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MARGARINE AS A VALUE-ADDED PRODUCT FROM FISH OIL, A BY-PRODUCT OF LEMURU FISH CANNERIES

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ABSTRAK: Lemuru fish (*Sardinella lemuru*) is rich in omega-3 so it is widely used as raw material for fisheries production such as fish canning which produces by-products in the form of crude fish oil which needs to be purified by adding adsorbents so that it becomes pure fish oil. The adsorbent that can be used in the purification of fish oil is activated charcoal. Fish oil can be used as raw material for making added value products such as margarine. The aim of this study is to analyse the characteristics of margarine as a value-added product made from fish oil. Research shows that purification with activated charcoal produces fish oil with %FFA and PV that are in accordance with IFOS. Fish oil margarine in this study showed organoleptic value of colour 7.4 and aroma 7.07, emulsion stability 100%, %FFA content 0.28 ± 0.08, PV 1.6 ± 0.173, and higher 0.39% EPA and 2.58% DHA content compared to commercial margarine. In previous study showed margarin from sardine fish oil contains 19.5% EPA and 10.5% DHA which is also not found in commercial margarine from palm oil. The difference in EPA and DHA content between this study and previous study may be due to the fact that the fish oil in this study was obtained from by-products of canning processing with high temperatures which may cause a decrease in fatty acid levels due to protein denaturation.

Keywords: Fish Oil Refining, Activated Charcoal, Fish Oil Margarine.

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INTRODUCTION

The Lemuru fish (*Sardinella lemuru*) has a high omega-3 content of 36.96%, which is attributed to its food source, marine phytoplankton. According to Suprakto et al. (2022), marine phytoplankton is the primary producer of omega-3. The fatty acid content of Lemuru fish is 19.37%, with the most abundant types being Eicosapentaenoic Acid (EPA, C20:5ω-3) at 14.46% and Docosahexaenoic Acid (DHA) at 4.60%, as reported by Suseno et al. (2014). Lemuru fish is commonly used as raw material in various processing industries, such as canning. The canning process produces liquid waste from washing and heating, which contains crude fish oil. This oil is rich in omega-3 Polyunsaturated Fatty Acids (PUFA), specifically eicosapentaenoic (EPA) and Docosahexaenoic (DHA) (Damaiyanti et al., 2021; Safnowandi, 2021). Ratih et al. (2016), reported that fish oil by-products from fish canning contain approximately 29,68% fatty acids, with 15% EPA and 11% DHA.

The by-products of lemuru fish canning must through a purification process that includes degumming, neutralisation, and bleaching stages. The aim of this process is to remove impurities such as non-triglyceride compounds, dyes, odours, and toxic compounds. The degumming stage in the fish oil purification process is intended to separate phospholipid compounds, while the neutralisation stage aims to neutralise fatty acids and reduce the oil's acidity. The objective of the bleaching stage in fish oil purification is to absorb contaminants and pigment substances. This is achieved by adding adsorbent materials, such as activated charcoal (Suseno et al., 2014), which actively filter unwanted colours, aromas, sulfur compounds, heavy metals, and oxidation states. Activated charcoal is an inexpensive material. It is a highly developed form of coconut shell charcoal with a large surface area that can be easily activated physically and chemically. This makes it an effective adsorbent for purifying fish oil (Waluyo et al., 2020).

Refined fish oil is widely used by the public as a dietary supplement. However, it has not been widely diversified for use in other processed products. Research has shown that margarine derived from catfish oil has a higher nutritional value than commercial margarine derived from vegetable oil (Hasanah et al., 2017). Margarine is a water-in-oil (W/O) emulsion product made from vegetable or animal oils. Margarine typically contains between 80-90% fat, with milk fat content limited to no more than 3%.This emulsion is capable of maintaining a solid form at 20°C.The manufacturing process involves mixing lipids and water to create a W/O emulsion, with at least 80% of the mixture consisting of fat and the remaining 20% consisting of skimmed milk, whey, or an aqueous solution containing salt and flavourings (Guillen et al., 2016).

The difference between butter and margarine lies in the source of the fats they contain. Butter is made from milk fat, while margarine was originally made from vegetable oil (Israel, 2015). According to Srinovia (2016), butter contains high levels of saturated fatty acids that can increase cholesterol in the body. Therefore, margarine was developed as a product that could replace butter using oil with a high content of unsaturated fatty acids. Vegetable oil is typically used as the raw material for making margarine due to its high unsaturated fatty acid content. However, fish oil, which is rich in unsaturated fatty acids such as EPA and DHA (Damaiyanti *et* al., 2021), can also be used as an alternative ingredient for margarine. The aim of this study is to analyse the characteristics of margarine as a value-added product made from fish oil.

