Effect of light intensity on the photo-oxidation stability of red and yellow palm olein mixture

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ABSTRACT

Palm oil is an edible oil derived from the mesocarp of oil palm fruit (Elaeis guineensis), which has a high content of carotenoids and tocopherol components. This research aimed to study the effects of light intensity on the photo-oxidation stability of a red and yellow palm olein mixture. The red and yellow palm oleins were mixed into 100, 200, and 350 ppm carotene content. The photo-oxidation stability of the palm olein mixture was investigated under fluorescent light intensities of 5,000 and 10,000 lux at 31±2°C for 7 days. Changes in the content of chlorophyll, carotene, tocopherols, and peroxide value (PV) were evaluated daily. The results showed that an increase in carotene and tocopherol contents effectively improved the photo-oxidative stability of the palm olein mixture. Degradation of chlorophyll, tocopherols, and increased PV were proportional to light intensity during photo-oxidation. There were no significant changes in carotene content at 5,000 lux light intensity exposure. The degradation rates of chlorophyll and tocopherols can be described as first-order reaction kinetics. In contrast, the increase rate of PV can be described as a zero-order kinetics model with k-values of 6.6 x 10^{-2}, 4.9 x 10^{-2}, 3.7 x 10^{-2} mequiv.kg^{-1}.h^{-1}, and 8.3 x 10^{-2}, 6.8 x 10^{-2}, and 5.6 x 10^{-2} mequiv.kg^{-1}.h^{-1} in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure, respectively. These results suggested that carotene protected tocopherol in palm olein and that tocopherol and carotene synergistically acted as singlet oxygen quenchers during photo-oxidation.

Keywords: carotenoids, intensity, palm olein, photo-oxidation, tocopherol

INTRODUCTION

Indonesia is one of the world's largest palm oil (PO) producing countries besides Malaysia [1]. As one of the world's most produced and consumable edible oils, PO has a high content of carotenoids and tocopherols. Generally, PO in Indonesia is consumed in the form of cooking oil, which is packaged in transparent plastic. Packaging palm cooking oil in transparent plastic allows photo-oxidation reactions during storage and distribution.

The palm oil used as cooking oil has undergone a refining and bleaching process so that it contains limited carotene and tocopherol, indicated by its transparent yellow colour. However, palm oil can also be processed into red palm oil (RPO) to retain β-carotene and vitamin E. Red palm oil is processed crude palm oil from the fruit mesocarp using pre-treatment of deacidification and deodorization via short-path distillation and without bleaching process [2]. In refining without bleaching, RPO also maintains chlorophyll content. The amount of chlorophyll in refined palm oil is 582.9-579.4 µg.kg^{-1}, the highest among cottonseed, rapeseed, safflower, sunflower, corn, and soybean oils [3]. Studies have shown that the amount of retained carotenoids after refining ranges from 500 to 786 parts per million, depending on the condition of crude (red) palm oil before refining. About 80% of the carotenoids retained were found to consist of 0.2% phytoene, 0.6% phytolflue, 41.3% α-carotene, 10.2% cis-α-carotene, 41.0% β-carotene, cis-β-carotene, 0.6% ζ-carotene, 0.8% γ-carotene, 0.8% δ-
carotene, 0.2% neurosporene, 0.5% α-zeacarotene, 1.3% β-zeacarotene and 1.0% lycopene [4]. Among the 13 carotenoids retained, only α-carotene, β-carotene, and γ-carotene can show provitamin A activity and have 15, 44, and 300 times more retinol than carrots, leafy vegetables, and tomatoes, respectively [4]. The meta-analysis study demonstrated that RPO might effectively prevent or alleviate vitamin A deficiency [5].

Other than carotenoids, tocopherols and tocotrienols also contribute to the oxidative stability of red palm oil. About 85% of tocopherols and tocotrienols are retained after refining, ranging from 600 to 1000 parts per million [2]. The tocopherols and tocotrienols present are in the form of 19% α-tocopherol, 29% α-tocotrienol, 41% γ-tocotrienol and 10% δ-tocotrienol with 70% are tocotrienols, and 30% are tocopherols. As an antioxidant, it may prolong food products' shelf-life, stabilize oils and fats, and lower free radical damage [6].

