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Physicochemical and Fatty Acid Profile of Fish Oil from Head of Tuna (*Thunnus albacares*) Extracted from Various Extraction Method

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Abstract- **By-product of tuna fish processing industry has the potential to be developed into fish oil rich in omega. Fish by-products are the main natural source of omega-3 polyunsaturated fatty acids, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) with a great importance in food industry and pharmacy. The purpose of this research is to see the effect of fish oil extracting method from the head of tuna fish as by-product of tuna fish processing industry to the physicochemical properties and the profile of fatty acid fish oil. There are several methods of extraction to produce of fish oil. This research was run in triplicate with a completely randomized design (CRD) with three treatments: pre-cooked wet rendering, acid silage, and solvent extraction. It can be concluded that the extraction method has an effect on physicochemical properties and fatty acid profile of fish oil. The wet rendering extraction method is the most effective and most potential extraction method to be applied because it produces the highest yield (12.80%) compared to the silage process (6.16%) and solvent extraction method (8.49%). PUFA produced from wet rendering was 44.34%, statistically not different with solvent extraction method (44.49%), but it higher than silage process method (32.77%).**

***Keywords*—fish oil extraction; tuna (*Thunnus albacares*); wet rendering; silage process; solvent extraction; poly unsaturated fatty acid (PUFA); EPA; DHA**

I. INTRODUCTION

It is estimated that more than 70% of the total processing of processed fish fishery produces large amounts of solid waste and by-products. Fish fillet industry can produce waste that sometimes reaches 50% of the total weight of processed fish [1]. Most by-products from fish processing are further processed into fish flour for feed. However, the by-products of processing products still have the potential to be developed into more economically valuable products, one of which is fish oil.

Junker et al. [2] said that the fish waste obtained from the processing industry is still very rich in omega fatty acids. The demand for fish oil products continues to increase, since it is known that fish oil is a good source of polyunsaturated fatty acids such as omega. Polyunsaturated long chain fatty acids Unsaturated fatty acids (PUFAs) are important substances for maintaining health and growing human development [3]. This oil is an important source of essential dietary components such as eicosapentaenoic acid and docosahexaenoic acid [4] Epidemiological and experimental facts has shown that n-3 polyunsaturated fatty acids (n-3 PUFA), especially eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are among the main components responsible for the health benefits resulting from fish consumption [5]

Fish oil from by-product of various fish sources has been extensively studied by many researchers, such as study on the quality of fish oil from precooked and non-precooked tuna heads [6], fish oil from by-products of herring fillet [7] salmonella skin [8], mackerel fish waste [9], By-products of trout [10] fish waste *Cyprinus carpio* [11] and catfish waste [12]. There are several kinds of fish oil extraction methods. Extraction methods that are usually used are wet rendering and dry rendering methods. Those methods do not require chemicals during process. Wet rendering extraction involves cooking of fish with steam to damage the structure of cell and pressing the oil from the cooked fish. This is the most common method used for fish oil production which involves three basic steps: cooking at high temperatures (85-95 °C), pressing and centrifuging [13]. High volumes of crude fish oil could be obtained from this process. For edible purposes, subsequent refining steps are required. According to Ref.[14], the optimum temperature for wet rendering was 80 °C.

Other processes, such as, the study for obtaining crude oil from fish by-products by enzymatic reaction with proteases

[15]. Supercritical fluid extraction (SFE) method has become an attractive technology for obtaining high quality fish oil from some by-products [16,17]. It is not only because it uses moderate temperatures and provides an oxygen free media, which aim to reduce oxidation of omega-3 during the extraction, but also because it selectively allows extracting low polar lipid compounds, avoiding the co-extraction of polar impurities such as some inorganic derivatives with heavy metals. High production cost is the main limitation of the SFE process [17].

The other methods are the acid silage and the solvent extraction, both methods are the process of separating a substance from its mixture by dividing a solute between two non-mixed solvents to extract the solute from one solvent to another. Fish silage is defined as a liquid product obtained from the whole or parts of fish, where acids, enzymes or lactic-acid-producing bacteria are added. Liquefaction is then stimulated by the action of enzymes from the fish [18].

