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The Nutritional Lipid Profiles of Marine Fish from Medan, Indonesia

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Abstract

BACKGROUND: Evaluation of the nutritional value of fish is very important because fish contains essential fatty acids. Fatty acids substances in fish are very important for brain growth and the immune system of the fetus and baby, are also useful as cardioprotective, antihypertensive, anti-thrombolytic, cognitive development, fat metabolism, and anti-inflammatory.

AIM: The aim of this research is to determine the nutritional index of marine fish based on the fatty acid composition that is circulated in Medan City.

METHODS: Fish samples were collected from traditional markets; those is Thunnus spp., Lutjanus argentimaculatus, Sardina pilchardus, Rastrelliger kanagurta, and Euthynnus affinis. While, fish oil was produced from extracted with n-hexane and characterization by determining the acid, saponification, iodine, and peroxide value, respectively. The analysis of fatty acid composition was carried out by gas chromatography method.

RESULTS: The result of research shows, the proportion of fatty acids in marine fish samples was saturated fatty acid (SFA) >monounsaturated fatty acid >polyunsaturated fatty acid. From the nutritional index based on fatty acid composition, the best nutritional fish is Thunnus spp. based on the index value of atherogenic index (IA) and hypocholesterolemic/hypercholesterolemic.

CONCLUSION: As for the ratio of omega-6/omega-3, polyunsaturated/SFA (P/S), and thrombogenic index (IT), that is in the second rank category, so it can conclude that Thunnus sp. has the best nutritional index among the five types of marine fish.

Introduction

Fish is very important for pregnant women as a source of protein and especially omega-3 because fish contains docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). These two fatty acids, especially DHA, are very important for fetal and infant brain growth [1], [2]. Premature babies are very sensitive to deficiency of essential fatty acids (Linoleic and Linolenic) and EPA and DHA because of limitations in diet and greater nutritional requirements at rapid growth [3], [4]. So it is very dependent on the intake of EPA and DHA from food for tissue/muscle growth. Therefore, EPA and DHA supplementation is safe for premature infants [5], [6]. During growth and development, the fetus obtains DHA through the placenta for the function of cholinergic neurotransmission [7], [8], [9]. In addition, DHA can also protect the brain from free radicals and reactive oxygen species [10], [11]. Omega-3 intake, especially EPA and DHA after birth, is less effective in improving the condition due to a lack of omega-3 that has occurred before the fetus in the womb [12], [13].

The fatty acid composition of a pregnant mother and lactation plays an important role in providing essential fatty acids to support development and metabolism for the fetus and neonate [11], [12]. The fetus has a limited capacity to convert linolenic acid to EPA and DHA and therefore relies almost exclusively on DHA and EPA transferred across the placenta from the maternal circulation [13], [14]. DHA is transported across the placenta in the last trimester of pregnancy and during this period, there is a rapid accumulation of DHA in fetal tissues, especially in retinal membrane synapses and neural cortex tissues [14], [15].

The dose of omega-3 administration during pregnancy to achieve optimal results varies between individuals depending on dietary background, intake of dietary habits during pregnancy, and various other factors. The most critical is at the dietary level of omega-6. The increase in omega-6 in the western diet compared to the traditional diet resulted in an imbalanced ratio of omega-6 and omega-3–10–15:1 versus 2:1. The resulting imbalance has implications for important physiological/biological functions because omega-6 competes with omega-3 to entry into the cells and tissues [10], [11].

Other effects of fish oil on health also provide a suppressive effect in preventing cardiovascular disease, Type 2 diabetes, hypertension, cancer, inflammatory and autoimmune diseases, and neurodegenerative diseases [16], [17], [18]. The American Heart Association recommends that every individual should consume fish twice a week and those with coronary heart disease should take 1 g/day of EPA and DHA as an additional supplement [11], [19], [20].

