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RESEARCH ARTICLE

Stability of Tocopherols and Lutein in Oil Extracted from Roasted or Unroasted Mustard Seeds During the Oil Oxidation in the Dark

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Abstract This study compared stabilities of tocopherols and lutein in oil extracted from roasted mustard seeds (RMSO) with those in oil from unroasted seeds (URMSO) during oil oxidation at 60ºC in the dark for 12 days. Tocopherols and lutein were determined by high performance liquid chromatography, and the oil oxidation was monitored with conjugated dienoic acid (CDA) content and fatty acid composition by gas chromatography. The rate of CDA increase was lower in RMSO (0.038%/day) than in URMSO (0.047%/day) during 12-day oxidation in the dark, with little change in fatty acid composition in both oils. Tocopherols and lutein were more abundant in RMSO, initially 465.38 and 100.55 μg/g, respectively, than in URMSO, and their stability was higher in RMSO (–4.63 and –5.94%/day of degradation in tocopherols and lutein, respectively) than in URMSO during 12-day oxidation of oil, both of which contributed to higher autoxidative stability of RMSO than URMSO.

Keywords: mustard oil oxidation, seed roasting, tocopherol, lutein, stability

Introduction

Tocopherols are beneficial compounds which are commonly found in edible oils and are more sensitive to temperature than to light (1). Tocopherols are self-protection systems against oxidation to preserve the oil quality by donating hydrogen to peroxy radicals and retard production of off-flavors. Carotenoids are also found in unrefined oil and degraded during storage of oil (2).

Mustard oil is one of edible plant oils which were not undergone refining before consumption, and can naturally contain tocopherols and carotenoids (3-5). Tocopherols and carotenoids also have some physiological functions in our body; tocopherols inhibit formation of reactive oxygen species to retard aging (6) and carotenoids protect retinal tissues and retard photooxidative damage (7). Mustard oil is obtained from both unroasted and roasted mustard seeds, and the roasted seed oil gives a characteristic good odor. Roasting of seeds is usually performed at high temperature above 150ºC and very often accompanies Maillard reaction and caramelization (8,9), which gives desirable flavors. However, minor compounds of which are phytochemicals might be damaged at high temperature and thus affect their functionality and oxidative stability of the oil during storage after oil extraction.

Increasing interests of consumers on good flavor and functional compounds such as tocopherols and carotenoids in oils may enable mustard oil to be utilized more extensively in food industry. Although there are many studies on the stability of antioxidants in edible oil during cooking or storage (10-13), antioxidants in mustard seed oil, especially roasted mustard seed oil, have been excluded since consumption of mustard oil as cooking oil was very low and still limited in specific area of the world such as Nepal. Therefore, we compared degradation of tocopherols and carotenoids in oils obtained by pressing roasted mustard seeds with those in the unroasted seed oil during the oil oxidation in the dark, and provided scientific evidence of seed roasting on the improvement of autoxidative stability of mustard oil.

Materials and Methods

Materials and chemicals Oriental mustard seeds (*Brassica*

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juncea) were purchased from Ottogi Co., Ltd. (Seoul, Korea) and stored in polyethylene bags in the dark below −20°C until use. Standard fatty acid methyl esters, heptadecanoic acid, tocopherols, β-carotene, and lutein were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Isooctane, acetonitrile, methanol, tetrahydrofuran, and hexane of high performance liquid chromatography (HPLC) grade were purchased from J.T. Baker (Phillipsburg, NJ, USA), and other chemicals were of reagent grade.