Solvent extraction is typically used to obtain components of a solid or liquid material. Solvent contact with samples which will dissolve the desired solute. The success of solvent extraction depends on how quickly the compound to be extracted is dissolved, and at equilibrium is achieved in the solvent phase. Solvent extraction is considered a process of mass transfer between solid phases (insoluble solid matrix), liquid phase is clogged in the pores within the article, and the miscella that travels through space between particles [19]

Selection of the extraction process to obtain omega-3 rich oils is essential to get the best oil quality. This is because the method of extraction not only affects the oil extraction results, but also the quality of the oil [20]

The purpose of this research is to see the effect of fish oil extraction method on the physicochemical properties and fatty acid profile of fish oil from head of tuna (*Thunnus albacares*).

II. Materials and Methods

A. Raw material and pre-treatment

The raw materials studied in this study are by-products of yellow fin tuna (*Thunnus albacares*) processing which is still fresh in the form of head. Raw materials taken directly from the Tuna Fish Processing Industry in Bungus Padang, West Sumatra-Indonesia. Raw material came from a unique batch (related to a certain place, season of fish capture and processing batch), which was delivered frozen at -2°C. Each batch received in the laboratory was cut in small pieces (1–10 mm equivalent diameter) with a cutter, packed in individual plastic bags under vacuum and kept frozen until the experiments were performed.

B. Oil extraction methods

Oil from each fish by-product was obtained in parallel by three different methods: solvent extraction (SE) or centrifuging, wet rendering (WR), acid silage extraction (AS). The amount of raw material used in each extraction method was approximately 100 g. The scheme of extraction procedure in this research can be seen in Figure 1.

The precooked-wet rendering extraction process are as follow: a) washing raw material of by-product of fish processing with running water, b) steaming at 105 °C for 30 minutes, c) pressing; (d) separating liquid and cake, e) liquid (oil and water) separated by separating funnel, f) centrifuging the oil in top layer (10,000 rpm) for 10 minutes, g) crude fish oil was produced. Homogenized by-product was added with distilled water at a ratio of 1: 1. [21].

The acid silage extraction process are as follows: a) washing raw materials of by-product b) soaking with 3% formic acid, c) stand-by at room temperature for 4-7 days, d) separating the liquid and cake using by filtering, e) centrifuging at 10,000 rpm for 10 minutes, f) pressing the cake to produce the oil-water mixture and centrifuging at 10,000 rpm for 10 minutes. (g) collecting crude oil.

In a ***solvent extraction*** process, 5 g of solid sample was placed on a cotton-coated porous thimble. The solvent used is hexane with a boiling point of 60-80°C. Hexane was used as a solvent. Thimble which already filled with samples was placed in soxhlet. Soxhlet was connected to a flask and placed on an electric heater and condenser. The cooler was connected with soxhlet. Heat was applied to heat the solvent to its boiling point for 1 hour. As the warming continues, the solvent in the flask begins to boil in just 5 minutes of warming and the water begins dropping from the top to the sample in the thimble. When the solvent reaches the top of the tube, it sucks into the flask and thereby removes the oil part that has been extracted in the reflux process. The solvent used is then recovered by applying heat and collected on top of a round bottom flask into the soxhlet apparatus while the extracted oil is collected and measured.

C. Yield Determination

Yield was expressed as a percentage of oil extracted from tuna heads. Yield was calculated as follows:

$$\text{Yield} = \frac{\text{Extracted fish oil}}{\text{Weight of sample}} \times 100\% \qquad (1)$$

D. Oil Refining [22]

1) Degumming: Degumming is a process of separation of gums consisting of phosphatides, proteins, residues, carbohydrates, water and resins without reducing fatty acids in oil. The oil is shaken or stirred first, weighed and heated to 70°C. The oil is put into a separator flask. Add hot water as much as 10-20 percent oil volume and then stand for 10 minutes till the mixture form three layers of oil, gum, and water. Water and gum are removed from the separating flask. The pH check is performed by measuring the pH of the separated water. This separation of gums and water is carried out until the pH of water reaches neutral.

