Table 3). The data indicated that the amount of total lipid varied widely among the species analyzed.

The EPA and DHA concentrations of selected marine fish species from the East Coast of Peninsular Malaysia are shown in Table 4. Generally, DHA concentrations (55.48-165.21 µg/g) are significantly higher than EPA (17.74-55.38 μ g/g) levels in the fish species analyzed. Among the different marine fish species, sixbar grouper recorded the highest concentration of DHA (165.21 µg/g) while barramundi had the highest concentration of EPA (55.38 μ g/g). There are seven marine fish species containing DHA exceeded 100 µg/g. These are hardtail pomfret, black pomfret, delagoa threadfish bream, anchovy, spotted mackerel, Indian scad, barramundi and sixbar grouper. This data indicated that these fish species could be considered as an excellent source of DHA and represent a very precious essential nutrient choice for the maintenance of a healthy body. The other fish species recorded the DHA concentrations between 50 and $100 \mu g/g$.

In previous study, out of 100 percent fatty acid compositions of extracted fish oils, hardtail pomfret and yellow striped scad were two fish species had highest percentage of DHA (28.6 and 27.3%, respectively) (Osman *et al.*, 2001).

The values of EPA and DHA of selected freshwater fish species from East Coast of Peninsular Malaysia are listed in Table 5. Generally, freshwater fish had lower amounts of DHA (less than 9.2 μ g/g) and EPA (less than 2.00 μ g/g), respectively. However, the concentrations of EPA in freshwater fish in our study

Table 1. Total fat content of selected freshwater fish species from East Coast of Peninsular Malaysia

Common name	Local name (species)	Fat content (%)
Catfish	Keli (Clarias batrachu)	10.60 ± 1.83
Eel	belut (Synbranchus bengalensis)	0.71 ± 0.08
Silver catfish	Patin (Pangasius pangasius)	14.07 ± 0.76

Table 2. Total fat content of selected shellfish species from East Coast of Peninsular Malaysia

Local name (species)	Fat content (%)
Ketam (Liocarcinus vernalis)	2.49 ± 0.36
Sotong (Sepia officinalis)	2.06 ± 0.57
Kerang (Arca granosa)	1.08 ± 0.45
Udang (Metapenaeus affinis)	1.62 ± 0.32
	Ketam (Liocarcinus vernalis) Sotong (Sepia officinalis) Kerang (Arca granosa)

Table 3. Total fat content of selected commercial canned fish available in East Coast of Peninsular Malaysia

Common name	Local name (species)	Fat content (%)
Sardine (Ayam brand TM)	Sardin (Clupeoides spp.)	15.83 ± 1.12
Mackerel (Ayam brand TM)	Makeral (Scomberomorus spp.)	8.97 ± 1.64

Table 4. EPA and DHA concentration of selected marine fish species from East Coast of Peninsular Malaysia

Common name	Local name (species)	EPA	DHA	DHA/EPA
		$(\mu g/g)$	$(\mu g/g)$	ratio
Barramundi	Siakap (Lates calcarifer)	55.38 ± 7.38	109.43±6.63	1.9:1.0
Small tuna	Aya kecil (Thunnus tonggol)	24.63 ± 1.12	76.02 ± 1.51	3.1:1.0
Large tuna	Aya besar (Euthynnus affinis)	22.83 ± 0.28	88.64 ± 1.81	3.9:1.0
Hardtail pomfret	Cencaru (Magalapsis cordyla)	34.56 ± 1.00	145.92 ± 2.81	4.2:1.0
Small yellow striped scad	Selar kuning kecil (Selarides leptolejus)	36.58 ± 1.32	95.85 ± 1.21	2.6:1.0
Yellow striped scad	Selar besar (Selarides spp.)	17.22 ± 0.58	55.48 ± 2.81	3.2:1.0
Black pomfret	Bawal hitam (Parastromateus niger)	24.47 ± 2.09	100.88 ± 9.98	4.1:1.0
Silver pomfret	Bawal putih (Pampus argenteus)	29.89 ± 3.75	83.74 ± 8.85	2.8:1.0
Delagoa threadfish bream	Kerisi (Nemipterus japonicas)	32.55 ± 1.74	115.45 ± 6.38	3.6:1.0
Anchovy	Bilis (Stolephorus commersonii)	45.13 ± 0.69	117.21 ± 1.53	2.6:1.0
Spotted mackerel	Tenggiri (Scomberomorus guttatus)	33.74 ± 1.12	118.01 ± 4.36	3.5:1.0
Indian scad	Selayang (Decapterus russelli)	35.38 ± 1.60	116.25 ± 9.27	3.3:1.0
Indian mackeral	Kembung (Rastrelliger kanagurta)	17.74 ± 4.18	67.46 ± 5.90	3.8:1.0
Sixbar grouper	Kerapu (Epinephelus sexfasciatus)	33.13 ± 2.28	165.21 ± 6.19	5.0:1.0

