Effect of Tocopherols, Tocotrienols, β -Carotene, and Chlorophyll on the Photo-oxidative Stability of Red Palm Oil

Dewi Fortuna Ayu^{1,2}, Nuri Andarwulan^{1,3,*}, Purwiyatno Hariyadi^{1,3}, and Eko Hari Purnomo^{1,3}

¹Department of Food Science and Technology, Bogor Agricultural University, IPB Darmaga Campus, Bogor 16680, Indonesia

²Department of Agricultural Technology, Riau University, Bina Widya Campus Km 12.5 Simpang Baru, Pekanbaru 28293, Indonesia

³Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University, IPB Darmaga Campus, Bogor 16680, Indonesia

Received August 5, 2015
Revised December 27, 2015
Accepted January 1, 2016
Published online April 30, 2016

*Corresponding Author
Tel/Fax: +62-251-8629535
E-mail: andarwulan@yahoo.com

pISSN 1226-7708
eISSN 2092-6456

© KoSfoST and Springer 2016

Abstract Effect of tocopherols, β -carotene, and chlorophyll on photo-oxidative stability of red palm oil (RPO) were studied. Model systems of triacylglycerols+tocopherols, triacylglycerols+ β -carotene, triacylglycerols+tocopherols+ β -carotene, and triacylglycerols+tocopherols+ β -carotene+chlorophyll were exposed to fluorescent light at intensities of 5,000, 10,000, and 15,000 lux for 7 h at $30 \pm 2^\circ\text{C}$. Changes in concentrations of tocopherols, tocotrienols, β -carotene, chlorophyll, and peroxide values were evaluated every hour. Light intensity accelerated degradation of tocopherols in the triacylglycerols+tocopherols system and β -carotene in the triacylglycerols+ β -carotene system. Gamma-tocotrienol showed the highest degradation rate and β -carotene was the most sensitive compound to changes in light intensity, indicated by the lowest light intensity coefficient (z_i) value. The presence of tocopherols and β -carotene together showed protective effects for the photo-oxidative stability of RPO. The presence of chlorophyll increased the rate of photo-oxidation at high light intensities. Interactions between tocopherols and β -carotene contributed to the photo-oxidative stability of RPO.

Keywords: tocopherols, β -carotene, chlorophyll, photo-oxidation, red palm oil

Introduction

Red palm oil (RPO) is a refined palm oil with a high carotene content of approximately 524–542 mg/kg (1,2). In addition, RPO contains vitamin E at 953–955 mg/kg in the form of α -tocopherol (α -T), and α -, γ -, and δ -tocotrienol (α -T3, γ -T3, and δ -T3) (1,2). RPO after refining still contains a low content of chlorophyll, which may act as a photosensitizer (3,4). The presence of tocopherols, tocotrienols, β -carotene, and chlorophyll were associated with the photo-oxidative stability of edible oils (5,6).

Chemical structures of tocopherols, tocotrienols, and β -carotene affect roles during photo-oxidation. Beta-carotene has 11 conjugated double bonds which, associated with a capability to quench singlet oxygen or a sensitizer triplet, are excited via energy transfer (4–7). According to Hernandez-Marin *et al.* (8), β -carotene exposed to UV light can transfer electrons to peroxy radicals. Meanwhile, tocopherol acts as a physical quencher of singlet oxygen, for which α -tocopherol has the highest activity, followed by γ - and δ -tocopherol (5,6). The structure of the aromatic ring in tocopherol was associated with donation of a phenol hydrogen to peroxy and alkyl radicals (6). Although β -carotene acts as a pro-oxidant in sunflower and rapeseed oils, a combination of tocopherol and β -carotene showed effective

inhibition of photo-oxidation (9,10).

According to Yi *et al.* (1), RPO stability during auto-oxidation was lower than the stability of yellow palm olein, and a slight decrease in the carotene content of RPO was associated with regeneration of carotene by tocopherols or tocotrienols. In the absence of tocopherol, β -carotene rapidly degraded (11). Schroeder *et al.* (12) and Rossi *et al.* (13) reported that the thermal stability of RPO was higher than the stability of yellow palm oil and also noted that tocopherols, tocotrienol, and β -carotene showed synergistic anti-oxidant activities.