Although RPO contains chlorophyll, characterized as a photosensitizer, the high content of β-carotene and tocopherol compounds in RPO has antioxidant activity in photo-oxidation [7]. The mechanism of singlet oxygen quenching or another excited sensitizer compound by β-carotene was done via energy transfer [8]. Meanwhile, tocopherol acts as a physical quencher of singlet oxygen, for which α-tocopherol has the highest activity, followed by γ- and δ-tocopherol [8]. Because of that, red palm oil had the rate of chlorophyll degradation slower than virgin olive oil, and a mixture of olive oil and perilla [9].

Light intensity triggers photo-oxidation reactions in food containing photosensitisers and influences its nutritional quality [10]. Meanwhile, the presence of β-carotene has an important role in the photo-oxidation stability of RPO, which also contains chlorophyll and tocopherols [7], [9], [10]. Even though palm cooking oil might be exposed to light intensity during storage and distribution, photo-oxidation in palm cooking oil has not been reported yet. This research objective was to study the effect of light intensity on the photo-oxidation stability of red and yellow palm olein mixture. The deterioration rate during photo-oxidation can be used to obtain suitable storage conditions for palm cooking oil.

Scientific hypothesis
The bioactive components in palm oil (carotenoids, tocopherol, and chlorophyll) significantly affected the photo-oxidation stability of palm cooking oil. Information about the influence of light intensity on photo-oxidation stability and the changes of bioactive compounds during photo-oxidation storage can be used to apply suitable storage conditions for palm cooking oil.

MATERIAL AND METHODOLOGY
Samples
Crude palm oil (CPO) was obtained from Salim Ivmosas Pratama Ltd., Jakarta. Crude palm oil was processed into red palm olein (RPO) and yellow palm oil (YPO) in March 2022. The RPO and YPO were used as materials in this study.

Chemicals
Beta-carotene and tocopherol standards were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). N-hexane, toluene, isooctane, methylene chloride, ethanol, 2,2 bipyridine, and FeCl₃·6H₂O were obtained from JT Baker (Phillipsburg, NJ, USA). All chemicals were analytical grade.

Instruments
Chlorophyll, β-carotene, and tocopherol changes in mixed oil were analyzed by Shimadzu UV-2450 spectrophotometer (Shimadzu Co., Tokyo, Japan). The photo-oxidation stability of palm oil during storage was carried out using a mirror glass box (60 × 50 × 30 cm) equipped with nine fluorescent cool white lights 18 watts as a light source. The box was placed in a wooden box (70 cm × 60 × 50 cm) equipped with a heater, thermocouple, and 4 blowers relayed at the bottom of the glass box. Light intensity and temperature during storage were controlled using a lux meter (Digital Lightmeter QM1587, China) and thermocouple digital (Digital LED Display DC 12V K-type, China), respectively.

Laboratory Methods
Peroxide value (PV) was evaluated using the AOCS method Cd 8-53 [11]. The chlorophyll content was determined using the AOCS method Ce 13i-96 [12]. The absorbances of samples were measured at 670, 630, and 710 nm using CH₂Cl₂ as blank. Chlorophyll content was calculated as Equation 1.

\[
\text{Chlorophyll content (ppm) } = \frac{34.5 \times (A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710})}{L}
\]  

Where:
A = absorbance at each wavelength; L = cuvette thickness (mm).
Carotene content in oil was determined using the PORIM method [13]. As much as 4 mg of palm oil was dissolved with hexane and diluted into 10 ml. The solution was transferred into a 1 cm quartz cuvette, and the absorbance was measured at 446 nm against hexane. The carotene content in oil was expressed as ppm (molar absorption coefficient was $1.40 \times 10^3 \text{L.mol}^{-1}.\text{cm}^{-1}$).

