Effects of Roasting on Pyrazine Contents and Oxidative Stability of Red Pepper Seed Oil Prior to Its Extraction

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Red pepper seeds were roasted with constant stirring for 6, 9, 10, and 12 min at 210 °C, and oils were extracted from the roasted red pepper seeds using an expeller. The iodine values and fatty acid compositions of red pepper seed oils did not change with roasting time. The fatty acid composition of the oil obtained from the red pepper seeds roasted for 6 min was 0.24% myristic acid, 13.42% palmitic acid, 0.33% palmitoleic acid, 2.07% stearic acid, 10.18% oleic acid, 73.89% linoleic acid, and 0.37% linolenic acid, showing a fatty acid composition similar to that of high-linoleate safflower oil. Thirteen alkylpyrazines were identified in the roasted red pepper seed oils: 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2,6-diethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-isobutyl-3-methylpyrazine, and 3,5-diethyl 2-methylpyrazine. The pyrazine content increased markedly as the roasting time increased, showing 2.63, 5.01, 8.48, and 13.10 mg of total pyrazine/100 g of oils from the red pepper seeds roasted for 6, 8, 10, and 12 min, respectively, at 210 °C. 2,5-Dimethylpyrazine in the roasted red pepper seed oil seemed to be the component most responsible for the pleasant nutty aroma of the oils. The oxidative stabilities of oils increased greatly as the roasting time increased.

Keywords: *Pyrazine; red pepper seed; oil; oxidative stability; roasting*

INTRODUCTION

Red pepper seed oil is a widely used condiment oil along with sesame seed oil and perillar seed oil in Korea and many other nations. Red pepper seeds are obtained as byproducts in the preparation of red pepper powder. Traditionally, red pepper seed oil is prepared by extraction with a mechanical press after the seeds have been roasted for the appropriate time. During roasting, nut-like or peanut butter like-aroma is developed, and the aroma is also transferred along with the oil during extraction. The taste of the oil is hot, with a pleasant nut-like aroma. We assumed that the aroma is due to the pyrazines formed during the roasting process becuase pyrazines impart a reportedly nut-like aroma in many different types of foods roasted, baked, or otherwise thermally processed (Maga, 1982; Maga and Sizer, 1975; Fors and Erickson, 1986; Masuda and Mihara, 1986; Shibamoto, 1986; Alli et al., 1990). The formation of pyrazine compounds in many thermally processed foods results from Maillard-type nonenzymatic reactions between reducing sugars and free amino acid or amide (Koehler et al., 1969; Koehler and Odell, 1970). The mechanisms by which pyrazines are formed have been proposed (Newel et al., 1967; Koehler et al., 1969; Shibamoto et al., 1979).

Although the conditions are favorable for the formation of pyrazine compounds in the preparation of red pepper seed oil, a systematic attempt to identify pyrazines in red pepper seed oil has not been reported in the literature. The oxidative stability of the seed oil is also important information for predicting the quality deterioration of the oil during storage and marketing. However, the oxidative stabilities of the oils extracted from the red pepper seeds after roasting for different time have not been studied.

The objectives of this research were (1) to isolate, identify, and quantify pyrazines present in the roasted red pepper seed oils and (2) to determine the oxidative stability of the oils prepared from red pepper seeds roasted for 6, 8, 10, and 12 min at 210 °C.

MATERIALS AND METHODS

Materials. Red pepper seeds were obtained from a local red pepper powder mill. Pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3-methylpyrazine, 2,3-diethylpyrazine, tetramethylpyrazine, 2-methyl-3-*n*-propylpyrazine, 2,3-diethyl-5-methylpyrazine, and 2-isobutyl-3-methylpyrazine were purchased from Tokyo Kasei (Tokyo, Japan).

Preparation of Roasted Red Pepper Seed Oil. Red pepper seeds were heated in an oven equipped with a stirrer and a temperature controller. Red pepper seeds were roasted with constant stirring for 6, 8, 10, or 12 min at 210 °C. The oils were then extracted using an expeller. The extracted red pepper oils were filtered to remove any particles. The oils stood for 2 days at 4 °C until two distinct layers (a clear top layer and a viscous gummy layer) had separated. The top layer was collected and used as oil sample. The lower viscous gummy layer was discarded.

