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# Inventory and Characterization of Sardine (*Sardinella* Sp.) Oil from Java Island-Indonesia

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**Abstract:** Parameter of fish oil quality could be observed from an omega-3 fatty acid content, antioxidant activity, Peroxide Value (PV), Free Fatty Acid (FFA) value, total oxidation (totox) value, density and viscosity, level of toxicity and its heavy metals content. This study aimed to determine the quality of sardine oils which was obtained as by product of fish meal and fish canning industry. Sardine A and B had different fatty acid composition. Based on toxicity test, it was known that sardine oil B (572.02 ppm) had higher toxicity than sardine oil A (726.03 ppm). Heavy metal analysis results showed that sardine oil A had Pb concentration at 0.118 ppm, it passed a standard limit (≤0.1 ppm) and it might be harmful for consumption. Peroxides value of sardine oil A and B was 13.33 meq/kg and 5.00 meq/kg, respectively. The highest FFA value was sardine oil B (3.948%) then followed by sardine oil A (0.423%). Value of p-anisidine of sardine oil A and B was 1.09 and 0.88 meq/kg. The highest totox value was sardine oil A (27.76 meq/kg) then followed by sardine oil B (10.88 meq/kg). Viscosity of sample B (270 cPs) was higher than A (69 cPs) and sample B (1.02 g/cm$^3$) was denser than sample A (0.92 g/cm$^3$).

**Keywords:** By products, chemical quality, omega-3, physical quality, sardine oil, *Sardinella* sp

## INTRODUCTION

Omega-3 fatty acids especially EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) can affect human health. The mentioned fatty acids are needed in the development of the brain and the retina (Spector, 1999; Crawford *et al*., 1999), the development of the premature baby brain (Uauy and Hoffman, 2000), prevent variety of cancer disease (Terry, 2003; Hardman, 2004), lowering side effects of chemotherapy on cancer patients (Hardman, 2004), prevent atherosclerosis and give positive impacts on lipid metabolism (Mori *et al*., 1999), lowering the depressive symptoms (Tiemeier, 2003), fix tolerance against glucose on diabetics (Mori *et al*., 1999) and prevent lupus and athritis (Jolly *et al*., 2001).

One of fish species which has a great potential as a source of omega-3 in Indonesia was sardines (*Sardinella* sp.). Sardine is included to high fatty fish with a various fat content. The different of fat content depend on the size, maturity, season and food. Sardine oil which usually found in Indonesia is a byproduct of fish meal and fish canning industry, it has not been optimized as a source of an omega-3 fatty acid. Yunizal (2002) recorded that in 1996 the number of sardine oils as by product from fish processing industry was about 4,300 tons. According to Khoddami *et al*. (2009), mostly sardine oil contained high concentration of omega-3. Characterization of sardine oil is important, because it can be a consideration for further use.

## MATERIALS AND METHODS

**Materials and equipments:** The main material that used in this study was sardine oils from fish meal and fish canning industry in Muncar (East Java) and Pekalongan (Central Java). Other materials which used were 96% ethanol, phenolphthalein indicator, KOH 0.1 N, chloroform, glacial acetic acid, saturated solution of potassium iodida, aquades, 1% starch, $Na_2S_20_3$ 0.1 N, isooctane, p-anisidine reagent, (hexana) and the materials which used for fatty acid composition analysis.

Some equipments whice were used in this study were alumunium foil, stop watch, digital scale, burettes, glasses, UV-Vis spectrophotometer 2500 (LaboMed), gas chromatography GC 2010 plus AFA PC (SHIMADZU), syringe 10 μL, water bath, analytical scale and micro pipette.

