Kinetic Study of Free Fatty Acid Adsorption Using Adsorbent in Sardine (Sardinella sp.) Oil Refining

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ABSTRACT— ***In Fish oil can be produced from fish meal industry by-products, but its fish oil has uninteresting color and high free fatty acids content so it needs to be purified. This study aimed to determine fatty acid profile of sardine oil, to determine free fatty acid adsorption kinetics model, and to determine sardine oil clarity after purification. Pseudo second order (k2) of attapulgite, bentonite, and chitosan treatment was 0.0344 g mg-1min-1, 0.0222 g mg-1min-1, and 0.0460 g mg-1min-1, respectively. The equilibrum adsorption capacity (qs) of attapulgite, bentonite, and chitosan treatment was 56.53 mg/gram, 42.40 mg/gram, and 47.11 mg/gram. The external film mass-transfer coefficient (KsA) was 0.0018 mL/s for attapulgite treatment, 0.0015 mL/s for chitosan treatment, and 0.0013 mL/s for bentonite treatment. The intraparticular diffusion coefficient (Kw) was 0.1542 mg mL-1 min-0.5 for attalpugite treatment, 0.1285 mg mL-1 min-0.5 for chitosan treatment, and 0.1156 mg mL-1 min-0.5 for bentonite treatment. Clarity for all treated fish oil increase with the increasing of adsorption time, and the equilibrum point has been reached at 60 minutes. Results indicated that attapulgite was the best adsorbent than chitosan and bentonite for free fatty acid removal from sardine oil.***

Keywords— adsorption, free fatty acid, kinetics model, purification, pseudo-second order, sardine oil

1. INTRODUCTION

Fish oil is an important source of polyunsaturated fatty acids (PUFA), especially eicosapentanoic acid (EPA, 20:5 ω=3) and docosahexaenoic acid (DHA, 22:6 ω=3) as beneficial for physiological activity. The content of EPA and DHA in fish oil varies at 5-26% and 6-26% of total fatty acids [2]. EPA is beneficial for the heart and cardiovascular healthy, while DHA is beneficial for pregnancy, newborn development , nerve and brain development [10].

Fish oils in Indonesia are generally derived from fish meal industry or canned fish industry by products. Utilization of fish oil that obtained from fish meal industry is very limited, because of its low quality of fish oil, e.g. oil unattractive color and its high level in free fatty acid. This is due to the raw materials which are used in fish meal industry are fish with low quality, or the head, skin, and viscera which are removed from the fish canning industry. Damaged fats in low quality fish can increase its free fatty acids content [8]. Therefore, to optimize utilization of fish oil which derived from fish meal industry, it needs to be purified by some treatments.

Fish oil generally contains triacylglycerol, but unpurified fish oil still contains non-triacylglycerol components such as free fatty acids, water, and prooxidant components that can degrade the quality of fish oil [10]. Purification of fish oil can remove impurities, so it can increase the acceptability and shelf life of fish oil [16]. Adsorption of fish oil impurities using adsorbent can be done as one technique of fish oil purification.

Adsorbent can adsorb impurities components, pigments , and free fatty acids in fish oil. Adsorbents which are potential to be used in purification process are attapulgite, bentonite, and chitosan. Characteristic of adsorbent ability to adsorb free fatty acid component can be studied through adsorption kinetic studies. Kinetic study of attapulgite,

bentonite, and chitosan in fish oil purification are still limited. Sathivel et al. [17] stated that the study of fish oil purification was only focused in removing impurities from fish oil, on the other hand, the information of kinetic studies in imputity adsorption is important to produce the most effective process in terms of both quality and economy. The purpose of this study was to determine sardine oil fatty acid profile, to determine the kinetics model of adsorption process in decreasing levels of free fatty acids, and to determine sardine oil clarity after purification process using adsorbent.

2. MATERIALS AND METHODS

2.1 Materials and Equipments

Materials used in this study were sardine oil as fish meal industry by product and adsorbents such as chitosan, attapulgite, and bentonite. Materials used for the analysis included 96% neutral alcohol, PP indicator (phenolphthalein) 1%, KOH 0.1 N, n-hexane, and materials for fatty acid analysis using gas chromatography (GC).