METHOD

Tools and Materials

The instruments used in this research were an oven, a 220g analytical balance, a stopwatch, a 100ml beaker, a 250ml beaker, a 500ml beaker, a 50ml measuring cup, a 100ml measuring cup, a 10ml test tube, a test tube rack, a 1ml graduated pipette, a 5ml graduated pipette, a 10ml graduated pipette, The equipment used includes a 5 mL and 10 mL pipette, 250 mL and 100 mL Erlenmeyer flasks, a porcelain cup, a baking sheet, a watch glass, a bulb, a glass stirring rod, a glass funnel, a fume hood, a thermometer, a dropper pipette, an iron spatula, a burette, a stand clamp, a hot plate, a magnetic stirrer, and a refrigerator. This study utilises crude oil by-products from canning of lemuru fish at a factory in Banyuwangi. The materials used in this study included HVS paper, activated charcoal, distilled water, aluminium foil, NaCl 30%, NaOH 9.5%, citric acid 3%, glacial acetic acid, chloroform, potassium iodide (KI) solution, Na2S2O3 0.01 N, phenolphtalein (PP) indicator, ethanol 95%, KOH 0.1 N, n-hexane, water, Virgin Coconut Oil (VCO), salt, flavourings, lecithin, and BHA.

Research Methods

Purification of Fish Oil

The crude fish oil that was used was the fish oil by-product from the pre-cooking process of fish canning. The crude fish oil was purified using a modification of the research method of Aini et al. (2021). The oil purification stage consists of three stages, namely degumming, neutralisation and bleaching with activated carbon adsorbent. The degumming stage was carried out by heating 100 g of fish oil at 70 °C for 1 min using a hot plate and stirring rod. To the heated oil was added 3 mL of 3% citric acid and then heated again at 70°C for 1 minute. The heated oil was allowed to stand at room temperature and then centrifuged at 2600 rpm for 10 minutes.

The degummed oil was diluted with 9.5% NaOH to 50.3% (w/b) and then heated at 65°C for 20 minutes with stirring. The oil was cooled to room temperature and centrifuged at 2600 rpm for 10 minutes. The oil was rinsed three times with distilled water to remove soap. The oil obtained was then treated with activated charcoal. Fish oil added with activated charcoal adsorbent was heated at 80°C for 20 minutes with stirring. The oil was cooled to room temperature and then centrifuged at 2600 rpm for 10 minutes to obtain pure fish oil. The pure fish oil obtained was also tested for fatty acid profile, free fatty acid number, bioavailability and lipid content.The raw material for margarine production is fish oil that has undergone the purification process and has been tested for free fatty acid content (%FFA) and peroxide number (PV).

Fish Oil Margarine Formulation

Fish oil margarine was produced using a modified version of method, which consisted of 48% fish oil, 32% Virgin Coconut Oil (VCO), 16% water, 2% salt, and 0.2% lecithin. The organoleptic characteristics of fish oil margarine were evaluated based on SNI 3541:2014. The evaluation included colour and aroma parameters, free fatty acid value (%FFA), peroxide number (PV), emulsion stability (Yasumatsu et al, 1972), and EPA and DHA content, which were compared to those of commercial margarine.

Analytical Methods for Fish Oil and Margarine

1) Free Fatty Acid (%FFA)

Free fatty acids in fish oil were analysed by the aocs ca 5a-4 method with the modification of Suseno et al. (2013). Fish oil was weighed as much as 10 grams and then added with 95% neutral ethanol 25 ml in a 250 ml erlenmeyer. Fish oil was heated in a waterbath for 10 minutes and then the mixture was given a 2 ml drop of pp indicator. The mixture was shaken and titrated with 0.1 n koh until a pink colour appeared that did not disappear for 10 seconds.

$$\% \text{ FFA} = \frac{A \; x \; N \; x \; M}{10 \; G}$$

Description:
A : Volume of KOH titration (mL);
N : Normality of KOH;
G : Sample weight (gram); and
M : Molecular weight of the dominant fatty acid (oleic acid =282.5 g/mol).

