# Characterization of Fish Oil from Mackerel *(Scomber japonicus)* Canning By Product

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**ABSTRACT —** ***Fish oil of mackerel canning by product is a potential source of omega-3 but has not been fully utilized. Mackerel oil had higher levels of EPA and DHA, respectively 7.31% and 19.22%. The value of free fatty acids (FFA) 4%, acid value (AV) 7.95 mg KOH/g, peroxide value (PV) 6.27 meq/kg, anisidine value (AnV) 11.12 meq/kg and total oxidation value (Totox) 23.65 meq/kg. Determination of heavy metals Cd, Pb, Ni, As and Hg obtained results of Ni and Pb levels above the limit of IFOS standard ≤ 0.1 ppm, respectively 0.926 ppm of Ni and 13.52 ppm of Pb.***

**Keywords—** byproduct, DHA, EPA, fish oil, mackerel

## 1. INTRODUCTION

Canning is one of the fisheries industry with the largest production volume after freezing [11]. One of the species used is mackerel which had the volume of imports in 2011 amounted to 93,789,155 kg [16]. The process of fish canning commonly produce solid and liquid (oil mixture in high amount) byproduct. In the stage of pre-cooking, the meat will discharge oil mixed with water. Oil quality result is still a crude oil with feed quality due to the use of high temperatures on pre-cooking process.

Studies on mackerel oil has not been done intensively. In generally, process of canning mackerel and canning sardines side by side with the processing of by products that are in one unit. The resulting oil was labeled sardine oil, whereas larger production volume is mackerel on average up to 20 tons meat/day. According this amount can produce a byproduct of oil canning of 100 kg (0.5% w/w).

Utilization of canning mackerel oil by products potentially be used as a source of omega-3 fatty acids. Fish oil from Scombroidae family, Clupeidae and Salmonidae contains EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are the highest. EPA and DHA are essential dietary for human health, they are defined essential fatty acids that cannot be synthesized by the human body and consequently they must be provided from the diet [21]. Fish oil contains omega-3 higher than of vegetable oils [20]. This research will discuss the potential for fish oil of canning mackerel byproducts as a source of omega-3 fatty acids, which is expected to be used as a supplement or consumption of food additives to reduce dependence on fish oil imports.

## 2. MATERIALS AND METHODS

### 2.1. Material and Equipments

The main materials that used in this research was fish oil of canning mackerel by product from fish oil factory, Pekalongan, Central Java, Indonesia. Other supporting materials were KOH 0.1 N, sodium thiosulfate ($Na_2S_2O_{3)}$ 0.01 N, $CH_3COOH$, clorofoam, potassium iodide (KI), indicators phenolphthalein (indicators pp), starch, trimethylpentane, p-Anisidine solution, 96% ethanol, distilled water, HNO3, HClO4, and the materials used for fatty acids analysis.

The tools used are digital scales, burrets, glass tools, stop watch, aluminium foil, UV-VIS spectrophotometer brand Agilent 8453, water bath, micro pipette, gas chromatography device brand GC2010 plus AFA PC, with field type a cyanopropyl methyl silica (capillary column), Atomic Absorption Spectrometer device brand Analyst 100 Perkin Elmer HGA850.

### 2.2. Method

Mackerel meat nutrient content determined by proximate analysis. Byproduct of oil canning quality were analyzed. Fish oil quality parameters were observed, among others, peroxide value (PV), the value of free fatty acids (FFA), acid value (AV), anisidine value (AnV), the value of total oxidation (totox), fatty acid profile and content of heavy metals. Deuteronomy is done 3 times.

#### 2.2.1. Preparation of Samples

Fish oil of canning mackerel byproduct taken from fish oil factory use a dark airtight containers then stored in a freezer before use. Oil is taken directly from the company by collecting liquid product draining fish canning after pre-cooking stage. Oil is taken from a tank that had been separated from most of the non-oil components.

