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THE TREATMENTS COMBINATION (CENTRIFUGATION AND ADSORPTION) FOR REDUCING PRIMARY-SECONDARY OXIDATION PRODUCTS OF SARDINE OIL

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Abstract

Canned fish industry produces by-products such as fish oil. Fish oil as by products of fish meal and fish canning industry was purified by centrifugation and adsorption using carp scales, scallop shells, and attapulgite. Centrifugation treatment was done at 2.500 rpm for 45 minutes. There were 12 treatments of adsorbent combination as a further step of purification after centrifugation. The lowest peroxide value can be found in a treatment of addition of attapulgite (4.75±0.25 meq/kg). The lowest free fatty acid value can be found in attapulgite treatment too (2.33±0.70%). The lowest p-anisidine value can be found in a treatment of gradual addition of adsorbent, which there were three steps of purification, firstly refined fish oil was adsorbed using carp scales, and it was then purified using scallop shell, after that there was attapulgite addition as a last purification step. Its p-anisidine value was 0.15±0.46 meq/kg. The lowest total oxidation had been reached in a treatment of attapulgite addition (9.68±0.54 meq/kg). Single attapulgite addition was determined as the best treatment which resulting a good quality of fish oil based on primary and secondary oxidation products parameters, free fatty acids, and its clarity.

Keywords: *adsorbent, centrifugation, sardine oil, and quality.*

1. Introduction

Sardines are currently utilized as a raw material in some fish processed industries, such as canned fish industry, salted fish industry, and fish meal industri (Rostini 2007). Canned fish industry produces by-products such as fish oil. Fish oil is a fatty fraction obtained from the extraction of fish or as a by-products of the fish meal and canning fish industry.

Fish oil refining and purification with an effective and efficient method is very important for improving the quality of fish oil which suitable for consumption. Fish oil refining and purification can be carried out through a passive filtration and active filtration treatment. Treatments of passive filtration which commonly used are centrifugation and utilization of filter paper to separate the soap stocks, solid particles, and other impurities in fish oil. Suseno *et al*. (2011) showed that treatment of centrifugation in fish oil refining was more effective in reducing the soap stocks and other impurities if it was compared to filter paper treatment. Active filtration treatment is done by using a material that has the ability to adsorp impurities in fish oil, especially surfactant material which contain carbon group. Fish oil purification can be done by using various adsorbents. Active filtration treatment implementation aim to improve oil color, reducing unwanted odour components, sulfur compounds, heavy metals, and can reduce the production of fat oxidation, such as peroxides, aldehydes, and ketones (Estiasih 2009).

The combination of passive filtration and active filtration treatment can be carried out in the fish oil purification, so it can produce better quality of fish oil. Treatment of active filtration/depth filtration in fish oil that has been centrifuged is expected to improve the quality of fish oil. The purpose of this study is to choose the best treatment of adsorbent addition.

2. Materials and Methods

2.1. Materials and Equipments

Raw materials used in the study was fish oil which obtained from fish meal and fish canning industry in Bali. Fish oil which used in this study was a fish oil which had been purified using a treatment of centrifugation at 2.500 rpm for 45 minutes. Determination of centrifugation speed and centrifugation time had been done in a previous study. Adsorbents used were a carp scales from *Cyprinus carpio*, scallop shells form *Amusium pleuronectes*, and attapulgite. Carp scales and scallop shells were sun dried and crushed to the form of powder. Other supporting materials were distilled water, glacial acetic acid, chloroform, a solution of potassium iodine (KI), solution of sodium thiosulfate ($Na_2S_2O_3$) 0.1 N, 0.1 N KOH solution, phenolptalein indicator (indicator PP), ethanol 96 %, starch indicator 1 %, isooctan, reagent p-anisidine, n-hexane. Equipments which used were some glasses, magnetic stirrer, magnetic stirring bar, digital scale, high speed refrigerated centrifuge (HITACHI himac CR 21G), and 2500 UV-Vis spectrophotometer (LaboMed).