Tocopherol content was determined based on [14]. A total of 40 mg samples were put into a 10 ml volumetric flask, added 5 ml toluene, 3.5 ml 2,2-bipyridine (0.07% w/v in 95% ethanol), 0.5 mL FeCl$_3$·6H$_2$O (0.2% w/v in 95% ethanol), and appropriated 10 ml with 95% ethanol. The sample was shaken and allowed to stand for 10 minutes. The absorbance of the sample was measured at 520 nm. Tocopherol content was calculated based on the standard curve $\alpha$-tocopherol in the range of 250-2000 ppm.

**Description of the Experiment**

**Sample preparation:** Red palm oil was processed from CPO at the laboratory of the Pilot Plant of Oil and Fat, Southeast Asian Agricultural Science and Technology Center, Bogor, Indonesia. The RPO contains 4.36 ±0.03 ppm total chlorophyll, 559.39 ±4.26 ppm $\beta$-carotene, and 1262.47 ±2.31 ppm tocopherols, with the composition of fatty acids; 40.32% oleic, 35.81% palmitic, 11.00% linoleic, 3.92% stearic, and 0.30% linolenic acids. Yellow palm olein (YPO) was purified from CPO by bleaching and application processes in the chromatographic column to remove chlorophyll, carotene, and tocopherol compounds. Red palm oil and YPO were mixed according to the ratio, so each treatment had a carotene content of 100, 200, and 350 ppm. The carotene, tocopherol, and chlorophyll content were measured before and during photo-oxidation storage.

**Number of samples analyzed:** Six samples from forty-eight total samples were analyzed daily during photo-oxidation storage. The samples were exposed to light on intensities of 5,000 and 10,000 lux for 7 days at 31 ±2 °C.

**Number of repeated analyses:** The samples were collected in triplicate, and the changes in chlorophyll contents, carotene, tocopherol, and peroxide values were analyzed daily.

**Number of experiment replication:** Each study was carried out three times. The samples were eight for seven days of storage. Consequently, twenty-four repeated analyses were carried out for 5,000 and 10,000 lux intensities, respectively.

**Design of the experiment:** The study was conducted experimentally. Palm oil photo-oxidation was performed according to [10] with light intensity modification. Palm oil as much as 30 ml was poured into a 100 ml transparent serum bottle with 77% headspace. The bottle was tightly capped with rubber and sealed with plastic parafilm. The samples were placed in a mirror glass box and exposed to light on intensities of 5,000 and 10,000 lux for 7 days of photo-oxidation storage at 31 ± 2 °C. The samples were collected and analyzed every d in triplicate. The change rate of chlorophyll, $\beta$-carotene, tocopherol, and peroxide values in the palm olein mixture were determined during photo-oxidation. Evaluation of the kinetics model describes the reaction rate as a function of experimental variables, making it possible to predict changes in photooxidation. The general equation for studying chlorophyll, $\beta$-carotene, tocopherol, and PV changes can be given as Equation 2.

$$\frac{dQ}{dt} = -kQ^n$$  \hspace{1cm} (2)

Where:

- $k$ – represents the reaction rate constant;
- $Q$ – represents the content of chlorophyll, $\beta$-carotene, tocopherol (ppm), or PV mequiv.kg$^{-1}$ at time $t$;
- $n$ – represents the reaction order;
- $t$ – represents the time (h).

**Statistical Analysis**

The changes of chlorophyll, $\beta$-carotene, and tocopherol contents, and peroxide values in the palm olein mixture were analyzed by linear programming on Microsoft Excel 16 and IBM SPSS Statistics 24. All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. The accuracy of the experimental data was determined using the Student’s t-test with a confidence coefficient $p \leq 0.05$.