2) Peroxide Value (PV)

The peroxide number (pv) was analysed by the aocs cd-8b-90 method by determining the peroxide value. The peroxide value indicates oil damage calculated by the principle of titration of iodine released by potassium iodide compounds by peroxide using thiosulfate solution as titrant and starch solution as an acid indicator. This method is used to analyse all substances that oxidise potassium iodide under acidic conditions. Pv analysis was carried out in accordance with sni 8392-2: 2018. Fish oil samples as much as 5 grams were put in a 100 ml erlenmeyer flask then added with 18 ml glacial acetic acid and 12 ml chloroform in a ratio of 3:2. The mixture was then added with 0.5 ml saturated potassium iodide (ki) solution and allowed to stand for 1 minute then carefully homogenised to mix, after which it was added with 30 ml distilled water. The next step was titration using 0.01 n sodium thiosulfate (na2s2o3) solution until it turned yellow then added 0.5 ml 1% amylum indicator which changed the colour of the solution to light blue which marked the release of iodine from the chloroform layer. Titration is continued until the blue colour in the solution disappears.

$$\text{PV} = \frac{S \; x \; M \; x \; 1000}{sample \; (g)}$$

Description:
S : Volume of sodium thiosulfate (mL); and
M : Concentration of sodium thiosulfate (0.01 N).

3) Emulsion Stability (%) (Yasumatsu et al., 1972)

The emulsion stability test was conducted to determine the stability of the emulsion formed between oil and water. Margarine was heated at 80°C for 30 minutes, then 10 ml was taken and centrifuged with a 10 ml scaled tube at 2700 rpm for 10 minutes. The volume of the mixture that forms an emulsion is measured by the emulsion stability formula:

$$\text{Emulsion Stability (\%)} = \frac{V\text{Total volume of mixture} - \text{Separated oil}}{\text{Total volume of mixture}} \; x \; 100$$

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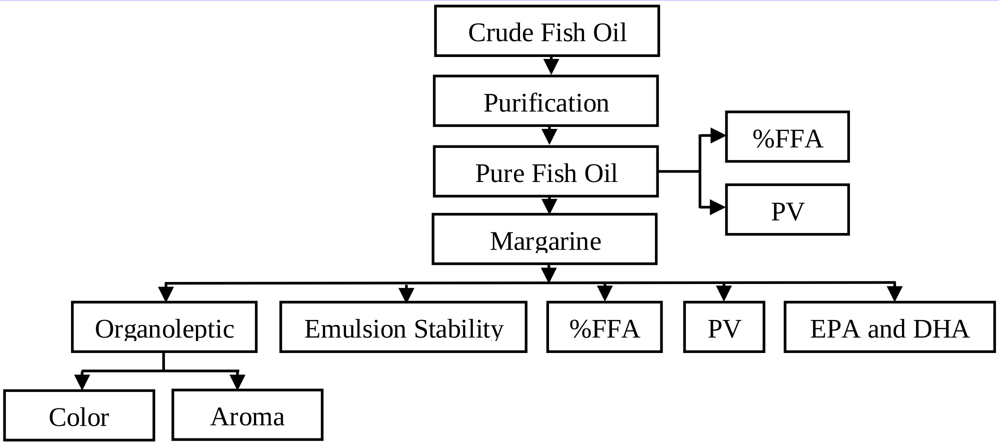

Figure 1. Methodology Flow Chart.

RESULT AND DISCUSSION

Table 1 shows the results of crude fish oil purification of fish canning by-products, which are in accordance with the International Fish Oil Standard (IFOS). According to IFOS, good fish oil should have a %FFA content of <2% and a PV of <3.75 mEq/kg. Sagita et al. (2020) stated that determining the free fatty acid number of fish oil is crucial in assessing its quality as it indicates the fatty acid content. Free fatty acids in fish oil are compounds produced due to oxidation and the breaking of double bonds of fatty acids. High levels of free fatty acids in fish oil indicate a decrease in quality. Activated charcoal can absorb molecules of fatty acids due to the presence of cellulose, which has numerous hydroxyl groups (-OH) and is electronegative (basic) and polar. The nature of cellulose allows it to interact with carboxylic acid groups (COOH) in free fatty acids, which are opposite to cellulose, namely electropositive (acidic) and polar (Waluyo et al., 2020). The nature of cellulose allows it to interact with carboxylic acid groups (COOH) in free fatty acids, which are opposite to cellulose, namely electropositive (acidic) and polar (Waluyo et al., 2020). The nature of cellulose allows it to interact with carboxylic acid groups (COOH) in free fatty acids, which are opposite to cellulose, namely electropositive (acidic) and polar (Waluyo et al., 2020).