Determination of Iodine Value. The iodine value of the oil was calculated with fatty acid composition of the oil according to AOCS Official Method Cd 1c-85 (AOCS, 1990).

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Fatty Acid Analysis. Oils were methyl-esterified at 70 °C for 20 min with 0.25 N sodium methoxide in methanol. Methyl esters of fatty acids were extracted with petroleum ether. One microliter aliquots of the extracts were then injected into a gas chromatograph (GC-14B; Shimazu, Kyoto, Japan) equipped with a flame ionization detector. The column used was an AT-SILA fused capillary column (30 m × 0.25 mm, 0.25 μm; Alltech Associates, Deerfield, IL). The injector, oven, and detector temperatures were 250, 180, and 250 °C, respectively.

Pyrazine Extraction from Oils. The extraction procedure described by Reineccius et al. (1972) was used. Each oil sample (20 g) was dissolved in 100 mL of diethyl ether. The sample solution was extracted (20 mL × 5) with a 1 M HCl solution containing 100 g of NaCl/L. The ether layer was discarded, and the aqueous layers were combined and washed (2 × 50 mL) with diethyl ether and then adjusted to pH 8.3 by dropwise addition of 5 M KOH solution. The aqueous solution was extracted (20 mL × 5) with dichloromethane. The dichloromethane extract was dried over anhydrous $MgSO_4$ and filtered. After addition of internal standard (dodecane) to the extract, the extract was concentrated under a stream of nitrogen.

Gas Chromatography for Pyrazines. The extracts were injected into a gas chromatograph (Hewllet-Packard 5890A) equipped with a flame ionization detector for the isolation and quantification of individual pyrazines. The column used was a Supecowax 10 fused silica capillary column (60 m × 0.32 mm, 0.25 μm, Supelco Inc., Bellefonte, PA). The temperatures of the injection port and detector were 240 and 250 °C, respectively. The oven temperature was held at 50 °C for 5 min and then programmed at 2 °C min^{-1} to 230 °C. For the quantification of pyrazines, extracts obtained by dichloromethane extraction were analyzed and the peak areas were compared with those of authentic samples to calculate the contents of pyrazines. We found that the detector response was closely related to the molecular weight of pyrazines. Thus, the contents of some pyrazines, for which we did not have authentic samples, were calculated by comparing the peak areas with those of other pyrazines with similar molecular weights.

Gas Chromatography/Mass Spectrometry (GC/MS). For identification, a DS 6200 gas chromatograph (Dongnam, Seoul, Korea) coupled to a mass spectrometer (JMS-SX102A, JEOL, Japan) was used. Mass spectra were obtained by electron ionization at 70 eV. The analytical conditions were identical to those used for the gas chromatography.

Oxidative Stability of Roasted Red Pepper Seed Oils. To study the oxidative stabilities of red pepper seed oil, 60 g of oil was transferred, in triplicate, to a 100 mL capacity beaker. The samples were stored in a forced-draft air oven at 60 °C for 31 days. The oxidation stabilities of oils were studied by measuring the increase in peroxide content (AOCS Official Method Cd 8-53) and conjugated diene content in the oils. For the determination of conjugated diene, samples were diluted with 2,2,4-trimethylpentane. The absorptivities of the prepared samples were then measured at 233 nm, and the contents of the conjugated diene were expressed as absorptivities of the 1% red pepper seed oils in 2,2,4-trimethylpentane at 233 nm.

Statistical Analysis. Statistical analysis was accomplished using an SAS method (Statistical Analysis Systems Institute Inc., 1988). Duncan's multiple-range test was used to ascertain the treatment effect on the oxidative stabilities of roasted red pepper seed oils.

RESULTS AND DISCUSSION

Iodine Value and Fatty Acid Composition. The iodine value of the red pepper seed oil did not change with roasting time (data not shown). The iodine value of the red pepper seed oil was 137, which is higher than that (125−130) of soybean oil, indicating the high unsaturation of the oil.

