

# Interaction of Light and Temperature on Tocopherols During Oxidation of Sunflower Oil

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**Abstract** Light and temperature effects on tocopherols during the oxidation of sunflower oil were studied. The oxidation was performed at 40, 60, or 80 °C for 30, 15, and 6 days, respectively, in the dark or under 1,700 lux light. Oil oxidation was analyzed with peroxide values and conjugated dienoic acid contents, and tocopherols in the oil were separated and quantified by HPLC. The oxidation of sunflower oil was increased with temperature increase, and the light decreased the temperature dependence of the oil oxidation. Sunflower oil before oxidation contained tocopherols with a total of 737.96 mg/kg, with  $\alpha$ - and  $\gamma$ -tocopherol at 726.41 and 11.56 mg/kg, respectively, and the tocopherol contents decreased during the oil oxidation. Degradation of tocopherols increased with the temperature increase, and its dependence on the temperature was lower under light than in the dark.  $\gamma$ -Tocopherol showed higher stability than  $\alpha$ -tocopherol during oxidation of the oil in the dark and under light. Residual amounts of tocopherols showed a relatively good correlation with the degree of oil oxidation, and the dependence of  $\alpha$ -tocopherol degradation on the oil oxidation was higher than that of  $\gamma$ -tocopherol, and light decreased the dependence on the oil oxidation in both tocopherols.

**Keywords** Light · Temperature · Tocopherols · Storage · Sunflower oil

## Introduction

Tocopherols, monophenolic compounds and derivatives of chromanol, are one of the most important antioxidants naturally found in food materials including fats and oils. Tocopherols are powerful antioxidants to decrease the oxidation of nutrients and useful components in foods, and are beneficial in reducing the risk of cardiovascular diseases, prostate cancer, glaucomatous damage, and Parkinson's disease [1–4]. Tocopherols give hydrogen to peroxy radicals, producing hydroperoxide and tocopheroxyl radicals which are more stable than peroxy radicals [5]. Lower reduction potential of tocopherols than that of lipid (alkyl) radicals results in faster reaction of tocopherols with peroxy radical than the reaction between lipids and peroxy radicals [6]. The antioxidant action of tocopherols usually brings degradation of tocopherols with the loss of vitamin E activity. Tocopherols may increase the oil oxidation instead of acting as antioxidants [7, 8]; tocopherols abstract hydrogen from lipids at a very low concentration of lipid peroxy radicals and produce lipid radicals to react with atmospheric triplet oxygen [9]. Thus tocopherols decrease or increase the oil oxidation depending on the environment, and the resulting degradation products of the oil affect the fate of tocopherols.

Since the oil oxidation is dependent on the temperature and light [10], tocopherols during oil oxidation should also be affected by them. Considering that fats and oils are one of the major sources of vitamin E from foods, efficient control of oil quality via storage conditions is very crucial for maximum retainment of tocopherols which help provide good antioxidants to our body and preserve the quality of oil. Most studies have been on the effects of temperature or light on oil oxidation [10–13] and tocopherol degradation [14] separately, however, there are few reports on the

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light effects on the degradation of tocopherols interrelated with the oil oxidation at various temperatures. This study, therefore, investigated the effects of oil oxidation on the tocopherol isomers present in the oil at different temperatures in the dark and under light to determine the light effects on the temperature dependence of each tocopherol isomer with respect to oil oxidation.

## Experimental Procedures

### Materials and Chemicals

Sunflower oil was regular RBD oil purchased from a local market in Incheon, Korea. Standard fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids) methyl esters and tocopherols, and 14 %  $\text{BF}_3$  in methanol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). *n*-Hexane, isopropanol, and isooctane in HPLC grade were purchased from J. T. Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade.

### Sample Preparation and Oxidation of Oils

Ten grams of sunflower oil was poured into a 20-mL serum vial (Supelco Inc., Bellefonte, PA, USA), and the vials were capped with *hanji* (Korean paper) to allow the air to pass through the vials and tightened with a rubber ring. The vials containing oils were stored in the dark by wrapping them with aluminum foil or under 1,700 lux light at 40, 60, and 80 °C for 30, 15, and 6 days, respectively. All samples were prepared in duplicate.