Roasting of mustard seeds and oil extraction Mustard seeds were roasted in a Gene Café coffee bean roaster (Genesis Co., Ltd., Suwon, Korea) at 165°C for 30 min by the method of Wijesundera *et al*. (4). Unroasted or roasted mustard seeds were placed in a screw-type expeller (NJE-2500; NUC Electric Co., Ltd., Daegu, Korea) to get oil extracted. The extracted oil was placed at room temperature in the dark for 24 hr followed by filtration through Whatman filter paper No. 42 (Whatman International Ltd., Kent, England). The filtered oil was stored in capped bottles covered with aluminum foil under nitrogen at temperature below −20°C until use. The oils extracted from unroasted mustard seeds and from roasted seeds were designated as oil from unroasted seeds (URMSO) and roasted mustard seeds (RMSO), respectively. Color of URMSO and RMSO was determined by measuring 3 color scales, L, a, and b using Hunter Lab colorimeter (Nippon Denshoku Ind., Tokyo, Japan); lightness from 0 (dark) to 100 (light); a, redness from −a (green) to +a (red); and b, yellowness from −b (blue) to +b (yellow).

Preparation and oxidation of samples The URMSO or RMSO (25 g) was put into 50-mL bottles wrapped with aluminum foil, capped with *hanji* (Korean traditional paper) to allow the air to pass, and fastened with rubber tape. The bottles were then placed in a 60°C oven for 12 days, and taken out at the interval of 3 days for analyses. All samples were prepared and analyzed in duplicate.

Analysis of tocopherols and carotenoids in oils Tocopherols in oils were determined by HPLC (14). The oil was dissolved in hexane and filtered through a 0.2-μm PTFE membrane filter (Tokyo Rashi Kaisha, Tokyo, Japan). The filtrate (20 μL) was injected into a HPLC (Waters Alliance 1525; Waters Co. Ltd., Milford, MA, USA) with a Phenomenex Luna 5 μm NH_2 column (100Å, 250×4.5 mm; Phenomenex Co., North Hills, CA, USA) and a fluorescence detector with an excitation wavelength of 298 nm and emission of 325 nm. The mobile phase was 2%(v/v) isopropanol in *n*-hexane. Tocopherols were identified by comparing retention times with those of standard tocopherol isomers, and the content was determined by using respective calibration curves.

Carotenoids in oils were also determined by HPLC (15) after extraction with saponification of oil according to the AOAC method (970.64) (16). Carotenoids extracted were dissolved in tetrahydrofuran, of which 20 μL was then injected into HPLC (Hewlett Packard 1050 Series; Agilent Technologies Inc., Santa Clara, CA, USA). The column was a Develosil reversed-phase column C30-UG-5 (4.6× 150 mm; Normura Chemical Co., Ltd., Seto, Japan) and an ultraviolet/visible (UV-Vis) detector with wavelength of 470 nm was used. The eluting solvent was a mixture of acetonitrile-methanol-tetrahydrofuran (40:56:4, v/v/v) with a flow rate of 1.5 mL/min. Carotenoids were identified by comparing retention times with those of standard β-carotene and lutein, and the concentration was calculated using respective calibration curves.

Analysis of fatty acid composition and oxidation of oil Fatty acid composition of oil was analyzed by gas chromatography after esterification with 14% BF_3 in methanol (14). A gas chromatograph (M600L; Younglin Co, Seoul, Korea) equipped with a Supelcowax capillary column (30 m×0.53 mm, 1.0 mm thick, Supelco Inc., Bellefonte, PA, USA) and a flame ionization detector was used. Temperatures of the column oven, the injector, and the detector were 230, 270, and 280°C, respectively. 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 of standard fatty acid methyl esters and quantified by peak areas. Heptadecanoic acid was an internal standard. The oxidation of oil in the dark was also determined by conjugated dienoic acid (CDA) content by AOCS method (Ti 1a-64) (17).

Statistical analysis Data were analyzed by the SAS statistical system (SAS version 9.1; SAS Institute Inc., Cary, NC, USA) and MS Office Excel 2003 (Microsoft Co., Redmond, WA, USA) which included Duncan's multiple range test at 5% significance level, *t*-test, and linear regression analysis as well as determinations of mean and standard deviation.