2) *Neutralization:* Degummed oil is weighed and heated to 80°C. Add caustic soda according to the calculation and stirred with stirrer for 2 minutes. After that, the oil is put into the separator flask and then washed with hot distilled water as much as 5 percent of the weight of the oil. After forming three layers that consist of oil, soap stock and water, then soap and water are separated from oil. The separation is stopped until the pH of the separating water becomes neutral. Once the pH becomes neutral, add 1 percent of the

anhydrous salt to the oil to reduce the water content of the oil.

3) Bleaching: The neutralized oil is weighed and heated to 80-100ºC. Then add the active charcoal as much as 1 percent of the oil weight and stirred for 10 minutes. After mixing well, the oil is filtered with filter paper.

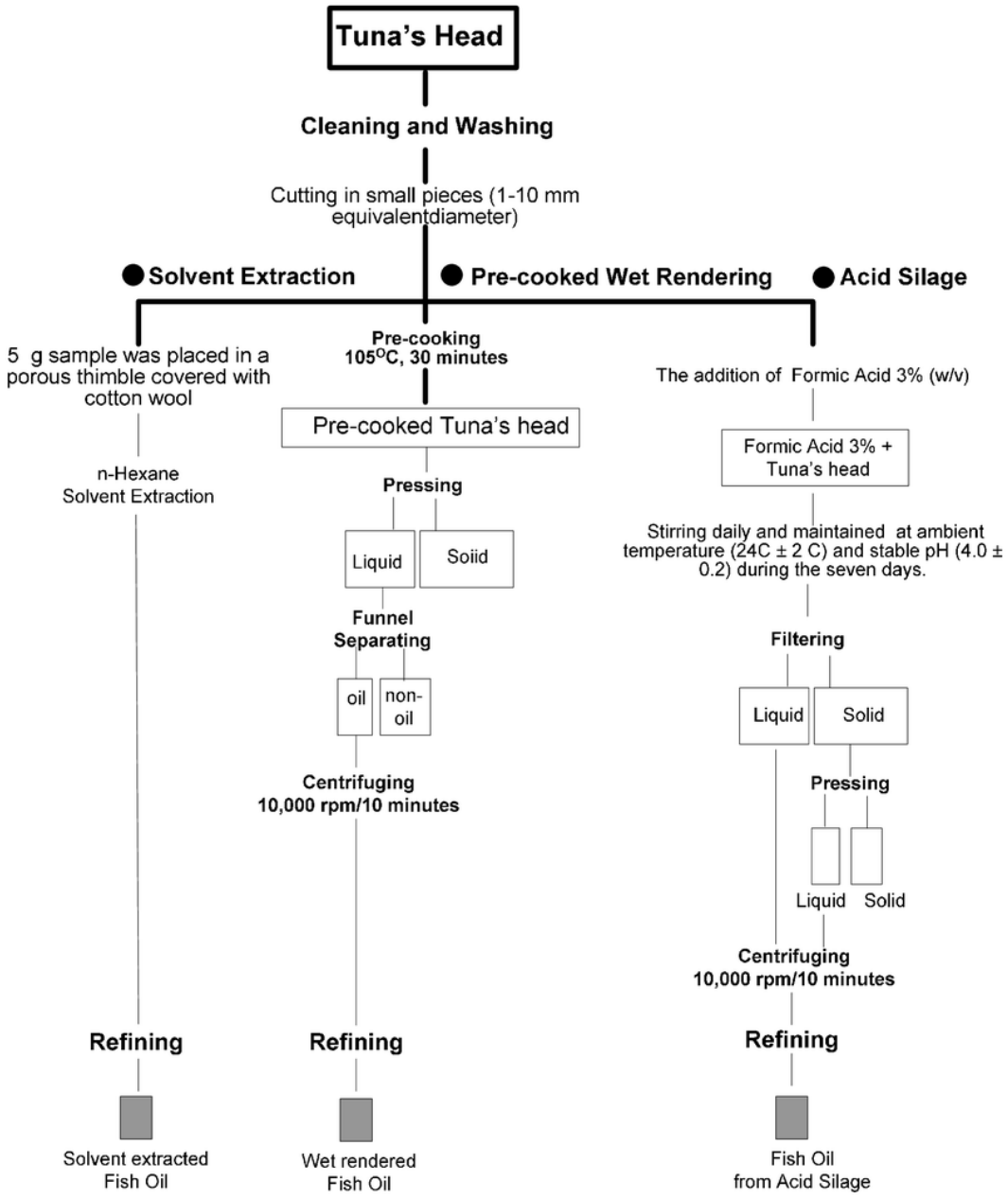

Fig 1. Scheme of the different fish oil extraction procedures studied in this work

