The Comparison Extraction Methods of Crude Fat Content and Fatty Acid Profile of Eels (*Anguilla marmorata* (Q.) Gaimard) from Lake Poso

Jamaluddin*, Ayu Putu Putriasih, Yonelian Yuyun and Agustinus Widodo

Department of Pharmacy, Mathematics and Natural Science of Faculty, Tadulako University, Palu, Central Sulawesi, (Postal: 94118), Indonesia

Abstract: Eels (*Anguilla marmorata* (Q). Gaimard) from Lake Poso contain nutrition such as fatty acids EPA and DHA that are good for health. Extraction processes can be carried out to get the fats of these eels (*Anguilla marmorata*). This research aims to determine which one of the following extraction methods, namely: maceration and soxhletation, which is more appropriate to extract eels (*Anguilla marmorata*) so as to produce crude fats and the fatty acid profile with a higher content. The fatty acid profile was examined using the method of gas chromatography by converting results of the fat extraction into volatile fatty acid methyl esters. Research findings suggest that the obtained mean of crude fats was equal to 3.704% for the method of maceration and 28.872% for the method of soxhletation. Furthermore, results of the statistical testing analysis of the fatty acid profile generated a sig. a value greater than the 5% significance level, meaning that the content of fatty acids generated using the extraction method of maceration and that generated using the extraction method of soxhletation were not significantly different. This can be seen from the means of the fatty acid content, namely by 4.6956%, 9.6496%, and 1.5765% for the maceration method, and by 4.7287%, 9.338%, and 1.6646% for the soxhletation method. Based on the foregoing, it can be concluded that soxhletation is the appropriate extraction method.

Keywords: *Anguilla marmorata*, crude fat, fatty acid, fatty acid profile.

INTRODUCTION

Indonesia is rich in fish diversity, in which there are 8,500 species of fish, 1,300 species of which live in freshwater ecosystems [1]. Among the areas in Indonesia with plenty of endemic freshwater fish is Sulawesi.

Central Sulawesi is an area with lots of fresh water, among of which is Lake Poso. Besides a tourist attraction, Lake Poso is also a habitat for various types of fish, such as mujair fish, carp, cook fish, eels locally known as *sogili* fish or *masapi* fish, and so on that are often caught by local fishermen. It is well known that fish are a source of nutrition for humans. Thus, their body contains various types of nutrition and so do eels. Among the nutrition which is vital for human bodies is fatty acids. Eels (*Anguilla marmorata*) contains palmitic fatty acids (*Saturated fatty acid*/SAFA) by 48.55%, oleic acids (*Mono Unsaturated Fatty Acid*/MUFA) by 51.44% [2] and omega 3 (*Docosaheksaenoat acid*/DHA and *Eicosapentaenoic acid*/EPA) that can strengthen brain functions [3].

Fat extraction processes can be done to obtain EPA, DHA, and other fatty acids important for human bodies from eel fish. Extraction is a separate one or many materials of a solids or liquids use dissolving [4]. Among the common extraction methods used for fish are rendering, mechanical expression, and solvent extraction.

The extraction method through rendering is used for fish containing a high content of fats and in large quantities. The method of mechanical expression is more appropriate for oil extraction from grains containing a high oil content. Lastly, solvent extraction is the method of oil/fat extraction using a suitable solvent. This method is usually employed for substances with a low fat/oil content or with a high economic value. Based on the foregoing, the method of solvent extraction was chosen to be used as the extraction method because it was more suitable for the sample used.

The structure of saturated fatty acids EPA and DHA consists of numerous double bonds making them easy to oxidize and result in damage to these fatty acids. The oxidation reaction will be accelerated by heating which is generally part of the fish processing procedure before these fish are consumed. To anticipate the said damage, a method that reduces the heating process using high temperatures during the extraction process, such as maceration and soxhletation which have been modified.

