The Effects of Roasting Temperatures on the Formation of Headspace Volatile Compounds in Perilla Seed Oil

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ABSTRACT: Volatile compounds of perilla seed oils roasted at different temperatures (150–190°C) were analyzed by dynamic headspace gas chromatography–mass spectrometry. The headspace volatiles in roasted perilla seed oils (RPSO) were composed of thermally produced flavors and compounds originating from the raw perilla seeds. The roasting temperatures significantly affected the production of thermal reaction flavors. Oils from parilla seeds roasted below 170°C had relatively high concentrations of aldehydes, whereas pyrazines and furans were the predominant volatiles above 170°C. In all of the RPSO, the contents of both perilla aldehyde and perilla ketone remained almost constant and might be used to discriminate perilla seed oils from other roasted vegetable seed oils.

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KEY WORDS: Headspace volatiles, perilla aldehyde, perilla ketone, perilla seed oil, roasting.

Perilla seed [*Perilla frutescens* (L.) Britt.] is composed of 40–45% (w/w) oil with a high α-linolenic acid (n-3 fatty acid) content (1). Roasted perilla seed oils (RPSO) are widely used as condiment oils in Asian countries, especially in Korea and China, because of their roasted, nutty, and distinctive aromas, reminiscent of perilla aldehyde. Similar to sesame seed oils, RPSO are traditionally tailor-made by roasting, mechanical pressing, and simple refining from the raw perilla seeds. The roasting process may be a critical step for producing perilla seed oils because many aromatic compounds may be produced by the heat treatment that has an effect on the flavor quality of RPSO. Flavor profiles of RPSO are generally changed under different roasting temperatures (2). There have been no detailed reports yet, however, on the volatile components of RPSO. Hence, the objective of the present study was to identify the volatile compounds of RPSO and to investigate the effects of roasting temperature on the production of headspace volatile flavor components of RPSO.

MATERIALS AND METHODS

Materials. Perilla seeds were obtained from local areas in Korea. Total lipids of perilla seeds extracted with diethyl ether in a Soxhlet apparatus for 12 h were 44.5 % (w/w). Standard chemicals for identification of volatiles in gas chromatography (GC) and mass spectra were purchased from Aldrich Chemical Company (Milwaukee, WI) Sigma Chemical Co. (St. Louis, MO), and Fluka Chemie AG (Buchs, Switzerland). Iso-octane, used as a dilution solvent of standard chemicals was obtained from Fisher Scientific (Norcross, GA).

Preparation of RPSO and nonroasted perilla seed oils (NPSO). Perilla seeds were washed and dried to 6.4% (w/w) moisture content prior to roasting. The seeds (400 g) were roasted at 150, 160, 170, 180, and 190°C for 3 min using a continuous and circular monolayer roasting machine (Taewhan Automatic Instrument Co., Seoul, Korea). The roasted seeds were fed into the hopper immediately to minimize heat loss and expelled with a screw-type press at 60 rpm and 500 kg/cm^2. The barrel temperature of a press during each run was maintained at 120°C. This process produced about 120 to 125 g of oil and cakes in the form of flakes with 0.6-mm thickness. Four replications for RPSO at each roasting temperature were randomly conducted. The screw-pressed oils were filtered through a clean gauze sheet and kept overnight at 4°C to allow sedimentation of the small portion of debris. The supernatants of all the RPSO were separated from the debris and stored in amber glass bottles under nitrogen at −20°C until used for GC analysis. For the isolation of volatile compounds, the RPSO stored at −20°C were transferred to 4°C and held for several hours to melt. NPSO was extracted from the raw perilla seeds by simple pressing without roasting. Therefore, NPSO was prepared by the same procedures as described above except without heat.

Dynamic headspace-GC. Modified Thermal Desorption Equipment (Supelco, Inc., Bellefonte, PA) was used for analysis of the headspace volatile compounds of RPSO and NPSO by GC. We modified the purge and trap extraction systems (3–5) to eliminate the foam formation caused by high phospholipid levels of perilla seed oils. Magnetic stirring was performed to avoid foam production and to enhance the recovery of the volatile compounds. The apparatus used to collect the volatile compounds from the oil samples is shown in Figure 1. A round bubbler (170 mL) was applied outside the oven chamber of the Thermal Stripper (Supelco, Inc.), and the temperature of the bubbler was controlled using a water bath and hot plate stirrer.

