Quality characteristics of Bali sardinella (Sardinella lemuru) oil purified with bentonite as an adsorbent

U Nadhiro¹, S Subekti², W Tjahjaningsih² and Patmawati²

¹ Aquaculture Study Program, Faculty of Fisheries and Marine, Universitas Airlangga.
² Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga.

E-mail: patmawati.wahyudin@gmail.com

Abstract. Crude fish oil extracted from fish canning industry is of low quality, therefore refining process is required to obtain feasible fish oil for food purposes. Purification of fish oil can be done through steps of degumming, neutralization, and bleaching by using bentonite as the adsorbent. This study aims to analyze the results of the purification process of crude fish oil by-product of canning industry of lemuru fish by using bentonite adsorbent with different concentrations. The method used was an experimental method by descriptive data analysis. The results showed that the highest yield (33.418 %) obtained from oil purification of lemuru with bentonite concentration of 6 % are classified as follows: free fatty acid content of 0.265 %, peroxide value of 6.343 mEq / kg, produce clarity 60.275 % T, 88.075 % T, 87.5 % T, 87.425 % T, 87.975 % T at a wavelength (λ) of 450 nm, 550 nm, 620 nm, 665 nm, 700 nm, para-anisidine value of 3.725 mEq / kg; and value of oxidation total of 16.41 meq / kg.

1. Introduction
Fish canning industry produces a low quality liquid by-product, which is a mixture of oil fraction, water fraction and suspended solids obtained in the steaming stage (pre-cooking) [1,2]. To increase the value of Indonesia’s fish oil exports, purification of fish oil should be performed [3].

Purification of fish oil consists of the stages of degumming, neutralization and bleaching [1]. According to Rubio-Rodriguez et al. [4], degumming aims to eliminate phospholipids by adding phosphoric or citric acid, and neutralization aims to eliminate free fatty acid with sodium hydroxide, while bleaching aims to absorb oxidation products and color with active clay (activated clay). In this study, bentonite was used in the fish oil bleaching stage. According to Faisal [5], bentonite (aluminosilicate mineral is widely used as a catalyst, buffer, bleaching agent and adsorbent.

The use of bentonite as an adsorbent has an advantage because bentonite has an interlayer structure that can be modified easily, which will improve its absorption properties. This study aimed to analyze the results of the purification process of crude Bali sardinella oil in the fish canning industry using bentonite as an adsorbent at different concentrations.

2. Methodology
The research materials consisted of oil of Bali sardinella (S. lemuru) from the Canary of Bali; bentonite, H₂SO₄ 5N, distilled water, 3 % citric acid, 9.5 % NaOH, 95 % ethanol, KOH 0.1N, indicator of phenolphthalein, acetic acid, chloroform, potassium iodide, sodium thiosulfate (Na₂S₂O₃) 0.1N, 1 % starch solution, isooctane (2,2,4-trimethylpentanes), and p-anisidine.
This research used experimental method with completely randomized design (CRD), and the data were analyzed descriptively. The data obtained included free fatty acid value, peroxide value, para-anisidine value, oxidation number and clarity, and the results were compared with the fish oil standard Codex Alimentarius Commission (CAC) [6] or International Fish Oil Standard (IFOS).

The study began with the activation of bentonite chemically by mixing bentonite and H$_2$SO$_4$ 5N (1 gram of bentonite:10 mL of acid) in a Beaker glass. The activation was done in water bath for two hours at 70°C. The following step was filtering and washing with water at a temperature of 75 °C until the pH of the wash water became neutral. The bentonite was subsequently dried using an oven at 105°C until it reached a constant weight [7].

Before the purification was done, initial characterization of Bali sardinella oil was carried out, and the characteristics consisted of free fatty acid value, peroxide value, para-anisidine value, oxidation number and clarity. The procedure of crude fish oil purification was based on the study of Sari et al. [2] that was modified.