*Address correspondence to this author at the Department of Pharmacy, Mathematics and Natural Science of Faculty, Tadulako University, Palu, Central Sulawesi, (Postal: 94118), Indonesia; Tel: +6281355198799;
E-mail: jamal_farmasi02@yahoo.co.id

ISSN: 2223-3806 / E-ISSN: 1927-5951/19

Based on the foregoing, this research aims to determine which one of the following extraction methods, namely: maceration and soxhletation, which is more appropriate to produce crude fats and the fatty acid profile with a higher content from eels (*Anguilla marmorata*) from Lake Poso.

MATERIALS AND METHODS

Materials

The materials used were wet eels *Anguilla marmorata*, aquadest, filter paper, anhydrous sodium sulfate, 25% HCl solution, boiling stone, paper thimble, hexane, boron trifluoride (BF3), saturated sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and standard fatty acid solution (*Fatty Acid Methyl Esters*/FAME)

Sample Preparation

The sample preparation stage began with washing the sample using running water to remove mucus, followed by removing the internal organs (entrails) before washing the sample once again. After the sample was clean, they were cut into a smaller size and then put into the blender until their texture was smooth and homogeneous [5]. Now, they were ready for extraction.

Sample Extraction

Maceration (Modified from [6])

20 grams of the sample was weighed and then put into the maceration vessel with the addition of 100 ml of hexane. The sample was macerated for 1 day and filtered. The pulp was macerated and this was repeated three times. The obtained extracts were collected and separated using a separating funnel. The oil layer was removed and filtered using filter paper coated with anhydrous sodium sulfate. The extracts were rotated at the temperature of 35-40°C before their weight and content were measured.

Soxhletation [7,8]

The weight of 2 grams of the wet sample was measured and then it was put into a breaker glass with the addition of 30 ml of 25% HCl and 20 ml of water along with several boiling stones, then the breaker cup was closed using a watch glass, boiled for 15 minutes, and filtered while it was still hot and washed using hot water until it was no longer acid. After that, filter paper and with its contents were dried at the temperature of 105°C before they were put on the thimble paper and extracted using hexane for 2-3 hours at the temperature of 80°C. Using the hexane solution during the distillation, the fat extracts which were dried at the temperature of 100-105°C were left to cool and afterwards, the weight was measured. This drying process was repeated until the fixed weight was obtained.

Fatty Acid Analysis (Angler Biochem Lab's Laboratory)

The sample preparation was carried out as follows. The weight of oil sample was measured at 30-40 mg, 2 ml of 0.5 N methanolic NaOH was added to it, and then they were incubated at the temperature of 85°C for 10 minutes and left to cool. Next, 2 ml of 14% methanolic BF3 was added before they were put into the vortex and underwent incubation once again at the temperature of 85°C for 60 minutes. Subsequently, the sample was left to cool at room temperature and 10 ml of hexane and 2 ml of saturated NaCl were added to it before it was vortexed. The result was then centrifuged and its top layer was removed, then anhydrous Na2SO4 was added. The sample was vortexed once again, put into the shaker, and then centrifuged. The centrifugation results were evaporated at the temperature of 40°C, followed by the addition of hexane, and then they were vortexed and filtered again.

Before injecting the methyl ester hexane layer into the gas chromatography tool to analyze the fatty acid composition of the sample, the standard FAME mix solution was firstly diluted and crushed with hexane into a 10-ml volumetric flask containing 500 µl of standard solution. Afterwards, 1 µl of standard solution and 1 µl of sample solution were injected into the gas chromatography tool.

The condition of the GC tool while it was being analyzed is explained as follows:

The type of the column was *Polyethylene glycol* (30 m x 0.25 mm x 0.25 µm), the program oven was at 120^{O}C with an increase of 4^{O}C to 240^{O}C/minute, the injection volume was 1 µl, the injector temperature was 260^{O}C, the FID detector was at 260^{O}C, and the flow rate of N_2 was 1 ml/minute

Data Analysis

The obtained data were statistically analyzed using a paired T-test to determine the appropriate extraction method to produce a higher content of crude fats and the fatty acid profile.

