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DETERIORATION KINETICS OF NEUTRALIZED RED FRUIT (*Pandanus conoideus* L.) OIL DURING STORAGE

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Graphical abstract

Abstract

This study's goal was to obtain a kinetics model of neutralized red fruit oil (NRFO) quality change during storage. The NRFO was stored in dark bottles at 60 °C, 75 °C and 90 °C for ± 15 days to evaluate the quality. The results showed that the quality of NRFO decreased along with the increase in temperature and storage time. This was indicated by increasing moisture content, Free Fatty Acid (FFA), peroxide number, as well as decreasing of the total carotenoids and total tocopherols. The FFA content and peroxide number of NRFO increase followed the kinetics zeroth order model, with activation energy (Ea) of 6372 cal/mol^{o}K and 9954 cal/mol^{o}K, respectively. Meanwhile, the decrease in carotenoid and tocopherol content followed the kinetics first order model, with Ea 9581 cal/mol^{o}K and 14242 cal/mol^{o}K, respectively. Estimation of shelf life of NRFO was based on peroxide number equation. The shelf life of NRFO is 398.5 days or 13.3 months if it is stored at 20 °; and the shelf life is 277.6 days or 9.3 months if it is stored 30 °C.

Keywords: Kinetics; deterioration, red fruit (*Pandanus conoideus* L.) oil, neutralization, shelf life

1.0 INTRODUCTION

Natural red fruit oil (*Pandanus conoideus* L.) is crude oil which is rich in both natural antioxidants and unsaturated fatty acids. The antioxidants comprise of α-carotene, β-carotene, β-cryptoxanthin, and α-tocopherol whereas the unsaturated fatty acids include oleic, linoleic, linolenic and palmitoleic acids [1,2]. Indeed, it still contains impurities either non-triglycerides, *i.e.*, gums (phospholipids), carbohydrates, proteins, and pigments; or several other minor components. The purification of crude red fruit oil (CRFO) is intended to remove impurities in order to improve the taste but suppress the

deterioration risks of the oil during storage and processing while minimizing loss in refining step. General conventional refining steps of crude vegetable oils includes degumming, neutralization, bleaching and deodorization process. However, during these steps, a large amount of micronutrients and antioxidant polyphenols, tocopherols, sterols, and carotenoids are unintentionally lost thus the nutritional value and total quality of vegetable oils are substantially reduced [3]. The purification of CRFO can be carried out in two stages, namely, degumming and neutralization [4]. It is expected that all compounds which interfere flavour, color, stability, and safety of the final refined oil products are

removed but the active components are well preserved.

Prior to the neutralization process, the oil needs to be degummed. Degumming process of vegetable oils can be carried out in various ways, but the use of acids has been proven to be cost effective and many variations have been developed to improve consistency of the oil obtained [5]. Hadi *et al.* reported that acid degumming removed phosphorus from Moringa oleifera kernel oil reaching 20.33 ± 1.37 mg/kg, which was much greater than that of water degumming (31.18 ± 0.90 mg/kg) [6]. Sarungallo *et al.* [7], reported that degumming CRFO using citric acid could reduce phosphorus from 51.68 g/ml to 0.20 mg/kg, but did not affect the decrease in moisture content, FFA, peroxide number, iodine number, β-carotene and α-tocopherol [7]. However, the neutralization process using alkali is the most widely used method in the oil industry because it is cheaper and more efficient in reducing crude oil FFA contents [8]. Engelmann *et al.* [9], reported that a study of neutralization of rice bran oil under various temperatures and amount of NaOH solution treatments resulted in the best condition at 60°C and addition of 20 % excess soda, where 0.41 % of oleic acid and 1.10 % of γ-oryzanol was obtained [9]. Furthermore, Sarungallo *et al.* [7], reported that the process of neutralization of degummed red fruit oil (DRFO) using 0.75 % of 1.25 N NaOH did not affect moisture content, iodine value, carotenoids, tocopherols, and α-tocopherol; but significantly decreased the FFA, peroxide, phosphorus and β-carotene levels [7].

Purified red fruit oil, like other vegetable oils, will continuously undergo chemical changes such as hydrolysis and oxidation. Hydrolysis converts triglycerides into FFA and glycerol leading to oil deterioration which occur in the presence of a certain amount of water in the oil [10]. On the other hand, the oil can also undergo oxidation. The oxidation process occurs when oxygen contacts unsaturated oil or fat, which increase the number of peroxides in the oil or fat, thus it begins to form peroxides and hydroperoxides [11]. The CRFO is dominated by unsaturated fatty acids around 70.33 g/100g, by which the main components are oleic acid ≥61.65 g/100g, linoleic ≥7.01 g/100g and palmitoleic acid ≥1.31 g/100g [7]. Meanwhile, the degumming and neutralization processes tend to increase unsaturated fatty acids in red fruit oil by around 67.49 g/100g and 64.54 g/100g [7]. The high level of unsaturated fatty acids in red fruit oil facilitates oxidation because their double bonds can bind oxygen, consequently, it reduces the quality of red fruit oil during storage. In addition, the stability of vegetable oils decreases with increasing amounts of polyunsaturated fatty acids. The initial concentration of FFA does not significantly affect the stability of the oil. It was observed that hydroperoxides began to change to some secondary oxidation products during the monitoring period when sunflower oil and grapeseed oil are used because of the poorest stability amongst oils [12].