**RESULTS AND DISCUSSION**

**Chlorophyll, $\beta$-carotene and tocopherol changes during photo-oxidation of palm olein mixture**

The presence of minor components, especially chlorophyll, contributed to the photo-oxidation reactions that occur in oil. According to [15], light exposure can lead to chlorophyll being excited and acting as a photosensitizer type II that reacts with triplet oxygen and transforms into highly reactive singlet oxygen. Singlet oxygen directly attacks the double bond in chlorophyll-a between the fifth and sixth carbon, resulting in a subsequent shift of
Position of the double bond and formation of hydro-peroxides, which are then further cleaved through oxygen-oxygen linkage and form degradation products [16]. Figures 1a and 1b showed that the higher light intensity exposure accelerated the degradation rate of chlorophyll in the palm olein mixture, especially on the lowest carotene content (100 ppm). Chlorophyll content in palm oil, which contains 100, 200, and 350 ppm carotene, decreased from 0.41, 1.19, 1.96 to 0.13 (68.29%), 0.57 (52.10%), and 1.19 ppm (39.28%) after 7 days of photo-oxidation storage on light intensity of 5,000 lux.

Meanwhile, on light intensity storage of 10,000 lux, chlorophyll content in palm oil decreased faster than 5,000 lux, starting from 0.33, 1.08, 1.84 to 0.09 (72.73%), 0.47 (65.88%), and 0.92 ppm (50%) after 7 days of photo-oxidation storage which contains 100, 200, and 350 ppm carotene, respectively. The degradation rate of chlorophyll content in PO can be described as a first-order kinetics model. Exposure to the light intensity of 5,000 lux caused degradation of chlorophyll in palm oil, which contains 100, 200, and 350 ppm carotene with a reaction rate constant (k values) of $6 \times 10^{-3} \ (r^2 = 0.77)$, $3.5 \times 10^{-3} \ (r^2 = 0.81)$, and $2.7 \times 10^{-3} \ h^{-1} \ (r^2 = 0.86)$, respectively. Meanwhile, exposure to light intensity of 10,000 lux caused degradation of chlorophyll faster than 5,000 lux in palm oil, which contains 100, 200, and 350 ppm carotene with k values of $6 \times 10^{-3} \ (r^2 = 0.66)$, $4 \times 10^{-3} \ (r^2 = 0.80)$, and $3.5 \times 10^{-3} h^{-1} \ (r^2 = 0.82)$, respectively (Figure 1b). This was similar to the photo-degradation of chlorophyll in RPO, which followed the first-order kinetics model [9].

![Figure 1](image_url)  
**Figure 1** The first-order kinetics of chlorophyll degradation rate in red and yellow palm olein mixture which contain (o) 100 ppm, (□) 200 ppm, and (△) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.
The concentration of carotene showed the role of maintaining chlorophyll content in oil, which indicated the antioxidant activity of carotene. The study conducted by [17] stated that carotenoids added to soybean oil could singlet oxygen quencher to protect the soybean oil from chlorophyll photo-sensitized oxidation. The greater the number of double bond conjugations, the greater the effect of protection against oxidation of singlet oxygen.

According to [18], 1 molecule of β-carotene can quench 250-1000 molecules of singlet oxygen with \( k = 3 \times 10^{10} \text{ M}^{-1}\text{s}^{-1} \) via energy transfer. The excitation energy of the electron is transferred from the singlet oxygen to the singlet state carotenoids (CAR), producing carotenoid triplet state \( ^3\text{CAR} \) and triplet oxygen, called quenching singlet oxygen. Energy is also transferred from the excited triplet sensitizer state \( ^3\text{Sen}^* \) to the carotenoid singlet state \( ^1\text{CAR} \), called quenching triplet sensitizer.

Changes in carotene contents in palm oil during storage when exposed to light at intensities of 5,000 and 10,000 lux are presented in Figure 2. Figure 2a showed no significant changes in carotene content in palm olein mixture \( (p > 0.05) \) during 7 days of 5,000 lux light intensity exposure. This indicated that exposure to light at an intensity of 5,000 lux could not cause carotene degradation in the palm olein mixture. According to [19], visible light causes the degradation of carotene, which is slower, as much as 1/100, than UV rays. The photo-degradation rate of β-carotene was slow in this research, similar to β-carotene in pure olive oil up to 22.5 h on exposure to the light intensity of 12100 lux [20]. An increase in amounts of β-carotene in RPO during photo-oxidation storage was unlikely [7], probably associated with tocopherol was acting as an antioxidant for the protection of β-carotene via a delay in the isomerization process of trans-β-carotene [21]. 13,15-di-cis-β-carotene is the main isomer formed due to light exposure with a molar extinction coefficient higher than trans-β-carotene [16], [22].