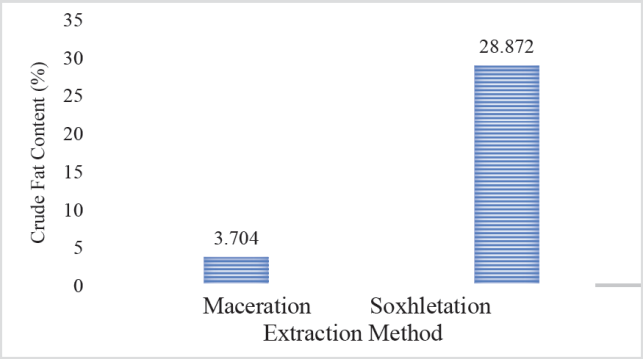

Figure 1: The content of crude fats of eels *Anguilla marmorata* was extracted using two methods.

Table 1: The Fatty Acid Profile of Eels *Anguilla marmorata*

Fatty Acid	Number of Carbons (C)	Fatty Acid Content Using Maceration (%)		Mean (%)	Fatty Acid Content Using Soxhletation (%)		Mean (%)
		Rep 1	Rep 2		Rep 1	Rep 2	
Saturated Fatty Acid							
Lauric Acid	C12:0	0.5648	0.5556	0.5602	0.5305	0.5733	0.5519
Myristic Acid	C14:0	3.4801	3.5382	3.5092	3.3223	3.4389	3.3806
Pentadecanoic Acid	C15:0	0.5169	0.5336	0.5253	0.5087	0.5185	0.5136
Palmitic Acid	C16:0	23.5001	24.3906	23.9454	23.3713	24.3471	23.8592
Margaric Acid	C17:0	0.7364	0.7628	0.7496	0.7581	0.7628	0.7605
Stearic Acid	C18:0	6.7251	6.9740	6.8496	7.0783	7.2564	7.1674
Arachid Acid	C20:0	0.2596	0.2653	0.2625	0.2835	0.2888	0.2862
Lignoseric Acid	C24:0	1.2807	1.0447	1.1627	1.4065	1.2132	1.3099
Monounsaturated Fatty Acid							
Miristoleic Acid	C14:1	0.1482	0.1468	0.1475	0.1338	0.1366	0.1352
Palmitoleic Acid	C16:1	5.1995	5.2882	5.2439	4.8041	4.8821	4.8431
Cis-10-Heptadecanoic Acid	C17:1	0.6173	0.6064	0.6119	0.5885	0.5892	0.5889
Oleic Acid	C18:1n9c	40.5868	41.6645	41.1257	39.5996	40.4375	40.0186
Cis-11-Eicosenoic Acid	C20:1	1.1106	1.1277	1.1192	1.0924	1.1159	1.1042
Polyunsaturated Fatty Acid							
Linolenic Acid	C18:3n3	1.1412	1.0261	1.0837	1.1026	0.9566	0.5296
Gamma Linolenic Acid	C18:3n6	0.2527	0.2524	0.2526	0.2636	0.2448	0.2542
Linolenic Acid	C18:3n3	1.1412	1.0261	1.0837	1.1026	0.9566	0.5296
Cis-11,14-Eicosadienoic Acid	C20:2	0.5280	0.5009	0.5145	0.5386	0.5203	0.5295
Cis-11,14,17-Eicosatrienoic Acid	C20:3n3	0.2089	0.1986	0.2038	0.2083	0.2002	0.2043
Cis-8,11,14- Eicosatrienoic Acid	C20:3n6	0.3639	0.3269	0.3454	0.3757	0.3506	0.3632
Arachidonic Acid	C20:4n6	1.7741	1.4868	1.6305	1.9729	1.7545	1.8637
Cis-5,8,11,14,17-Eicosapentaenoic Acid	C20:5n3	0.9516	0.7648	0.8582	1.0275	0.8725	0,95
Cis-4,7,10,13,16,19-Dicosahexaenoic Acid	C22:6n3	6.1875	4.7244	5.4559	7.0909	5.7057	6.3983

RESULTS

The content of crude fats of eels *Anguilla marmorata* was extracted using two methods. The extraction method soxhletation generated a higher content of crude fats than the extraction method *maceration* did. It can be seen in Figure **1**.