Light intensity is a factor that triggers photo-oxidation in food containing photosensitizer (4,6). Effects of tocopherols, tocotrienols, and β -carotene, and of interactions during photo-oxidation of red palm oil containing chlorophyll, have not been reported. The objective of this study was investigation of the effects of tocopherols, tocotrienols, β -carotene, and chlorophyll during photo-oxidation in red palm oil model systems exposed to high light-intensities.

Materials and Methods

Materials and chemicals Refined bleached deodorized (RBD) palm olein and crude palm oil (CPO) were obtained from Salim Ivomas

Pratama Ltd., Jakarta, Indonesia in March of 2014. CPO was processed into red palm oil (RPO) in a laboratory of the Pilot Plant of Oil and Fat, Southeast Asian Food and Agricultural Science and Technology Center, Bogor, Indonesia. Standards of β -carotene, α -T, γ -T, and neutral oxide alumina were obtained from Sigma-Aldrich (St. Louis, MO, USA) while standards of α -T3, γ -T3, and δ -T3 were obtained from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Chemicals for analysis were purchased from JT Baker Chemical Co. (Phillipsburg, NJ, USA). All chemicals were of analytical grade.

Preparation of model systems Model systems used were triacylglycerols (TAG)+tocols (Toc), TAG+ β -carotene (Car), TAG+Toc+Car, and TAG+Toc+Car+chlorophyll (Chlor). TAG+Toc was prepared from RBD palm olein that contained tocols at 704.72 mg/kg. TAG+Car was prepared from RBD palm olein from which tocols were stripped (14), followed by addition of β -carotene. TAG+Toc+Car was prepared from RBD palm olein with added β -carotene. TAG+Toc+Car+Chlor was prepared from RPO that contained tocols, β -carotene, and chlorophyll. Beta-carotene was added to TAG+Car and TAG+Toc+Car at approximately 530 mg/kg.

RBD palm olein was stripped of tocol compounds via adsorption chromatography using a glass column (750x22 mm i.d.) packed with 150 g of activated alumina (100°C for 16 h, then 220°C for 8 h), followed by suspension in *n*-hexane. RBD palm olein (100 g) was dissolved in 100 mL of *n*-hexane, passed through the column, then washed with 100 mL of *n*-hexane. The purification process was performed in a dark room and TAG was collected in a dark bottle. The solvent was removed from TAG using a rotary evaporator at 40°C for 45 min. TAG was void of tocol compounds (<10%), based on use of an LC 2040 HPLC apparatus (Shimadzu, Kyoto, Japan). TAG in dark bottle was passed with N₂ gas for 1-2 min and stored in Sansio tropicalized freezer (Sansio, Copenhagen, Denmark) at -20°C.

Photo-oxidation of oils Photo-oxidation of oils was performed according to AOCS recommended practice Cg 6-01 (15) with modification of the light intensity. Thirty mL of RPO was poured into a 100 mL transparent serum bottle (77% headspace), then the bottle was tightly capped with rubber and sealed with plastic parafilm to prevent leakage. Bottles were placed in a 60x50x30 cm mirror glass box equipped with 9 fluorescent cool white lights at 18 watts each (Fig. 1). The glass box was placed in a 70x60x50 cm wooden box equipped with a thermocouple, a heater, and 4 blowers (Karya Mitra Usaha Ltd., Bogor, Indonesia) attached to the bottom of the glass box. Bottles were exposed to light at intensities of 5,000, 10,000, and 15,000 lux at 30±2°C for 7 h. Palm oil samples from bottles were collected and analyzed every h in triplicate.