Fish with different fatty acid profiles is likely to make different contributions to human health, especially fetuses and infants. EPA and DHA are very important for health because they are essential fatty acids, but evaluating the nutritional value of fats by comparing only the omega-6: omega 3 or P/S ratio is only a simple dietary advice. Hence, the index IA, IT, and hypocholesterolemic/ hypercholesterolemic (HH) based on the functional effects of fatty acids are also used [19], [20]. Hence, the evaluation of fish nutrition based on fatty acid composition was carried out by calculating polyunsaturated/saturated (P/S), omega 3: omega 6, calculating the atherogenic index (IA) value, thrombogenic index (IT), and the ratio of fatty acids causing HH. According to the UK Department of Health, the ratio between omega-6: omega-3 in the range 1.0–4.0 in the diet helps to prevent coronary heart disease by lowering cholesterol in the blood. Meanwhile, increasing levels of this ratio can increase the risk of cardiovascular disease [21], [22].

The P/S ratio reflects to the effects of polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA). Foods that have a P/S ratio below 0.45 are considered undesirable for the diet because of their potential effect to increase blood cholesterol. IA and IT index values were associated with fatty acid caused pro- and anti-atherogenic and pro- and anti-thrombogenic to the blood vessels. High IA and IT values can stimulate platelet aggregation and thrombus and atheroma formation in the cardiovascular system [19], [20]. Thus, lower IA and IT values are desirable to prevent cardiovascular disorders. The HH index refers to the ratio of fatty acids caused hypocholesterolemia and is related to the specific effect of fatty acids on cholesterol metabolism. Nutritionally, higher HH values are considered more beneficial for health. The IA and IT indicate the potential effect to stimulate platelet aggregation. Thus, the smaller the IA and IT values, the greater the protective potential for coronary artery disease. With a range of expected values are 1 and 0.5, respectively [23], [24]. The composition of fatty acids in fish can be influenced by the type of fish, food intake, and the environment of the seawater. The purpose of this study was to determine the nutritional index by determining the composition of fatty acids in the fat molecules of several marine fish circulated in the market of Medan City.

Materials and Methods

Sample

The samples in this study were five types of marine fish taken from traditional markets that are often consumed by the Medan community, that is *Thunnus* spp., *Lutjanus argentimaculatus*, *Sardina pilchardus*, *Rastrelliger kanagurta,* and *Euthynnus affinis*. Once taken from the market, the sample is saved in a cold container and then measured the length and weight in the laboratory. Fish is cleaned, filleted, and cut into small pieces.

Extraction of fish oil

The fish is mashed with the help of a blender. Then, 500 g of each sample was weighed and dried in a vacuum oven at a temperature of 50–55°C to constant weight. Extracted using n-hexane solvent at a temperature of 25–30°C for 5 × 24 h. Then, the solvent was evaporated from each sample at a temperature of 50–60°C. Then, the yield was calculated according to the research of Adawyah *et al*.; Ainiwati *et al*.; Apituley *et al*.; Messias *et al*. with modifications [25], [26], [27], [28]. Then, the characterization was carried out, by determining the acid, saponification, peroxide, and iodine value [29].

Fatty acid analysis using gas chromatography

Analysis of the fatty acid profile using the Association of Analytical Chemist (2005) reference with the principle of gas chromatography which converts fatty acids into their derivatives, namely, methyl esters. The initial stage in the analysis of the fatty acid profile is the methylation process by refluxing the fat in a water bath with 0.5 N NaOH reagent (in methanol), BF_3 and n-hexane [30], [31].

Fish oil sample of 25 mg was saved into a closed tube 1 mL of 0.5 N NaOH (in methanol), then heated in a water bath for 20 min. The mixture was added 2 mL of 20% BF_3 and 5 mg/mL of internal standard, heated again for 20 min. The next step is the addition of 2 mL of saturated NaCl and shaken, 5 mL of hexane is added and shaken again. So that two layers are formed that is the water and n-hexane layer. The n-hexane layer formed is separated so that what remains is only a layer of water. The aqueous layer was extracted again with 1 ml of n-hexane. The n-hexane layer formed is taken and combined with the first n-hexane layer. The n-hexane extract was added with 50 mg of anhydrous $NaSO_4$ and left for 15 min. Water-free liquid phase was injected as much as 1 μl for analysis with gas chromatography. Gas chromatography uses a flame ionization detector (FID) or a FID. So that the retention time and peak

height of each fatty acid chromatogram are obtained. The retention time and peak of each component were calculated. The retention time value obtained is compared with the standard to obtain information about the types of components in the sample.

