Analysis Quality Fish Oil (Decapterus sp.) Containing Omega-3 Using Extraction Method (Steam Press) and (Bligh and Dyer)

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ABSTRACT

Fat or oil contained in some types of fish can be used as a source of fatty acids for the human body if the component has been separated from other elements such as water or extracted from fish meat. Ways that can be used to produce oil or fat of materials suspected to contain oil or fat is by way of extraction. This study was conducted to analyze the quality of fish oil, yield and fatty acid composition of fish oil kites using two different extraction methods, namely the method (Bligh and Dyer) and method (Steam and Press). The material used is fresh fish kite. This research is an experimental laboratory. Parameters observed that the composition of omega-3 fatty acids, peroxide analysis, avidin numbers, the total yield of oxidation and extracts. Data were analyzed descriptively and presented in the form of tables and figures. The results of the analysis of peroxide, avidin numbers, total oxidation at elevated fish oil using the method (Bligh and Dyer and) and method (Steam and Press) IFOMA still meet standards (International Fishmeal and Oil Manufactures Association). The yield of the extract value Bligh and Dyer method was 4.05% while the yield of extract value using Steam and Press is 3.70%. The composition of fatty acids omega-3 fish kites are analyzed, namely EPA and DHA. The composition of fatty acids omega-3 fish kite by using the method of Bligh and Dyer: EPA 26.98% and DHA 16.10%, while the results of the analysis using the Steam and Press: EPA 4.77% and DHA 15.29%.

INTRODUCTION

Important role polyunsaturated fatty acids (PUFA-polyunsaturated fatty acids) omega-3 in an increase in the value of human nutrition has been recognized since the 1930s use of omega-3 fatty acids as functional food continues to increase along with the increasing public awareness of the important role of These fatty acids. Division of market research group, Packaged Facts in 2011 reported that the market potential of omega-3 fatty acids in the United States has been in the form of a medical prescription, while in Europe already in the form of dietary supplements.

New innovations research on omega-3 fatty acids performed in 2005 by Cole will report an important role of omega-3 fatty acids help improve memory in people with Alzheimer’s for. Further [1] stated that the omega-3 fatty acids may play a role in brain development, most builders cerebral cortex of the brain, visual function, and growth of a normal organ. Furthermore, [2] and [3] stated that the omega-3 fatty acids was found to play an important role in the development of clinical psychology and healing various mental illnesses, such as depression, attention deficit hyperactivity, and dementia. It was also reported by [4] that the omega-3 fatty acids can help in the development of the field of psychology, which is to determine the level of growth, development and behavior and growth of early age children, especially for children with autism spectrum disorders.

Fish oil is a source of essential fatty acids, especially polyunsaturated fatty acids n-3 or better known as omega-3, which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [5].

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The oil content of fish with high omega-3 found in fish that live at high salt levels. The cold of environment an fish does not make an indication in determining the amount of omega-3 [6].

The amount of fatty acids in fish oil is different depending on the type of fish, fish food, fish habitat, and others. The amount of omega-3 biggest found in fish. Fish oil is the best source of omega-3 fatty acids. According [7], the content of unsaturated fatty acids (PUFA) were higher in the fish oil causes susceptible to oxidative damage and produce bad odor.

From the data that has been issued by the Institute of Nutrition Department of Health, several species of marine fish Indonesia has content / levels of omega-3 fatty acids is high (there are up to 10.9 g / 100 g) as lemuru fish, eel, mackerel, overpasses and tuna [8].

Fish Kite (Decapterus russelli) is one of the small pelagic fisheries communities are important in Indonesia. Fish belonging to Carangidae and his tribe clustered so that its size reaches 15 cm and 25 cm. The characteristic that is often found in fish kite is there a small fin behind the dorsal fin and anal fins and scales are thick (lateral scute) to the scales thick line.

The problem of fish oil is easily damaged by oxidation and the effect of heating temperature, so that when applied in the food industry to consider the quality and stability of the fish oil fish oil especially unsaturated fatty acids is high including EPA and DHA are highly susceptible to oxidation.