In the degumming stage, 100 grams of fish oil was heated at 70°C for one minute and then 3 mL of 3% citric acid solution was added. This was reheated to a temperature of 70°C for one minute while being stirred. Next, the oil was kept at room temperature and centrifuged at a speed of 2,600 rpm for 10 minutes.

In the neutralization stage, the degummed oil was added with 9.5% NaOH solution as much as 50.3% (w/w) and heated at 65°C for 20 minutes while being stirred. The next step was keeping it at a room temperature and centrifuging it at a speed of 2,600 rpm for 10 minutes. The result of centrifugation of fish oil was rinsed with distilled water three times to obtain oil and soap.

In the bleaching stage, some of the result of oil neutralization was added with bentonite at concentrations of 2%; 4%; 6%; 8% of the oil weight in different treatments, and some other was not added with bentonite (control). After the addition of bentonite, the fish oil was heated at 80°C for 20 minutes while being stirred. The fish oil that was heated and centrifuged at a speed of 6,500 rpm for 10 minutes became pure fish oil. The result of the purification process of Bali sardinella oil was analyzed in terms of: free fatty acid value, peroxide value, para-anisidine value, oxidation number, clarity, yield [8, 9, 10].

3. Results and Discussion

The Bali sardinella oil used was the rough, dark brown by-product of the canning process in pre-cooking stage. The results of the baseline characteristics of crude Bali sardinella oil in the form of free fatty acid value, peroxide value, para-anisidine (p-anisidine) value, and oxidation number did not meet the IFOS or CAC standard, where the free fatty acid value was < 2%, the peroxide value was ≤ 5 meq/kg, the para-anisidine (p-anisidine) value was ≤ 20 meq/kg and the oxidation number was ≤ 26 meq/kg (table 1). The results of the baseline characteristic of clarity of crude Bali sardinella oil did not meet the commercial fish oil standard due to low percent transmittance (%T) (table 2). The results of the final characteristics of Bali sardinella oil (S. lemuru) after purification are listed in table 5.3 and table 5.4. The results of the final characteristics of Bali sardinella oil (S. lemuru) after purification are listed in table 3 and table 4.

**Table 1.** The results of the baseline characteristics of lemuru fish oil.

| Characteristic                            | Value   |
|------------------------------------------|---------|
| Free fatty acid content (%)              | 32.25   |
| Peroxide value (meq/kg)                  | 10.95   |
| Paranisidine (p-anisidin) value (meq/kg) | 41.83   |
| Value of oxidation total (meq/kg)        | 63.72   |
Table 2. The result of the baseline characteristics of the clarity of lemuru fish oil.

| Fish Oil                          | λ450 nm       | λ550 nm       | λ620 nm       | λ665 nm       | λ700 nm       |
|-----------------------------------|---------------|---------------|---------------|---------------|---------------|
| Commercial fish oil (standard)    | 89.5%T ± 0.71 | 96.05%T ± 0.91| 97.1%T ± 0.99 | 97.45%T ± 0.92| 97.95%T ± 1.34|
| Baseline of fish oil              | 12.5%T ± 0.90 | 57.2%T ± 9.76 | 71.05%T ± 4.03| 70.05%T ± 6.15| 78.5%T ± 1.70 |

Table 3. The results of the final characteristics of lemuru fish oil.

| Characteristics                  | Treatments  |
|----------------------------------|-------------|
| Free fatty acid content(%)       | P0          | P2          | P4           | P6           | P8           |
| P0                               | 0.38 ± 0.13 | 0.33 ± 0.06 | 0.365 ±0.16  | 0.265 ± 0.10 | 0.245 ±0.10  |
| Peroxide value(meq/kg)           | 4.213 ± 2.24| 3.815 ± 1.60| 8.553 ±4.40  | 6.343 ± 3.00 | 10.16 ±5.54  |
| Paranisidine (p-anisidine) value(meq/kg) | 3.545 ±2.64 | 5.32 ±6.47  | 7.29 ±2.32   | 3.725 ±3.72  | 7.84 ±4.27   |
| Value of oxidation total (meq/kg) | 11.97 ±6.43 | 12.95 ±5.81 | 24.395 ±7.97 | 16.41 ±7.07  | 28.17 ±11.79 |
| Rendement (%)                    | 28.763±6.00 | 29.228±7.78  | 27.87 ±7.46  | 33.418 ±1.22 | 27.305±5.50  |

Description: P0 = 0% bentonite, P2 = 2%, P4 = 4% bentonite = P4, P6 = 6% bentonite, P8 = 8% bentonite. * IFOS; ** CAC standard.