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Perilla oil (0.5 g) was placed in a bubbler, which had been flushed with nitrogen for 40 min, and immersed in a water bath adjusted to 40°C. Three microliters of a 1-ppm solution of 2-octanone was added to the sample flask as an internal standard for estimating the relative content of each compound. A glass tube (11.5 cm × 4 mm) packed with Tenax-TA (200 mg, 60–80 mesh; Supelco, Inc.) was attached immediately. The tube had been conditioned for 30 min at 250°C using high-purity nitrogen flow of 150 mL/min in the Tube Conditioner (Supelco, Inc.) prior to trapping volatiles. The sample vessel was preheated for 5 min to equilibrate with the water bath temperature. The headspace volatiles were then purged with nitrogen (156 mL/min), using a Dynamic Thermal Stripper (Supelco, Inc.), for 15 min into a glass tube with magnetic stirring. The Tenax-TA tube temperature was maintained at 30°C. Desorption of volatile compounds trapped in the Tenax-TA tube into the GC was conducted using a Thermal Tube Desorber (Supelco, Inc.). The volatile compounds were thermally desorbed at 250°C for 9 min and then directly injected into the gas chromatograph (HP 5890 series II plus, Hewlett-Packard Co., Palo Alto, CA). The gas chromatograph was equipped with a flame-ionization detector (FID) and a fused SE-54 silica capillary column (60 m, 0.32 mm i.d., 0.25-µm film thickness; Supelco, Inc.). The helium carrier gas flow rate was 1.3 mL/min, with the injection splitter at a split ratio of 50:1. Blank runs with clean sample tubes were periodically carried out during the study. The oven temperature in the gas chromatograph was programmed as follows: the oven temperature was maintained at 35°C for the first 5 min and then raised to 95°C at 1°C/min rate, followed by a 2-min holding and then another raise to 250°C at 8°C/min rate with a final holding time of 5 min. The total running time was 88.38 min. Injector and detector temperatures were set at 220 and 250°C, respectively. The GC peak areas were calculated with an HP Chemstation data system (Hewlett-Packard Co.).

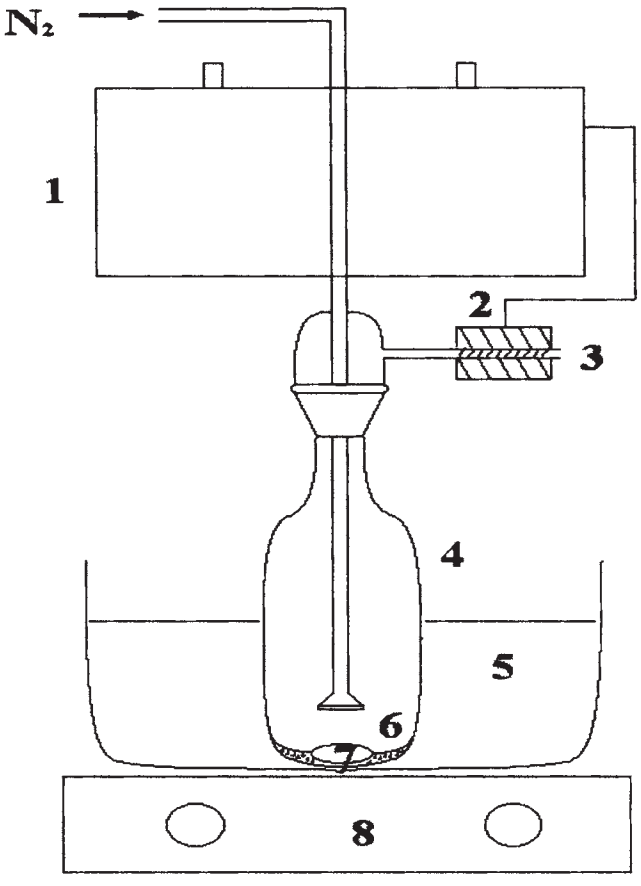

FIG.1. Purge and trap apparatus for collection of volatile compounds. 1, Dynamic Thermal Stripper (Supelco, Bellefonte, PA); 2, temperature controller; 3, Tenax-TA trap (Supelco); 4, bubbler; 5, water bath; 6, perilla oil; 7, magnetic bar; 8, hot plate stirrer.