The fatty acid composition of oil can be an indicator for its oxidative stability, crystalline properties, and nutritional quality (Zeitoun et al., 1993). Each fatty acid has a different nutritional value and health significance. The fatty acid compositions of red pepper seed oils with different roasting times were also not different (data not shown). Red pepper oil obtained after 6 min of roasting consisted of 0.24% myristic acid, 13.42% palmitic acid, 0.33% palmitoleic acid, 2.07% stearic acid, 10.18% oleic acid, 73.89% linoleic acid, and 0.37% linolenic acid. It is interesting to note that the red pepper seed oil has exceptionally high linoleic acid content and the fatty acid composition was similar to that of high-linoleate safflower oil, which contains 0.1% myristic acid, 6.7% palmitic acid, 0.1% palmitoleic acid, 2.9% stearic acid, 13.8% oleic acid, and 75.8% linoleic acid (Padly et al., 1986).

Pyrazines in Roasted Red Pepper Seed Oils. Gas chromatograms of the dichloromethane extracts of red pepper seed oils prepared from red pepper seed roasted for 6, 8, 10, and 12 min at 210 °C are shown in Figure 1. The individual pyrazine contents in the oils are shown in Table 1. As the roasting time increased, more pyrazines were formed and their contents increased. In the red pepper seed oil obtained after 6 min of roasting, only 6 pyrazines were identified. However, the oils obtained after 8, 10, and 12 min of roasting contained 11, 13, and 13 pyrazines, respectively. By use of the GC retention time and/or GC/MS data, the following pyrazines were identified in the red pepper seed oils, in increasing order of retention time in the gas chromatograph: 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2,6-diethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-isobutyl-3-methylpyrazine, and 3,5-diethyl-2-methylpyrazine. Analysis with authentic pyrazine samples showed that trimethylpyrazine and 2-ethyl-3-methylpyrazine were coeluted at the same retention time under the tested chromatographic condition. However, the mass spectrum of peak 7 obtained from gas chromatograms of the roasted red pepper seed oils was exactly same as that of the trimethylpyrazine obtained from the library (Figure 2). Thus, it was concluded that the peak 7 in Figure 2 was trimethylpyrazine and that 2-ethyl-3-methylpyrazine was not present in the sample. By comparing the retention times of authentic samples, it was also concluded that there were no 2,3-dimethylpyrazine, 2,3-diethylpyrazine, and 2-methyl-3-*n*-propylpyrazine in the roasted red pepper seed oils. Johnson et al. (1971) suggested that alkylpyrazines contributed to the roasted peanut flavor. Most of the identified alkylpyrazines in our present research were previously identified in roasted peanut volatiles (Walradt et al., 1970). The total pyrazine contents increased greatly with increasing roasting time, showing 2.63, 5.01, 8.48, and 13.10 mg/100 g of oil obtained from red pepper seeds after roasting for 6, 8, 10, and 12 min at 210 °C, respectively. In the oil from red pepper seeds roasted for 12 min at 210 °C, 2,5-dimethylpyrazine (3.48 mg/100 g of oil) was the most abundant pyrazine, followed by 2-methylpyrazine (2.45 mg/100 g of oil), trimethylpyrazine (1.44 mg/100 g of oil), and 2,6-dimethylpyrazine (1.33 mg/100 g of oil). It is also interesting to note that three pyrazines (tetramethylpyrazine, 2-ethyl-6-methylpyrazine, and 3,5-diethyl-2-methylpyrazine) did not increase with increasing roasting time.