**Methods:** Sardine (*Sardinella* sp.) oils as fish meal and fish canning industry by-product from Muncar and Pekalongan were preapred to be analyzed to see its quality. Some analysis which were conducted include fatty acid composition analysis (AOAC, 1999), antioxidant activity analysis (Beirao and Bernardo-Gil, 2005), peroxide value (PV) analysis, Free Fatty Acids (FFA) value analysis (AOAC, 2000 with Method No. 965.33b), p-anisidine value (PAV) analysis (Watson, 1994), total oxidation (totox) value analysis (Perrin,

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1996), density analysis which according to BSN (1992), viscosity analysis (O'Brien *et al*., 2000), toxicity analysis which according to Meyer *et al*. (1982) and heavy metal content (BSN, 2009).

## RESULTS AND DISCUSSION

**Fatty acid composition:** Based on the data obtained, the composition of the fatty acids in sample A was dominated by saturated fatty acids/SFA (28.96%) then followed by polyunsaturated fatty acid/PUFA (26.79%). Fatty acid composition of sample B was similar to the sample A, it was dominated by SFA (31.16%) then followed by PUFA (24.21%). Its monounsaturated fatty acid/MUFA was 15.96%. The fatty acid compositions are difference between one species to another species. The different composition of fatty acid depend on various factors such as temperature, season, place of growing, fish species, age, sex and dietary habits (Saito *et al*., 1997; Bandarra *et al*., 1997; Tanakol *et al*., 1999). Fatty acid composition of sample A and B is shown in Table 1.

Palmitic acid was the highest SFA in sample A (15.90%) and also sample B (16.81%). Both samples containing C16:0, but its content in sample A was fewer compared than sample B. It showed that there was a difference in the ability of the fatty acids biosynthesis and the intake of fatty acids which were consumed by fish (Iverson *et al*., 2002). Oleic acid dominated the MUFA content of sample B (8.61%) and it was about 8.43% in sample A.

Based on Table 1, the percentage of EPA+DHA of sampe A and B was 22.77 and 19.62%, respectively. The content of omega-3 fatty acids in fish are not the result of pure synthesis in fish body, but it can be caused by food intake of fish such phytoplankton, zooplankton, algae, copepods and shellfish which are known contain omega-3 (Ackman, 1980). Environmental parameters also affect the composition of the unsaturated fatty acid compounds (Ould *et al*., 2003). Meanwhile, the content of PUFA on sample a (26.79%) was higher than sample B (24.21%).

**Antioxidant activity:** Rancimat method was used in this study. The principle of this test is the process of oxidation is accelerated by the presence of air flow and heat (temperature was 100°C). Induction time is measured as the time required reaching the end point of oxidation associated with a detectable level of rancidity or sudden changes in the level of oxidation and is usually associated with the shelf life of the product (Pressa-Owens *et al*., 1995). Test results of antioxidant activity can be seen in Table 2.

Based on the data obtained, sample A had shorter induction time (0.18 h) than sample B (4.085 h). The longer induction time indicated that the sample had

