Effect of Roasting Temperature and Time on the Chemical Composition of Rice Germ Oil

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ABSTRACT: Compositional changes of rice germ oils prepared at different roasting temperatures (160–180°C) and times (5–15 min) from rice germ were evaluated and compared with those of unroasted rice germ oil. The color development and phosphorus content of oils increased significantly as roasting temperature and time increased, whereas the FA compositions of rice germ oils did not change with roasting temperature and time. Four phospholipid classes, i.e., PE, PI, PA and PC, were identified. PE had the lowest stability under roasting conditions. There were no significant differences in γ-oryzanol levels of rice germ oils prepared at different roasting temperatures and times. Four tocopherol isomers (α-, β-, γ-, and δ-tocopherol) and three tocotrienol isomers (α-, γ-, and δ-tocotrienol) were identified, but no β-tocotrienol was detectable. The content of α- and γ-tocopherol in rice germ oil gradually increased as roasting temperature and time increased.

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KEY WORDS: γ-Oryzanol, phospholipid, phosphorus, rice germ oil, roasting, tocopherol, tocotrienol.

Rice germ is a by-product of the rice milling process which is included in rice bran. In addition rice germ, which is produced by sieving and vibrating the rice bran, has a higher oil content (30%) than rice bran does (18%) (1). Because rice germ accounts for 20% or more of the weight of rice bran, the removal of rice germ can affect the oil content of rice bran (2).

Recently, rice germ oil has been used in Korea as a condiment oil along with sesame oil and perilla oil. Traditionally, these condiment oils are prepared by extracting the roasted seed with a mechanical press or expeller after the seeds have been roasted at the appropriate temperature and for the appropriate time (3,4). During the roasting process, a pleasant aroma or taste (nutlike or peanut butter-like) that transfers to the oil during extraction is developed. The conventional method for preparation of condiment oils such as sesame oil, perilla oil, and red pepper oil involves cleaning, roasting, grinding, and pressing but not refining (5). The roasting process is the key step for making condiment oil since the color, composition, and quality of the oil are all influenced by the conditions of the process. Some researchers (6,7) reported that the chemical composition of an oil is independent of the roasting temperature used for preparing it. However, to our knowledge, little investigation has been conducted on the effects of roasting on the chemical composition of rice germ oil.

The objective of this study was to investigate changes in color, FA composition, and minor components, such as phosphorus, tocopherol, tocotrienol, γ-oryzanol and phospholipids, of rice germ oil roasted at different temperatures and times.

EXPERIMENTAL PROCEDURES

Rice germ and reagents. Rice germ used in this study was obtained from Kimpo Agricultural Cooperative Federation (Kimpo, Korea). Tocopherol isomers were purchased from Merck (Darmstadt, Germany) and tocotrienol isomers from Calbiochem (Calbiochem-Novabiochem Co., San Diego, CA). Phospholipid standards were purchased from Sigma Chemical Company (St. Louis, MO). Other chemicals used in this study were analytical grade.

Preparation of rice germ oil. Rice germ (200 g) was roasted in an electric roaster equipped with a stirrer and a temperature controller. Rice germ was roasted with constant stirring at 160, 170, and 180°C for 5, 10, and 15 min, respectively. After roasting, the roasted rice germ was allowed to cool to ambient temperature. The roasted rice germ was then pressed (600 kg/cm^2) using a mechanical press (Carver Inc, Wabash, IN) to obtain the rice germ oil. Unroasted rice germ oil was prepared by the same procedure as described above but without roasting. The extracted rice germ oils were filtered to remove particles.

Determination of color development. As an index of color development (8), the absorbance at 420 nm of a 5.0% (wt/vol) solution of oils in chloroform was determined with a spectrophotometer (UV-900; JASCO, Tokyo, Japan).

FA composition. Oils were esterified according to AOCS standard method Ce 2-66 (9). Methyl esters of FA were extracted with hexane. Then 1 μL aliquots of the extracts were injected into a gas chromatograph (Varian 3800; Varian Inc., Walnut Creek, CA) equipped with an FID. The column used was a Supelcowax 10 fused-silica capillary column (30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA). The carrier gas was helium, and the total gas flow rate was 20 mL/min. The

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injector, oven, and detector temperatures were 240, 190, and 260°C, respectively.