![Figure 2](image-url)

**Figure 2** The zero-order kinetics of β-carotene degradation rate in red and yellow palm olein mixture which contain (○) 100 ppm, (□) 200 ppm, and (◇) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.
In the case of 10,000 lux light intensity exposure, carotene in the palm olein mixture significantly decreased \( (p < 0.05) \) during 7 days of photo-oxidation storage (Figure 2b). After 7 days of storage, exposure to the light intensity of 10,000 lux caused degradation of 100, 200, and 350 ppm carotene in palm olein mixture as much as 58.93\% (42.81 ppm), 19.35\% (165.29 ppm), and 4.98\% (333.49 ppm), respectively. Figure 2b also shows that the degradation rate of carotene in palm olein mixture during exposure to 10,000 lux is described as a zero-order kinetics model. Light intensity accelerated degradation of carotene in palm olein mixture \( (p < 0.05) \), which contains 100, 200, and 350 ppm with \( k \) values of 0.39 \( (r^2 = 0.98) \), 0.25 \( (r^2 = 0.96) \) and 0.11 ppm.h\(^{-1} \) \( (r^2 = 0.96) \), respectively. The appearance of red and yellow palm olein mixture during exposure to 5,000 and 10,000 lux for 7 days is shown in Figure 3.

Figure 3 The red and yellow palm olein mixture which contain 100 ppm, 200 ppm, and 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux for seven days.

The slow rate of carotene photo-degradation in RPO was also shown by [9]. This study measured carotene degradation after storage for 12, 11, and 10 days at light intensities of 5,000, 10,000, and 15,000 lux, respectively. Exposure to low light intensity (5,000 lux) during 7 days of storage was not strong enough to cause carotene degradation, which can be observed using a spectrophotometer at a wavelength of 446 nm. The isomerization process of trans-\( \beta \)-carotene into 13,15-di-cis-\( \beta \)-carotene increased absorbance in the measurement of total carotene [22]. Because of that, carotene was relatively stable during dark storage, and the degradation rate of \( \beta \)-carotene in RPO followed the zero-order kinetics model with \( t_{1/2} \) for 12 months [23].

The photo-degradation of carotene in the palm olein mixture had a different pattern from the auto or thermal oxidation reaction in RPO. Temperature treatments of 60, 75, and 90 °C during storage increased the degradation rate of \( \beta \)-carotene in RPO, which followed the first-order reaction kinetics with \( k \) values of 9.13 x 10\(^{-3} \), 2.15 x 10\(^{-2} \), and 4.79 x 10\(^{-2} \) h\(^{-1} \) at 60, 75, and 90 °C storage, respectively [24]. The high energy exposure to carotene caused a higher rate of carotene degradation.

In the case of tocopherol, exposure to light and carotene concentration resulted in changes in tocopherol content in the palm olein mixture (Figure 4). Figure 4 showed that light intensity accelerated the degradation of tocopherol in palm olein mixture \( (p < 0.05) \), especially on the low content of carotene (100 and 200 ppm). Tocopherol content in palm oil, which contains 100, 200, and 350 ppm of carotene, decreased from 670, 911.13, and 1027.47 to 46.82 (93.01%), 201.92 (77.84%), and 288.56 ppm (71.92%) after 7 days of photo-oxidation storage on light intensity of 5,000 lux. Meanwhile, on light intensity storage of 10,000 lux, tocopherol content in palm olein mixture decreased from 746.14, 944.78, 1189.81 to 53.76 (92.79%), 170.68 (81.93%), and 274.49 ppm (69/69%) after 7 days of photo-oxidation storage which contains 100, 200, and 350 ppm carotene, respectively.