Analysis of oils Tocol compound compositions in oil were determined using an LC 2040 HPLC apparatus (Shimadzu) (15). A total of 0.04 g oil was dissolved in 1 mL of methanol, vortexed (Vortex-Genie 2; Scientific Industries Inc., Bohemia, NY, USA) for 3

min at room temperature, passed through a 0.45 mm nylon syringe filter (Sartorius, Goettingen, Germany), and injected into the HPLC that was equipped with a Shimadzu LC-20 AD pump, a Combi Develosil RP-5 column (50x4.6 mm, 5 mm i.d.) (Nomura Chemical, Tokyo, Japan), and a UV-Vis Shimadzu SPD-20A detector at an excitation wavelength of 292 nm. The mobile phase was methanol: water (95:5, w/w) with a flow rate of 1.0 mL/min. Tocopherol isomers were identified based on comparison of retention times with standards of α -T (2.5-200 mg/kg), γ -T (0.1-14 mg/kg), α -T3 (25-1,000 mg/kg), γ -T3 (4-1,000 mg/kg), and δ -T3 (2-250 mg/kg). Concentrations of tocopherol isomers in oil samples were calculated based on calibration curves of α -T, γ -T, α -T3, γ -T3, and δ -T3 standards.

The β -carotene content in oil was determined using HPLC (Shimadzu) (16). One hundred mg of oil was dissolved in 10 mL of ethanol and 2.5 mL of KOH 50% (w/v) was added. The samples solution was heated at 80°C in water bath for 1 h. After cooling at room temperature, oil samples received 2.5 mL of glacial acetic acid, vortexed (Scientific Industries Inc.) for 1 min at room temperature, and then made up to 25 mL in a volumetric flask with a solvent mixture of ethanol:tetrahydrofuran (1:1, v/v). The solution was passed through a nylon 0.45 mm syringe filter (Sartorius) and injected into a Shimadzu LC 2040 HPLC. A symmetry C18 ODS column (15x4.6 cm, 5.0 mm i.d.) (Waters, Milford, MA, USA) and a Shimadzu SPD-20A UV-Vis detector set at 450 nm were used. The elution solvent was a mixture of dichloromethane:acetonitrile: methanol (2:1:3, v/v/v) at 1.0 mL/min. Beta-carotene in oil samples was identified based on comparison of retention times with a β -carotene standard and quantified by using calibration curve of β -carotene standard.

Chlorophyll and thiobarbituric acid reactive substance (TBARS) contents were determined spectrophotometrically while peroxide values and free fatty acid (FFA) contents were determined gravimetrically using AOCS methods Cc 13d-55, Cd 19-90, Cd 8-53, and Ca 5a-40 (15), respectively.

Photo-degradation kinetics The general equation for study of changes in tocopherol, tocotrienol, and β -carotene contents in oil samples was:

$$dQ/dt = -kQ^n$$

where *k* represents the reaction rate constant, *Q* represents the tocopherol, tocotrienol, or β -carotene content at time *t* (mg/kg), *n* represents the reaction order, and *t* represents time (h). Coefficient of determination (*r*²) was also calculated to assess how close the data are to the fitted regression line.

The influence of light intensity on the reaction rate constant (*k*) was evaluated based on plotting the value of log *k* vs. light intensity. The light intensity coefficient (*z_i*), as a dependence indicator of the *k* value to changes in light intensity (lux), was then calculated.

Statistical analysis Tocopherol, tocotrienol, and β -carotene contents in oil samples at initial and final exposure times to light were

statistically analyzed using an analysis of variance (ANOVA) and Duncan's multiple comparison test at $p < 0.05$.

Results and Discussion

Chemical characteristics of model systems Chemical characteristics of model systems used in this study are shown in Table 1. Model systems had low levels of oxidation, indicated by a low peroxide value (PV), and TBARS, and free fatty acid (FFA) values. PV and FFA values of model systems met the standard of Indonesia quality cooking oil (17) of a maximum of 10 mequiv/kg and 0.3%, respectively. Major fatty acids in TAG+Toc, TAG+Car, and TAG+Toc+Car model systems were palmitic (31.45%), stearic (3.9%), oleic (36.80%), and linoleic (11.0%) acids with a saturated/unsaturated fatty acid ratio of 43:57. Major fatty acids in the TAG+Toc+Car+Chlor model system were palmitic (35.8%), stearic (3.17%), oleic (40.3%), and linoleic (10.36%) acids with a saturated/unsaturated fatty acid ratio of 45:55. Consequently, oil model systems were susceptible to photo-oxidation due to an abundance of electrons in double bonds of unsaturated fatty acids (4).

