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Effects of Seed Roasting on Tocopherols, Carotenoids, and Oxidation in Mustard Seed Oil During Heating

Bipin Vaidya · Eunok Choe

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Abstract Seed roasting is practiced in the mustard oil industry in some areas of the world, and can affect the physicochemical properties of the oil for further applications. This research studied the differences in oxidative stability, tocopherols, and carotenoids during heating at 160 °C between oil extracted from roasted mustard seeds and that from unroasted seeds. The content of free fatty acids, polar compounds (PC), and lutein were not significantly different between the roasted and unroasted seed oils before heating. The fatty acid compositions of both oils were also similar, with high amounts of erucic, linoleic, and oleic acids, moderate amounts of linolenic and eicosenoic acids, and low amounts of palmitic and stearic acids. However, the levels of tocopherols and conjugated dienoic acids (CDA) were higher in the roasted seed oil. Heating increased the content of CDA and PC in both oils, but decreased tocopherols and lutein. The rates of increase in CDA and PC and the degradation rates of tocopherols and lutein during heating were lower in the roasted than in the unroasted seed oil. Overall, the increased thermo-oxidative stability of the mustard oil by roasting the seeds before oil extraction was highly correlated with improved heat stabilities for both tocopherols and lutein.

Keywords Mustard oil · Seed roasting · Thermo-oxidative stability · Tocopherol · Lutein

B. Vaidya · E. Choe (✉)
Department of Food and Nutrition, Inha University,
253 Yonghyun-Dong, Nam-ku, Incheon 402-751, Korea
e-mail: eochoe@inha.ac.kr

Introduction

Mustard seeds are important oilseeds in several parts of the world, and are used for various purposes, including frying oil, condiments, and preservatives for pickles, as well as for massage purposes for newborn children and lactating mothers, and to alleviate joint pain [1]. Roasting mustard seeds prior to oil extraction has been practiced for a long time in Nepal, where the oil is then used for frying. Seed roasting before oil extraction gives it a characteristic good flavor and is reported to improve the stability of some oils such as sesame [2] and safflower [3], partly due to certain reactions such as the Maillard reaction occurring during roasting. Maillard reaction products were reported to lengthen the induction period of oil oxidation and decrease the rate of oxidation at the propagation step [4–6].

Mustard seeds contain tocopherols [7, 8] and carotenoids such as lutein and β-carotene [9]. Tocopherols are the most important natural antioxidants present in vegetable oil. The antioxidant activity of tocopherols is dependent on their isomers and concentrations; α-tocopherol was more effective at decreasing oil oxidation than γ-tocopherol up to 200 ppm, but was then less effective above this concentration [10]. Tocopherols sometimes show prooxidant activity at high concentrations in oil [11]. Although carotenoids in oils are generally considered to be antioxidants, β-carotene, lutein (free lutein and lutein dimyristate), and lycopene can accelerate oil oxidation under specific conditions [12, 13]. The synergistic effect of β-carotene and α-tocopherol was reported for lipid peroxidation [14], whereas γ-tocopherol inhibited the prooxidant activity of lutein by decreasing the hydroperoxide formation of triglycerides [15].

Oil undergoes oxidation upon heating and the mechanism is principally the same as autoxidation with a very

high reaction rate. During heating, thermolytic and oxidative reactions occur, with the formation of both volatile and nonvolatile decomposition products. The volatile decomposition products include hydrocarbons, aldehydes, ketones, furans, and carboxylic acids, and contribute greatly to the odor of the oil. Oxidative polymerization results in the production of nonvolatile decomposition products, which include polar and non-polar cyclic and noncyclic monomers, dimers, trimers, and high molecular weight compounds [16].