### Analysis of Oils

The fatty acid composition of the oil was analyzed by gas chromatography after esterification with 14 %  $\text{BF}_3$  in methanol [15]. The instrument used was a Younglin M600D gas chromatograph (Younglin Co., Ltd., Anyang, Korea) equipped with a Supelcowax capillary column (30 m  $\times$  0.53 mm, 1.0  $\mu\text{m}$  thick; Supelco Inc.) and a flame ionization detector. The temperatures of the oven, the injector, and the detector were 200, 270, and 280 °C, respectively. The nitrogen flow rate was 5 mL/min, and the split ratio was 33:1. Each fatty acid in the chromatogram was identified by comparing the retention times with those of standard fatty acid methyl esters, and quantified by the peak areas. The degree of the oil oxidation was evaluated by measuring the peroxide values (POV) and conjugated dienoic acid (CDA) values by the AOCS Cd 8-83 and Ti 1a-64 methods [16], respectively.

The tocopherol content of the sunflower oil was determined by HPLC [17] with a Younglin SP 930D HPLC

equipped with a  $\mu$ -porasil column (330 mm  $\times$  3.9 mm, 10  $\mu\text{m}$  size; Waters Co., Milford, MA, USA) and a fluorescence detector at an excitation and emission wavelength of 290 and 330 nm, respectively. The mobile phase was 0.2 % isopropanol in *n*-hexane (v/v) with a flow rate of 2.0 mL/min. Each tocopherol was identified by comparing the retention times with those of standard tocopherols, and quantified by using respective calibration curves.

### Data Analysis

All measurements for each sample were replicated, and SAS (Version 8.2; SAS Inst. Inc., Cary, NC, USA) and Microsoft Excel 2003 (Microsoft Corporation, Seoul, Korea) were used for the statistical treatment of the data. Statistical treatment included Duncan's multiple range test at the 5 % significance level and the regression analysis as well as the determination of means and standard deviations.