Evaluation of the nutritional value of marine fish

The nutritional value of the studied marine fish oil was evaluated with five nutritional indicators based on the fatty acid composition [19], [22], [23], [24].

1. The omega-6/omega-3 ratio index refers to the ratio of omega-6 PUFAs to omega-3 PUFAs
2. The P/S ratio index refers to the PUFA fraction compared to SFA
3. IA = [(C12:0 + (4 × C14:0) + C16:0)]/ monounsaturated fatty acid (MUFA + n-6 PUFA + n-3 PUFA)
4. IT = (C14:0 + C16:0 + C18:0/[(0.5 × MUFA) + (0.5 × n-6 PUFA) + (3 × n-3 PUFA) + (n-3 PUFA/n-6 PUFA)]
5. HH = (C18:1 n-9 + C18:2 n-6 + C20:4 n-6 + C18:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3)/(C14:0 + C16:0)l.

Statistical analysis

The comparison of fatty acid composition was performed using one-way analysis of variance (ANOVA) with the Tukey's test to identify significant differences with $p < 0.05$ significance level through software IBM SPSS Statistics version 26.

Results and Discussion

Fish sample

Marine fish obtained from traditional markets can be seen in terms of weight and length in Table 1.

Table 1: Fish size characterization

Sample	Weight (g)	Length (cm)
Sardina pilchardus	164.1	25.8
Lutjanus argentimaculatus	1500	53.6
Euthynnus affinis	1000	31.3
Thunnus spp.	5000	105.8
Rastrelliger kanagurta	150.2	21.0

S. pilchardus, 7 *R. kanagurta,* and *E. affinis* are pelagic fish that live in coastal and offshore waters. These fish live in groups and enter estuarine waters to find food in the form of plankton, copepods, and crustaceans, which can live at a depth of 10–50 m of sea water. While *L. argentimaculatus* and *Thunnus* spp. are usually hunter fish, they can consume fish that are smaller than them. Lives at a depth of 50–100 m deep sea waters [32], [33].

Marine fish oil

Marine fish oil obtained from the maceration process is expected to reduce the heating time of the sample to avoid damaging the PUFA fatty acids in the sample [27], [28]. The yield (%) of the marine fish oil from the sample is shown in Table 2.

Table 2: Yield sample of marine fish oil

Marine fish	Wet sample (g)	Extract weight (g)	% Yield
Sardina pilchardus	500	77.153	15.431
Lutjanus argentimaculatus	500	84.206	16.841
Euthynnus affinis	500	74.590	14.918
Thunnus spp.	500	90.369	18.074
Rastrelliger kanagurta	500	60.153	12.031

The extraction time by maceration was 5 days, with stirring every 6 h for 5 min. This is so that there is an equilibrium between the fatty acids inside the cell and the outside of the cell is achieved. Extraction is carried out without heating, it is hoped that PUFA which has EPA and DHA essential fatty acids will not be damaged, because PUFAs, PUFAs have more than one double bond which can be damaged by heating [34], [35].

From the research of Pandiangan *et al*., 2018 which used soxhlet extraction with heating, the iodine value was low compared to the maceration method in this study. So that the maceration method is more appropriate in protecting the PUFAs in fish oil. Although it still has the same high acid number as previous studies, it indicates that the fatty acids in the triacylglyceride molecule are independent of the fat structure [34], [35].

Fish oil characterization of samples

After obtaining fish oil, the fish oil was characterized by determining the acid, iodine, saponification, peroxide value, and the results obtained are in Table 3.

The obtained acid number does not meet the requirements, namely, <0.6 mg potassium hydroxide (KOH)/g, so that a lot of fatty acids are released from the triacylglyceride structure. The saponification number of fish oil is the smallest in *L. argentimaculatus* which shows the larger the fatty acid molecules contained in the oil because the longer the C chain, compose the fatty acids on the lipid structure of fish oil. Moreover, the biggest one is *S. pilchardus*, which shows the shorter the C chain of fatty acids in the oil. The low saponification value indicates the formation of longer chain fatty acids in the oil, so it has a large molecular weight and a small saponification value.