Table 1. Characteristic of Fish Oil.

Parameters	Fish Oil	IFOS Standard
%FFA	0.057 ± 0.02	< 2%
PV	2 ± 0.38	< 3.75 mEq/kg

Note: %FFA = Free Fatty Acid; and PV = Peroxide Value.

Purified fish oil is used as a raw material for margarine, which is made by adding Virgin Coconut Oil (VCO), water, BHA, lecithin as an emulsifier, salt, sugar, and flavouring. According to Hasanah et al. (2017), margarine from fish oil can produce margarine with higher nutritional value than commercial margarine such as vitamin A, vitamin D, vitamin E, essential fatty acids in the form of omega 9, omega 6, omega 3 which are quite high, and low saturated fatty acid content. A score of one to nine was used to conduct an organoleptic assessment of fish oil margarine. Organoleptic assessment is a method commonly used in the food industry to evaluate quality, and generally assesses colour, taste, aroma, and texture parameters. This involves either three trained panelists or 20 untrained panelists. The organoleptic test on margarine, based on SNI 3541:2014, consists of sensory parameters of colour, aroma, and taste. The study did not evaluate the flavour of the margarine in the shelf life test. However, the margarine was assessed organoleptically by 30 untrained panellists.

Table 2. presents the characterisation of fish oil margarine based on organoleptic tests, emulsion stability, %FFA, and PV. The organoleptic assessment of colour and aroma in fish oil margarine showed lower values compared to commercial margarine. The colour of the margarine, based on SNI 3541:2014, is bright yellow, typical of margarine. The lower colour value of fish oil margarine compared to commercial margarine may be due to the pale yellow colour of fish oil margarine. The colour of fish oil margarine may appear pale yellow due to the colourless nature of the vegetable oil mixture with Virgin Coconut Oil (VCO), which reduces the brightness of the yellow colour of fish oil. This aligns with SNI 7381:2008 on virgin coconut oil, which specifies that virgin coconut oil (VCO) is clear or colourless and has a distinct aroma of fresh coconut (Notriawan et al., 2023).

Commercial margarine is produced with the addition of natural dyes such as beta-carotene and curcumin so that it has a bright yellow colour in accordance with SNI compared to margarine in fish oil in this study which was formulated without the addition of coloring agents. Raharjo et al. (2017), research on the manufacture of margarine using coconut oil which is clear white showed that the addition of beta carotene turmeric showed an increase in the colour of margarine so that beta carotene is thought to be able to increase the colour organoleptic value.

Table 2. Characteristic of Fish Oil Margarin Compared with Commercial Margarin.

Parameters	Fish Oil Margarin	Comercial Margarine
Organoleptic		
Color	7.4 ± 0.29	9 ± 0
Aroma	7.07 ± 0.16	9 ± 0
Emulsion Stability	100% ± 0	100% ± 0
%FFA	0.28 ± 0.08	0.057 ± 0.11
%PV	1.6 ± 0.173	4 ± 0.4

Note: %FFA = Free Fatty Acid; and PV = Peroxide Value.

The aroma organoleptic results also showed a lower value compared to commercial margarine. Based on the organoleptic assessment, some panellists liked the smell of fish oil margarine and some panellists disliked the distinctive smell of fish oil in margarine. The distinctive odour of fish oil margarine is caused by fish content in the form of free amino acids from protein and free fatty acids from fish fat. The smell and taste of fish oil margarine can also be caused by odour compounds extracted during the purification process of fish oil with organic solvents (Hasanah et al., 2017), making it less preferred by some panellists. Some panellists stated that they disliked the fish oil margarine because there was a

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Volume 12, Issue 1, June 2024; Page, 116-125
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distinctive smell of coconut oil. The low aroma organoleptic value is due to the fact that this aroma parameter is a subjective assessment of each panellist's sense of smell (Lamusu, 2018).

