Kinetic oxidation of protein and fat in snapper (Lutjanus sp) fillet during storage

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KEYWORDS

Reaction kinetics modeling
ABSTRACT

Fillet of snapper (*Lutjanus sp*) contains oil and proteins that are easily oxidized during storage. The kinetic model can determine oxidation reaction rate. The objective of this research was to evaluate kinetic oxidation reaction of both oil and protein in fillet of snapper. Fillet of snapper was stored at 0, 10, 20, 30 and 40 °C. The reaction rate constant (*k*) and activation energy were further determined. Results showed that the value of *k* increased from 0.28 mg/kg per day to 10.27 mg/kg per day at temperatures from 0 to 40 °C for the peroxide value. For the acid value, TBA value and carbonyl value were rate constants 0.05 mg/kg per day becomes 3.56, mg/kg per day, 0.29 mg/kg per day to 8.91 mg/kg per day, and 0.38 nmol/gr per day becomes 12.06 nmol/gr per day, respectively. The activation energy (*Ea*) of oxidation reaction peroxide value 65.69 kJ/mol.K, while the acid value, the TBA value and carbonyl value, 84.80 K/mol.K, 62.99 K/mol.K and 63.64 K/mol.K, respectively. Fish fillet started to deterioration after 20 days when it was stored at 0 °C or after 12 days when it was stored at 10 °C. Kinetics studies show that oxidative reaction of snapper fillet (*Lutjanus sp*) during storage followed zero order reactions.

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Kinetic Oxidation of Protein and Fat in Snapper (*Lutjanus* *sp*) Fillet During Storage

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ABSTRACT. Fillet of snapper (*Lutjanus sp*) contains oil and proteins that are easily oxidized during storage. The kinetic model can determine oxidation reaction rate. The objective of this research was to evaluate kinetic oxidation reaction of both oil and protein in fillet of snapper. Fillet of snapper was stored at 0, 10, 20, 30 and 40 °C. The reaction rate constant (k) and activation energy were further determined. Results showed that the value of k increased from 0.28 mg/kg per day to 10.27 mg/kg per day at temperatures from 0 to 40 °C for the peroxide value. For the acid value, TBA value and carbonyl value were rate constants 0.05 mg/kg per day becomes 3.56, mg/kg per day, 0.29 mg/kg per day to 8.91 mg/kg per day, and 0.38 nmol/gr per day becomes 12.06 nmol/gr per day, respectively. The activation energy (Ea) of oxidation reaction peroxide value 65.69 kJ/mol.K, while the acid value, the TBA value and carbonyl value, 84.80 K/mol.K, 62.99 K/mol.K and 63.64 K/mol.K, respectively. Fish fillet started to deterioration after 20 days when it was stored at 0 °C or after 12 days when it was stored at 10 °C. Kinetics studies show that oxidative reaction of snapper fillet (*Lutjanus sp*) during storage followed zero order reactions.

INTRODUCTION

Fish can be considered as a source of high quality of animal protein and oil (Poly Unsaturated Fatty Acid, PUFA) [1]. However, the high content of oil and protein are one of the main sources of fish oxidation [2]. Lipid oxidation caused quality loss [3]. The primary product of lipid oxidation is hydroperoxide. It is determined as peroxide value (PV). Hydroperoxide can be subsequently oxidized to aldehydes, ketones, and alcohols. Oxidations of peroxide products cause product rancidity. Thiobarbituric acid (TBA) and PV are the main chemical index of oxidative rancidity [4, 5]. Protein is also susceptible to oxidation, which is primarily triggered by free radicals components in fish fillet [6, 7]. Protein oxidation triggered damage in biological systems, and it has been extensively studied over the past two decades [8-10].

Nutrient degradation can be calculated in the same way by microbe inactivation. In general, the kinetics nutrient degradation followed the zero-order and first-order kinetics [11]. Kinetics has been used in food sciences to illustrate how quickly the reaction changes when the product is stored at high temperatures. If the kinetic parameters are known, then they can be used to predict the shelf life of the product [11]. Generally, nutrient degradation under isothermal conditions can be represented by Eqs. 1:

\[
\frac{dc}{dt} = -k(C)^n
\]  

(1)

where k is the rate constant, C is the quantitative indicator of a quality attribute at time t, and n is the order of reaction. The integrated form for zero-, first- and second-order kinetic models is listed in Eqs. (2) and (3), respectively.

\[
\text{zero order : } C_t = C_0 - k \cdot t 
\]  

(2)
\[ \text{first-order: } \ln \frac{C_t}{C_0} = -k \cdot t \quad (3) \]

where \( C_0 \) represents the initial value at time zero, \( C_t \) is the value at time \( t \), \( k \) is the rate constant. The Arrhenius equation (Eq. 4) is usually applied to describe the reaction rate constant temperature dependence:

\[ k = k_0 \cdot e^{-\frac{E_o}{RT}} \quad (4) \]

A plot of the rate constant on the semi-logarithmic scale as a function of reciprocal absolute temperature \((1/T)\) should give a straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant \( R \).