Peroxide value is a value that indicates the level of damage to fish oil. From the results obtained, *Thunnus* spp. has the highest level of damage. However, it still meets the requirements of the SNI 01-3555-1998 standard, which is a maximum of 5 mEq/kg. The highest iodine value in the sample was in *Thunnus* spp. and the lowest was in *R. kanagurta* fish. The higher the iodine value, the more double bonds in fish oil because iodine will bind to the double bonds in fatty acids. Then, the fatty acids in the fish will be more unsaturated in the fish.

Table 3: Chemical characteristic of marine fish samples

Sample	Acid value (standard: <0.6 mg KOH/g)	Saponification value (standard: 196–206 mg KOH/g)	Peroxide Value (standard: ≤5 mEq/kg)	Iodine Value (standard: 45–46 mg/100 g)
Sardina pilchardus	4.211 ± 0.288^{a}	210.111 ± 2.326^{a}	3.634 ± 0.014^{a}	37.375 ± 2.572^{ab}
Euthynnus affinis	3.810 ± 0.563^{a}	205.067 ± 2,536^{a}	2.731 ± 1.293^{a}	46.733 ± 1.720^{c}
Rastrelliger kanagurta	4.679 ± 0.145^{a}	214.276 ± 4.882^{a}	4.548 ± 1.285^{a}	36.734 ± 0.986^{a}
Lutjanus argentimaculatus	4.517 ± 0.343^{a}	203.522 ± 4,423^{a}	4.536 ± 1.276^{a}	44.903 ± 1.350^{bc}
Thunnus spp.	4.380 ± 0.014^{a}	205.146 ± 3.545^{a}	4.553 ± 1.277^{a}	47.025 ± 2.786^{c}

Data mean with different superscript indicates different significant statistically (p ≤ 0.05). Data means ± standard deviation.

The parameters in determining the characteristics of fish oil include acid, saponification, peroxide, and iodine value [35], [36], [37]. The acid value is the mg of KOH required to neutralize the free fatty acids in 1 g of oils. The saponification value is the value of mg of KOH to completely saponify the oil from 1 g of oil. The peroxide value is the value of milli-equivalents of peroxide in every 1000 g (1 kg) of oils. Iodine value is the amount of iodine to be absorbed by 100 g of oil [37].

Composition of fatty acids in marine fish

The fatty acid ingredients of fish samples are shown in Table 4. The SFA ingredients for *Thunnus* sp., *L. argentimaculatus*, *S. pilchardus*, *R. kanagurta* and *E. affinis* are ranged from 55.690 to 77.044%; MUFA composition ranged from 18.494% to 28.860% and PUFA composition ranged from 4.462 to 20.228%. Then, the proportion of fatty acids in the fish sample is SFA > MUFA > PUFA. This is not in accordance with the previous studies, with the proportion of fatty acids is MUFA > SFA > PUFA in Fernandez, 2019 and Zhang, 2020. The composition of SFA is the most dominant in fish samples. It is stated by WHO that SFA can increase LDL in the blood vessels. The most SFAss among the five samples were C 16:0, that is, palmitic acid, and the largest was in *R. kanagurta* (43.066%). Palmitic acid can provide hypercholesterolemic, thrombogenic, and atherogenic effects. So that it has a negative impact for the fish to consumed. Meanwhile, the most dominant MUFA in fish samples was omega-9, that is oleic acid (C 18:1). Omega-9 in oleic acid acts as a hypocholesterolemic found in most *Thunnus* spp. (18.706%). Unlike omega-3 and omega-6, omega-9 can be produced in the body naturally. The source of omega-3 and omega-6 as essential fatty acids is PUFA fatty acids. The most dominant PUFA fatty acids are omega-3 (C 22:6), that is in *Thunnus* spp. (17.760%) and omega-6 (C 20:4) in *Thunnus* spp. to (2.990%). Omega-3 and omega-6 have many functions for heart and blood vessel health. lowering fat levels in the blood, lowering high blood pressure in hypertensive patients, increasing HDL levels in the blood, as anti-atherogenic and preventing blood clots [13], [16], [17].