The degradation rate of tocopherol during light exposure in red and palm olein mixture is described as a first-order kinetics model, as shown in Figure 4. Exposure to light intensity of 5,000 lux caused degradation of tocopherol in palm oil, which contains 100, 200, and 350 ppm carotene with \( k \) values of 1.5 x 10\(^{-2} \) \( (r^2 = 0.99) \), 9.0x10\(^{-3} \) \( (r^2=0.99) \), and 7.4x10\(^{-3} \) h\(^{-1} \) \( (r^2=0.98) \), respectively (Figure 4a). Meanwhile, exposure to the light intensity of 10,000 lux caused degradation of tocopherol faster than the light intensity of 5,000 lux, which contains 100, 200, and 350 ppm carotene with \( k \) values of 1.9 x 10\(^{-2} \) \( (r^2 = 0.92) \), 9.70 x 10\(^{-3} \) \( (r^2 = 0.90) \), and 7.4 x 10\(^{-3} \) h\(^{-1} \) \( (r^2 = 0.99) \), respectively (Figure 4b). High-light-intensity supplied more energy to result in singlet oxygen for further progression of oxidation. The degradation of tocopherol during photo-oxidation was due to its chemical quenching of singlet oxygen and free-radical scavenging [8]. Light also supplies energy to break the O–H bond and ether linkage in the structure of tocopherol, resulting in semiquinone radicals and quinones [25].
The photo-degradation rate of tocopherol in 10,000 lux was higher than 5,000 lux light intensity, especially on the low carotene content (100 and 200 ppm) (Figure 4). This result indicated that carotene had a protective effect on tocopherol in palm oil during photo-oxidation. Although the light intensity accelerated the degradation of tocopherol, the presence of carotene was more protective of tocopherol due to light exposure. At the highest carotene content (300 ppm), exposure to light intensities of 5,000 and 10,000 lux showed the same rate of tocopherol degradation ($7.4 \times 10^{-3} \text{h}^{-1}$). According to [7], changes in tocopherol to the tocopheroxyl radical occurred at a slower rate in the TAG+Toc+Car model system and became measurable due to the antioxidant activity of β-carotene. Beta-carotene is excited by light energy, becomes more active, and donates hydrogen, thus protecting tocopherol against free radicals [25].

**Figure 4** The first-order kinetics of tocopherol degradation rate in red and yellow palm olein mixture which contain (a) 100 ppm, (b) 200 ppm, and (c) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.

**Effect of light intensity and β-carotene on photo-oxidation stability of palm oil**

The effect of light intensity and carotene during photo-oxidation storage on PV in palm olein mixture is shown in Figure 5. Exposure to 5,000 and 10,000 lux light intensities directly increased PV in the palm olein mixture ($p < 0.05$). Peroxide value in palm oil which contains 100, 200, and 350 ppm carotene, increased from 0.00, 0.69, 1.39 to 11.17, 8.91, and 7.51 mequiv.$\text{kg}^{-1}$ after 7 days of photo-oxidation storage on light intensity of 5,000 lux (Figure 5a). Meanwhile, on light intensity storage of 10,000 lux, PV in palm olein mixture increased higher than 5,000 lux, starting from 0.49, 0.99, 1.68 to 14.52, 12.46, and 11.05 mequiv.$\text{kg}^{-1}$ after 7 days of photo-oxidation storage which contains 100, 200, and 350 ppm carotene, respectively (Figure 5b). The high PV in the palm olein
mixture after 7 days of light storage made the oil no longer fit for consumption as cooking oil, the quality standard for cooking oil in Indonesia at a maximum PV of 10 mequiv.kg\(^{-1}\) [26]. This result indicated that light exposure was very effective in initiating photo-oxidation. The higher the light intensity, the faster the PV in palm oil increases. Light accelerates oil oxidation, especially if the oil contains sensitizers such as chlorophylls [27].

