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Short Communication

Effect of Solvent Type and Temperature Variation on Yield and Quality Parameters of Oil Extracted from Eel Fish (*Anguilla marmorata* [Q.] Gaimard) Using Soxhletation Method

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ABSTRACT

Fish oil is a lipid, soluble in non-polar solvents such as ether, hexane, chloroform, petroleum ether, and benzene. Oil of eel (*Anguilla marmorata* [Q.] Gaimard) contains a high level of unsaturated fatty acids that have health benefits. The aim of this study was to determine the best solvent and optimal temperature required for the extraction of eel fish oil by the soxhletation method. Fish oil was extracted with the soxhletation method using n-hexane and diethyl ether as extraction solvent at 60, 70, and 80ºC for 5 hours. The extracted fish oil was analyzed for organoleptic properties, percentage yield, peroxide value, and free fatty acid content. The results of the organoleptic analysis showed that extraction with n-hexane at 60ºC produced the best oil and the highest percentage yield was obtained with diethyl ether at 60ºC. It was observed that the highest peroxide value was obtained for oil extracted with diethyl ether at 80ºC, while the lowest peroxide value was in the n-hexane at 70ºC. The highest free fatty acid level was found in the oil extracted with n-hexane and the lowest in diethyl ether at 60ºC. Analysis of the results indicated that the best condition for extracting eel fish oil was the use of diethyl ether at 60ºC, which produced a clear oil extract, with a typical aromatic odour of fish oil and a golden-brown colour, with a percentage yield of 36.49%, a peroxide value of 5.460 meq/kg, and a free fatty acid level of 41.595%.

Keywords: *Anguilla marmorata* [Q.] Gairmard, Fish oil, Soxhletation, Solvent, Temperature.

Introduction

Fat or oil is a class of lipid composed of three fatty acids and a glycerol (triacylglycerols). Based on the level of saturation, fats are divided into saturated and unsaturated fats. Lipids include non-polar organic compounds, which are readily soluble in non-polar solvents such as ether, hexane, petroleum ether, and benzene. They can be obtained by extracting plant or animal tissues containing fats. The selection of suitable solvents for extraction from specific materials may affect the concentration and quality of the oil produced.[1]

Fish oil contains saturated and unsaturated fatty acids approximately 25 and 75%, respectively.[2] Saturated fatty acids do not have double bonds, whereas unsaturated fatty acids have double bonds. Based on preliminary studies on saturated and unsaturated fatty acids, soxhlet extraction and analysis showed that fatty acid composition of eel fish (*Anguilla marmorata* [Q.] Gaimard) in the yellow eel phase collected from Palu river and Lake Poso were respectively: 2.766 g/100 g and 0.275 g/100 g; mono-unsaturated fatty acids were 4.029 g/100 g and 0.276 g/100 g, while polyunsaturated fatty acids were 0.541 g/100 g and 0.102 g/100 g.[3] In a similar study, analysis of fatty acid content in eel fish (*Anguilla bicolour*) in the elver eel phase obtained from Palu river and Lake Poso were respectively: saturated fatty acids were 6.085 g/100 g and 5.543 g/100 g; mono-unsaturated fatty acids were 5.157 g/100 g and 7.817 g/100 g, while polyunsaturated fatty acids were 1.265 g/100 g and 1.950 g/100 g. Meanwhile, in the silver eel phase, the values obtained were respectively 3.829 g/100g and 4.177 g/100 g (for saturated fatty acids): 2.035 g/100 g and 2.859 g/100 g (for mono-unsaturated fatty acids), while 3.370 g/100 g and 0.524 g/100 g (for polyunsaturated fatty acids).[4]

Fish oil is a component of fat in fish tissue that has been extracted into oil form.[5] Based on previous research that was carried out on eel (*A. marmorata* [Q.] Gaimard), extraction of fish oil using soxhletation method produced a lot more fatty acids compared to other methods.[6] Extraction with soxhletation or thermal method is most effective and efficient to determine the number of oil in a material because the solvents used can be recovered and less time is required. Several factors that affect the amount and quality of the oil obtained when soxhletation extraction method is used include particle size of the samples, type of solvent, time, and temperature at the time of extraction.[7]

Therefore, the present research was conducted to determine the best solvent and temperature for extraction of oil from eel fish (*A. marmorata* [Q.] Gaimard) using soxhletation method.

