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Characteristics and Fatty Acid Profile of Refined Fish Oil from Byproduct of Yellowfin Tuna (*Thunnus albacares*) Meal Processing

I Gusti Ayu Budiadnyani*, Teti Estiasih and Yunianta
Agricultural Technology Faculty, University of Brawijaya, Indonesia
*Corresponding author's e-mail: igustiayu678@gmail.com

ABSTRACT: Most of the by-product of tuna meal processing is fish oil. Tuna fish oil is a source of polyunsaturated acid (PUFA), especially omega-3 fatty acids such as docosahexaenoic acid (DHA, C22: 6 Ω-3) and eicosapentaenoic acid (EPA, C20: 5 Ω-3). This research aimed to study fish oil refining from byproduct of tuna meal processing, to know the characteristics and fatty acid profile of the by-product oil and the refined fish oil. Degumming with phosphoric acid 85%, neutralization with NaOH 14.36% and bleaching with zeolite adsorbent about 10% (w/w) are steps on byproduct oil refining. Characteristics of the by-product oil and pure oil has been verified by its acidic value, free fatty acid rate, peroxide value, anisidine value and total oxidation (TOTOX) and also its color. The results showed that refining steps were able to improve the quality of fish oil from the byproduct of tuna meal processing to meet international fish oil standards (IFOS) and the color from the refined fish oil was similar to tuna fish oil sold in the market. Dominant fatty acid identified from the byproduct oil and refined oil was unsaturated fatty acid about 63.81% consisting of oleic, linoleic, DHA and EPA. For that reason, the byproduct oil of Yellowfin-tuna meal processing can be considered as fish oil source which is rich in omega-3 essential fatty acid.

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INTRODUCTION

Indonesia is an importer of Ω-3 fatty acid, although actually it has potentials for local source of Ω-3 acid which has not been fully optimized. One source of that Ω-3 fatty acids is the byproduct oil of tuna meal processing [1]. In the meal processing, fish oil is produced as byproduct and it contains Ω-3 fatty acid [2]. Raw material of meal processing industry is the solid waste from the canning process, and this solid waste consists of the head, intestines, and liver of tuna fish. It is known that those organs are sources of polyunsaturated fatty acid (PUFA) especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [3].

Byproduct oil of tuna meal processing has dark colored and contains dirt and other compounds, such as free fatty acid, monoglycerides and diglycerides, phosphatide, hydrocarbon, carbohydrate, protein and degradation product which is not good for human [4]. Therefore, this oil should be refined in order to meet the standards of quality fish oil for human consumption [5].

Common method used to improve the oil quality is by chemical refining. The steps of chemical refining are degumming, neutralization and bleaching [6]. Studies on fatty acid profile of some tuna fish species had been conducted, but only a few researches were about fatty acid profile of tuna meal processing's byproduct and other byproduct materials [7]. Considering that fact, a study on fatty acid profile of byproduct oil from tuna meal processing and oil resulted from each step of refining was needed. This research was aimed to study the refining of byproduct fish oil from tuna meal processing, to know the characteristics and fatty acid profile of byproduct oil and the refined fish oil.

MATERIAL AND METHODS

Material used in the research was the byproduct oil of tuna meal processing acquired from Yellowfin-tuna meal processing industry in Muncar, East Java, Indonesia. Substances used in the refining and the analysis of byproduct fish oil quality and refining result were phosphate acid 85%, NaOH solution 14.36%, 60 mesh size active zeolite, KOH (Merck), NaOH (Merk), chloroform (Merk), ammonium thiocyanate (Merk), $BaCl_2$ (Sigma Aldrich), trimethylpentane (merk), p-Anisidine solution (Sigma Aldrich).

Refining was conducted in three steps; degumming, neutralization and bleaching [8]. Degumming was done by adding phosphoric acid 85% (v/v) as much as 1% of oil weight and then mixing at 500 rpm for 30 minutes in 80 ^{o}C temperature. Then the oil was centrifuged for 20 minutes at 5000 rpm. Neutralization of oil from degumming was conducted by adding sodium hydroxide solution (NaOH 14.36% w/w, using 0.20% of excess related to free fatty acid content of oil after degumming process) in 70 ^{o}C and mixing at 500 rpm for 10 minutes. When the mixing finished, oil was left to cool in room temperature until its temperature was 40 ^{o}C, oil and soap was separated with centrifugation for 15 minutes at 5000 rpm. Washing was performed by adding water as much as 5% of oil weight for 10 minutes and mixed at 500 rpm. This step was repeated for three times. Bleaching of oil from neutralization process was by adding zeolite as adsorbent 15% of oil weight and then heated at 70^{o}C and stirred at 500 rpm for 20 minutes. Centrifugation was done at 5000 rpm for 20 minutes to separate the oil from the adsorbent.