Table 4. The results of test of clarity of lemuru fish oil after purified.

| Fish Oil                          | λ450 nm       | λ550 nm       | λ620 nm       | λ665 nm       | λ700 nm       |
|-----------------------------------|---------------|---------------|---------------|---------------|---------------|
| Commercial fish oil (standard)    | 89.5 %T ± 0.70| 96.0%T ± 0.92 | 97.1%T ± 0.99 | 97.45%T ± 0.92| 97.95%T ± 1.34|
| P0                               | 49.4%T± 2.13  | 87.2%T± 4.76  | 89.2%T± 4.51  | 82.5%T ± 5.09 | 90.12%T± 6.00 |
| P2                               | 40.7%T± 24.22 | 89.8%T± 8.12  | 89.7%T±6.27   | 86.9%T ± 5.43 | 90.35%T± 6.74 |
| P4                               | 58.9%T±2.12   | 88.0%T± 4.00  | 88.4%T±3.74   | 85.6%T ± 3.15 | 90.28%T± 3.28 |
| P6                               | 60.3%T± 3.77  | 88.1%T± 2.45  | 87.5%T±2.43   | 87.4%T ± 5.07 | 87.98%T± 2.69 |
| P8                               | 64.6%T± 5.27  | 90.7%T± 0.93  | 89.5%T±4.50   | 89.1%T ± 3.49 | 90.25%T± 2.98 |

Description: P0 = 0% bentonite, P2 = 2%, P4 = 4% bentonite = P4, P6 = 6% bentonite, P8 = bentonite 8%.

After the purification of Bali sardinella oil with bentonite at concentrations corresponding to different treatments, the results showed that the free fatty acid values in all treatments met the International Fish Oil Standards (IFOS), that were <2 %. The lowest free fatty acid value (0.245%) was obtained at a bentonite concentration of 8 %. The free fatty acid value at maximal decreased from the baseline by 32.245 %. According to Bahri [11], the higher the mass of bentonite in the bleaching stage, the lower the free fatty acid value. It was also stated by Okolo and Adejumo [12] that the free fatty acid value, iodine value, peroxide value, refractive index and viscosity decrease during bleaching. According to Arita et al. [13], Additional NaOH can attach free fatty acids, which leads to decreased levels of free fatty acids (FFA). Estiasih [1] also argued that free fatty acids will react to form colored compounds that affect the color of the fish oil. According to Aditia et al. [14], the carbon chain with a
The double bond in unsaturated fatty acids will react to heat and form free fatty acids which can affect the quality of fish oil.

The lowest value of peroxide of Bali sardinella oil after being purified with bentonite at a concentration of 2 % is 3.815 mEq/kg. This value has yet to meet the CAC standard, i.e. <5 mEq/kg, but this value has the greatest decrease from the baseline characteristics of peroxide of Bali sardinella oil, amounting 10.945 mEq/kg. According to Dewi and Hidayati [15], bentonite contains alumina and silicate that are effective in attracting adsorbates. Palanisamy et al. [16] also stated that the effectiveness of an adsorbent to reduce peroxide depends on the type of fish oil being purified, time, temperature and the concentration of adsorbent being used. According to Estiasih [1], purification using alkali can also reduce fat oxidation products such as peroxides. According to Andarwulan et al. [9], peroxide is a product that is formed at the beginning of fat oxidation process. The decomposition of peroxide occurs in several steps. The first step is the dissolution of the oxygen bond in peroxide group, which will produce alkoxyl and hydroxyl radicals, while the second step is the breaking of carbon bond.