Phosphorus and phospholipid analysis. Phosphorus contents of the rice germ oils were determined by AOCS standard method CA 12-55 (9). To analyze phospholids by HPLC, phospholipids were isolated from the oils as follows. Triplicate samples of 2 g of oil were fractionated on a 20 g column of silicic acid (100–200 mesh, Sigma) with sequential elution by 200 mL chloroform, 100 mL acetone, and 200 mL methanol. Methanol was removed in a rotary evaporator at 35°C; the sample residues were then frozen (–20°C) until analysis. Phospholipid analysis was performed with an HPLC (PU-1580; JASCO) connected to a Rheodyne injector with a 20 µL sample loop and an ELSD (Sedex 55; Richard Scientific, Novato, CA). Phospholipids isolated by silicic acid column were analyzed on a normal-phase column, Lichrospher Si-60 (250 × 4.6 mm i.d.; Merck Co.), and a linear gradient elution from (A) chloroform/tertiary-butyl-methyl ether (75:15, vol/vol) to (B) methanol/ammonium hydroxide/chloroform (92:7:1, by vol) at 0.5 mL/min for 30 min and held at (B) for 10 min. This was followed by a reverse linear gradient to the starting solvent (A) at 0.5 mL/min for 10 min.

γ-Oryzanol contents. γ-Oryzanol content of the rice germ oils was determined by using a spectrophotometer (UV- 900; JASCO) at 315 nm, according to the method of Kim and Kim (10). The analytical reference standard of γ-oryzanol was obtained from Il-Dong Pharmaceutical Company (Seoul, Korea).

Tocopherol and tocotrienol contents. To determine tocopherol and tocotrienol isomers of rice germ oil, 1 g of the oil, 4 mL 5% pyrogallol solution in ethanol, and a few boiling chips were placed in a 120 mL round-bottomed flask fitted with a reflux condenser and heated on a hot plate. When the mixture started boiling, the condenser was removed and 1 mL 50% aqueous potassium hydroxide solution was added. The sample was saponified for 5 min. After saponification, the flask was placed in an ice bath, and 20 mL water and 30 mL diethyl ether were added. The mixture was transferred to a 250 mL separatory funnel. Extraction of the sample with 30 mL diethyl ether was repeated twice. The pooled diethyl ether layer was washed three times with 20 mL distilled water, filtered through anhydrous sodium sulfate, and then evaporated at 30°C. The remaining sample was diluted with 10 mL *n*-hexane and filtered through a Millipore 0.45 µm FH membrane and injected into the liquid chromatograph (LC). The LC system consisted of an HPLC (PU-1580; JASCO) connected to a Rheodyne (Rohnert Park, CA) injector with a 20 µL sample loop and a fluorescence detector (FP-1520; JASCO) with excitation set at 298 nm and emission set at 325 nm. A Lichrospher Si-60 column (250 × 4.6 mm i.d.; Merck Co.) was used. The mobile phase was *n*-hexane/2-propanol (99:1) at 1.0 mL/min.

Statistical analysis. Each reported value is the mean of determinations for triplicate samples prepared from each roasting condition, and the data are analyzed by ANOVA and Duncan's multiple range test (Duncan's test). Statistical significance was accepted at a level of $P < 0.05$ (11).

RESULTS AND DISCUSSION

Color development. Color formation in the oil was influenced by the extent of roasting. Color development of rice germ oil prepared at different roasting temperatures and times is shown in Figure 1. With an increase in roasting time and temperature, browning substances were developed, resulting in a significant ($P < 0.05$) increase of the absorbance at 420 nm. The formation of browning substances in several thermally processed foods results from Maillard-type nonenzymatic reactions between reducing sugars and free amino acid or amide (12).