Photo-degradation of α -tocopherol (α -T), and α , γ , and δ -tocotrienol (α -T3, γ -T3, and δ -T3) Changes in α -T, α -T3, γ -T3, and δ -T3 contents in oil model systems during storage with exposure to light at intensities of 5,000, 10,000, and 15,000 lux are presented in Fig. 2. In the absence of carotene, as illustrated by the TAG+Toc model system (Fig. 2A), amounts of α -T, α -T3, γ -T3, and δ -T3 all decreased during storage, and the extent of tocols degradation was associated with the light intensity. For example, the amount of α -T decreased as much as 14.56, 19.53, and 20.67% after 7 h of storage under light intensities of 5,000, 10,000, and 15,000 lux, respectively. Similar phenomenons were observed for α -T3 with decreases of 12.83, 14.25, and 24.67%, γ -T3 with decreases of 5.37, 11.97, and 22.55%, and δ -T3 with decreases of 5.82, 10.03, and 13.32% after 7 h

of exposure to light at intensities of 5,000, 10,000, and 15,000 lux. According to Choe and Min (6) and Choe (18), degradation of tocopherols during photo-oxidation was related to actions as anti-oxidants. Light also supplies energy to break the O-H bond and the ether linkage in the tocopherol structure, resulting in the semoquinone radical and quinones (19).

Further kinetics analysis showed that the photo-degradation rate of tocols followed a first order kinetics model (Table 2). Gamma-tocotrienol had the highest rate of degradation, followed in order by α -T > α -T3 > δ -T3. This phenomenon was also observed during deep fat frying of RPO at 175 and 165°C, which showed that γ -T3 had the lowest stability among isomers due to the highest activity of free radical scavenging (12,13).

The presence of β -carotene at 529.47 mg/kg in the TAG+Toc+Car model system showed protective effects for α -T, α -T3, γ -T3, and δ -T3, amounts of which did not significantly ($p > 0.05$) decrease for up to 7 h of exposure to light, compared with controls (Fig. 2B). An increase of tocol content during exposure to light was unlikely because there was no synthesis of tocols, and the observed increase in absorbance value might have been due to formation of tocopheroxyl radicals from tocopherol during early stages of photo-oxidation (Fig. 2B). The tocopheroxyl radical has a higher molar extinction coefficient than tocopherol on the UV-vis spectrum (20). In the TAG+Toc+Car model system, change of tocopherol to the tocopheroxyl radical occurred at a slower rate and became measurable due to the anti-oxidant activity of β -carotene. According to Hernandez-Marin *et al.* (8) β -carotene, which is excited by light energy, becomes more active and donates a hydrogen and, thus, protects tocol compounds against free radicals. An observed increase in the tocol absorbance value was visible for α -T, followed in intensity by the α -T3, γ -T3, and δ -T3 isomers (Fig. 2B).

The role of chlorophyll in photo-oxidation is shown in Fig. 2C. Amounts of α -tocopherol, α -T3, γ -T3, and δ -T3 in the TAG+Toc+Car+Chlor system decreased in the first h of storage, followed by an increase during the remaining duration of storage probably

Table 1. Chemical characteristics of model systems¹⁾

Chemical characteristics	Model systems			
	TAG+Toc	TAG+Car	TAG+Toc+Car	TAG+Toc+Car+Chlor
β -Carotene (mg/kg)	3.40±0.36	535.73±41.00	529.47±23.36	518.61±8.65
Tocols compound (mg/kg)	704.72±30.57	64.44±10.90	641.02±8.02	698.76±3.56
α -T (mg/kg)	191.30±1.35	14.83±7.64	166.18±3.04	190.82±16.59
γ -T (mg/kg)	2.37±0.20	ND ²⁾	1.70±0.28	ND
α -T3 (mg/kg)	144.75±8.99	28.80±6.93	133.05±3.71	115.09±2.52
γ -T3 (mg/kg)	276.00±18.91	ND	259.69±9.03	290.28±15.09
δ -T3 (mg/kg)	90.29±2.15	20.81±1.66	80.40±2.40	102.01±2.82
Chlorophyll (mg/kg)	ND	ND	ND	3.44±0.12
PV (mequiv/kg)	0.30±0.00	1.62±0.14	0.44±0.01	1.97±0.16
TBARS value (mg/kg)	0.01±0.00	0.03±0.00	0.02±0.00	0.03±0.00
FFA (%)	0.09±0.01	0.14±0.02	0.15±0.01	0.24±0.02

