Roasting Effects on the Distribution of Tocopherols and Phospholipids Within Each Structural Part and Section of Soybeans

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ABSTRACT: To clarify the effects of microwave roasting on the distribution of tocopherols and FA of phospholipids within soybeans, whole soybeans (*Glycine max*) were treated by microwave and further evaluted as compared to a raw sample. Tocopherol homologs, measured using HPLC, and phospholipid profiles, quantified with GC, were determined in the seed coat, the embryonic axis, and sections of cotyledons separated from three cultivars. The tocopherols were predominantly detected in the axis, followed by the cotyledons, and then very little in the coat. As much as 25% of the individual tocopherols originally present in the coat were lost at 12 min of roasting, whereas <25% was lost in the cotyledons and the axis after 20 min of roasting. The greatest rate of phospholipid loss ($P < 0.05$) was observed in PE, followed by PC and PI, and their changing patterns were more pronounced in the coat than in the cotyledons or the axis. Thus, tocopherol content and phospholipid profiles change with microwave roasting according to tissue.

Paper no. J10423 in *JAOCS 80,* 665–674 (July 2003).

KEY WORDS: Cotyledons, embyronic axis, FA distributions, microwave roasting, phospholipids, seed coat, soybean, tocopherol homologs.

Soybeans are rich in protein and oil. Many East Asian countries, including Japan, have a long history of utilizing soybeans in great quantities as essential protein foods for humans. However, soybeans contain antinutritional factors such as trypsin inhibitor (1), which is thought to reduce protein digestibility and to cause pancreatic hypertrophy. Several processes, e.g., heat treatment (steaming or boiling), dry roasting (2), homogenization, and hydrolysis (3) can be used to increase the biological utilization and digestibility of soybeans. For heating purposes, microwave energy is more efficient than traditional methods (4). The characteristic feature of microwave heating is that it ensures homogeneous operation in the whole volume of substance, with great penetrating depth (5). However, the differential heating behavior of food components can result in severely uneven heating of certain foods rich in fats and protein (6). We suggested that microwave heating is an effective means for inactivating trypsin inhibitors in whole soybeans and also for making full-fat soy flour from raw beans with a high vitamin E content (7).

Microwave energy penetrates a food material and produces heat internally, which leads to faster heating rates and shorter processing times. In fact, microwaves are used in the food industry for thawing, drying, and baking as well as for other applications such as sterilizing and pasteurizing many types of foods (8). Microwave ovens are credited with rapid heating rates and high efficiency, because of their high penetration power (6). Phospholipids are the major constituents of cell membranes in soybeans, and they have a high degree of unsaturation. However, very little work has been reported on how microwave energy affects distribution of tocopherols in relation to FA composition of various acyl lipids within the three structural parts of a soybean.

The objective of this research was to evaluate the composition of tocopherols and FA distribution of major phospholipids in the seed coat, axis, and sections of cotyledons in whole soybeans when roasted in a microwave oven.

EXPERIMENTAL PROCEDURES

Materials. Commercially available soybeans (*Glycine max* L.) used in this work were from three Japanese cultivars—Mikawajima, Fuki, and Shishiou—that were grown in Japan during the summer of 2001. Cultivars were purchased from Takii Seed Co. (Kyoto, Japan) and selected for uniformity based on bean weights of 330 to 379 mg for Mikawajima, 350 to 399 mg for Fuki, and 400 to 449 mg for Shishiou. The soybeans were hand-selected to eliminate those with cracked or otherwise damaged seed coats. All the beans were divided into three groups for storage in stainless steel containers at 4°C and 52.0% RH until needed, respectively.

