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Physicochemical Characteristics and Quality Parameters of Alkali-Refined Lemuru Oil from Banyuwangi, Indonesia

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Abstract: Lemuru oil which is a by-product of fish meal industry is potential to be developed in various fields, such as the pharmaceutical, food and feed industry. Characterization of lemuru oil needs to be done to obtain basic information about crude fish oil. This study aimed to determine fatty acid composition, soapstock content, tocopherols, chemical and physical quality parameters, heavy metal and PCB content of alkali-refined lemuru oil. The content of omega-3 in lemuru oil was 29.09%. The content of soapstock and vitamin E in lemuru oil was 20% and 1.742 mg/100 g. Free fatty acid level, acid value, peroxide value, p-anisidine value and the total oxidation of this oil were 1.25%, 2.49 mg KOH/g, 4.40, 31.79 and 40.59 meq/kg, respectively. Viscosity and density value were 51.73 pas (pascal second) and 22.75 g/cm^3. Result of atomic absorption spectrometry (AAS) test of lemuru oil showed that heavy metals and PCB content in alkali-refined lemuru oil were not detected.

Key words: Alkali-refined lemuru oil, characterization, omega-3, quality, soapstock

INTRODUCTION

Fish oil is considered as an easily available and invaluable source of long chain omega-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are recognized to play an essential role in human health and nutrition (Newton, 1996). The chemical structure of fish oil consists of triacylglycerols (TAG) that contain a glycerol molecule esterified with three fatty acids. The fatty acids have an even number of carbon atoms. They are usually unbranched (saturated or unsaturated) (Taylor, 1973). Fish oil is useful for food, health and industry.

Many of the fatty acids can be synthesized by the human body but there is a group of polyunsaturated fatty acids (PUFA), the essential fatty acids, that the human body cannot produce: omega-3 (n-3) and omega-6 (n-6) fatty acids. A major source of omega-3 fatty acids is fish, whether marine or fresh water.

Sardinella lemuru appears to have great potential as a raw material for fish oil and n-3 fatty acid. This particular fish species is apparently found in large numbers in the Bali Strait and Banyuwangi, East Java, Indonesia. The volume of catch was 371,325 tonnes in 2008 and 338,537 tonnes in 2009. Sardine-based oil usually contains approximately 15-20% lipids and a high concentration of n-3 fatty acid. A processing plant in East Java, Indonesia produces about 20,000-30,000 tonnes of crude lemuru oil annually as a by-product from the processing of fish meal (Department of Marine and Fisheries, 2007). This great potential of crude lemuru oil in Indonesia can be developed for various field such as pharmaceutical, food and feed industry. Characterization of crude fish oil needs to be done in order to determine an appropriate utilization in the future. The physicochemical characteristics of crude fish oil as the basic information can help to determine some actions for improving quality of fish oil. This study aimed to determine fatty acid composition, soapstock content, tocopherols, chemical and physical quality parameters, heavy metal and PCB (polychlorinated biphenyls) of alkali-refined lemuru oil.

MATERIALS AND METHODS

Alkali-refined lemuru (*Sardinella lemuru*) oil was the sample that would be analyzed in this study. It was a by-product from fish meal processing industry and it was obtained from a fish meal plant in Banyuwangi, East Java, Indonesia. Some chemicals were used in quality analysis of fish oil such as chloroform, distilled water, isooctane (2,2,4-trimethylpentane), glacial acetic acid, p-anisidine, alcohol, potassium hydroxide, phenolphthalein indicator, sodium thiosulfate, potassium iodide, starch, tocopherol and 0.5% isopropanol, n-hexane and other chemicals used in determination of fatty acid composition and determination of heavy metal and PCB. All chemicals were analytical grade.

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Chemical and physical analysis: The oil was analyzed to determine its physicochemical characteristics and quality parameters. These include the determination of fatty acid composition, soapstock content, tocopherols, chemical and physical quality parameters (such as peroxide value, free fatty acid, acid value, p-anisidine value, total oxidation, viscosity, density and colour), heavy metal and PCB content.

