The Use of “Jambal” Fish (*Pangasius hypophthalmus*) and Grouper (*Cromileptes* sp) Oils for Infant Biscuit Formulation

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Abstract. The objective of this study was to determine the effect of fish oil use the combinations of against nutritional value infant biscuits which confectionery meet the standards of omega 3 and omega 6 (1 : 5) according to WHO standards and ISO 01-7111.2-2005. The combination of fish oil used from belly fat “Jambal” fish (*Pangasius hypophthalmus*) and Grouper (*Cromileptes* sp). The design experiment used by complete random design (RAL). The treatment attempting is a combination of fish oil use belly fat making infant biscuits: M0 (0 mL fish oil), M1 (7 ml fish oil), M2 (14 ml fish oil), and M3 (21 ml fish oil). The results of the analysis of the nutritional value biscuits are generated compared with quality terms SNI (Indonesia National Standard) 01-7111.2-2005 infant biscuits. Based on the findings of research it was known that treatment which is the best fish oil use for a combination of 21 ml of water contents of 4.42% (standard maximum of 5%), 10.88% protein (the minimum standard of 6%), fat 13.74% (minimum standard 6%), carbohydrates 69.91% (standard maximum of 70%), ash 2.15% (standard maximum 3.5%), and the ratio of omega 3 : omega 6 is 1 : 4.3.

1. Introduction

“Jambal” fish (*Pangasius hypophthalmus*) contain components that include protein, fat, minerals and vitamins. Belly fat cath fish fatty acids omega 3 and omega 6 are beneficial for human health. So far waste fish entrails jambal conjoined especially abdominal fat is not used optimally. In addition as an added value in the field of fisheries can also overcome the problem of environmental pollution is caused by waste generated due to the increasing demand for jambal fish conjoined. According Hwang et al, [1] and Hastarini et al, [2], the stomach contents jambal conjoined including the gastrointestinal tract, liver, gall bladder and stored fat (abdominal fat) is a potential source of fats with omega 3 are relatively high. Research profile and fatty acid composition of the waste catfish have been done by several researchers Sathivel et al, [3], Klemeyer et al, [4], Maulana et al, [5], but for the types of catfish in Indonesia have not been made, either for catfish types of Siam and the types Jambal which are the two most consumed types of catfish in Indonesia.

According Kaban and Daniel [6] and Panagan et al, [7], the fish entrails and heads are quite a lot of oil content. fish oil used as an additive in industrial manufacture of biscuits and other food industry. Okada and Michael [8] suggested that freshwater fish species potentially produce the fish oil, which generally have quite high EPA content. In catfish oil (*Pangasius pangasius*) found levels of omega 3 and omega 6 from 1.6 to 12.44% from 12.27 to 15.96%. Simopaulus [9] argued that the interest to consume fish that progressively increase with the level of health gained from consuming fish oil, especially from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fatty acids are a class...
of compounds that have a long chain carboxylic acid, either saturated or unsaturated. Saturated fatty acids have only single bonds, each carbon atom in the chain of the two atoms of hydrogen. Examples of saturated fatty acids are lauric acid, meristat acid, palmitic acid, stearic acid, arakidat acid, and behenic acid. The nature of saturated fatty acids can be synthesized in the body is a solid at room temperature, and no-carbon double bond.

Unsaturated fatty acids have one or more double bonds between two carbon atoms bonded to carbon atoms are bonded duplicate each other. Examples of unsaturated fatty acids are meristoleat acid, palmitoleic acid, oleic acid, acid eikosanoat, mervanoat acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, and the acid dokosahexanoat. The properties of unsaturated fatty acids are essential nature, can’t be produced by the body, a liquid at room temperature, a substance derived from animal and vegetable sources, and there is a double bond.

In human nutrition, fatty acids such as linoleic and linolenic acids regarded as essential fatty acids because it is needed by the body, while the body can’t synthesize. Unsaturated fatty acids such as linoleic and linolenic acid can prevent the onset of various diseases and accelerate the growth of toddlerhood. If the ratio of omega-6 fatty acids is higher than omega 3 will be a negative effect on cognition, mood and behavior [10], [11], [12]. Omega 6 fatty acids, especially linoleic acid and arachidonic acid are a constituent of body fat is very important, especially in the process of growth and development of children [13].