Material and Methods

Sources of eel fish and chemicals

Eel fish (*A. marmorata* [Q.] Gaimard) in the yellow eel phase used for this study was obtained from the Palu river, Palu, Central Sulawesi, Indonesia. The chemical materials included n-hexane, diethyl ether, 95% ethanol, chloroform, water (aquabidest), potassium iodide, glacial acetic acid, sodium thiosulfate, sodium hydroxide and phenolphthalein indicator used were purchased from Intraco Makssar (South Sulawesi, Indonesia).

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Sample preparation
Blood and mucus were removed from the fish and the fish was chopped into small pieces. They were dried in an oven at 60ºC for 24 hours, then blended using a laboratory blender and stored at room temperature.

Sample extraction
Fifteen grams of the blended fish sample was placed in an extraction thimble and placed in the sohxlet extractor fitted into a round-bottom flask containing the solvent (diethyl ether or n-hexane, 150 mL). Each was extracted at a temperature of 60, 70, or 80ºC for 5 hours. The solvent was removed by evaporation. Then, the fat obtained was placed in an oven at 105ºC for 1 hour. The beakers containing the fat were cooled in a desiccator for 30 minutes. The round-bottom flask containing the fat was weighed to a constant weight. The extraction process were conducted at least in duplicate.[8]

Organoleptic tests
Organoleptic tests which included determination of turbidity, odour, and colour of the extracted fish oil were carried out as previously described.[9]

Determination of yield of extracted fat
Based on ISO procedure 01-2354.3-2006, an empty round-bottom flask was weighed (A). Fifteen grams (15 g) of sample (B) were inserted into the sheath of fat. An aliquot of 150 mL solvent was added into the round-bottom flask. The extraction was done at the test temperatures for 5 hours. The mixture of fat and solvent were evaporated from the round-bottom flask to dryness and then placed in an oven with a temperature of 105ºC for 1 hour. The flask with the fat was cooled in a desiccator for 30 minutes. The round-bottom flask containing the fat (C) was weighed to constant weight.[8] The Percentage yield was calculated using the formula:

$$\%\ Yield = \frac{(C - A)}{B} x\ 100\ \%$$

Where:
A = Weight of empty round-bottom flask (g)
B = Sample weight (g)
C = Weight of round-bottom flask and extracted fat (g)

Determination of peroxide value of fish oil
Based on SNI (Standar Nasional Indonesia) 01-3555-1998 procedure, 1 g sample of fish oil was placed into a 250-mL Erlenmeyer flask and 18 mL of acetic acid, 12 mL of chloroform, and 0.5 mL of potassium iodide (KI) solution were added. The solution was carefully shaken to mix thoroughly and 30 mL aquabidest was added. Also, 0.5 mL of 1% starch indicator was added. Then, the solution was titrated with 0.01 N sodium thiosulfate until the solution turns yellow.[10] The Peroxide value was calculated using the formula:

$$Peroxide\ value\ \left(\frac{meq}{Kg}\right) = \frac{(V1 - V0)\ xMx\ 1000}{G}$$

Where:
V1 = Volume of titrant sample (mL)
V0 = Volume of titrant blank (mL)
M = Normality of standard solution of sodium thiosulfate (N)
G = Weight of the sample (g)

Determination of free fatty acid content of fish oil
Based on SNI 01-3555-1998 procedure, a sample of 0.5 g was placed into a 250-mL Erlenmeyer flask. An aliquot of 25 mL of 96% ethanol was added and heated at a temperature of 40°C, after which 2 mL phenolphthalein indicator was added. The solution was titrated with 0.05 M NaOH until a pink colour appeared, which did not disappear for 30 seconds.[10] Percentage free fatty acids (% FFA) was calculated with the formula:

$$\%\ FFA = \frac{mlNaOHxMNaOHxBMx}{Berat\ Sampel\ x\ 1000}\ x\ 100\%$$

Where:
mL NaOH = Volume of titrant NaOH (mL)
M NaOH = Molarity of NaOH
BM = Molecular weight of oleic fatty acid (282 g/mol)

Statistical analysis
The experiments were conducted in triplicates and the experimental data were presented as mean + SEM. Statistical data analysis was performed using IBM SPSS Statistics 26 trial version by Version 1 Solutions Limited (England). Student's t-test and ANOVA analysis were applied for comparing means. $P < 0.05$ indicated a statistically significant difference.