Although mustard seed oil contains a large amount of erucic acid and it is not generally regarded as a good fatty acid for better health [17], some areas of the world still value mustard seed oil as a frying oil. Few studies have been carried out on the oxidative stability of roasted mustard seed oil. The interests of consumers for good flavor and functional compounds such as tocopherols and carotenoids in oils are increasing. This may enable mustard oil to be utilized more extensively in the food industry all over the world, beyond a limited area such as Nepal, and information on the behavior of mustard seed oil with regard to its oxidation at high temperature should be provided. Therefore, this work was performed to provide information on the physicochemical characteristics, including antioxidants and the oxidative stability, of mustard seed oil during heating as affected by roasting the seeds prior to oil extraction.

Experimental Procedures

Materials and Chemicals

Oriental mustard seeds (*Brassica juncea*) were purchased from Ottogi Co. Ltd. (Seoul, Korea) and stored in polyethylene bags in the dark at −18 °C until use. Standard fatty acid methyl esters, tocopherols, β-carotene, and lutein were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and silica gel 60 (63–200 μm) for column chromatography was purchased from Merck Corporation (Darmstadt, Germany). Isooctane, acetonitrile, methanol, tetrahydrofuran, and hexane of high-performance liquid chromatography (HPLC) grade were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of reagent grade.

Oil Extraction From Mustard Seeds

The oil was extracted from unroasted and roasted mustard seeds using an oil extractor. Roasting was performed in duplicate by placing the mustard seeds (300 g) in a Gene Café coffee bean roaster (Genesis Co. Ltd., Suwon, Korea) at 165 °C for 30 min, according to the method of Wijesundera et al. [18]. The roasted and unroasted seeds were put into a screw-type expeller (model no. NJE-2500, NUC Electric Co. Ltd., Daegu, Korea) to extract the oil. The extracted oil was placed at 15 °C in the dark for 24 h to get rid of some of the non-triacylglycerol sediments and filtered through Whatman filter paper Grade No. 42 (Whatman International Ltd., Kent, England). The filtered oil was stored in capped bottles covered with aluminum foil under nitrogen at a temperature below −20 °C until use.

Preparation and Thermal Oxidation of Samples

The roasted or unroasted mustard seed oils (25 g) were put into 50-mL beakers capped with *hanji* (Korean paper) to allow air to pass, fastened with rubber tape, wrapped with aluminum foil, and then placed in a 160 °C oven. Oil samples were collected at intervals of 0, 2, 4, 7, and 12 h for analyses. All samples were prepared and analyzed in duplicate.

Analysis of Carotenoids and Tocopherols in Oils

Carotenoids were determined in the samples by HPLC [19] after saponification of the oil according to the AOAC official method 970.64 [20]. The carotenoids extracted and saponified were redissolved in tetrahydrofuran, of which 20 μL was then injected into the HPLC system (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; Nomura Chemical Co. Ltd., Seto, Japan) and a UV–Vis detector with a 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. The carotenoids were identified by comparing their retention times with those of standard carotenoids, and their concentrations were calculated using a respective calibration curve of standard carotenoids.

Tocopherols were determined by using HPLC [21]. The oil was dissolved in hexane and filtered through a 0.2-μm PTFE membrane filter (Toyo Roshi Kaisha, Tokyo, Japan). The filtrate (20 μL) was injected into an HPLC system (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., Torrance, CA, USA) and a fluorescence detector with an excitation wavelength of 298 nm and emission wavelength of 325 nm. The mobile phase was 2% isopropanol in n-hexane (v/v). The tocopherols were identified by comparing their retention times with those of standard tocopherol isomers, and their contents were determined by using respective calibration curves.

Analysis of Oil Oxidation and Fatty Acid Composition

The oxidation of the oil during heating was determined by a combination of conjugated dienoic acid (CDA) and polar compound (PC) content using the AOCS official method Ti 1a-64 [22] and AOAC method 982.27 [20], respectively.

The fatty acid compositions of the oils were determined by gas chromatography after esterification with 14% BF_3 in methanol. A gas chromatograph (M600L gas chromatograph; Younglin Co., Seoul, Korea) equipped with a Supelcowax capillary column (30 × 0.53 mm, 1.0 mm thick, Supelco Inc., Bellefonte, PA, USA) and a flame ionization detector was used. The temperatures of the column oven, the injector, and the detector were 230, 270, and 280 °C, respectively. The nitrogen flow rate was 5 mL/min, and the split ratio was 33:1. The individual fatty acids in the chromatogram were identified by comparing them to the retention times of standard fatty acid methyl esters and were quantified by the chromatogram peak areas. Heptadecanoic acid was an internal standard and was added to the oil prior to esterification.

