PRELIMINARY STUDY OF FISH OIL FROM MILKFISH SATAY BY PRODUCT USING DRY RENDERING EXTRACTION

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ABSTRACT

Milkfish satay processing has been left viscera waste that may causes environmental pollution. The viscera waste has contained omega-3 which can be extracted as fish oil. Dry rendering is a method of fish oil extracting using temperature without water addition. The temperature of extraction greatly affects to quality of fish oil. The purpose of this study was to determine the optimal temperature of extraction and characterized fish oil quality extracted from milkfish viscera. This study used dry rendering extraction method with three different temperatures (40ºC, 50ºC, and 60ºC) and tested the yield, free fatty acids, peroxide value, p-anisidine and total oxidation, for the best fish oil will be tested for its fatty acid profile. The best treatment for extracting fish oil from milkfish viscera used extraction temperature of 50ºC with yield (6.88%), free fatty acid (4.89%) peroxide value (29.35 mEq/kg), anisidine value (4.61 mEq/kg), and total oxidation (63.53 mEq/kg). The fatty acid profile of fish oil was dominated by palmitic acid (31.17%) and also contains omega-3 such as linoleic acid, docosahexaenoate acid (DHA), and eicosapentaenoate acid (EPA).

Keywords: dry rendering extraction, milkfish visceral, temperature

INTRODUCTION

Serang City, Banten is one of the centers for milkfish product processing with milkfish raw material that needs 133.14 tons/year (DKP 2017). One of the popular products is milkfish satay. The milkfish satay processing produced viscera waste. According to DKP (2017) the amount of viscera waste that produced in milkfish satay processing in Serang were of 49.6 kg/day.

Milkfish satay processing waste has not used optimally, if not managed properly will have a negative impact on the environment. The most effective and efficient alternative to solve this problem is optimizing the utilization of viscera waste to be fish oil. Suseno (2014) viscera of fish contain omega-3 such as Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA).

Fish oil is a fat component in fish body tissue and produced by extraction, one of the fish oil extraction methods is the dry rendering method which uses temperature treatment without the addition of water (Estiasih 2009). According to Putra (2018), temperature greatly affects the fish oil extraction process and the use of high temperatures (>70ºC) can cause quality degradation that affects the quality of fish oil that does not comply with the International Fish Oil Standard (IFOS (2011). The purpose of this study was to determine the optimal temperature and characterize the quality of fish oil extraction from milkfish viscera.
MATERIALS AND METHODS

**Materials**

Viscera of milkfish collected from the processing industry of milkfish satay, located at Serang, Banten. The chemical material used in this study are ethanol 96%, n-heksan (Merck), phenolphthalein indicator, aquades, NaOH (Merck) 0.1 N, chloroform (Merck), acetic acid glacial (Merck), anisidine reagent (Aldrich chemistry), Bromine Cresol Green-Methyl Red indicator, KOH (Merck) 0.1 N, KI, starch 1%. The tools used in this study are oven, sieve, blender, knife, measuring cup, tray, aluminun foil and others.

**Sample Preparation**

The viscera of milkfish were taken from the milkfish satay processing industry. The viscera transported to the laboratory by being put into a cool box with the addition of ice (1:1 w/w). The sample washed with water and crushed using blender, then weighed.

**Extraction Methods**

This extraction used dry rendering extraction method according to Rozi et al. (2016). Viscera milk fish was heated in an oven with a temperature variation of 40°C, 50°C and 60°C for 8 hours and then pressed for collected the fish oil. The extract of fish oil was stored in the refrigerator for 5 minutes at 15 °C, then the fish oil was centrifuged at 4.000 rpm for 15 minutes. After that, the fish oil was weighed and put into a dark glass bottle that had been coated with aluminum foil, then stored at low temperature. The optimum extraction temperature was determined from the results of the yield test, peroxide value (PV), free fatty acid (FFA), anisidine value (p-AV) and total oxidation. The best temperature treatment would be characterized by the fatty acid profile.