Table 5. EPA and DHA concentration of selected freshwater fish species from East Coast of Peninsular Malaysia

Common name	Local name (species)	EPA	DHA	DHA/EPA
		$(\mu g/g)$	(μg/g)	ratio
Catfish	Keli (Clarias batrachu)	1.45 ± 0.12	*na	
Eel	Belut (Synbranchus bengalensis)	1.03 ± 0.01	9.18 ± 0.42	8.9:1.0
Silver catfish	Patin (Pangasius pangasius)	1.55 ± 0.18	7.13 ± 0.43	4.6:1.0

*na : not available

is comparatively lower than that reported by Suriah *et al.* (1995) which could again be due to geographical variations in the fishes sourced. The lower levels of DHA and EPA detected in all selected freshwater fishes used in the present study was in agreement with Wang *et al.* (1990) who reported that freshwater fish are not good sources of ω -3 fatty acids. Freshwater fish normally consist of more ω -6 polyunsaturated fatty acid. However, they are still providing very vital essential nutrients of the Malaysian diet, constituting 60-70% of the nation animal protein intake (Suriah *et al.*, 1995). Similar trend was also seen in Chedoloh *et al.* (2011) article. Recently, they found that compared

Table 6. EPA and DHA concentration of Selected Shellfish Species from East Coast of Peninsular Malaysia

Common	Local name (species)	EPA (μg/g)	DHA (μg/g)	DHA/EPA
name				ratio
Crab	Ketam (Liocarcinus vernalis)	51.71 ± 3.82	74.97 ± 4.92	1.5:1.0
Cuttlefish	Sotong (Sepia officinalis)	19.62 ± 0.18	86.07 ± 2.99	4.4:1.0
Cockles	Kerang (Arca granosa)	25.30 ± 0.53	19.02 ± 0.58	0.8:1.0
Shrimp	Udang (Metapenaeus affinis)	51.98 ± 2.61	73.72 ± 6.41	1.4:1.0

Table 7. EPA and DHA concentration of selected canned fish available in East Coast of Peninsular Malaysia

Common name	Local name (species)	EPA (μg/g)	DHA (μg/g)	DHA/EPA ratio
Sardine (Ayam brand TM)	Sardin (Clupeoides spp.)	11.12 ± 0.16	50.50 ± 1.33	4.5:1.0
Mackerel (Ayam brand TM)	Makeral (Scomberomorus spp.)	52.64 ± 6.14	76.60 ± 6.51	1.5:1.0

to marine fish, freshwater fish had significantly lower DHA and EPA contents. In addition, Chedoloh *et al.* (2011) found that catfish, with an EPA fraction of 1.5% was a good source of ω -3.

EPA and DHA concentrations of selected shellfish species from East Coast of Peninsular Malaysia are listed in Table 6. Unlike freshwater fishes, the amounts of EPA in shellfish were less than 52.00 µg/g while both crab and shrimp recorded high values (51.71 and 51.98 µg/g, respectively. On the other hand, cockles had 25.30 µg/g while squid recorded 19.62 µg/g EPA values. However, like marine fish species, some selected shellfish species recorded DHA content larger than EPA concentrations except for cockle. Cuttlefish had the highest amounts of DHA (86.07 μ g/g) followed by crab (74.97 μ g/g) and shrimp (73.72 μ g/g), respectively. On the other study, Chedoloh et al. (2011) found that squid and shrimp had less ω-3 fatty acids concentration than marine fish species, but still had a high ω -3 content, in a range of 9.9-21.7%.