The analysis of the fatty acid profile in eels *Anguilla marmorata* from Lake Poso using the extraction methods *maceration* and soxhletation showed that the fatty acid content of eels can is classified into three, namely *Saturated Fatty Acids* (SAFA), *Monounsaturated Fatty Acids* (MUFA), and *Polyunsaturated Fatty Acids* (PUFA). The fatty acid profile of eels *Anguilla marmorata* using the extraction methods *maceration* and soxhletation can be seen in Table **1** above.

DISCUSSION

This research aims to determine which one of the following extraction methods, namely: maceration and soxhletation, which is more appropriate to extract eels (*Anguilla marmorata*) so as to produce crude fats and the fatty acid profile with a higher content, thus it can be used as a source of information for society and researchers concerning the appropriate extraction method to extract eels *Anguilla marmorata* by still maintaining their nutritional value. Besides, it is also expected to provide information about the nutritional value of eels *Anguilla marmorata*.

In this research, extraction of eels *Anguilla marmorata* from Lake Poso was carried out using two different methods, namely maceration, and soxhletation. The maceration method was chosen based on the fatty acid content of the sample used, eels contain several components of essential fatty acids [2,3]. Essential fatty acids are fatty acids required by the body and containing double bonds, that the body cannot synthesize them [9]. These essential fatty acids have numerous double bonds, making them easy to oxidize, which are also accelerated by heating, and this oxidation causes damage to fatty acids contained in fish oil [10]. The extraction method soxhletation was the second method used. Use soxhletation tool is efficient and effective extraction method to determine the oil or fat content of a material because the solvent used can be recovered and in which it only spends relatively short time [11].

As compound as hydrocarbon, oil or fat generally insoluble in water but soluble in organic solvent, therefore on the two fat extraction method utilized by hexane dissolving. On extraction using the maceration used the wet sample, so oil coat generated from partition was filtered using filter paper coated with anhydrous sodium sulfate to tying-up water that stills to be contained on extracts [12].

The sample used in the extraction using the soxhletation method was the wet sample and therefore prior to the extraction process, they were hydrolyzed by heating in the acidic atmosphere. It aims to release fats bound in the sample and this hydrolysis reaction will produce glycerol and fatty acids. The material used to create the acidic atmosphere is 25%HCl.

The fatty acid profile of the extraction-generated fish oil can be determined by performing an analysis using gas chromatography. The selection of this tool is based on its principle, i.e. converting the fatty acid component contained in fats/oil into volatile compounds in the form of fatty acid methyl esters [7], which fits the properties of fats which have different volatility based on the fatty acid composition they contain.

Fatty acids which at the beginning are not volatile can be converted into volatile fatty acid methyl ester compounds through the fatty acid methylation process. Basically, this fatty acid methylation process consists of two stages, namely alkali hydrolysis, i.e. removing fatty acids from glyceride ester bonds with a strong base (NaOH), and transmethylation, i.e. replacing hydrogen groups of fatty acids with methyl groups from methanol using BF3/methanol as the catalyst [7]. The use of a catalyst aims to accelerate the reaction.

Polyethylene glycol was the column used in this analysis because this stationary phase was polar like the sample (methyl esters) used. Furthermore, in order to give a chance to the fatty acid component to be able to separate itself according to its volatility level at a certain temperature, the column temperature used was increased gradually until the desired final temperature is reached.