This study was conducted to analyze the quality, yield and fatty acid composition of fish oil kite using two extraction methods namely method (Bligh and Dyer) and method (Steam and Press).

**MATERIAL AND METHODS**

**Tools and Materials:**

1. **Tool:**
   - Volumetric Pipette 3 ml, 8 ml, 9 ml, 20 ml, and 40 ml. Beaker glass and 100 ml. Pumpkin 50 ml round bottom 100 ml. Separating funnel, Centrifuge, homogenizer, Scales, funnel and filter paper, Distillation, Evaporators, gas chromatography unit, mixer, gas stove.

2. **Ingredients:**
   - The materials used to make fish oil transform kite: fish oil from fish kite (Decapterus sp), bentonite, NaOH, EDTA dilute alcohol, ethanol, HCl, n-hexane, hot urea, methanol, HCl, octyl gallate. Chemicals for analysis of fatty acid: 0.5 N NaOH, NaCl, methanol (CH 3 OH), Isoctane, BF3 20%, NaCl, Isooctane, anhydrous Na2SO4, Petroleum benzine (40-60°C), distilled, anhydrous sodium sulfate (Na2SO4), neutral alcohol KOH, pp indicator, HCl, acetic acid, chloroform.

**Research Methods:**

The method used in this study is the experimental laboratory and the data were analyzed descriptively and then displayed in the form of tables and figure. for compare the quality of the fish oil extraction method (steam and press) and the results of the extraction method (Bligh and dyer), acid composition analysis fats in omega-3 concentrates, it can be seen briefly in the study.

**Fish Oil Processing Procedures:**

a. **Oil extraction [9]:**
   - Chopped fish meat free of bone and skin weighed as much as 20 ml of chloroform and 40 ml of methanol. Then homogenized by means of a blender for 2 minutes, the homogenate was added into 20 ml of chloroform and blend again for 30 seconds. To the homogenate was added 20 ml of distilled water and blend again for 30 seconds. The addition of distilled water adjusted to the moisture content of the sample, when the water content of 80%, then the addition of 20 ml distilled water enough, but if the water content is less than 80, then distilled water is added should be 20 ml, so that the proportion ratio of chloroform: methanol: water = 1: 2 : 0.8 to 2: 2: 1.8. Furthermore, the homogenate was filtered with filter paper in a funnel which assisted with a vacuum pump, and the filtrate is collected in a 500 ml Erlenmeyer flask, glass blender was rinsed with 5 ml of chloroform, then filtered with residue. Residue which still contains a little fat blend again for 30 seconds after being added with 20 ml of chloroform. Homogenates filtered and the filtrate was put together with the first filtrate. The filtrate was then inserted into the measuring cup volume of 1000 ml. let some time until there is a separation and purification that will form two layers. The top layer consisted of methanol-water, the bottom layer consists of a solution of oil in chloroform. The volume of each note, then the top layer was taken by aspiration using a pipette, to the left is really a layer of oil in a known volume of chloroform. Chloroform was then evaporated using a vacuum evaporator.
b. Fish Oil Extraction Method Steam and Press:
The steamed fish with cooking (steam) approximately 90°C for 15 minutes, then cook fish wrapped in fabric to be pressed, separating the liquid fraction (containing fish oil) and fish meal. Crude fish oil is heated at 60°C. Do blanching temperature of 60°C, 60 rpm for 60 minutes with the addition of bentonite 2.5% (w/v) were activated in a muffle temperature of 300 °C for 3 hours. Filtration with filter paper to separate the dirt and residue adsorbent to produce fish oil purification results.

c. Transesterification:
Transesterification method using the acid catalyst BF3 (Boron Triflourida). A total of 0.33 g of oil added to a beaker containing 10 ml of BF3 methanol, then stirred and refluxed at 40 °C for 1 h on a hot plate. Results reflux cooled, after it is inserted into a separating funnel and add 25 ml of distilled water. Subsequently extracted with the addition of 20 ml of n-hexane. After the two layers which formed the bottom layer containing glycerol separated and the upper layer containing the methyl esters extracted again with 10 ml of n-hexane, then added distilled water until neutral pH. After it was added 10 grams of anhydrous Na₂SO₄ to eliminate the possibility of water remaining in solution. Furthermore, the separation and the filtrate obtained was evaporated with a vacuum pump. Methyl ester mixture was analyzed by GC-MS to determine the type and content of fatty acids.