#### 2.2.2. Analysis of Proximate Composition

Proximat composition was determined by the method [2]. Water content was measured by drying the sample in an oven at a temperature of 105 $^0$C to constant weigh. Fat content measured by Soxhlet method, protein content was measured by the Kjeldahl method using a conversion factor of 6.25. Ash content was measured by ashing in an oven at a temperature of 550 $^0$C for 24 hours.

#### 2.2.3. Analysis of Free Fatty Acid (FFA)

Analysis of free fatty acids [3] with no. method Ca 5a-40 based on a % free oleic acid in the sample. A total of 2 g of oil was dissolved in 25 ml of 96% neutral alcohol (200ml erlenmeyer), heated for 10 minutes, then the mixture spilled indicator PP 2 ml. The mixture is shaken and titrated with 0.1 N KOH until the pink color arises not lost in 10 seconds. The percentage of FFA was calculated by the following equation:

$$\% \text{ FFA} = \frac{V \times N \times 282{,}5}{10\ G}$$

V : Number of KOH titration (ml)
N : Normality of KOH
G : Weight of sample
282.5 : Molecular weight of oleic acid

#### 2.2.4. Analysis of Acid Value (AV)

Determination of acidity was carried out according to the AOCS Official Method Ca 5a-40 [3] by KOH titration the sample, which uses the principle of the necessary amount of KOH (mg) to neutralize 1 g of fat. The equation to obtain the degree of acidity (mg KOH/ g lipid) :

$$\text{Acid Value} = \frac{V \times N \times 56.1}{G}$$

V : Number of KOH titration (ml)
N : Normality of KOH
G : Weight of sample
56.1 : Molecular weight of KOH

#### 2.2.5. Analysis of Peroxide Value (PV)

The peroxide value (PV ) of the oil was carried out according to the AOCS Official Method Cd-8b-90 [3]. 5 g sample included 250 ml erlenmeyer, add 30 ml of acetic acid and chloroform (3:2), then added 0.5 ml of a solution of saturated potassium iodide (KI) with stirring, add 30 ml of distilled water. Further to the titration with 0.01 N sodium thisulfate ($Na_2S_2O_{3)}$ until the solution changes color to yellow, after it was added 0.5 ml of 1% starch indicator solution will change color to blue solution, followed by titration continues to shuffle solution carefully until the blue color of the solution disappeared. Peroxide value calculation is done with the following equation:

$$\text{Peroxide value} = \frac{V \times N \times 1000}{G}$$

V: The number of $Na_2S_2O_3$ titration (ml)
N: Normality $Na_2S_2O_3$ (0.01)
G: Weight of sample (g)

### 2.2.6. Analysis of Anisidine Value (ANV)

The anisidine value (AV ) of the samples was determined according to AOCS official method Cd 18-90 [3].Test 1 solution is created by dissolving 0.5 g of sample into 25 ml of trimethylpentane. Then the solution of compensation made by using trimethyl-pentane. test 2 solution is made by adding 1 ml of p-anisidine (2.5 g/l) into 5 ml of test solution 1 and 5 ml of trimethylpentane solution as a reference solution, then shaken and averted from the light. Then the solution was measured at 350 nm absorbance value of exactly 10 minutes after the solution was prepared.

Anisidine value determined by the following equation:

$$\text{Anisidine Value} = \frac{25 \times (1.2\ A2 - A1)}{G}$$

A1 : Absorbance of test solution 1
A2 : The absorbance of the test solution 2
G : Weight of sample (g)

### *2.2.7. Total Oxidation (Totox)*

Determination of total oxidation (Totox) according to AOCS official method Cd 18-90 [3] performed with the following equation:

Total Oxidation = (2PV + ANV)

### 2.2.8. Analysis of fatty acid profiles

Analysis of fatty acid methyl esters (FAME) using AOCS Official Method Ce 2-66 [3] with the injection of the gas chromatography under the following conditions: type of gas chromatography instrument used was a Shimadzu GC 2010 Plus. The gas is used as mobile phase is nitrogen gas with a flow rate of 30 mL / min and a gas burner is hydrogen and oxygen, the column used was Capilary brand Quadrex column with an inner diameter of 0.25 mm.