Statistical Analysis

The 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 the 5% significance level and linear regression analysis, as well as the determination of means and standard deviations.

Results and Discussion

Initial Characteristics of Mustard Seed Oils

The characteristics of the roasted and unroasted mustard seed oils before heating are shown in Table 1. The unroasted mustard seed oil contained high amounts of linoleic, oleic, and erucic acids, moderate amounts of eicosenoic and linolenic acids, and low amounts of palmitic and stearic acids, resulting in 24.8, 24.7, 21.6, 12.8, 11.1, 3.8, and 1.3% of the total fatty acids, respectively. A similar fatty acid composition was reported for different genotypes of *B. juncea* seeds found in China [23] and Egypt [24]. The relative concentrations of palmitic (3.9%), stearic (1.4%), oleic (24.4%), linoleic (24.8%), linolenic (11.0%), eicosenoic (12.8%), and erucic (21.7%) acids in the roasted mustard seed oil were not significantly different from those in the unroasted oil, although the absolute amounts were slightly decreased. This indicates that roasting the seeds did not affect the fatty acid composition of the mustard seed oil. It was reported that the fatty acid composition did not change with roasting in sesame seeds [25] or in hazelnuts [26].

Table 1 Characteristics of unroasted and roasted mustard seed oils before heating ($n = 4$)

	Unroasted	Roasted
Fatty acid composition, mg/100 mg (relative %)		
16:0	3.3 ± 0.1 (3.8)	3.1 ± 0.1 (3.9)
18:0	1.1 ± 0.0 (1.3)	1.1 ± 0.0 (1.4)
18:1	21.3 ± 0.2 (24.7)	19.5 ± 0.3 (24.4)
18:2	21.4 ± 0.5 (24.8)	19.8 ± 0.3 (24.8)
18:3	9.6 ± 0.1 (11.1)	8.8 ± 0.1 (11.0)
20:1	11.0 ± 0.5 (12.8)	10.2 ± 0.1 (12.8)
22:1	18.6 ± 1.0 (21.6)	17.3 ± 0.1 (21.7)
Conjugated dienoic acid value (%)	0.09 ± 0.0	0.16 ± 0.0
Free fatty acid, % as oleic acid	0.32 ± 0.02	0.30 ± 0.01
Total polar compound (%)	2.18 ± 0.02	2.02 ± 0.0
Tocopherol (μg/g)		
α-Tocopherol	145.25 ± 2.64	179.03 ± 3.34
γ-Tocopherol	431.95 ± 3.46	437.68 ± 13.82
δ-Tocopherol	24.89 ± 1.22	25.06 ± 1.91
Total	602.09 ± 4.88	641.78 ± 19.08
Lutein (μg/g)	78.15 ± 4.3	80.77 ± 6.4

The CDA values of the unroasted and roasted mustard seed oils before heating were 0.09 and 0.16%, respectively, which indicates that roasting the seeds at 165 °C for 30 min caused slight oxidation of the seed lipid. A similar increase in the content of conjugated dienes by seed roasting was reported in sunflower oil [27]. The free fatty acid content of the roasted and unroasted mustard seed oils were not significantly different, with 0.30 and 0.32% as oleic acid, respectively. Similar results for unchanged acid values by seed roasting were observed in sesame oil [28]. PCs were present at 2.02 and 2.18% in the roasted and unroasted mustard seed oils, respectively.