FA composition. The FA composition of an oil can be an indicator of its stability, physical properties, and nutritional value. There were no differences in FA composition of rice germ oils prepared at different roasting temperatures and times (data not shown). Rice germ oil (unroasted) consisted of 0.11% myristic acid, 19.11% palmitic acid, 0.16% palmitoleic acid, 2.04% stearic acid, 35.42% oleic acid, 41.10% linoleic acid, 1.12% linolenic acid, 0.59% arachidic acid, and 0.35% eicosenoic acid. In the case of rice bran oil, the content of oleic acid (41%) was higher than that of linoleic acid (38%) (13). On the other hand, our data showed that the content of linoleic acid (41%) was higher than that of oleic acid (35%) in rice germ oil.

Phosphorus content and phospholipid distribution. Phospholipids are integral structure elements of all kinds of membranes in living organisms and are essential for the growth, maturation, maintenance, and functional capacity of cells of the animal and human body (14). The phosphorus contents and

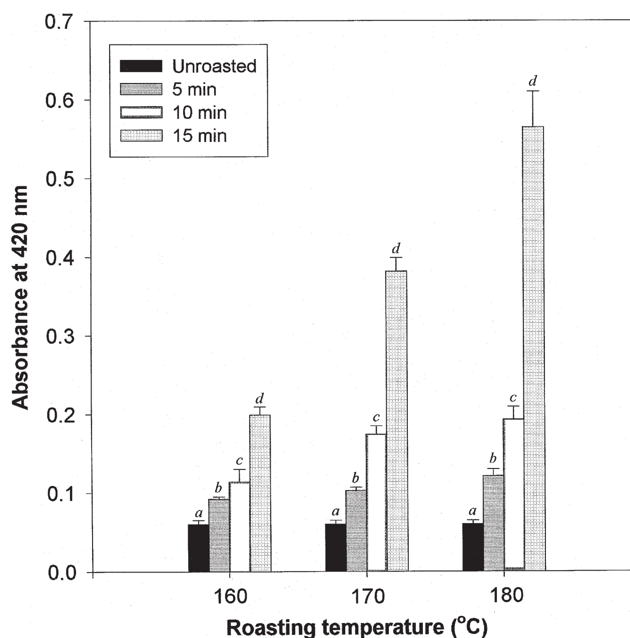

FIG. 1. Changes in the color (absorbance at 420 nm) of rice germ oil prepared by roasting at different temperatures and times. Each value represents the mean value of determinations for triplicate samples, and the vertical bars represent the SD of the replicates. Values in each temperature grouping with different letters (*a–d*) are significantly different ($P < 0.05$) as measured by Duncan's test.

TABLE 1
Phosphorus Content and Phospholipid Composition of Rice Germ Oil Prepared by Roasting at Different Temperatures and Times[a]

Roasting temperatue (°C)	Roasting time (min)	Phosphorus content (ppm)	Phospholipid classes (%) PE	PI	PA	PC
Unroasted		70.4 ± 0.7[c]	32.6 ± 0.8[c]	2.5 ± 0.1[c]	59.9 ± 2.5[c]	4.9 ± 0.3[c]
160	5	220.4 ± 4.2[d]	51.9 ± 1.5[d]	29.7 ± 2.1[d]	13.6 ± 0.6[d]	4.7 ± 0.2[c]
	10	272.9 ± 3.9[e]	37.4 ± 1.1[e]	38.7 ± 0.9[e]	12.6 ± 1.2[d]	11.4 ± 0.6[d]
	15	350.6 ± 4.2[f]	10.4 ± 0.7[f]	58.5 ± 3.4[f]	14.1 ± 0.8[d]	17.0 ± 1.0[e]
170	5	232.9 ± 2.3[d]	51.6 ± 2.3[d]	29.7 ± 1.3[d]	13.5 ± 0.8[d]	5.2 ± 0.6[c]
	10	312.5 ± 3.1[e]	23.7 ± 0.7[e]	54.4 ± 2.2[e]	15.2 ± 1.4[d]	6.7 ± 1.4[c]
	15	377.6 ± 1.7[f]	1.5 ± 0.2[f]	60.5 ± 5.8[e]	9.9 ± 0.7[e]	28.2 ± 2.1[d]
180	5	262.3 ± 4.1[d]	44.9 ± 3.1[d]	31.5 ± 2.3[d]	17.0 ± 1.0[d]	6.7 ± 0.2[d]
	10	322.7 ± 2.6[e]	4.8 ± 0.5[e]	60.5 ± 7.1[e]	8.9 ± 0.4[e]	29.2 ± 1.6[e]
	15	460.4 ± 1.7[f]	ND	56.8 ± 3.1[e]	3.2 ± 0.6[f]	40.0 ± 2.7[f]