**MATERIALS AND METHODS**

**Raw Material**

Snapper (*Lutjanus sp*) was obtained from the fish market Kobong market, Kaligawe village, Ganyamsari subdistrict, Semarang, province of Jawa Tengah. HCl, acetic acid, thiobarbituric acid, ethanol, phenolphthalein, KOH, chloroform, ammonium thiosulphate, ferrous chloride were obtained from Merck KGaA (Darmstadt, Germany).

**Preparation of Snapper Fillet**

Fresh snapper (*Lutjanus sp*) was skinned. The fish meat was then separated from gill, head, bone, and other fish parts. It was washed and then rinsed with ice water to remove blood and other debris. The fish fillet was analyzed for peroxide value, TBA value, acid value and carbonyl value.

**Effect of Temperature during Storage on Peroxides Value, TBA, Acids Value, and Carbonyl Value**

Approximately 50 g of fish fillet were stored at 0, 10, 20, 30, and 40 °C. Samples were analyzed at various time for each storage temperature. Sampling was conducted every 5 days during 45 days, 3 days during 27 days, every 1 day during 9 days, 12 h during 108 h and 6 h during 54 h at storage temperature 0 °C, 10 °C, 20 °C, 30 °C and 40 °C, respectively. Samples were analyzed for acid value, peroxide value, TBA value and carbonyl value.

**Analysis of Peroxide Value, Acid Value, TBA Value and Protein Carbonyls Value**

Peroxide value was determined according to Adnan [12]. Sample (0.5 g) was put into a test tube, further it was added with 0.1 mL of ammonium thiocyanate and 0.1 mL ferro chloride. The test tube was shaken for 5 sec and heated at 50 °C for 2 min. It was then cooled to 25 °C. Sample absorbance was measured using spectrophotometer at 510 nm. The blank solution was prepared by mixing all reagents without a sample. Peroxide value was calculated as milli-equivalent peroxide per kg of sample using following equation:

\[ \text{Peroxide value} = \frac{X + DF}{g \text{ sample} \times \frac{1}{MW} \text{ Fe}} \quad (5) \]

where \( X \) is mg Fe per 10 mL, DF is dilution factor, and MW is molecular weight of Fe.

The acid value was determined using the acid number titration method [13]. Approximately 0.5 g of oil was added to 50 mL of 95% alcohol then it was heated for 10 min in water bath. It was further cooled, and then phenolphthalein indicator was added before titration using 0.1 N KOH. Titration was ended when pink color just appears.

\[ \text{Acid Value} = \frac{V \text{ KOH} \times N \text{ KOH} \times MW \text{ KOH}}{\text{Sample Weight (g)}} \quad (6) \]
TBA value analysis was performed according to the method by Tokur and Korkmaz [10]. About 0.5 g of oil, 50 mL of distilled water and 2.5 mL of N HCl were mixed sequentially prior to distillation. Distilled liquid was collected up to 50 mL. About 5 mL of distillate was added into 5 mL of TBA and heated for 30 min and cooled. Absorbance was measured at 528 nm. TBA value was expressed as mg malonaldehyde/kg of oil.

Analysis of carbonyl value was performed according to the method of Lappin and Clark [14], about 0.5 g sample was diluted with 10 mL of distilled water. About 1 mL of the final diluted sample was put into a test tube. About 7.5 mL of 1 N KOH and 1 mL of 2.4 dinitrophenylhydrazines were added before mixing. Absorbance was measured at 480 nm.

**RESULTS AND DISCUSSION**

**Effect of Temperature and Period on Peroxide Value**

The concentration of protein and fat in fish fillet were 17.77 % and 1.95 %, respectively. Peroxide is a primary product of snapper (Lutjanus sp) fillet oxidation during storage. It is an indicator for fat stability during storage [15]. Fig. 1 shows peroxide value at various temperatures and time. An increase in peroxide value tended to linear during fish fillet storage at cold temperature (0 °C) for 45 days. Peroxide values increased 7.5 times after 45 days when it was stored at 0 °C, whereas they increased 9.19 times after 27 days, 10.38 times after 9 days, and 11.72 times after 108 h at storage temperature 10 °C, 20 °C, 30 °C and 40 °C, respectively. Peroxide value at a storage temperature of 40 °C was 14.15 times higher compared with at a storage temperature 0 °C after 54 h. Thus the rate of peroxide forming increased, when storage temperature increased from 0 °C to 40 °C. Oxidation of fish fillet occurs easily because fish oil has a high concentration of PUFAs. According to Salam [17], peroxide value limit was 10-20 Meq/kg sample, while Huss [18] mentioned about 3-25 Meq/kg sample.