E. Design Experiment

The experiment was run in triplicate with a completely randomized design (CRD) with three treatments: pre-cooked wet rendering, acid silage, and solvent extraction. The data were subjected to analysis of variance (ANOVA). The differences among the treatments were determined using Duncan's New multiple range test (DNMRT) [23].

F. Physicochemical Properties of the tuna oils

The free fatty acid content (FFA) and Acid number (AN). FFA and AN were determined according to AOCS Official method Ca 5a-40. 19. [24] A well mixed oil sample (7.05 ± 0.05 g) was accurately weighed into a 250 mL Erlenmeyer flask and 75 mL of hot neutralized 95% ethanol and 2 mL of 1% phenolphthalein indicator solution were added to the oil sample. The hot neutralized 95% ethanol was prepared by heating 75 mL of 95% ethanol with 2 mL of 1% phenolphthalein indicator solution to incipient boiling. The ethanol was neutralized by adding 0.25N sodium hydroxide solution until a faint permanent pink colour appeared. The oil samples were then titrated against 0.25N sodium hydroxide until the appearance of the first permanent pink colour of the same intensity as that of the neutralized ethanol before the addition of sample. The permanent pink colour persisted for at least 30 seconds during titration. The Free Fatty Acids content (%FFA) and acid number were calculated using Equations:

$$\text{FFA (\%)} = \frac{\text{mL of alkali x N x } 28.2}{\text{w}}$$

Where:
N = Normality of NaOH solution
W = Weight of oil (g)

Acid number (mg KOH/g) = 1.99 X FFA (%)

Saponification Value. SV was determined according to AOCS Method cd 3-25 (1993) [25] A 0.002 kg of the oil sample was weighed into a volumetric flask. Then 25mL of 0.5N alcoholic KOH was pipetted and allowed to drain for about 1 minute into the mixture. A condenser was connected to the flask and the mixture sample allowed to boil gently but steadily for 45 minutes for complete saponification. The flask and the condenser were then cooled but not sufficiently to form a gel, the inside of the condenser was washed down with about 1ml of distilled water. The condenser was disconnected and 1ml of phenolphthalein indicator added. The solution was titrated with 0.5N hydrochloric acid (HCl) until the pink colour just disappeared. A blank determination was conducted simultaneously with the sample. The saponification value was calculated using the formula below:

$$\text{Saponification Value} = \frac{56.1 \text{ x N x (V2-V1)}}{\text{w}}$$

Where,
N = normality of HCl,
V1= volume of HCl used in the test, (mL)
V2 = volume of HCl used in the blank, (mL)
W = weight of sample, (g)

Peroxide value (PV). Measurement Peroxide value is a measure of peroxides contained in the oil. PV is determined by measuring iodine released from potassium iodide.The peroxide value was determined by AOCS official method Cd8-53. The test oil sample was filtered through a Whatman No. 40 filter paper to remove moisture and impurities. A 5 g sample of filtered oil was accurately weighed to 0.05 g in a 250 mL Erlenmeyer flask. 30 mL of 3:2 acetic acid-chloroform was added and swirled to mix well. Two blank samples were simultaneously prepared without the addition of fish oil. To the samples, 0.5 mL of saturated potassium iodide solution was added and allowed to stand for exactly 1 min. Saturated potassium iodide solution was prepared by adding 10 g potassium iodide to 6 mL boiled distilled water so that undissolved potassium iodide crystals were present during analysis. After the standing time 30 mL of distilled water was immediately added to the oil samples and swirled to mix. The samples were titrated against 0.1 N sodium thiosulfate until the yellow iodine color disappeared. Starch indicator (2 mL) was added and the titration was continued against 0.1 N sodium thiosulfate until the blue color disappeared. The blank titration value must not exceed 0.1 mL and the peroxide value was calculated by using Equation 1 [26].