Tocopherol concentration was 602.09 and 641.78 μg/g in unroasted and roasted mustard seed oil, respectively. These values were much higher than the reported values of 55.64 ~ 87.71 μg/g in hexane–isopropanol-extracted oil [7] and lower than in hexane-extracted oil [29]. The difference could be due to the different species and extraction methods. Roasting increased the tocopherol content in soybeans [30]; however, it showed a negligible increase in hazelnuts [31]. Three tocopherol isomers, α-, γ-, and δ-tocopherol, were detected in the mustard seed oil, with the most abundant tocopherol being γ-tocopherol, followed by α-tocopherol, and δ-tocopherol had the lowest concentration. The content of γ- and δ-tocopherols in the unroasted oil were not significantly different from those in the roasted

seed oil, although there was a tendency for slightly lower amounts in the unroasted seed oil. α-Tocopherol was present at a significantly higher concentration in the roasted mustard seed oil than in the unroasted seed oil. Increased α-tocopherol content by seed roasting was also observed in safflower and canola oils, which was due to membrane damage causing the tocopherol to be released from the seeds to the oil by roasting [3, 18].

Among the carotenoids, lutein was predominant in the mustard seed oil, with only a trace amount of β-carotene and both the roasted and unroasted mustard seed oils contained similar amounts of lutein. These values were higher than the values (7.72 μg/g) reported by other researchers for refined mustard (*B. nigra*) oil [32], possibly due to the differences in species and oil processing. The result suggested that roasting did not affect the lutein content of mustard seed oil, which might be due to the protection effect of phospholipids on lutein from degradation during the roasting of mustard seeds [33]. Also, carotenoids are bound to proteins, so they could keep their natural state, which provides heat stability [34].

Effects of Seed Roasting on the Stability of Carotenoids and Tocopherols in Mustard Seed Oil During Heating

The changes in lutein content in the roasted and unroasted mustard seed oils during heating at 160 °C are shown in Fig. 1. The lutein content of the roasted mustard seed oil was 80.77 μg/g at room temperature; however, it decreased to 29.83 μg/g by 2 h of heating at 160 °C, and no lutein was detected after 7 h of heating. This indicates that lutein gradually degraded during heating at 160 °C. Lutein, a dihydroxy carotenoid, has a highly unsaturated structure, enough to cause instability against heat [35]. The heat sensitivity of lutein was similarly reported in corn oil [13]. The unroasted mustard seed oil showed a similar trend for changes in lutein content during heating. The degradation

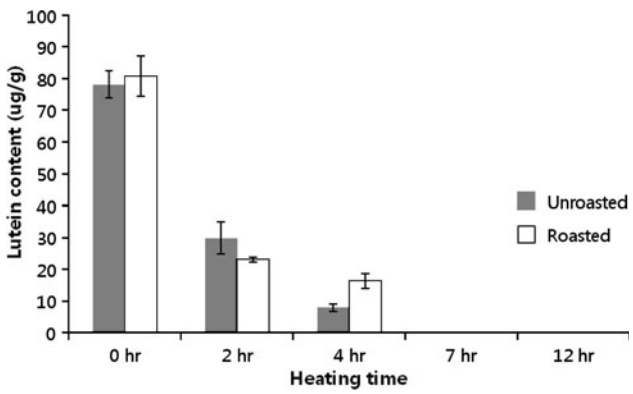

Fig. 1 Changes in the lutein content of unroasted and roasted mustard seed oils during heating at 160 °C

of lutein during heating at 160 °C was well-correlated with heating time in both the roasted (correlation coefficient, $r = 0.9343$) and unroasted ($r = 0.9451$) mustard seed oils. The degradation rate of lutein during heating tended to be slightly lower in the roasted (7.782 ppm/h) than in the unroasted (8.174 ppm/h) seed oil. This suggests that the roasted mustard seed oil contained certain beneficial compounds to increase the stability of lutein during heating.