Table 1: Fatty acid composition of sardine (*Sardinella* sp.) oil

| | Result (%w/w) | |
|---|---|---|
| Fatty acid | A | B |
| Lauric Acid, C12:0 | 0.090 | 0.120 |
| Myristic Acid, C14:0 | 8.000 | 5.800 |
| Pentadecanoic Acid, C15:0 | 0.570 | 0.900 |
| Palmitic Acid, C16:0 | 15.90 | 16.81 |
| Heptadecanoic Acid, C17:0 | 0.500 | 1.010 |
| Stearic Acid, C18:0 | 3.200 | 5.420 |
| Arachidic Acid, C20:0 | 0.490 | 0.650 |
| Heneicosanoic acid, C21:0 | 0.040 | 0.120 |
| Behenic Acid, C22:0 | 0.130 | 0.250 |
| Tricosanoic Acid, C23:0 | 0.040 | 0.080 |
| ΣSFA | 28.96 | 31.16 |
| Miristoleic Acid, C14:1 | 0.030 | 0.020 |
| Palmitoleic Acid, C16:1 | 8.430 | 6.530 |
| Elaidic Acid, C18:1n9t | 0.090 | 0.150 |
| Oleic Acid, C18:1n9c | 7.570 | 8.610 |
| Cis-11-Eicosenoic Acid C20:1 | 0.360 | 0.370 |
| Erucic Acid, C22:1n9 | 0.080 | 0.070 |
| Acid Nervonat, C24:1 | 0.170 | 0.210 |
| ΣMUFA | 16.73 | 15.96 |
| Linolelaidic Acid, C18:2n9t | 0.040 | 0.050 |
| Linoleic Acid, C18:2n6c | 0.960 | 1.100 |
| Y-Linoleic Acid, C18:3n6 | 0.250 | 0.180 |
| Linoleic Acid, C18:3n3 | 0.530 | 0.720 |
| Cis-11,14-Eicosedienoic Acid C20:2 | 0.120 | 0.230 |
| Cis-8,11,14-Eicosetrienoic Acid, C20:3n6 | 0.190 | 0.180 |
| Cis-11,14,17-Eicosetrienoic Acid C20:3n3 | 0.040 | 0.070 |
| Arachidonic Acid, C20:4n6 | 1.890 | 2.060 |
| Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3 | 14.13 | 8.210 |
| Cis-4,7,10,13,16,19-Docosahexaenoic Acid, C22:6n3 | 8.640 | 11.41 |
| ΣPUFA | 26.79 | 24.21 |
| Unidentified | 27.52 | 28.67 |

Table 2: Antioxidant activity of sardine oil

| Sample | Induction time (h) |
|---|---|
| A | 0.18±0.00 |
| B | 4.08±0.12 |

(A) Sardines Muncar and (B) Sardines Pekalongan

Table 3: Physico-chemical characterteristic of sardine (*Sardinella* sp.) oil

| | | Value | |
|---|---|---|---|
| | Properties | A | B |
| 1 | Free Fatty Acid (% oleic) | 0.4200 | 3.9500 |
| 2 | Peroxide value (meq/kg) | 13.330 | 5.0000 |
| 3 | P-anisidine value (meq/kg) | 1.0900 | 0.8800 |
| 4 | Totox (meq/kg) | 27.760 | 10.880 |
| 5 | Viscosity (cPs) | 69.000 | 270.00 |
| 6 | Density (g/cm³) | 0.9200 | 1.0200 |
| 7 | LC50 (ppm) | 726.03 | 572.02 |

(A) Sardines Muncar and (B) Sardines Pekalongan

better stability, since it can be inferred, the antioxidant activity of sample B was better than sample A. If it is compared to the PUFA content of sardine oils, sample B has a lower PUFA than A, so sample A is potential to be oxidized rapidly.

**Peroxide value:** Result of peroxide value analysis can be seen in Table 3. Peroxide value of sample A and B was 13.33 meq/kg and 5.00 meq/kg. The lower peroxide value means the better the quality of fish oil. Peroxide value in the fish oil should be valued at about ≤5.00 meq/kg (IFOS, 2011). Huss (1998) stated that the content of unsaturated fatty acid compounds in fish oil can stimulate the onset of oxidative damage and the speed of oxidation of fish oil is higher than other types of fat or oil. Based on data, PUFA content of sample A was greater than sample B, it meant that sample A was more labile than B, so it can be oxidized faster and then formed peroxide products. Some factors which can enhance oxidation process are the presence of oxygen, peroxydase enzymes, heat, radiation (light) and the presence of monovalent or bivalent ion (Kusnandar, 2011).

**P-anisidine value:** P-anisidine value indicates the estimation of the further decomposition of fat/oil which is induced by advanced oxidation (Irianto and Giyatmi, 2009). Result of p-anisidine value can be seen in Table 3. The value of anisidine in sample A (1.09 meq/kg) was higher than sample B (0.88 meq/kg). The value of p-anisidine should be lower than 15 meq/kg (IFOS, 2011).