¹⁾Value is mean and standard deviation of triplicates, $n=3$

²⁾ND, not detected

Fig. 1. Schematic diagram of a light box for controlled photo-oxidation experiments

because, at the initial exposure time, singlet oxygen directly oxidized tocopherols and tocotrienols. However, the presence of β -carotene eventually inhibited further degradation of tocol compounds, similar to the TAG+Toc+Car model system (Fig. 2B). Formation of singlet oxygen was indicated by a decline in chlorophyll content during the first h of light exposure (data not shown). Thus, β -carotene was an active anti-oxidant, especially at high light-intensities of 10,000 and 15,000 lux.

Photo-degradation of β -carotene Changes in β -carotene contents in oil model systems during storage with exposure to light at intensities of 5,000, 10,000, and 15,000 lux are presented in Fig. 3. In the absence of tocols, as illustrated by the TAG+Car model system (Fig. 3A), the amount of β -carotene decreased during storage, and the extent of degradation was directly associated with the light intensity. Amounts of β -carotene decreased 2.03, 5.26, and 13.54% after 7 h of exposure to light at intensities of the 5,000, 10,000, and 15,000 lux, respectively. Using a first order kinetics model, degradation rate constant (k) values of β -carotene stored and exposed to light at intensities of 5,000, 10,000, and 15,000 lux were 0.37×10^{-2} ($r^2=0.75$), 0.72×10^{-2} ($r^2=0.87$), and 2.19×10^{-2} per h ($r^2=0.65$), respectively. An increase in light intensity accelerated formation of free radicals, which probably accelerated degradation of β -carotene.

As observed for β -carotene, the presence of tocols together with β -carotene in the TAG+Toc+Car system did not significantly ($p>0.05$) decrease after 7 h of exposure to light, compared with controls (Fig. 3B), indicating a protective effect of β -carotene. An increase in amounts of β -carotene during storage was unlikely because there was no synthesis of β -carotene, so the observed increased level of β -carotene's absorbance was probably associated with tocol compounds acting as anti-oxidants for protection of β -carotene via a delay in the

isomerization process of trans- β -carotene (21,22). According to Chen and Huang (22), 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 (23).

The same phenomenon was also observed in changes of β -carotene amounts in the TAG+Toc+Car+Chlor system after 7 h of exposure to light (Fig. 3C). Formation of singlet oxygen might cause fast degradation of β -carotene in the early stages of light exposure. However, an increase in the content of β -carotene after 2 h of exposure to light at intensities of 10,000 and 15,000 lux was unlikely due to anti-oxidant activities of tocol compounds in the TAG+Toc+Car model system.

Effect of light intensity on degradation of tocopherols, tocotrienols, and β -carotene

The influence of light intensity on degradation rates of tocopherols and tocotrienols in TAG+Toc and β -carotene in TAG+Car model systems was examined based on measurement of the light intensity coefficient z_i as an indicator of k value dependence on changes in light intensity (Table 2). Beta-carotene had the most sensitive degradation rate to changes in light intensity, followed in order by γ -T3> α -T3> δ -T3> α -T. Beta-carotene has 11 conjugated double bonds while tocotrienols have 6 conjugated double bonds and tocopherols have 3 conjugated double bonds, which may be associated with sensitivity to light intensity, indicated by the lowest z_i value of 12,500 lux.

Effect of tocols, β -carotene, and chlorophyll on the photo-oxidative stability of RPO

Photo-oxidation of oil with exposure to light at intensities of 5,000, 10,000, and 15,000 lux was indicated as an increase in the PV. An increase in PV relative to the initial value (Δ peroxide value) of oil during storage was monitored (Fig. 4). In the

Fig. 2. Changes in α -T, α -T3, γ -T3, and δ -T3 contents in (A) TAG+Toc, (B) TAG+Toc+Car, and (C) TAG+Toc+Car+Chlor model systems during exposure to light at intensities of 5,000, 10,000, and 15,000 lux. Bar indicates the mean value of three replicate experiments and standard error. Different letters on bars indicate significant differences at $p<0.05$ based on Duncan's multiple comparison test.