The changes in tocopherol content in the mustard seed oils during heating at 160 °C are shown in Table 2. The tocopherol content decreased in both oils during heating, in which the total tocopherols in the unroasted and roasted seed oils decreased from 602.09 to 199.97 μg/g and from 641.78 to 495.13 μg/g, respectively, after 12 h of heating. The greater degradation of tocopherols in the unroasted seed oil (66.8% after 12 h heating) compared to the roasted seed oil (22.8% after 12 h heating) shows that roasting the mustard seeds improved tocopherol stability during heating of the oil. Among the tocopherol isomers, δ-tocopherol showed the highest retention, and, thus, higher heat stability, compared to the other isomers; 79.8, 75.5, and 87.7% of the amounts of α-, γ-, and δ-tocopherols, respectively, remained in the roasted seed oil after 12 h of heating. The unroasted mustard seed oil showed 21.8, 34.4, and 79.0% retentions of α-, γ-, and δ-tocopherols, respectively, after the same period of heating.

The correlation between tocopherol retention and heating time was high, with the coefficient of determination (r^2) ranging between 0.8294 and 0.9703 (Table 3). This suggests that tocopherol degradation was well-correlated with heating time. Among the tocopherol isomers, δ-tocopherol showed the lowest degradation rate in both the roasted and unroasted seed oils, and α- and γ-tocopherols were more heat-sensitive. It was previously reported that δ-tocopherol was the most stable among tocopherol isomers during heating at 180 °C for 10 h in partially hydrogenated soybean oil [36]. A high degradation rate for α-tocopherol was also reported in a model system of triolein and trilinolein at 180 °C [37]. The high stability of δ-tocopherol is thought to be partly due to its higher bond dissociation energy for O–H as compared to γ- or α-tocopherols [38]. The degradation of individual tocopherol isomers was faster in the unroasted mustard seed oil than in the roasted seed oil. This indicates that roasting the mustard seeds improved tocopherol stability during heating, and certain products formed during seed roasting, such as Maillard reaction products, may have partly contributed to the improved heat stability of the tocopherols. Maillard reaction products act as antioxidants in oil [39], and, thus, they can preserve tocopherols as antioxidants. The roasted mustard seed oil showed a higher absorption at 450 nm, a characteristic maxima of Maillard reaction products [40], as compared to

Table 2 Changes in the tocopherol content in μg/g (retention %) of unroasted and roasted mustard seed oils during heating at 160 °C

Heating time (h)	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Unroasted				
0	145.25 ± 2.64[a] (100.0)	431.95 ± 3.46[a] (100.0)	24.89 ± 1.22[a] (100.0)	602.09 ± 4.88[a] (100.0)
2	103.2 ± 14.06[b] (71.1)	335.10 ± 48.99[b] (77.6)	22.13 ± 1.68[abc] (88.9)	460.44 ± 64.72[b] (76.5)
4	89.15 ± 1.00[b] (61.4)	314.80 ± 7.27[b] (72.9)	23.20 ± 1.34[ab] (93.2)	427.15 ± 9.62[b] (70.9)
7	64.85 ± 1.42[c] (44.7)	239.23 ± 5.92[c] (55.4)	21.81 ± 0.18[bc] (87.6)	325.88 ± 7.51[c] (54.1)
12	31.66 ± 3.81[d] (21.8)	148.67 ± 6.79[d] (34.4)	19.65 ± 0.30[c] (79.0)	199.97 ± 10.90[d] (33.2)
Roasted				
0	179.03 ± 3.34[a] (100.0)	437.68 ± 13.82[a] (100.0)	25.06 ± 1.91[a] (100.0)	641.78 ± 19.08[a] (100.0)
2	175.86 ± 4.25[a] (98.2)	425.35 ± 2.46[a] (97.2)	24.33 ± 0.58[a] (97.1)	625.55 ± 6.12[a] (97.5)
4	163.08 ± 7.33[ab] (91.1)	395.11 ± 10.23[ab] (90.3)	23.97 ± 0.16[a] (95.7)	582.16 ± 17.40[ab] (90.8)
7	151.16 ± 16.20[b](84.4)	359.71 ± 34.40[bc] (82.2)	22.38 ± 1.18[a] (89.3)	533.26 ± 51.78[bc] (83.1)
12	142.86 ± 0.33[b] (79.8)	330.30 ± 3.60[c] (75.5)	21.97 ± 1.18[a] (87.7)	495.13 ± 5.12[c] (77.2)