$$\frac{\text{(S-B).N.1000}}{\text{mass of sample, g}}$$

Where:
B = volume of titrant, mL. of blank
S = volume of titrant, mL. of sample
N = normality of sodium thiosulfate solution

Iodine value (IV), Measurement A known weight of the oil sample is treated with an excess of iodobromine (IBr) in glacial acetic acid. Unreacted iodobromine is reacted with potassium iodide which converts it to iodine. The iodine concentration is then determined by titration with standard sodium thiosulphate.

$$\text{IV= (b-v) x N x 126.9x 100/w x 1000 (1)}$$

Where b is the mL of sodium thiosulphate used for blank, v is the mL of thiosulphate for sample, N is the normality of thiosulphate solution, w is the weight of oil sample and 126.9 is the molecular weight of iodine. [27]

Thio Barbituric Acid (TBA). Weigh 3 g of oil and put in flask and 50 m distilled water, then transferred into 1000 ml distillation flask while washed with 48.5 ml distilled water and 1.5 ml 4 N HCl. Add boiling stones and antifoam. The distillation is run as high as possible so as to obtain 50 ml of distillate during 10 minute heating. The destilate obtained was stirred, filtered and as much as 50 ml. Move it into a closed erlenmeyer and add 5 ml of TBA reagents (0.02 M thiobarbituric-acid solution in 90% glacial acetic acid).

The solution is mixed in a closed erlenmeyer and inserted into boiling water for 35 minutes. The reaction tube was cooled with running water and then measured its absorbance at 528 nm wavelength with blank solution as a zero boiling point. TBA number were calculated and expressed as mg of malonaldehyde / kg sample [28].

G. Analysis of Fatty Acid Profile

For determining the fatty acid profile, the tuna oils were subjected to methylation or derivatization, as described by Ref. [29]. The extracted oil is methylated into fatty acid methy ester (FAME) [30]. Put 1 mL of hexane into 0.1 mL of oil, and 1 mL of sodium methoxide solution (1.55 g NaOH in 50 mL methanol) is added to the oil solution. The solution was stirred with a hard spin using a Vortex stirrer for 10 seconds. The solution is allowed for 10 minutes to separate the clear- coloured FAME solution from a cloudy aqueous layer. The top layer is carefully collected. The collected oil was measured using a UV-Vis DAD detector at a predetermined wavelength. The analysis was carried out using GC-MS Shimadzu QP 2010. A 1 µL sample is injected into GC-MS operated using a 30-meter long glass column M, 0.25 mm diameter and 0.25 µm thickness with CP-Sil 5CB stationary phase with a pre-programmed oven temperature of 60-220°C with a temperature rise rate of 10°C / min. The carrier gas is 12 kPa pressurized Helium with a total rate of 30 mL / min, and a split ratio of 1:50. From the chromatogram it can be determined the type and content of fatty acids belonging to saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).

III. Result and Discussion

A. Effect of Extraction Method on Yields of Fish Oil

Yields obtained after different treatments with different extraction method shown in Table 1. The silage extraction method was the lowest yield (6.16%), followed by solvent extraction method (8.48%). The highest yield was found in pre-cooked wet rendering extraction method. Cooking can coagulate the protein of fish, so that mechanically oil and solid materials from fish can be separated [6].

TABLE I
EFFECT OF EXTRACTION METHOD ON YIELD OF OILS

Extraction Method	Yield (%)
Wet rendering	12.80[c a]
Solvent extraction	8.49[b]
Acid Silage	6.16 [c]

[a]"Different superscripts indicate a significant difference at $P < 0.05$ for DNMRT (Duncan's New Multiple Range Test)

Yields variation relates to whether the fish is cooked or not before it is extracted [6], how high the contact temperature during extraction [17] and whether there is contact with a particular solvent or not [8]. The wet rendering method is the best method for the extraction of fish oil in line with Rodriguez et al [17] because it involves three basic principles of extraction: cooking, pressing and centrifugation.