Oil quality deterioration reduces the shelf life of food, but it can be controlled through manipulation on their mechanism pathways and chemical reaction rates. The mechanism is based on a kinetics model using Arrhenius equation which can explain the rate of quality degradation as a function of time and temperature [13]. The kinetics method can be used to determine degradation rates in oil quality due to hydrolysis and oxidation during storage which are influenced by temperature and storage time. Sarungallo *et al.* [14], reported that the increase in FFA content and peroxide number of CRFO followed the zeroth order reaction, with activation energy (Ea) values of J/mol °K and 29437 J/mol °K, respectively, whereas the decrease in carotenoid content followed the first order reaction with an Ea value of 66783 J/mol °K [14]. These results indicate that the CRFO is most sensitive to oxidative attacks during storage because it has the lowest Ea value. Furthermore, it was reported that changes in FFA content and peroxide number of the DRFO also followed the zeroth order reaction, with Ea values of 22416 J/mol °K and 34839 J/mol °K, respectively, while the decrease in carotenoid content followed the first order reaction with Ea 48504 J/mol °K. This indicates that the lowest Ea was found for FFA content so that DRFO is most sensitive to hydrolysis deterioration during storage [15]. What is the oxidation kinetics behavior in NRFO during storage as a function of time. This is a highly important quality parameter in order to control its stability. This study aimed to obtain kinetics model of the quality changes of NRFO during storage at high temperatures.

2.0 METHODOLOGY

Materials

The main raw material used in this study was crude red fruit oil (CRFO) extracted through steaming process, followed by pressing and centrifugation; a preparation method by Sarungallo *et al.* [16]. Chemicals with analytical grade levels used for the analysis included phosphate buffer, p-nitrophenilbutyrate and p-nitrophenol from Sigma-Aldrich, Inc. (MO, USA), sodium hydroxyde, phenolphthalein, butylated hydroxyl toluene (BHT), hexane, toluene, ethanol, 2,2 bipyridine and FeCl$_3$.6H$_2$O were obtained from Merck, Darmstadt, Germany. While α-tocopherol standard was purchased from Wako Pure Industries, Ltd. Tokyo, Japan.

Refining Process of Crude Red Fruit Oil (CRFO)

The refining process of CRFO was conducted with degumming stage and followed by neutralization

stage. The degumming stage began with mixing the CRFO with 2 % (by mass) of citric acid for 10 minutes. Then it was washed with water (50°C) and centrifuged (800-1 Centrifugal Machine, Made in China) at 112 x g for 5 minutes in ambient temperature. Furthermore, washing was repeated until neutral pH [15]. The DRFO was stored in a dark glass bottle until neutralization stage.

The DRFO was neutralized referring to Santoso *et al.* Method [4]. The neutralization process was started by mixing DRFO with 1.25 N NaOH 0.75 % and stirred using a magnetic stirrer at 8,4 x g for 5 minutes, then added with distilled water with a ratio of 1:2 (oil: water). Removal of NaOH was conducted using distilled water with a ratio of oil and water 1:1 until neutral. The NRFO was placed in dark glass bottles and ready for analyzed.

Quality Stability Test of NRFO During Storage

The NRFO was tested for oxidation stability using the method by Ayustaningwarno [17]. Sample of NRFO was placed in dark bottles and stored for ±15 days at temperatures of 60 °C, 75 °C and 90 °C. The period for observing the quality of NRFO at each temperature was carried out as presented in Table 1. The research design was a Completely Randomized Design (CRD) with 2 replications. The combination of time and temperature in the observations was justified according to the degradation risks of each treated sample therefore the time intervals of sampling were unequal. For instance, the higher temperature degrades the oil sample severely thus the shorter time scale of sampling was chosen. These combinations have been proven useful for red palm oil by Ayustaningwarno [17]. It is important to recognize the critical time scale as it strongly affects the dynamics reaction curve obtained during observation of kinetics model.