Fatty acid composition of fish oil was determined using gas chromatography, it was according to Ichibara *et al.* (1996). Tocopherol content was determined by high-performance liquid chromatography (HPLC) (IUPAC, 1987) using a Hewlett-Packard 1100 HPLC system. Peroxide value was determined according to AOAC (2000). Free fatty acid was determined using official method Ca 5a-40 (AOCS, 1998) and acid value (AV) was determined using official method AOAC (2000). The p-anisidine value (PAV) was determined by spectrophotometric method described by IUPAC (1998). Total oxidation (TOTOX) was determined according to AOCS (1997). The colour of lemuru oil was determined using a colourimeter based on the method of Sathivel *et al.* (2003). The oil viscosity was measured using Ostwald viscometer, it was according to Joslyn (1950). The density of fish oil was determined according to Nielsen (1998).

Heavy metals parameter, analyzed using atomic absorption spectrophotometer, were lead, arsenic, nickel, cadmium and mercury. Lead was analyzed according to Kendrikse *et al.* (1991). Arsenic was analyzed according to AOAC (2000). Nickel was analyzed according to Kendrikse *et al.* (1988). Cadmium was analyzed according to Lacoste *et al.* (1999). Mercury was analyzed according to AOAC (2000). Polychlorinated biphenyls (PCBs) was analyzed according to Freijer *et al.* (2001).

RESULTS

The fatty acid composition for alkali-refined lemuru oil is shown in Table 1. The sample fractions contained saturated fatty acids (41.43%), monounsaturated fatty acids (15.07%) and polyunsaturated fatty acids (32.43%). The fatty acid composition of lemuru oil was dominated by the saturated fatty acid (SFA), palmitic acid C16:0 (20.25%); the monosaturated fatty acid (MUFA), palmitoleic acid C16:1 (12.02 %) and the polyunsaturated fatty acid (PUFA), Eicosatrienoic acid C20:3 n3 (15.36%).

Referring to the Table 1, alkali-refined *Sardinella lemuru* oil showed the high content of omega-3. The oil was then analyzed to determine its physicochemical characteristics and quality parameters. These include the determination of soapstock content, tocopherols, chemical and physical quality parameters, heavy metal and PCB content, viscosity and density, colour and fatty acid composition.

Alkali-refined lemuru (*Sardinella lemuru*) oil, obtained from a fish meal plant in East Java (Indonesia), was a by-product from fish meal processing. The crude fish oil had 20% soapstock. The crude fish oil had a vitamin E content of 2.6 IU/100 g or 1.742 mg/100 g. Chemical and physical parameters of alkali-refined lemuru can be seen in Table 2. The colour characteristics of lemuru oil were L = 91.43, a = -1.2, b = 49.01, C = 49.03 and H = 91.41. Result of atomic absorption spectrometry (AAS) test of lemuru oil showed that heavy metals and PCB content in alkali-refined lemuru oil were negligible (not detected). Results of its test can be seen in Table 3.

DISCUSSION

Fatty acid compsition: The fatty acid compostion (Table 1) showed that alkali-refined lemuru oil was high in omega-3 content and its value was 29.09%. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are vital nutrients and may be taken to maintain healthy functions of the cardiovascular system, human growth and intellectual development (Pike and Jackson, 2010) but the content of more highly unsaturated polyenoic acids make the fish oil more susceptible to deterioration (Ackman, 1988).

The PUFA/SFA ratio demonstrated that the percentage of saturated fatty acids was higher relative to polyunsaturated fatty acids but the [MUFA+PUFA]/SFA ratio showed that the percentage of the combined mono- and poly-unsaturated fatty acids was higher than that of the saturated fatty acids. The concentration of omega-3 PUFAs differs among species and can be influenced by a number of factors. Lipid levels and fatty acid composition will vary with species, sex, age, season of the year, food availability, salinity and water temperature (Stansby, 1981; Monsen, 1985).

Soapstock: The crude fish oil had 20% soapstock. The soaps are made by the action of sodium or potassium hydroxide on fats with glycerol as a by-product (Uvarov and Chapman, 1979). Soaps formation process is known as saponification (Head *et al.*, 1995). Soapstocks are mainly composed by moisture and solvent, free fatty acids and sterols. They can contain organic phosphates, monoacylglycerols (MAG), diacylglycerols (DAG), triacylglycerols (TAG), polyalcohols, carbohydrates and other minor miscellaneous components. As they are rich sources of free fatty acids, they find useful applications as an animal feed additive. Treating (refining) crude vegetable oils with alkali produces a sodium soap, which is then separated from the oil by centrifugation. Typically, soapstock will account for 5-10% of the crude oil mass, although higher values are possible if the crude oil is "colour set" or has a high concentration of free fatty acids. Soapstock from oilseed refining has been the primary source of fatty acids and glycerol (Dumont and Narine, 2007).