Results and Discussion

Chemical characteristics of mustard seed oil Chemical characteristics of URMSO and RMSO are shown in Table 1. There was no big difference in fatty acid composition between URMSO and RMSO. Both oils contained the highest amount of erucic acid, followed by linoleic, oleic, linolenic, and eicosenoic acid. Total content of palmitic, stearic, arachidic, and eicosadienoic acid was less than 7% in both oils. Content of unsaturated fatty acids in URMSO

Table 1. Chemical characteristics of mustard oils from unroasted seeds (URMSO) and roasted seeds (RMSO)

	URMSO	RMSO
Fatty acid composition[1] (relative %)		
16:0	2.5±0.1	3.1±0.2
18:0	1.3±0.0	1.4±0.0
18:1	18.9±0.3	19.2±0.0
18:2	20.8±0.4	21.4±0.0
18:3	13.3±0.2	13.1±0.1
20:0	0.7±0.0	0.7±0.1
20:1	13.4±0.1	12.9±0.1
20:2	1.0±0.0	1.1±0.1
22:1	28.2±0.8	27.1±0.1
US	95.6	94.8
MUFA	60.5	59.2
PUFA	35.1	35.6
Tocopherol (μg/g)		
α-	75.51±1.41	105.58±0.14
γ-	318.30±33.37	345.25±2.63
δ-	16.01±3.46	14.55±0.18
Total[2]	409.82±38.25[b]	465.38±2.68[a]
Lutein (μg/g)	96.87±4.66	100.55±9.8
Conjugated dienoic acid (%)[2]	0.08±0.00[b]	0.16±0.002[a]
Color reading (Hunter)[3]		
L	86.71±0.09	46.42±1.57
a	-6.12±0.08	8.15±0.11
b	131.19±0.06	84.66±2.01

[1]US, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids
[2]Different superscript means significant difference between unroasted and roasted oils by *t*-test at 5% significance level.
[3]L, lightness from 0 (dark) to 100 (light); a, redness from –a (green) to +a (red); b, yellowness from –b (blue) to +b (yellow)

and RMSO was 95.6 and 94.8%, respectively. It was reported that fatty acid composition was not affected by seed roasting in canola (4) and safflower (12) oil. Concentration of tocopherols was slightly higher in RMSO than in URMSO; URMSO contained α- (75.51 μg/g), γ- (318.30 μg/g), and δ-tocopherol (16.01 μg/g) while concentration of α-, γ-, and δ-tocopherols in the RMSO was 105.58, 345.25, and 14.55 μg/g, respectively. Higher concentration of tocopherols in RMSO than in URMSO was partly because the roasting caused membrane rupture and more tocopherols could be released, as reported previously with safflower (12) and canola (4) seed oil. Among carotenoids, lutein was predominant in mustard oil with trace amount of β-carotene, and no other peak was observed. Content of lutein was not significantly different between two oils, at 96.87±4.66 and 100.55±9.80 μg/g in URMSO and RMSO, respectively.

CDA values of URMSO and RMSO were 0.08 and 0.16%, respectively, possibly due to CDA formation at high temperature during seed roasting. Increased content of CDA by seed roasting was reported in sunflower oil (9), too.

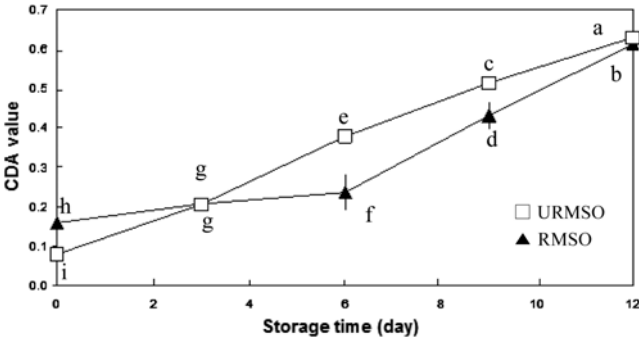

Fig. 1. Changes in CDA value of mustard oils from unroasted seeds (URMSO) and roasted seeds (RMSO) during oxidation in the dark at 60°C. Different letter in each point means a significant difference by Duncan's multiple range test at 5% significance level.