Table 1 Storage duration of the NRFO at different temperatures and storage lengths

Temperature (°C)	Duration of storage (hours)				
60	0	10	34	106	202
75	0	8	24	120	180
90	0	5	24	72	120

Analysis of the Chemical Quality of the NRFO

Quality parameters of the NRFO observed were moisture content using gravimetric method [18], FFA content was analyzed by titration method [18]; and peroxide number using acetic acid-chloroform method [18]. Total carotenoid content was determined using the method of Knockaert *et al.* [19]. A total of 1 mg of each sample was dissolved in 0.1 % of butylated hydroxyl toluene (BHT) solution in hexane. The absorbance of the sample solution was measured spectrophotometrically (Shimadzu UV-2450, Kyoto, Japan) at a wavelength of 470 nm with 0.1 % BHT in hexane as a blank. Total carotenoids were calculated using Equation 1:

$$\text{Carotenoid conc.} \left(\frac{mg}{kg}\right) = \frac{A \times volume\ (ml \times 10^4)}{E_{1\ cm}^{1\%} \times weigth\ sample\ (g)} \quad \text{......(1)}$$

Where A is the absorbance value, V is the total volume of sample solutions; $E_{1cm}^{1\%}$ is the extinction coefficient = 2,560 for β-carotene in hexane [20].

Total tocopherol of the NRFO was determined using the method of Wong *et al.* [21]. Approximately 0.01 g of oil was filled in the volumetric flask (10 mL), then added with 5 mL of toluene, 3.5 mL of 2,2 bipyridine (0.07 % w/v in 95 % ethanol) and 0.5 mL $FeCl_3.6H_2O$ (0.2 % w/v in 95 % ethanol). The solution was added with 95 % ethanol to 10 mL and the absorbance was measured at a wavelength of 520 nm using spectrophotometer (Shimadzu UV-2450, Kyoto, Jepang). A blank was similarly made without sample. The total tocopherol concentration was calculated based on a standard curve of α-tocopherol (100-1,500 mg/kg in toluene).

Data Analysis

Data analysis of quality changes in the NRFO during storage was carried out using Arrhenius model with Microsoft Excel 2007 software. Response surface and counter plots were obtained from Minitab version 16. The quality changes in the NRFO could be explained by Equation 2 for zeroth order reaction and Equation 3 for the first order reaction; where A = concentration (mg/kg), k = reaction rate constant, t = reaction time (days) (22). From Equation 2 and Equation 3, the value of the reaction rate constant (k) is obtained as a function of temperature (T). The representation of the NFRO quality change was chosen based on the highest coefficient of determinant (R^2) from the regression equation. The appropriate reaction order of the NRFO quality deterioration was selected based on the highest value of R^2 from the regression equation.

$$A = Ao + kt \quad \text{.............(2)}$$

$$ln\ A = \ln Ao + kt \text{.........(3)}$$

The effect of temperature on the rate reaction of changes in the NRFO quality during storage was calculated using Arrhenius equation (Equation 4); where k = reaction rate constant, A = pre-exponential factor (mol/Ls), Ea = activation energy (kJ/mol), R = ideal gas constant (1.986 cal/mol °K), and T = temperature (°K) [22].

$$\ln k\ = \ln A - \frac{Ea}{RT} \quad \text{.........(4)}$$

The activation energy (Ea) and the pre-exponential factor or frequency factor (A), was determined from the slope and intercept, respectively, and the line is generated by linear regression between ln k and 1/T.

3.0 RESULTS AND DISCUSSION

Changes in NRFO Quality During Storage

There are several parameters that influence oil quality, such as moisture content, FFA, and peroxide number [23]. Especially for red fruit oil, it is also necessary to consider carotenoids and tocopherols as active compounds that can be degraded during storage.

Based on contour plots (Figure 1a & b), it can be seen that moisture contents increase after 75-130 hours of storage at temperatures of 60-80 °C and reach peaks 115 hours at 60-62.5 °C, whereas at 90 °C it shows relatively stable at around 1-2 %. Thus, there are two peaks of increasing risks of moisture content during storage at temperature 60-80 °C. These tested temperatures are categorized as an accelerated experiment above 55 °C. By using Q_{10} law of Arrhenius thus the lower storage temperature can be deduced from these accelerated tests.
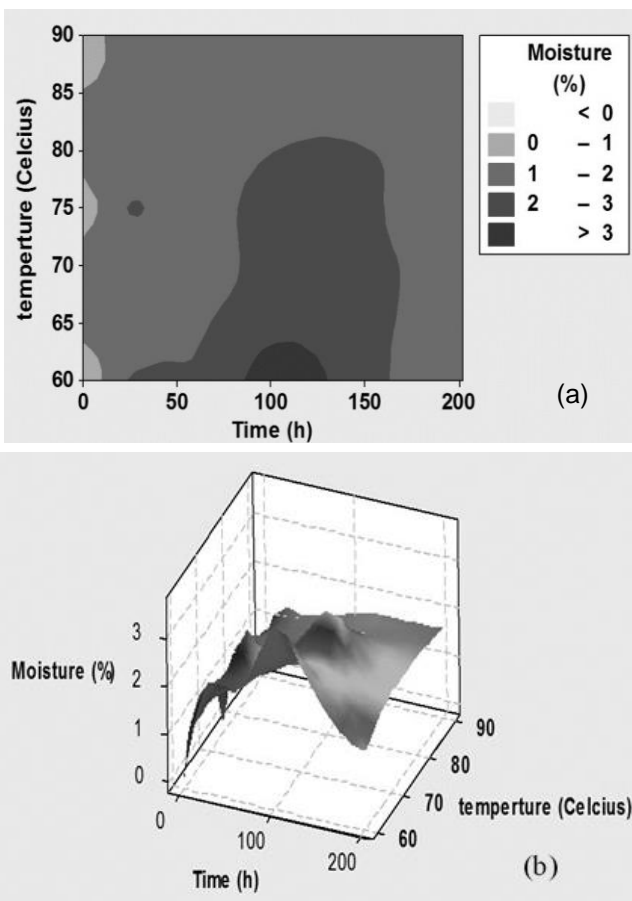
Figure 1 Changes of moisture content of the neutralized red fruit oil (NRFO) during storage at 60, 75 and 90 °C: (a) a contour plot shows critical moisture contents at 60-80 °C stored for 25-160 hours and (b) response surface plot for all data observation