Nutrient index based on fatty acids in marine fish oils is shown in Table 5.

From this study, it can be concluded that the best nutritional index values based on fatty acid composition are *Thunnus* sp.*, E. affinis, L. argentimaculatus, S. pilchardus*, and *R. kanagurta* fish, this is clarified in Figure 1.

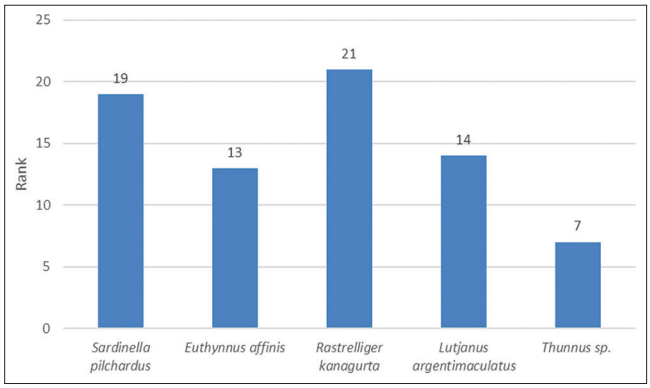

Figure 1: Nutritional index values based on fatty acid composition

Table 4: Fatty acid composition in marine fish oil

Fatty acid	Marine Fish (x□ ± SD)				
	Sardina pilchardus	*Euthynnus affinis*	*Rastrelliger kanagurta*	*Lutjanus argentimaculatus*	*Thunnus spp.*
C 14:0	6.356 ± 0.011^{b}	5.852 ± 0.016^{b}	5.611 ± 0.021^{b}	3.853 ± 0.011^{a}	3.973 ± 0.605^{a}
C 15:0	1.447 ± 0.005^{b}	1.501 ± 0.023^{b}	2.637 ± 0.004^{c}	1.223 ± 0.004 ^{a}	1.245 ± 0.033^{a}
C 16:0	41.645 ± 0.000^{d}	33.354 ± 0.029^{b}	43.066 ± 0.021^{e}	34.122 ± 0.083^{c}	33.107 ± 0.076^{a}
C 17:0	2.149 ± 0.006^{b}	2.081 ± 0.018^{b}	4.280 ± 0.038^{d}	1.861 ± 0.003^{a}	2.445 ± 0.089^{c}
C 18:0	15.006 ± 0.033^{c}	12.882 ± 0.036^{b}	20.127 ± 0.003^{a}	14.172 ± 0.032^{c}	13.811 ± 0.156^{d}
C 20:0			1.324 ± 0.019	0.704 ± 0.004	0.586 ± 0.006
C 21:0					0.524 ± 0.022
C 22:0				0.556 ± 0.001	
SFA	66.602 ± 0.023^{c}	55.669 ± 0.063^{a}	77.044 ± 0.030^{d}	56.490 ± 0.136^{b}	55.690 ± 0.388^{a}
C 16:1	6.859 ± 0.042^{d}	7.115 ± 0.008^{e}	5.791 ± 0.013^{b}	6.546 ± 0.010^{c}	4.167 ± 0.130^{a}
C 17:1				0.666 ± 0.004	0.988 ± 0.059
C 18:1 ω9	17.417 ± 0.013^{c}	11.920 ± 0.005^{b}	10.581 ± 0.006^{a}	17.232 ± 0.025^{c}	18.706 ± 0.457^{d}
C 20:1 ω9				1.425 ± 0.002	0.932 ± 0.029
C 22:1 ω9	2.437 ± 0.003^{b}	5.069 ± 0.009^{c}	2.123 ± 0.004^{a}	2.441 ± 0.004^{b}	2.447 ± 0.009^{b}
C 24:1				0.551 ± 0.000	0.530 ± 0.021
MUFA	26.713 ± 0.026^{b}	24.103 ± 0.030^{b}	18.494 ± 0.016^{a}	28.860 ± 0.037^{b}	27.769 ± 0.240^{b}
C 18:2 ω6				0.635 ± 0.055	1.640 ± 0.103
C 18:3 ω3					0.976 ± 0.047
C 20:4 ω6	1.607 ± 0.006^{a}	2.469 ± 0.009^{b}	2.556 ± 0.004^{bc}	2.750 ± 0.001^{c}	2.990 ± 0.127^{d}
C 20:5 ω3				0.495 ± 0.003	0.664 ± 0.001
C 22:6 ω3	5.079 ± 0.004^{b}	17.760 ± 0.023^{d}	1.906 ± 0.008^{a}	10.772 ± 0.118^{c}	10.273 ± 0.350^{c}
PUFA	6.686 ± 0.002^{b}	20.228 ± 0.032^{e}	4.462 ± 0.013^{a}	14.651 ± 0.171^{c}	16.542 ± 0.629^{d}
Total	100.000	100.000	100.000	100.001	100.000