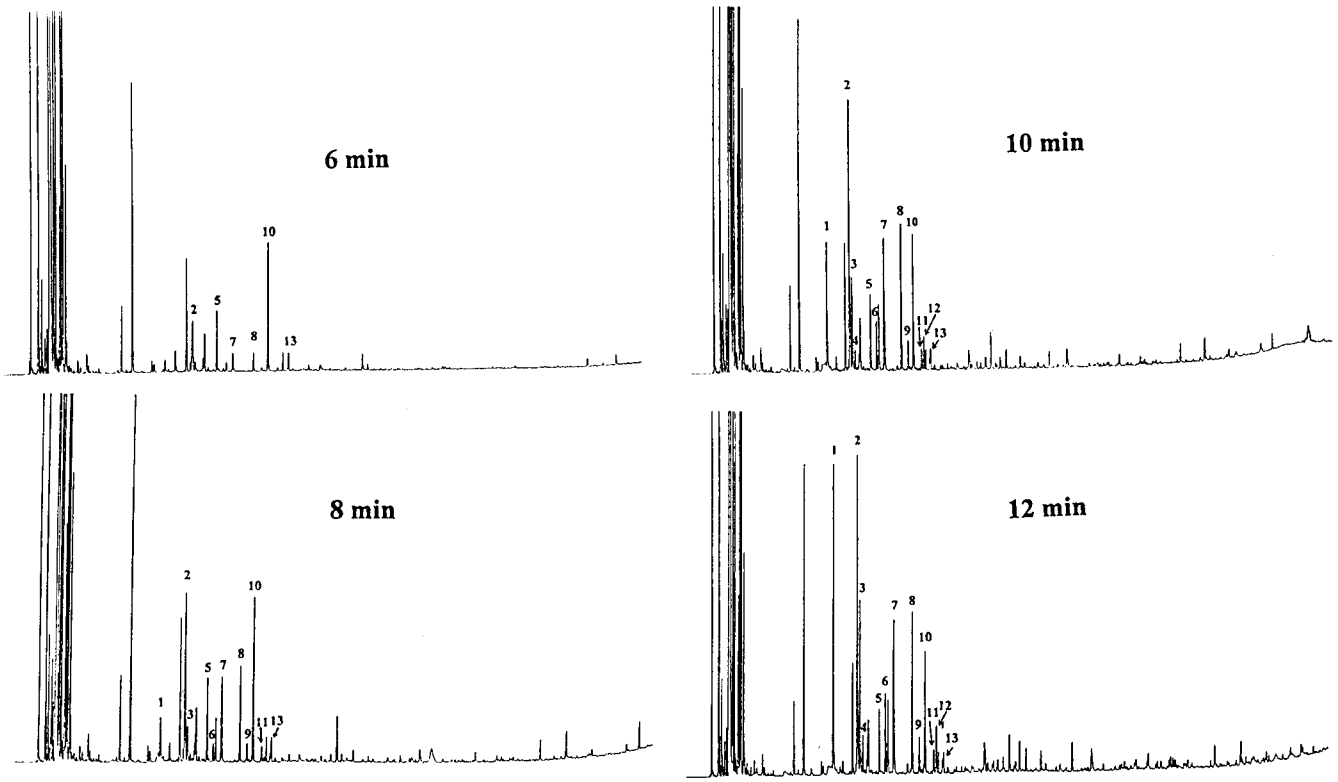

Figure 1. Gas chromatograms of the dichloromethane extract of red pepper seed oils prepared from red pepper seeds roasted for 6, 8, 10, and 12 min at 210 °C: 1, 2-methylpyrazine; 2, 2,5-dimethylpyrazine; 3, 2,6-dimethylpyrazine; 4, 2-ethylpyrazine; 5, 2-ethyl-6-methylpyrazine; 6, 2-ethyl-5-methylpyrazine; 7, trimethylpyrazine; 8, 2,6-diethylpyrazine; 9, 2-ethyl-3,5-dimethylpyrazine; 10, tetramethylpyrazine; 11, 2,3-diethyl-5-methylpyrazine; 12, 2-isobutyl-3-methylpyrazine; 13, 3,5-diethyl-2-methylpyrazine.

Table 1. Pyrazine Contents in Red Pepper Seed Oils Extracted from Red Pepper Seeds Roasted for 6, 9, 10, and 12 min at 210 °C

	pyrazine content (mg/100 g of oil)			
	6 min	8 min	10 min	12 min
2-methylpyrazine[a]		0.37	0.99	2.45
2,5-dimethylpyrazine[a]	0.68	1.33	2.14	3.48
2,6-dimethylpyrazine[a]		0.26	0.69	1.33
2-ethylpyrazine[a]			0.14	0.28
2-ethyl-6-methylpyrazine[b]	0.43	0.42	0.43	0.42
2-ethyl-5-methylpyrazine[b]		0.11	0.34	0.61
trimethylpyrazine[a]	0.18	0.61	1.08	1.44
2,6-diethylpyrazine[b]	0.15	0.57	0.92	1.19
2-ethyl-3,5-dimethylpyrazine[b]		0.12	0.21	0.28
tetramethylpyrazine[a]	1.05	0.97	1.01	0.92
2,3-diethyl-5-methylpyrazine[a]		0.12	0.18	0.22
2-isobutyl-3-methylpyrazine[a]			0.22	0.34