GC–mass spectrometry. The identification of the volatile compounds in RPSO was performed by a GC 8000 series 8060 gas chromatograph coupled with a VG Platform mass spectrometer, and a VG Masslynx data system (VG Analytical, Manchester, United Kingdom). The separation procedure for volatiles was the same as described for GC analysis. The mass spectrometer was operated at an ionization voltage of 70 eV, an ion source temperature of 200°C, and a mass scan range of 40–300 *m/z*. The compounds were tentatively identified by comparing their mass spectra with Wiley/NIST Mass Spectral Database, or from published data (6,7). Volatile compound identification was confirmed by GC retention time and by mass spectral data for authentic compounds.

Statistical analysis. Area ratios of individual peaks to the internal standard were used for the statistical analysis. The data was analyzed by one-way analysis of variance for evaluating the roasting temperature effect in each volatile compound (8). Significant differences between RPSO in specific volatile compounds were determined by Duncan's multiple-range test ($P < 0.05$).

RESULTS AND DISCUSSION

Dynamic headspace analyses. Figure 2 shows a typical ion chromatogram of RPSO volatiles roasted at 180°C (RPSO180). More than 80 peaks were obtained in the gas chromatogram. The reproducibility of dynamic headspace GC analysis was evaluated by analyzing RPSO190 in quadruplicate, one per day for 4 d, and calculating the relative standard deviations (RSD) for the area ratios of individual peaks and total peaks to the internal standard. The RSD of 10 major volatiles and total peaks are listed in Table 1. The RSD for most of the volatile compounds did not deviate more than 10%, suggesting that our modified method afforded good reproducibility for the analysis of headspace volatile compounds in RPSO (9). The maintenance of constant temperature in the Tenax-TA trap during headspace volatile extraction was important so as to keep the low RSD of the volatiles.

Influence of roasting temperature on distribution of headspace volatile compounds of RPSO. The volatiles and mean values for area ratios of individual compounds to the internal standard are listed in Table 2. RPSO150, RPSO160, RPSO170, RPSO180, and RPSO190 are the denotations for RPSO prepared at 150, 160, 170, 180, and 190°C under the

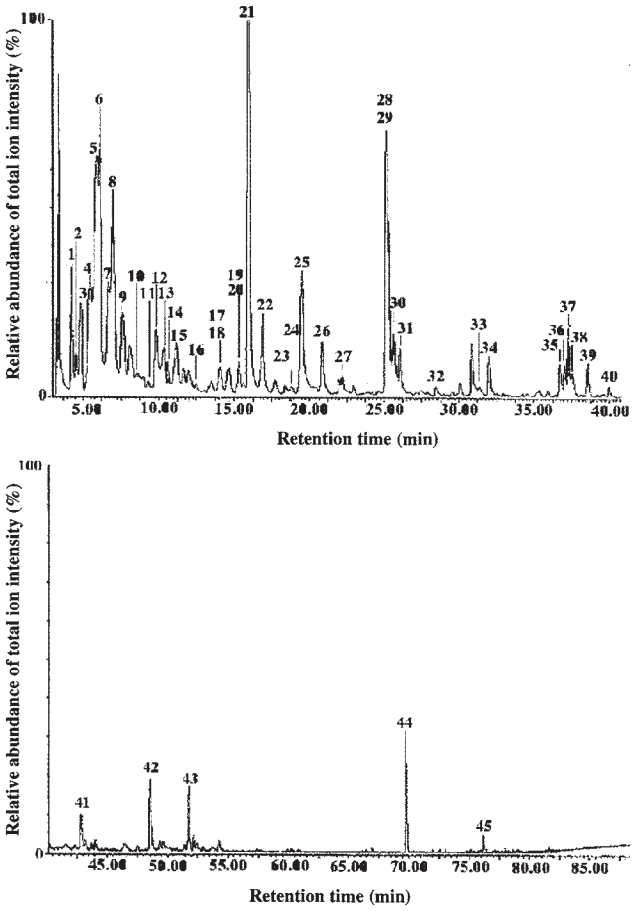

FIG. 2. Total ion chromatogram for the headspace volatile compounds of roasted perilla seed oil prepared at 180°C. Numbered peaks are identified in Table 2.