[a]Mean values ± SD of determinations for triplicate samples. Value of the unroasted sample and values in the same column (within the same subgroup) with different superscript letters (c–f) are significantly different ($P < 0.05$) as measured by Duncan's test. ND, not detected.

phospholipid distributions determined by HPLC-ELSD for rice germ oils prepared following different roasting temperatures and times are presented in Table 1. There were significant ($P < 0.05$) differences in the phosphorus content of oils prepared at different roasting conditions. With increasing roasting time, phosphorus content significantly ($P < 0.05$) increased. The phosphorus contents of oils prepared from rice germ roasted for 5, 10, and 15 min at 160°C were 220, 272, and 350 ppm, respectively, whereas that of oil prepared from unroasted rice germ was 70 ppm. A similar trend was observed at other roasting temperatures (170 and 180°C). Veldsink *et al.* (15) reported that the phosphorus content of rapeseed and sunflower oils significantly increased as the preheating temperature of the oilseeds increased. Clark and Snyder (16) also reported that at a higher pretreatment temperature, a larger amount of phosphorus was extracted. Our results confirmed these observations. HPLC chromatograms of phospholipids isolated from rice germ oil prepared at 160°C and different roasting times are shown in Figure 2. Four phospholipid classes—PE, PI, PA, and PC—were identified. The major

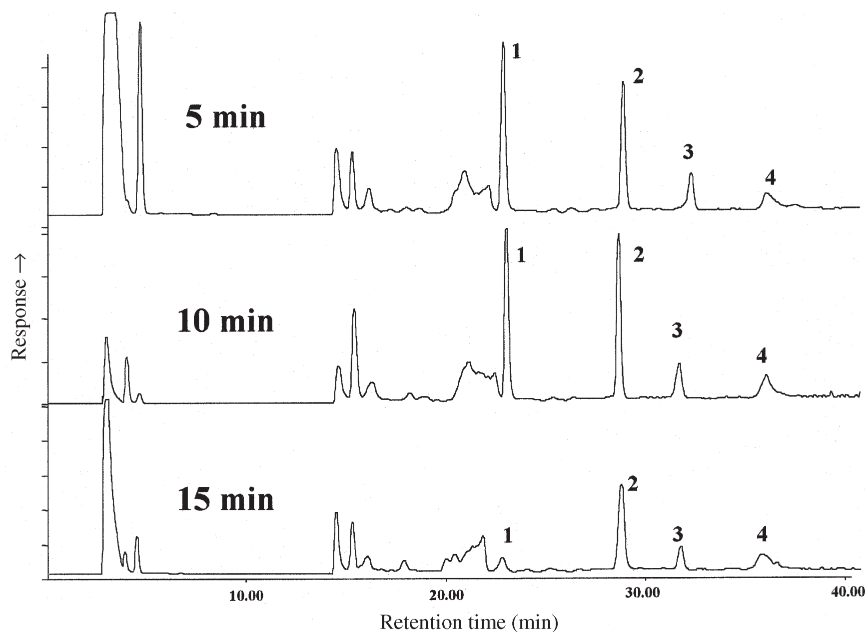

FIG. 2. HPLC chromatograms of phospholipids isolated from rice germ oils prepared from rice germ roasted for 5, 10, and 15 min at 160°C. Chromatographic conditions: Lichrospher Si-60, 250 × 4.6 mm, 5 μm column (Merck, Darmstadt, Germany); mobile phase, A. chloroform/tertiary-butyl-methyl ether (75:15), B. methanol/ammonium hydroxide/chloroform (92:7:1): linear gradient elution 0 to 100% (B) in 30 min, 10 min at 100% (B), and 0 to 100% (A) in 10 min; flow rate, 0.5 mL/min; detection, evaporative light scattering; peaks: 1, PE; 2, PI; 3, PA; 4, PC.