The color scales, L, a, and b of URMSO were 86.71, -6.12, and 131.19, respectively. The color scale L, a, and b of oil were changed into 46.42, 8.15, and 84.66, respectively, by seed roasting. The decrease in L color scale showed that the color of roasted oil changed into darker than the unroasted oil. The initial negative a color scale (-6.12), representing greenness was markedly increased to positive color scale (8.15) representing redness. The color change of RMSO to dark red was thought to be due to several reactions such as Maillard reaction, caramelization, and phospholipids degradation during roasting of seed as reported in the oil extracted from roasted safflower (12) and sunflower seeds (9).

Oxidation of mustard seed oil in the dark affected by seed roasting CDA values of URMSO and RMSO increased with oxidation time in the dark at 60°C, as shown in Fig. 1. The increase in CDA value is resulted from formation of more stable conjugated dienes from nonconjugated polyunsaturated fatty acids (PUFA) during oxidation (18). Initial CDA value of URMSO was lower than that of RMSO, however, its rate of CDA value increase with time was sufficiently higher (0.047%/day, r^2=0.996) than RMSO (0.038%/day, r^2=0.893) to exceed the CDA value of RMSO after 3-day oxidation at 60°C in the dark. This indicates that CDA formation in RMSO due to the autoxidation of oil was slow compared to that in URMSO.

Changes in fatty acid composition of URMSO and RMSO during oil oxidation in the dark at 60°C were not evident (Table 2), however, there was a tendency of decreasing content ratio of PUFA to saturated fatty acids (PUFA/S) in both oils. Although the correlation was not very high, PUFA/S changed at rates of -0.072%/day (r^2=0.861) and -0.034%/day (r^2=0.601) in URMSO and

Table 2. Changes in fatty acid composition (relative %) of mustard oils from unroasted seeds (URMSO) and roasted seeds (RMSO) during the oil oxidation in the dark at 60°C

Mustard oil	Oxidation time (day)	Fatty acid composition (relative %)									PUFA/S[1)]
		16:0	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:1	
RMSO	0	3.1±0.2	1.4±0.0	19.2±0.0	21.4±0.0	13.1±0.1	0.7±0.1	12.9±0.1	1.1±0.1	27.1±0.1	6.85
	3	2.7±0.0	1.4±0.0	18.6±0.1	20.8±0.1	12.8±0.0	0.8±0.0	13.3±0.1	1.2±0.0	28.3±0.2	7.10
	6	3.1±0.1	1.5±0.0	19.5±0.4	21.8±0.5	13.2±0.4	0.7±0.0	12.9±0.1	1.1±0.0	26.2±1.2	6.81
	9	3.3±0.0	1.5±0.0	19.8±0.0	22.1±0.1	13.3±0.2	0.7±0.0	12.8±0.2	1.0±0.1	25.4±0.2	6.62
	12	3.0±0.0	1.5±0.0	19.4±0.2	21.2±0.3	12.6±0.3	0.8±0.0	13.2±0.0	1.1±0.0	27.2±0.7	6.58
URMSO	0	2.5±0.1	1.3±0.0	18.9±0.3	20.8±0.4	13.3±0.2	0.7±0.0	13.4±0.1	1.0±0.0	28.2±0.8	7.80
	3	2.7±0.0	1.4±0.0	18.9±0.2	20.8±0.2	13.3±0.2	0.7±0.0	13.1±0.1	1.2±0.1	27.9±0.6	7.35
	6	2.5±0.0	1.4±0.0	18.9±0.1	20.5±0.1	13.0±0.0	0.8±0.0	13.3±0.0	1.2±0.1	28.3±0.2	7.38
	9	2.6±0.0	1.4±0.1	19.2±0.0	20.7±0.1	13.0±0.1	0.8±0.1	13.2±0.0	1.1±0.1	28.0±0.4	7.25
	12	2.9±0.3	1.5±0.0	19.5±0.4	20.9±0.5	13.1±0.3	0.8±0.0	13.1±0.3	1.2±0.0	27.1±1.1	6.77