**Determination of Yield**

The fish oil yield was calculated based on the following equation:

\[
\% \text{ Yield} = \frac{\text{Fish oil weight (g)}}{\text{Viscera weight (g)}} \times 100\%
\]

**Determination of Peroxide Value (BSN, 2018)**

Peroxide value was calculated based on the following equation:

\[
\text{Peroxide value (mEq/kg)} = \frac{S \times M \times 1000}{\text{Sample weight (g)}}
\]

where:
- \( S \) = Amount of sodium thiosulfate (mL)
- \( M \) = Sodium thiosulfate concentration (0.01 N)

**Determination of Free Fatty Acid (AOAC, 2005)**

Free fatty acid was calculated based on the following equation:

\[
\% \text{ Free fatty acid} = \frac{A \times N \times M}{10G}
\]

where:
- \( A \) = Amount of KOH titration (mL)
- \( N \) = Normality of KOH (0.1 N)
- \( G \) = Sample weight (g)
- \( M \) = Dominant fatty acid molecular weight

**Determination of p-anisidine value (Watson, 1994)**

Determination of anisidine value was calculated based on the following equation:

\[
\text{Anisidine value (mEq/kg)} = \frac{25 \times (1.2 A2 - A1)}{\text{weight of sample}}
\]

where:
- \( A1 \) = Absorbance of test solution 1
- \( A2 \) = Absorbance of test solution 2

**Determination of Total Oxidation Value (AOCS, 1998)**

The total oxidation value was obtained by adding twice the peroxide number plus the anisidine value. The total oxidation value is calculated based on the following equation:

\[
\text{Total oxidation} = 2PV + p-AV
\]

where:
- \( PV \) = Peroxide value (mEq/kg)
- \( p-AV \) = Anisidine value (mEq/kg)
Determination of Fatty Acid profile (AOAC, 2005)

Analysis of the fatty acid profile using a gas chromatograph. This tool uses the principle of converting fatty acids into methyl esters which are more volatile. The transformation of this research sample was carried out through methylation to obtain Fatty Acid Methyl Ester (FAME) which was then analyzed by gas chromatography.

Data Analysis

The experiment was run in triplicate with a completely randomized design. Fish oil data from dry rendering extraction (yield, PV, FFA, p-anisidine, total oxidation value) were subjected to analysis of variance (ANOVA). The differences among the data were determined using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Yield

The results showed that the difference in extraction temperature had a significant effect (p<0.05) on the percentage of fish oil yield (Table 1). The higher of extraction temperature would increase the yield of fish oil produced. This is presumably because the temperature in the heating process affects the protein denaturation of the raw material. According to Nugroho et al. (2014) high temperature heating can damage protein and cell membranes, making it easier for deposited fat to come out more easily. According to Triyono (2010) protein denaturation will occur in the heating process at a temperature of 50°C-80°C and every 10°C increase in temperature the protein denaturation rate can reach 600 times, so the higher the temperature in the extraction process the greater the yield of oil produced.

Free Fatty Acid Value (FFA)

The results showed that the extraction temperature had a significant effect (p<0.05) on the value of free fatty acids in the fish oil produced (Table 2). The value of free fatty acids of fish oil in this study does not meet the standard of free fatty acids in consumption fish oil set by IFOS (2011), which is <1.13%. Free fatty acids are produced due to hydrolysis of triglycerides so that fatty acids are released from bonds with glycerol and also caused by splitting and oxidation of fatty acid double bonds (Crexi et al. 2010; Deepika et al. 2014). Increased hydrolysis can increase the potential for damage oil so that the oil smells rancid (Kamini et al. 2016).

| Table 1. Yield of viscera fish oil |
|-----------------------------------|
| **Temperature (°C)** | **Yield (%)** |
| 40 | 2.88 ± 0.35<sup>a</sup> |
| 50 | 6.88 ± 0.32<sup>b</sup> |
| 60 | 7.54 ± 0.81<sup>b</sup> |

*Different superscript letters in the same column indicate a significant difference (p<0.05)

| Table 2. Free fatty acid value of viscera fish oil |
|-----------------------------------------------|
| **Temperature (°C)** | **Free Fatty Acid Value (FFA) (%)** |
| 40 | 10.47 ± 0.36<sup>c</sup> |
| 50 | 4.89 ± 0.36<sup>a</sup> |
| 60 | 5.86 ± 0.18<sup>b</sup> |