[a] Identified by both comparison of mass spectra with those in the mass library and comparison of GC retention time with that of authentic sample. [b] Identified by comparison of mass spectra with those in the mass library.

2-Methylpyrazine, 2,5-dimethylpyrazine, and trimethylpyrazine reportedly exhibit a nutty odor, and their reported odor thresholds in water were 60, 1.8, and 9 ppm, respectively (Maga and Sizer, 1975; Maga, 1982). Because the odor threshold of 2,5-dimethylpyrazine was low and its concentration was highest among the pyrazines identified in the oils, we concluded that the 2,5-dimethylpyrazine was the most responsible component for the nut-like odor of the oils obtained from roasted red pepper seeds. The identified pyrazine compounds are commonly found in numerous thermally processed (heated, roasted, baked, cooked) foods (Maga, 1982) and are therefore not unique to red pepper seed oil. However, this represents the first report of their identification and quantification in red pepper seed oils.

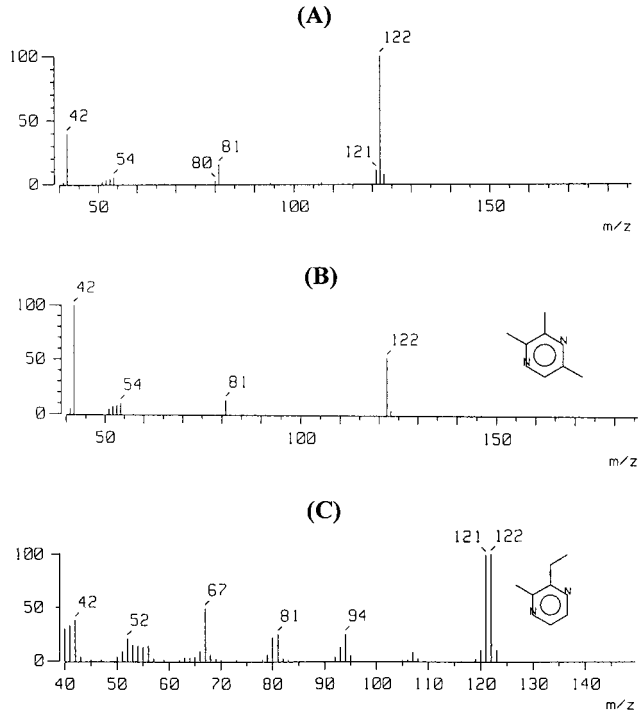

Figure 2. Mass spectra of peak 7 (A) (from the gas chromatogram of dichloromethane extract of roasted red pepper seed oil), trimethylpyrazine (B), and 2-ethyl-3-methylpyrazine (C) (from the mass library).

Oxidative Stability of Red Pepper Seed Oil. The oxidative stability tests clearly showed that, as the roasting time increased, the oxidative stability of the red pepper seed oil increased greatly (Figures 3 and 4). Peroxide value in red pepper seed oil extracted from red

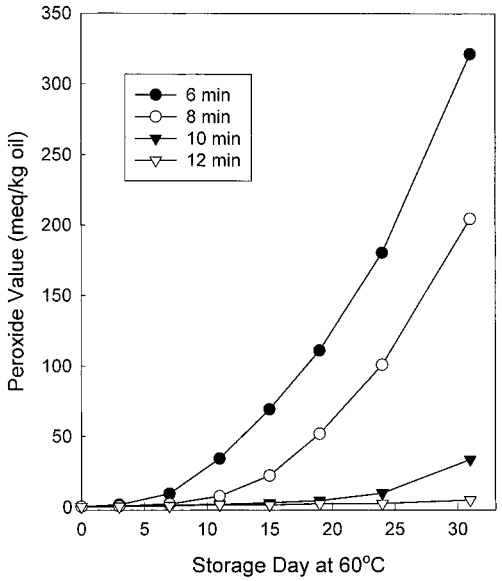

Figure 3. Changes in peroxide values in red pepper seed oils extracted after roasting the seeds for 6, 8, 10, and 12 min at 210 °C during storage at 60 °C.