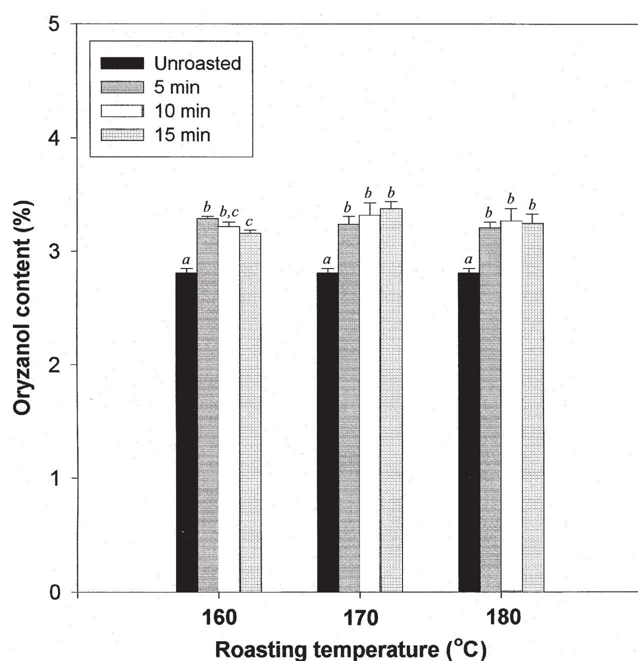

FIG. 3. Changes in γ-oryzanol contents of rice germ oil prepared by roasting at different temperatures and times. Each value represents the mean value of determinations for triplicate samples, and the vertical bars represent the SD of the replicates. Values in each temperature grouping with different letters (*a–c*) are significantly different ($P < 0.05$) as measured by Duncan's test.

phospholipid component of rice germ oil prepared at the shortest roasting time (5 min) was PE. However, the proportion of PE in the rice germ oil decreased significantly ($P < 0.05$) as roasting time increased. PE in rice germ oil roasted for 15 min at 180°C was completely degraded, suggesting that PE has the lowest stability among phospholipids in rice germ. Previous studies (17,18) have reported that an increase in the roasting time of seed, such as sesame and soybean, resulted in significant reductions in PE, which is consistent with our results.

γ-Oryzanol contents. Crude rice bran oil contains about 2–3% γ-oryzanol, a group of ferulate esters of triterpene alcohols and phytosterols. Physiological effects associated with γ-oryzanol intake are decreased plasma cholesterol (19), decreased platelet aggregation (20), and decreased cholesterol absorption (21). γ-Oryzanol has also been used to treat nerve imbalance and disorders of menopause (22). The contents of γ-oryzanol in rice germ oils prepared following different roasting temperatures and times are given in Figure 3. The content of γ-oryzanol in unroasted rice germ oil was 2.81%, whereas that in roasted rice germ oil was 0.5% or less higher. However, there were no significant differences in γ-oryzanol level of rice germ oils prepared at different roasting temperatures and times.