TAG+Toc model system, the increase of PV was significantly ($p<0.05$) affected by light intensity, compared with controls (Fig. 4A). The initial PV was 0.3 mequiv/kg and increased up to 2.47, 3.84, and 4.81 mequiv/kg after 7 h of exposure to light at intensities of 5,000, 10,000, 15,000 lux, respectively. Using a zero order kinetics model, an increase in the PV during storage was associated with k values of 0.29 ($r^2=0.97$), 0.53 ($r^2=0.99$), and 0.72 ($r^2=0.97$) mequiv/kg/h at intensities of 5,000, 10,000, and 15,000 lux, respectively. The quantity of hydroperoxide formed during photo-oxidation was directly proportional to the total amount of light absorbed (24).

Changes in the PV in the TAG+Car model system also showed protective effects of β -carotene against oil oxidation (Fig. 4B), consistent with observations above (Fig. 2 and 3). The k values associated with PV in the TAG+Car system of 0.13 ($r^2=0.83$), 0.18 ($r^2=0.96$), and 0.21 ($r^2=0.98$) mequiv/kg/h at intensities of 5,000, 10,000, and 15,000 lux, respectively, were 3 times lower than for the TAG+Toc model system. Beta-carotene, which is rich in conjugated double bonds, can absorb and transfer more light energy than

tocotrienols and tocopherols, thus, reducing the amount of energy available for hydrogen abstraction of unsaturated fatty acids and preventing formation of hydroperoxide. According to Matsushita and Terao (25), β -carotene has a physical quenching rate of singlet oxygen of 1.5×10^{10} M/s, which is 58 times higher than the α -tocopherol rate of 2.6×10^6 M/s.

The presence of tocol compounds and β -carotene together in the TAG+Toc+Car system provided effective protection during photo-oxidation at light intensities up to 15,000 lux (Fig. 4C). The PV in the TAG+Toc+Car system showed the lowest increase among the model systems after 7 h of exposure to light. According to Haila and Heinonen (10), the mechanism of cooperation between tocopherols and β -carotene during photo-oxidation was chemical quenching of singlet oxygen and free radicals by tocopherols, and physical quenching of singlet oxygen through energy transfer by β -carotene. The presence of chlorophyll in the TAG+Toc+Car+Chlor system caused an increase in PV that was 3 times higher than for the TAG+Toc+Car model system, especially at a light intensity of 15,000 lux.

Table 2. Photo-degradation kinetic parameters of tocopherols and tocotrienols in a TAG+Toc model system and β -carotene in a TAG+Car model system

Minor compounds	Light intensity (lux)	Reaction rate constant		Light intensity coefficient	
		k^1 (per h)	r^2	$z_i^{2)}$ (lux)	r^2
β -Carotene	5,000	0.37×10^{-2}	0.75	12,500	0.98
	10,000	0.72×10^{-2}	0.87		
	15,000	2.19×10^{-2}	0.65		
α -Tocopherol	5,000	2.40×10^{-2}	0.91	50,000	0.98
	10,000	3.41×10^{-2}	0.91		
	15,000	4.21×10^{-2}	0.63		
α -Tocotrienol	5,000	1.87×10^{-2}	0.73	25,000	0.93
	10,000	2.38×10^{-2}	0.87		
	15,000	4.70×10^{-2}	0.89		
γ -Tocotrienol	5,000	1.55×10^{-2}	0.62	20,000	0.98
	10,000	3.00×10^{-2}	0.71		
	15,000	4.94×10^{-2}	0.81		
δ -Tocotrienol	5,000	1.58×10^{-2}	0.88	33,333	0.98
	10,000	2.09×10^{-2}	0.91		
	15,000	3.30×10^{-2}	0.63		

¹⁾The reaction rate constant was calculated based on first order reaction kinetics.

²⁾The light intensity coefficient was calculated based on plotting the value of $\log k$ vs. light intensity.