[1)]Content ratio of polyunsaturated fatty acids to saturated fatty acids

RMSO, respectively, during 12-day oxidation, which possibly suggests a slower oxidation in RMSO than in URMSO. PUFA is oxidized faster than saturated fatty acid and thus PUFA/S decreases with oxidation time, which enables to evaluate the oil oxidation (19). The results clearly indicated that the oxidation proceeded more slowly in the RMSO than in the URMSO, and suggests that oxidative stability of mustard oil could be improved by seed roasting before oil extraction. It was similarly reported that roasting of safflower seeds before oil extraction improved oxidative stability of the oil (12). RMSO could have Maillard reaction products (MRP) produced during seed roasting as in peanut (20), safflower (12), and sesame seeds (8) and released into the oil during oil extraction by pressing, but URMSO could not. MRP have been known as antioxidant in the oil oxidation (21).

Degradation of tocopherols and lutein in mustard seed oil during the oil oxidation Tocopherol content of URMSO and RMSO decreased during the oil oxidation in the dark at 60°C (Table 3), indicating that tocopherols were degraded during autoxidation of oil. Degradation of tocopherols tended to be higher in the later period of oil oxidation than in the beginning. Contents of tocopherols in URMSO decreased continuously during 12-day storage, while tocopherols in RMSO were relatively stable (>90% retention) up to 6-day storage. This indicates that induction period of tocopherol degradation during the oil oxidation at 60°C in the dark was lengthened to 6 days in RMSO. The induction period of tocopherol degradation in URMSO, however, was not clearly observed. The overall correlation between tocopherol retention (%) and oxidation time was high, with coefficient of determination (r^2) ranging from 0.737 to 0.960. Total content of tocopherols in URMSO decreased significantly from 409.82 to 41.72 μg/g at rate of −8.30%/day (r^2=0.960) during 12-day oxidation of oil in the dark, while RMSO showed a decrease from 465.38 to 180.89 μg/g at −4.63%/ day (r^2=0.766) during the same period. This indicates higher retention with lower degradation rate of tocopherols in RMSO during autoxidation of oil than those in URMSO, and suggests that roasting of seeds before oil extraction increased tocopherol stability in the mustard oil during oxidation. Retention of total tocopherols after 12-day oxidation at 60°C in the dark, 10.2 and 38.9% in URMSO and RMSO, respectively, were lower than reported values by Lee *et al.* (13) in soybean (45.6%), sunflower (76.1%), and olive oil (78.3%). This could be related with the extent of respective oil oxidation. Degradation of tocopherols in oil is strongly related with their role as antioxidant in the oil oxidation (13). Tocopherols donate hydrogen to peroxy radicals produced by the reaction of oil with oxygen and are oxidized, resulting in their degradation (22,23). As more peroxy radicals are present in oils, more tocopherols are consumed. The content ratio of unsaturated fatty acids to saturated fatty acids (U/S) of our mustard seed oil was 20-21, while those of soybean, sunflower and olive oil in their work were 5.57, 10.58, and 6.21, respectively. The oil having higher amount of unsaturated fatty acids thus with higher U/S ratio is more susceptible to the oxidation than the oil having lower U/S ratio (18,24), and more tocopherols could be consumed to decrease the oil oxidation with lower retention, as shown in our case.

Stability of tocopherols in the mustard seed oil during oil oxidation in the dark was different among isomers. Content of γ- and δ-tocopherol of URMSO were initially 318.30 and 16.01 μg/g, and decreased to 31.18 and 10.54 μg/g after 12-day oxidation with rates of −8.32 and −2.81%/day, respectively; however, α-tocopherol was not detected at all on the 9th day of oil oxidation. After 12-day autoxidation of oil, RMSO showed decrease in α-, γ-, and δ-tocopherol from 105.58, 345.25, and 14.55 μg/g to 18.90, 148.94, and 13.05 μg/g with rates of −6.12, −4.34, and −0.76%/day,

Table 3. Tocopherol content in μg/g (% retention) of mustard oils from unroasted seeds (URMSO) and roasted seeds (RMSO) during the oil oxidation in the dark at 60°C