Reagents. All chemicals were analytical grade (Nacalai Tesque, Kyoto, Japan). Vitamin E homologs (α, β, γ, and δ) were purchased from Eisai Co. (Tokyo, Japan). All tocopherols were the D-form (*RRR*-), and each tocopherol had a purity of greater than 98.5% as determined by HPLC. Precoated Silica Gel G 60 plates (20×20 cm^2, 0.25 mm layer thickness) for TLC were purchased from Merck (Darmstadt, Germany). The phospholipid standards were obtained from a phospholipid kit

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(Serdary Research Lab, Ontario, Canada). FAME standards (mixture #3, AOCS) were purchased from Applied Science (State College, PA). Methyl pentadecanoate ($C_{15:0}$, 100 mg; Merck) was dissolved in a 20-mL glass volumetric flask with *n*-hexane and used as internal standard. Boron trifluoride (BF_3) in methanol (14.0%; Wako Pure Chemical Ind., Osaka, Japan) was used to prepare FAME (9).

Roasting of soybeans. AOAC methods were used to determine the chemical composition of the seeds before roasting (10). In accordance with previous methods (11), whole soybeans were arranged in a single layer in Pyrex petri dish (12.0 cm i.d.). Three dishes, containing 100 seeds each, were placed on the turnable plate in a commercial microwave oven (Model R-5550; Sharp, Osaka, Japan). The total weight of the 300 seeds thus treated was 96.5 ± 1.3 g. Microwave treatment of soybeans for 6 min was optimal to prepare full-fat soy flour without a burnt odor; this treatment time retained about 90% of the tocopherols (7). Therefore, the seeds in this study were microwaved for 6, 12, or 20 min at 2,450 MHz (high-power setting; capable of generating 0.5 kW power). As soon as they were removed from the oven after each treatment, the internal temperature of the treated seeds was determined as previously described (7). After microwave roasting, the soybeans were allowed to cool to ambient temperature prior to lipid extraction.

Seed sectioning and lipid extraction. After microwave roasting of soybeans, whole beans were first dissected into the three structural parts—seed coat, axis, and cotyledons—with a razor blade. The two cotyledons were then halved and designated as cotyledon I, the section closer to the axis areas, and cotyledon II, the section opposite the axis area. Each pair of cotyledons was separated medially, then transversely, to produce four sections. Samples (1000 beans) for each genotype were separated and sectioned. Procedures were carried out in duplicate. Each sample (cotyledon sections: 200, seed coats, and axes: 500, respectively) were crushed with a Maxim homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan) at high speed for 15 min (0°C under ice), and the lipids were further extracted at 0°C with 50–200 mL of chloroform/methanol (2:1, vol/vol) fortified with 0.01% BHT (12), which was added to inhibit the oxidative degradation of lipids during analysis. The homogenate was vaccum filtered through defatted filter papers on a Büchner funnel, and the filter residue rehomogenized with a second volume of chloroform/methanol. The filtrates were combined and dried in a rotary vacuum evaporator at 35°C. The residue was dissolved in 100 mL of chloroform/methanol (2:1, vol/vol), 20 mL of a 0.75% (wt/vol) aqueous potassium chloride solution was added (12), and the phases were vigorously mixed (13). After phase separation, the chloroform layer was withdrawn, dried under anhydrous sodium sulfate, and filtered, and the organic phase concentrated under vacuum. Finally, the chloroform was completely removed under a stream of nitrogen. The extracted lipids were weighed to determine the lipid content within each soybean section, carefully transferred to a 5- or 25-mL brown glass volumetric flask with chloroform/methanol (2:1, vol/vol) solutions, and stored under nitrogen in the dark at –25°C until analyzed. By using the same procedures, lipids were extracted from three structural parts of raw soybeans for use as controls.

Color measurement. As an index of color development (14), the absorbance at 420 nm of a 5.0% (wt/vol) solution of total lipids in chloroform was determined using a Shimadzu UV-2500 PC spectrophotometer (Shimadzu, Kyoto, Japan).