Different oil extraction results in this study were suspected because of differences in solvents (solvent and silage methods) and wet rendering methods that did not use solvents.

B. Effect of Extraction Method on Chemical Properties of Oils

Chemical Properties of Tuna Oils from Various Extraction Method is shown in Table 2.

Acid Value. Based on the data obtained from Table 2, the largest acid number was obtained on wet rendering method (1.26%) and the silage method (1.09%). The smallest acid value was obtained on solvent extraction method. Acidity of oil is an important quality parameter related to the presence of Free Fatty Acids (FFA) and other non-lipid acid compounds. FFA is primarily produced by the hydrolysis reaction of triacylglycerides, whereas non-lipid acid compounds, such as acetic acid, can be produced during the decay of the feedstock. Thus, the acidity of the oil depends on several factors relating to the oil composition, the extraction procedure and the freshness of the raw material [17].

The high number of acid numbers in the wet rendering method is suspected due to the hydrolysis process at the time of cooking the fish before it is extracted. Meanwhile, the silage method also produces a high acid number, presumably because the material is soaked in 3% formic acid solution which also causes hydrolysis of the material.

Free Fatty Acid. Carbon chains that have double bonds on unsaturated fatty acids will react with heat to form free fatty acids that can affect the quality of fish oil. The statement is in accordance with the opinion of unsaturated fatty acids will decompose due to the surface of the hot oil and direct contact with air, so that free fatty acids increase [34].

Free fatty acids are closely related to flavors that are less attractive to oil. In the fish oil processing industry the FFA value is closely related to the amount of alkali used in the purification process. Free fatty acids occur due to the hydrolysis process of triglycerides [6]. The Hydrolysis will occurs if oil in contact with water and heat [33].

Peroxide Value. Peroxide rate is the most important value to determine the degree of oil damage. Unsaturated fatty acids can bind oxygen to their double bonds to form peroxides, while saturated fatty acids can not react with oxygen to form peroxides because the bonds are saturated. The smaller the peroxide number means the better the quality of the oil. Oil damage can occur due to the oxidation process by oxygen from the air to unsaturated fatty acids in the oils that occur during processing or storage. Peroxide value (PV) determines the extent to which the oil has undergone rancidity during storage and it can be used to monitor the fats and oils quality and stability [35]. Pre-cooked wet-rendered oils and oils derived from acid silage extraction process have higher PV than oil derived from solvent extraction process (Table 2).

TABLE II
PHYSICOCHEMICAL PROPERTIES OF TUNA OILS FROM VARIOUS EXTRACTION METHOD

Methods	Physicochemical Properties					
	Acid Value	FFA	Peroxide Value	Saponification Value	Iodine value	TBA
Wet rendering	1.05 [b]	0.55[a]	2.93 [b]	126.46 [a]	109.10 [a]	0.79[a]
Acid Silage	1.09[b]	0.58	2.96 [b]	121.26 [b]	110.25 [a]	0.86[b]
Solvent Extraction	0.88[a]	0.46 [b]	1.44 [a]	107.10 [c]	104.74 [c]	0.79[a]

[a-c]Different superscripts indicate a significant difference at $P < 0.05$ for DNMRT (Duncan's New Multiple Range Test)

Heat denatures ion-containing proteins causing more free ion released. Precooked samples contained more free iron resulting in a higher oxidation rate [36]. The higher oxidation rate in acid silage extraction is predicted caused by protein denaturation.

Saponification Value. The high number of saponification value indicates that the oil has a lower molecular weight of fatty acid than fish oil with low-saponification value [37]. However, it can not be used in the identification process of tuna oil [6].

Iodine Value. According to Chantachum et al [6], the double bonds of the fatty acids are oxidized due to the use of the temperature at the time of cooking prior to being extracted. This is supposedly the cause of the high iodine number in wet rendering extraction method (Table 2)

Thio Barbituric Acid (TBA). According to Tokur et al. [38], the acceptable TBA value is less than 2 mg of malonaldehyde / kg of oil. Based on the value of TBA obtained in this study, all fish oil from various methods of extraction and from various types of raw materials is edible oil.