The EPA concentrations for all fish species however was less than 56 μ g/g. There were two fish species and one commercial fish product that recorded EPA value exceeding 40 μg/g. These are barramundi, anchovy (Table 4) and canned mackerel (Table 7). The majority of marine fish species had EPA in the range from 30-40 µg/g namely hardtail pomfret, small yellow striped scad, delagoa threadfish bream, spotted mackerel, Indian scad and sixbar grouper. On the other result, black pomfret, small tuna and large tuna recorded EPA concentrations ranging from 20-30 μg/g. The other two marine fish species namely yellow striped scad and Indian mackerel (Table 4) and canned sardine (Table 7) had EPA concentration lower than 20 µg/g. Previously, Osman et al. (2001) reported that black pomfret, hardtail pomfret, Indian mackerel and yellow striped scad had EPA content less than 1.00%.

The present study showed that several Malaysian coastal marine fishes and a few selected freshwater fish species are rather rich in EPA and DHA levels. Thus, both our marine and fresh water fish species provide various alternative omega-3 sources to the community especially those living in the East Coast of Peninsular Malaysia. In addition, the wide range of EPA and DHA levels offered from all fresh- and saltwater fish species analyzed indicated that consumer have wider option in choosing their preferred source of EPA and DHA. Apart from that, the data derived from the present study can be used by various nutrition, health and medicinal groups especially in planning interventional programs and also for initiating long term campaigns towards the consumption of local fishes as major protein, EPA and DHA sources.

Sufficient intake of EPA and DHA is vital in maintaining an individual's health. A total intake of EPA plus DHA (1200 mg/day) is recommended through the diet that comes from fish/marine sources in a considerable portion (Loukas $et\ al.$, 2010). Another important fact is that DHA is vital for the growth and functional development of the brain in infants and is also necessary for the maintenance of normal brain function in adults (Sidhu, 2003). Since the brain has a very limited capability to synthesize new ω -3 fatty acids, it is important to consume fish/marine organisms to maintain healthy EPA and DHA levels.

The highest DHA/EPA ratio among coastal marine fish species was 5.0:1.0 (Table 4) in sixbar grouper (Siakap) while the lowest in barramundi (1.9:1.0). The high DHA/EPA ratio recorded by sixbar grouper in the present study was slightly lower than Lavie et al. (2009) who documented that grouper had DHA/ EPA ratio of 6.1:1.0. The different may be due to the different species of grouper being investigated in previous and present study. However, our data shows that small tuna had DHA/EPA ratio (3.1:1.0) similar to what Lavie et al. (2009) have found in Bluefin tuna. It is indicated that, the DHA/EPA ratios found in the present study was comparable to Lavie et al. (2009). According to them, both DHA and EPA are present in most all fish species studied, particularly oily fishes, generally in a 2.0:1.0 ratio.

Meanwhile, among freshwater fishes, eel and silver catfish had higher DHA/EPA ratios than marine fishes at 8.9:1.0 and 4.6:1.0, respectively. On the other hand, all 4 selected shellfish species used in the present study except cuttlefish recorded lower DHA/EPA ratios than freshwater fishes. They had the ratios ranging from 0.8:1.0 – 4.4:1.0 (Table 6). The DHA/EPA ratio of shrimp was slightly higher than what

Lavie *et al.* (2009) determined. In the present study shrimp had DHA/EPA ratio at 1.4:1.0 while Lavie *et al.* (2009) reported the DHA/EPA ratio at 1.0:1.2. The different is thought to be due to the different geographical area of sources and shrimp species used. On the other result, among commercial canned fish products, sardine had higher DHA/EPA ratio at 4.5:1.0 while mackerel recorded 1.5:1.0 (Table 7).

Conclusion

From our data, most of the fish investigated had lipid amounts lower than 10% of their total body weights. Marine fish species known as sixbar grouper recorded the highest concentration of DHA while barramundi had the highest concentration of EPA. In general, the level of DHA in all marine species analyzed was significantly higher than EPA levels. The geographical areas from where the species were sampled also play a vital role in influencing the levels of EPA and DHA. In conclusion, all fish and shellfish species could be considered as a good source of EPA as well as DHA and represent a very valuable essential nutrient choice for the maintenance of a healthy body.

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