Increasing moisture content in NFRO during storage might be caused by the oxidation reaction, especially at the propagation stage, which is a bimolecular reaction of hydroperoxide compounds that release water and increase the moisture content of the oil [9]. This increase in water content was in line with the increase in peroxide number during storage (Figure 3). Furthermore, the moisture content in the NFRO decreases at the end of storage which might be due to the breakup of a number of chemical bonds making bound water evaporating. An increase in the moisture content of the oil during storage has also been obtained by several other researchers, *i.e.*, crude red fruit oil [14]; coconut oil [24]; as well as crude sunflower oil and crude canola flower oil, which concomitantly increase peroxide number [25].

Changes in FFA content of the NRFO tended to increase during storage at 60°, 75°, and 90 °C (Figure 2). Storage of the NRFO at high temperatures for a long time can trigger a hydrolysis reaction of triglycerides into fatty acids, which is supported by an increase in the moisture content of NFRO during storage (Figure 1). Musafira *et al*. [24], also reported that the moisture content and FFA content of coconut oil increased with increasing storage time [24]. Phenomenon of increasing FFA content during storage at 60°-90 °C also occurs in CRFO ((moisture content from 1.9 % to 4.3 %) [14] and DRFO ((moisture content from 3.9 % to 4.34 %) [15]; where the increasing of FFA in NRFO ((moisture content from 1.29 % to 1.66 %) is lower than DRFO, but higher in CRFO.

Peroxide number of the NRFO was 0.213 meq/kg, lower than the Indonesia standard for cooking oil of 10 meq O_2/kg [23], and the DRFO of 0.39 meq O_2/kg [14]. This indicates that the neutralization process can reduce the oxidation rate of the red fruit oil. Sarungallo *et al*. [7] also reported that the neutralization process could reduce the peroxide number of CRFO from 0.48 meq/kg to 0.26 meq/kg [7].