Testing Procedure:
Analysis of concentrated omega-3 fatty acids include fatty acid analysis by Gas Chromatography (GC) (to determine the content of omega-3 fatty acid), peroxide analysis [10], and the number toks [10]. The parameters analyzed refers to IFOMA (International Fishmeal and Oil Manufactures Association) standards fish oil consumption. In addition, the calculation of the yield of fatty acid omega-3 fish oil extraction results.

1 The yield [11]:
The yield of concentrated omega-3 fatty acids is determined by comparing the weight (g) extraction results obtained by the weight (g) of fish oil used multiplied by 100%

Sucrose content (%) = \( \frac{\text{Weight of extracted (g)} \times 100\%}{\text{The weight of the starting material (g)}} \)

2. Numbers peroxide [12]:
Peroxide analysis performed by weighing 5 grams of sample in a 250 ml Erlenmeyer flask was added 30 ml of solvent (60% acetic acid and 40% chloroform) and shaken until the sample is dissolved. Then added 0.5 ml of saturated KI then allowed to stand for 2 minutes in a dark room while occasionally shaken. Add 30 ml of distilled water, then to titration with Natriumthiosulfat 0.01 N. The calculation as follows:

\[ \text{Peroxide (meq/kg)} = \frac{\text{ml of titrant (sample – blanko)} \times N \ Na_{2}C_{2}O_{4} \times 1000}{\text{grams of sample}} \]

Analysis of the blank is done in the same way. Samples were replaced with distilled water as a blank.

3. Determination of Value Anisidin/p-AV [13]:
First make a test solution by dissolving 0.5 g of sample into 25 mL of trimethylpentane. Then make a second test solution by adding 1 mL of p-anisidin (2.5 g/l) into 5 mL of test solution 1, then shake and keep it away from light. Then make a reference solution by adding 1 mL of p-anisidin (2.5 g/l) into 5 mL of trimethylpentane, then shake it and keep it away from light. Then measure the absorbance values, the test solution 1 at 350 mm exactly 10 minutes after preparing the solution, using the reference solution as compensation. Anisidin value determined by the following equation:

\[ \text{Value anisidin} = 25 \times (1.2 \text{A1} - \text{A2}) \]
\[ \text{M} \]
\[ \text{A1} = \text{absorbance of test solution 1} \]
\[ \text{A2} = \text{absorbance of test solution 2} \]
\[ \text{M} = \text{Mass of the sample used in the test solution 1} \]

4 Total Oxidation Numbers (Perrin, 1996)[14]:
Total oxidation value obtained by adding together the 2 PV with PAV, where PV (Peroxide Value) peroxide and p-AV (P-anisidin Value) is the number of p-anisidin.
Total Oxidation = 2PV + p-AV

RESULTS AND DISCUSSION

A. The Yield and Quality Of Maracelie Scud Oil Fresh:

Table 1: The yield and Quality of Fresh Fly Fish Oil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Bligh and Dyer method</th>
<th>Methods Steam and Press</th>
<th>Quality standards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IFOMA</td>
</tr>
<tr>
<td>1.</td>
<td>The yield of</td>
<td>4.05</td>
<td>3.07</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Peroxide</td>
<td>8.50</td>
<td>8.20</td>
<td>3-20</td>
</tr>
<tr>
<td>3.</td>
<td>Anisidin</td>
<td>9.34</td>
<td>8.56</td>
<td>4-60</td>
</tr>
<tr>
<td>4.</td>
<td>Totox</td>
<td>26.34</td>
<td>24.96</td>
<td>10-60</td>
</tr>
</tbody>
</table>

Source: Primary data IPB Integrated Laboratory, 2014

The yield of fish oil:
The yield of fish oil that is extracted by the method of Bligh and Dyer were 4.05% and 3.70% for the method of Steam and Press.