**Effect of Storage and Time on Acid Value**

The acid value is an important parameter for measuring fat quality. It is an indicator for lipid hydrolysis, in which it can be determined by measuring free fatty acids (FFA) [16]. FFA formation during fish fillet storage can be seen in Fig 2. Acid value increased 2.35 times during storage at 0 °C for 45 days. It increased 2.44 times, 2.97 times, 3.51 times and 4.28 times at storage at 10°C for 27 days, 20°C for 9 days, 30°C for 108 h and 40°C for 54, respectively. FFA formation tended to linear when fillet was stored at cold temperature (0°C) during 45 days. According to Salam [17], acid value standard was 7 mg KOH/g sample, whereas Huss [18] suggested that the acid value was in the range of 7-8 mg KOH/g sample.

![FIGURE 1. Effect of temperature and time on peroxide value](image-url)
FIGURE 2. Effect of temperature and time on acid value of snapper (Lutjanus sp) fillet during storage of snapper (Lutjanus sp) fillet during storage.

Effect of Storage Temperature and Time on TBA Value

TBA value is a parameter for measuring secondary products of lipids oxidation, particularly derived from PUFA [19] and also an indicator for rancidity level, especially in high PUFA-containing fats [1]. The formed TBA, as secondary products of snapper fillet oxidation during storage, can be seen in Fig 3. TBA value increased 6.34 times at 0 °C, when storage time increased from 0 to 45 days, whereas TBA values increased 7.46 times, 8.80 times, 9.55 times and 10.22 times at storage temperature 10 °C for 27 days, 20 °C for 9 days, 30 °C for 108 h, and at 40 °C for 54 h, respectively. According to Salam [17] and Huss [18], the standard of TBA value for fish fillets about 10-15 mg malonaldehyde/kg fish samples.

Effect of Storage Temperature and Time to Carbonyl Value

Carbonyl value is one of protein oxidation biomarker [20] and used as oxygen radicals induced protein damage indicator [21]. Carbonyl value increased 4.15 times at 0 °C for 45 days. It increased 4.71 times at storage temperature 10 °C for 27 days, 5.14 times at 20 °C for 9 days, 5.51 times at 30 °C for 108 h, and 6.09 times at 40 °C for 54 h (Fig 4). It is suggested that an increase in carbonyl value was catalyzed by the iron (Fe) content of snapper protein [22]. Lipid oxidation, which is catalyzed by Fe, can naturally occur. Since fat content in fish fillet was very low, then it is assumed to be insignificant. Thus, an increase in carbonyl value is expected due to protein oxidation. An increase in carbonyl value tended to linear.
Kinetic of Peroxide Value Changes

The reaction rate constant (k) of peroxide formation increased with an increase in storage temperature. They were 0.35, 0.85, 2.88, 7.21 and 18.88 Meq/Kg.day at 0, 10, 20, 30 and 40 °C for the zero-order reaction, respectively. Reaction rate constant for the first-order reaction were 0.05, 0.09, 0.27, 0.59, and 1.28 day^{-1}, respectively. The activation energy (Ea) for the peroxide formation was 57.48 kJ/mol.K for the first-order reaction. While Ea for the zero-order reaction was 65.69 kJ/mol.K. Prediction of peroxide value of fish fillets according to zero- and a first-order reaction can be seen in Fig 5. A linear increase in peroxide value indicated that the reaction tends to follow zero-order reaction. It is due to slow oxidation rate at room temperature. Auto-oxidation may be dominated the reaction since samples were stored in a dark room. Thus photo-oxidation was neglected. According to the peroxide value standard (7 mg Meq/kg sample), it is suggested that fish fillet starts to deterioration when peroxide value exceeds the standards, namely after 20 days when it was stored at 0 °C or after 12 days when it was stored at 10 °C.