The mobile phase used in this analysis was the nitrogen gas. It was used because this gas is suitable for use in FID detectors. Detectors are an electronic sensor whose function is to convert the signal of the carrier gas and the components it contains into electronic signals that will be useful for qualitative and quantitative analyses. In this research, FID (Flame Ionization Detector) detectors were used. These detectors were used as because they are common detectors for almost all organic compounds (high fluoro

compounds and carbon disulfide cannot be detected), in addition to their high sensitivity, linearity viewed from the sample size, and accuracy [13].

The research data on the sample of eels *Anguilla marmorata* after conducting the statistical analysis showed that results of the discrimination test of two treatment media generated a mean by 3.704% for the maceration method while the mean of the crude fat content produced by the soxhletation method was equal to 28.872%. Based on the above results, it is revealed that the average crude fat content produced by the soxhletation method was higher than that of the maceration method. This is because during the heating in the process of drying the sample, not all water was evaporated and thus emulsion was formed that increased the weight of crude fats obtained and made the crude fat content generated by the soxhletation method higher than that generated by the maceration method. In addition, the soxhletation method carried out the heating process at the reflux stage. This heating process results in inclots of cell membrane proteins and water evaporation so that oil can be easily separated from the tissues. Conversely, the maceration method used only low temperatures (room temperature). A low heating temperature causes only a few proteins are denaturated making it harder for oil contained in the sample to penetrate the cell membrane [14] and in the extraction method using soxhletation, the solvent used was also not easily saturated because the extraction was carried out intermittently, where the solvent will wet the sample and will be trapped in the sleeve until the height of the solvent in the chiffon pipe is equal to the height of the solvent in the sleeve, then the whole solvents will flow back into the boiling flask and this process repeats until the color of the solvent dissolving the sample is clear. This is contrary to the extraction using maceration where the solvent used will be easily saturated so as to produce a few extracts.

The analysis of the fatty acid profile showed that eels *Anguilla marmorata* extracted using these two methods contained 3 groups of fatty acids, namely *saturated fatty acids* (SAFA), *monounsaturated fatty acids,* (MUFA), and *polyunsaturated fatty acids* (PUFA). Moreover, it also had the fatty acid composition of the same quantity and type, namely as many as 22 types and with different concentrations, for the two methods used. After conducting the analysis of the statistical data on saturated fatty acids, it is revealed that the value of sig. was equal to 0.354, which was greater than the 5% significance level, meaning that the H_0 was accepted. Thus, it is indicated that the saturated fatty acid content generated by the maceration method and that produced by the soxhletation method does not differ significantly. Likewise, this also applies for the monounsaturated and polyunsaturated fatty acids. The discrimination test of two treatment medians to determine the difference between the extraction methods *maceration* and soxhletation in producing the monounsaturated fatty acid content obtained generated the value of sig. by 0.057, which was greater than the 5% significance level, meaning that the H_0 was accepted. Thus, it is indicated that the monounsaturated fatty acid content generated by the maceration method and that produced by the soxhletation method does not differ significantly. Results of the statistical analysis of polyunsaturated fatty acids obtained by the extraction method also did not have a significant effect. This is indicated by the value of sig. by 0.060, which was greater than the 5% significance level, meaning that the H_0 was accepted. Based on these results, it can be concluded that extraction methods do not significantly affect the content of polyunsaturated fatty acids produced.

The average fatty acid content in the fatty acid profile produced by the soxhletation method was higher than that of the maceration method, except for monounsaturated fatty acids. The obtained fatty acid content was 4.6956% for saturated fatty acids, 9.6496% for monounsaturated fatty acids, and 1.5765% for polyunsaturated fatty acids using the maceration extraction method. As for the extraction method using soxhletation, the average fatty acid content obtained was 4.7287% for saturated fatty acids, 9.338% for monounsaturated fatty acids, and 1.6646% for polyunsaturated fatty acids. These research findings suggest that soxhletation is the better extraction method to choose.