### 2.2.9. Analysis of heavy metals Cd, Pb, Hg, Ni and As

Heavy metals analysis was performed by [4] using 1 g of sample, then put in a 100 ml flask destruction, add 15 ml of concentrated $HNO_3$ and 5 ml of $HClO_4$, then allowed to stand 24 hours. Next destruction to clear samples, cooled and added 10-20 ml of deionized water, made warming ± 10 minutes, removed, and chill. The solution was transferred into a 100 ml measures flask (flask destruction rinsed with deionized water and put into measures flask). Solution was added water to the extent of calibration marks. Then shaken and filtered with Whatman filter paper No. 4. Samples were prepared and analyzed in accordance with the testing of heavy metals (Cd, Pb, Hg, Ni, As) in water analysis (APHA 3110 for the metals Cd, Pb, and Ni; method 3114 for As, and method 3112 for Hg).

## 3. RESULTS AND DISCUSSION

### 3.1. Proximate Analysis

Proximate analysis was conducted to determine nutrient content and chemical composition of a substance, including water content, fat, protein, ash and carbohydrates from the sample. The results of the determination of nutrient content of fish meat mackerel *(Scomber japonicus)* are presented in Table 1.

**Table 1**: Percentage of the nutritional content of mackerel meat

| Composition | (%) |
|---|---|
| Water | 67.32 ± 1.02 |
| Ash | 1.69 ± 0.16 |
| Fat | 9.93 ± 0.77 |
| Proteins | 20.59 ± 1.20 |
| Carbohydrate | 0.47 ± 0.29 |

The measurement results showed that the water, fat, protein and ash content of mackerel meat has a high fat and protein content, which fat content of 9.93% and protein content of 20.59%, this is according to research conducted by [17] which states that the mackerel meat fat content 9.04% and according to [7] chub mackerel protein content is between 20.61 to 22.16%.

Water and ash content respectively 67.32% and 1.69%, according to research [17] who measured the water content of chub mackerel (*S. japonicus*) of 67.71% and the ash content by 1.31 to 1.77%

Results calculated by the method of carbohydrate content by difference suggests that own a mackerel fish meat carbohydrate content of 0.49%. The results of calculations with carbohydrates by difference method is a method of determining the levels of carbohydrates in food are rude, crude fiber which is also counted as carbohydrates.

Mackerel meat proximate levels is strongly influenced by the environment and habitat residence of the fish [5, 6, 7, 17], age, ration level and growth of the species also affect the levels of proximate fish [13, 15, 18], [10] adds that the species-specific differences in the location and arrest also affect the proximate content of mackerel meat.

### 3.2. Free Fatty Acid

Free fatty acid levels of fish oil canning mackerel by product in value by 4%. This value according to the standard levels of free fatty acids of fish oil based IFOMA 1-7, but exceeds IFOS standard (≤ 1.13). This is because the raw materials used fish oil derived from the draining fish canning meat is heated at a temperature below 100 °C, and was taken immediately after the canning process.

**Table 2**: Observations of mackerel oil oxidation parameters

| Parameter | Result | Standard | |
|---|---|---|---|
| | | IFOS [14] | IFOMA [8] |
| FFA | 4.00 ± 0.45 | ≤ 1.13 | 1-7 |
| AV | 7.95 ± 0.61 | ≤ 2.25 | - |
| PV | 6.27 ± 1.73 | ≤ 3.75 | 3-20 |
| AnV | 11.12 ± 0.90 | ≤ 15 | 4-60 |
| Totox | 23.65 ± 1.42 | ≤ 20 | 10-60 |

### 3.3. Acid Value

The degree of acidity is closely related to the value of free fatty acids (FFA). Acidity values obtained using the principle of the necessary amount of KOH (mg) to neutralize 1 g of fat or can be obtained by multiplying a constant value of 1.99 with free fatty acids (FFA). Acidity values obtained from this study of 7.95 mg KOH/g This result is higher than IFOS standards that states must have oil consumption decent acidity value below 2.25 mg KOH/g.