2.2. Procedure of the Experiment

Fish oil which obtained from fish canning industry and fish meal industry in Bali was refined and purified. Refining step was done by separating soap stocks through centrifugation treatment at 2.500 rpm for 45 minutes. Supernatants which were formed then purified using adsorbents. There were 12 combination treatments (control, carp scales treatment (CS), scallop shells treatment (SS), attapulgite treatment (A), carp scales and scallop shells (CS+SS), carp scales and attapulgite (CS+A), scallop shells and attapulgite (SS+A), carp scales, scallop shells, and attapulgite (CS+SS+A),

gradual addition of carp scales and scallop shells (CS→SS), gradual addition of carp scales and attapulgite (CS→A), gradual addition of scallop shells and attapulgite (SS→A), gradual addition of carp scales, scallop shells, and attapulgite (CS→SS→A). Concentration of adsorbent that used in this study was 3% (%w/w).

Fish oil and adsorbent was mixed using magnetic stirrer (20 minutes, 29 °C), after that the mixture of fish oil and adsorbent was separated using high speed refrigerated centrifuge (10.000 rpm, 30 minutes, 10 °C). Fish oil which had been refined and purified was then analyze. Some analysis which conducted in this study were peroxide value (PV) analysis (AOAC 1990), free fatty acid value (%FFA) analysis (AOAC 1995), p-anisidine value (p-AV) analysis (Watson 1994), total oxidation value analysis (Perrin 1996), and clarity test at various wavelengths (450 nm, 550 nm, 620 nm, 665 nm, and 700 nm) (AOAC 1995). Data would be analyzed using a Completely Randomized Design (CDR) treatment with type adsorbent. The data obtained and processed with SPSS 16.0 software.

3. Results and Discussions

3.1. Peroxide Value (PV)

Peroxide value is the most important value to determine degree of deterioration of oil or fat. The peroxide value of control and treated fish oils can be seen in Figure 1

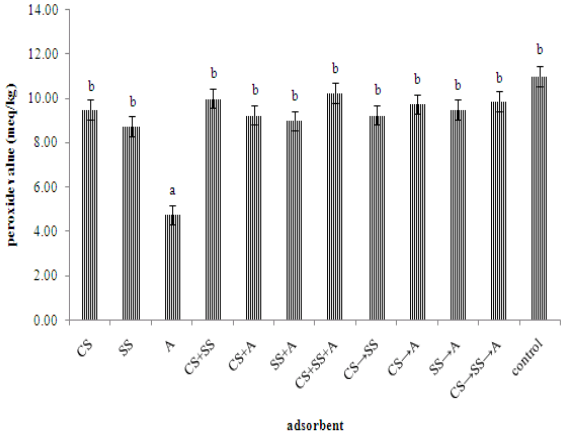

Figure 1 Peroxide value of fish oil

Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results showed that the highest value was in a treatment CS+SS+A (10.25±0.75 meq/kg) and the lowest value was in treatment A (4.75±0.25 meq/kg). Statistical analysis showed that treatment A give a significant effect to peroxide value ($p<0.05$). The peroxide value of each treated fish oils was not suitable with International Fish Oil Standard (IFOS 2011) (≤ 3.75 meq/kg). Maximum value of peroxide according to the Council for Responsible Nutrition (CRN 2006) is 5 meq/kg. Fish oil contains unsaturated fatty acids with double bonds are quite high (polyunsaturated fatty acids/PUFA) and easily oxidized when it reacts with oxygen. The increase in peroxide value is an indicator of the increasing amount of peroxide which can cause damage and lead to fish oil rancidity (Almunady *et al.* 2011). The highest of peroxide value indicate that fat or oil has been oxidized, but the lowest value is not always indicate that the oxidation is still early. Boran *et al.* (2006) reported the storage conditions of fish oil and fatty acid profiles contained in fish oil affect oxidative damage. The quality of the fish used for oil extraction, oil extraction process, and storage conditions will affect crude oil peroxide value of raw fish (EFSA 2010).

3.2. Free Fatty Acid Value (%FFA)

Free fatty acid analysis is conducted for determining the amount of free fatty acids that are formed as a result of fat damage which caused by hydrolysis or biologically. The free fatty acid of control and treated fish oils can be seen in Figure 2

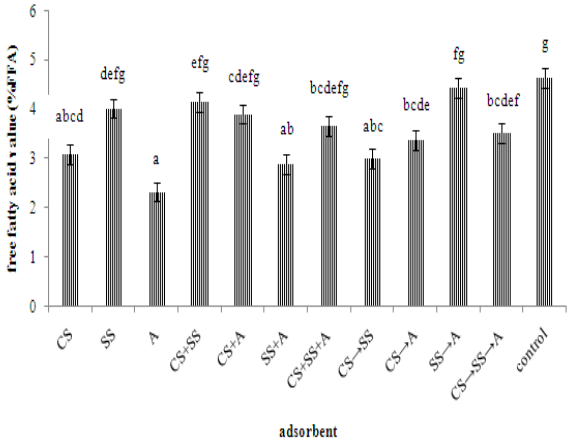

Figure 2 Free fatty acid value of fish oil.

Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results of the analysis showed the highest value can be found in treatment SS→A (4.44±0.21%) and the lowest value can be found in treatment A (2.33±0.70%). Statistical analysis showed that attapulgite give significant effect ($p<0.05$) to the free fatty acid value. The free fatty acid value of all treated fish oils were not suitable with Indonesian Pharmacopoeia Standard (≤ 2%). Bimbo (1990) suggested that free fatty acid standard for crude fish oil is about 1 to 7%, but typically ranges between 2 to 5%. The different of decrease level of free fatty acid levels may occur by the different of adsorbents type. Different types of adsorbents will have different polarity, active surface, surface area, porosity, particle size, pH, and different of water content (Zhu *et al.* 1994). Natural adsorbents such as chitosan have been used the purification of catfish as effectively absorb free fatty acids in crude oil (Huang and Sathivel 2010). Lipid molecules interact with chitosan in two steps, namely the carboxylic group of fatty acids bound the amine (NH_3^{+}) groups of chitosan via electrostatic interactions and hydrophobic interactions occur between the fatty acid and chitosan. Oxidation of volatile components especially aldehyde group can form a carboxylic acid group that will increase the acid value of oil (Feryanto 2007).

3.3. Anisidine Value (p-AV)

Analysis of p-anisidine value is conducted for measuring the secondary oxidation products (carbon component) (AOCS 1994). The p-anisidine value of

control and treated fish oils can be seen in Figure 3

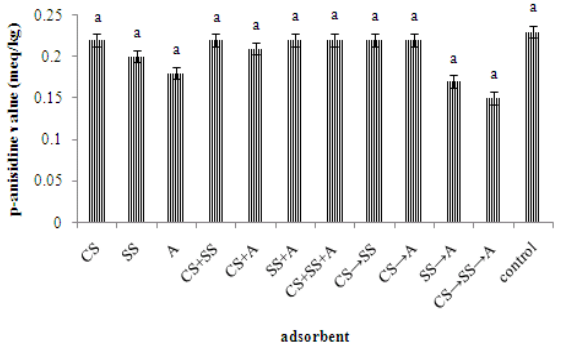

Figure 3 P-anisidine value of fish oil

Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results showed the highest value of p-anisidine value can be found in treatment CS (0.22±0.58 meq/kg) and the lowest value was in oil which treated by treatment CS→SS→A (0.15±0.46 meq/kg). Statistical analysis showed that all treatments did not give a significant effect ($p>0.05$) to p-anisidine value. The p-anisidine value of each treated fish oil was suitable to the International Fish Oil Standard (IFOS 2011) (≤ 15 meq/kg). Unsaturated fatty acids very easily oxidized when interacting with oxygen and high temperatures, it produces aldehydes compound, ketones, and other derivatives (Krishnamurthy and Vernon 1996). P-anisidine test is performed to measure the carbon components that influence the formation of unpleasant odors *(*off-flavor*)* results from the oxidation process (EFSA 2010).

3.4. Total Oxidation Value (Totox)

Analysis of total oxidation is conducted for determining the presence of oxidation products such as hydroperoxides, aldehydes, ketones, mainly produced by the degradation of PUFA in pro-oxidant conditions, particularly high temperature, oxygen, and light metal compounds. Total oxidation value of control and treated fish oils can be seen in Figure 4

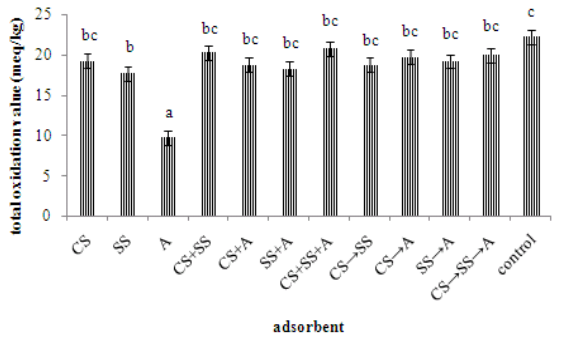

Figure 4 Total oxidation value of fish oil.

Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results showed that the highest value was found in treatment CS+SS+A (20.72±1.64 meq/kg) and the lowest value can be found in treatment of attapulgite addition (9.68±0.54 meq/kg). Statistical analysis showed that the treatment A give significant effect ($p<0.05$) to the total oxidation value. The total oxidation value of best treatment (9.68±0.54 meq/kg) was still in International Fish Oil Standard (IFOS 2011) (≤ 20 meq/kg). Total oxidation value is total of twice the peroxide value and p-anisidine value. The pore size is quite important role in adsorption process. Vitaria (2007) states that molecules with large size was difficult to get into the pores or voids contained in the adsorbent pore size is smaller when compared to the molecule. Factors affecting the adsorption capacity of adsorbent are surface area, pore size, solubility of the adsorbate, pH and temperature. The main constituent of the shells of shrimp or shellfish is chitin, a natural polysaccharide which has many uses such as a chelating agent, emulsifier, and adsorbent (Bhuvana 2006).

Based on Figure 4, it is known that the best treatment is the adsorbent attapulgite treatment. Attapulgite has a particle size of 150 mesh and granular powder as its form. Granular adsorbent powder is best choice for adsorption process in liquid mixture. The presence of a lot of pores and large surface area on attapulgite can make adsorption process become faster and effective. Ketaren (1986) stated that adsorption capacity to the oil color will be more effective if the adsorbent has a low specific gravity, high water content, fine particle size and adsorbent near neutral pH.

3.5. Clarity

Measurement of fish oil purity performed at 5 wavelengths (450 nm, 550 nm, 620 nm, 665 nm, and 700 nm). Fish oil clarity indicated by percent transmission which can be read in spectrophotometer instrument. High percent transmission and close to 100% indicates that fish oil which observed has good clarity. The results of fish oil's clarity test in all wavelength can be seen in Figure 5, 6, 7, 8, and 9

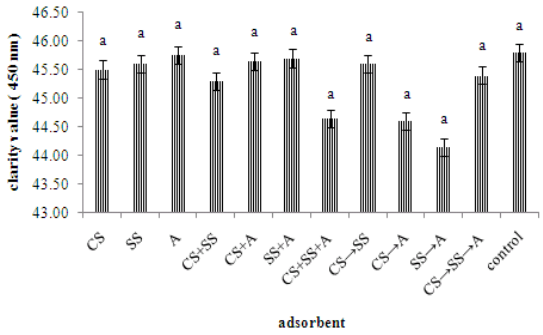

Figure 5 Percent transmission of light to fish oil (450 nm)

Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results showed that the highest value of light transmission can be found in treatment A (45.75±1.45 nm) and the lowest value was found in treatment SS→A (44.15±0.05 nm). Statistical analysis showed that type of adsorbent did not give a significant effect ($p>0.05$) to percent of light transmission. Figure 5 shows that attapulgite was the most effective adsorbent which give a most clear oil. Attapulgite has very good colloidal properties, such as dispersible, resistant to high temperature, resistant to salt and alkaline and has a high adsorption power, and able to remove color. Attapulgite ability to perform bleaching mainly caused by the large surface area of attapulgite and low capacity cation displacement (Huang *et al.* 2007).

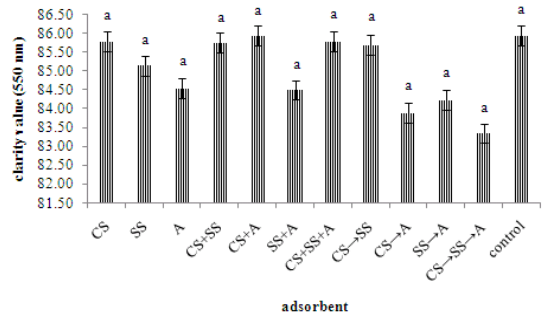

Figure 6 Percent transmission of light to fish oil (550 nm).
Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results showed that the highest value was found in treatment CS+A (85.95±0.35 nm) and the lowest value was found in treatment CS→SS→A (83.35±3.55 nm). Statistical analysis showed that type of adsorbent did not give significant effect ($p>0.05$) to the value of clarity. Figure 6 shows that carp scales and attapulgite are the most effective adsorbent in clarifying fish oil seen from the greatest percent transmission. Attapulgite has a small pore size and smooth shape while carp scales have big pore size and rough shape. Therefore, the combination of fine pore size and rough are expected to have high adsorption power and is able to remove color.