Characterization of byproduct oil and oil from the refining process was conducted to know the oil's quality by analyzing acid value with KOH titration [9], free fatty acid content (FFA) with titration method where the result was presented as % oleic acid [10], peroxide value (PV) with ferric chloride method and presented in meq/kg [11]. Anisidine value (p-AV) of fish oil was measured with spectrophotometer (20 D Plus "LaboMed") based on the reaction between unsaturated α and β aldehyde (especially 2-alkenal) and reagent p-anisidine [12]. Total oxidation value (TV) was addition of two times of peroxide value (PV) with anisidine value (p_AV) [12]. Color analysis with method CIE- L* a* b* using Color Reader device (Model CR 300, Minolta Instrument System) [8]. Analysis results were presented as L*, a* and b* value, L* was lightness level (0 = black and 100 = white); +a* values represent redness and –a* values represent greenness; +b* values represent yellow and –b* values represent blue. Hue degree was calculated with a formula: (h) [tan $^{-1}$ (b*/a*)]. Hue degree showed the actual color of the oil. As comparison, tuna oil fish sold in the market was used. Acid value, FFA, PV, p-AV, TV and color analyses were repeated twice (duplicate) for the byproduct oil, oil resulted from degumming, oil resulted from neutralization and oil resulted from bleaching.

Fatty acid profile analysis of byproduct oil and refining step resulted oil was conducted using Gas Chromatography Mass Spectrometry (GCMS). Before being analyzed with GCMS, oil fish sample was trans esterified into methyl ester (FAME) using BF_3 Methanol [13]. Fatty Acid Methyl Esters (FAME) was identified using GC-MS (Shimadzu QP 2010 S) with column type Agilent DB-1, 30 m column length, ID 0.25 mm, helium carrier gas, Electron impact (EI) ionization system, 70 eV ionization energy, 100 ^{o}C column temperature, 300 ^{o}C injector temperature, split injection mode, 12 kPa gas pressure carrier, 0.5 mL/minute gas flow velocity and 250 ^{o}C detector temperature. Spectra MS analysis results of various fish oil sample were compared with existing spectra MS in NIST.LIB and WILEY.LIB.

RESULTS

Quality test of fish oil from tuna meal processing's byproduct oil refining in the research was based on chemical parameters consisting of free fatty acid (FFA), acid value (AV), peroxide value (PV), p-Anisidine value (p-AV) and total oxidation value (TV) and referred to food grade fish oil standards of International Fish Oil Standards (IFOS). Fatty acid profile analysis was determined from relative percentages quantification affected by sum and width of fatty acid peak area which appeared in the chromatogram. Table 1 shows characteristics of byproduct oil, oil resulted from each step of refining, and international fish oil standards (IFOS). Table 2 shows color characteristics of byproduct oil and oil resulted from each step of refining and Table 3 shows fatty acid profile based on relative percentages.

Table 1. Characteristics of Tuna Meal Processing's Byproduct Oil and Oil Resulted from Each Step of Refining

Parameter	Byproduct Oil (Raw Oil)	Refining Step			IFOS
		Degumming	Neutralization	Bleaching	
Acid Value (AV) (mg KOH/g)	9.21 ± 0.05	10.25 ± 0.04	0.58 ± 0.04	0.25 ± 0.04	< 3
Free Fatty Acid (FFA) (% oleic)	4.63 ± 0.03	5.13 ± 0.03	0.29 ± 0.02	0.13 ± 0.06	< 1
Peroxide Value (PV) meq/kg	19.62 ± 0.11	17.64 ± 0.06	7.10 ± 0.08	2.12 ± 0.31	< 5
Anisidine Value (p-AV)	30.82 ± 0.05	14.64 ± 0.06	6.51 ± 0.02	2.01 ± 0.15	< 20
TOTOX Value (TV)	70.05 ± 0.17	44.72 ± 0.18	20.71 ± 0.18	6.24 ± 0.26	< 26

Mean Value ± standard (in duplicate)

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Table 1 presents acid value (AV), free fatty acid percentages (FFA), peroxide value (PV), p-Anisidine value (p-AV), TOTOX value (TV) in tuna meal processing's byproduct oil, degumming, neutralization, and bleaching resulted oil and also the international fish oil standards. Every step of refining improved the oil quality and the best quality obtained after bleaching. After bleaching, all quality parameters meet the International Fish Oil Standards (IFOS).