### 3.4. Peroxide Value

Peroxide analysis was conducted to determine the level of damage to the oil. Peroxide showed a new level of oxidation that occurs as the primary oxidation products. Peroxide mackerel fish oil in this study was 6.27 meq / kg. Peroxide value of mackerel fish oil standards based IFOMA [8], amounting to 3-20 meq / kg, but not for IFOS standard is ≤ 3.75. Oxidation values are very important as an indicator of oil quality, the lower the value of primary and secondary oxidation, the quality of oil produced, the better. Indications are the primary oxidation peroxide values important to know the quality of the oil as the first stage of formation of hydroperoxide oxidation is generally measured as peroxide. Peroxide value is highly dependent on the temperature of extraction [1].

### 3.5. Anisidine Value

Anisidine value is a parameter oxidation of fat that measures the oxidation secondary products that are non-volatile. Based on the research results, the anisidin value of fish oil from mackerel canning byproducts is 11.12 meq/kg. These results match the quality of the fish oil based IFOMA standard that is 4-60 and IFOS ≤ 15 meq/kg. According [12], the anisidine value is a measure of carbonyl compounds resulting from decomposition of the hydroperoxides that have medium molecular weight and are less volatile. Carbonyl compounds in oxidized lipids are the secondary oxidation products.

### 3.6. Total Oxidation (Totox)

Totox value is the total number of primary and secondary oxidation with obtained summing twice the peroxide values and anisidine values [19]. Totox value of fish oil from mackerel canning byproduct in this study is 23.65, still makes standard fish oil based IFOMA is 10-60.

### 3.7. Fatty Acid Profile

Fatty acid profile analysis is intended to determine the fatty acid composition of fish oil from mackerel canning byproduct. Based on the analysis of fatty acid profile (Table 3) shows that the mackerel fish oil containing saturated fatty acids (SAFA) was 27.15% higher than monounsaturated fatty acids (MUFA) was 15.54%, while PUFA was 30.99 % DHA dominated by 19.22%. These results are consistent research [6, 17] that the meat chub mackerel (*Scomber japonicus*) has a dominant unsaturated fatty acids, namely DHA with a value of 13.61 to 27.58%.

Unsaturated fatty acids in fish oil is the largest component in fish oil. Component fatty acids contained in the oil can be determined through analysis of Gas Chromatography (GC). The amount of unsaturated fatty acids in fish oil are high, amounting to 46.51% of the total components. Unsaturated fatty acids with many double bonds (PUFAs), namely omega-3 fatty acids (27.44%) and EPA (7.31%) and DHA (19.22%); omega-6 (3.19%); and omega-9 fatty acids (10.19%).

**Table 3**: Fatty acid composition of mackerel oil

| Fatty Acid | Result (% w / w) |
|---|---|
| Lauric Acid (C12: 0) | 0.07 |
| Tridecanoid Acid (C13: 0) | 0.08 |
| Myristic Acid (C14: 0) | 3.44 |
| Pentadecanoic Acid (C15: 0) | 0.94 |
| Palmitic Acid (C16: 0) | 16.34 |
| Heptadecanoic Acid (C17: 0) | 0.93 |
| Stearic Acid (C18: 0) | 4.24 |
| Arachidic Acid (C20: 0) | 0.60 |
| Heneicosanoic Acid (C21: 0) | 0.11 |
| Behenic Acid (C22: 0) | 0.23 |
| Lignoceric Acid (C24: 0) | 0.17 |
| **Total SAFA** | **27.15** |
| Myristoleic Acid (C14: 1) | 0.02 |
| Palmitoleic Acid (C16: 1) | 3.49 |
| Elaidic Acid (C18: 1n9t) | 0.12 |
| Oleic Acid (C18: 1n9c) | 10.19 |
| Eicosenoic Acid (C20: 1) | 1.00 |
| Erucic Acid (C22: 1n9) | 0.33 |
| Nervonic Acid (C24: 1) | 0.39 |
| **Total MUFA** | **15.54** |
| Linoleic Acid (C18: 2n6c) | 1.18 |
| Linolelaidic Acid (C18: 2n9t) | 0.06 |
| Linolenic Acid (C18: 3n3) | 0.91 |
| γ-Linolenic Acid (C18: 3n6) | 0.13 |
| Eicosedienoic Acid (C20: 2) | 0.25 |
| Eicosetrienoic Acid (C20: 3n6) | 0.11 |
| Arachidonic Acid (C20: 4n6) | 1.77 |
| Eicosapentaenoic Acid (C20: 5n3) | 7.31 |
| Docosadienoic Acid (C22: 2) | 0.03 |
| Docosahexaenoic Acid (C22: 6n3) | 19.22 |
| **Total PUFA** | **30.97** |
| **Total Fatty Acid** | **73.66** |