**Free fatty acid:** The formation of free fatty acids occurs due to hydrolysis and oxidation of oil which is caused by the presence of free radicals and parsing double bonds during heating (Paul and Mittal, 1997). Free fatty acid value of samples is shown in Table 3. Free fatty acid value of sample A and B was 0.42 and 3.95%. Free fatty acid value of fish oil should be lower than 1.5% (IFOS, 2011). An indication of the degree of hydrolysis occurs in oil can be determined by free fatty acid content (Berger, 1997). Results showed that the value of free fatty acid in sample A didn't match to standard but the value of free fatty acid in sample B appropriated to standard limit.

**Total oxidation (TOTOX) value:** Total oxidation is the summation between the twice of peroxide value and p-anisidin value.

The totox value in sample A and B was 27.76 meq/kg and 10.88%, respectively. Fish oil should have a totox value which is lower or equal to 20 meq/kg (IFOS, 2011). The total oxidation value can be used to measure the progressivity of deterioration process that occurs in oil and provides information on the formation of primary as well as secondary oxidation products (Hamilton and Rossell, 1986).

**Density:** Density is the amount of a substance on a volume unit. Density of sample B was 1.02 g/cm$^3$ while the density of sample A was 0.92 g/cm$^3$. The higher density value indicated the more components were contained in the sample. Marcia *et al*. (2002) stated that temperature and shear rate will effect to changes in the density and the viscosity of the CPO (Crude Palm Oil). The density of CPO tended to decrease when temperature was increasing. However, changes in density because of temperature changes are relatively smaller compared to its viscosity changes.

**Viscosity:** Matuszek (1997) stated that the force required to start fluid flow on certain speed is related to the fluid viscosity. Based on Table 3, viscosity value of sample B was higher than sample A. The viscosity value of sample B was 270 cPs while viscosity value of sample A was 69 cPs. Similar conditions also occur in the density value of the two samples of fish oil. Gentry (2013) stated that the viscosity of the fluid was caused by changes in temperature; the viscosity of the fluid will decrease with increasing temperature. Sample A was predicted had been exposed to very high temperature during process, so it can affect the lower value of viscosity.

**Toxicity:** Toxicity analysis can be used as an introduction on research that leads to cytotoxic assay (Meyer *et al*., 1982). Parameter used to indicate the presence of a compound which has biological activity that can cause the death of *Artemia salina* (Meyer *et al*., 1982). The result of BSLT (Brine Shrimp Lethal Test) can be seen in Table 3. It was known that sample B was more toxic than B. LC 50 value of sample A and B was 726.03 and 572.02 ppm.

**Heavy metal:** Heavy metals are harmful for body, because it can damage or lose the central nervous system functions, damage composition of blood,

Table 4: Heavy metal content of sardine (*Sardinella* sp.) oil

| | Heavy metal | | | | | |
|---|---|---|---|---|---|---|
| Sample | Cd | Pb | Ni | As | Hg | Standard |
| A | <0.005 | 0.118 | 0.051 | 0.0720 | 0.003 | ≤0.1 ppm |
| B | <0.005 | 0.095 | 0.018 | <0.005 | 0.008 | ≤0.1 ppm |

(A) Sardines Muncar and (B) Sardines Pekalongan

damage the lungs, kidneys and other vital organs (Darmono, 1995). Based on research results, most of heavy metals concentration in fish oil is still lower than standard limit (<0.01 ppm), except the Pb concentration in sample A. So it may be concluded that both of samples were relatively safe for consumption (Table 4).

## CONCLUSION

Sardine oil A and B had different fatty acid composition. Based on toxicity test, it was known that sample B was more toxic than sample A. Heavy metal testing result showed that both of samples were harmless and a safe for consumption because of the heavy metals content was under limit standard (≤0.1 ppm). The highest peroxides can be found in sample A (13.33 meq/kg). The highest FFA value was in sample B (3.95%). The highest p-anisidin value and totox value can be found in sample A (1.093 meq/kg and 27.76 meq/kg). Refining and purification process can be done to improve the quality fish oil, so it can give the value-added to the fish oil as fish meal and fish canning industry by product which has great potential as a source of omega-3.

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