TABLE III
FATTY ACID PROFILE OF FISH OIL FROM VARIOUS EXTRACTION METHOD

Extraction Method	Saturated Fatty Acid (SFA)														
	C6:0	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C18:0	C20:0	C22:0	C24:0				∑SFA
Wet rendering	0.03	0.66	0.22	0.78	2.18	2.12	10.80	7.03	1.24	1.85	1.05				27.96[a]
Silage	0.01	0.30	0.06	0.49	2.84	2.46	12.83	9.13	1.68	4.84	3.18				37.82[c]
Solvent Extraction	0.01	0.30	0.01	0.42	3.34	3.21	10.35	6.26	1.39	3.34	2.08				30.61[b]
Extraction Method	Monounsaturated Fatty Acid (MUFA)														
	C12:1	C14:1n5	C16:1n7	C18:1n9	C20:1n9										∑MUFA
Wet rendering	1.25	0.08	5.79	20.45	0.14										27.71[b]
Silage	3.23	1.08	6.37	18.57	0.10										29.41[c]
Solvent Extraction	2.43	2.08	4.58	15.72	0.08										24.89[a]
Extraction Method	Polyunsaturated Fatty Acid (PUFA)														
	C18:2n6	C18:3n3	C18:3n6	C18:4n3	C20:3n3	C20:3n6	C20:4n6	C20:5n3	C22:5n6	C22:5n3	C22:6n3	ALA	EPA	DHA	∑PUFA
Wet rendering	5.16	3.16	3.19	7.13	2.93	1.25	1.55	6.12	3.30	1.90	8.65	3.16	6.12	8.65	44.34[b]
Silage	0.22	0.17	2.95	5.32	1.10	2.18	1.27	5.21	5.53	3.27	5.55	0.17	5.21	5.55	32.77[a]
Solvent Extraction	6.36	2.10	2.53	6.30	2.25	2.23	2.39	3.28	4.21	6.40	6.44	2.10	3.28	6.44	44.49[b]

∑SFA= sum of Saturated Fatty Acid; ∑MUFA= sum of Monounsaturated Fatty Acid; ∑PUFA= sum of Polyunsaturated Fatty Acid
*Mean indicated by different letters in the same column differ according to the DNMRT at 5% probability
[a-c]Different superscripts indicate a significant difference at $P < 0.05$ for DNMRT (Duncan's New Multiple Range Test)

C. Effect of Extraction Method on Fatty Acid Profile of Fish Oil

The method of oil extraction has a significant different effect on the content of these three types of fatty acids. It is seen that the extraction with the wet rendering and solvent extraction methods is better in extracting PUFA than the acid silage method. However, the wet rendering method works best in extracting DHA and EPA compared to the other two methods (Table 3). The presence of this variation is consistent with earlier studies which suggest that this variation is due to the variation of polar and non-polar fats in the fish portion [39,40] and the variation in chain length and saturation level [40] and whether the extraction using solvent or not. Saturated Fatty Acid (SFA) is a fatty acid that does not have a double bond. Monounsaturated Fatty Acid (MUFA) having a single double bond, Polyunsaturated Fatty Acid (PUFA) is a fatty acid having double bonds of more than one. The third is a group of fatty acids based on the double bond. The method of oil extraction has a different effect on the content of these three types of fatty acids.

In contrast to the extraction method of fish oil derived from the skin it is seen that extraction with silage method is better in extracting PUFA especially EPA, wet rendering method is better in extracting DHA (Figure 3). The interaction between this extraction method of the fish oil portion is in line with previous studies which suggest the variation of polar and non-polar fats in the fish portion

[39,40] the variation in chain length and saturation level [40] and whether the extraction using a solvent or not .

IV. Conclusion

It can be concluded that extraction method has an effect to physicochemical properties and fatty acid profile of fish oil. The wet rendering extraction method is the most effective and most potential extraction method to be applied because it produces the highest yield (12.80%) compared to the acid silage process (6.16%) and solvent extraction method (8.49%). PUFA produced from wet rendering 44.34%, statistically not different with solvent extraction method (44.89%), but it higher than acid silage process (32.77%).

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