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Omega-3 fatty acid profiles of chub mackerel fish oil (*Rastrelliger* sp.) during cold storage

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Abstract
Marine fish and pelagic fish have contain of fatty acids. Fat or fish oil can be used as the source of fatty acid for human body if that component has been separated from other substances such as water or extracted from fish flesh. Pelagic fish such as Indonesia Mackerel has the potential of high fatty acid content. Fatty acids contained in fish oil are classified as unsaturated fatty acids (omega-3). Fish oil stability is very low so it need to storage at low temperatures. The aims of this study was to determine the omega-3 fatty acids in Indonesia Mackerel Fish oil during storage at low temperatures. The method of extracting fish oil used is wet rendering. Materials used in this research was Indonesian Mackerel (*Restrelliger* sp). This research was experimental laboratoris with three repetitions. Analysis of the parameters observed during storage of fish oil are fatty acid profiles, yields, peroxide value, and organoleptic value such as color and aroma. The results showed a decrease in the amount of Omega-3 fatty acids content during storage by 6,382% relative, peroxide value increased by 1,86 meq/kg, Organoleptic value of color and aroma decreased by 0,01 and 0,09. The yield of fish oil is 0,62%.

Keywords: fatty acids, Indonesia Mackerel fish, omega-3, storage, wet rendering

1. Introduction

Omega-3 is an unsaturated fatty acid contained in fish oil. The need for omega-3 fatty acids is very important for the health of the human body. The health benefit of Omega-3 is to reduce cholesterol level in the blood. In addition, foods that contain unsaturated fatty acids can reduce the risk of hyperlipidemia which can potentially cause heart disease [1].

The increasing need for omega-3 fatty acids as drugs to prevent heart disease, other degenerative diseases, and the presence of synergism between nutritional components (vitamins and omega-3 fatty acids) in fish oil as a prevention against these diseases causes fish oil to be very important [2].

The fat content in fish is an unsaturated fatty acid that is beneficial to the body. The composition of unsaturated fatty acids is 75%, while 25% is the content of saturated fatty acids. One of the unsaturated fatty acid content in fish is omega-3. Omega-3 content can be found in mackerel, milkfish, lemuru, flying fish, and mackerel tuna [3]. DHA (Docosahexaenoic acid), EPA (eicosapentaenoic acid), and Linoleic are kind of Omega-3 [(n-3)] long-chain polyunsaturated fatty acid [4]. But, EPA and DHA are more dominant in fish oil [5].

Fish oil sources are divided into two groups, such as fish oil derived from the liver (fish liver oil) and fish oil from the body of the fish (fish body oil). In addition to omega-3, fish oil also contains vitamins A and D. In fish liver oil contains a number of vitamins A and D. Fish oil can be processed into body supplements, food and feed mixtures as well as for non-food industrial raw materials. Fish oil, especially omega-3 fatty acids has an important role in health and intelligence because omega-3 such as EPA (Eicosa Pentaenoic Acid), DHA (Docosa Hexaenoic acid) and Linoleic are useful to increase brain intelligence [5].

Pelagic fish such as lemuru is usually processed into canned fish, boiled fish, salted fish and fish meal. To increase the added value of fish that are included in the low economy can be done by utilizing the oil as a source of omega-3 fatty acids [6].

Fish oil is very sensitive to the oxidation process so that it can affect its stability. This can occur because of the high content of unsaturated fatty acids in fish oil, even in fish oil there are unsaturated fatty acids that have 5 to 6 double bonds, such as EPA and DHA [7]. Efforts to improve fish oil stability can be done by storing at low temperatures and adding oxidant chains [8].

Chub mackerel is potential as a source of fish oil intake and omega-3 sources. Therefore, this research will be carried out the process of oil extraction with the wet rendering method and carried out storage at low temperatures, to determine the content of omega-3 fatty acids in chub mackerel oil fish produced.