### 3.8. Analysis of heavy metals

The results of the determination of heavy metal residue of fish oil mackerel canning byproduct showed most of heavy metals contained is still within the threshold set [4] except for Pb content. The high content of lead (Pb) in the mackerel oil, thought to be caused by contamination during the canning process in which steam and water and used cans can cause a high content of Pb in the oil produced. According to [9], heavy metal contamination in canned products influenced the process water contamination, fish contamination during handling and process, [22] adds that quality lacquer coating cans also affect the level of heavy metals in canned products.

**Table 4**: Heavy metal residue of mackerel oil

| Heavy Metals | Result (mg / l) | Standard [4] | IFOS Standard |
|---|---|---|---|
| Pb | 13.52 | 1 | ≤ 0.1 |
| Cd | 0.005 | 0.5 | ≤ 0.1 |
| Hg | 0.001 | 1 | ≤ 0.1 |
| Ni | 0.926 | 1 | ≤ 0.1 |
| As | 0.002 | 1 | ≤ 0.1 |

Characteristics of mackerel raw material used can also affect the high content of heavy metals depending on the fishing area, season, feed, water temperature and salinity [5].

The results of this study showed levels of heavy metals cadmium (Cd), mercury (Hg) and arsenic (As) is still below the threshold set by [4] and IFOS [14]. Cd can accumulate in the human body and trigger heart problems, bone damage and reproductive disorders. Mercury can affect changes in normal brain development in infants and in high amounts can interfere with the development of adult nerve. Arsenic can increase the incidence of cancer through food consumed.

Heavy metals nickel in mackerel oil is 0.926 mg/l. This result is in accordance SNI but exceeds the threshold limit for IFOS standards. Variations of heavy metals in aquatic products influenced by the results of the habitat, place of capture and season [5, 7].

## 4. CONCLUSIONS

Fish oil of mackerel canning by product have potential as a source of omega-3 with EPA and DHA levels, respectively 7.31% and 19.22%. Observations indicate that the quality parameters of the oxidation of mackerel oil still exceeds IFOS standards that need to be purified and further processing to be used as food additives source of omega 3.