As shown in Figure 5, the increased rate of PV can be explained by the zero-order kinetics model. Exposure to light at an intensity of 5,000 lux increased k values of PV to 6.6 x 10\(^{-2}\) (\(r^2 = 0.99\)), 4.9 x 10\(^{-2}\) (\(r^2 = 0.99\)), and 3.7 x 10\(^{-2}\) (\(r^2 = 0.99\)) mequiv.kg\(^{-1}.h^{-1}\) in palm olein mixture which contains 100, 200 and 350 ppm carotene, respectively (Figure 5a). Exposure to light at an intensity of 10,000 lux also proportionally increased k values to 8.3 x 10\(^{-2}\) (\(r^2 = 0.99\)), 6.8 x 10\(^{-2}\) (\(r^2 = 0.99\)), and 5.6 x 10\(^{-2}\) (\(r^2 = 0.99\)) mequiv.kg\(^{-1}.h^{-1}\) in palm olein mixture which contains 100, 200, and 350 ppm carotene, respectively (Figure 5b). Exposure to high light intensity accelerated the chlorophyll excitation and transferred more energy onto adjacent triplet oxygen to form active singlet oxygen [15]. Electrophilic singlet oxygen directly reacts with the double bond's high electron density, producing conjugate and non-conjugate hydro-peroxides [15], [27], [28]. The quantity of hydro-peroxides formed during photo-oxidation was proportional to the amount of light absorbed [27].

In this research, carotene could inhibit the increase of PV in palm olein mixture on either the light intensities of 5,000 or 10,000 lux (Figure 5). The higher carotene content slowed the increase of PV during exposure to light. This was because of carotene activity as a singlet oxygen quencher during photo-oxidation in RPO [7]. However, mixing red and yellow palm olein will also increase the tocopherol content in the olein mixture. Tocopherol in the palm olein mixture suggested cooperation with carotene as an antioxidant during photo-oxidation [7], [21]. Carotene had a protective effect on tocopherol due to exposure to light intensity, while tocopherol could be an antioxidant in photo- and auto-oxidation [8]. The palm olein mixture is used as cooking oil, which has high carotene and tocopherol content and can be stored in dark conditions to maintain its photo-oxidation stability.

Figure 5 The zero-order kinetics of peroxide value increase rate in red and yellow palm olein mixture which contain (a) 100 ppm, (b) 200 ppm, and (c) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.
CONCLUSION

The degradation of chlorophyll and tocopherols and an increase in PV were proportional to light intensity during photo-oxidation in red and yellow palm olein mixtures. The degradation rates of chlorophyll contents can be described as first-order reactions with $k$-values of $6 \times 10^{-3}$, $3.5 \times 10^{-3}$, $2.7 \times 10^{-3}$, and $6 \times 10^{-3}$, $4 \times 10^{-3}$, $3.5 \times 10^{-3}$, respectively in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure. The degradation rates of tocopherols contents can be described as first-order reaction with $k$-values of $1.5 \times 10^{-2}$, $9.0 \times 10^{-3}$, $7.4 \times 10^{-3}$, and $1.9 \times 10^{-2}$, $9.70 \times 10^{-3}$, and $7.4 \times 10^{-3}$, respectively in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure. Meanwhile, the increase rate of PV is described as a zero-order kinetics model with $k$-values of $6.6 \times 10^{-2}$, $4.9 \times 10^{-2}$, $3.7 \times 10^{-2}$ mequiv.kg$^{-1}$h$^{-1}$, and $8.3 \times 10^{-2}$, $6.8 \times 10^{-2}$, and $5.6 \times 10^{-2}$ mequiv.kg$^{-1}$h$^{-1}$ in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure.

The carotene content in the palm olein mixture showed no decrease at low light-intensity storage conditions. The carotene content at high concentrations might act as a singlet oxygen quencher, decrease tocopherol degradation, and eliminate the effect of differences in light intensity up to 10,000 lux. The palm olein mixture used as cooking oil should be stored in dark packaging to delay the rancidity and degradation of the carotene and tocopherol contents.

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**Ethical Statement:**
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