HPLC. A measured quantity (50 to 200 mg) of lipids was carefully transferred to a 2- or 5-mL brown volumetric flask, and the solvents were evaporated under nitrogen. The residue was dissolved with the mobile phase (for HPLC as described below). The chromatographic system was the same as previously described (15), and the mobile phase was a mixture of *n*-hexane/1,4-dioxane/ethanol (490:10:1, by vol) at 2.0 mL/min. An aliquot (5–10 μL) was injected with a fully loaded 20 μL loop. Each tocopherol was monitored with a fluorescence detector (Shimadzu RF-535). Levels of tocopherols were quantified using separate calibration curves for α-, β-, γ-, and δ-tocopherols. The other HPLC conditions were as reported previously (15).

Lipid class analysis. Total lipids were fractionated by TLC into the following two fractions: TAG and polar lipids (16). The crude lipid extracts were applied to the TLC plates as 7-cm bands (approximately 20 mg per plate) with a microsyringe (Hamilton Co., Reno, NV). The TLC standard mixture was applied as a reference on one side of each plate, and the plate was developed in a mixture of *n*-hexane/diethyl ether/acetic acid (80:30:1, by vol) as previously described (17).

A part of the polar lipid extracts was further separated by TLC into glycolipids and phospholipids with acetone/acetic acid/water (100:2:1, by vol) as the mobile phase. After separation on TLC, the plates were covered with another glass plate, leaving the reference zone exposed to be visualized by exposure to iodine vapor. Bands corresponding to TAG, polar lipids, and phospholipids were scraped separately into test tubes (10.5 cm × 16 mm) with Teflon-coated screw caps. Methyl pentadecanoate solution (50 or 100 μg) was added as internal standard to the total lipids and each fraction. The FAME prepared by transesterification (9) were analyzed by a Shimadzu Model GC-14A (gas chromatograph) and quantified with methyl pentadecanoate as previously described (13).

Samples of the extracted polar lipid, obtained as descibed above, were further separated by TLC into several fractions with chloroform/methanol/acetic acid/deionized water (170:30:20:7, by vol) as the mobile phase. Phospholipid classes were detected by iodine vapor and were consistent with the authentic standards. Bands corresponding to PE, PC, PI, and others were carefully scraped into test tubes, and methyl pentadecanoate (25 μg) was added as just described. FAME were prepared for the isolated lipids by heating the samples at 80°C for 2 h in BF_3/methanol as just described. The samples were cooled to 0°C in an ice bath, 5 mL of *n*-hexane was added, and the mixture was vigorously stirred for 15 s by vortex mixer. The organic layer with the FAME was separated and washed several times with deionized water, then dried under anhydrous sodium sulfate. The solvent was removed and the residue quantified as previously described (13). The detection limit was 0.05% of total

FA for each FA in a FA mixture, and the data were expressed as weight percentages of total FA.

Statistical analyses. All experiments were carried out in triplicate, and subjected to ANOVA for a completely random design as described by Steel *et al.* (18), to determine the least significant difference among means at the level of 0.05. These data were then compared applying Duncan's multiple range method (19).

RESULTS AND DISCUSSION

Proximate analyses showed the composition of seeds before roasting to be as follows: moisture 9.4 to 9.6%, fat 16.8 to 17.3%, and protein 37.8 to 38.3%. There were no significant differences ($P > 0.05$) in these contents among the three cultivars.

Structural parts and sections of soybeans. Each value is an average of three determinations and expressed as an individual weight percentage per 200 beans. Each cotyledon constituted the predominant fraction at 45.9 to 46.3% of the seed mass, whereas the coat was a minor fraction at 5.6 to 6.7%, and the axis was a very small part at 1.6 to 2.0%. No significant differences ($P > 0.05$) in these percentages were observed, not only among the three cultivars, but also at each time after microwave roasting (data not shown).

Tocopherol distributions within each structural part of soybeans during microwave roasting. The temperature of the whole seed samples was 25°C before roasting and increased from 80.2 to 100.5 to 123.0°C, at 6, 12, or 20 min after microwave roasting, respectively. The color of the lipids extracted from each tissue changed gradually from yellow-brown at 6 min of roasting to brown at 12 min of roasting, and finally deep-brown at 20 min of roasting, in agreement with previous results (14).