Table 1: Fatty acid composition of alkali-refined lemuru oils from fish meal by products (Area%)

Fatty acid composition (% methyl ester)	Area (%)
C 10:0	0.26±0.01
C 11:0	0.13±0.01
C 12:0	0.33±0.01
C 13:0	0.19±0.02
C 14:0	10.04±0.02
C 15:0	0.73±0.02
C 16:0	20.25±0.03
C 17:0	1.25±0.01
C 18:0	5.48±0.01
C 20:0	1.65±0.03
C 21:0	0.24±0.00
C 22:0	0.38±0.00
C 23:0	0.44±0.01
C 24:0	0.45±0.00
Σ SFA	41.43±0.09
C 14 :1	0.03±0.00
C 15 :1	0.11±0.01
C 16: 1	12.02±0.01
C 17: 1	0.78±0.01
C 18: 1 n9	2.13±0.01
Σ MUFA	15.07±0.02
C 18: 2 n6 cis	0.7±0.01
C 18: 3 n3	2.5±0.03
C 20: 2	1.89±0.01
C 20: 3 n3	15.36±0.01
C 20: 4 n6	0.32±0.01
C 20: 5 n3	7.31±0.00
C 22: 2	0.43±0.00
C 22: 6 n3	3.92±0.01
Σ PUFA	32.43±0.03
PUFA/SFA	0.78±0.01
Σ n6	1.02±0.01
Σ n3	29.09±0.05
n6/n3	0.04±0.00
Unidentified	11.07±0.08

Values are Means ± Standard Deviations of triplicate determinations

Table 2: Oxidation quality, viscosity, density of alkali-refined lemuru oil

Properties	Value	IFOS standards
Peroxide value (meq/kg)	4.40±0.79	Max 5.00
Free fatty acid (% oleic)	1.25±0.07	Max 1.50
Acid Value (mg KOH/g)	2.49±0.07	Max 3.00
Totox (meq/kg)	40.59±0.08	Max 26.0
P-anisidine (meq/kg)	31.79±0.09	Max 20.00
Viscosity (Pa.s)	51.73±0.1	-
Density (g/cm^3)	22.75±0.02	-

Values are means±standard deviations of triplicate determinations

Table 3: Heavy metal and PCB content of alkali-refined lemuru oil

Heavy metal	Concentration (ppm)	CRN standard
Nickel	<0.01 ppm (not detected)	Max 0.1 ppm
Cadmium	<0.01 ppm (not detected)	Max 0.1 ppm
Arsenic	<0.001 ppm (not detected)	Max 0.1 ppm
Lead	<0.01 (not detected)	Max 0.1 ppm
Mercury	<0.001 ppm ((not detected)	Max 0.1 ppm
PCB	<0.05 (not detected)	Max 0.09 ppm

CRN: Council for Responsible Nutrition

Vitamin E (tocopherols): Vitamin E content of this alkali-refined lemuru oil was lower than the values reported for herring (marinated), mackerel (autumn) and shrimp which were 0.43, 1.3 and 0.9 mg/g tissue, respectively. The tocopherol content of foods is influenced by a large number of factors, e.g., seasonal differences and significant losses may occur during processing and storage of foods (Deshpande *et al.*, 1996). The main function of vitamin E is as an antioxidant (Niki, 1996) and it is the principal component of the secondary defence mechanisms against free radical-mediated cellular injuries (Deshpande *et al.*, 1996).

According to Niki (1996), the mechanism of inhibition of oxidation by alpha-tocopherol has been studied extensively. Alpha-tocopherol (TOH) acts as an antioxidant by scavenging lipid peroxyl oxygen radical (LOO●):

$$LOO\bullet + TOH \rightarrow LOOH + TO\bullet$$

It scavenges the peroxyl radical about 10 times faster than the rate at which the lipid reacts with the radical.