The different superscripts indicate significant differences in samples with different heating time by Duncan's multiple range test at the 5% significance level

Table 3 Regression analysis between tocopherol retention (%) and heating time (h) in roasted and unroasted mustard seed oils during heating at 160 °C

	Unroasted			Roasted		
	a	*b*	r^2	*a*	*b*	r^2
α-Tocopherol	−6.05	90.04	0.9430	−1.80	99.71	0.9449
γ-Tocopherol	−5.16	93.84	0.9649	−2.15	99.77	0.9703
δ-Tocopherol	−1.50	97.24	0.8294	−1.08	99.32	0.9218
Total	−5.22	93.07	0.9596	−2.01	99.74	0.9645

Tocopherol retention (%) = $a \times$ heating time (h) + b, r = correlation coefficient

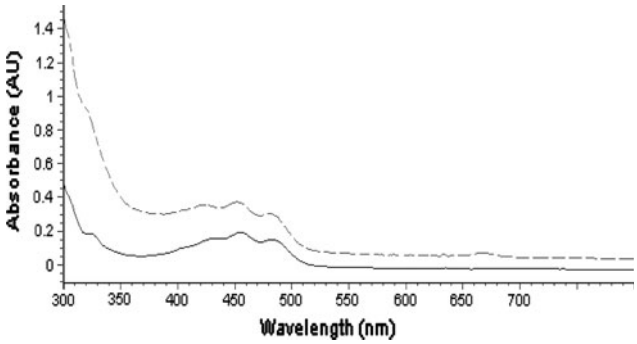

Fig. 2 UV–Vis absorption spectrum of roasted (*dotted line*) and unroasted mustard seed oil (*solid line*)

the unroasted oil (Fig. 2), which indicates the possible presence of Maillard reaction products in the roasted mustard seed oil. Maillard reaction occurred between reducing sugars and amino acids or peptides during the roasting of safflower seeds [3] and cocoa beans [41].

Effects of Seed Roasting on the Oxidation of Mustard Seed Oil During Heating

The changes in CDA values in the roasted and unroasted mustard seed oils during heating at 160 °C are shown in Fig. 3. The CDA values were 0.19 and 0.12% when the roasted and unroasted seed oils reached at 160 °C, respectively, and increased to 0.58 and 0.70%, respectively, after 12 h of heating. Increases in CDA values result from the transformation of non-conjugated linoleic acid to more stable conjugated linoleic acid during heating [42]. The CDA values of both oils were highly correlated with heating time; r^2 of 0.9583 and 0.9904 for roasted and unroasted oil, respectively. The rate of increase in CDA with heating time was lower in the roasted (0.032%/h)

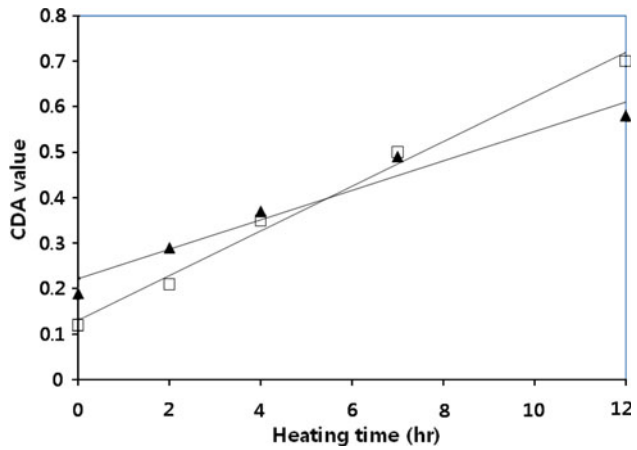

Fig. 3 Changes in the conjugated dienoic acid (CDA) values of unroasted and roasted mustard seed oils during heating at 160 °C (*filled triangles* roasted, $y = 0.032x + 0.222$, $r^2 = 0.958$; *open squares* unroasted, $y = 0.049x + 0.131$, $r^2 = 0.990$)