*Different superscript letters in the same column indicate a significant difference (p<0.05)

| Table 3. Peroxide value of vicera fish oil |
|-------------------------------------------|
| **Temperature (°C)** | **Peroxide Value (mEq/kg)** |
| 40 | 25.58 ± 0.26<sup>a</sup> |
| 50 | 29.35 ± 0.76<sup>b</sup> |
| 60 | 46.50 ± 0.88<sup>c</sup> |

*Different superscript letters in the same column indicate a significant difference (p<0.05)

Peroxide Value

The results showed that the extraction temperature had a significant effect (p<0.05) on the the peroxide value of the fish oil produced (Table 3). The higher of extraction temperature will affect increasing peroxide...
value. The fish oil peroxide value from milkfish satay waste does not meet the consumption fish oil standard set by IFOS (2011), which is 3.75 mEq/kg.

According to Aidos et al. (2002) and Suseno et al. (2015) temperature is one of the supporting factors that can accelerate the oxidation process in fish oil. The value of peroxide is very dependent on the extraction temperature, the higher the temperature used, the faster the oxidation process occurs. The higher peroxide value indicates the level of damage to fish oil. Peroxide levels are closely related to the quantity of hydroperoxides. Hydroperoxides can occur due to the presence of double bonds (unsaturated fatty acids) in the oil that binds oxygen from the surrounding air. Other factors that affect the peroxide value are the type and freshness of the raw materials used, the length of storage and the extraction method used.

Table 4. Anisidine value of viscera fish oil

| Temperature (°C) | Anisidine Value (mEq/kg) |
|------------------|--------------------------|
| 40               | 10.67 ± 0.87b            |
| 50               | 4.61 ± 0.04a             |
| 60               | 13.68 ± 0.94c            |

*Different superscript letters in the same column indicate a significant difference (p<0.05)

Table 5. Total oxidation value of fish oil

| Temperature (°C) | Total oxidation value (mEq/kg) |
|------------------|-------------------------------|
| 40               | 61.90 ± 0.48a                 |
| 50               | 63.53 ± 0.96b                 |
| 60               | 107.36 ± 0.83c                |

*Different superscript letters in the same column indicate a significant difference (p<0.05)

Anisidine Value

The results showed that temperature had a significant effect (p<0.05) on the anisidine value (Table 4). The lowest anisidine value was obtained at the extraction temperature of 50 oC. The anisidine value in all fish oil produced in this study met the IFOS (2011) standard, which is 15 mEq/kg. The anisidine value is an indicator of secondary oxidation, so the higher the peroxide value produced from the primary oxidation process, the faster it decomposes into secondary oxidation products. (Panagan et al. 2012, Deepika et al. 2014).

Total Oxidation Value

The results showed that the extraction temperature had a significant effect (p<0.05) on the total oxidation value of fish oil (Table 5). The higher of extraction temperature causes increasing of total oxidation value. The total oxidation value in this study did not meet the consumption fish oil standard set by IFOS (2011), which is 20 mEq/kg. According to Kamini et al. (2016), the total oxidation value was used to estimate lipid oxidative damage. The total oxidation value is influenced by the primary oxidation value (peroxide number) and secondary oxidation value (anisidine). The higher of peroxide value and anisidine value causes increasing of total oxidation value.

Fatty Acid Profile

The fish oil selected for the fatty acid profile test was fish oil extracted using a temperature of 50 oC. This selection was based on the fact that extraction at 50 oC produced the best yield, free fatty acids and anisidine values. The fatty acid profile of viscera fish oil showed in Table 6. The fatty acid composition of milkfish viscera fish oil consists of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The fatty acid composition of viscera fish oil was dominated by SFA>MUFA>PUFA. SFA are dominated by palmitic acid with the highest percentage of 31.17%. Estiasih (2009) stated that palmitic acid is one of the two dominant fatty acids and is most easily obtained in fish bodies with high enough levels, exceeding 30% or more in all types of fish.
Table 6. Fatty acid profile of viscera fish oil