Changes in peroxide number during storage tended to increase with increasing temperature and storage time (Figure 3). The increase in the peroxide number of NRFO indicates that an oxidation reaction occurs in NRFO during storage because it is dominated by unsaturated fatty acids [7]. The increasing number of peroxides is caused by the decomposition of unsaturated fatty acids, in which the carbon chain is broken then binds to oxygen to form free radicals that produce peroxides [26]. Widodo *et al*. [27], reported that palm oil is very susceptible to oxidation process [27]. Therefore, the peroxide number is an important parameter to determining the shelf life of palm oil. Ayustaningwarno [17] explained that the presence of FFA greatly affected the oxidation of neutralized red palm oil (NDRPO), which occurs at the beginning of the oxidation and is influenced by the presence of water at high temperature [17]. Furthermore, Ghazali *et al*. [28] explained that through heating palm oil at 135 °C and storing it in the dark in the first 100 hours there were formation of hydroperoxides from unsaturated fatty acids as a result of fat oxidation and increased the peroxides number.
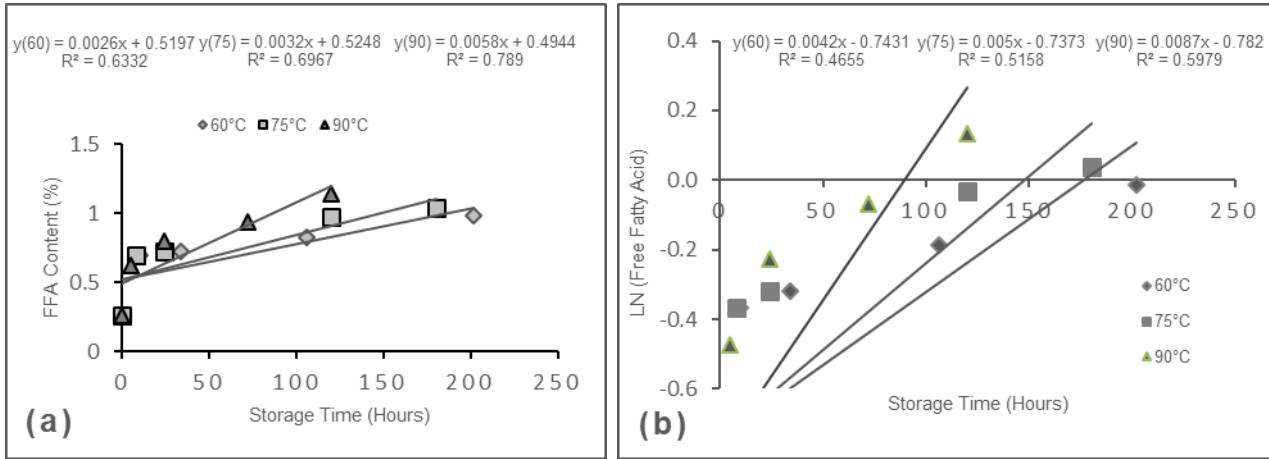
Figure 2 Reaction rates of free fatty acid changes in the NFRO: (a) the zeroth order and (b) the first order reaction at 60, 75, and 90 °C
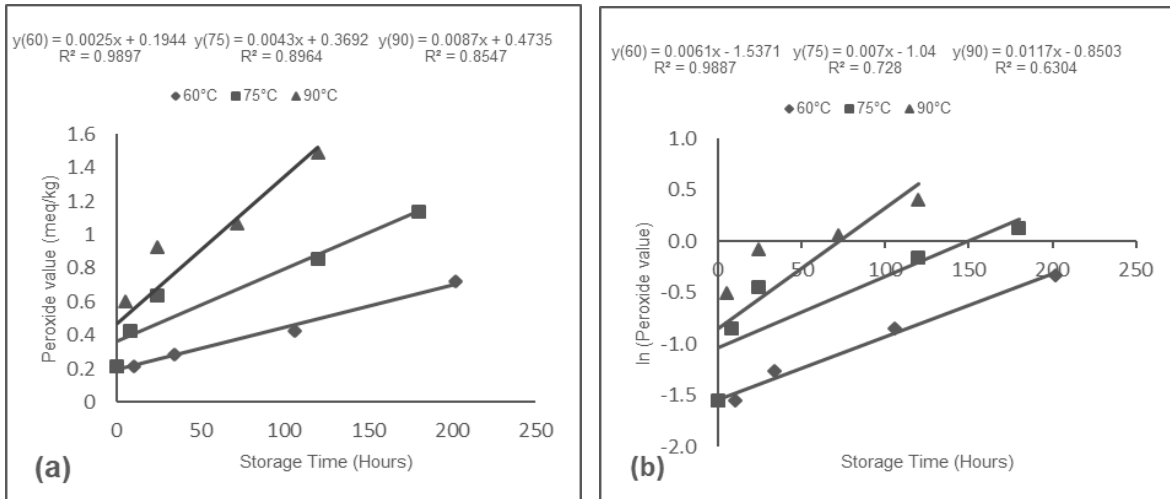