Equipments used for the refining process were erlenmeyer glass, pipette, digital scales, aluminum foil, magnetic stirrer, magnetic stirring bar, stop watch, and high-speed refrigerated centrifuge HIMAC (HITACHI CR 21G). Some equipments used in the analysis were burette, electric stove, gas chromatography instrument (Shimadzu GC2010), and UV-VIS spectrophotometer (UV-2500 RS LaboMed).

2.2 Scope of Research

This research was conducted in three stages, i.e. characterization of sardine oil as raw material, preliminary study, and primary study. Characterization of sardine oil was done by analyzing fatty acid profiles using GC and free fatty acids (FFA) content. Fish oil quality were observed after refining process including free fatty acid levels, and levels of fish oil clarity.

2.2.1 Fish Oil Refining

Preliminary research was done in the form of fish oil refining using adsorbents to determine the best adsorbent concentration. The best adsorbent concentration was used in the primary study. About 10 grams of unrefined sardine oil was put into erlenmeyer glass and adsorbent (chitosan, attapulgite, and bentonite) with a concentration of 1%, 3%, and 5% was added to weighed sardine oil. Adsorption process was performed with constant stirring using a magnetic stirrer at room temperature for 20 minutes, then a mixture of sardine oil and adsorbent was centrifuged (10,000 rpm, 10 ° C for 10 minutes) to separate the adsorbent. FFA analysis was conducted to see the best concentration of adsorbent which give good quality. Treatment in preliminary study was performed in three replicates.

The primary study was done to obtain a kinetic model of each adsorption process using adsorbent with choosen concentration. About 50 grams of sardine oil was put into erlenmeyer and adsorbent (chitosan, attapulgite, and bentonite) with the choosen concentration was added to the weighed sardine oil. Adsorption process was performed with constant stirring using a magnetic stirrer at room temperature. All treatments were conducted for 100 minutes. Free fatty acid level was analyzed in a sample which was refined for 0 minute, 20 minutes, 40 minutes, 60 minutes, 80 minutes, and 100 minutes. Refined sardine oil was then centrifuged (10,000 rpm, 10ºC for 10 minutes) to separate the adsorbent from fish oil. Final quality of refined fish oil was analyzed, and quality parameter were free fatty acids (FFA) and the level of clarity.

2.2.3 Analysis of Fish Oil Quality

Some analysis conducted in this study were fatty acid profile analysis using gas chromatography [4] with no. method 969.33, free fatty acid analysis [5] with no. method Ca 5a-40, and the oil clarity test [3].

2.2.4 Data Analysis

Result of refining process was statistically processed using SPSS software version 16.0 to see the regression parameter coefficients, percent significance (confidence interval), and the pattern of interaction of factors that significantly influence the response. Tukey test was used to see significant effects of factors. Study of free fatty acid adsorption kinetics in this research was according to the pseudo second order Ho [11]. Transforming the data to the pseudo second order model was done to determine kinetic parameters, i.e. k_2 (constant rate of pseudo second order) and qe (amount of free fatty acids are adsorbed at equilibrium). Equation of Kadirvelu et al. [14] was made to determine external film mass-transfer coefficient (KsA), intraparticular diffusion coefficient (Kw) , and the equilibrium adsorption capacity (qs).

3. RESULT AND DISCUSSIONS

3.1 The Sardine Oil Fatty Acid Profile

Fish oil contained varied fatty acids with the dominant fatty acids werer unsaturated carbon atom number 20 (C20) and 22 (C22). The dominant fatty acids belong to a group of omega-3 fatty acids. Results of fatty acid profile analysis of fish oil from fishmeal industry's by products presented in Table1.