ACKNOWLEDGMENTS

The authors are grateful to the Head, Faculty of Mathematics and Natural Sciences, Tadulako University. Laboratory of Nutrition Faculty of Fishery and Livestock and Laboratory Angler Biochemlabfor providing necessary laboratory facilities.

REFERENCES

[1] Budiman A, Arief AJ, Tjakrawidjaya AH. The Role of Zoology Museum In Research And Conservation Of Biodiversity (Fish). Journal of Ictiology Indonesia 2002; 2(2): 51.

[2] Sandita A, Maulana IT, Syafnir L. Comparison of Fatty Acid Composition Between Eels (*Monopterus albus*) and Palm Oil

(*Anguilla Sp*.) By Kg-Sm Method. Proceedings of SpeSIA Unisba Research 2015; 392.

[3] Effect of Adding Flour Fish Sidat (*Anguilla* spp.) On Making Tortilla Chips Against Nutritional Value, Water Content And Acceptance Of Organoleptik. Journal of Sains Peternakan Indonesia 8(2): 141.

[4] Novia, Yuliyati H, Yuliandhika R. Utilization of Rubber Seeds As Semi Drying Oil With Extraction Method Using N-Hexane Solvent. Journal of Chemical Engineering 2009; 4(16): 2.

[5] Jamaluddin, Atina N, Yuyun Y. The Analysis of Protein Level and Amino Acid Profile in Eels (Anguilla marmorata (Q.) Gaimard and Anguilla bicolor) of Lake Poso . Journal of Pharmacy and Nutrition Sciences 2019; 9(1): 32-37.

[6] Maryana R. The Extraction of Sedentary Oil Massage-Vacuum Maceration Method. Journal of Technology and Food Proceedings of the National Seminar on Chemistry and Chemistry Education 2009; 656.

[7] Andarwulan N, Kusnandar F, Herawati D. Food Analysis. Jakarta: Dian Rakyat 2011.

[8] Jamaluddin , Mappiratu , Septiawan , and Yuyun Y. Analysis of Fatty Acid and Amino Acid Profile of “Meti” Mussels (Batissa violacea L. von Lamarck, 1818) In La'a River of Petasia District North Morowali Regency. Rasayan Journal of Chemistry 2016; 9(4): 673-679.

[9] Panangan AT, Yohandini H, Wulandari M. Qualitative and Quantitative Analysis of Unsaturated Fatty Acids Omega-3, Omega-6, and Characterization of Patin Fish Oil (Pangasius pangasius). Journal of Science Research 2012; 15(3C): 103.

[10] Hadipranoto N. Study of EPA and DHA Thermal Stability in Mujahir Fish Oil (*Oreochromis moss ambitus*). Indo J Chem 2005; 5(2): 152. https://doi.org/10.22146/ijc.21823

[11] Sahriawati, Daud A. Optimization of Fish Oil Extraction Process Soxhletation Method with Different Variations of Solvent And Temperature. Galung Tropika Journal 2016; 5(3): 166.

[12] Sutapa IW, Rosmawaty. The Effect of Catalyst Weight, Temperature And Time Reaction Against Biodiesel Products Of Cow Fat. Proceedings of the National Science Basic VI Seminar 2014; 243.

[13] Gandjar IB, Rohman A. Pharmaceutical Chemical Analysis. Yogyakarta: Student libraries 2007.

[14] Nugroho AJ, Ibrahim R, Riyadi PH. Effect of Steam Jacket Difference on Quality of Oils from Tilapia Fish Waste (*Oreochromis niloticus*). Journal of Fishery Products Processing and Biotechnology 2014; 3(1): 27.

Received on 20-09-2018 Accepted on 28-02-2019 Published on 14-03-2019

DOI: https://doi.org/10.29169/1927-5951.2019.09.02.11