Figure 3 Reaction rate of peroxide number changes in the NFRO: (a) zeroth order and (b) the first order at 60, 75, and 90 °C
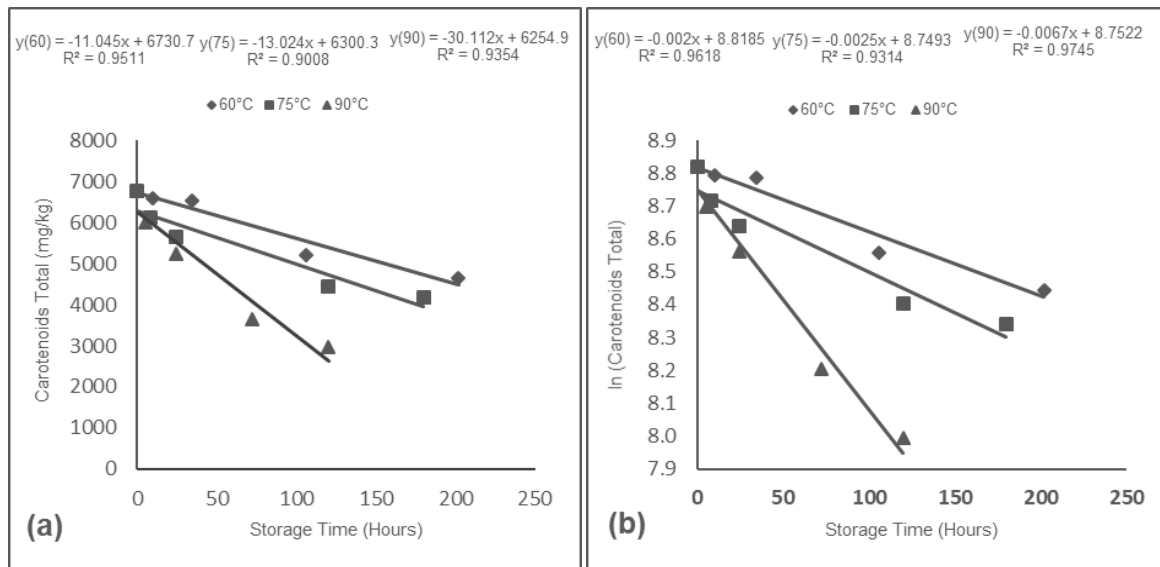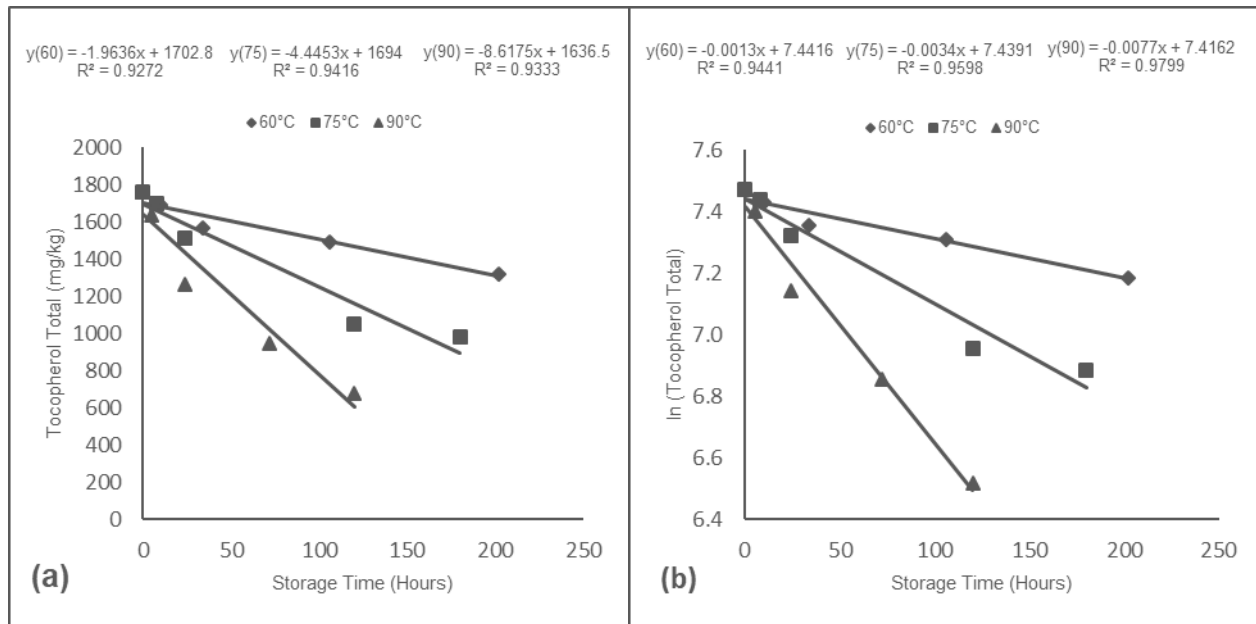

Figure 4 Reaction rates of total carotenoid changes of the NFRO: (a) the zeroth order reaction and (b) the first order reaction at 60, 75, and 90 °C

Figure 5 Reaction rates of total tocopherol changes of the NFRO: (a) the zeroth order and (b) the first order at 60, 75, and 90 °C

Total carotenoids decreased during storage at 60°, 75° and 90 °C (Figure 4). It is also seen that the degradation of carotenoids in the NRFO increases with increasing storage temperature. The high decreasing of carotenoid content in NRFO occurred at 90 °C storage, the initial total carotenoids of 6767 mg/kg decreased to 2964 mg/kg after 120 hours of storage. This phenomenon is influenced by characteristic of carotenoids which have a structure with a conjugated double bond system so that they contain many reactive electrons and are easily degraded by oxygen, light, temperature, enzymes and chemicals [29]. In addition, oxidation of carotenoids resulted in changes of carotenoids from the formation of epoxy compounds to the formation of new carotenoid compounds with lower activity [30]. The reduction of total carotenoid content also occurred in the storage of CRFO for 15 days of storage at 90 °C from 7,857 mg/kg to 2,000 mg/kg [14]. In this study, the carotenoid was continuously decreased during storage. Similar trend was found for the carotenoid in sweet potato peel extract [31].

The tocopherol contents declined during storage at 60°, 75° and 90 °C (Figure 5). The total tocopherol of NRFO decreased from 1,760 mg/kg to 676 mg/kg after storage at 90 °C for 120 hours. On the other hand, tocopherol shows smaller reductions than carotenoids (Figure 4) at the same temperature. This statement is supported by the data in Table 2, which shows that the Ea value of tocopherols is greater than that of carotenoids. According to Choe and Min, tocopherols as antioxidants inhibit free radicals and prevent the formation of peroxides from propagation process [32]. It was also explained that tocopherols had the ability to react with free radicals higher than carotenes to lower potential for tocopherol reduction. Based on these data, the total

tocopherol in NRFO degrades more slowly than total carotenoids.