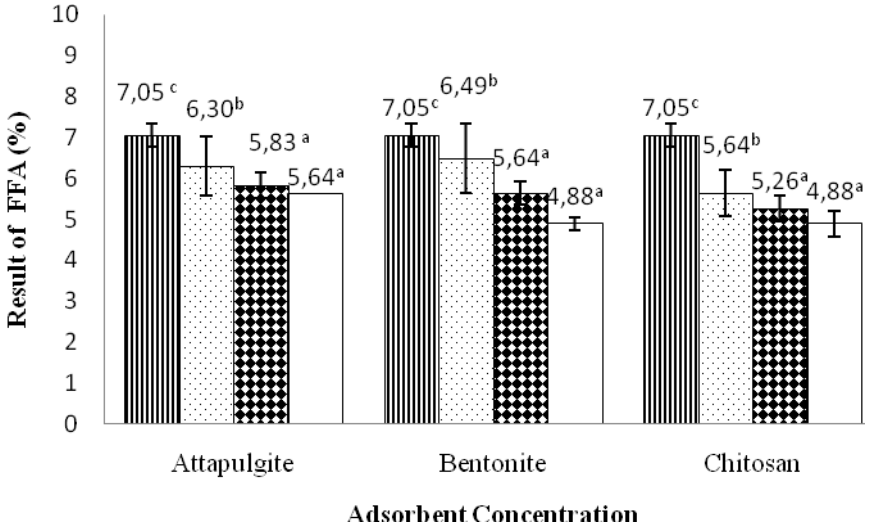
Table 1: Fatty acid composition of unrefined sardine oil

Fatty Acid	Result (% w/w)
Lauric Acid (C12:0)	0.09
Tridecanoic Acid (C13:0)	0.04
Myristic Acid (C14:0)	7.76
Pentadecanoic Acid (C15:0)	0.46
Palmitic Acid (C16:0)	14.13
Heptadecanoic Acid (C17:0)	0.4
Stearic Acid (C18:0)	2.76
Arachidic Acid (C20:0)	0.52
Heneicosanoic Acid (C21:0)	0.05
Behenic Acid (C22:0)	0.13
Tricosanoic Acid (C23:0)	0.03
Total SFA	**26.37**
Myristoleic Acid (C14:1)	0.03
Palmitoleic Acid (C16:1)	9.87
Elaidic Acid (C18:1n9t)	0.1
Oleic Acid (C18:1n9c)	6.58
Eicosenoic Acid (C20:1)	0.24
Erucic Acid (C22:1n9)	0.18
Total MUFA	**17**
Linolenic Acid (C18:3n3)	0.53
Linolelaidic Acid (C18:2n9t)	0.06
Linoleic Acid (C18:3n3)	1.01
γ-Linoleic Acid (C18:3n6)	0.33
Eicosedienoic Acid (C20:2)	0.09
Eicosetrienoic Acid (C20:3n6)	0.24
Eicosetrienoic Acid (C20:3n3)	0.08
Arachidonic Acid (C20:4n6)	2.41
Eicosapentaenoic Acid /EPA (C20:5n3)	15.83
Docosaheksaenoic Acid /DHA (C22:6n3)	11.36
Total PUFA	**31.94**
Total Fatty Acid	**75.31**

Table 1 shows the greatest fatty acids eicosapentaenoic acid (EPA) that is equal to 15.83%, followed by 14.13% palmitic acid, docosahexaenoic acid (DHA) was 11.36%, and 9.87% of palmitoleic acid. Omega-3 fatty acids are needed for brain development, eye, fetal growth during pregnancy and for health maintenance. Omega-6 fatty acids play a crucial role in brain function and heart health. Beneficial omega 9 fatty acids support heart health, balance cholesterol levels, and improve immune function [7].

3.2 Free Fatty Acid (FFA)

Levels of free fatty acids is one of the indicators used to assess the quality of fish oil. Free fatty acids produced when the hydrolysis of the triglyceride, and the fatty acids are apart from the glycerol structure [1]. Result of preliminary study can be seen in Figure 1.