2. Materials and methods

Tools and Materials

The tools used in the study which include the extraction and testing process are dark brown glass bottles and covered with brown paper, analytical scales, gas stoves, separating funnels, autoclaves, presses, centrifuges, fish oil organoleptic score sheets, and spectrophotometer GC. The raw material for making fish oil is chub mackerel (*Restrellige*r sp). The ingredients used in the omega-3 test are BF3-Methanol, hexane, saturated NaCl, fatty acid capsules, Benzene-methanol, ferrochloride, chloroform, KI, $Na_2S_2O_3$ 0.1 N, 1% starch solution, and \ ethyl ether

Research Method

The research method used was experimental laboratories. This research was conducted by extracting fish oil by wet temperature rendering method with high temperature. The extraction temperature used in this study was 105ºC for 30 minutes. The temperature and time used are based on the temperature used in the extraction process with the rendering method [9]. In this extraction process, 5% of the weight of the sample was added. Comparison of water volume and sample weight. Oil or fat that has been extracted will go through a storage process in the freezer for 30 days with a test interval at the beginning and end of storage.

Quality Testing

The main parameters tested are chemical tests, such as omega-3 fatty acid profiles using gas chromatography, PV (Peroxide Value) based on AOAC (2006), and physical tests were organoleptic based on SNI.01-2346-2006. While the supporting parameters tested are yield based on AOAC (2006) [10].

Omega-3 Fatty Acids

Omega-3 fatty acid content was identified from the chromatogram (peak) from the fractionation of fatty acid ester metals by capillary gas chromatography. The methyl esters are obtained from the fat methylation process in accordance with the modified British Standard method, while the fat for methylation is extracted from the material by the modified Bligh-Dryer method. Fatty acids were identified by comparing the peak of the analysis results with the peak of the standard fatty acids Oil samples were taken from 0.1 to 0.2 g transesterified with BF3-methanol then cooled and dissolved in n-hexane then the samples taken 2 μL were injected in the GC injector, the results of the order were chromatogram, from the most% EPA and DHA. To find out the retention time of EPA and DHA, it is first injected in the GC of fatty acid esters from omega-3 capsules containing EPA and DHA made by Deep sea as standard, the presence of EPA and DHA samples can be seen by equalizing the standard EPA and DHA retention times

Peroxide Number

The methods for testing peroxide numbers are as follows: (1) Weigh 5 ± 0.005 g of sample in a 250 ml Erlenmeyer lid and add 30 ml of acetic acid and chloroform solution (3: 2). Shake the solution until all the ingredients are dissolved. Then 0.5 ml of KI saturated solution is added. (2) Leave it in the dark for 1 minute and occasionally shake it then add 30 ml of distilled water. (c) Titrate with 0.1 N Na2S2O3 until the yellow color disappears. Add 0.5 ml of 1% starch solution. Continue the titration until the blue color begins to disappear. (d) Peroxide figures are expressed as the equivalent value of peroxide in every 100 grams of the sample

$$\text{Peroxide number} = \frac{\text{ml Na2S2O3 x N Thio x 1000}}{\text{sample weight (gr)}}$$

Organoleptic Test *(SNI.01-42346-2006)*

Organoleptic test is conducted to determine the quality and preferences of consumers for the color and aroma produced from the oil. In this study using an organoleptic test with a nine-hedonic scale. Organoleptic testing is carried out as follows: (a) Samples of the oil to be tested are marked. (b) panelists are asked to look at the color of the oil alternately in the choices provided according to the state of the oil. (c) The choices to be given a value from each sample are then searched for an average value.

The Yield

The yield is the percentage of the main raw material that becomes the final product or the comparison of the final product with the main raw material. Can be expressed in decimal or percent.

$$\text{The Yield (\%)} = \frac{A}{B} \text{ x } 100\%$$

Data Analysis

Data were analyzed by Analysis of Variance (ANOVA). If the F count shows a significant difference at the 95% test level and a very significant difference at the 99% test level then proceed with the Tukey HSD test. Whereas data processing for organoleptic tests uses nonparametric statistics with Friedman test [11]. To find out the influence of interactions between different time periods, a correlation and regression test will be performed.

3. Results & Discussion

Fatty Acid Profile

Fatty acids in fish oil consist of three groups, namely fatty acids Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA) and Polyunsaturated Fatty Acid (PUFA). Chub mackerel fish oil contains all three classes of fatty acids. The relative percentage of total SFA fatty acids is higher than those of MUFA and PUFA fatty acids. Based on other studies of fatty acid content states that the fatty acid content of SFA group is mostly contained in tuna mackerel fish oil, spanish mackerel, and layur [12], and elevated fish oil [5].

