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Low Temperature Extraction and Quality of Oil from Spotted Sardinella (*Amblygaster sirm*) and Goldstrip Sardinella (*Sardinella gibbosa*)

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Abstract: Fish belonging to the family Clupeidae have high fatty acid content. This high fatty acid content can be utilized for the fish oil production. Production of fish oil in this study was conducted by wet rendering, Bligh and Dyer and soxhletation method. The results of this study showed that the highest yield of fish oil from some parts of two samples (spotted sardinella and goldsrtrip sardinella) was resulted from viscera. Oil produced by Bligh and Dyer method have yield of fish oil was 0.60-23.44%, soxhletation method was 0.9-39.00% and wet rendering method was 0.00-10.00%. For quality of fish oil, the peroxide value in viscera oil of spotted sardinella and goldstrip sardinella produced from wet rendering method was 15-25 meq/kg, free fatty acid value was 0.80-16.43%, anisidine value was 5.91-33.67 meq/kg and total oxidation was 35.91-73.67 meq/kg. Lipids contained in the viscera oil of goldstrip sardinella were triglycerides and cholesterol and in viscera oil of spotted sardinella were cholesterol, cholesterol esters and fatty acid methyl esters.

Key words: Extraction · Fish Oil · Goldstrip Sardinella · Quality · Spotted Sardinella · Wet Rendering

INTRODUCTION

Fish belonging to the family Clupeidae (herrings, shads, sardines, menhadens) have high fatty acid content [1]. This high fatty acid content can be utilized for the fish oil production. Fish oil is an important source of polyunsaturated fatty acids (PUFA), especially eicosapentanoic acid (EPA, 20:5 ω=3) and docosahexaenoic acid (DHA, 22:6 ω=3) as beneficial for physiological activity. The content of EPA and DHA in fish oil varies from 5-26% and 6-26% of total fatty acids [2]. Polyunsaturated fatty acid (PUFA) is a substance that is essential for maintaining the human health, growth and development [3]. DHA is proven to help the formation of the human retina and brain during development, while the EPA has anti-inflammatory characteristics, reduces disturbance in patients with obesity, helps in the treatment of tumors and disorders due to depression [4]. Study of Ranasinghe and Attygalle [5] about fatty acid composition in the three length classes of whole *Amblygaster sirm* showed that dominant saturated fatty acid (SFA) was palmitic acid, their values were 29.04% for species with body length < 15 cm, 27.32% for species with body length 15-20 cm and 15.37% for species with body length >20 cm. Oleic acid (monounsaturated fatty acid/MUFA) in species with body length <15 cm was 7.45%, species with body length 15-20 cm was 11.6% and species with body length >20 cm was 15.09%. The highest content of PUFA was DHA, which is in length class <15 cm of 24.49%, 15-20 cm of 15% and >20 cm of 6.21%, while the content of EPA was 9.76% in length class <15 cm, 10.33 % in length class at 15-20 cm and 6.27% in length class >20 cm. Study of Nisa and Asadulla [6] reported that in January, total lipid in *S. gibbosa* was 7.5% with the predominant SFA was palmitic acid (24.0%), its predominant MUFA was oleic acid (9.5%) and its predominant PUFA was DHA (19.1%) and EPA (13%). These fatty acids content in the small pelagic fish can be utilized for fish oil production, particularly as a source of omega 3. The production of fish oil is very likely to be conducted and developed. Zuta *et al.* [7] succeeded in extracting fish oil from fish skin of mackerel with a high

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yield amounted to 38%. The quality of the fish oil is also a special concern in fish oil production process. The aims of this study were to produce small pelagic fish oils with various methods of extracting and to analyze the quality of fish oil which was produced.

MATERIALS AND METHODS

Main materials used in this study were spotted sardinella (*Amblygaster sirm*) and goldstrip sardinella (*Sardinella gibbosa*). These materials were obtained from Muara Angke, North Jakarta, Indonesia. Other materials were solvent for extraction (distilled water, methanol, chloroform and hexane)

Production of fish oil was conducted by several methods, namely wet rendering, Bligh and Dyer [8] method and soxhletation. But the main method which its result would be further analyzed to see its quality was wet rendering extraction method, while the method of Bligh and Dyer and soxhletation were conducted as comparison of fish oil yield.

Extraction of wet rendering was conducted in a water bath using different temperature (40, 50 and 60 °C). Each temperature treatment was performed for 30 minutes. Ratio of the sample and distilled water was 1: 1. Then samples were filtered to obtain a liquid fraction. The liquid fraction was then centrifuged at a speed of 10,000 rpm for 20 min at 10 °C to separate water and oil fractions. After that, the samples were stored at -4 °C until it was analyzed.

