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by E Hastarini , M Nabilla , A Permadi C P Adi, R Nurbayasari

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Characteristic of margarine with ingredient mixed of catfish (*Pangasius* sp.) oil and vegetable oil

E Hastarini^{1*}, M Nabilla², A Permadi², C P Adi², R Nurbayasari¹

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¹Research centre for Marine and Fisheries Product Processing and Biotechnology, Jakarta, Indonesia

²Marine and Fisheries Polytechnic, Karawang, West Java, Indonesia

*Corresponding author e-mail: emahastarini@gmail.com

Abstract. The use of fish oil is still not optimal whether it is crude or refined fish oil. One of the alternative uses is processing into food ingredients or as an ingredient for food product enrichment. The purpose of this study was to evaluate the effect of the combination of catfish oil and vegetable oil on the characteristics of margarine. Refined catfish oil was analyzed for peroxide content, free fatty acids, iodine number, and saponification number. Margarine products are made with the main ingredient of stearin and a mixture of refined catfish oil and corn oil with a ratio of 100:0; 75:25 and 50:50. The analysis carried out included proximate, peroxide value, color, sensory and microbiology. The results showed that refined catfish oil had a peroxide value of 1.74%, free fatty acids 0.21%, iodine value 50.48% and saponification value 102.10%. Based on the quality characteristics of margarine, the treatment with a ratio of 50:50 has the best result with a moisture content of 9.03%, 78% fat content, 1.75% peroxide value and have a bright yellow color, conform the margarine standard SNI 01-3541-2002. The results of the analysis of total plate count (ALT) ranged from 1.0×10^2 to 4.0×10^2 , *Staphylococcus aureus* showed that the colony did not grow up to 4.0×10^1 . The hedonic test results for the color, taste and aroma of the margarine, panelists preferred the fish oil concentration 50:50 while the panelists preferred margarine with the addition of fish oil 75:25 for the texture.

1. Introduction

The by-product of the catfish fillet industry, namely belly meat, has a fairly high fat content of about 28.52% (Hastarini et al., 2012) so that it has the potential to be extracted into fish oil. The utilization of fish oil is still not optimal, either crude or purified fish oil. One of the alternative uses is to be processed into food ingredients or enrichment and fortification materials. Oil-based processed products are generally emulsion products, either in the form of oil in water (o/w) or water in oil (w/o).

One of the popular emulsion products is margarine. Margarine is a food product in plastic form which is generally made from vegetable or animal fat raw materials (Ketaren, 2005). According to ((SNI, 2002) Margarine is a food product in the form of a solid or semi-solid emulsion made from vegetable fat and water with or without the addition of other permitted ingredients. In general, margarine production is vegetable oil which is high in saturated fatty acid content, so research is needed on the use of animal oil in this case is fish oil as an ingredient for making margarine where fish



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oil has high unsaturated fatty acid content. The high fat content of this margarine can be used as an alternative potential for developing catfish oil-based products that are rich in fatty acids and vitamins so that a final product with high nutrition is obtained.

Margarine is one of the bread spread products which is also very popular and widely used. Margarine is an artificial butter consisting of vegetable and animal oils. Margarine contains at least 80% fat by weight, the rest (approximately 17-18%) consists of skim milk derivatives, water, or liquid soy protein and 1-3% is salt, which is added as a flavor (Noraini and Teah, 1994; Hui, 1996; O'Brien, 2004; Hasibuan, 2009; Sahri and Idris, 2010). Margarine is a type of emulsion. The most common emulsion system is a mixture of oil and water. Oil and water are immiscible liquids because they have different polarity and specific gravity properties. Water is polar and has a greater specific gravity than oil (Suryani et al., 2002). To avoid trans fatty acids that endanger health from margarine, a blending process can be carried out between solid, semisolid and liquid oils (Hui, 1996; Berger & Idris, 2005; O'Brien, 2004). One type of solid oil is stearin fraction palm oil (Hasibuan et al., 2009). Stearin is a solid fraction of palm oil and is a coproduct or by-product obtained from palm oil together with the olein fraction. Stearin has a slip melting point in the temperature range of 45-56°C, while olein has a temperature range of 23°C. The impure fractionated stearin is a mixture of various saturated fatty acids and unsaturated fatty acids with the most component being palmitic acid (Ketaren, 2008). Stearin has plastic properties and freezes at room temperature due to its high palmitic fatty acid content. Stearin is generally used as a raw material for the manufacture of shortening, margarine, and pasta (Ketaren, 2008).