Kinetics of Deterioration of the NRFO During Storage

The linear or logarithmic kinetic pattern of deterioration of food can be determined based on the order of the reaction, that are zeroth order and first order. The appropriate reaction order is selected based on the highest R^2 coefficient value, so that the mathematical model obtained will be more accurate in predicting the actual quality value [33]. In this research, determining the deterioration model of NFRO was based on the increase in FFA and peroxide number as well as the decrease in total carotenoids and total tocopherols.

The appropriate reaction order of FFA and peroxide number were zeroth order reaction (Figure 2a and Figure 3a). According to Labuza [33], the pattern of change of the zeroth order for food quality is constant during storage. Types of quality deteriorations which follow the zeroth order reaction kinetics include enzymatic reactions, enzymatic browning and oxidation. Sarungallo *et al.* [14] reported that the increase in FFA content and peroxide number of CRFO also followed the zeroth order [14], and similarly for the DRFO [15]. Ayustaningwarno [17] explained that FFA content of the neutralized red palm oil decreased steadily following zeroth order during storage [17], which indicates that the reaction rate was not affected by concentration.

Changes of carotenoid and tocopherol content in NRFO during storage followed first order reaction (Figure 4b and Figure 5b). The reduced carotenoid content from the CRFO and DRFO were also reported following the first order reaction [14, 15], where the

reaction rate was concentration dependent. Šeregelj *et al.* [31] also reported that the carotenoid degradation kinetics of sweet potato peel extract followed first-order kinetics [31], which is indicated by linear regression of ln (carotenoid retention) with negative slope when plotted on a natural logarithmic scale.

The quality degradation constant (k) was obtained from the linier regression equation, which was obtained from the slope in the linear regression of each order (Table 2). In Table 2 also shows that the k value for FFA content is the highest (0.0026) at 60 °C compared to the peroxides number, carotenoids, and tocopherols. It means that the FFAs in the NRFO are very easily formed due to the hydrolysis reaction which are influenced by temperature and are readily deteriorating during storage. It is in agreement with the highest critical moisture content in Figure 1 that the availability of moisture apparently responsible for

the FFA kinetics. It is also triggered by the lowest Ea value (6,372.1 cal/mol °K). The higher the storage temperature of NRFO causes the higher deterioration rate (k value) for each quality parameter observed. Sarungallo *et al.* [14] reported that changes in the quality of the CRFO stored at the same temperature gave the highest oxidation reaction rate compared to hydrolysis reactions and carotenoid degradation [14]; while the fat hydrolysis in DRFO which produced FFA had the highest reaction rate because it had the lowest Ea value [15]. But it is not the case for NRFO when the temperature storage is 75 and 90 °C despite the lowest Ea values because of water evaporation at high temperatures. Hence, there was a significant contribution of neutralization step in kinetics of FFA content compared to other quality parameters observed, not always be the highest reaction rates.

Table 2 The regression of k and ln k values from changes in FFA content, peroxide number, total carotenoids and tocopherols of red fruit oil at different temperature during storage

Temp., T (°C)	Temp., T (K)	1/T	k value	ln k	Regression ln k and 1/T	
					Linier equation	Ea value (cal/mol °K)
Changes of FFA content						
60	333	0.003002	0.0026	-5.952		
75	348	0.002872	0.0032	-5.745	y = -3208.5x +3.6157[a]	6,372.1[b]
90	363	0.002754	0.0058	-5.150		
Change of peroxide number						
60	333	0.003002	0.0025	-5.991		
75	348	0.002872	0.0043	-5.449	y = -5012.2x + 9.0256[c]	9,954.2[d]
90	363	0.002754	0.0087	-4.744		
Change of carotenoids						
60	333	0.003002	0.0020	-6.215		
75	348	0.002872	0.0025	-5.991	y = -4824.1x + 8.1424[e]	9,580.7[f]
90	363	0.002754	0.0067	-5.006		
Change of tocopherol						
60	333	0.003002	0.0013	-6.645		
75	348	0.002872	0.0034	-5.684	y = -7171.4x+14.901[g]	14,242.4[h]
90	363	0.002754	0.0077	-4.867		

[a] The intercept is the A value (3.6157) then the change of FFA, Ao value = exp. 3.6157

[b] Slope is the - *Ea*/R value (3,208.5), where R: gas constant (1.986 cal/mol °K), then for change of FFA, value - *Ea* = 3208.5 x 1.986 cal/mol °K = 6,372.1 cal/mol °K

[c] Change of peroxide number, *Ao* value = exp. 9.0256

[d] Change of peroxide number, - *Ea*/R value is 5,012.2, then *Ea* value = 9,954.2 cal/mol °K

[e] Change of carotenoids, *Ao* value = exp. 8.1424

[f] Change of carotenoids, - *Ea*/Rvalue is 4,824.1, then *Ea* value = 9,580.7 cal/mol °K