Some analysis conducted in this study were analysis of free fatty acid level [9], analysis of peroxide value [10], analysis of p-anisidine value [11], determination of the total oxidation/TOTOX [12] and lipid fractionation of fish oil [13]. Analysis data of quality fish oil was statistically analyzed by One Way ANOVA method using SPSS 16.0, then further tested using Duncan's test.

RESULTS AND DISCUSSSION

The Yield of Fish Oil: The percentage yield of oil from spotted sardinella and goldstrip sardinella was obtained by comparing the fish oil produced with the initial weight of each part of fish. The yield of fish oil from some parts of spotted sardinella and goldstrip sardinella can be seen in Table 1.

Based on Table 1, the results showed that the highest fish oil yield was produced by the method of soxhletation and Bligh and Dyer. In wet rendering method, the results of fish oil yield tended to increase with the increasing of extraction temperature. Chantachum *et al*. [14] stated that heating may cause coagulation of proteins which facilitate the separation of the solid fraction and liquid fraction (oil). The results (Table 1) also showed that the highest yield of fish oil from some parts of the two samples (spotted sardinella and goldsrtrip sardinella) was resulted from viscera. In viscera oil of spotted sardinella and goldstrip sardinella, temperature which could produce highest oil yield was 60 °C. Study Khoddami et al. [15] reported that the yield of fish oil in head, intestine and liver of *Sardinella lemuru* was respectively 5.67 %, 5.08 % and 5.80 %.

Peroxide Value: Peroxide value becomes the most important to determine the degree of fat damage. Unsaturated fatty acids are able to bind oxygen and the oxygen molecule can attack the double bond of the chain, then formation of peroxides is occured. Peroxide determination based on the reaction between potassium iodide with peroxide under acidic conditions. Liberated iodine was then titrated with a standard solution of sodium thiosulfate [16]. The peroxide values of fish viscera oil from wet rendering method for spotted sardinella oil and goldstrip sardinella can be seen in Figure 1.

Figure 1 showed the lowest peroxide value of viscera oil was shown in treatment SS60 (viscera oil of spotted sardinella with treatment of 60 °C), GS40 (viscera oil of goldstrip sardinella with treatment of 40 °C) and GS60 (viscera oil of goldstrip sardinella with treatment of 60 °C). Peroxide values in fish oil resulted from all treatment still appropriated to the fish oil standard specified by International Association of Fish Meal and Oil Manufactures, except viscera oil of goldstrip sardinella which was extracted at 50 °C (GS50). IFOMA standard for peroxide value is 3-20 meq/kg [17]. However, these results were not in accordance with the International Fish Oil Standard (IFOS) standards. Standard of peroxide value according to IFOS was 3.75 meq/Kg for maximum limit [18]. High peroxide value indicates the oil has been oxidized, but low value does not necessarily indicate the condition of premature oxidation. The low peroxide value may occur as a result of new peroxide formation rate which is smaller than the rate of degradation into other compounds. There are three different mechanisms that play a role in the formation of the lipid peroxide, i.e. autooxidation by free radical reactions, reactions involving enzymes and photo-oxidation [19]. Aminah [20] stated that there are several factors that can affect the oxidation; they were oxygen presence, high temperature and light exposure.

Table 1:Comparison of fish oil yield

	Spotted sardinella (*Amblygaster sirm*)				Goldstrip sardinella (*Sardinella gibbosa*)			
Methods	Whole (%)	Meat (%)	Viscera (%)	Head (%)	Whole (%)	Meat (%)	Viscera (%)	Head (%)
Soxhletation	3.51	0.90	7.30	2.10	6.86	7.01	39.00	14.76
Bligh and Dyer	0.80	0.60	8.20	3.30	2.00	2.30	23.44	8.20
Wet rendering 40°C	0.20	0.00	5.40	1.5	1.30	0.20	5.00	2.40
Wet rendering 50°C	0.40	0.00	6.10	3.50	1.20	0.00	8.50	1.90
Wet rendering 60°C	0.60	0.00	7.00	6.00	0.00	0.20	10.00	4.70

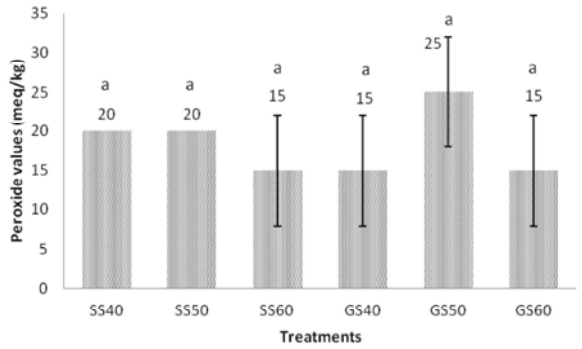

Fig. 1: Peroxide values of viscera oil from spotted sardinella and goldstrip sardinella

