Thermal degradation kinetic study of Pangasius fish oil

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Abstract. Pangasius fish oil is rich in unsaturated fatty acids that are beneficial for health. However, unsaturated fatty acids are easy degraded due to temperatures and oxidation. This study aimed to determine the changes in the quality of pangasius fish oil during heating and the result of oxidation value based on activation energy. Pangasius fish oil was heated at three different temperatures which were 70°C, 80°C, and 90°C for 15, 30, 45, 60, and 75 min. The observation includes peroxide value, TBA value, FFA, and fatty acid profiles. The results showed that higher temperatures and prolonged heating increased the peroxide value, TBA value, and FFA. The rate of quality degradation in pangasius fish oil proved by each activation energy of PV 111.433 kJ/mol, TBA 7.368 kJ/mol, and FFA 83.971 kJ/mol and unsaturated fatty acid, especially linolenic acid decreased. It showed that the oxidation during heating reduced the quality of pangasius fish oil.

1. Introduction
Pangasius is a freshwater fish with high nutrient content. The fat content in pangasius is around 14.07%, and it is higher than catfish (Clarias sp.) and eel, which contains respectively 10.60% and 0.71% of fat [1]. Pangasius has potential as raw material for fish oil according to its fat content. Pangasius fish oil rich in saturated fatty acid and unsaturated fatty acid. The saturated fatty acid dominated by palmitic acid, single unsaturated fatty acid by oleic acid (ω-9), and double unsaturated fatty acid by linoleic acid (ω-6) and linolenic acid (ω-3) [2].

Fatty acids, such as ω-3, ω-6, and ω-9 contained in fish oil are useful for health. For example, ω-9 helps to prevent cardiovascular disease, cancer, and works as an anti-inflammatory agent to prevent inflammation after enteral injection [3]. ω-6 is useful in reducing oxidative stress, inflammation, and atherosclerosis [4]. While ω-3 helps to prevent cardiovascular disease, diabetes mellitus, and coronary heart disease [5], also helps the brain growth and development [6].

Fish oil is also suitable for children, adults, and the elderly due to its unsaturated fatty acid contents. However, the problem of fish oil is its fishy odor. Usually, fish oil is consumed in the form of capsules and its application into dairy products and bakeries is still limited. However, several modification such as fish oil nanoemulsion has been used for in yogurts [7], fish oil microcapsules in biscuits [8], and fish oil in snacks [9]. The limited application of fish oil into the foodstuff is caused by the perishable properties of the unsaturated fatty acid.

The double bonds in unsaturated fatty acids, particularly ω-3, are susceptible to oxidation and the products of oxidation are hazardous to health [10]. The unsaturated fatty acids in ω-3 contain multiple bonds that easily broken due to oxidation reactions [11]. The oxidation of unsaturated fatty acids creates peroxide and secondary oxidizing products that reduce the benefits of fatty acids [12]. The oxidation of unsaturated fatty acids is caused by the presence of oxygen, temperature, light, prooxidant [13].
Most of the foods are processed at high temperatures, so the application of fish oil in foodstuff is also limited. Therefore, further study about the kinetics of degradation in fish oil due to the temperature is needed in order to provide information on how quickly oxidative damage reactions occur in fish oil. This study aimed to determine the changes in the quality of pangasius fish oil during heating and determine the level of oxidation based on its activation energy.

2. Methods

2.1. Material

The primary material in this research was *Pangasius* sp. It was purchased from a local market in Semarang, Central Java, Indonesia.

2.2. Extraction of Fish Oil

Samples were dried rendering at 100°C for 30 min, then pressed with a hydraulic press to collect the liquid by product. The fish oil was obtained by centrifugation.

2.3. Degradation Kinetic of Pangasius Fish Oil

The kinetics of degradation was observed at three different temperature. *Pangasius* fish oil was heated by oven at 70°C, 80°C and 90°C for 75 min and observed every 15 min for each temperature parameters. The kinetic reaction followed the Arrhenius equation [14]. Generally, chemical reaction rates that influence foodstuffs degradation are following zero and first-order rule. The data then plotted to the graph of the time (x-axis) and the parameters at each heating temperature. From the graph, the slope of each heating temperature defined as $k$ for each temperature. Then, from the correlation graph between $1/T$ ($k$) and $\ln k$, the activation energy value ($E_a$) and the Arrhenius constant (A) were obtained. The Arrhenius equation as follows:

$$\ln k = \ln A - \frac{E_a}{(R \cdot T)}$$

2.4. Peroxide Value

Peroxide value [15] measured by dissolving the oil to the acetate acid solution – chloroform (3:2) then reacted with saturated KI. Then the sample titrated by Na$_2$S$_2$O$_3$, 0.01 N until the yellow color almost disappeared. Then added a 1% starch solution as the indicator and titrated again until the blue color almost disappeared. The peroxide value expressed in milli-equivalent peroxide per 1000 g of sample.