versus unroasted (0.049%/h) mustard seed oil, even though the initial CDA value of the roasted seed oil was higher than that of the unroasted seed oil. This clearly indicates

that roasting the seeds increased the oxidative stability of the mustard seed oil during heating and confirms the production of useful compounds such as Maillard reaction products during seed roasting and their transfer to the oil. In addition, the higher stabilities of the tocopherols and lutein in the roasted seed oil as compared to the unroasted seed oil could have contributed to reduced oxidation in the roasted seed oil versus the unroasted seed oil.

The correlations between CDA values and the content of lutein or tocopherols in the roasted and unroasted mustard seed oils during heating are shown in Table 4. The lutein and tocopherols contents were correlated with the CDA values ($r^2 > 0.785$) in both oils during heating. The lower the lutein or tocopherol content, the higher the CDA values, which means that lutein and tocopherols clearly decreased oxidation of the mustard seed oil during heating. δ-Tocopherol, which showed the highest slope in the regression equation between the content of lutein or tocopherols and CDA values, had the greatest effect on delaying CDA formation in oil during heating. It was reported that the antioxidant activity of δ-tocopherol was higher than that of other tocopherol isomers [43].

Table 4 Regression analysis between CDA values and the content of lutein or tocopherols in unroasted and roasted mustard seed oils during heating at 160 °C

	Unroasted			Roasted		
	a	*b*	r^2	*a*	*b*	r^2
Lutein	−0.0062	0.4498	0.8553	−0.0049	0.4544	0.9064
α-Tocopherol	−0.0053	0.8374	0.9514	−0.0099	1.9843	0.9742
γ-Tocopherol	−0.0021	1.0029	0.9618	−0.0034	1.7211	0.9818
δ-Tocopherol	−0.1066	2.7564	0.7852	−0.1163	3.1216	0.9729
Total tocopherols	−0.0015	0.9826	0.9592	−0.0025	1.8200	0.9813

CDA value (%) = $a \times$ lutein or tocopherol content (μg/g) + b, r = correlation coefficient

Changes in the fatty acid compositions of the roasted and unroasted mustard seed oils during heating at 160 °C are shown in Table 5. During heating, the linoleic and linolenic acid content decreased in both oils, and these decreases tended to be higher in the unroasted oil, although there were no significant differences in the fatty acid composition between the two oils.

The total PC content of the roasted and unroasted mustard seed oils also increased during heating at 160 °C (Fig. 4); the total PC contents were 2.02 and 2.18% in the unheated roasted and unroasted mustard seed oils, respectively, and they increased to 8.11 and 8.40%, respectively, after 12 h of heating. The total PC content linearly increased with heating time, and the roasted seed oil (0.476%/h, $r^2 = 0.983$) tended to increase at a lower rate than the unroasted seed oil (0.497%/h, $r^2 = 0.994$), although the difference was not significant. All of these results clearly indicate that roasting the mustard seeds before oil extraction was helpful for retaining larger amounts of beneficial antioxidants, tocopherols, and lutein, and to increase the stability of the tocopherols and lutein, leading to improved oxidative stability in the mustard seed oil during heating.

Conclusions

Roasting mustard seeds increased the tocopherol content in the mustard seed oil, with no significant differences in the

Table 5 Changes in fatty acid compositions of unroasted and roasted mustard seed oils during heating at 160 °C