Data mean with different superscript indicates different significant statistically (p ≤ 0,05). Data means ± standard deviation.

Table 5: Nutrient index based on fatty acids in marine fish oils

Nutrient index	Marine fish (rank)					Standard
	Sardina pilchardus	*Euthynnus affinis*	*Rastrelliger kanagurta*	*Lutjanus argentimaculatus*	*Thunnus sp.*	
ω 3	5.079 ± 0.004	17.760 ± 0.023	1.906 ± 0.008	11.267 ± 0.115	11.912 ± 0.400	
ω 6	1.607 ± 0.006	2.469 ± 0.009	2.556 ± 0.004	3.385 ± 0.056	4.630 ± 0.230	
ω 6/ω 3	0.316 ± 0.001 (3)	0.139 ± 0.000 (5)	1.341 ± 0.004 (1)	0.300 ± 0.002 (4)	0.389 ± 0.006 (2)	1 – 4
P/S	0.100 ± 0.000 (4)	0.363 ± 0.001 (1)	0.058 ± 0.000 (5)	0.259 ± 0.004 (3)	0.297 ± 0.013 (2)	<0.45
IA	2.008 ± 0.000 (4)	1.280 ± 0.003 (3)	2.854 ± 0.008 (5)	1.138 ± 0.006 (2)	1.106 ± 0.066 (1)	<1
IT	1.935 ± 0.003 (4)	0.706 ± 0.001 (1)	4.050 ± 0.012 (5)	0.979 ± 0.009 (3)	0.934 ± 0.029 (2)	<0.5
HH	0.502 ± 0.000 (4)	0.820 ± 0.002 (3)	0.309 ± 0.001 (5)	0.840 ± 0.006 (2)	0.951 ± 0.022 (1)	>1
Total rank	(19)	(13)	(21)	(14)	(7)	

ω6/ω3: omega-6/omega-3, P/S: Polyunsaturated/saturated fatty acid, IA: Atherogenic index, IT: Thrombogenic index, HH: Hypocholesterolemic/hypercholesterolemic.

From this study, it can be seen that the SFA value is high compared to the MUFA and PUFA values. This is likely due to the food consumed by the fish. *E. affinis* fish is rich in PUFA because omega-3, namely, C 22:6 is 17.760%, that *E. affinis* fish consumes a lot of PUFA-rich phytoplankton. This is similar to the research conducted by Zhang *et al*., 2020, which examined the effect of river conditions in China on the nutritional value of the fish that live in it.

Conclusion

The value of the omega 6: omega 3 ratio of the five marine fish samples ranged from 0.139 to 1.341. From the value of ratios, omega 6 and omega 3 that meet the best requirements of the five fish are *R. kanagurta* fish. The value of the P/S ratio is between 0.058 and 0.363 and the best is *E. affinis* fish. IA values ranged from 1.106 to 2.854 and none of them met the IA requirements but the best among the five marine fish was *Thunnus* spp. The IT value ranged from 0.706 to 4.050 and none of them met the IT requirements but the best among the five marine fish was *E. affinis*. As for the HH values ranging from 0.309 to 0.502, none of them met the HH requirements but the best among the five marine fish was *Thunnus* spp. From the nutritional index based on composition of fatty acid, the best is *Thunnus* spp. based on the index of IA and HH values. As for the ratio omega 6/3, P/S and IT, *Thunnus* spp. is in the second rank category, so it can be concluded that *Thunnus* spp. has the best nutritional index among the five types of marine fish.

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