2.5. TBA Value

TBA value was measured according to the intensity of the red color from the reaction between 2-thiobarbituric acid and malonaldehyde [16].

2.6. Free Fatty Acid

Free fatty acids in fish oils was measured by dissolving the oil into 95% ethanol then heated at 40°C. The value of free fatty acids calculated by titrating the sample, which has been added with a PP indicator with 0.1N KOH to create a pink color that did not disappear for 30 seconds [15].

2.7. Fatty acid analysis

Oleic, linoleic, and linolenic fatty acids were measured using Gas Chromatography-Mass Spectrometer (GCMS-QP2010S Shimadzu) using Rtx 5 MS column (30 m x 0.25 mm ID; 0.25 um film thickness) with helium as the carrier gas. A 1 microliter sample injected into the column at 70°C using the split mode method (1:49). The column temperature then set to increase by 5°C per minute until it reached the final temperature (300°C) and the detector set at 250°C. Methyl ester fatty acids then separated by pressing at 13.7 kPa. The formed fatty acid peaks were identified by comparing the obtained results with mass spectra data based on the Willey 229 library [17].
3. Results and Discussions

3.1. Peroxide Value

Peroxide value measured the primary oxidation output, such as hydroperoxide, peroxide, and the other peroxide polymer from the lipid oxidation process, particularly in unsaturated fatty acid. Oxidation occurred due to the presence of light, increased temperature, and metal elements [18, 19]. Pangasius fish oil heated at 70-90ºC from 0-75 min generated a high peroxide value. It indicated from the equation of zero and first order as a function of temperature resulted in positive k value (reaction rate constant), which means that the prolonged heating could increase the peroxide value. Moreover, the higher temperature could increase the k value. The higher the temperature, the higher the peroxide value. Another study on canola oil that heated at 180ºC for 12 h resulting in increased peroxide value [20]. The increasing temperature causing oxygen insoluble in lipid and water due to partial oxygen pressure [19].

The enhancement in peroxide value was linear to the heating duration, presented by R-value with a score of 0.98. The linearity of peroxide value also occurred on corn and olive-pomace oil [21]. Based on the Arrhenius plot, the slope for PV and activation energy was 13403 and 111.433 kJ/mol, respectively. Activation energy is minimal energy used for initiating some reaction. The activation energy in this study was higher than in walnut oil of 79.57 kJ/mol [22]. The difference due to the heating temperature, the formation rate of peroxide value was influenced by temperature [23].

Table 1. Quality Degradation of Pangasius Fish Oil According to Zero and First Order Reaction

| Quality attribute | Temp (°C) | Temp (°K) | Zero Order Reaction | First Order Reaction | k | ln k | 1/T |
|-------------------|----------|-----------|---------------------|----------------------|---|------|-----|
| PV                | 70       | 343       | y = 0.0032x + 1.3252 | y = 0.0023x + 0.2831 | 0.0032 | -5.7446 | 0.0029 |
|                   | 80       | 353       | y = 0.0097x + 1.3340 | y = 0.0058x + 0.2992 | 0.0097 | -4.6356 | 0.0028 |
|                   | 90       | 363       | y = 0.0467x + 1.3806 | y = 0.0169x + 0.4174 | 0.0467 | -3.0640 | 0.0027 |
| TBA               | 70       | 343       | y = 0.0789x + 1.8574 | y = 0.0165x + 0.8587 | 0.0165 | -4.1044 | 0.0029 |
|                   | 80       | 353       | y = 0.0900x + 1.9928 | y = 0.0172x + 0.9356 | 0.0172 | -4.0628 | 0.0028 |
|                   | 90       | 363       | y = 0.1203x + 2.0596 | y = 0.0197x + 1.0208 | 0.0197 | -3.9271 | 0.0027 |
| FFA               | 70       | 343       | y = 0.0036x + 1.2215 | y = 0.0026x + 0.2035 | 0.0026 | -5.9522 | 0.0029 |
|                   | 80       | 353       | y = 0.0046x + 1.2418 | y = 0.0033x + 0.2178 | 0.0033 | -5.7138 | 0.0028 |
|                   | 90       | 363       | y = 0.0509x + 0.7610 | y = 0.0196x + 0.1097 | 0.0196 | -3.9322 | 0.0027 |