| Fatty acid                                      | Percentage (%) |
|------------------------------------------------|----------------|
| Capric Acid, C10:0                             | 0.04           |
| Lauric Acid, C12:0                             | 1.60           |
| Myristic Acid, C14:0                           | 2.55           |
| Pentadecanoic Acid, C15:0                      | 0.27           |
| Palmitic Acid, C16:0                           | 31.17          |
| Heptadecanoic acid, C17:0                      | 0.20           |
| Stearic Acid, C18:0                            | 6.59           |
| Arachidic Acid, C20:0                          | 0.20           |
| Heneichoclyc Acid, C21:0                       | 0.02           |
| Behenic Acid, C22:0                            | 0.08           |
| Tricosanoic Acid, C23:0                        | 0.02           |
| **Total SFA**                                  | **42.74**      |
| Myristolic Acid, C14:1                         | 0.05           |
| Palmitoleic Acid, C16:1                        | 5.42           |
| Cis-10-Heptadecanoic Acid, C17:1               | 0.23           |
| Elaidic Acid, C18:1n9t                         | 1.14           |
| Oleic Acid, C18:1n9c                           | 23.56          |
| Cis-11-Eicocenoic Acid, C20:1                  | 2.08           |
| Erucic Acid, C22:1n9                           | 0.13           |
| Nervonic Acid, C24:1                           | 0.04           |
| **Total MUFA**                                 | **32.65**      |
| Linoleic Acid, C18:2n6c                        | 6.38           |
| Y-Linolenic Acid, C18:3n6                      | 0.15           |
| Linolenic Acid, C18:3n3                        | 0.83           |
| Cis-11,14- Eicosadienoate Acid, C20:2          | 0.57           |
| Cis-8,11,14- Eikocetrienoate Acid, C20:3n6     | 1.12           |
| Arachidonic Acid, C20:4n6                      | 0.52           |
| Cis-13,16- Docosadienoate Acid, C22:2          | 0.03           |
| Cis-5,8,11,14,17- Eicosapentaenoate Acid, C20:5n3 | 0.74         |
| Cis-4,7,10,13,16,19- Docosahexaenoate Acid, C22:6n3 | 0.32         |
| **Total PUFA**                                 | **10.66**      |
| **Total identified fatty acids**               | **86.04**      |
| **Total unidentified fatty acids**             | **13.96**      |
| **Total omega-3**                              | **1.89**       |
| **Total omega -6**                             | **8.17**       |
| **Total omega -9**                             | **24.83**      |

MUFA are dominated by oleic acid (23.56%) which is included in omega 9 fatty acids. Oleic acid (MUFA) has a better role than omega 3 and omega 6 in lowering blood cholesterol levels. Oleic acid plays a role in lowering LDL blood cholesterol and increasing HDL blood cholesterol (Sartika 2008).

Omega 3 and omega 6 are included in PUFA fatty acids. Omega 6 is dominated by linoleic acid with a percentage of 6.38%. Omega 6 plays an important role in fat transport and metabolism, immune function,
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maintaining the function and integrity of cell membranes (Sartika 2008). Omega 3 is dominated by linolenic acid by 0.83%. Omega 3 plays an important role for intellectual development (Suseno and Saraswati 2015).

Omega fatty acids in fish oil from milkfish viscera consist of omega 9 (24.83%), omega 6 (8.17%) and omega 3 (1.89%). Based on these results, the highest percentage is omega 9 fatty acid. In accordance with Hafiludin's research (2015) the composition of omega fatty acids in milkfish is dominated by omega 9 fatty acids. Factors that affect the fatty acid content of milkfish are the type of food and fish habitat.

CONCLUSION

The best treatment for extracting fish oil from milkfish viscera used extraction temperature of 50 ºC. This was because the extraction using a temperature 50 oC produced the best yield, free fatty acids and anisidine values. Fish oil from milkfish viscera was dominated by palmitic acid and still contains relatively small amounts of omega-3 such as linoleic acid, docosahexaenoate acid (DHA) and eicosapentaenoate acid (EPA).

The fish oil in this study needs to be further purified, so that its quality can be improved and meet the fish oil standards set by IFOS.

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