Fig. 3. Changes of β -carotene contents in (A) TAG+Car, (B) TAG+Toc+Car, and (C) TAG+Toc+Car+Chlor model systems during exposure to light at intensities of 5,000, 10,000, and 15,000 lux. Bar indicates the mean value of three replicate experiments and standard error. Different letters on bars indicate significant differences at $p < 0.05$ based on Duncan's multiple comparison test.

The k value associated with a PV of 0.10 mequiv/kg/h ($r^2 = 0.92$) in the TAG+Toc+Car+Chlor system at a light intensity of 15,000 lux was significantly ($p < 0.05$) higher than k values at light intensities of 10,000 and 5,000 lux (Fig. 4D). Chlorophyll acted as a photosensitizer that accelerated photo-oxidation. However, the increase in the PV of RPO in the TAG+Toc+Car+Chlor model system was lower than for virgin coconut oil with a chlorophyll content and unsaturated fatty acid composition lower than for RPO (24). The photo-oxidative stability of RPO was also higher than for virgin olive oil after exposure to UV rays (26), higher than for sunflower oil at a light intensity of 1,700 lux (18), and higher than a mixture of olive and perilla oil at a light intensity of 2,500 lux (27,28). Virgin coconut, virgin olive, sunflower oil, and a mixture of olive and perilla oils have tocopherols and β -carotene that are lower than for RPO (18,24,26-28).

Light intensity accelerated degradation rates of tocol compounds and β -carotene. The existence of tocol compounds and β -carotene together in model systems showed a protective effect against photo-oxidative changes, measured as PV, with minimum values in the presence of both tocol compounds and β -carotene. The presence of tocol compounds and β -carotene can counter the activity of chlorophyll as a photosensitizer in RPO and, thus, chlorophyll increased the rate of photo-oxidation only at high-light intensities.

Acknowledgments This research was supported by a Doctorate Research Grant (contract No. 346/UN.19.2/PL/2014) from Directorate General of Higher Education, the Ministry of National Education, the Republic of Indonesia in 2014.

Disclosure The authors declare no conflict of interest.

Fig. 4. Influence of light intensity on the photo-oxidative stability of (A) TAG+Toc, (B) TAG+Car, (C) TAG+Toc+Car, and (D) TAG+Toc+Car+Chlor model systems. Bar indicates the mean value of three replicate experiments and standard error.