Kinetic of Acid Value Changes

Kinetic rate constant (k) of acid formation increased with an increase in storage temperature. They were 0.05, 0.09, 0.44, 1.04, and 0.89 mg/g.a day at storage temperature 0, 10, 20, 30 and 40 °C for zero-order reaction, respectively. They were 0.02, 0.03, 0.13, 0.24, and 0.17 day^{-1} at storage temperature 0, 10, 20, 30 and 40 °C for the first-order
reaction, respectively. The activation energy for the forming of acids was 84.80 kJ/mol.K and 65.88 kJ/mol.K for zero-order reaction and the first-order reaction, respectively. Prediction of snapper fillet acid value under zero- and the first-order reaction can be seen in Fig 6. The pattern of acid value increased linearly indicated that oxidation followed zero-order reaction. This was due to the slow hydrolysis rate of fat at room temperature. According to the acid value standard (7 mg KOH/g sample), it is suggested that snapper fillet, which was stored at 40 °C, was no longer available for consumption after 24 h. Thus, the acid value was not a critical point for the deterioration of fish fillet compared with peroxide value.

![Figure 5](image5.png)
**FIGURE 5.** Prediction of peroxide value under zero- and first-order reaction in snapper (Lutjanus sp) fillet

![Figure 6](image6.png)
**FIGURE 6.** Prediction of acid value under zero- and first-order reaction in snapper (Lutjanus sp) fillet

**Kinetic of TBA Value Changes**

The k value of TBA formation increased with an increase in storage temperature. They were 0.17; 0.45; 0.84; 1.24 and 3.11 mg/kg.a day at storage temperature 0, 10, 20, 30 and 40°C for zero-order reaction, respectively. For first-order reaction, k values were 0.02; 0.05; 0.08; 0.11 and 0.25 day⁻¹ at 0, 10, 20, 30 and 40 °C, respectively. Ea values for TBA formation were 62.9 kJ/mol.K and 48.08 kJ/mol.K for the zero-order and first-order reaction, respectively. Prediction of TBA value of snapper fillet for zero- and the first-order reaction can be seen in Fig 7. TBA value pattern tended to follow zero-order reaction since oxidation was solely caused by auto-oxidation and photo-oxidation was neglected. According to Salam [17], the standard for TBA value was 10-15 mg malonaldehyde/kg fish. It is suggested that fish fillet is unavailable for consumption when TBA value exceeded the standard, namely after 25 days when it was stored at 0 °C or after 15 days when it was stored at 10 °C.
Kinetic of Carboxyl Value Changes

The k value of carboxyl formation increased from 0.0015 to 0.0504 nmol/mg.day with an increase in storage temperature from 0 to 40 °C. Ea values of carboxyl formation for zero- and first-order reactions were 58.86 kJ/mol.K and 63.64 kJ/mol.K, respectively. Prediction of snapper fillet carboxyl value for zero- and first-order reactions can be seen in Fig 8. It is suggested that the hydroxyl radicals were formed by H₂O₂ degradation in the presence of Fe²⁺ or Cu²⁺. It attacked protein and led to protein hydrolysis. As a consequence, protein carboxyls were formed [21]. An increase in temperature and time of storage might lead to increase in protein carboxyl formation [23, 24]. Fig. 8 shows that an increase in carboxyl value tended to follow zero-order reaction due to the slow rate of reaction at room temperature. It is suggested that photo-oxidation dominated the reaction since the sample was stored in a dark condition.

**FIGURE 7.** Prediction of TBA value under zero- and first-order reaction in snapper (Lutjanus sp) fillet

**FIGURE 8.** Prediction of carboxyl value under zero- and first-order reaction in snapper (Lutjanus sp) fillet

**CONCLUSION**

From the kinetic evaluation, it was found that Ea values of the formation of peroxide, free fatty acids, TBA and carboxyl for first order reaction were 57.48 kJ/mol.K, 84.80 kJ/mol.K, 62.99 kJ/mol.K and 58.86 kJ/mol.K, respectively. For zero-order reaction, Ea values of the formation of peroxide, free fatty acids, TBA, and carboxyl were 65.69 kJ/mol.K, 65.88 kJ/mol.K, 56.21 kJ/mol.K, and 63.64 kJ/mol.K, respectively. Based on the calculation of peroxide value and TBA value, fish fillet started to deterioration when peroxide value exceeds the standards, namely...
after 20 days when it was stored at 0 °C or after 12 days when it was stored at 10 °C. It was also unavailable for consumption, when TBA value exceeded the standard, namely after 25 days when it was stored at 0 °C or after 15 days when it was stored at 10 °C. The forming of peroxide, acid, TBA and carbonyl during storage of snapper fillet (Lutjanus sp) followed zero-order reaction.

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