Margarine can basically be divided into two types according to its use, namely margarine for household use and margarine for industrial purposes. One of the properties that must be possessed by margarine for household purposes is its plasticity and easy to melt at body temperature and has good greasing power. The main quality requirement for margarine is the fat and water content in margarine because it will affect the texture quality of margarine. Margarine quality requirements are a guideline used in the resulting product which is useful for determining the quality of the product so that it is safe for human consumption. The margarine quality requirements are regulated in SNI 01-3541-2002.

Corn oil is an oil that is rich in unsaturated fatty acids, namely linoleic and linolenic acids. Both of these fatty acids can lower blood cholesterol and reduce the risk of coronary heart attack. Corn oil is also rich in vitamin E which functions for stability against rancidity (Puspaningrum, 2015). The stable and easily compacted properties of corn oil provide an advantage for producers to diversify or develop corn oil into other forms not only in liquid form (Hongwei Si, 2014). Based on the content of corn oil, many people choose it because corn oil does not contain carbohydrates and protein and contains vitamins, especially vitamin E.

Good quality fish oil must have levels of free fatty acids, impurities and water, levels of oxidation, color and metal content that do not exceed the maximum limit set based on fish oil standards (Estiasih, 2009). Pure oil standards according to the International Fish Oil Standard (IFOS), namely peroxide value <3.75 meq/kg, anisidin number <15 meq/kg, free fatty acid content <2%, total oxidation number <20 meq/kg.

The by-products of the catfish filet processing industry by 55% such as belly meat, entrails, abdominal fat, tailbone head, and trimming have not been used optimally. The high fat content in almost all parts of the body makes this catfish a potential source as animal oil that can be applied to fat/oil-based food products such as margarine. So far, the fats that make margarine generally come from vegetable oils, so the issue of trans fats which are harmful to health because of the high saturated fatty acids in vegetable oils is often heard. This study aims to see the effect of using catfish oil as an ingredient on the quality of the margarine produced. The use of catfish oil will be combined with corn oil to improve the overall quality of margarine.

2. Materials and Methods

2.1. Ingredients

The raw materials used are stearin from palm oil and corn oil and refined fish oil extracted from the belly of catfish. The chemicals used include aquades, 8% NaCl, 1N NaOH, bentonite, BHT, soy lecithin, salt, beta-carotene. Materials for chemical analysis are 37% HCl, aquades, ether, H₂SO₄, catalyst grains, 0.1 N KOH, ether: 95% ethanol, phenolphthalein, chloroform, wajs, KI, starch, 0.5 N HCl, ethyl alcohol, acid acetate:chloroform (3:2), saturated KI, Na₂S₂O₃ and pH paper. Materials for microorganism analysis are butterfield's phosphate buffered (BFP), Plate Count Agar (PCA), aquades, BPA, egg yolk and BFP.

2.2. Equipment

The tools used in the study were fish oil extractor, glass separatory funnel, thermometer, scales, glass bottles, stirrer, measuring cup, meat cutter, hot plate, hand mixer, centrifuge. Tools for testing include crucibles, desiccators, ovens, furnaces, erlenmeyer, scales, measuring pipettes, beakers, soxhlet, kjeltec, coloni counters, colorimeters.

2.3. Research Method

In this study, there is two step of process: (1) characterization of the raw materials used, including belly meat and catfish oil, (2) manufacture of margarine by combination treatment of fish oil concentration with vegetable oil (com) to obtain a formulation with specifications according to the requirements of the Indonesian National Standard.

(1) The process of extracting catfish oil was carried out using the method of Hastarini et al., 2012. The crushed belly meat was added with water in a ratio of 1: 3 and heated at a temperature of about 70 C for 30 minutes. After boiling, filtered with a cloth to obtain a liquid yield. The liquid obtained is still in the form of an emulsion, which is a mixture of oil, water and solids, so a separation process is carried out to separate the oil from other ingredients. The solid obtained from the filtering is pressed, then the liquid obtained is mixed in the separation process.