Chemical and physical parameters

Peroxide value (PV): Peroxide value for alkali-refined lemuru oil was 4.40 meq/kg. This value is below the IFOS (International Fish Oil Standard) limit of 5 meq/kg. Several factors such as exposure to oxygen, heat and light can accelerate the oxidative processes in oils. It implies on the rancidity of the products (Shahidi and Wanasundara, 1992; Ulu, 2004). Due to its high content of polyunsaturated fatty acids, fish oil is highly susceptible to oxidative spoilage (Huss, 1988) and the rate of fish oil oxidation is significantly higher than that of most other oils.

Free fatty acid (FFA): Unrefined lemuru oil contains non-triglycerides (non-TG), such as free fatty acids (FFA) and oxidized components that reduce its quality. The removal of impurities and non-TG components from alkali-refined lemuru oil is necessary to produce refined oil with a desirable and acceptable shelf life. The content of FFA in fish oil is affected by extraction process parameters such as temperature, oxygen exposure and, chemicals. Free fatty acids are formed in large quantities as a result of hydrolysis, oxidation due to free radical formation and due to cleavage of double bonds during frying (Paul and Mittal, 1997). Table 2 shows that FFA content of alkali-refined lemuru oil was 1.25±0.07 (% oleic acid) and acid value (AV) was 0.67±0.00 (mg KOH/g). The acceptable limit for AV is reported to be <2.25 mg KOH/g oil (IFOS, 2006).

P-anisidine value (PAV): The anisidine value is used to determine the amount of secondary oxidation products (such as alpha and beta-alkenals) that are in a sample due to lipid oxidation. However, the rate of hydroperoxide production does not always coincide with the production of secondary oxidation products (Guillen and Cabo, 2002). PAV of crude lemuru oil was 31.79 meq/kg which is much higher than the limit stated (15 meq/kg) in the IFOS. This is probably due to its high content of polyunsaturated fatty acids, which makes the oil highly susceptible to oxidative spoilage (Huss, 1988). High PAV values may also be a result of the effects of fish oil processing.

Viscosity and density: The viscosity of alkali-refined lemuru oil was 51.73 Pas (pascal second) at 25°C. This value is affected by soapstock and impurities content in the alkali-refined lemuru oil. The lower purity of the fish oils will increase the viscosity value and otherwise. Adsorbent treatment could cause viscosity to decrease (Farag and Basuny, 2009). The density of the lemuru oil was 22.75 g/cm^3. Purification steps will affect the density value. Density value is contributed by impurities in the fish oil.

Colour: The colour characteristics of lemuru oil were L = 91.43, a = -1.2, b = 4 9.01, C = 49.03 and H = 91.41. The alkali-refined lemuru oil was lighter (higher L*) than catfish oil (30.09). It had a negative a* value, indicating a slight greenish colour and a positive b* (yellowish) value. Hue angle values of lemuru oils were higher than 90°. Oils with a hue angle value between 90 and 180° were more greenish-yellow in colour. Commercially refined menhaden oil had the highest hue angle (227.34°). The colour is contributed by pigment content, impurities, oxidation compound and the processing process (Sathivel, 2005).

According to EFSA (2010), colour of refined fish oil should be pale yellow. Final refined sardine oil possessed a brilliant and transparent light yellow colour (20A84.1R). CRN (2006) stated that the colour of fish oils should vary from pale, light yellow to orange.

Heavy metals and PCB: Atomic absorption spectrometry (AAS) test of lemuru oil showed that heavy metals and PCB content in alkali-refined lemuru oil were negligible (not detected). So, there was no treatment requirements to remove heavy metals. According to Maes *et al.* (2005) and Eppe *et al.* (2005), the activated carbon (AC) can be used to reduce polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) and PCB by 90% in cod liver oil. It is well-known that intake of PCB can cause a broad range of toxic effects in the human body, including teratogenicity and carcinogenicity (Barlow, 1992).

Conclusion: The characterization of alkali-refined *Sardinella lemuru* oil showed that it contained a large amount of soapstock and secondary oxidation compounds, which were not complying with International Fish Oil Standard. Free fatty acid level, acid value and peroxide value of alkali-refined lemuru oil were below maximum limit the International Fish Oil Standard. This fish oil was high in omega-3 content. Atomic absorption spectrometry (AAS) test of lemuru oil showed that heavy metals and PCB content in alkali-refined lemuru oil were negligible. High viscosity and density can be affected by high content of impurities in crude fish oil. The quality of the oil can be improved by reducing these undesirable constituents and the use of physical treatments for this purpose.

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