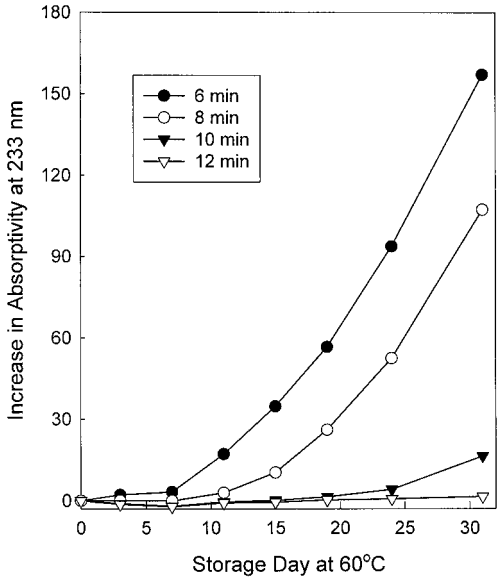

Figure 4. Changes in conjugated diene content in red peppers seed oils extracted after roasting the seeds for 6, 8, 10, and 12 min at 210 °C during storage at 60 °C.

pepper seeds roasted for 6 min increased drastically as the storage time increased, resulting in 321 mequiv/kg of oil after 31 days of storage at 60 °C (Figure 3). As the roasting time increased, the peroxide value increment of the oils greatly decreased ($p < 0.05$). The peroxide values of oils extracted from red pepper seeds roasted for 6, 8, 10, and 12 min were 321, 204, 34, and 5 mequiv/kg of oil, respectively. The oxidative stability of the oil obtained from the red pepper seeds roasted for 12 min was extremely high. Note that the oxidative stability test was done at 60 °C without cover. The 31 days of storage at 60 °C was roughly comparable to 16 months at room temperature (20 °C), when the general concept of 2 times the oxidation reaction rate increment with 10 °C increment ($Q_{10} = 2$) was applied.

Becuase the initial absorptivities of the oils changed with the time of roasting red pepper seed prior to oil extraction, the changes of the conjugated diene were determined by the difference in absorptivity between oils stored at 60 °C and initial original oils. The absorptivity difference at 233 nm was used to measure the increase in conjugated diene. As the roasting time increased, the increment of conjugate diene decreased greatly (Figure 4). The absorptivity increments of the oil extracted from red pepper seed roasted for 6, 8, 10, and 12 min were 157.0, 107.3, 16.4, and 1.4, respectively. The increment of conjugated diene in oil extracted from red pepper seed oil roasted for 12 min was almost negligible, indicating the extremely high oxidative stability of the oil. The present results were inconsistent with previously reported ones (Jung et al., 1997; Yen and Shyu, 1989) in that as the roasting time and temperature increased, the oxidative stability of roasted soybean oil or sesame oil increased. Probably the greater antioxidative stability of red pepper seed oils extracted from the red pepper seeds for longer roasting time was due to the nonenzymatic reaction products formed during the roasting process. Maillard reaction products, formed through the interaction of proteins and reducing sugars, reportedly have strong antioxidant activities (Elizalde et al., 1991, 1992; Lee, 1992; Beckel and Waller, 1983).

Conclusion. Iodine value and fatty acid composition of roasted red pepper seed oils did not change with roasting time. The red pepper seed oil contained an exceptionally high amount of linoleic acid (73%), and the fatty acid composition was very close to that of the high-linoleate safflower oil. Thirteen alkylpyrazines were identified in the roasted red pepper seed oils. The pyrazine content increased considerably as the roasting time increased. It was concluded that 2,5-dimethylpyrazine in the roasted red pepper seed oil was the component most responsible for the pleasant nutty aroma of the oils. The oxidative stabilities of oil increased greatly as the roasting time increased.

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