Results and Discussion

The research was conducted using the commonly consumed eel fish (*A. marmorata* [Q.] Gaimard), which was in the yellow eel phase. It was collected from the Palu river, Palu, Central Sulawesi, Indonesia. Method, solvent, temperature, and time of extraction affect the concentration and quality of fish oil recovered from the extraction process. Extraction of fish oil using several solvents and different temperatures revealed that the best treatment was the diethyl ethyl solvent and a temperature of 80ºC.[8] Therefore, in the current research, soxhletation method was employed with variation in temperature and the type of solvents used. The parameters determined were organoleptic properties which included turbidity, odour, and colour. Other analyses performed were the determination of percentage yield, peroxide, and free fatty acid values to know the best eel fish oil extract. Analysis of eel (*A. marmorata* [Q.] Gaimard) oil is important to determine the quality of fish oil produced through the extraction process. Oil from fish is a source of omega-3, especially EPA (*Eicosapentaenoic acid*) and DHA (*Docosahexaenoic acid*) that play important roles in human health.[11] Fish oil with chemical and physical properties that meet required standards can be used as food or raw materials in pharmaceutical preparations such as fish oil supplements and other products. However, the quality of fish oil is considered not satisfactory if its physicochemical properties did not meet certain standards. This can make the fish oil rancid, resulting in unwanted flavour or toxic effect. Also, degraded fat and oil can reduce the nutritional values of products containing them, thereby making these products unsafe for consumption.[12]
Sampling technique using the purposive sampling method is a technique used for choosing a sample in a population following the study's purpose so that the sample can represent the characteristics of the population. When purposive sampling is applied, the sampling can be adjusted to the researcher's desire, based on the type of weight, size, and the sampling site.[13] In this study, the type of sample used was eel (*A. marmorata* [Q.] Gaimard) in the yellow eel phase and sampling site was the Palu river. The yellow eel phase is an adult fish category with a size of 30 - 40 cm. This phase of eel fish is characterized by a high fatty acid content and is commonly called the consumption stage. Extraction of eel (*A. marmorata* [Q.] Gaimard) oil was initiated by crushing the dried fish sample into powder form to increase the surface area for the soxhlet extraction method. This method is an effective and efficient way to extract oil or fat from a material. Previous research has shown that the soxhletation method was more effective than maceration method because the solvents used could be easily recovered and the entire process occurred within a relatively short time.[4] The extraction process by soxhletation method was carried out using two solvents; n-hexane or diethyl ether, which are non-polar solvents suitable for extracting fish oil or fat that are also non-polar. Besides, the solvents used were based on previous research which found that diethyl ether was the best solvent and the optimal temperature was 80ºC in the soxhletation process of milkfish (*Chanos chanos* Forsk).[8] Other study that was conducted on the extraction and characterization of skin catfish oil investigated temperature variations (50, 60, 70, 75, 85, and 95°C) and the findings revealed that the best temperature was at 60°C with n-hexane as the extraction solvent.[14] Therefore, variations in temperature (60, 70, and 80ºC) and solvent types (n-hexane or diethyl ether) on fat content and quality of extracted eel fish oil were investigated in this study.

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The results of the organoleptic tests which included determination of turbidity, smell, and colour of fish oil for both the n-hexane or diethyl ether solvent are presented in Table 1. A temperature of 60°C was observed to be the optimal extraction temperature. Also, at this temperature, the n-hexane solvent produced fish oil with a typical odour and golden-brown colour. Meanwhile, at higher temperatures, the fish oil became blackish-brown and feculent. Previous research has shown that an extraction temperature of 60ºC produced more fish oil than at higher temperatures, while the oil colour changed from golden-yellow to reddish yellow.[15] There were changes in the turbidity of oil from clear at 60ºC extraction temperature to slightly feculent or feculent with an increase in temperature, which was an indication of the presence of oil degradation products or leftover materials in the oil. In this study, the dark fish oil extract was a result of the oven and pressing processes undertaken to reduce the water level which caused the oil to have a brown appearance. Generally, the smell of fish oil has a specific characteristic fishy smell that is not rancid. Rancidity is directly proportional to peroxide level in an oil; hence the higher the peroxide level, the more rancid is the oil.[9] When the organoleptic test parameters do not meet certain standards, the quality of the fish oil, especially the appearance, taste, and texture is affected, thereby causing the fish oil to become rancid with a bitter taste. In this case, the oil is not suitable for consumption and cannot be processed into food products.[16]

Percentage yield is often used to determine the effectiveness of the method, solvent, and temperature used in an extraction process of fish oil. Any procedure with more percentage yield can be inferred as a good method of extraction. A higher value of percentage yield indicates a lot more fish oil production. Table 2 shows the results obtained for the percentage yield of oil with variations in extraction temperature (60, 70, or 80ºC) and types of solvent (n-hexane or diethyl ether). The highest percentage yield of 36.49 ± 4.03% was obtained when diethyl ether solvent was used for fish oil extraction at 60ºC, while the lowest value of 31.89 ± 0.68% was recorded for extraction with n-hexane solvent at 80ºC.