The profile of fatty acids found in mackerel oil during cold storage is presented in Table 1.

Table 1: Fatty Acid profile in oil fish chub mackerel (%)

Fatty Acie	Day 0	Day 30
SFA		
Myristic acid (C14:0)	12,573	1,081
Palmitic acid (C16:0)	25,111	30,800
Stearic acid (C18:0)	10,865	8,756
Arachidic acid (C20:0)	1,996	1,319
Total SFA	50,545	41,956
MUFA		
Miristoleic acid (C14:1)	0,412	0,410
Palmitoleic acid (C16:1)	14,011	5,609
Oleic acid (C18:1)	18,361	28,494
Total MUFA	32,784	34,513
PUFA		
Linoleic acid (C18:3n-3)	1,173	-
EPA (C20:5n-3)	15,044	10,071
DHA (C22:6n-3)	0,585	0,349
Total PUFA	16,802	10,420

The amount of omega-3 in chub mackerel oil decreased during storage at cold temperatures, whereas the amount of omega-3 in mackerel oil decreased in the amount of omega-3 from 16.02% to 10.42%. This result is higher than male chub mackerel fish (*Rastrelliger kanagurta*) which produces omega-3 fatty acids by 1.5% [13]. The difference in the amount of omega-3 content is thought to be influenced by the use of different types of fish and fat content in fish and different extraction methods. Differences in the amount of omega-3 fatty acids may be due to the different quality of raw materials. In addition, the difference in omega-3 fatty acid content is thought to be due to the higher oxidation activity in fish oil so that it can damage the fatty acids present in fish oil [4].

Anova test results stated that the storage time of day 0 had a very significant effect ($P < 0.01$) on the content of omega-3 fatty acids on storage on day 30. This can be seen from the average difference in omega-3 fatty acids.

The content of fatty acids of chub mackerel oil stored stored changes. SFA and PUFA fatty acids decreased, while MUFA fatty acids increased. This change is thought to be a reaction to fatty acids during storage. Instability of omega-3 fatty acids in fish oil during storage, it is suspected that there is a breakdown of the double chain in unsaturated fatty acids so that changes in fish oil fatty acid levels occur [4]. This is also shown by changes in the peroxide number which is used as an indicator of changes in fish oil. During the low temperature storage process there will be a reaction between SAFA and PUFA so that the levels of SAFA fatty acids increase while PUFA has decreased [14, 15].

Peroxide Number

One indicator of fish oil damage can be seen from changes in peroxide numbers. The peroxide number in chub mackerel oil on the 0^{th} day was 13.59 ± 0.24 meq /kg and 15.45 ± 0.22 meq/kg on the 30^{th} day. During cold storage, the peroxide number increases 1.86%. However, this peroxide number is still limited to the acceptance of fish oil quality. The maximum limit of peroxide numbers on the quality of crude fish oil is 3-20 meq/kg [4].

Changes in fish oil can be determined from increases in fish oil peroxide number [14]. This difference in peroxide value is thought to be due to the higher oxidation activity of fish oil. Storage time will affect the process of fat oxidation. Peroxide number will increase with storage. Peroxide number is a determination of hydroperoxide formation which causes rancidity in fish oil during storage [17].

Different extraction methods can also affect fish oil peroxide numbers during storage. The extraction time is directly proportional to the increase in the peroxide number [18]. The increase in peroxide number is thought to be due to the temperature factor and the storage time. The higher the temperature and the duration of storage, the peroxide number increases 7 times at room temperature [14].

In the extraction process allows the presence of impurities that are still carried into fish oil such as protein, water, and Fe. These impurities can accelerate the process of rancidity in fish oil [9]. The process of rancidity occurred due to the inclusion of peroxide numbers [19]. Peroxide numbers can increase due to the breakdown of the double bonds in fish oil due to heating. The more double bonds, the higher the peroxid value. There are several factors that influence peroxide numbers, namely the quality of raw materials, extraction methods, packaging, and storage [4].