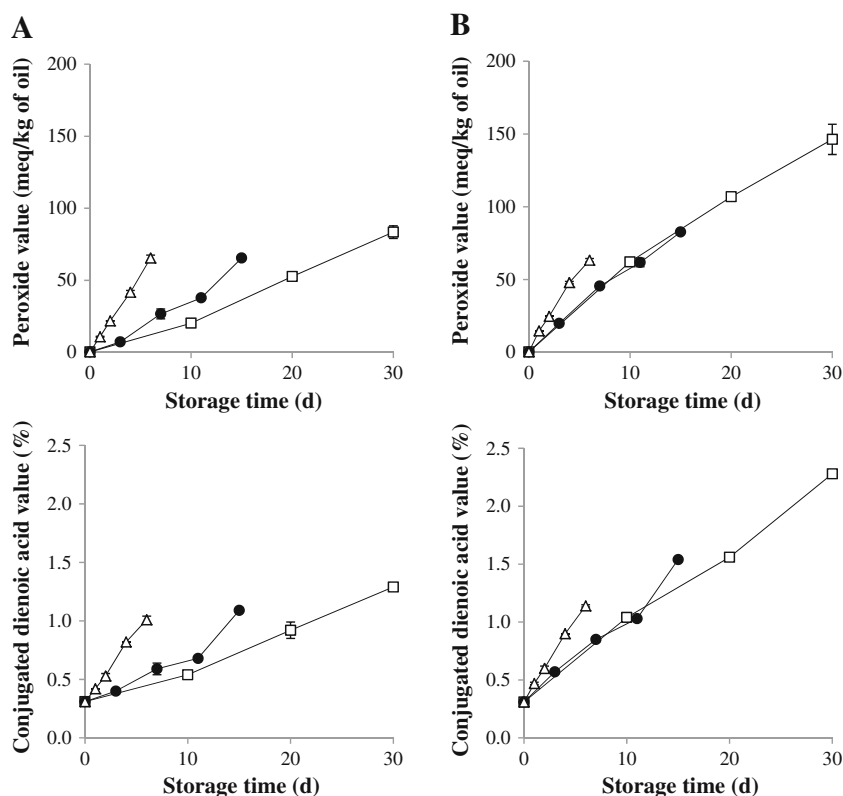
## Results and Discussion

### Effects of Light on the Oxidation of Sunflower Oil at Different Temperatures

The sunflower oil consisted of palmitic, stearic, oleic, linoleic, and linolenic acid at  $6.95 \pm 0.05$ ,  $3.48 \pm 0.11$ ,  $26.47 \pm 0.10$ ,  $63.00 \pm 0.30$ , and  $0.10 \pm 0.04$  %, respectively, which was similar to the previous report [18]. The POV and CDA content of sunflower oil before oxidation were 0.27 mequiv/kg oil and 0.31 %, respectively, and they were increased as the storage time and temperature increased as shown in Fig. 1. This indicates the occurrence of oil oxidation during storage and the oil oxidation was increased by temperature increase. Increased temperature supplied more energy for the hydrogen abstraction from the oil to give lipid (alkyl) radicals, resulting in a higher oxidation of the oil. Light even increased the POV and CDA contents of the oil at the same temperature; the POV of the oil in the dark was 52.61 mequiv/kg after 20 days storage at 40 °C, 26.66 mequiv/kg after 7 days storage at 60 °C, and 41.62 mequiv/kg after 4 days storage at 80 °C, while the POV under light was 106.87, 45.50, and 47.91 mequiv/kg, respectively. The CDA content of sunflower oil in the dark was 0.92 % after 20 days storage at 40 °C, 0.59 % after 7 days storage at 60 °C, and 0.82 % after 4 days storage at 80 °C, while under light it was 1.56, 0.85, and 0.90 %, respectively. These results confirmed the increase in oil oxidation by both light and temperature increases as shown in previous reports [13, 19].

The POV and CDA contents of sunflower oil were highly correlated with storage time ( $r^2 > 0.93$ ) as shown in Table 1. The rates of increases in POV at 40, 60, 80 °C in

**Fig. 1** Temperature effects on peroxide value and conjugated dienoic acid contents of sunflower oil during storage in the dark (a) and under 1,700 lux light (b) (open squares 40 °C, filled circles 60 °C, open triangles 80 °C)



**Table 1** Regression analysis between peroxide values (POV) and conjugated dienoic acid (CDA) values of sunflower oil and storage time in the dark or under 1,700 lux light at 40, 60, and 80 °C for 30, 15, and 6 days, respectively

Light	Temperature (°C)	Regression parameter <sup>a</sup>					
		POV			CDA contents		
		<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>
No	40	2.819	−3.152	0.990	0.033	0.267	0.989
	60	4.257	−3.180	0.973	0.049	0.263	0.930
	80	9.296	1.809	0.992	0.120	0.306	0.995
Yes	40	4.829	6.468	0.989	0.064	0.333	0.996
	60	5.426	2.990	0.993	0.077	0.307	0.974
	80	10.48	2.887	0.990	0.139	0.323	0.998

<sup>a</sup> POV (mequiv/kg oil) or CDA (%) contents = *a* × storage time (days) + *b*, *r*<sup>2</sup> determination coefficient

the dark was 2.819, 4.257, and 9.296 mequiv/kg/day, respectively, and 4.829, 5.426, and 10.48 mequiv/kg/day, respectively, under light. The rates of CDA content increases at 40, 60, 80 °C were 0.033, 0.049, and 0.120 %/day, respectively, in the dark, and 0.064, 0.077 and 0.139 %/day, respectively, under light. These results clearly indicate that the rate of oil oxidation increased with temperature increase and light accelerated it. The temperature dependence of the oxidation rate of oil, obtained from the regression equation (*r*<sup>2</sup> > 0.82), was 0.1619 and 0.1413 mequiv/kg/day/K in the dark and under light, respectively (Table 2). This indicates that the dependence of the peroxide formation rate on the temperature was lower under light than in the dark. The same tendency was shown with the rate of CDA production, and thus the light

decreased the effects of temperature on the acceleration of oil oxidation. Velasco and Dobarganes [13] reported decreased light effects on the oxidation of olive oil by temperature increase.