3.2. TBA Value

TBA value used to measure the result of secondary oxidation by a fatty acid. Results of secondary oxidation are aldehyde, 2-enal, and 2-dienal from the termination phase in the oxidation process, where these products are detected when it reacted with 2-thiobarbituric acid. TBA and peroxide value used to measure the rancidity of lipids or oil [24]. In this study, the increase of TBA value was equal to the temperature and heating duration. The k value for TBA at 70ºC, 80ºC dan 90ºC were 0.0789; 0.0900; and 0.1203, respectively. Linear R-value indicated a significant increase and amounted to be 0.96 at 90ºC. The TBA value also increased in groundnut seed oil that heated for 9 h and linear to the increase of peroxide value [25]. Based on the Arrhenius plot, the TBA value was 886.29, so the activation energy value was obtained for 7.368 kJ/mol. The activation energy value for TBA was lower than the energy activation for peroxide. It indicated that the TBA value was more sensitive in the initial step of oxidation and the establishment of the oxidation product [21].
3.3. FFA Value

FFA value expresses the hydrolysis rate of lipid or oil. The increase of FFA value in pangasius fish oil was equal to the increase in temperature and heating duration. The FFA value in vegetable oil also increased due to high-temperature treatment [21]. It showed that the increased temperature led to an increase of hydrolysis and lipolysis activity as its activity could break the chemical bond in oil, then produced free fatty acid and glycerol [26, 27]. Generally, the fatty acid is in the form of triglyceride, but it can degrade during processing, such as the increasing temperature, presence of water in oil, and the presence of lipase as contaminant microorganisms [28]. Based on the Arrhenius plot for FFA, the slope value was 10100, so the activation energy was obtained for 83.971 kJ/mol.

Table 2. Reaction and Activation Energy Equation

| Quality attribute | Reaction Equation | Slope | R (ideal gas constant) | Activation energy kJ/mol |
|-------------------|-------------------|-------|-----------------------|-------------------------|
| PV                | \( y = -13403x + 33.047 \) | 13403 | 8.314 | 111.433 |
|                   | \( R^2 = 0.9902 \) |       |                       |                         |
| TBA               | \( y = -886.29x - 1.5498 \) | 886.29 | 8.314 | 7.368 |
|                   | \( R^2 = 0.914 \) |       |                       |                         |
| FFA               | \( y = -10100x + 23.081 \) | 10100 | 8.314 | 83.971 |
|                   | \( R^2 = 0.8371 \) |       |                       |                         |

3.4. Unsaturated Fatty Acid

Pangasius fish oil contains unsaturated fatty acid MUFA and PUFA with high oleic acid content [29, 30]. Unsaturated fatty acids content in this study that detected by Gas Chromatography-Mass Spectrometer, such as 9-hexadecenoic acid, methyl ester (palmitoleic acid), 9-octadecenoic acid, methyl ester (oleic acid), 9,12-octadecadienoic acid, methyl ester (linoleic acid), and 6,9,12-octadecatrienoic acid, methyl ester (gamma-linolenic acid) (Table 3). Based on the percent area of each unsaturated fatty acid, the composition of 9-octadecenoic acid had the highest value.
The heating treatment altered the unsaturated fatty acids content of pangasius fish oil. The 9-hexadecenoic acid, 9,12-octadecadienoic acid, and 6,9,12-octadecatrienoic acid decreased along with the increased temperature and heating duration, while there was no visible decreased in the percent area of 9-octadecenoic. It showed that 9-hexadecenoic acid, 9,12-octadecadienoic acid, and 6,9,12-octadecatrienoic acid were damaged during the heating process. Another study stated that palmitoleic acid and linoleic acid had decreased by 14% and 19% due to the heating, while oleic acid did not change much [31]. The decrease in unsaturated fatty acids was due to oxidation. Polyunsaturated fatty acids were more easily oxidized due to the number of double bonds. Unsaturated fatty acids 6,9,12-octadecatrienoic acid experienced the most significantly decreased during the heating since it was not detected at the end of heating at 80°C and 90°C for 75 min. It followed the TBA value, which increased along with the increased temperature and heating duration. Unsaturated fatty acids, primarily linolenic acid, created the oxidation products from the oxidation process of unsaturated fatty acids, i.e., malonaldehyde that detected with TBA value [21].

### 4. Conclusion

It can be concluded that heating treatment can reduce the quality of pangasius fish oil. It indicated by the peroxide value, TBA value, and FFA that increased during the heating process. The quality degradation of pangasius fish oil expressed by each activation energy, including PV 111.433 kJ/mol, TBA 7.368 kJ/mol, and FFA 83.971 kJ/mol. Unsaturated fatty acid, especially linolenic acid, decreased significantly related to malonaldehyde formation as the product of oxidation.

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