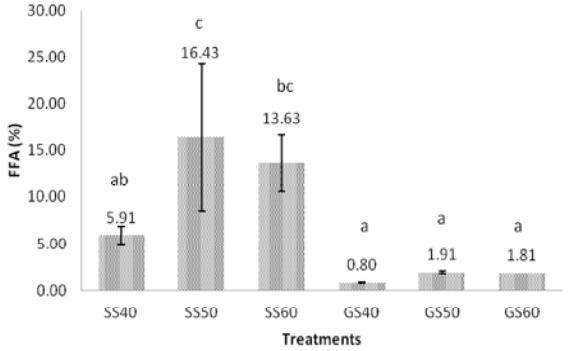

Fig. 2: Free fatty acid values of viscera oil from spotted sardinella and goldstrip sardinella

Note: SS40= viscera oil of spotted sardinella with treatment of 40 °C, SS50= viscera oil of spotted sardinella with treatment of 50 °C, SS60= viscera oil of spotted sardinella with treatment of 60 °C, GS40= viscera oil of goldstrip sardinella with treatment of 40 °C, GS50= viscera oil of goldstrip sardinella with treatment of 50 °C, GS60= viscera oil of goldstrip sardinella with treatment of 60 °C

Note: SS40= viscera oil of spotted sardinella with treatment of 40 °C, SS50= viscera oil of spotted sardinella with treatment of 50 °C, SS60= viscera oil of spotted sardinella with treatment of 60 °C, GS40= viscera oil of goldstrip sardinella with treatment of 40 °C, GS50= viscera oil of goldstrip sardinella with treatment of 50 °C, GS60= viscera oil of goldstrip sardinella with treatment of 60 °C

Free Fatty Acid Value: Free fatty acid analysis is generally performed to measure the rate of hydrolysis of fatty acid esters that has been freed from the glycerides as parent molecule [21]. The presence of free fatty acids indicates the occurrence of hydrolytic rancidity. The results of the free fatty acid analysis can be seen in Figure 2.

Figure 2 showed that the lowest value of free fatty acid was found in a treatment of GS40 (viscera oil of goldstrip sardinella with treatment of 40 °C). In general, the higher cooking temperature, the higher of free fatty acid value resulted. According to Chantachum *et al*. [14], higher temperature allowed the release of free fatty acids volatile components and then followed by the decline in the value of free fatty acids. Oxidation will increase the amount of free fatty acids which is contained in fish oil [22]. Free fatty acid value of fish oils which is suitable for IFOMA standard ranges from 1-7% [17]. The results showed that viscera oil from all treatments has free fatty acid value which is in accordance with the IFOMA standard, except viscera oil with treatment SS50 and SS60. But according to IFOS, viscera oil which met the standard was only viscera oil with treatment of GS40. The amount of free fatty acids for fish oil specified by the International Fish Oil Standards in 2011 must be =1.13% [18].

Anisidine Value: Peroxide in oil that has been oxidized is an intermediate products that will be broken down into a variety of carbonyl and other components. The faster rate of decomposition occurs when the temperature is raised [21]. Anisidine values are values stating the presence of aldehydes as secondary products of primary oxidation products (hydroperoxides). Aldehydes supposedly formed during the process of separation and storage as well as a volatile compound [19]. The results of anisidine value analysis can be seen in Figure 3.

Figure 3 showed that the lowest anisidine value was resulted from a treatment of GS40 (viscera oil of goldstrip sardinella with treatment of 40 °C). Anisidine values contained in viscera oil resulted from all treatments were

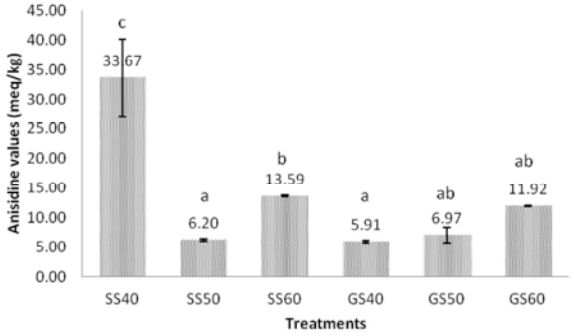

Fig. 3: Anisidine values of viscera oil from spotted sardinella and goldstrip sardinella