Table 2. Color Characteristics of Byproduct Oil and Oil Resulted From Each Step of Refining

Oil	L*	a*	b*	H
By product (raw)	20.40 ± 0.57	2.05 ± 0.07	1.55 ± 0.07	37.09 ± 0.31
Degummed Oil	20.65 ± 0.79	1.84 ± 0.06	1.45 ± 0.07	38.08 ± 0.29
Degummed and Neutralized Oil	26.00 ± 0.71	1.55 ± 0.07	10.00 ± 0.57	81.17 ± 0.89
Degummed, Neutralized and Bleached Oil	36.50 ± 1.70	1.03 ± 0.04	13.59 ± 0.54	85.69 ± 0.02
Tuna Oil	36.77 ± 0.52	2.15 ± 0.07	13.75 ± 0.21	81.22 ± 0.15

Mean Value ± standard (in duplicate)

Table 2 presents lightness level (L*), reddish (a*), yellowish (b*) and hue degree of by product oil, oil resulted from each step of refining and commercial tuna fish oil. L* value of byproduct oil was increasing in every step of refining. b* value was increasing with the declining on a* value so it caused hue degree of the fish oil improved after each step of refining. Overall, the color characteristics of refined oil from the refining was approximate to the quality of commercial tuna fish oil.

Table 3. Fatty Acid Profile (%) of Byproduct Oil and Oil Resulted From Each Step of Refining

Fatty Acid	Tuna Meal Processing Byproduct Oil	Refining Step		
		Degumming	Neutralization	Bleaching
C14:0 (Myristic acid)	7.04	6.68	5.10	4.20
C16:0 (Palmitic acid)	20.96	20.97	22.80	22.20
C16:1 Ω-7 (Palmitoleic acid)	9.35	8.63	8.19	7.76
C18:0 (Stearic acid)	8.79	7.81	5.42	5.49
C18:1 Ω-9 (Oleic acid)	22.31	22.17	23.70	22.18
C18:2 Ω-6,9 (Linoleic acid)	3.77	3.11	2.83	2.82
C18:3 Ω-3 (α-Linolenic acid)	0.97	0.96	1.60	0.63
C20:1 Ω-9 (Eicosenoic acid)	2.18	2.10	1.41	1.79
C22:1 Ω-9 (Erucic acid)	1.68	3.21	2.36	2.30
C20: 5 Ω-3 (Eicosapentaenoic acid(EPA))	8.35	9.59	11.51	12.03
C22:6 Ω-3 (Docosahexaenoic acid (DHA))	14.60	14.77	15.08	18.25
Σ SFA*	36.79	35.46	33.32	31.89
Σ MUFA**	35.52	36.11	35.66	34.03
Σ PUFA***	27.69	28.43	31.02	33.73
Σ Ω-3****	23.92	25.32	28.19	30.91
Total Unsaturated Fatty Acid	63.21	64.54	66.68	67.76

* Σ SFA: sum of unsaturated; ** Σ MUFA: sum of monounsaturated; *** Σ PUFA: sum of polyunsaturated; **** Σ Ω-3: sum of omega-3

Table 3 presents fatty acid profile of byproduct oil and oil resulted from each step of refining. Dominant fatty acids in byproduct oil and oil resulted from refining were palmitic acid C16:0, oleic acid C18:1 and docosahexaenoic acid C22:6 Ω-3. Palmitic acid was the highest among saturated fatty acid with 20.96-22.80% followed by stearic acid ranged between 5.49-8.79%. The highest monounsaturated fatty acid (MUFA) was oleic acid ranged between 22.17-23.70%. The biggest proportion from polyunsaturated fatty acid (PUFA) was DHA

ranged between 14.60-18.25%, followed by EPA ranged between 14.60-18.25%.Total SFA in byproduct oil and oil resulted from each step of refining ranged between 31.89-36.79%. Total MUFA was 34.03-36.11%, Total PUFA was 27.69-33.73%, while total sum of unsaturated fatty acid (MUFA + PUFA) ranged between 63.21-67.76%. The sum of omega-3 fatty acid found in tuna meal processing's byproduct oil and oil resulted from each step of refining ranged between 23.92-30.91%.