There has been a report that fish oil processing under 70ºC produced a yield of 30 – 40%.[15] In another study, it was found that the yield of oil in the liver silky shark (*Carcharhinus falciformis*) at an extraction temperature of 60°C was 20.16%.[17] The difference in extracted crude fish oil yields is dependent on fish fat levels;[18] the higher the fat level, the more amount of oil obtained. Based on the two-way ANOVA test using SPSS, there were significant differences ($p<0.05$) in the percentage yield among the three test temperatures in the n-hexane solvent treatment, whereas the diethyl ether solvent showed no significant difference ($p > 0.05$). The t-test results between the two types of solvent indicated no significant difference ($p > 0.05$) in percentage yield. Also, temperature plays an important role in the extraction process of fish oil, as heating at high-temperature will cause more protein denaturation, leading to the production of more fish oil. High percentage yield indicates the production of more fish oil and polyunsaturated fatty acid (omega-3), contained in fish oil which has lots of health benefits and found usefulness in pharmaceutical preparations.[15]

Determination of free fatty acids is a parameter for detecting the quality of oil. Free fatty acids are fatty acids that are not bound as triglycerides, which are produced through hydrolysis and oxidation. The higher the value of free fatty acids, the lower the quality of the fish oil. Results of previous research on comparing the quality of crude fish oil extracted from a variety of fishes which included catfish, tuna and milkfish revealed free fatty acid values of 2.44, 1.49 and 2.61%, respectively.[18] In a similar study, the level of free fatty acids from fish oil extracted from a red snapper (*Lutjanus malabaricus*) using the wet rendering method was found to be 1.02%.[19] In a study conducted by Sari and coworkers showed that in refined oil from catfish, free fatty acid values of 0.27 to 0.83% were obtained after a decline, resulting in 50.30%.[0] Percentage of free fatty acids obtained in the present study were between 41.595 - 54.919 (Table 3). The lowest value was obtained when diethyl ether was used as extraction solvent at a temperature of 60ºC, while the highest value was recorded for n-hexane at a temperature of 80ºC. The differences observed in these values may be attributed to the extraction and purification methods used, fatty acid profile/oil produced, type, and freshness of raw materials.[20] Based on the two-way ANOVA test using SPSS, there were significant differences ($p<0.05$ [$p=0.341$]) and ($p=0.001$) in free fatty acids for the three test temperatures and solvent types. The t-test results between the two solvents indicated a significant difference ($p<0.05$) in free fatty acid between them. It was observed that the average free fatty acids of n-hexane solvent were higher than that of diethyl ether. The SNI numerical quality standard for free fatty acid value is between 15 – 75% for a sample of 0.5 g. In the current research, the value of free fatty acids obtained met the required standard.[8]

Table 1: Organoleptic tests on oil extracted from eel (*A. marmorata* [Q.] Gaimard)

Temperature	Solvent	Replication	Turbidity	Odour	Colour
60ºC	n-hexane	A	Clear	Typical Aromatic Fish Oil	Golden brown
		B	Clear	Typical Aromatic Fish Oil	Golden brown
	diethyl ether	A	Clear	Typical Aromatic Fish Oil	Golden brown
		B	slightly Feculent	Typical Aromatic Fish Oil	Dark brown
70ºC	n-hexane	C	Feculent	Typical Aromatic Fish Oil	Dark brown
		D	Feculent	Typical Aromatic Fish Oil	Dark brown
	diethyl ether	C	slightly Feculent	Typical Aromatic Fish Oil	Reddish-brown
		D	Clear	Typical Aromatic Fish Oil	Reddish-brown
80ºC	n-hexane	E	slightly Feculent	Typical Aromatic Fish Oil	Golden brown
		F	slightly Feculent	Typical Aromatic Fish Oil	Golden brown
	diethyl ether	E	Feculent	Typical Aromatic Fish Oil	Dark brown
		F	Clear	Typical Aromatic Fish Oil	Reddish-brown
Standard			Clear	Aromatic Fish Oil	Golden yellow

*A = replication 1st at 60°C; B= replication 2sd at 60°C; C replication 1st at 70°C; D= replication 2sd at 70°C; E = replication 1st at 80°C; F = replication 2sd at 80°C

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Table 2: Percentage yield of eel (*A. marmorata* [Q.] Gaimard) oil