Infant is one of the age groups that is vulnerable to malnutrition. Nationally, from 2007 to 2010 the prevalence of baby malnutrition and undernourishment decreased 0.5%. In 2012 there are approximately 900 thousand (4.5%) of the 22 million children under five in Indonesia malnourished and malnutrition (Republic Indonesia Health Department 2012). According to Health Research in 2013 trends in each of the provinces of malnourished children amounted to 19.6% which increased from 18.4% in 2012. When the conversion is done in absolute numbers, then when the number of infants in 2013 was 23,708,844, the number of children malnutrition amounted to 4,646,933 baby, in contrast areas of highest number of underweight children under NTT is approximately 34%. In 2014, the number of malnourished children under five malnutrition of 5.7% and 13.9%, 2015 amounts to 17.9%, although the number dropped but still below the MDG target of 15% (Ministry of Health Republic Indonesia, 2016). Alternatives can be done to solve the problem is to increase the intake of nutrients needed to meet nutrient adequacy. One of the extra food that can be consumed by children under five are the biscuits. Simopaulus [14] states that the biscuits are good for infant is a biscuit enriched with omega 3.

The biscuits were given as food additives in children under five is generally in the form of biscuits made from butter. Simopaulus [14] states that margarine contain trans fatty acids ("Trans Fatty Acid") that can cause adverse health impact is as a trigger for coronary heart disease. The intake of trans fatty acids are high can affect the metabolism of omega 3 fatty acids and essential fatty acids, for the children of butter is not recommended. The influence of trans fatty acids is highly dependent on the levels of intake; high levels (above 6% of total energy) and moderate levels (4.5% of total energy) will not be harmful if taken together with polyunsaturated fatty acids. The negative influence of trans fatty acids is increased if the intake of essential fatty acids linoleic low as trans fatty acids inhibit fatty acid biosynthesis arachidonat that is needed for tissue growth ([16], [17]). By meeting the standards of omega 3 and omega 6 in food will have a positive effect [14] on health [. Since the content of omega 3 and omega 6 oils jambal fish (Pangasius hypophthalmus) below the standards are set by WHO, to meet the standard of 1: 5 can be combined with oil grouper (Cromileptes sp). The result of the combination of fish oil is expected to meet the standards of omega 3 and omega 6 and SNI 01-7111.2-2005. This study aimed to determine the effect of using a combination of fish oil on the nutritional value of the biscuits baby who meet the standards of omega 3 and omega 6 (1:5).

2. Methodology
Raw materials used in this study are fat and abdominal fat grouper fish jambal conjoined. Grouper can be obtained from cultivation in Batam, Riau Islands province and abdominal fat fish jambal conjoined from the rest of the fish processing in Kampar, Province Riau. The chemical used is diethyl ether, methylene blue red, distilled water, NaOH, H₂SO₄, H₂BO₃, HCL and 1 gram of catalyst (Cu complex) and the standard of omega 3 and omega 6. The materials used for the manufacture of biscuits are oily fish (grouper fish oil combination and “jambal siam”), wheat flour, sugar, butter, egg yolks, cocoa powder, baking soda, and salt. Equipment used for laboratory analysis is oven, destruction pumpkin, pumpkin Kjeldahl, Soxlet, furnaces, glass tools, analytical balance, GC, and other tools that are needed in this study. The equipment used in the manufacture of biscuits are; knives, cutting boards, mixers, spoons, trays, molds biscuit, oven, measuring cups, scales, blenders, and paper labels.

3. Procedure research
1. Fatty acids, omega 3 and omega 6 [16]
2. Procedure making biscuit [17] with modification.
3. Composition nutrition biscuit (moisture, protein, fat, ash, and carbohydrate) [15].

   Results nutrition (moisture, protein, fat, ash, and carbohydrate) compared with the quality requirements of ISO 01-7111.2-2005 infant biscuits. From this comparison it can be seen that the biscuits are produced standards established qualify or do not qualify.