DISCUSSION

Degumming step was able to lower the oxidation product represented by PV, p-AV and TV. The decrease of this oxidation level was caused by absorption of primary and secondary oxidation compound by hydrated gums. Phosphoric acid left trail in the oil after degumming step caused rising acidity in the process [4]. Neutralization and bleaching were able to lower the acid values and FFA content in the oil. Function as expected, neutralization step was to diminish most of free fatty acid (FFA) [6]. Decreasing AV and FFA content in the bleaching process was caused by residue nullification of residual soap and water content in the oil. Adsorbent were able to decrease water in the fish oil which was one cause of hydrolysis [14].

Neutralization and bleaching were able to lower PV, p-AV and TV values of tuna meal processing's by product oil. The first phase of oxidation process was marked by hydroperoxide production, which was commonly measured as peroxide value [15]. P-AV was related with the second phase of oxidation which was triggered by degradation of hydroperoxide caused by free radicals [15]. Refining especially bleaching process was able to lower PV, p-AV and TV values. Adsorbent was able to adsorb dirt and oxidation product. This dirt and oxidation product adsorption can improve the oxidative quality and stability of the refined oil [14]. Table 1 shows refining steps on tuna meal processing's byproduct oil can improve the quality of fish oil which was marked by the decreasing AV, FFA, PV, p-AV and TV values especially bleaching process which was able to produce pure fish oil out of tuna meal processing's byproduct and met the international fish oil standards.

Clarity value was increasing (Table 2) because of the elimination of non oil fraction and dark color causes of tuna meal processing's byproduct oil. During refining using phosphoric acid and NaOH, heme structure would be damage because Fe atom would dissolved by alkali. Red color intensity in fish oil was related with heme structure which was in myoglobin and hemoglobin protein [8]. Decreasing red color intensity in fish oil caused the yellow color increase. Hue degree analysis from the fish oil showed color intensity change where the reddish byproduct oil turned into yellow-orange after refining step were done. Hue value ranged between 79.53-90.76 that showed yellow orange color intensity [8]. Bleaching was aimed to improve the color, because in bleaching process, zeolite was able to adsorb impurities and residual soap re caused by soaping process in the neutralization and caused the oil became so clear and transparent that it had the same color intensity as tuna oil sold in the market.

Fatty acid profile of tuna (*Thunnus sp)* meal processing's byproduct oil which had ever been reported were SFA about 30.5%, MUFA 30.37%, PUFA 39.13% and total unsaturated fatty acid was 69.50% with DHA about 27.76% (1). Overall, tuna waste fatty acid composition showed high content of palmitic acid, oleic acid and DHA [3]. Oil from pacific bluefin tuna (*Thunnus thynnus orientalis*) usually possessed 30% DHA [16]. Differences on profile of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid in Yellow fin tuna or other tunas were caused by season and environmental effect which was different between the types of fish [17].

SFA value of tuna meal processing's byproduct fish oil seemed decreasing during the chemical refining process, started at 36.76% in byproduct oil and ended at 31.89% in pure fish oil (bleaching) (Table 2). On the other hand, PUFA showed increase from 27.69% to 33.73%, which was caused by nullification of substances like residue and dirts which was damaging fatty acid so SFA kept decreasing and PUFA kept increasing. Same thing happened at Hybrid Sorubim oil refining where PUFA increased from 32.26% to 33.02% and SFA decreased from 32.56% to 30.24% [18].

CONCLUSION

Fish oil refining refining by degumming, neutralization and bleaching improved the quality of Yellow fin tuna (*Thunnus albacares)* from by-product of meal processing's. The oil quality met the quality of international fish oil standards (IFOS) and the color intensity was similar to commercial tuna fish oil that was yellow orange. Refining steps were also able to lower saturated fatty acid value and improve unsaturated fatty acid especially omega-3 fatty acid and DHA. Tuna fish oil from meal processing's byproduct is essential fat source which is good for human body.

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