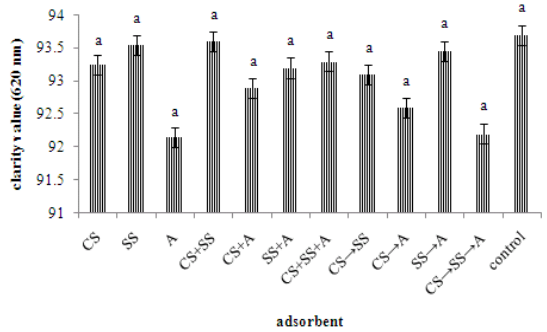

Figure 7 Percent transmission of light to fish oil (620 nm).
Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results showed the highest value of percent of light transmission was found in treatment CS+SS (93.60±0.40 nm) and the lowest value was found in a treatment of attapulgite addition (92.15±0.55 nm). Statistical analysis showed that type of adsorbent did not give significant effect ($p>0.05$) to the value of clarity at 620 nm as a light wavelength).

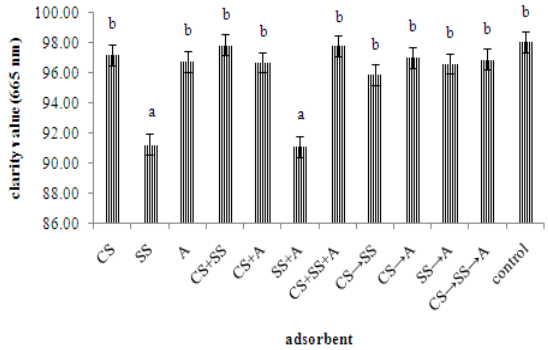

Figure 8 Percent transmission of light to fish oil (665 nm).
Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results which can be seen in Figure 8 showed that the best treatment was CS+SS treatment (97.85±1.25 nm). Statistical analysis showed that the type of adsorbent give significant effect ($p<0.05$) on the clarity value at 665 nm as a wavelength. Based on Figure 7 and 8, CS+SS are the most effective adsorbent to purify fish oil that can bee seen from the greatest percent transmission at the wavelengths of 620 and 665 nm. Carp scales and scallop shells which has a surface area of porous can be used to physically adsorb an adsorbate through direct contact surfaces, based on Scanning Electron Microscope (SEM) on the structure of carp scales and shells of the mussels (Esmaeli *et al.* 2012) and Checa *et al.* (2007).

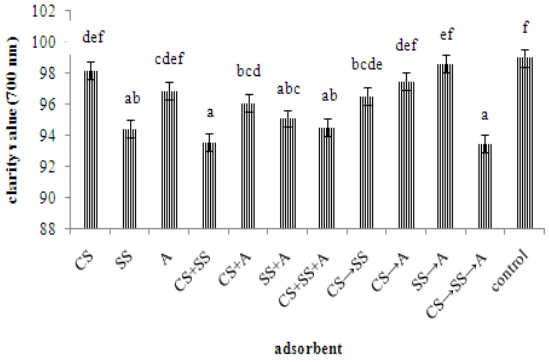

Figure 9 Percent transmission of light to fish oil (700 nm).
Ex: CS (carp scales *Cyprinus carpio*), SS (scallop shells *Amusium pleuronectes*), and A (attapulgite). sign → (gradual addition of adsorbent).

The results in Figure 9 showed that the best treatment was a treatment of SS→A (98.63±0.68 nm). Statistical analysis showed that the type of adsorbent give significant effect ($p<0.05$) to the clarity value. Figure 9 shows that a tretament of SS→A was the most effective treatment in clarify fish oil. Gradual step of purification capable to increase the capacity to adsorb impurities and removing color. Primary and secondary oxidation products can affect the color and turbidity of fish oil, the higher content of primary and secondary oxidation products in fish oil can cause the appearance of the observed fish oil become darker, so the level of clarity decreases (Estiasih 2009).

4. Conclusion

Attapulgite addition was the best treatment based on the total oxidation parameters and its clarity, so the treated fish oil using attapulgite can result a fish oil which suitable for consumption. Based on the analysis of free fatty acids and oil clarity, all adsorbents can work effectively in reducing the impurities in fish oil. The quality of all treated fish oils is closer to IFOS standard (2011) (International Fish Oil Standard) and the Indonesian Pharmacopoeia Standard.

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