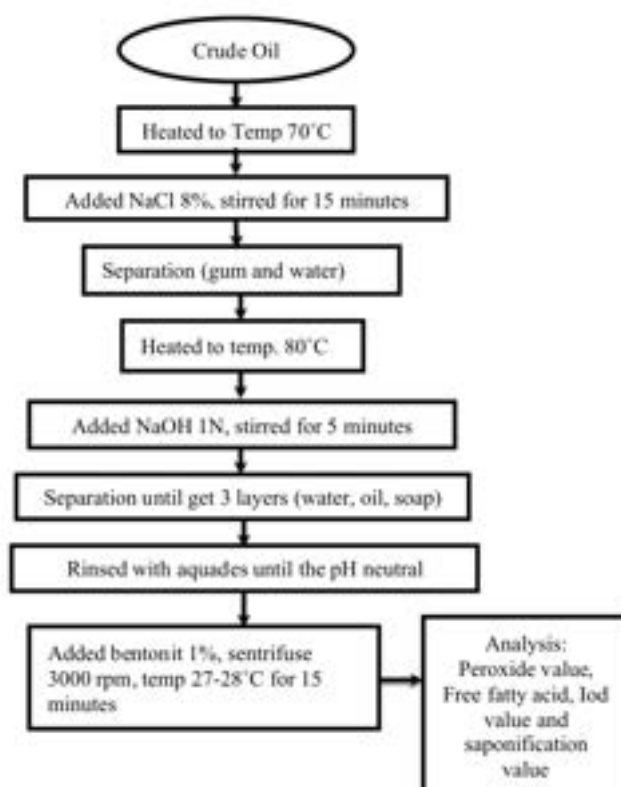


Figure 1. Refining process of catfish oil (Hastarini *et al.*, 2012 mod.).

The separation process is carried out using a separatory funnel until the oil and water are completely separated. The results obtained in the form of crude catfish oil. Furthermore, crude catfish oil was purified using the modified Hastarini *et al.*, 2012 method. The crude fish oil refining stage can be seen in the figure 1. Fish oil characterization was carried out including the number of peroxides, free fatty acids, iodine and saponification (AOAC 2005). (2) The process of making margarine is carried out using a blending method between the oil phase and the water phase based on the modified Dewi *et al.*, 2012 method. In the manufacture of margarine, the right composition is determined for the ingredients for making margarine with the addition of oil with different concentrations. The composition of margarine can be seen in the table below.

Table 1. Margarine formulation.

Ingredients	Concentration
Stearin	48%
Catfish oil and corn oil	30%
Brine	10%
Salt	2%
Flavour	8%
Lecithin	1%
BHT	0,5%
Beta carotene	0,5%

Based on the formulation, the amount of catfish oil and corn oil is 30% of the total amount of ingredients needed in the manufacture of margarine, with a combination ratio of catfish oil to corn oil treatment of:

KM1 = 50% : 50%
KM2 = 75% : 25%
KM3 = 100% : 0%

The process of making margarine includes mixing the water phase and the oil phase which is carried out by heating 60°C and stirring using a hand mixer for 5 minutes. Stirring was carried out for 10 minutes until homogeneous between the oil and water phases. Then added flavouring and beta carotene as a colorant. Stir until the color is evenly mixed about 5 minutes of stirring. Furthermore, tempering for 2x24 hours in temperature 5-7 °C, until the dough thickens. After thickening, the dough is poured into a plastic cup container and tightly closed, then the dough is stored in the chilling room. Packaging is done by using a plastic cup container covered with a layer of aluminium foil.

The analysis includes color analysis using a Chromameter; chemical analysis (proximate SNI 2006 method, peroxide number AOAC 2005 method), sensory analysis (hedonic quality test), microbiological analysis (TPC and *Staphylococcus aureus*).

3. Result and Discussion

3.1. Raw Material Characteristic

The raw material used in the manufacture of fish oil is catfish belly which is a by-product of processing catfish fillets. Belly Patin comes from a company in the Karawang area. Belly catfish is packaged using HDPE plastic and then stored in cold storage at -18°C until used. Sample preparation was done by cutting the belly of frozen catfish into small pieces using a bandsaw into dice. Belly that has been cut is then crushed using a silent cutter for ± 10 minutes. Proximate results of belly patin meat can be seen in the table 2.