References

- Yi J, Andersen ML, Skibsted LH. Interactions between tocopherols, tocotrienol and carotenoids during autooxidation of mixed palm olein and fish oil. *Food Chem.* 127: 1792-1797 (2011)
- Dauqan E, Sani HA, Abdullah A, Muhammad H, Top AGM. Vitamin E and beta-carotene composition in four different vegetable oils. *Am. J. Appl. Sci.* 8: 407-412 (2011)
- Usuki R, Usuki T, Endo Y, Kande T. Residual amounts of chlorophylls and pheophytins in refined edible oils. *J. Am. Oil Chem. Soc.* 56: 785-788 (1984)
- Min DB, Boff J. Chemistry and reaction of singlet oxygen in foods. *Compr. Rev. Food Sci. F.* 1: 58-72 (2002)
- Choe E, Min DB. Mechanisms and factors for edible oil oxidation. *Compr. Rev. Food Sci. F.* 5: 169-186 (2006)
- Choe E, Min DB. Mechanisms of anti-oxidants in the oxidation of foods. *Compr. Rev. Food Sci. F.* 8: 345-358 (2009)
- Kim HJ, Min DB. Chemistry of lipid oxidation. 3rd edition. pp. 299-320. In: *Food Lipids, Chemistry, Nutrition, and Biotechnology*. Akoh CC, Min DB (eds). CRC Press, Inc., Boca Raton, FL, USA (2008)
- Hernandez-Marin E, Galano A, Martínez, A. Cis carotenoids: Colorful molecules and free radical quenchers. *J. Phys. Chem. B* 117: 4050-4061 (2013)
- Yanishlieva NV, Marinova EM, Raneva VG, Partali, V, Sliwka HR. β -Apo-8'-carotenoic acid and its esters in sunflower oil oxidation. *J. Am. Oil Chem. Soc.* 78: 642-644 (2001)
- Haila K, Heinonen M. Action of β -carotene on purified rapeseed oil during light storage. *Lebensm-Wiss Technol.* 27: 573-577 (1994)
- Puspitasari-Nienaber NL. Stability of carotenoids in red palm oil and its effects in their bioavailability, provitamin A activity and toxicity. PhD thesis, The Ohio State University, Columbus, OH, USA (2002)
- Schroeder MT, Becker EM, Skibsted LH. Molecular mechanism of anti-oxidant synergism of tocotrienols and carotenoids in palm oil. *J. Agr. Food Chem.* 54: 3445-3453 (2006)
- Rossi M, Alamprese C, Ratti S. Tocopherols and tocotrienols as free radical-scavengers in refined vegetable oils and their stability during deep-fat frying. *Food Chem.* 102: 812-817 (2007)
- Lampi AM, Kamal-Eldin A. Effect of α - and γ -tocopherols on thermal polymerization of purified high-oleic sunflower triacylglycerols. *J. Am. Oil Chem. Soc.* 75: 1699-1703 (1998)
- AOCS. Official Methods and Recommended Practices of AOCS. 5th ed. Methods Ce 8-89, 13d-55, Cd 19-90, Cd 8-53, Ca 5a-40. American Oil Chemists' Society, Champaign, IL, USA (2003)
- AOAC. Official methods of Analysis of AOAC Intl. 18th ed. Method 2001.13. Association of Official Analytical Communities, Gaithersburg, MD, USA (2005)
- SNI. Minyak Goreng Sawit. SNI 7709. BSN, Jakarta, Indonesia. pp. 1-2 (2012)
- Choe E. Interaction of light and temperature on tocopherols during oxidation of sunflower oil. *J. Am. Oil Chem. Soc.* 90: 1851-1857 (2013)
- Sabliov CM, Fronczek C, Astete CE, Khachatryan M, Khachatryan L, Leonardi C. Effect of temperature and UV light on degradation of α -tocopherol in free and dissolved form. *J. Am. Oil Chem. Soc.* 86: 895-902 (2009)
- Mukai K, Kohno Y, Ouchi A, Nagaoka S. Notable effects of metal salts UV-Vis absorption spectra of α -, β -, γ -, and δ -tocopheroxyl radicals in acetonitrile solution, the complex formation between tocopheroxyls and metal cations. *J. Phys. Chem. B* 116: 8930-8941 (2012)
- Wrona M, Korytowski W, Rózanowska M, Sarna T, Truscott TG. Cooperation of anti-oxidants in protection against photosensitized oxidation. *Free Radical Bio. Med.* 35: 1319-1329 (2003)
- Chen BH, Huang JH. Degradation and isomerization of chlorophyll α - and β -carotene as affected by various heating and illumination treatments. *Food Chem.* 62: 299-307 (1998)
- Zechmeister L, Polgár A. Cis-trans isomerization and cis-peak effect in the α -carotene set and in some other stereoisomeric sets. *J. Am. Oil Chem. Soc.* 66: 137-144 (1944)
- Rukmini A, Raharjo S. Pattern of peroxide value changes in virgin coconut oil (VCO) due to photo-oxidation sensitized by chlorophyll. *J. Am. Oil Chem. Soc.* 87: 1407-1412 (2010)
- Matsushita S, Terao J. Autooxidation in Food and Biological Systems. Plenum Press, New York, NY, USA. pp. 27-44 (1980)
- Poulli KI, Mousdis GA, Georgiou CA. Monitoring olive oil oxidation under thermal and UV stress through synchronous fluorescence spectroscopy and classical assays. *Food Chem.* 117: 499-503 (2009)
- Kim N, Choe E. Singlet oxygen-related photooxidative stability and anti-oxidant changes of diacylglycerol-rich oil derived from mixture of olive and perilla oil. *J. Food Sci.* 77: C1185-C1191 (2012)
- Kim N, Choe E. Contribution of minor compound to the singlet oxygenated photooxidation of olive and perilla oil blend. *Food Sci. Biotechnol.* 22: 315-321 (2013)