Mustard oil	Oxidation time (day)	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
URMSO	0	75.51±1.41$^{d1)}$ (100)$^{2)}$	318.30±33.37^{abc} (100)	16.01±3.46^{a} (100)	409.82±38.25^{bc} (100.0)
	3	61.98±0.22^{e} (82.1)	301.17±0.21^{bc} (94.6)	13.70±0.32^{abc} (85.6)	376.86±0.31^{c} (92.0)
	6	17.28±4.43^{f} (22.9)	218.45±11.52^{d} (68.6)	12.68±0.30^{bcd} (79.2)	248.41±16.26^{d} (60.6)
	9	UD	81.31±25.89^{f} (25.5)	11.16±0.02^{cd} (69.7)	92.47±25.88^{f} (22.6)
	12	UD	31.18±6.49^{g} (9.8)	10.54±0.20^{d} (65.9)	41.72±6.69^{g} (10.2)
	a$^{3)}$	-9.40	-8.32	-2.81	-8.30
	b$^{3)}$	97.4	109.6	96.9	106.9
	r^2	0.898	0.946	0.960	0.960
RMSO	0	105.58±0.14^{a} (100)	345.25±2.63^{a} (100)	14.55±0.18^{ab} (100)	465.38±2.68^{a} (100.0)
	3	101.83±0.95^{a} (96.5)	336.66±2.42^{ab} (97.5)	14.30±0.06^{ab} (98.3)	452.79±3.43^{a} (97.3)
	6	95.30±0.99^{b} (90.3)	318.39±2.96^{abc} (92.2)	14.27±0.13^{ab} (98.1)	427.96±4.08^{ab} (92.0)
	9	81.31±1.04^{c} (77.0)	280.11±2.33^{c} (81.1)	13.99±0.05^{ab} (96.1)	375.41±3.32^{c} (80.7)
	12	18.90±0.42^{f} (17.9)	148.94±28.22^{e} (43.1)	13.05±0.07^{bcd} (89.7)	180.89±27.86^{e} (38.9)
	a$^{3)}$	-6.12	-4.34	-0.76	-4.63
	b$^{3)}$	113.1	108.8	101.0	109.5
	r^2	0.737	0.778	0.807	0.766

$^{1)}$Different superscript means significant differences within the same column by Duncan's multiple range test at 5% significance level; UD, undetectable
$^{2)}$Retention (%) based on the value of 0 day
$^{3)}$Regression parameters of tocopherol retention (%)=a×oxidation time (day)+b, *r*=correlation coefficient

respectively. The results indicated that δ-tocopherol was more stable than α- or γ-tocopherol in both oils during autoxidation of oil. Similar result of more stable δ-tocopherol than α-tocopherol was reported in rapeseed (25) and soybean (26) oil during storage in the dark at 40-50°C. Low stability of α-tocopherol was partly due to its low reduction potential (270 mV) compared to 405 and 350 mV for δ- and γ-tocopherol, respectively (27), and so α-tocopherol was a stronger hydrogen donor and more vulnerable to the oxidation (25). The fully methylated structure of α-tocopherol could have contributed to easier hydrogen donor than γ- or δ-tocopherol (28). Also lower activation energy for the oxidative degradation of α-tocopherol than that of δ- and γ-tocopherol (29) contributed to lower stability of α-tocopherol. Degradation of each tocopherol was also slower in RMSO than in URMSO during autoxidation of the oil, and thus it is very obvious that roasting of seeds improve the stability of tocopherols during oil oxidation in the dark.