4. Methods of research
The method used in this study is an experimental method that attempted use of a combination of fish oil (which meets standard omega 3 and omega 6 (1: 5) in the manufacture of biscuits baby who meet SNI 01-7111.2-2005. According to Swanson et al, [22] a combination of fish oil can be added to 39 mL in the formula biscuit dough. The result obtained with such an attractive biscuit (shiny) but the taste less like a baby, and low protein content of SNI. Based on these results a combination of fish oil use is reduced to 21 mL. The design used was a completely randomized design (CRD). The treatments were: M0 (0 mL fish oil), M1 (7 mL fish oil), M2 (14 ml fish oil), and M3 (21 ml fish oil). The treatment was repeated 3 times, the amount is 12 units experiment.

5. Data analysis
Data nutritional value (moisture, protein, fat, carbohydrate, ash, omega 3 and omega 6) obtained first tested for normality, when the normal data distribution of the analysis is followed by analysis of variance (Anova). If the distribution of the data is not normal then it needs to be transformed first. Based on the analysis of variance, if obtained F count > F table at the 95% confidence level, the null hypothesis is rejected. If the null hypothesis is rejected, then continued with Duncan test to see any difference in treatment.

6. Result and Discussion
Characteristics of the fish oil and fish oil conjoined grouper fish. The yield is the percentage of key raw materials that can be used as a final product or a product comparison with the end of the main raw material, and can be expressed as a percent or decimal. Grouper fish oil yield obtained in this study was 25%. The low heating temperature causes the denatured protein only slightly so it will make it more difficult to penetrate the cell wall by the oil contained in the material being heated. While at higher temperatures much protein is defective due to denaturation process, so it is more penetrated easily by the oil resulting in the more oil produced. “Jambal” fish oil yield adequate enough to be used as a source of oil.

   The results of the analysis of the content of fatty acids oil grouper (Cromileptes sp) oil and fish jambal siam (Pangasius hypophtalmus) can be seen in Table 1 and Table 2. In Table 1 we can see the content of fatty acids groupers are: saturated fatty acids 45.26%, unsaturated fatty acids 54.76%, monounsaturated fatty acids 0.59%, fatty acids 54.15% polyunsaturated fatty acids omega 3 and omega 6 5.38% 14.99%. Ratio of omega 3 to omega 6 oils grouper is (1:2.8).
In Table 2 can be seen the content of the fatty acids are fish belly conjoined jambal: 51.73% saturated fatty acids, unsaturated fatty acids 49.27%, monounsaturated fatty acids 17.63%, polyunsaturated fatty acids 31.64% fatty acids omega 3 and omega 6 18.70%. Ratio of omega 3 to omega 6 oils jambal Siam fish's stomach is (1: 6.8). Based on the content of omega 3 and omega 6 oils and oil grouper fish belly conjoined jambal can be made of fish oil combination that meet the standards of omega 3 and omega 6 according to the WHO 1: 5.

Omega 3 fatty acids contained in fish oil combination is linolenic acid (C 18: 3 n-3 / ALA), arachidonic acid (C 20: 4 n-6 / AA), acid eicosapentenoic/EPA (C 20: 5 n-3), and dokosaheksanoat acids / DHA (C 22: 6 n-3). While omega 6 fatty acids contained in fish oil combination is linolenic acid (C 18: 3, n-6 / ALA), arachidonic acid (C 20: 4 n-6 / AA) and linoleic acid (C 18: 2, n-6 / LA). EPA contained in the grouper fish oil DHA 2.42% and 0.89%. EPA contained in fish oil jambal belly conjoined 1.01% and 0.60% DHA. EPA and DHA are omega 3 types most calculated to be formulated in accordance WHO standard infant biscuits. Formula recommended (Harli, 2007) for infant consumption is 0.1% EPA, DHA 0.35%, and 1.5% ALA. Omega-3 fatty acids are by and large (> 60%) is required as a component of the cell wall constituent neurons. While the rest of the necessary building-blocks of the cup (container) rhodopsin a vital compound sensing and delivery of the signal received through the eye to the brain. Yui et al, (2016) suggested that the increased provision of omega 3 in the diet can increase the toddler sensing arrangement and delivery of the signal received through the eye. DHA is needed for the growth and function of neural networks toddlers [23]. Brenna et al, [24] suggested that the fatty acids which are components of fat is biomolecules needed to regulate metabolism and body health. Buckley and Howe [25] reported that the omega 3 and omega 6, which is a product of the fish, can be used as a food source baby that are needed to regulate the function of physiology, growth, and development of a normal toddler. According to Glick and Fisher [26] stated that omega 3 and omega 6 are essential fatty acids that need to be added to food toddlers to promote growth and development of malnourished children/malnutrition.