Table 2. Proximate analysis of catfish belly flap as raw material.

Content	(%)
Moisture	10,69
Ash	0,08
Fat	28,18
Protein	21,69

The results of the proximate analysis showed that the belly of catfish has a high fat content because the belly flap is the lower part near the stomach so it looks enlarged because of the large fat deposits. The high fat content of the belly flap has the potential to be used as a raw material for the manufacture of fish oil. The characteristics of crude and refined fish oil from the belly of catfish are shown in the following table.

Table 3. Characteristic of crude oil and refined catfish oil from catfish belly flap.

Parameters	Crude Oil	Refined Oil
Peroxide value (meq/kg sampel)	10,84	1,74
Free fatty acid (%)	0,83	0,21
Iod number	56,20	50,48
Saponification number	106,83	102,10

The results of peroxide analysis showed that after the purification process there was a significant decrease from 10.84% to 1.74%. Oil peroxide levels can decrease due to heating factors and the addition of adsorbent material in the form of bentonite, Suseno et al. (2013) stated that the bleaching process or with the addition of bentonite can reduce the peroxide value in the oil. Bentonite can absorb odors, colors, and oxidation products so that bentonite can reduce oil peroxide levels. The peroxide number indicates the quality of the oil, the lower the peroxide number, the better the quality of the oil. The initial stage of the oxidation reaction is to form free radical compounds which will then produce peroxide compounds when reacted with oxygen (Andarwulan, 2011). The peroxide value is the most important number in determining the degree of oil damage. Damage to fat or oil can occur due to the oxidation process by oxygen from the air to unsaturated fatty acids in oil that occurs during storage or processing (Panagan et al, 2012).

The content of free fatty acids can be triggered due to the high temperature used during the purification process. The free fatty acid content in Figure is 0.21%, the result is lower than crude oil which is 0.83%, the decrease in free fatty acid levels in pure oil is due to the addition of 1N NaOH during the purification process with the neutralization stage, heating so that the NaOH can reduce the levels of free fatty acids contained in fish oil. These results are in accordance with Suseno's research (2017) which states that the degumming stage followed by neutralization can reduce free fatty acid levels in the oil while the degumming stage can only separate gum or mucus consisting of protein, phosphatides, water, carbohydrates without reducing the amount of acid, fat free. During the neutralization process, NaOH was mixed with oil at a temperature of 80°C and then neutralized by rinsing using warm water until the pH was neutral so that the process could reduce the free fatty acid levels in the oil (Yulianti et al 2012).

Iod number analysis was carried out to determine the degree of unsaturation of the fatty acids that make up catfish oil. The number of iodine bonds indicates the number of double bonds contained in the oil. From the results of the test the iod number in purified catfish oil was 50.48. The iodine number is determined by the level of unsaturation of the oil, if the level of unsaturation is high, it will bind to larger amounts of iodine so that the iodine number is greater. The high level of unsaturation of the oil causes the oil to be more easily oxidized.

The analysis of the saponification number is the number of milligrams of KOH required to saponify one gram of oil or fat. The results of the analysis of the saponification number obtained a value of 106.83-102.10. The number of saponification in catfish oil is quite high to the high content of fatty acids with short carbon chains. According to Saptu (2011), the higher the saponification number of the oil, the better the quality of the oil because if the carbon chain is shorter, the fatty acids will be easily metabolized by the body.

3.2. The Making of Margarine

Research on the manufacture of margarine was carried out to obtain the best combination of concentration of catfish oil and vegetable oil (corn oil). Saturated fatty acids in corn oil are 13% while unsaturated fatty acids are 86% (Kataren, 1986). In the process of making margarine, mixing is carried out between the oil phase and the water phase with the addition of an emulsifier which aims to make the margarine produced can be homogeneous between oil and water and margarine gets melted in the mouth. The manufacture of margarine is also carried out with a cooling phase which aims to form good margarine crystals because if the cooling process is not carried out the resulting margarine has a dense texture. According to Rizki (2016), the margarine emulsion crystallization process generally consists of a cooling process and the application of mechanical force to the emulsion. In the cooling process, the emulsion will go through a sequence of cooling steps gradually so that crystallization will occur at different temperature levels. Cooling also aims to remove the heat formed during the crystallization process.



