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THE OIL EXTRACTION FROM EEL (*ANGUILLA BICOLOR BICOLOR*) BONE AS BY-PRODUCT FROM KABAYAKI PROCESSING INDUSTRY

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ABSTRACT

Fish oils have been recognized as good sources of polyunsaturated fatty acids (PUFA) which are widely used for pharmaceutical purposes and as food supplements. In this study, fish oil from eels (*Angguilla bicolor bicolor*) bone were extracted using a Bligh and Dyer methods. The fatty acid composition of the oil was analyzed and quantified using gas chromatography. Results showed that the total yield of by-product of Eel (*Anguilla bicolor bicolor*) reached 26,38 %, the lipid content of eels bone was 17.33 ± 0.58 g/100 g. Yield of eel bone oil extracted by Bligh and Dyer method was 17.12%. In the fatty acid analysis of eel bone oil, it was discovered that SFA was 19.87%, MUFA was 25.84%, and PUFA was 13.84%. The major fatty acids in the oil from the bone were palmitic acid (13.58%), oleic acid (20.94%), linoleic acid (4.01%), EPA (1.57%), and DHA (4.84%).

Key words: bligh and dyer methods, eel bone, fatty acid composition, fish oil

INTRODUCTION

Fish bone is one kind of by-product from fish processing which is potentially utilized to developed its nutrition value**.** Research showed that fish bone could be used to produce many kinds of products such as collagen extract, cartilage elements, oligopeptide, bone protein, and bone oil (Yin *et al.,* 2007).

Large amount of eels by-products are produced in Indonesia every year. In 2012 about 3,500 kg of eel by-products produced in PT Java Indah State. These eels by-products comprise eel heads, bone, an**d** viscera. Production and purification of fish oil from eels bone as by-products can give benefit to Indonesian fisheries development.

High quality food grade oils should have a balanced fatty acid (FA) composition containing high levels of valuable minor compounds such as vitamins and natural antioxidants. Fish oil is an excellent source of n-3 fatty acids (n-3 FAs), mainly eicosapenta-enoic acid (EPA) and docosa-hexaenoic acid (DHA). The n-3 FAs

are claimed to be essential for normal growth and development and play an important role in the prevention and treatment of hypertension, cancer, and several inflammatory and autoimmune disorders (Razak 2001).

The objectives of this study were: (1) to determine the yield of by-product of eel, (2) to extract oil from eel bone by-product using Bligh and Dyer methods, (3) to analyze fatty acids composition of the eel oil.

MATERIALS AND METHODS

Samples: Eels (*Anguilla bicolor bicolor*) and eels bones obtained from kabayaki processing plant at PT Java Suisan Indonesia, Palabuhan Ratu Sukabumi, West Java, Indonesia. Eels were brought alive to experiment place for determining the yield of eel by-product. The eels bone was frozen and kept in ice box during transport to experiment place.

Chemicals: All solvents and chemicals used in extraction procedures were analytical grade and obtained from Merck (Darmstadt, Germany). Chloroform and Methanol. Sodium sulphate anhydrous (Merck, Darmstads, Germany) were used as drying agent. Distilled water **Procedure**

Determination the yield of by-product*:* The yield of by-product wasdetermined by separating eel meats, head, bone, and viscera. Yield of by-product was then calculated as percentage to the total weight.

Sample preparation*:* All bones were washed using water to remove the residual blood. Bones were then weighed into three groups of 100 gr, there were whole bone, cutted bone (reduced to small particle), and blended bone (reduced to smaller particle than cutted one). Bones were then stored at freezer until used in extraction.

Proximate analysis of Eel bone: Proximate analysis was performed by standard methods of AOAC (2005).

Extraction: Extraction of Eel bone oil was carried out according to Bligh and Dyer (1959) methods. After being thawed at ambient temperature, the bone (100 g) was put into a beaker. About 100 mL distilled water was added and mixed. 200 mL chloroform and 400 mL methanol were then added and the mixture was homogenized for two minutes while being cooled in ice. More chloroform (200mL) was added and homogenized for one minute, followed by the addition of distilled water (200 mL), and finally homogenized for 30 seconds. The mixture was centrifuged at 3000 rpm for 20 minutes. The aqueous layer was removed by suction. The chloroform fraction was dried with sodium sulphate anhydrous, filtered and the chloroform was evaporated under vacuum on rotary evaporator. The oil fraction remaining was stored in a bottle being wrapped in aluminium foil to exclude light.