Tocopherol and tocotrienol contents. Biological activities of tocopherol and tocotrienol are generally believed to be due to their antioxidant action, which inhibits lipid peroxidation in biological membranes. α-Tocopherol is generally recognized as one of the most efficient antioxidants for breaking free radical-driven chain reactions in the body. However, recent results by a chemiluminescence assay in a liposome membrane system indicate that α-tocotrienol is at least threefold more efficient as a scavenger of peroxyl radicals than is α-tocopherol (23). Furthermore, the tocotrienols differ substantially in their capacity to suppress tumor cell proliferation (24). The contents of the individual tocopherol and tocotrienol isomers in rice germ oils prepared at different roasting temperatures and times are given in Table 2. Four tocopherol isomers, i.e., α-, β-, γ-, and δ-tocopherol, and three tocotrienol isomers, i.e., α-, γ-, and δ-tocotrienol, were identified, whereas no β-tocotrienol was detected (Fig. 4). The major tocopherol and tocotrienol homologs in rice germ oil were α- tocopherol and α-tocotrienol, respectively. The content of α-tocopherol in rice germ oil gradually ($P < 0.05$) increased as roasting temperature and time increased. For example, the contents of α-tocopherol in rice germ oils roasted for 5, 10, and 15 min at 160°C were 1404, 1432, and 1457

TABLE 2
Tocopherol and Tocotrienol Isomers Contents of Rice Germ Oil Prepared by Roasting at Different Temperatures and Times[a,b]

Roasting temperature (°C)	Roasting time (min)	Tocopherol and tocotrienol isomers (mg/kg oil)						
		α-T	β-T	γ-T	δ-T	α-T_3	γ-T_3	δ-T_3
Unroasted		1307.6 ± 10.5^{c}	55.0 ± 1.8^{c,d}	115.3 ± 2.5^{c}	6.8 ± 0.5^{c}	76.0 ± 1.5^{c,d}	49.5 ± 2.1^{c}	4.4 ± 0.4^{c}
160	5	1404.0 ± 8.6^{d}	54.4 ± 2.2^{c,d}	118.8 ± 4.6^{c}	6.5 ± 0.2^{c}	76.2 ± 2.0^{c,d}	45.2 ± 1.7^{c,d}	4.8 ± 0.8^{c}
	10	1432.2 ± 9.5^{e}	57.1 ± 0.8^{c,d}	127.2 ± 2.8^{d}	6.9 ± 0.7^{c}	76.3 ± 2.3^{c,d}	46.2 ± 2.8^{c,d}	4.9 ± 0.6^{c}
	15	1457.5 ± 14.3^{f}	56.6 ± 1.1^{c,d}	137.9 ± 3.3^{e}	6.4 ± 0.6^{c}	73.6 ± 1.8^{c,d}	44.5 ± 3.0^{d}	4.0 ± 0.7^{c}
170	5	1428.7 ± 7.3^{d}	57.0 ± 2.4^{c}	121.6 ± 3.7^{c}	6.3 ± 0.5^{c}	73.4 ± 0.7^{c,d}	46.1 ± 1.5^{c,d}	4.4 ± 0.6^{c}
	10	1435.9 ± 4.6^{d}	53.1 ± 0.7^{d}	135.3 ± 1.5^{d}	6.4 ± 0.6^{c}	73.1 ± 2.4^{c,d}	43.9 ± 1.6^{d}	4.2 ± 0.5^{c}
	15	1478.6 ± 12.1^{e}	56.2 ± 0.9^{c,d}	146.6 ± 5.5^{e}	6.3 ± 0.4^{c}	74.3 ± 1.4^{c,d}	47.4 ± 2.3^{c,d}	4.3 ± 0.6^{c}
180	5	1445.0 ± 8.6^{d}	53.6 ± 2.8^{c,d}	128.7 ± 4.1^{d}	6.5 ± 0.3^{c}	73.2 ± 0.8^{c}	47.6 ± 2.5^{c,d}	4.2 ± 0.7^{c}
	10	1468.9 ± 7.9^{e}	54.5 ± 1.9^{c,d}	144.6 ± 3.3^{e}	6.4 ± 0.8^{c}	73.6 ± 1.7^{c}	45.1 ± 0.9^{d}	4.0 ± 0.1^{c}
	15	1520.2 ± 4.3^{f}	55.5 ± 3.0^{c,d}	160.7 ± 3.2^{f}	6.7 ± 0.4^{c}	78.7 ± 2.7^{d}	48.4 ± 1.7^{c,d}	4.3 ± 0.2^{c}

[a]Mean values ± SD of determinations for triplicate samples.
[b]Abbreviations: T, tocopherol; T_3, tocotrienol. Value of the unroasted sample and values in the same column (within the same subgroup) with different superscript letters (c–f) are significantly different ($P < 0.05$) as measured by Duncan's test.