### Table 1. Content fatty acids grouper

| Content Fatty Acid Grouper | % FA/Total FA Detection |
|----------------------------|-------------------------|
| 1. Saturated Fatty Acid    | 45.26                   |
| 2. Unsaturated Fatty Acid  | 54.74                   |
| MUFA                       | 0.59                    |
| PUFA                       | 0.34                    |
| Omega-6                    | 14.99                   |
| 9, 12,15-oktadetkaterinoat (C 18:3, n-6) Acids, alpha linolenat acids /ALA, n-6 | 0.34 |
| 5,8,11,14-eikosatetranato (C 20:4 n-6) acids, arachidonat acids/AA n-6 | 0.28 |
| 9,12-oktadekadienoat ( C 18:2, n-6) Asam, linoleat acids/LA, n-6 | 14.37 |
| Omega -3                   | 5.38                    |
| 9, 12, 15-oktadetkaterinoat (C 18:3, n-3) Acids, Alpha linolenat acids/ALA, n-3 | 0.51 |
| 9,12, 15-eikosanoat (C 20:3 n-3) acids, arachidonat acids/AA, n-3 | 1.56 |
| Eikosapentanoat acids/EPA (C 20:5 n-3) | 2.42 |
| Dokosaheksanoat acids/DHA (C22:6, n-3) | 0.89 |

### Table 2. Fatty Acid Belly Fat "Jambal" fish
Content fatty Acids belly fat "Jambal" Fish

| % FA/Total FA Detection |
|-------------------------|
| 1. Saturated Fatty Acid |
| 2. Unsaturated Fatty Acid |
| MUFA | 51.73 |
| PUF | 49.27 |
| Omega -6 |
| 9, 12,15-oktadetkaterinoat (C 18:3, n-6) Acid, alpha linolenat/ALA, n-6 acid | 17.63 |
| 5,8,11,14-eikosatetranoot (C 20:4 n-6) acid, asam arachidonate/AA n-6 acid | 31.64 |
| Omega-3 |
| 9, 12, 15-oktadetkaterinoat (C 18:3, n-3), alpha linolenat/ALA, n-3 | 18.70 |
| 9, 12, 15-eikosanoat (C 20:3 n-3), arachidonate/AA, n-3 | 2.35 |
| eikosapentanoat/EPA (C 20:5 n-3) | 14.24 |
| dokosaheksanoat/DHA (C22:6, n-3) | 2.11 |

Glick and Fisher [26] suggested that the addition of fish oil in food can increase the content of omega-3 are beneficial for improving the body's metabolism. According Innis [27] state that fish oil containing omega-3 is a functional food. Beside that Innis, Lee et al, [28] reported that the balance of omega 3 and omega 6 are taken into account in the manufacture of biscuits is a therapeutic food for children malnutrition. Deficiency of omega-3 fatty acids can lead to neurological disorders and sight of children under five. In infants who lack omega-3 fatty acids can lead to the process of cell formation inhibited neurons so that the baby can be defective, of poor quality and the process of growth and development of brain cells are not normal or suboptimal [29].

Omega-3, when consumed in excess will also be negatively impacted, among other causes the body smelled of fish oil, causing indigestion and bleeding at the time of injury, surgery, or when is attacked by a nosebleed will take longer to heal because the blood clotting process slow [30]. Increase Ratio of omega 3 and omega 6 can increase the risk of obesity [30]. The exact ratio can prevent obesity [31].