Peroxide:
Peroxide test is intended to see how the content of hydroperoxides in oils. The greater the oil content of hydroperoxide on the more shows the more damage that occurs in the oil. Hydroperoxide is a product of oxidation in fish oil that occurs when otooxidation termination reaction. The peroxide is a primary oxidation products in the oil that occurred during the process extraction. Test peroxide aimed to determine the level of damage to the fish oil extraction[15].

Peroxide value in food products that contain lots of unsaturated fatty acids during storage will increase with increasing free radical formation, and after reaching the maximum value it will tend to decrease due to degradation processes that generate peroxide compounds aldehydes, alcohols, hydrocarbons, and other volatile compounds. Peroxide value can not be used in determining the shelf life of a food product because it can be used to determine the level of oxidative damage to a product [16].

The results of the analysis of peroxide as shown in Table 1 above shows that descriptively seen that peroxide at concentrations of omega-3 fish oil that is extracted kite with Bligh and Dyer method is higher than using Steam and Press.

Based IFOMA (International Fish Meal and Oil Manufacturers Association) standard fish oil on consumption (food grade fish oil), peroxide has a standard of 3-20 meq / kg. This shows that the 2nd extraction method used is very good because of the peroxide value obtained in fish oil IFOMA kite still meet the standards. Therefore, this extraction method can be used to produce concentrated omega-3 fish oil consumption as a nutritional supplement.

According [17] oil containing unsaturated fatty acids are high susceptible to oxidation. Damage to fish oil that begins by breaking of unsaturated fatty acids to form free radicals caused by light, heat, fat peroxide, heavy metals, hematin, hemoglobin, myoglobin, chlorophyll and enzyme lipoksidase. These free radicals then react with oxygen to form a hydroperoxide compound is active which ultimately affects the physical and chemical properties of fish oil as unwelcome odor and murky brown color.

Numbers p-Anisidin:
Numbers p-anisidin a number of secondary outcome measures of fat oxidation products to determine the amount of aldehydes (especially 2- alkenal and 2,4-dienal) in fat. Aldehydes react with p-anisidin form a chromogen which absorbs at a wavelength of 350nm, which can be measured by spectrophotometry [18].

The results of the analysis of p-anisidin numbers as shown in Table 1 above shows that descriptively seen that the number of p-anisidin at concentrations of omega-3 fish oil that is extracted kite with Bligh and Dyer method is higher than using Steam and Press.

Based IFOMA (International Fish Meal and Oil Manufacturers Association) standard fish oil on consumption (food grade fish oil), p-anisidin numbers have a standard of 4-60 meq / kg. This shows that the 2nd extraction method used is very good because the p-value obtained anisidin on fish oil IFOMA kite still meet the standards.

Totoks Value (Total Oxidation):
Total fat oxidation in Table 1 shows the results of measurements of peroxide value as the primary product and the number of p-anisidin as secondary products simultaneously in order to obtain the total amount of oil oxidation products were expressed as Totoks [18].
The results of the analysis Totoks values as shown in Table 1 above shows that descriptively seen that the value Totoks at concentrations of omega-3 fish oil that is extracted kite with Bligh and Dyer method is higher than using Steam and Press.

Based IFOMA (International Fish Meal and Oil Manufacturers Association) standard fish oil on consumption (food grade fish oil), has a standard of value Totoks 10-60 meq / kg. This shows that the 2nd extraction method used is very good because Totoks values obtained in fish oil IFOMA kite still meet the standards. 26 is the maximum acceptable value for the parameter Totoks[19]. The value of Totoks can be used to determine the level of damage caused by oxidation of fat. Thus Totoks value can be used later as a critical parameter for estimating quality deterioration shelf life of fish oil [18].