Emulsion stability is important in margarine because margarine is water-in-oil (W/O) emulsion. The process involves mixing lipids and water to achieve a W/O emulsion with a minimum fat content of 80%. The remaining 20% comprises skimmed milk, whey, or an aqueous solution containing salt and flavourings (Guillen et al., 2016). Emulsion stability analysis is conducted to determine the instability of emulsions, which occurs due to the tendency of emulsion particles to aggregate and cause the emulsion to break down (Malau & Ardiansah, 2022). In this study showed that the fish oil margarine has similar emulsion stability compared to the commercial margarine. In the previous study about catfish fish oil also shown good emulsion stability at different temperatures (Hasanah et al., 2017). Several factors, including temperature, stirring time, stirring speed, oil content, moisture content, and processing processes such as stirring speed, can influence emulsion stability in margarine. The balance of the emulsion is also affected by the ratio of oil to water content. When the concentration of oil is lower than that of water, the oil is unable to bind to the water molecules, resulting in an unstable emulsion (Prasetya and Evanuarini, 2019). However, further research is required regarding the emulsion stability of fish oil margarine during the shelf life. This is due to that the fish oil margarine in this study did not go through a hydrogenation process like commercial margarine in general, which can affect emulsion stability. The hydrogenation process in commercial margarine is carried out with the aim of hydrogen gas addition to the oil. The hydrogenation process converts oil in liquid form into solid fat, making the oil or fat more stable (Zaeromali et al., 2014).

The %FFA analysis results indicate that the %FFA value of fish oil margarine was higher than commercial margarine. However, the PV analysis of fish oil margarine showed a lower PV than that of commercial margarine. The analysis of %FFA and PV is used to determine the level of damage in fish oil margarine caused by fat oxidation. An increase in %FFA and PV indicates the occurrence of the fat oxidation process. Fat oxidation in margarine can cause rancidity. It is important to note that an increase in ALB and PV can contribute to this process. It is crucial to prevent rancidity as it is one of the consequences of the oxidation process in fats and proteins. Rancidity in margarine is caused by the lipid autooxidation reaction, which is characterised by an increase in peroxide number (PV) and free fatty acids (%FFA) formed in fat hydrolysis. (Narwati et al., 2021). The fatty acid content and peroxide number in fish oil margarine showed good values when compared to commercial margarine and the International Fish Oil Standard where the acid number limit is 3% and the peroxide number is less than 5 mEq/kg (IFOS, 2017).

Table 3. EPA and DHA Content of Fish Oil Margarin (%)

Parameters	Fish Oil Margarine	Commercial Margarine
EPA (C20:5n-3)	0.39	nd*
DHA (C22:6n-3)	2.58	nd*

Source: *Pangestu et al. (2023). **Note:** nd = not detected.

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Volume 12, Issue 1, June 2024; Page, 116-125
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The test results of EPA and DHA content of fish oil margarine are shown in Table 3, which shows that fish oil margarine contains EPA and DHA while commercial margarine did not detect EPA and DHA. This is thought to be due to the content of lemuru fish oil which is rich in fatty acids that have many health benefits (Suseno et al., 2014). Lemuru fish contains 19.37% fatty acid content with the most types of fatty acids including 14.46% eicosapentaenoic acid (EPA, C20:5ω-3) and 4.60% docosahexaenoic acid (DHA) (Suseno et al., 2014). According to Arellano et al. (2015), the formulation of fish oil margarine is based on fish oil that is high in omega-3 polyunsaturated fatty acids such as EPA and DHA. In this research, margarin from sardine fish oil contains 19.5% EPA and 10.5% DHA which is also not found in commercial margarine from palm oil. High levels of omega-3 in fish oil margarine is expected to have beneficial effects on cardiovascular disease (CVD) because it can reduce blood triglycerides, reduce low-density lipoprotein (LDL) cholesterol, and increase high-density lipoprotein (HDL) cholesterol in people with CVD.

The difference in EPA and DHA content between this study and Arellano et al. (2015), may be due to the fact that the fish oil in this study was obtained from by-products of canning processing so that the nutrient content is lower than fish oil purified from fresh fish. The oil by-product of canning fish obtained from the pre-cooking process with high temperatures which may cause a decrease in fatty acid levels due to protein denaturation at high temperatures (Abraha et al., 2018). This study shows that by-products of fish canning production still contain nutritional content so that they can still be utilised into value-added products such as margarine so as to increase economic value and reduce fishery production waste.

CONCLUSSION

The conclusion obtained from this research is that fish oil, a by-product of fish canning, can be utilised as an Added Value Product such as fish oil margarine after going through a purification process. Fish oil margarine in this study showed organoleptic value of colour 7.4 and aroma 7.07, emulsion stability 100%, %FFA content 0.28 ± 0.08, PV 1.6 ± 0.173, and higher EPA and DHA content compared to commercial margarine.

SUGGESTION

The research suggests the addition of food additives to improve the colour and aroma organoleptic values. Further observations are needed regarding the stability of fish oil margarine emulsions during the storage process.

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