Analysis of Fatty Acid: Analysis of fatty acid composition was performed to the fresh eel bone and the eel bone oil. Fatty acid composition was analyzed according to AOAC method (2005). A total of 20-40 mg of fat or oil was taken in a teflon-covered tube

followed by adding 1.0 mL of NaOH in methanol and then heated in a water bath for 20 minutes. Later on 2.0 mL of 20% BF_3 and 5 mg/ml of internal standard were added to the mixture, and the mixture was heated again for 20 minutes. The mixture was cooled and then 2.0mL of saturated NaCl and 1.0mL isooctane were added, subsequently the mixture was shaken well. Isooctana layer formed was trans-ferred with the aid of pipette into a tube containing approximately 0.1 grams of anhydrous Na_2SO_4, and then awaited for 15 minutes. Liquid phase formed was separated, while 1.0 mL of oil phase was injected, previously injection of FAME standard mixture was performed. Retention time and peak of each component was measured and compared with the standard retention time to obtain information about the types and fatty acid components in the sample. Determination of fatty acid content in the samples can be calculated by using the formula as follows

$$\text{Component content of samples} = \frac{Ax/As \times Cstandard \times Vsample/100}{100\ \%\ \text{Sample weight}} \times$$

Ax: Sample area; As: Standard area; Cstandar: Standard concentration Vsample: Sample volume

RESULTS AND DISCUSSION The yield of by-product: The yield of by-product was obtained by calculating the percentage of the total weight of eel (*Anguilla bicolor bicolor*) and results are shown in Table-1. According to data from Table 1, the total yield of by-product of eel (Anguilla bicolor bicolor) reached 26.38± 1.04%. Therefore eels by-product are potential to have added value that can optimize the Indonesian eels (*Anguilla bicolor bicolor*) processing industry.

Table-1: Yield of eel (*Anguilla bicolor bicolor*) by-product

By-product	Percentage of yield (%)
Head	15.04 ± 3.05
Viscera	6.34 ± 1.05
Bone	5.04 ± 0.22

Widyasari *et al.,* (2013) used eel's by-product to produce flour that can be used as raw materials or additive in food diversification products. Meizhen *et al.,* (1996) extracting eel oil from waste eel bone as raw material and purifying the poly-unsaturated fatty acids (PUFA) concentrates using salt forming with urea complexation, the complete yield for extracting eel bone oil was above 76%, the contents of EPA and DHA in PUFA purified from eel bone oil increased from 7.4% to about 45%, the recovery of EPA plus DHA was 64%.

Yan *et al.,* (2011) was used Trypsin in the study to extract oil from

the eel bone. The extraction rate of oil was 23.87%. The eel oil composed of 18 kinds of fatty acid, which were between C14 and C30.6 kinds of saturated fatty acids (SFA), 6 kinds of monounsaturated fatty acids (MUFA), 6 kinds of polyunsaturated fatty acids (PUFA). SFA was 30.02%, MUFA was 55.82%, PUFA was 14.18%. Oleic acid (C18:1(9)) was 42.68%, palmitic acid (C16:0) was 20.35%, palmitoleic acid (C16:1(9)) was 9.61%, DHA (C22:6) was 7.23%, EPA (C20:5) was 4.7%.

Chemical composition: Chemical composition analysis of eel bone performed by standard methods of AOAC (2005). The proximate analysis results are shown in Table-2.

Table-2: Chemical composition (*Anguilla bicolor bicolor*) of eel bone

Parameter	Value (% w/w)
Moisture	45.21 ± 0.20
Ash content	8.63 ± 0.16
Fat content	17.33 ± 0.58
Protein content	13.19 ± 0.63
Carbohydrate content	15.65 ± 0.07

Proximate analysis showed that eel bone had moisture content 45.21%, ash 8.63%, fat 17.33%, protein 13.19% and carbohydrate 15.65%. Widyasari *et al.,* (2013) showed that fresh eel had 42.03% moisture content, 3.93% ash, 28.29% fat, 17.68% protein and 9.53% carbohydrate. Eel bone by product have higher ash than fresh eel, in the other side have lower fat and protein. In this case, proximate composition affected by source of sample. Fresh eel dominated by edible portion of eel such as a meat, while eel bone by-product dominated by bone. Eel bone as by-products and frsh eel had fat content more than 15%, it was similar to fresh eel. Therefore eel bone can be used as source of oil fish.

Extraction of Eel oils: Extraction of eel oils was done according to the Bligh and Dyer methods (1959) with some modification performed by Kinsela *et al.,* (1977). The results of extraction are showed in the Table 3. The result shows that the oil yield from cutted bone and blended bone was not significantly different and whole bone had a lowest value of yield. Extracted oil was obtained from fat located in spinal cord. When the bone is blended or cutted, fat inside the bone can interact with solvent more effective than whole bone. This result was parallel with analysis of fat content also. Except whole bone, the yield of extracted eel bone oil is closed to the fat content of eel bone on the Table 2. The solvent exctraction methods such as Bligh and Dyer methode (1959) depend on interaction between solvent and sample but the surface area size is important factor. Cutted and blended bone have larger surface area than whole bone. So, the yield of exctraction higher than whole bone.