TABLE 1
Reproducibility for GC Analysis of the RPSO190[a]

Peak no.[c]	RT (min)	Compound	Peak area ratio[b] Mean (n = 4)	RSD (%)
1	4.24	2-Propanone	1.85	9.66
4	4.85	2-Butanone	4.78	1.74
8	6.98	2-Methyl butanal	2.60	3.71
15	11.20	1H-Pyrrole	2.40	3.09
21	15.98	2-Methyl pyrazine	15.83	1.90
25	19.54	Furfuryl alcohol	4.55	6.58
28	25.13	2,5(6)-Dimethyl pyrazine	6.83	2.31
37	37.26	Trimethyl pyrazine	1.02	4.54
43	51.80	Perilla aldehyde	0.19	7.93
44	69.74	Perilla ketone	0.18	6.45
Total peaks			90.47	1.14

[a]Calculated from quadruplicate gas chromatography (GC) determinations of a roasted perilla seed oil prepared at 190°C (RPSO190.
[b]Area ratios of individual peaks and total peaks to the internal standard.
[c]Peak numbers correspond to numbers in Figure 2. RT, retention time; RSD, relative standard deviation.

roasting process, respectively. Among the 80 individual peaks detected in the RPSO180, 45 peaks could be identified; they were classified as 7 aldehydes, 4 ketones, 1 alcohol, 6 furans and oxazoles, 14 nitrogen-containing compounds, 6 sulfur-containing compounds, and 7 miscellaneous compounds. Most of the volatile compounds identified in RPSO were considered to be very similar to those of roasted sesame seed oils (RSSO) (10,11). The roasting process conditions seemed to affect the production of many headspace volatile compounds of RPSO. The area ratios of most of the individual peaks and of the total peaks to the internal standard increased as the roasting temperature increased. The perilla ketone and trace amounts of perilla aldehyde, hexanal, and octane also could be found in the NPSO. The relative contributions of volatile compounds in RPSO are shown in Figure 3. The relative contributions of aldehydes in RPSOs were decreased with the increase of roasting temperature whereas nitrogen-containing compounds were significantly ($P < 0.001$) increased.

Three straight-chain aldehydes, three branched-chain aldehydes, and perilla aldehyde could be identified in RPSO. The straight-chain aldehydes (C5–C9) seemed to be breakdown products of unsaturated fatty acids. 2,4-Heptadienal was detected in the RPSO190 and RPSO180, whereas 2-heptenal was found only in RPSO190. The branched-chain aldehydes are suggested to be derived from Strecker degradation of amino acids such as 2-methyl propanal from valine, 2-methyl butanal from isoleucine, and 3-methyl butanal from leucine. These branched-chain aldehydes are commonly found in roasted sesame pastes (12), roasted peanuts (13), and virgin olive oil (14). The flavor note of 2-methyl butanal was described by several authors to be fermented and fruity, whereas 3-methyl butanal is pungent with a fruity flavor (3,12,14). All the RPSO contained relatively high contents of the three branched aldehydes in comparison to the straight-chain aldehydes. A distinctive feature of perilla seed oil volatile components was the presence of characteristic compounds such as 4-(1-methylethenyl)-1-cyclohexene-1-carboxaldehyde (perilla aldehyde) with or without roasting. Kameoka and Nishikawa (15) reported that perilla aldehyde and perilla alcohol were major compounds in the essential oil of *P frutescens* L. Brit. var. *acuta* Thunb. Kudo seed. It seemed that perilla aldehyde was not generated from the roasting process but originated from raw perilla seeds, since this compound was also identified in the NPSO. The perilla aldehyde contents of the RPSO190 and RPSO180 were almost twice as much as that from NPSO. The amounts of straight- and branched-chain aldehydes were significantly ($P < 0.001$) increased with the increase of roasting temperature. The relative contributions of aldehydes to the total volatiles was approximately 40–50% in the RPSO150, RPSO160, and RPSO170, whereas aldehydes in total volatiles of RPSO accounted for 27 and 16% in the RPSO180 and RPSO190, respectively.