[g] Change of tocopherol, *Ao* value = exp. 14.901

[h] Change of tocopherol, - *Ea*/R value is 7,171.4, then *Ea* value = 14,242.4 cal/mol °K

Based on the facts, the CRFO are very sensitive to oxidation reactions, so that for post-production handling it needs to be immediately packaged; while the DRFO and the NRFO were very sensitive to the hydrolysis reaction which produce FFAs. Therefore, in the purification process, both degumming and neutralization could trigger a hydrolysis reaction especially due to washing steps [7]. Meanwhile, Andarwulan *et al.* [34] reported that during storage of palm cooking oil which passed through the refining process, *i.e,* degumming, neutralization, bleaching and deodorization with a very low water content, the formation rate of peroxide is higher than that of FFAs [34]. The presence of minute water in the final refined oil is a critical point in quality assurance of oil. Therefore, it is highly recommended that NRFO handling requires drying step to suppress hydrolysis attack by residual moisture from washing step.

Changes of antioxidant components in red fruit oil, *i.e.* carotenoids and tocopherols, took a relatively longer time to degrade due to higher Ea values than that of FFA and peroxide number (Table 2), that it requires high activation energy to start oxidation. The same phenomenon also occurs in the CRFO [14] and the DRFO [15]. Edge and Trustcott [35] explained that carotenoids can prevent radical singlet oxygen from quenching by converting singlet oxygen into triplet oxygen which is not reactive. Based on Ea, it is more likely that the hydrolysis took place first then it facilitated the oxidation reactions for carotenoids and tocopherols when the packaging already controlled with tight sealing and dark protection from lights.

In Table 2 shows that the Ea value of carotenoid changes in the NRFO is 9,581 cal/mol °K, much lower than that of CRFO is 78,113 J/mol °K or 18,653 cal/mol °K [14], and similarly for DRFO is 48,504 J/mol °K or 11,583 cal/mol °K [15]. Moreover, the k values at storage temperatures of 75 and 90 °C show higher values than that of FFA contents. These data indicate that red fruit oil that has undergone a refining process has lower carotenoid stability than crude oil yet more vulnerable for deterioration. In high purity oil has lower the stability of the bioactive components. However, the purification stage can increase the oxidative stability of red fruit oil during storage, which can be seen from the Ea of peroxide value of NRFO is higher than that of FFA (Table 2).

The data in Table 2 also shows that the Ea value of the total tocopherol is greater than that of total carotenoids, which indicates that tocopherols in NRFO are more stable than carotenoids during storage. Ogan *et al.* [36], stated that oxidation inhibition in oil will use the stronger antioxidant, thus carotenoids sacrifice themselves so that they will be oxidized first. Also, Liu *et al.,* stated that α-tocopherol was the most effective as antioxidation of β-carotene at a concentration of 0.10 % under light exposure [37]. However, the coexistence of tocopherol and β-carotene was reported giving a protective effect on red palm oil from oxidative stability risks [38]. Similarly,

in the present research tocopherol best storage is at 60 °C with the lower k value (0.0013) than β-carotene (0.0020).

Estimation of the NRFO shelf life can be drawn based on the data in Table 2, using the formula in Equation 3, then the resulting equation for increasing FFA content and peroxide number, using the equation: $K = 37.1773[e^{-3208.5 \ (1/T)}]$ and $K = 8,313.2009[e^{-5012.2 \ (1/T)}]$ whereas the decreasing of the total carotenoids and tocopherols using the equation: $K = 3,437.16[e^{-4824.1 \ (1/T)}]$ and $K = 2,960,889[e^{-7171.4 \ (1/T)}]$, respectively. The Ea value of the peroxide number was the lowest compared to the other parameters (Table 2), therefore the estimation of the shelf life of NFRO was using the peroxide number equation. The shelf life of NRFO is 398.5 days or 13.3 months if stored at 20°C, and the shelf life is 277.6 days or 9.3 months if stored at 30°C.

4.0 CONCLUSION

The quality of NRFO tends to decrease with increasing temperature and storage time, which is indicated by increases in moisture content, FFA, and peroxide number but simultaneously show decreases in total carotenoids and total tocopherol. The increasing FFA and peroxide number in NRFO followed the zeroth order kinetics model, with Ea of 6,372.08 cal/mol °K and 9,954.23 cal/mol °K, respectively. Meanwhile, the decreasing carotenoid and tocopherol content followed the first order kinetics model, with Ea of 9581 cal/mol °K and 14242 cal/mol °K, respectively. There was a relationship between activation energy (Ea) and rate of quality change (K). Peroxide number is more sensitive to temperature increase than other parameters. While tocopherol is relatively more resistant to increasing temperature. Based on the present study, it can be drawn a recommendation to add drying step for neutralized red fruit oil to eliminate residual moisture due to washing step prior to packaging in a dark bottle in order to suppress the hydrolytic attack which further facilitate the oxidative deterioration.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

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