Table-3: Yield of extracted eel bone oil (%)

No.	Sample	Weight (gr)	Oil weight (gr)	Yield of extraction (%)
1	Whole Bone	100	5,9706	5,97
2	Cutted bone	100	17,1223	17,12
3	Blended bone	100	17,3459	17,35

Fatty Acid Composition: The fatty acid composition of the fresh eel bone and eel bone oil is shown in Table 4. The result was in row with the study of Widyasari *et al.,* (2013) utilizing by-product of Indonesian eel (*Anguilla bicolor*) as flour while Yan *et al.,* (2011) using trypsin for extracting oil from the eel bone.

Table 4. The fatty acid composition of fresh eel bone and eel bone oil

Fatty Acid	Structure	Fresh Eel Bone (g/100g lipid)	Fish Oil of Cutt bone (g/100g lipid)
Lauric Acid	C12:0	0.10	0.12
Tridecanoic Acid	C13:0	0.07	0.07
Myristic Acid	C14:0	2.27	2.03
Pentadecanoic Acid	C15:0	0.61	0.47
Palmitic Acid	C16:0	17.12	13.58
Heptadecanoic Acid	C17:0	0.65	0.53
Stearic Acid	C18:0	3.51	2.84
Arachidic Acid	C20:0	0.14	0.13
Heneicosanoic Acid	C21:0	0.02	0.02
Behenic Acid	C22:0	0.04	0.04
Lignoceric Acid	C24:0	0.05	0.04
TOTAL of SFA		**24.48**	**19,87**
Myristoleic Acid	C14:1	0.04	0.05
Palmitoleic Acid	C16;1	3.11	2.84
Cis-10-Heptadecanoic Acid	C17:0	-	0.08
Elaidic Acid	C18:1n9c	0.16	0.15
Oleic Acid	C18:1n9c	26.59	20.94
Cis-11-Eicosenoic Acid	C20:1	1.90	1.09
Erucic Acid	C22:1n9	0.20	0.25
TOTAL of MUFA		**32.00**	**25.40**
Linoleic Acid	C18:2n6c	6.09	4.01
ɣ-Linolenic Acid	C18:3n6		0.10
Linolenic Acid	C18:3n3	0.98	1.10
Cis-11,14-Eicosedienoic Acid	C20:2	0.64	0.46
Cis-8,11,14,17-Eicosetrienoic Acid	C20:3n6	0.37	0.24
Arachidonic Acid	C20:4n6	1.14	0.98
Cis-5,8,11,14,17-	C20:5n3	1.56	1.57

Eicosapentaenoic Acid			
Nervonic Acid	C24:2		0.03
Cis-4,7,10,13,16,19-Docosahexaenoic Acid	C22:6n3	5.10	4.84
TOTAL of PUFA		**15.88**	**13.84**
Fatty Acid Total		72.44	56.12
Not detection		27.56	43.88

Total saturated fatty acid (SFA) of fresh eel bone was 24.48%, monounsaturated fatty acid (MUFA) was 32.00%. While, polyunsaturated fatty acid (PUFA) was 15.88% with EPA 1.56% and DHA 5.10%. This result was closed to the study of Widyasari *et al.,* (2013) results showing fresh Indonesian eel consisted of 22.78 % SFA, 32.84% MUFA and 11.4% PUFA with EPA 1.15% and DHA 5.16%.

In the fatty acid composition of Eel bone oil was SFA 19.87%, MUFA was 25.40% and PUFA was 13.84% with EPA 1.57% and DHA at 4.48%. There is a little different with study done by Yan *et al.,* (2011) which declared that eel bone oil contained SFA at 30.02%, MUFA at 55.82% and PUFA 14.18% with EPA 4.7% and DHA 7.23%. Fatty acid composition of eel bone oil is vary depend on nutrition from living enviromental, food intake, and species.

The most abundant saturated fatty acid (SFA) in the both of sample was palmitic acid. This result was appropriated to study of Crexi *et al*., (2010) which explained that palmitic acid was most abundant saturated fatty acid which can reach 50% of total saturated fatty acid. The dominant monounsaturated fatty acid (MUFA) was oleic acid, it was 26.59% for fresh eel bone and 20.94% for eel bone oil. While, the dominant polyunsaturated fatty acid (PUFA) was linoleic acid, it was 6.09% for fresh eel and 4.01% for eel bone oil.

CONCLUSION

Eel by-product are potential to be developed as value added products that can optimum utilization of Indonesian eel (*Anguilla bicolor bicolor*) in processing industry. The total yield of by-product of eel (*Anguilla bicolor bicolor*) reached 26.38%. One of utilization is extraction oil fish from eel bone by-product. The yield of extracted oil resulted from Bligh and Dyer method (1959) was 17.12%. Eel bone oil contained saturated fatty acid (SFA) 19.87%, monounsaturated fatty acid (MUFA) 25.84%, and polyunsaturated fatty acid (PUFA) 13.84%. The major fatty acids in the oil from the bone were palmitic acid (13.58%), oleic acid (20.94%), linoleic acid (4.01%), EPA (1.57%), and DHA (4.84%).

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