The p-anisidine values in all treatments are below the CAC standard, i.e. <20 mEq/kg. The p-anisidine values of Bali sardinella oil after purification has decreased as the activated bentonite absorbed the oxidation products. The lowest p-anisidine value of Bali Sardinella oil (3.545 mEq/kg) was obtained from the treatment without bentonite. According to Guillen and Cabo [17], the para-anisidine (p-anisidine) value is not always in line with high peroxide value.

Total oxidation is the relationship between peroxide value and anisidine value that indicates the level of oxidation of fats/oils [1]. The lowest total oxidation value was obtained with bentonite concentration of 0 %, whereas the bentonite concentration of 8 % yielded the highest total oxidation value (28.17 mEq/kg). This value did not meet the CAC standard i.e. <26 mEq/kg. This is due to the fact that the activated bentonite adsorption was not perfect in the bleaching stage. According to O’Brien [18], the factors that affect the bleaching process are the procedure, the dosage of adsorbent, temperature, time, humidity and filtration. Similarly, the pore size plays an important role in the process of adsorption [19].

As a result, the quality and quantity of the result of fish oil purification is directly influenced by the condition of the purification process. The resulted Bali sardinella oil was the highest in the treatment with a bentonite concentration of 6 % (33.418 %). This value indicates that the higher the yield obtained, the higher the economic value of the product. The research of Kurniati and Susanto [20] that used 9.5 % NaOH in the neutralization process could produce a high yield. The research of Suseno et al. [21] showed that the centrifugation at a speed of 6,500 rpm produced the lowest yield. In this study, the centrifugation speed of 6,500 rpm for 10 minutes became a controlled variable, thus it did not affect the yields of all treatments.

According to Hastarini et al. [22], the decrease in the oil weight from the baseline weight is the result of the purification process that can eliminate the impurities found in crude Bali sardinella oil before the purification process. Estiasih [1] also stated that the decrease of yield of oil purification occurs because the amount of alkali given is excessive, which causes hydrolysis reaction of triglycerides and forms soap in an excessive amount. The type of alkali, concentration, the amount of dye, and the technique used (e.g. agitation and separation between saponified and unsaponified fractions) are the important factors that must be considered as they affect the purification process.

The highest clarity of Bali Sardinella oil from the purification process was obtained from the treatment using a concentration of 8 %, with the values at wavelengths (λ) of 450 nm, 550 nm and 665 nm being 64.625 %T, 90.7 %T and 89.1 %T, respectively (table 4). According to Hasibuan et al. [23], the percent transmittance of oil is used as an indicator of change in the levels of dye contained in the oil. The correlation between the percent transmittance and oil clarity level is that the higher the percent transmittance, the lower the level of dye contained in the oil.

The results showed that the clarity of the Bali sardinella oil after the purification was higher than that before purification because the activated bentonite was able to improve the color of the oil. According to Dewi and Hidayati [15], the activation of bentonite using mineral acids (H\textsubscript{2}SO\textsubscript{4}/HCl) produces clay with greater active site because the mineral acids dissolve and react with components in
the forms of tar, Ca salt and Mg covering the pores of the adsorbent, thus bentonite has a surface with high acidity that allows for higher adsorption capacity than its adsorption capacity prior to activation.

4. Conclusion
The purification of Bali sardinella oil with bentonite adsorbent at a concentration of 6 % produced the highest yield (33.42 %) with quality characteristics like free fatty acid value of 0.27 %, peroxide value of 6.34 mEq/kg, para-anisidine value of 3.725 mEq/kg, oxidation number of 16.41 mEq/kg, and clarity of 60.28 %T, 88.08 %T, 87.50 %T, 87.43 %T, 87.98 %T at wavelengths (λ) of 450 nm, 550 nm, 620 nm, 665 nm, 700 nm, respectively. This research revealed that the addition of activated bentonite can increase the quality of fish oil by-product from the Bali sardinella canning industry.

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