7. Formulation of infant biscuit

Besides meeting the ratio of omega-3: omega-6 WHO 1: 5, the use of fatty fish in the manufacture of biscuits using a combination of different doses. Formulation ingredients are made is based on preliminary experiments that have been done repeatedly until the resulting products favored by consumers both in terms of texture, color, flavor, and aroma. Based on the results of preliminary research that has been done obtained formulas in Table 3.

Table 3. Formulation biscuit of infant biscuit

| No | Component | M0 | M1 | M2 | M3 |
|----|-----------|----|----|----|----|
| 1  | Flour     | 260 g | 260 g | 260 g | 260 g |
| 2  | Sugar     | 100 g | 100 g | 100 g | 100 g |
| 3  | Margarine | 25 g | 25 g | 25 g | 25 g |
| 4  | Fish oil combinaitons ( grouper & jambal siam fish) | - | 7 mL | 14 mL | 21 mL |
| 5  | Salt      | 3 g | 3 g | 3 g | 3 g |
In the manufacture of baby biscuits, fish oil is used to meet the standard combination of omega 3 and omega 6 according to the WHO 1:5. Wheat flour as the main carbohydrate source, confectioners' sugar to give it a sweet taste, eggs and skim milk as a protein source. Margarine containing palmitic fatty acids which are relatively high, as well as a source of vitamins and minerals. Cocoa powder to provide flavor, odor, and color. Based on preliminary research results that have been done all formula biscuits have similarities in terms of smell, taste, and color controls. Compared with SNI 01-7111.2-2005 normal and no rancid odor, as well as normal and no rancid flavor, and normal color. Results of research Nazni and Parameshwari [32] states that the addition or substitution of materials in the manufacture of biscuits is not expected to affect the odor, taste and color of the biscuits. In terms of texture treatment of the most preferred by consumers is the addition of 21 ml of fish oil combination that produces a crisp texture when bite. Texture use a combination of oil can affect the texture than the control. According Mervina et al [33], the texture is one of the organoleptic attributes that affect the acceptance of panelists to biscuit. According Rustanti and Nurhidayati [34] organoleptic acceptance fish flour based biscuit formulations did not affect the smell, taste, and color, but the effect on the texture.

The addition of fish oil in the making aimed at reducing the impact of using butter, enriched nutritional value, and creating healthy meals for toddlers. In addition Ruxton et al, [35] suggested that the provision of supplementary food such as biscuits on a baby is one of the efforts to improve consumption patterns at the age of five to address malnutrition.

### 8. Nutritional composition Biscuits

Analysis of the composition of the nutritional value of the biscuits are presented in Table 4. The use of a combination of fish oil to produce 4.42- water content of 5.65%, from 8.40 to 10.88% protein content, fat content from 9.17 to 13.74%, and levels ash from 2.15 to 3.01%. Compared with controls (M0) overall combined use of fish oil can lower water levels, raising levels of protein, fat raise and lower the ash content.

| Treatment | Water (%) | Protein (%) | Fat (%) | Ash (%) |
|-----------|-----------|-------------|---------|---------|
| M0        | 5.35 a    | 7.84 a      | 9.51 a  | 2.05 a  |
| M1        | 5.65 a    | 8.00 b      | 9.17 a  | 2.36 a  |
| M2        | 4.79 b    | 8.48 b      | 13.56 b | 3.01 b  |
| M3        | 4.42 b    | 10.88 c     | 13.74 b | 2.15 a  |
| SNI       | Max 5%    | Min. 6%     | Min. 6% | Max 3.5%|
| (2005)    |           |             |         |         |

Description: The figure followed by the same letter in the same column are not significantly different by Duncan test 5%.

Based on the analysis of variance is no treatment effect on water content. M0 treatment was not significantly different to the treatment of M1, M1 treatment significantly different from the M2 and...
M2 treatment was not significantly different from the M3. The use of fish oil 14 mL has succeeded in reducing the water content of the biscuit. There is a growing trend of high oil dose combination given the lower the water content although M2 to M3 between treatments were not significantly different.