Solvent	Sample weight (g)	Yield average (%)			*P (Annova test)*	*P (T-Test)*
		60ºC	70ºC	80ºC		
n-hexane	15.0 g	34.16 ± 0.64	34.78 ± 0.39	31.89 ± 0.68	0.032**	0.197*
diethyl ether	15.0 g	36.49 ± 4.03	33.17 ± 1.17	36.18 ± 1.47	0.460*	
P (T-Test)		0.506*	0.207*	0.065*		

*P>0.005: insignificant difference; **P≤0.005: significant difference

Table 3: Determination of free fatty acids of eel (*A. marmorata* [Q.] Gaimard) oil

Solvent	Sample weight (g)	NaOH (M)	Oleic acid (g/mol)	FFA average (%)			*P (Annova-test)*	*P (T-test)*
				60ºC	70ºC	80ºC		
n-hexane	0.5	0.05	282	51.88 ± 4.43	53.79 ± 1.44	54.91 ± 1.18	0.341*	0.000**
diethyl ether	0.5	0.05	282	41.59 ± 1.90	48.08 ± 1.68	46.39 ± 1.16	0.001**	
P (T-Test)				0.005**	0.002**	0.000**		

FFA: Free fatty acids; *P > 0.005: insignificant difference; **P≤0.005: significant difference

Table 4: Measurement of peroxide value of oil extracted from eel (*A. marmorata* [Q.] Gaimard)

Solvent	Sample weight (g)	$Na_2S_2O_3$ (N)	Peroxide number average (meq/kg)			*P (Anova test)*	*P (T-test)*
			60ºC	70ºC	80ºC		
n-hexane	1.0	0.0091	5.46 ± 1.28	5.23 ± 1.36	5.91 ± 1.17	0.751*	0.642*
diethyl ether	1.0	0.0091	5.46 ± 0.74	5.68 ± 2.61	6.37 ± 2.29	0.808*	
P (T-Test)			1.000*	0.768*	0.730*		

*P > 0.005: insignificant difference; **P ≤ 0.005: significant difference

Measurement of peroxide value is used as an indication of the levels of peroxides and hydroperoxides formed in the early stage of the fat oxidation reaction. Peroxide is one of the quality indicator parameters of fish oil. Peroxide value is used to determine the extent of degradation or rancidity of fish oil,[8] whereby the higher the peroxide value, the lower the quality of the oil. In this case, the oil is not safe for consumption because it can be easily degraded and become rancid. Peroxide values obtained in this study for oil extracted with n-hexane or diethyl ether solvent ranged from 5.232 - 6.370 meq/kg (Table 4). The best condition was when n-hexane was used as an extraction solvent at a temperature of 70ºC. The highest level of peroxide was obtained in the diethyl ether solvent with a temperature of 80ºC. Based on previous studies, the lowest peroxide value obtained for skin catfish oil with an extraction temperature of 60°C was 38 meq/kg, while peroxide values of 4.88 to 6.08 meq/kg were recorded for steam-purified catfish oil.[14,20] In a similar research, it was revealed that crude fish oil from abdominal fat had a peroxide value of 8.23 meq/kg which decreased to 3.86 meq/kg during the process of purification.[21] Another study reported a slightly lower peroxide value of 3.49 meq/kg, when the heating method was employed for purification.[9] The difference in peroxide values was due to extraction methods, types of solvent, temperature and purification process of the fish oil. During purification, peroxide level decreased compared to crude oil extraction.[20] Test results from ANOVA showed that there were significant differences (p>0.05 [p=0.751] and p=0.808) in peroxide values of the three test temperatures and solvent types. T-test results between the two types of solvent showed that there was no significant difference in peroxide value (p=0.642). Therefore, the solvent and temperature differences did not significantly affect the results of the peroxide produced as a result of the non-polar nature of the solvent used. According to peroxide values obtained in this study, all the oil extracts met the international quality standard, IFOMA (International Fishmeal and Oil Manufactured Assosiation) of fish oil for peroxide value which is between 3 - 20 meq/kg. Peroxide level of oil increases during storage due to storage time, temperature and contact with light and air. When the peroxide value for oil does not meet the required standard (i.e. very high value of >100 meq/kg), the oil becomes extremely toxic. For fish oil to be used for nutritional purposes, it must meet standards of peroxide test.[12]

Conclusion

The results obtained in this study for the extraction of oil from eel (*A. marmorata* [Q.] Gairmard) using the soxhletation method indicated that the best extraction solvent was diethyl ether at a temperature of 60°C. At this condition, a fish oil extract that was clear, golden brown in colour with a distinctive aromatic fish smell was obtained. The yield was 36.489%, peroxide value of 5.460 meq/kg, and free fatty acid level of 41.595%.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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