**B. Fatty Acid Profile Fresh Maracelle Cud:**

The results of the analysis of fatty acid composition of fresh fish kite with GC-MS can be seen in Figure 1.

**Fig. 1:** Chromatogram of Methyl Esters of Fatty Acids Fish Fresh overpass (Method of Steam and Press).

**Fig. 2:** Chromatogram of Methyl Esters of Fatty Acids Fish Fresh overpass (Bligh and Dyer method).

From Figure 2 it can be seen that the extraction method of steam and oil press fresh fish kite detected 77 peaks while for Bligh and dyer method detected 92 peaks, of the peaks there are 25 peaks that were identified for the method of steam and press, while for the method of Bligh and dyer there are 22 peaks that were identified.

**3. Fatty Acid Composition of Fresh Maracelle Scud:**

Fatty acid profile is used to determine the types of fatty acids found in the oil either saturated fatty acids or unsaturated fatty acids. Fatty acid content of fish oil can be seen in Table 2. In Table 2, it can be seen fatty acids from fish oil there are 25 peaks that were identified for the method of steam and press, while for the method of Bligh and dyer there are 22 peaks that were identified in the fish oil fresh overpass.

In Table 2 it can be seen 25 fatty acids were identified for steam and press method while for the method of Bligh and dyer there are 22 fatty acids were identified in the fish oil fresh overpass. For the omega-3 EPA and DHA, EPA 26.98% for the method of Bligh and Dyer method while for Steam and Press 4.77%. As for the DHA 16.10% for the method of Bligh and Dyer and DHA for Steam and Press method is 15.29%.

**Conclusion:**

Based on the results it can be concluded that:

1. Quality of Fish Oil for both methods of Bligh and Dyer still meet the standards IFOMA
2. The yield for Bligh and Dyer extraction method were 4.05% and 3.70% for the method of Steam and Press. Composition 13 fatty acids omega-3 fish fresh kite is For Omega-3 EPA and DHA namely, 26.98% for the EPA method of Bligh and Dyer method while for Steam and Press 4.77%. As for the DHA 16.10% for the method of Bligh and Dyer method while for Steam and Press 15.29%. So the method of Bligh and Dyer is superior both in terms of yield as well as omega-3 (EPA and DHA)
Table 2: Fatty Acid Composition of Fresh Fish Kite by two methods (Steam and Press) and method (Bligh and Dyer).

<table>
<thead>
<tr>
<th>No</th>
<th>Fatty Acids</th>
<th>Symbols</th>
<th>Method of Bligh and Dyer</th>
<th>Methods Steam and Press</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saturated:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C12:0</td>
<td></td>
<td>C12:0 nd</td>
<td>C12:0 0.06</td>
</tr>
<tr>
<td>3</td>
<td>C13:0</td>
<td></td>
<td>C13:0 -</td>
<td>C13:0 0.04</td>
</tr>
<tr>
<td>4</td>
<td>C14:0</td>
<td></td>
<td>C14:0 0.28</td>
<td>C14:0 3.24</td>
</tr>
<tr>
<td>5</td>
<td>C15:0</td>
<td></td>
<td>C15:0 0.03</td>
<td>C15:0 0.75</td>
</tr>
<tr>
<td>6</td>
<td>C16:0</td>
<td></td>
<td>C16:0 3.14</td>
<td>C16:0 16.97</td>
</tr>
<tr>
<td>7</td>
<td>C16:1</td>
<td></td>
<td>C16:1 0.93</td>
<td>C16:1 4.16</td>
</tr>
<tr>
<td>8</td>
<td>C17:0</td>
<td></td>
<td>C17:0 0.29</td>
<td>C17:0 1.10</td>
</tr>
<tr>
<td>9</td>
<td>C17:1</td>
<td></td>
<td>C17:1 0.10</td>
<td>C17:1 0.22</td>
</tr>
<tr>
<td>10</td>
<td>C18:0</td>
<td></td>
<td>C18:0 4.43</td>
<td>C18:0 6.56</td>
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</table>

Source: Primary Data Integrated Laboratory IPB, 2014.

REFERENCES