Based on the analysis of variance is no treatment effect on protein content. Treatment significantly different from the treatment M0 M1, M1 treatment was not significantly different from the M2 and M2 treatments significantly different from the M3. The use of a combination of 21 ml fish oil can increase the protein content. Elevated levels of protein in the extraction allegedly because abdominal fat jambal fish conjoined into fish oil temperature are used to 60°C which cause not all of the cell wall in abdominal fat jambal Siam fish broke. The cell walls were not broken still contain protein so that the protein content of the biscuit is increased.

Based on the research results revealed that the best treatment is a combination of fish oil use 21 ml with water content of 4.42% (standard maximum of 5%), 10.88% protein (the minimum standard 6%), fat 13.74% (minimum standards 6 %), carbohydrates 69.91% (maximum 70% standard), and ash 2.15% (maximum standard of 3.5%). Carbohydrate levels are calculated based on the reduction (by difference). Biscuits produced meets the quality requirements SNI baby biscuits 01-7111.2-2005. In addition was successful combination fish oil increases fat content of biscuits. According Thomas et al, [36] reported that the addition of Virgin Coconut Oil (VCO), which contained 1.18% linoleic acid can increase the fat content of the biscuit on top SNI is 19.39 to 28.54%. The content of fatty acids best biscuits (21 mL with addition combination of fish oil. The results of the analysis of the content of fatty acids the best treatment biscuit can be seen in Table 5.

Table 5. The content of fatty acids the best treatment biscuits formulation (21 mL with combination of fish oil)

| Fatty acids te best biscuit formulation | % FA/Total FA | Detection |
|---------------------------------------|--------------|-----------|
| 1. Saturated Fatty Acid                | 47.23        |           |
| 2. Unsaturated Fatty Acid             | 52.77        |           |
| MUFA                                  | 18.13        |           |
| PUFA                                  | 34.64        |           |
| Omega 6                               | 14.20        |           |
| 9, 12,15-oktadetkaterinoat acid (C 18:3, n-6), alpha linolenat/ALA, n-6 | 2.11 | |
| 5,8,11,14-eikosatetranoto acid (C 20:4 n-6), asam arakidonat/AA n-6 | 2.05 | |
| 9,12-oktadekadienoat acid ( C 18:2, n-6), asam linoleat/LA, n-6 | 10.04 | |
| Omega 3                               | 3.25         |           |
| 9, 12, 15-oktadetkaterinoat acid (C 18:3, n-3), alpha linolenat/ALA, n-3 | 0.88 | |
| 9,12, 15-eikosanoat acid (C 20:3 n-3), arhacidonate acid/AA, n-3 | 0.76 | |
| Eikosapentanoat acid/EPA (C 20:5 n-3) | 1.01         |           |
| Dokosaheksanoat acid/DHA (C22:6, n-3) | 0.60         |           |

Table 5 viewable content of fatty acids biscuit are: 47.23% saturated fatty acids, unsaturated fatty acids 52.77%, monounsaturated fatty acids 18, 13%, polyunsaturated fatty acids 34.64%, omega 3 and omega 6 3.25% 14.20%. Ratio of omega 3 to omega 6 oils grouper is (1: 4.3). Based on the ratio of omega 3 and omega 6 are contained in a biscuit 1: 4.3 (above the WHO standard), it means biscuits produced meets the standards of omega 3 and omega 6. According Barlina [37] suggest that the omega 3 needs to be fortified on materials food child malnutrition.

9. Conclusion
Based on the research results revealed that the use of fish oil combined effect on the nutritional value of the biscuits. The best treatment is a combination of fish oil use 21 ml with water content of 4.42% (standard maximum of 5%), 10.88% protein (the minimum standard 6%), fat 13.74% (minimum standard 6%), carbohydrates 69, 91% (maximum 70% standard), ash 2.15% (maximum standard of 3.5%), and the ratio of omega 3 and omega 6 is 1: 4.3.

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