Note : ▮ = addition of 0% adsorbent (control), ⁝ = addition 1% of adsorbent, ◩ = addition of 3% adsorbent, and ☐ = addition of 5% adsorbent
Superscript a, b, c = significantly different to the concentration of adsorbent ($p<0,05$)

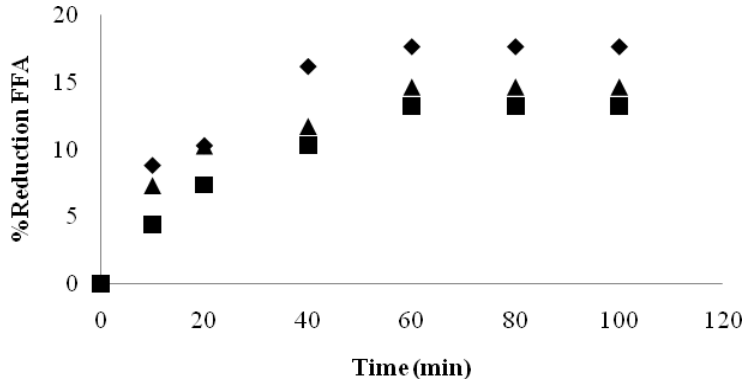
Figure 1: The Value of Free Fish Oil Fatty Acids with The Addition of Various Adsorbents at Different Concentrations

Based on the results, after the addition of the adsorbent with concentration of 0%, 1%, 3% and 5% in refining process (stirring for 20 minutes), fatty acids decreased, with a percentage of 10.64 to 20% for attapulgite treatment, 7.94 to 30.78% for bentonite treatment, and from 20 to 30.78% for chitosan treatmen. ANOVA analysis showed that the addition of adsorbent with different concentrations provided a significantly different effect on the value of free fatty acids. Tukey's test results showed that the concentration of 0% was significantly different adsorbents with a concentration of 1, 3 and 5%, while the concentration of 3% was not significantly different from the concentration of 5%. Therefore, the best treatment to reduce FFA content in the preliminary study was the addition of adsorbent concentration of 3%.

3.3 Kinetics Study of Free Fatty Acid Adsorption in Sardine Oil Refining

Kinetics model determination of free fatty acid adsorption in fish oil refining was done by adsorption of the time difference. Determination of the time difference was used to determine how fast the adsorption process takes place. The observation was based on the graph by making the relationship between percent reduction of FFA versus time. The graph is shown in Figure 2.

Figure 2 shows that the increasing of adsorption along with the increasing of free fatty acids adsorption until it reached equilibrium state. There was no increase in free fatty acids percent reduction after 60 min adsorption time. Therefore, it was estimated that the time of adsorption equilibrium had been reached, both in the addition of attapulgite, bentonite, and chitosan as adsorbent.



Note : ◆ = attapulgite, ■ = bentonite, ▲ = chitosan

Figure 2: Effect of Adsorption Time on Reduction of FFA (%)

Adsorption model change over time requires a kinetic model approach. From the data obtained in Figure 2, the adsorption kinetics studies performed with reference to the pseudo second-order kinetics model of Ho [11]. Table of kinetics calculation results using pseudo second-order kinetics model of Ho [11] is shown in Figure 3. Based on Figure 3, the line equation of y = mx + b could be obtained. The equation used to calculate k2 and qe . The kinetic parameters calculation results can be seen in Table 2

Table 2: Comparison of kinetic parameters on each adsorbent

Adsorbent	Pseudo Second-order Ho (2006)				
	k_2 (g $mg^{-1}min^{-1}$)	H (g $mg^{-1}min^{-1}$)	q_{e2} (mg g^{-1})	R^2	$q_{e.exp}$ (mg g^{-1})
Attapulgite	0.0344	0.1365	1.992	0.988	1.692
Bentonite	0.0222	0.0622	1.675	0.984	1.269
Chitosan	0.046	0.1216	1.626	0.993	1.410

Adsorbent	**Kadirvelu *et al.* (2000)**		
	K_sA (mL s^{-1})	K_w (mg mL^{-1} $min^{-0.5}$)	q_s (mg g^{-1})
Attapulgite	0.0018	0.1542	56.53
Bentonite	0.0013	0.1156	42.40
Chitosan	0.0015	0.1285	47.11

Note : k_2 = pseudo second-order adsorption rate
h = Initial adsorption rate
q_{e2} = amount of free fatty acids are adsorbed at equilibrium pseudo second-order
$q_{e.exp}$ = amount of free fatty acids are adsorbed at equilibrium results study
K_sA = the external film mass-transfer coefficient
K_w = intraparticular diffusion coefficient
q_s = equilibrium adsorption capacity