Figure 2. The making of catfish margarine.

3.2.1. Moisture content. Moisture content is the amount of water contained in food. Water in foodstuffs is an important component because it can affect the appearance, texture, and taste of foodstuffs. Determination of water content in a food product needs to be done because it affects the stability and quality of the product itself. From the results of the study obtained the water content of each margarine product with a different concentration of water content. The results of the moisture content can be seen in Figure 3.

The results of the analysis of water content in margarine with a concentration treatment of 50:50% (KM1) showed an average result of 9%. Margarine with 9% water content produces a soft margarine texture but still a little gritty. Margarine with a concentration of 75% produces a soft texture, while for margarine that has a concentration of 100% fish oil, the texture of margarine is rather hard. The addition of 1% lecithin as an emulsifier can reduce the water content in margarine. Lecithin has a higher hydrophilic group than the hydrophobic group. This hydrophilic group can bind water so that the water which was originally free water becomes unable to move freely because it is bound by the hydrophilic group of the emulsifier. The water content in a food ingredient needs to be determined, because the higher the water content in the food, the more likely the food will be damaged so that it cannot last long (Winarno, 1997).

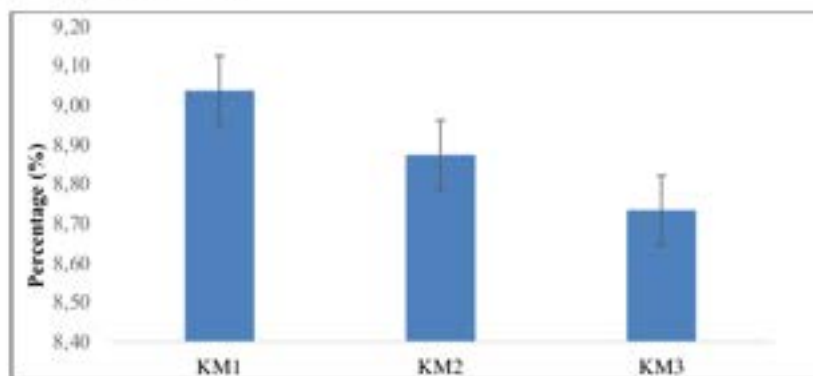


Figure 3. Moisture content analysis results.

The results of the analysis of the moisture content obtained in this study of about 9%, these results are included in the SNI 01-3541-2002 standard that the maximum moisture content of margarine is

16%. The results of this moisture content analysis are in line with the research of Lestari et al., 2010 where the catfish margarine produced has a moisture content value of 14.26%-15.16%.

3.2.2. *Fat content.* Fat content is the most important parameter in margarine because the largest component of margarine is fat. In general, margarine contains 80% fat which is composed of vegetable fat. The results of the analysis of the fat content of margarine in this study can be seen in Figure 4.

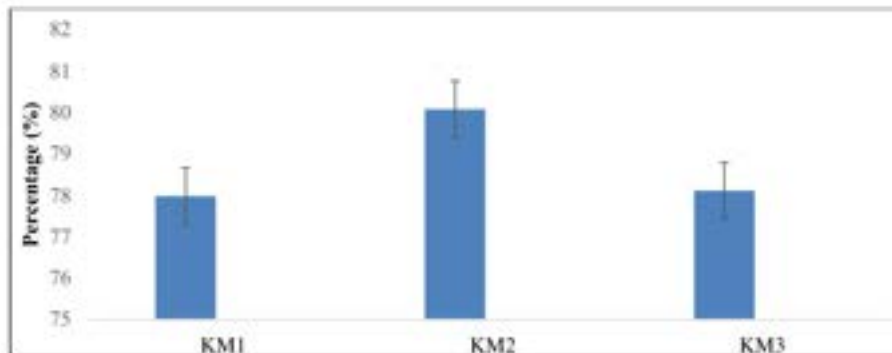


Figure 4. Fat content analysis of margarine.