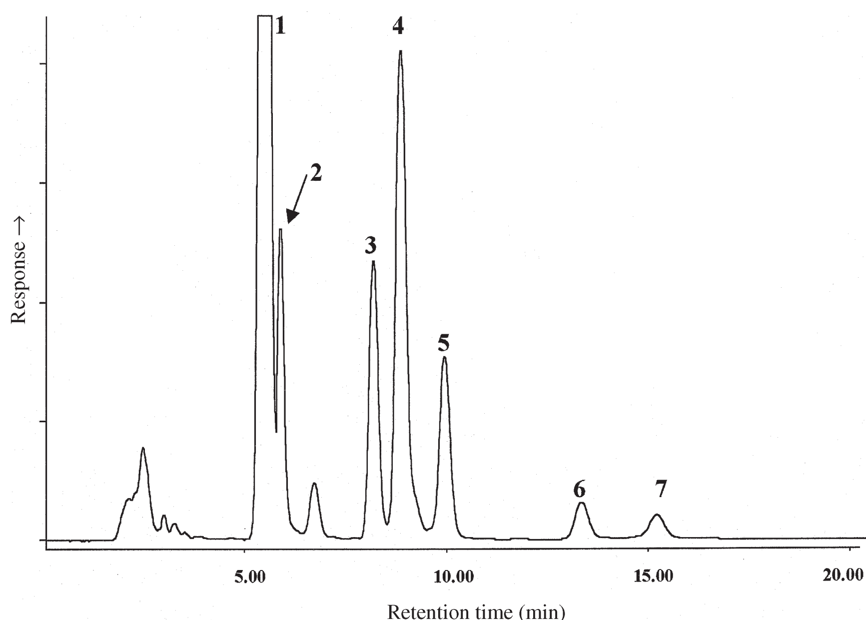

FIG. 4. HPLC chromatogram of saponified rice germ oil. Chromatographic conditions: Lichrospher Si-60, 250 × 4.6 mm, 5 μm column (Merck, Darmstadt, Germany); mobile phase, *n*-hexane/2-propanol (99:1); flow rate, 1.0 mL/min; detection, fluorescence, excitation 298 nm, emission 325 nm; peaks: 1, α-tocopherol; 2, α-tocotrienol; 3, β-tocopherol; 4, γ-tocopherol; 5, γ-tocotrienol; 6, δ-tocopherol; 7, δ-tocotrienol.

mg/kg, respectively, whereas those of α-tocopherol in rice germ oils roasted for 5, 10, and 15 min at 180°C were 1445, 1468, and 1520 mg/kg, respectively. A similar trend was observed in γ-tocopherol. However, there were no significant differences in the content of other tocopherol (β, δ) and tocotrienol homologs (α, γ, and δ) when roasting temperature and time were increased. Yoshida *et al.* (17) reported that the content of tocopherol in sesame oils prepared by microwave oven heating decreased over time. On the other hand, Yen (25) reported that the level of tocopherol in sesame oils prepared by electric oven heating was increased by roasting temperatures up to 200°C. Lane *et al.* (26) also reported that a heat pretreatment (over a range of 100–175°C) by a convection oven caused an increase in level and yield of tocopherol in rice bran oil. Moreau *et al.* (27) offered a possible explanation for the heat-induced increase in the levels of γ-tocopherol in corn hulls, suggesting that a significant amount of γ-tocopherol is bound to proteins or linked to phosphate or phospholipid and that heat breaks these bonds. It is possible that a similar phenomenon occurs in rice germ, in which bonds linking α-tocopherol and γ-tocopherol with phosphate or phospholipid are broken by roasting.

Changes in rice germ oil composition were observed at different roasting temperatures and times, except for FA and γ-oryzanol content. This study is the first report on chemical changes in rice germ oil with roasting temperature and time.

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