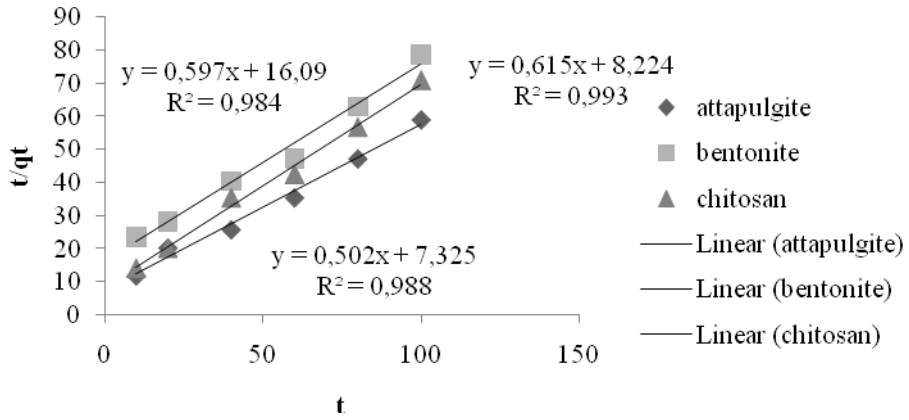


Figure 3: Ho Pseudo second-order model kinetics

Table 2 shows some differences in kinetic parameters of attapulgite, bentonite and chitosan treatment. Two pseudo order kinetics model has a very strong correlation (close to 1) with R_2 values above 0.95 in the third adsorbent. Therefore, the pseudo second-order kinetic model can explain the adsorption of free fatty acids in fish oil by a third adsorbent. Ho kinetics model based on the concentration of adsorbate on the adsorbent [6]. Table 2 shows that the adsorption of free fatty acids in fish oil using attapulgite had a pseudo second-order adsorption constant (k2) of 0.0344 g $mg^{-1}min^{-1}$, adsorption by bentonite has k_2 of 0.0222 g $mg^{-1}min^{-1}$, and adsorption by chitosan has k_2 of 0.0460 g $mg^{-1}min^{-1}$. Initial adsorption rate (h) of attapulgite, bentonite, and chitosan treatment were 0.1365 g $mg^{-1}min^{-1}$, 0.0622 g $mg^{-1}min^{-1}$, and 0.1216 g $mg^{-1}min^{-1}$, respectively. Attapulgite treatment had the highest value of q_{e2} than bentonite and chitosan treatment which was equal to 1.992 mg/g, whereas the bentonite treatment was 1.675 mg/g and chitosan treatment was 1.626 mg /g. The value q_{e2} was not different from the value $q_{e.exp}$ research . This suggests that the attapulgite has the largest adsorption capacity.