#### Effects of Light on Tocopherols During Sunflower Oil Oxidation at Various Temperatures

Sunflower oil contained tocopherols totally at 737.96 mg/kg, with  $\alpha$ - and  $\gamma$ -tocopherol at 726.41 and 11.56 mg/kg, respectively, and there was no  $\beta$ - and  $\delta$ -tocopherol detected. The contents of tocopherols were decreased during the oxidation of sunflower oil (Fig. 2), which indicates their degradation. Degradation of tocopherols during the oxidation of sunflower oil is thought to be related with their

action as antioxidants. Tocopherols donate phenolic hydrogen to peroxy or alkyl radicals of the oil, resulting in tocopheroxyl radicals which can be oxidized further to tocopheroxyl semiquinone [5]. The degree of tocopherol degradation in the dark was affected by the temperature. As the temperature increased, lower amounts of tocopherols were retained; retention of tocopherols after 30 days at 40 °C was in total 63.5 % (468.46 mg/kg) based on the initial value, and 59.6 % (439.56 mg/kg) and 42.2 % (311.73 mg/kg) of total tocopherols were remained after 15 days at 60 °C and after 6 days at 80 °C, respectively. Light further increased the degradation of tocopherols;

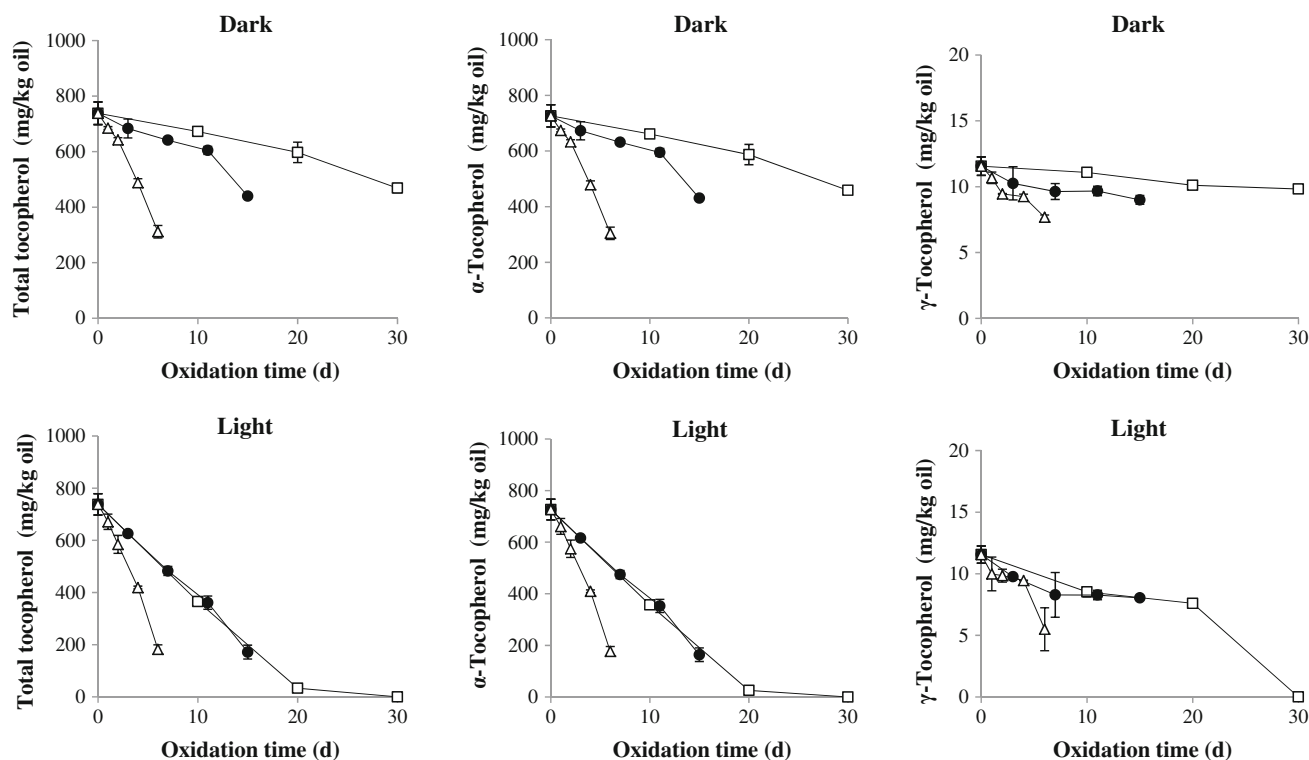
**Table 2** Effects of 1,700 lux light on the temperature dependence of the oxidation rate of sunflower oil at 40, 60, and 80 °C for 30, 15, and 6 days, respectively, on the basis of peroxide values (POV) and conjugated dienoic acid (CDA) values