Four ketone compounds were detected in the volatiles of all the RPSO. 2-Butanone was the most abundant and was likely produced by thermal degradation of sugars rather than by lipid oxidation (16). 2-Butanone, reported to be in virgin olive oil, has a fragrant and pleasant aroma (4). An unusual ketone, 1-(3-furanyl)-4-methyl-1-pentanone, i.e., perilla ketone, was detected in all the RPSO and also in the NPSO.

TABLE 2
Volatile Compounds Identified in Perilla Seed Oils Prepared at Different Roasting Temperatures

Peak no.[c]	Compound	Peak area ratio[a,b] RPSO 190	RPSO 180	RPSO 170	RPSO 160	RPSO 150	NPSO
Aldehydes							
3	2-Methyl propanal	2.81^{A}	2.61^{A}	2.86^{A}	1.67^{B}	1.09^{C}	—[d]
7	3-Methyl butanal	2.58^{A}	1.72^{A}	2.62^{A}	2.53^{B}	1.82^{B}	—
8	2-Methyl butanal	7.73^{A}	5.35^{B}	4.60^{C}	2.42^{D}	1.29^{E}	—
17	Hexanal	Trace[e]	Trace	Trace	Trace	Trace	Trace
33	2-Heptenal	0.37	—	—	—	—	—
39	(*E*,*E*)-2,4-Heptadienal	0.40^{A}	0.17^{B}	—	—	—	—
43	Perilla aldehyde	0.19^{A}	0.19^{A}	0.19^{A}	0.15^{B}	0.16^{B}	0.11^{C}
Ketones							
1	2-Propanone	1.72^{A}	1.07^{B}	0.52^{C}	0.30^{C}	0.30^{C}	—
4	2-Butanone	4.83^{A}	3.57^{B}	1.68^{C}	1.12^{D}	1.15^{D}	—
26	2-Pentanone	0.52^{A}	0.16^{C}	0.18^{C}	0.27^{B}	0.22^{BC}	—
44	Perilla ketone	0.18^{A}	0.14^{B}	0.14^{B}	0.14^{B}	0.16^{B}	0.06^{C}
Alcohol							
9	1-Penten-3-ol	2.48 A	1.63^{B}	1.20^{C}	0.80^{D}	0.73^{D}	—
Furans and oxazoles							
10	2,5-Dimethyl furan	0.37^{A}	0.25^{B}	0.19^{B}	0.15^{B}	0.22^{B}	—
22	2-Furfural	2.74^{A}	0.45^{B}	—	—	—	—
25	Furfuryl alcohol	4.24^{A}	0.58^{B}	—	—	—	—
34	5-Methyl-2-furfural	0.61	—	—	—	—	—
24	Trimethyl oxazole	0.19^{A}	0.13^{B}	—	—	—	—
41	3,5-Dimethyl	0.24	—	—	—	—	—
Nitrogen-containing compounds							
12	Pyrazine	2.75^{A}	0.51^{B}	—	—	—	—
21	2-Methyl pyrazine	15.86^{A}	4.62^{B}	1.10^{C}	0.29^{D}	—	—
28	2,5(6)-Dimethyl pyrazine	6.70^{A}	3.59^{B}	1.92^{C}	0.60^{D}	0.26^{D}	—
30	Ethyl pyrazine	1.35^{A}	0.45^{B}	0.17^{C}	—	—	—
31	2,3-Dimethyl pyrazine	0.92^{A}	0.45^{B}	0.18^{C}	—	—	—
35	2-Ethyl-6-methyl-pyrazine	0.58^{A}	0.25^{B}	0.12^{C}	—	—	—
36	2-Ethyl-5-methyl-pyrazine	0.38^{A}	0.24^{B}	0.16^{C}	—	—	—
37	Trimethyl pyrazine	1.08^{A}	0.74^{B}	0.48^{C}	0.18^{D}	0.12 E	—
38	2-Ethyl-3-methyl pyrazine	0.33^{A}	0.11^{B}	—	—	—	—
40	2-Acetyl pyrazine	0.24	—	—	—	—	—
42	2-Ethyl-2,5-dimethyl-pyrazine	0.42^{A}	0.29^{B}	0.20^{C}	—	—	—
14	Pyridine	0.84^{A}	0.27^{B}	0.12^{C}	—	—	—
15	1H-Pyrrole	2.44^{A}	0.68^{B}	0.22^{C}	0.16^{C}	0.15^{C}	
Sulfur-containing compounds							
13	Dimethyl disulfide	0.82^{A}	0.51^{B}	0.29^{C}	0.20^{D}	0.16^{D}	—
16	3-Methyl thiophene	0.23^{A}	0.14^{B}	—	—	—	—
20	4-Methyl thiazole	0.90^{A}	0.23^{B}	—	—	—	—
23	5-Methyl isothiazole	0.14	—	—	—	—	—
27	3,4-Dimethyl isothiazole	0.52^{A}	0.15^{B}	—	—	—	
32	4,5-Dihydro-2-methyl thiazole	0.20	—	—	—	—	—
Miscellaneous compounds							
2	Acetic acid methyl ester	0.40^{A}	0.22^{B}	0.14^{C}	—	—	—
5	Chloroform	1.45	—	—	—	—	—
6	Acetic acid	5.98^{A}	1.09^{B}	0.18^{C}	0.16^{C}	0.15^{C}	—
11	Phenol	0.32^{A}	0.25^{A}	0.24^{A}	0.29^{A}	0.29^{A}	—
18	Octane	0.55^{B}	0.33^{B}	0.38^{B}	0.84^{A}	0.87^{A}	Trace
19	2-Octene	0.72^{A}	0.25^{B}	—	—	—	—
45	*trans*-Caryophyllene	Trace	Trace	Trace	Trace	Trace	Trace
Unknown peaks		9.77^{A}	4.37^{B}	2.24^{C}	1.35^{D}	1.24^{D}	0.21^{E}
Total peaks		88.09^{A}	37.76^{B}	22.32^{C}	13.62^{D}	10.38^{E}	0.38^{F}