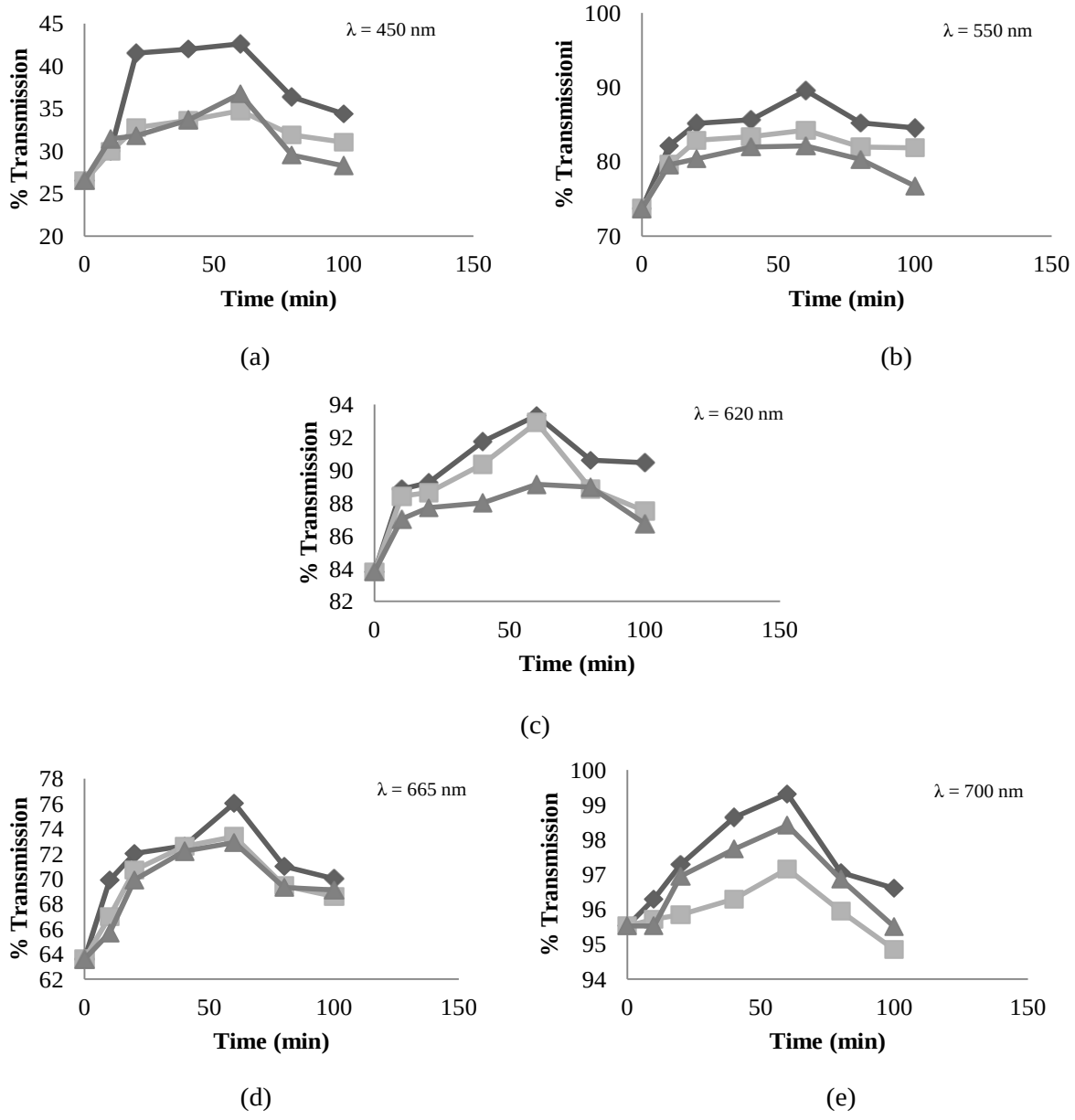
Based on Table 2, attapulgite treatment had the highest of equilibrium adsorption capacity (q_s) than bentonite and chitosan treatment, with the value of the adsorption of the FFA using this adsorbent was 56.53 mg/g, whereas the q_s values for bentonite and chitosan treatment were 42.40 mg/g and 47.11 mg/g. Si and Mg oxide structures on the surface of attapulgite can increase the adsorption capacity [9]. Many factors affect the adsorption capacity are initial adsorbate concentration, temperature, pH value, particle size, dose, and the characteristic of the solute [11]. These factors may also affect the adsorption rate constant of the adsorption process. Differences in adsorption capacity can also occur by the different types of adsorbents used. Different types of adsorbents have different polarity, the active surface, surface area, porosity, particle size, pH, and different of water content [20].

According to Mittal et al. [15], the steps involved in the adsorption of organic or inorganic compounds included: the external film mass transfer phase, is when an adsorbate transports into the external surface of the adsorbent, the intraparticular difussion phase, is when an adsorbate transports within the pores of the adsorbent except for a small amount of adsorption, it occurs in external surface, and the adsorption of adsorbate in the interior surface of the adsorbent. Ho and McKay [12] observed during the adsorption process occurs, there are four distinct stages, that are transport of adsorbate from the liquid phase (solution) surrounding the adsorbent particle surface (the first stage can be ignored if the system is perfectly agitate); external diffusion (second stage), the diffusion of intra-particles (the third stage), and the physical or chemical surface reaction (fourth stage), but only the external films diffusion and intraparticel difussion which are considered can control the adsorption kinetic.