Light	Regression parameter <sup>a</sup>					
	POV (mequiv/kg/day/K)			CDA contents (%/day/K)		
	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>
No	0.1619	−48.460	0.9066	0.0022	−0.6561	0.8788
Yes	0.1413	−40.127	0.8283	0.0019	−0.5261	0.8728

<sup>a</sup> Increasing rate of POV (mequiv/kg/day) and CDA contents (%/day) =  $a \times \text{temperature (K)} + b$ ,  $r^2$  determination coefficient

total retention of tocopherols after 15 days at 60 °C in the dark and under light was 59.6 % (439.56 mg/kg) and 23.3 % (171.59 mg/kg), respectively, and 42.2 % (311.73 mg/kg) and 24.7 % (182.25 mg/kg), respectively, after 6 days at 80 °C.  $\alpha$ -Tocopherol in sunflower oil was degraded to a higher extent than the  $\gamma$ -isomer in the dark at all temperatures; 59.3 % of  $\alpha$ -tocopherol and 77.8 % of  $\gamma$ -tocopherol were retained after 15 days at 60 °C. The same phenomena were observed under light. This clearly indicates that the stability of  $\gamma$ -tocopherol was higher than that of  $\alpha$ -tocopherol, and suggests that  $\alpha$ -tocopherol might have exerted a higher reactivity toward lipid and peroxy radicals than  $\gamma$ -tocopherol. Higher stability of  $\gamma$ -tocopherol than that of  $\alpha$ -tocopherol was shown previously during fish oil oxidation [20].

The degradation rate of tocopherols, the slope of the regression equation between tocopherol retention (%) and time ( $r^2 > 0.77$ ), became higher with temperature increases and it was higher under light than in the dark at the same temperature (Table 3); the degradation rate of tocopherols at 40, 60, and 80 °C during oxidation of sunflower oil in the dark was in total 1.197, 2.419, and 9.678 %/day, respectively, and it was 3.450, 4.987, and 12.48 %/day, respectively, under light. This clearly indicates that a temperature increase and light accelerated the degradation of tocopherols. Table 3 also shows that the degradation of



**Fig. 2** Temperature effects on tocopherol contents during oxidation of sunflower oil in the dark and under 1,700 lux light (open squares 40 °C, filled circles 60 °C, open triangles 80 °C)

**Table 3** Regression analysis between tocopherol retention based on the initial concentration and time during oxidation of sunflower oil in the dark and under 1,700 lux light at 40, 60, and 80 °C for 30, 15, and 6 days, respectively

Light	Tocopherol	Temperature (°C)	Regression parameter <sup>a</sup>		
			<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>
No	α-	40	-1.208	101.9	0.972
		60	-2.438	101.7	0.901
		80	-9.749	102.9	0.985
	γ-	40	-0.535	100.1	0.959
		60	-1.278	96.90	0.889
		80	-5.120	97.44	0.930
	Total	40	-1.197	101.8	0.973
		60	-2.419	101.6	0.902
		80	-9.678	102.8	0.986
Yes	α-	40	-3.456	90.02	0.905
		60	-5.326	104.6	0.982
		80	-12.56	102.8	0.991
	γ-	40	-3.081	106.1	0.875
		60	-1.893	93.09	0.779
		80	-7.468	99.61	0.849
	Total	40	-3.450	90.27	0.910
		60	-4.987	100.4	0.995
		80	-12.48	102.8	0.990