## 5. REFERENCES

[1] Aidos I., Jacobsen C., Jensen B., Luten J.B., Van Der Padt A., Boom R.M., "Volatile Oxidation Products Formed in Crude Oil Herring Under Accelerated Oxidation Conditions", Journal of Lipid Science Technology 4: 148-161, 2002.
[2] [AOAC] Association of Official Analytical Chemist, Official Method of Analysis of the Association of Official Analytical Chemist, Association of Analytical Chemist, Inc., Virginia, USA, 2005.
[3] [AOCS] American Oil Chemists' Society, Official Methods and Recommended Practices of the American Oil Chemists' Society, 5 th ed., AOCS Press, Champaign, 1998.
[4] [BSN] National Standardization Organization, SNI No 7387:2009, Maximum Limit of Heavy Metal Impurities in Food, Jakarta, 2009.
[5] Bae J.H., Yoon S.H., Lim S.Y., "Heavy Metal Contents and Chemical Compositions of the Atlantic (*Scomber scombrus*), Blue (*Scomber australasicus*), and Chub (*Scomber japonicus*) Mackerel Muscles", Food Sci. Biotechnol. 20:709-714, 2011.
[6] Bae J.H., Lim S.Y., "Effects of Season on the Heavy Metal Contents and Chemical Compositions of Chub Mackerel (*Scomber japonicus*) Muscle", J. Food Sci; 77: T52-T57, 2012.
[7] Bae J.H. Lim S.Y., "Comparative Study of the Concentration of Mercury and Lead and the Chemical Characteristics of Japanese and Korean Chub Mackerel (*Scomber japonicus* Houttuyn, 1782) in the East China Sea", Afr. J. Agric. Res. Vol. 8 (3), pp. 269-273, 2013.
[8] Bimbo A.P., Guidelines for Characterising Food Grade Fish Oil Volume 9. No. 5, International Fishmeal and Oil Manufacturers Association, 1998.
[9] Boadi N.O., Twumasi S.K., Badu M., Osei I., "Heavy Metal Contamination in Canned Fish Marketed in Ghana", Am. J. Sci. Ind. Res., 2 (6): 877-882, 2011.
[10] Boran G., Karacam H., "Seasonal Changes in Proximate Composition of Some Fish Species from the Black Sea", Turkish Journal of Fisheries and Aquatic Sciences 11: 01-05, 2011.
[11] [P2HP] Directorate General of Fishery Product Processing and Marketing, Data Calculation Large Industrial Production Volume, Jakarta, 2012.
[12] Gunstone F.D., Vegetable Oils in Food Technology: Composition, Properties and Uses. Blackwell Publishing Ltd., CRC Press, Oxford, UK, PP. 352. 2002.
[13] Huynh M., Kitts D.D., Hu C., Trites A.W., "Comparison of Fatty Acid Profiles of Spawning and Non-spawning Pacific Herring,*Clupea harengus pallasi*", Comparative Biochemistry and Physiology Part B, 146, 504–511, 2007.
[14] [IFOS] International Fish Oils Standard. Fish Oil Purity Standards. http://www.omegavia.com/best-fish-oil-supplement-3/ [April 2nd 2014], 2011.
[15] Kiessling A., Pickova J., Eales J.G., Dosanjh B., Higgs D., "Age, Ration Level, and Exercise Affect the Fatty Acid Profile of Chinook Salmon (*Oncorhynchus tshawytscha*) Muscle Differently", Aquaculture, 243, 345–356, 2005.
[16] [KKP] Ministry of Marine Affairs and Fisheries, Import Statistic of Fishery Product 2011, Jakarta, 2012.
[17] Lim S.Y., "Effects of Catch Areas on the Chemical Composition and Concentration of Heavy Metals Chub Mackerel (*Scomber japonicus*)', Life Science Journal; 9 (3), 2012.
[18] Nakamura Y., Ando M., Seoka M., Kawasaki K., Tsukamasa Y., "Changes of Proximate and Fatty Acid Compositions of the Dorsal and Ventral Ordinary Muscles of the Full-Cycle Cultured Pacific Bluefin Tuna *Thunnus orientalis* with the Growth", Food Chemistry, 103, 234–241, 2007.
[19] Perrin J.L., Determination of Alteration. In: Karleskind A, Wolff JP. (Eds.) Oils and Fats, Manual vol. 2. Lavoisier Publishing, Paris (France), 1996.

[20] Rodriguez N.R., Beltran S., Jaime I., Sara M., Sanz M.T., Carballido J.R., "Production of Omega-3 Polyunsaturated Fatty Acid Concentrates", Journal of Innovation Food Science and Emerging Technologies 11 (1): 1-12, 2010.
[21] Russo G.L., "Dietary n-6 and n-3 Polyunsaturated Fatty Acids: From Biochemistry to Clinical Implications in Cardiovascular Prevention", Biochemical Pharmacology, 77. pp.937– 946, 2009.
[22] Taha 'n J.E., Sanchez J.M., Granadillo V.A., Cubillan H.S., Romero R.A., "Concentration of Total Al, Cr, Cu, Fe, Hg, Na, Pb, and Zn in Commercial Canned Seafood Determined by Atomic Spectrometric Means After Mineralization by Microwave Heating", Journal of Agricultural and Food Chemistry 43, 910-915, 1995.