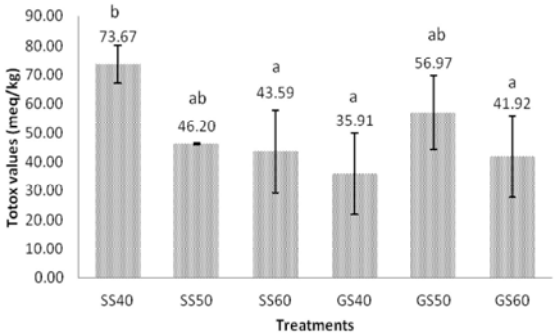

Fig. 4: Total oxidation values of viscera oil from spotted sardinella and goldstrip sardinella

Note: SS40= viscera oil of spotted sardinella with treatment of 40 °C, SS50= viscera oil of spotted sardinella with treatment of 50 °C, SS60= viscera oil of spotted sardinella with treatment of 60 °C, GS40= viscera oil of goldstrip sardinella with treatment of 40 °C, GS50= viscera oil of goldstrip sardinella with treatment of 50 °C, GS60= viscera oil of goldstrip sardinella with treatment of 60 °C

still included to the standard specified by IFOMA, it must be ranged from 4 to 60 meq/kg [17]. Viscera oil from all treatments had anisidine values which were still below the maximum amount of anisidine values set by IFOS standards, except a treatment of SS40. Maximum value for anisidine according to the International Fish Oil Standards (IFOS) is 15 meq/Kg [18]. Aldehydes are formed when a fatty acid of hydroperoxide is degraded, then leaving the non-volatile fatty acids which are still bound at glycerides molecule. Anisidine value examination was performed to measure the degraded fatty acid which is not volatile. The high anisidine values indicated that sample had undergone for further oxidation [19].

Total Oxidation Values (Totox): The total oxidation value overall indicates the oxidation state of the oil, with a maximum rate of 30 [23]. The total oxidation value is obtained by summing twice of peroxide value with the anisidine value. The results of the total oxidation value of spotted sardinella and goldstrip sardinella oil presented in Figure 4.

Figure 4 showed that the lowest value of total oxidation was shown in treatment of GS40 (viscera oil of goldstrip sardinella with treatment of 40 °C). Viscera oil from all treatments had total oxidation value which is in accordance with the IFOMA standard, except viscera oil from treatment of SS40. IFOMA standard assigned total oxidation for fish oil was at range of 10-60 meq/kg [17].

Table 2: Lipid fractionation of fish oil

Sample	Lipid type	Rf	Rf Standard
Viscera oil of goldstrip sardinella	Triglycerides	0.61	0.66
	Cholesterol	0.27	0.29
Viscera oil of spotted sardinella	Cholesterol	0.3	0.29
	Cholesterol esters	0.88	0.97
	Fatty acid methyl ester	0.75	0.84

Lipid Fractionation of Fish Oil: Fish oil was fractionated using thin-layer chromatography (TLC). Thin-layer chromatography is one example of method which can be used for lipid class analysis. The principle in the use of TLC is based on differences in affinity component in the stationary phase and mobile phase [24]. Fractionation performed was fractionation for non-polar lipids by eluent of n-hexane, ether and acetic acid as mobile phase. The results of fractionation can be seen in Table 2.

The result of fractionation showed that non-polar lipids contained in the viscera oil of goldstrip sardinella were triglycerides and cholesterol, while in viscera oil of spotted sardinella were cholesterol, cholesterol esters and fatty acid methyl esters. Study of Chaijan [25] about changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage showed that lipid in fresh sardine muscle were composed by triglyceride and phospholipids as major constituents. Small amount of free fatty acids and diglycerides was noticeable. During iced storage, both triglyceride and phospholipids contents decreased, while the free fatty acid, diglyceride and monoglyceride

contents increased, particularly with the increasing of storage time. This suggested that triglycerides and phospholipids were hydrolyzed into free fatty acid, diglyceride and monoglyceride during extended storage in ice. Components contained in fish oil are glycerides and phospholipids fraction. Other compounds contained in fish oil are hydrocarbons, vitamins, pigments and sterols. Cholesterol is a type of the most abundant sterols in fish oil [26].

CONCLUSION

The highest yield of fish oil from some parts of the two samples (spotted sardinella and goldsrtrip sardinella) was resulted from viscera. In viscera oil of spotted sardinella and goldstrip sardinella, temperature treatment which produced highest oil yield was 60 °C. If it is seen from all parameter of fish oil quality (peroxide value, free fatty acid, anisidine value and total oxidation value), the best quality of viscera oil was resulted from goldstrip sardinella with temperature treatment of 40°C. The result of fractionation showed that non-polar lipids contained in the viscera oil of goldstrip sardinella were triglycerides and cholesterol, while in viscera oil of spotted sardinella were cholesterol, cholesterol esters and fatty acid methyl esters.

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