<sup>a</sup> Tocopherol retention (%) = *a* × time (day) + *b*, *r*<sup>2</sup> determination coefficient

α-tocopherol was faster than that of the γ-isomer; the degradation rate of α-tocopherol in the dark at 40, 60, and 80 °C was 1.208, 2.438, and 9.749 %/day, respectively, and that of the γ-isomer was 0.535, 1.278, and 5.120 %/day, respectively. The same results were reported previously for perilla oil [21]. The same tendency was shown in the degradation of tocopherols under light. Higher and faster degradation of α-tocopherol than that of γ-tocopherol during sunflower oil oxidation clearly indicates lower a stability of α-tocopherol over that of γ-tocopherol. This is thought to be due to the differences in dissociation energy and the antioxidant activity among tocopherol isomers. The bond dissociation energy between phenolic oxygen and hydrogen in tocopherols decreases in the order of δ-, γ-, β-, and α-isomer [5]. The relative antioxidant activity of tocopherol isomers through radical scavenging is the highest in the α-isomer, followed by the β-, γ and δ-isomers, which is due to the difference in the methylation pattern and the number of methyl groups attached to the benzene ring [22]. α-Tocopherol has extra 3 methyl groups which can share the unshared electron in tocopheryl radical, while δ-tocopherol has only one methyl group in the benzene ring. β-Tocopherol has two methyl groups in *para*-position which is more stable than in *meta*-position of

**Table 4** Effects of 1,700 lux light on the regression analysis between the degradation rate of tocopherols and temperature during oxidation of sunflower oil at 40, 60, and 80 °C for 30, 15, and 6 days, respectively

Light	Tocopherol	Regression parameter <sup>a</sup>		
		<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>
No	α-	0.0522	9E-08	0.9655
	γ-	0.0565	1E-08	0.9828
	Total	0.0523	8E-08	0.9656
Yes	α-	0.0323	0.0001	0.9650
	γ-	0.0221	0.0022	0.4048
	Total	0.0321	0.0001	0.9427

<sup>a</sup> Tocopherol degradation rate (%/day) = *b* × *e*<sup>*a* × temperature (K)</sup>, *r*<sup>2</sup> determination coefficient

methyl groups in γ-tocopherol. Therefore, α-tocopherol with a lower energy requirement for the abstraction of phenolic hydrogen and a higher number of methyl groups for sharing the radical character would have donated hydrogen more easily, making it less stable compared to γ-tocopherol.

The temperature dependence of the degradation rate of tocopherols, by the regression equation, is shown in Table 4. The degradation rate of tocopherols was relatively well-fitted to an exponential function with the temperature, and the proportional constant was higher in the dark than under light; it was 0.0522 and 0.0323 %/day/K, respectively, for α-tocopherol and 0.0565 and 0.0221 %/day/K, respectively, for γ-tocopherol. This suggests that the degradation rate of tocopherols was dependent on the temperature to a higher extent during oxidation of sunflower oil in the dark than under light, although the degree of tocopherol degradation at the specific temperature was higher under light than in the dark. Wang and Choe [23] also reported a higher dependence of tocopherol degradation on the temperature in the dark than under light in safflower oil, but there have been no report on light effects on different temperature dependence among tocopherol isomers in edible oil so far. Increased temperature-dependence by light in degradation of tocopherols is thought to be related to the activation energy, *E*<sub>a</sub>, for tocopherol degradation; the *E*<sub>a</sub> is an energy barrier to overcome to initiate the reaction, and the energy supplied by the temperature increase is the only source in the dark, while light can supply extra energy in addition to temperature increase, and thus temperature increase would have less influenced the degradation of tocopherols under light than in the dark. The *E*<sub>a</sub> for degradation of tocopherols was reported to be 40.88 kJ/mol during oxidation of RBD corn oil [21]. The results also showed that higher difference in tocopherol degradation rates between in the dark and under light in γ-tocopherol (0.0565 vs. 0.0221 %/day/K) than in