The Yield

The physical characteristics observed in this study were the yield. The yield is the percentage of the main raw material that becomes the final product, or the comparison of the final product with the main raw material. Can be expressed in decimal or percent. Yield is the most important parameter to determine the economic value and effectiveness of a product or ingredient process. The greater the yield, the higher the value of the effectiveness of the product [20].

The amount of raw material (fish) used in the production of tuna mackerel oil, chub mackerel and lemuru is 3000 kg each. The yield of fish oil produced by the wet rendering method is 0.62 ± 0.08% with three replications. The yield obtained in this study is smaller when compared to the yield obtained from the extraction of catfish oil, tuna mackerel and milkfish with a temperature of 0-95ºC for 15 minutes which is 2.20%, 1.00% and 2.10% [21]. This is caused by the use of temperature and time in different extraction processes. The use of different presses, and the amount of oil produced are also based on the type of fish used.

There are two ways of mechanical presses namely hydraulic and threaded presses. 1). Hydraulic presses use a pressure of 136 atm [9]. The amount of oil depends on the pressure and duration of pressing. The amount of oil remaining in the cake is around 4-6%. 2). Threaded pressing (expeller pressing) requires a cooking or tempering process first. The cooking process takes place at a temperature of 240ºF (115.5ºC) with a pressure of 15-20 tons/inch^2. The content of the resulting cake still contains about 4-5% oil

The use of different types of fish relative to different yield values. This is due to differences in fat content and other non-mineral components, such as water and protein. The use of different extraction methods will affect the yield value produced [18]. Extraction with the wet rendering method will produce a higher yield value than the dry rendering method. The process of heating raw materials can separate the fat or oil content. Adding water to the extraction process will facilitate the process of separating oil from other solids because of the clumping of protein in fish. The principle of the wet rendering method is the existence of heating treatment, applying pressure, and centrifugation.

Organoleptic test

Organoleptic test of fish oil is determined from the assessment of color and aroma. The organolytic value of fish oil color was 5.78 ± 0.02 on day 0^{th} and 5.77 ± 0.00 on the 30^{th} day. Chub mackerel fish oil has a brownish yellow color. Organoleptic assessment on fish oil aroma was 3.97 ± 0.00 on the 0^{th} day and 3.88 ± 0.04 on the 30^{th} day. The aroma produced in chub mackerel oil is fishy and distinctive fish oil. There is a decrease in organoleptic value in fish oil during cold storage. This is thought to be due to an oxidation reaction during storage that affects the appearance of fish oil. The decrease in organoleptic value is also supported by the results of the peroxide number test which has increased.

The color of chub mackerel oil produced is thought to be influenced by the use of fish oil extraction methods and fish species. Impurities and dyes such as carotenoids and tocopherols affect the color of the oil produced [22]. The extraction method affected the color of fish oil produced [18, 23]. The longer the extraction process will produce fish oil that is dark in color. This is due to the use of temperature and pressure during extraction by the rendering method. The color of fish oil is influenced by natural pigment substances, such as xanthophyll, carotene, and anthocyanin [18]. It is also due to the presence of compounds resulting from the degradation of natural dyes to produce fish oil which is yellow or brownish yellow in color.

The odor produced in mackerel oil is thought to be influenced by the quality of fish oil during fish oil storage. This is also shown from the results of the peroxide number which has increased. The difference in aroma on day 0 to 30 did not differ significantly. The rancid odor in fish oil is a combination of chemical compounds or derivatives of highly complex unsaturated fatty acids [24]. Odor in oil, besides being naturally present, also occurs because of the decay of very short chain acids as a result of decomposition of oil damage [9]. The rapid oxidation process occurs due to the high unsaturated fatty acids contained in fish oil, giving rise to the distinctive aroma of fish oil [4]. In this study, the aroma of lemuru fish oil produced was fishy and specifically specific to fish oil with a little rancidity.

4. Conclusions

The low temperature storage process in fish oil has an influence on the profile of omega-3 fatty acids, peroxide numbers, and the appearance and color of fish oil. The decrease in total omega-3 fatty acids was 6.382%, the peroxide number increased by 1.86%, while the organoleptic test decreased the color value by 0.01 and the aroma by 0.09. The yield of mackerel oil produced is 0.62%.

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