Table 4. Changes in lutein contents of mustard oils from unroasted seeds (URMSO) and roasted seeds (RMSO) during the oil oxidation in the dark at 60°C

Oxidation time (day)	URMSO	RMSO
0	96.87±4.66$^{b1)}$ (100)$^{2)}$	100.55±9.80^{a} (100)
3	43.87±2.89^{d} (45.3)	51.59±5.31^{c} (51.3)
6	37.37±0.41^{f} (38.6)	41.77±2.40^{e} (41.5)
9	22.74±0.67^{g} (23.5)	37.37±7.72^{f} (37.2)
12	9.32±0.06^{i} (9.6)	18.12±0.15^{h} (18.0)
a$^{3)}$	-6.75	-5.94
b$^{3)}$	83.9	85.2
r^2	0.861	0.844

$^{1)}$Different superscripts mean significant differences among samples by Duncan's multiple range test at 5% significance level.
$^{2)}$Retention (%) based on the value of 0 day
$^{3)}$Regression parameters in 'lutein content (% retention)=a×oxidation time (day)+b, *r*=correlation coefficient

Changes in lutein content of URMSO and RMSO during the oil oxidation in the dark at 60°C are shown in Table 4. Lutein content continuously decreased with oxidation time and RMSO showed significantly higher content than URMSO throughout 12 days of oil oxidation. It is noteworthy that lutein content decreased rapidly in the beginning of the oil oxidation, which was a different pattern from degradation of tocopherols. Lutein degradation might be inter-related with tocopherol degradation as well as the oil oxidation; lutein is highly vulnerable to the oxygen (30) and it could exert faster antioxidant activity than tocopherols resulting in its high degradation in the beginning of oil oxidation (31). This could have saved tocopherols as antioxidant to ultimately retain more tocopherols in the earlier period of oil oxidation in this study. It is very well-known that one antioxidant can spare another antioxidant when there are more than 2 antioxidants in oil, resulting in synergistic antioxidant effect (32). Regeneration of tocopherols by carotenoids was reported

Table 5. Regression analysis[1)] between conjugated dienoic acid (CDA) value and residual content of lutein or tocopherols during oxidation of mustard oil from unroasted seeds (URMSO) and roasted seeds (RMSO) in the dark at 60 °C for 12 days

Minor compound	URMSO			RMSO		
	a	b	r^2	a	b	r^2
Lutein	-0.006	0.62	0.856	-0.005	0.57	0.626
α-Tocopherol	-0.006	0.55	0.931	-0.005	0.73	0.900
γ-Tocopherol	-0.002	0.68	0.943	-0.002	0.97	0.929
δ-Tocopherol	-0.100	1.64	0.962	-0.309	4.66	0.929

[1)]CDA value (%)=a×lutein or tocopherol content (μg/g)+b, r=correlation coefficient

previously (33). The overall rate of lutein content decrease in URMSO and RMSO during 12-day oil oxidation at 60°C in the dark was −6.75%/day (r^2=0.861) and −5.94%/day (r^2=0.844), respectively. This suggests that roasting of mustard seeds before oil extraction slightly increased the stability of lutein in the oil during oil oxidation in the dark, possibly by MRP in RMSO.

Correlations between CDA value and residual content of lutein or tocopherols in URMSO and RMSO are shown in Table 5. CDA content of both oils was negatively correlated with residual content of lutein or tocopherol in the oil after specific period of oxidation in the dark. This means that CDA content was higher in the oil with lower amount of residual lutein or tocopherols during oil oxidation in the dark, and both lutein and tocopherols contributed to the decreased oil oxidation. Thus both tocopherols and lutein acted as antioxidants in the autoxidation of URMSO and RMSO. It is well known that tocopherols decrease the oil oxidation by scavenging radicals (34), but the function of lutein as antioxidant in autoxidation is not still clear. Lutein was reported as anti- or pro-oxidant depending on the oil, concentration, and presence of tocopherols (30). Haila *et al*. (35) reported antioxidant activity of lutein in the autoxidation of low erucic rapeseed oil at 40°C in the co-presence of γ-tocopherol. However, lutein acted as prooxidant in purified corn oil during oxidation at 40°C in the dark (36). The correlation of CDA values with tocopherols was higher (r^2>0.965 in URMSO and r^2>0.920 in RMSO) than that with lutein (r^2=0.856 in URMSO and r^2=0.626 in RMSO), and this shows that concentration of tocopherols rather than lutein was more highly correlated with mustard oil oxidation. Thus it suggests a possibility that tocopherols were more important than lutein in decreasing autoxidation of mustard seed oil.

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