**Table 5** Effect of 1,700 lux light on the regression between the degree of oil oxidation at 40, 60, and 80 °C for 30, 15, and 6 days, respectively, and residual amounts of tocopherols

Light	Tocopherol	Regression parameter <sup>a</sup>					
		POV			CDA contents		
		<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>
No	α-	-4.1541	718.99	0.7363	-355.59	827.42	0.7798
	γ-	-0.0300	11.000	0.5049	-2.5220	11.752	0.5150
	Total	-4.1841	729.99	0.7354	-358.11	839.17	0.7787
Yes	α-	-5.7796	707.26	0.9313	-431.71	826.06	0.9144
	γ-	-0.0621	11.570	0.8014	-4.7435	12.941	0.8216
	Total	-5.8417	718.83	0.9333	-436.46	839.00	0.9169

<sup>a</sup> Residual amounts of tocopherols (mg/kg) =  $a \times \text{POV}$  (mequiv/kg of oil) or CDA (%) contents +  $b$ ,  $r^2$  determination coefficient

α-tocopherol (0.0522 and 0.0323 %/day/K), and thus lower light effects were exerted on α-tocopherol degradation than on γ-tocopherol degradation. This again could result from the higher energy required for hydrogen abstraction from γ-tocopherol than from α-tocopherol, and thus the presence of light would be more critical in γ-tocopherol to provide enough energy for degradation.

#### Effects of Light on the Relationship Between Oil Oxidation and Tocopherol Retention at Different Temperature

Table 5 shows a relationship between the degree of oil oxidation on the basis of POV and CDA contents and the residual amount of tocopherols for all data points at different temperatures and storage time. The correlation between the degree of oil oxidation and the residual amount of tocopherols was relatively good ( $r^2 > 0.50$ ), with a higher correlation with α-tocopherol than with γ-tocopherol. This suggests that the content of α-tocopherol rather than γ-tocopherol showed a better correlation with the progress of oil oxidation, possibly due to a higher amount of α-tocopherol than the γ-isomer and thus a higher chance of α-tocopherol with peroxy or alkyl radicals of oil. The negative sign of the slope in the regression equation indicates that the more the oil was oxidized, the lower was the tocopherol content and thus the higher was the tocopherol degradation. Higher value of the slope in the α-isomer than that of γ-isomer suggests that there is a higher dependence of α-tocopherol on the progress of oil oxidation than that of γ-tocopherol. As 1 mequiv hydroperoxide was increased in the oil as a result of oil oxidation in the dark, 4.1541 and 0.0300 mg/kg of α- and γ-tocopherol, respectively, was degraded. In the presence of light, the slope of the regression equation tended to increase especially for γ-tocopherol, and the dependence of tocopherol degradation on the oil oxidation was increased. Tocopherols absorb light in the ultraviolet region and cannot be directly excited by visible light [24], and the decreased tocopherol content would have resulted from their radical

scavenging activity during photooxidation of oil. Increased production of radicals in oils under visible light could increase the oxidation of oil and the degradation of tocopherols, and thus γ-tocopherol having a higher radical scavenging activity became more unstable under visible light than in the dark. Sabliov et al. [14] suggested that the degradation of α-tocopherol dissolved in hexane and methanol under UV light occurred via oxidation by superoxide anion radicals generated under light and hydroperoxy radicals converted from superoxide anion radicals, resulting in the tocopheroxy radical. Light also supplies the energy to break the O–H bond and ether linkage in the tocopherol structure, resulting in the semi-quinone radical and then quinones [14].

#### Conclusions

Temperature increases accelerated the oxidation of sunflower oil, and temperature dependence of the oil oxidation was decreased by light. Tocopherols in sunflower oil were degraded during the oil oxidation with a higher stability of γ-tocopherol than α-tocopherol. Degradation of tocopherols was dependent on both the temperature and the degree of oil oxidation, and light decreased their influences on the tocopherol degradation. The dependence of α-tocopherol on the degree of oil oxidation was higher than that of γ-tocopherol.

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