The results of the analysis showed that the fat content of the 50%, 75% and 100% fish oil concentration treatments were not significantly different. The range of margarine fat content in this study was 78% to 80.08%. The fat content of margarine based on the SNI 01-3541-2002 standard states that the maximum fat content is 80%. The fat content in margarine depends on the ratio of oil / fat and water in the formula. Therefore, in the manufacture of margarine, it is necessary to pay attention to the composition of oil/fat, water and lecithin (Jatmika, 1966).

3.2.3. *Peroxide value.* Analysis of the peroxide value in margarine showed that the results were not significantly different between treatments, namely 1.52 meq/kg sample to 1.75 meq/kg sample. The results of the peroxide number can be seen in Figure 5.

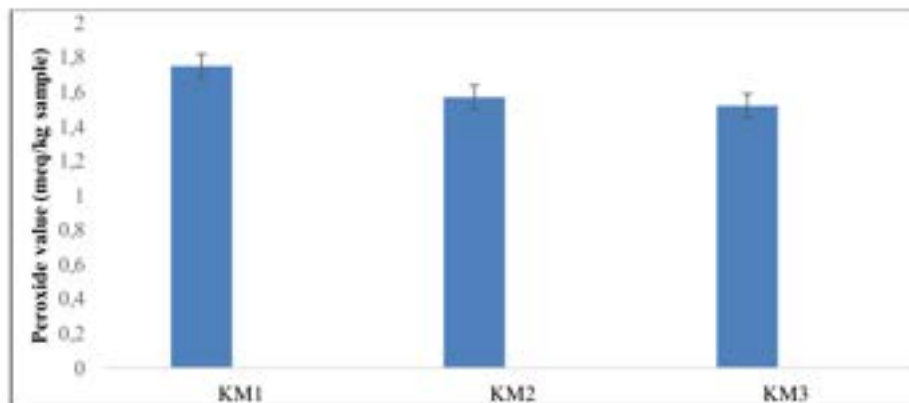


Figure 5. Peroxide value.

This peroxide value is quite low and is still below the standard peroxide value of 20 meq/kg sample. The results of the research of Ramadhana et al., 2016 showed that the peroxide value of margarine products in various proportions of tuna fish oil and palm stearin with the addition of BHA antioxidants ranged from 11.10 – 18.56 meq/kg. According to Sudarmaji (1996), the peroxide formation process is accelerated by the presence of light or heat, so if margarine is stored at room temperature, it is necessary to pay attention to closed and opaque packaging. The high fat content in margarine also makes this product susceptible to oxidation and easily rancid. Based on the pure oil standard according to the International Fish Oil Standard (IFOS), the peroxide value is <3.75 meq/kg (Estiasih, 2009).

3.2.4. Color analysis. The resulting margarine was analyzed for color using a Chromameter. The color notation system is characterized by three color parameters expressed by the notation L*, a* and b*. The value of L* represents brightness (range of values 0=black and 100=white), +a* represents a reddish color and -a* represents a green color, +b* represents a yellow color and -b* represents a blue color. The results of margarine color analysis can be seen in Table 4.

Table 4. Colour result analysis.

Treatment	Colour Content		
	L*	a*	b*
KM1	83,83	4,42	31,58
KM2	82,84	6,26	36,90
KM3	82,68	5,48	33,09

Based on the results of the color analysis, it shows that the catfish margarine has a high brightness value, but at each concentration it has a different brightness value. The highest brightness value in margarine with a concentration of 50:50% is due to the addition of corn oil which has a bright yellow color while catfish oil has a color that tends to be clear yellow compared to corn oil. The high brightness value in catfish oil margarine is because the catfish oil used has a high brightness value (Hastarini, 2012). The brightness value produced by catfish oil is due to the addition of an adsorbent at the purification process, where the adsorbent will absorb unwanted colors. The results of the analysis showed a positive b* value for all treatments which indicated the color of the product had an intensity of yellow with a value range of 31.58 to 36.90. The yellow color is obtained from the constituent oil of margarine derived from refined catfish oil and corn oil, both of which are yellow. The brightness and yellowish color are caused by the addition of 0.5% beta-carotene in each margarine formulation.

3.2.5. Sensory evaluation. Sensory test results based on hedonic quality showed that the color of margarine with fish oil concentration of 50% obtained the highest value and the lowest value with the addition of 100% fish oil. The results of the hedonic test can be seen in Table 5.