Heating time (h)	Fatty acid composition (mg/100 g)						
	16:0	18:0	18:1	18:2	18:3	20.1	22:1
Unroasted							
0	3.3 ± 0.1^{a}	1.1 ± 0.1^{a}	21.3 ± 0.2^{a}	21.4 ± 0.5^{a}	9.6 ± 0.1^{a}	11.0 ± 0.5^{a}	18.6 ± 1.0^{ab}
2	3.3 ± 0.0^{a}	1.1 ± 0.2^{a}	19.9 ± 0.6^{ab}	19.9 ± 0.5^{ab}	8.8 ± 0.2^{b}	10.1 ± 0.1^{b}	18.4 ± 0.6^{ab}
4	3.1 ± 0.3^{a}	1.1 ± 0.1^{a}	19.2 ± 1.2^{bc}	19.0 ± 1.1^{b}	8.7 ± 0.4^{b}	10.2 ± 0.2^{b}	18.8 ± 0.2^{ab}
7	3.1 ± 0.1^{a}	1.0 ± 0.1^{a}	19.6 ± 0.1^{b}	19.1 ± 0.1^{b}	8.4 ± 0.2^{b}	10.4 ± 0.0^{b}	19.1 ± 1.2^{a}
12	3.0 ± 0.0^{a}	1.1 ± 0.1^{a}	17.8 ± 0.2^{c}	17.1 ± 0.1^{c}	7.5 ± 0.1^{c}	9.8 ± 0.0^{b}	16.7 ± 0.9^{b}
Roasted							
0	3.1 ± 0.1^{b}	1.1 ± 0.0^{a}	19.5 ± 0.3^{a}	19.8 ± 0.3^{a}	8.8 ± 0.1^{a}	10.2 ± 0.1^{a}	17.3 ± 0.1^{b}
2	3.2 ± 0.1^{ab}	1.1 ± 0.1^{a}	19.5 ± 0.3^{a}	19.8 ± 0.3^{a}	8.6 ± 0.2^{ab}	11.2 ± 1.2^{a}	20.1 ± 1.5^{ab}
4	3.3 ± 0.1^{ab}	1.1 ± 0.1^{a}	19.8 ± 0.3^{a}	19.8 ± 0.2^{a}	8.3 ± 0.1^{bc}	11.5 ± 0.0^{a}	21.7 ± 1.9^{a}
7	3.3 ± 0.0^{ab}	1.2 ± 0.1^{a}	19.5 ± 0.3^{a}	19.5 ± 0.3^{a}	8.0 ± 0.2^{cd}	10.8 ± 0.7^{a}	18.2 ± 1.7^{ab}
12	3.4 ± 0.1^{a}	1.2 ± 0.0^{a}	20.0 ± 0.1^{a}	19.4 ± 0.1^{a}	7.9 ± 0.2^{d}	10.6 ± 0.5^{a}	19.0 ± 1.5^{ab}

The different superscripts indicate significant differences in samples with different heating time by Duncan's multiple range test at the 5% significance level

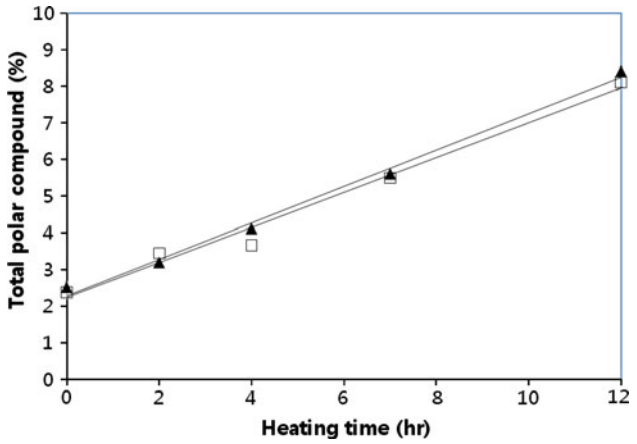

Fig. 4 Changes in the total polar compound (PC) content in unroasted and roasted mustard oils during heating at 160 °C (*filled triangles* roasted, $y = 0.476x + 2.236$, $r^2 = 0.983$; *open squares* unroasted, $y = 0.497x + 2.275$, $r^2 = 0.994$)

content of free fatty acids, polar compound (PC), and lutein, or in the fatty acid composition. The degradation of tocopherols and lutein in the mustard seed oil during heating at 160 °C was decreased by seed roasting. Overall, roasting the seeds prior to the oil extraction improved the heat stability of the mustard seed oil, along with tocopherols and lutein.

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