KsA values (the external film mass-transfer coefficient) depends on the concentration gradient between the adsorbate and the surface of the liquid phase adsorbent [16]. Table 2 shows the highest value of KsA is shown in attapulgite adsorbent treatment, and its value was 0.0018 mL/s, followed by chitosan treatment at 0.0015 mL/s, and bentonite treatment at 0.0013 mL/s. On a study of Sathivel and Prinyawiwatkul (2004), a value of KsA for chitosan treatment was 0.001 mL/s, activated carbon treatment was 0.001 mL/s, active earth treatment was 0,001 mL/s. According to Toro and Mendez [19], the external film mass-transfer coefficient is influenced by several factors, such as the magnitude of the driving force for the FFA, the adsorbent surface interaction with other lipids, as well as the interaction of FFA with other oil components in the liquid phase and on the surface of the adsorbent. Highest value of intraparticular diffusion coefficient (Kw) at Table 2 indicated by attapulgite treatment. Kw value for attapulgite treatment was 0.1542 mg mL^{-1} $min^{-0.5}$, chitosan treatment was 0.1285 mg $mL^{-1}min^{-0.5}$, and bentonite treatment was 0.1156 mg $mL^{-1}min^{-0.5}$. This suggests that attapulgite had faster intraparticle diffusion due to large tissue porosity. Attapulgite was in powder form so it had a subtle form pores that provide a large surface area to absorb free fatty acids

3.4 Clarity of Fish Oil

Measurement of fish oil clarity in the study conducted at a wavelength of 450, 550, 620, 665 and 700 nm. Results of fish oil clarity expressed by percent transmission can be seen in Figure 4. Based on figure 4, it can be inferred that the value of clarity (percent transmission) at all wave increased with the increasing of adsorption time to a maximum in the 60th minute. It had happened to all adsorbent (attapulgite, bentonite, and chitosan) treatment. Therefore, it was estimated that 60th minute of adsorption time was a equilibrium condition and it can limit the ability of the adsorbent to absorb impurities that affect the color or clarity of the fish oil.



Note : = attapulgite, = bentonite, = chitosan

Figure 4: Percent clarity of fish oil transmission after the addition adsorbent at a certain time with a wavelength of 450 (a), 550 (b), 620 (c), 665 (d), and 700 nm (e)

Figure 4 also shows the correlation of the level of clarity to the reduction of FFA in the oil. The increasing of adsorption time will increase the percent reduction of FFA, and it was in a line with the increasing of clarity level. Adsorbents can be used to reduce the pigment for oil refining and potentially adsorb FFA [18]. Color of fish oil can be caused by free fatty acids react to form colored compounds [8].

Figure 4 also shows that from three types of adsorbents, attapulgite is the most effective adsorbent which gave the highest light transmission on fish oil. Attapulgite has very good colloidal properties, that is easily dispersible, resistant to high temperature, resistant to salt and alkaline, has a high adsorption power, and able to remove the color. Attapulgite ability to perform bleaching mainly due to large surface area and low capacity of displacement cation [13].

4. CONCLUSION

Obtaining from fish meal industry, this sardine oil contained 26.37% of saturated fatty acids (SFA), 17% of monounsaturated fatty acids (MUFA), and 31.94% polyunsaturated fatty acids (PUFA). Polyunsaturated fatty acids were dominated by EPA and DHA, and its value was 15.83% and 11.36%, respectively.

The longer of adsorption time was in a row with the more free fatty acids were adsorbed. Equilibrium point in free fatty acids adsorption has been reached at 60 minutes. The best free fatty adsorption capacity can be shown in a treatment using attalpugite as adsorbent, and its adsorption capacity value was 56.53 mg / g.

Clarity value (percent transmission) of each treated fish oil will increase, with the increasing of adsorption time until the 60th minute. Sardine oil which was adsorbed by attapulgite showed better clarity than chitosan and bentonite.

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