Table 5. Hedonic test results for margarine products.

Treatment	Hedonic Quality			
	Colour	Aroma	Texture	Taste
50:50%	3,73	3,00	3,33	3,00
75:25%	3,67	2,60	3,60	2,40
100%	3,27	2,20	2,67	2,00

The results of the highest color analysis at 50:50% margarine concentration because the yellow color of margarine is supported by the addition of the most bright yellow corn oil. As for the lowest color at a concentration of 100% because the panelists considered that the margarine had a slightly pale color so that the color was influenced by the concentration of added corn oil.

The results of the taste analysis showed that the treatment with a concentration of 50:50% had the highest value of 3.00 because the fish oil taste was not too fishy and the margarine tasted salty like commercial margarine. The taste of margarine with a concentration of 100% and 75% fishy taste is still felt in margarine products.

Likewise for the aroma parameter, the panelists gave the highest value at the 50% concentration treatment while at the 100% and 75% concentration treatment the panelists gave the lowest value. The fishy smell that still appears in the final product is due to the fact that during the fish oil purification process it has not been completely deodorized so that the fish oil smell is still attached to refined catfish oil. Aroma is one of the important factors for consumers in choosing the most preferred food product. The aroma of food ingredients is a certain component that has several functions in food, namely improving and making it more acceptable (Winarno, 1997).

The texture of margarine is tested by spreading margarine on bread. For the power of smearing margarine, panelists prefer margarine with a concentration of 75:25% because margarine when spread on bread produces a long spread and sticks to the bread (plastic). According to Saparianti (2013) the plasticity of margarine is due to the effect of saturation of fat contained in the margarine. Texture is the nature of the material or product that can be felt by touching the skin or tasting. There is a direct relationship between the chemical composition of food, its physical structure and its physical or mechanical properties (Kartika et al., 1998).

3.2.6. *Microbiology*. The total plate count (TPC) is one of the methods for calculating the number of microbial colonies both bacteria contained in the test sample. Testing of *Staphylococcus aureus* bacteria was also carried out to meet SNI standards. The results of TPC and *S. aureus* testing performed on margarine products are shown in Table 6.

TPC test results on margarine showed results ranging from $1,0 \times 10^1$ until $4,0 \times 10^1$ which still met the maximum margarine quality requirement of 10^2 (SNI 01-3541-2002). The results of a similar study from Lestari et al., 2010 showed that the TPC results in catfish margarine were below 10^3 and there was no contamination of pathogenic bacteria such as *E. Coli*, *Salmonella*, *S. aureus* and *Enterococci*. Margarine products with fish oil formulation can be said to be suitable for consumption. The TPC content contained in the product is influenced by several factors, including the raw materials used and the place or handling during the product manufacturing process (Junianto, 2003). The results of the *Staphylococcus aureus* test showed that the colonies did not grow up to $4,0 \times 10^1$ which still met the requirements according to SNI margarine, namely a maximum of 10^2 .

Table 6. TPC test results on each margarine formulation.

Treatment	TPC	<i>Staphylococcus aureus</i>
50:50%	$1,0 \times 10^1$	No colonies grow
75:25%	$2,0 \times 10^1$	$1,0 \times 10^1$
100%	$4,0 \times 10^1$	$4,0 \times 10^1$

4. Conclusions

The test value for refined catfish oil has a peroxide value of 1.74%, FFA 0.21%, Iod 50.48% and saponification value of 102.10% the results meet the fish oil standards required by IFOS (International Fish Oil Standard). The treatment with a ratio of 50:50 has the best result with a moisture content of 9.03%, fat 78%, peroxide value 2.00% and a bright yellow color, meeting the margarine standard of SNI 01-3541-2002. The results of TPC analysis ranged from $1,0 \times 10^1$ until $4,0 \times 10^1$, *Staphylococcus aureus* showed that the results started not growing colonies up to $4,0 \times 10^1$ which still met the requirements according to SNI margarine, which is a maximum of 10^2 . The hedonic test results for color, taste and aroma, panelists prefer treatment with fish oil concentration: 50:50% corn oil, while for texture, panelists prefer margarine with 75:25% fish oil addition.

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