[a]All values are means calculated from area ratios of compounds to the internal standard in four different RPSO prepared at each roasting temperature. Means with same superscripts (*A*–*F*) within a row are not significantly different at $P < 0.05$.
[b]RPSO190, RPSO180, RPSO170, RPSO160, and RPSO150 indicate perilla seed oils roasted at 190, 180, 170, 160, and 150°C, respectively. NPSO represents nonroasted perilla seed oil extracted by simple pressing process without roasting.
[c]The peak numbers correspond to numbers in Figure 2. Trace, area ratio less than 0.02.

Similar to perilla aldehydes, the contents of perilla ketones in RPSO were two to three times greater than in NPSO, but perilla ketones remained nearly constant with roasting temperature for the RPSO tested. The reason for the higher content of perilla aldehydes and ketones of RPSO was that cell destruction during roasting resulted in greater extraction of these

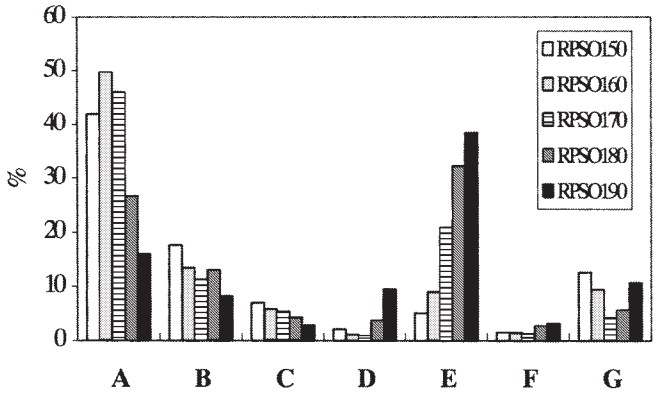

FIG. 3. Relative contributions of volatile compounds identified in roasted perilla seed oil (RPSO) prepared at 150 through 190°C. A, aldehydes; B, ketones; C, furans and oxazoles; D, alcohols; E, nitrogen-containing compounds; F, sulfur-containing compounds; G, miscellaneous compounds.

compounds (17). Both perilla compounds can be used as diagnostic compounds to distinguish RPSO from other roasted seed oils, because they are unique and characteristic compounds found in perilla seeds and have not been reported as aroma constituents of any other roasted vegetable seed oils except the essential oils of perilla (15) and orange juice (18).

Furans derived from sugar caramelization or degradation are related to pleasant and sweet flavors in many heated foods (12). 2,5-Dimethylfuran was detected in all the RPSO in trace amounts, whereas 2-furfural and furfuryl alcohol were found only in RPSO180 and RPSO190. These two furfuryl compounds contribute sweet, nutty, and caramel-like notes and have been reported in RSSO (5,11) and roasted sesame seed pastes (12). Umano *et al.* (16) suggested that furans could be precursors for other volatile compounds, thus RPSO volatile compounds may also be products of furan.

Fourteen nitrogen-containing compounds identified in the volatiles of RPSO have been classified as pyrazines, pyridines, and 1H-pyrrole. Pyrazines, formed *via* Maillard reactions, may be responsible for typical roasted and nutty aromas. Lee *et al.* (5) assumed that 2-methyl pyrazine and 2,6-dimethyl pyrazine were responsible for the sweet and roasted flavors of sesame seed oils. Shahidi *et al.* (12) also assumed 2-methyl pyrazine and 2,5-dimethyl pyrazine to be the major contributors to the roasted flavor of sesame seed paste. In the present study, 12 pyrazines were identified from RPSO. 2-Methyl pyrazine and 2,5(6)-dimethyl pyrazine represented about 70% of the total amount of pyrazines detected in RPSO180. In addition, ethyl pyrazines were also available in the volatiles of RPSO. Pyrazine production previously was strongly influenced by the roasting temperature (19). Figure 3 shows that the major thermal reactions took place at roasting temperatures above 170°C, because the majority of the pyrazines formed above this temperature. Pyrazines and total volatiles significantly ($P < 0.05$) increased with an increase in roasting temperature from 150 to 190°C. The area ratio percentage of pyrazines in the RPSO150, RPSO160, RPSO170, RPSO180, and RPSO190 were 3.66, 7.86, 19.40, 29.79, and 34.75%, respectively. Pyrazines seemed to impart roasted, burnt and nutty aromas to RPSO. Other nitrogen-containing compounds found in small amounts in the volatiles of RPSO were 1H-pyrrole and pyridine. All the RPSO had 1H-pyrrole in common, but pyridine was just detected in RPSO at and above the roasting temperature of 170°C. Pyridine might be formed *via* a cyclization of suitable amino sugar derivatives. Pyridine possessed less pleasant odors than did pyrazines and was noted as an irritating odor and flavor (20). Pyridine was widely reported in RSSO (11), roasted sesame seed paste (12), and roasted earth almond (21).

Various kinds of straight-chain and heterocyclic sulfur-containing compounds in RPSO were confirmed as dimethyl disulfide, thiazoles, and 3-methyl thiophene. Dimethyl disulfide, present in all the RPSO, is derived from the breakdown products of amino acids and affects overall food aroma (22). The thiazoles and thiophenes produced *via* the Maillard reaction were major heterocyclic sulfur compounds found only in RPSO180 and RPSO190. It is well known that the flavor notes of thiazoles are similar to that of pyrazines. The flavor of 2-acetyl thiazole is characterized as nutty, cereal-like, and popcorn-like flavor, and alkyl thiazoles are described as green, nutty, roasted, vegetable, or meaty (19). Hence, these heterocyclic sulfur-containing compounds frequently have been identified in roasted peanut (6), sesame (11,12), and almond (21) flavors.

Octane and *trans*-caryophyllene are components of raw perilla seed aroma as previously reported (15). Both compounds have been identified only in trace amounts in all the RPSO, but not in the NPSO. One of the most abundant components in the volatiles from RPSO was acetic acid, and it was positively correlated with the total volatiles of RPSO. Acetic acid in RPSO might be produced through the roasting process of raw perilla seeds and, therefore, responsible for the acidic aroma of RPSO. This observation was confirmed by the increase of acetic acid content in the RPSO190.

From the foregoing results, we concluded that the volatiles of RPSO were composed of compounds resulting from thermal reactions through Maillard reactions and lipid degradation as well as compounds originating from raw perilla seeds such as perilla aldehyde and perilla ketone.

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