The effects of microwave roasting on the total tocopherol contents were compared within each structural part and section of soybeans (Table 1). The amount of total tocopherols before microwave roasting varied significantly ($P < 0.05$), not only within each structural part and section but also between soybean cultivars. The tocopherol contents were highest in the axis, followed by the cotyledons, with very little in the coat, and these contents decreased in the following order: Mikawajima > Fuki and Shishiou, with a few exceptions in cotyledon I. As microwave roasting proceeded, the tocopherol concentration gradually decreased at a similar rate within each structural part and section in the three cultivars. The loss in cotyledon I was 8.0 to 10.9 mg at 12 min of roasting; and 15.5 to 19.4 mg at 20 min of roasting; there were no significant differences ($P > 0.05$) between cotyledons I and II among the three cultivars (data not shown). However, the losses in the axis and the coat were 8.4 to 20.6 mg at 12 min of roasting and 14.3 to 39.1 mg at 20 min of roasting, respectively. That is, the rate of tocopherol loss was more pronounced ($P < 0.05$) in the coat than in the cotyledons or the axis.

Figure 1 shows the changing patterns in the amount of the individual tocopherols within each structural part and section in soybeans of the three cultivars during microwave roasting. The types and amounts of individual tocopherols before microwave roasting depended on the cultivars and/or tissues. The possibility that sample variations, year, and place of seed production contributed to these differences cannot be excluded (20). With a few exceptions in the axis and/or the coat for Mikawajima, the predominant components in the cotyledons were γ- and δ-tocopherols, with much smaller amounts of α- and β-tocopherols in the four tissues before and during microwave roasting (Fig. 1). A similar pattern for the content of each tocopherol was observed between Fuki and Shishiou in all tissues. The changing patterns of tocopherol homologs in the three tissues for Shishiou were essentially the same as those found in Fuki. However, the distribution patterns of tocopherol homologs for Mikawajima differed significantly ($P < 0.05$) from those for the other cultivars. That is α-tocopherol was present at a higher concentration in Mikawajima than in the other two cultivars, particularly in the axis and the coat. The data for cotyledon I and axis at 6 min of roasting were omitted from Figure 1 because no significant differences ($P > 0.05$) were observed as compared to those for raw seeds.

TABLE 1
Total Tocopherol Contents in the Oils Prepared from Each Structural Part and Section of Soybeans Roasted in a Microwave Oven[a]

Cultivar	Roasting time (min)	Tissue		
		Cotyledon I	Axis	Coat
Shishiou	Unroasted	128.2^{e,f}	153.0^{g}	24.1^{h}
	6	125.0^{f}	148.4^{g,h}	20.3^{i}
	12	117.3^{g}	134.1^{i}	15.7^{k}
	20	108.8^{h}	115.7^{j}	8.2^{l}
Fuki	Unroasted	160.3^{b}	182.5^{e}	32.8^{e}
	6	157.0^{b,c}	177.5^{e}	29.2^{f}
	12	151.0^{c}	162.7^{f}	24.4^{h}
	20	143.8^{c,d}	143.4^{h}	18.5^{j}
Mikawajima	Unroasted	142.3^{d}	245.9^{b}	50.3^{b}
	6	139.9^{d,e}	239.1^{b}	46.0^{c}
	12	134.3^{e}	225.3^{c}	37.5^{d}
	20	126.8^{f}	207.1^{d}	27.3^{g}

[a]Each value is the average of three determinations and is expressed as mg per 100 g oil.
[b–l]Values in the same column with different indices are significantly different from those values in raw beans ($P < 0.05$).

With increased roasting times, as much as 25% of the individual tocopherols originally present in the coat was lost at 12 min of roasting. Therefore, the loss in the tocopherols for Fuki and Mikawajima was somewhat less than for Shishiou. On the other hand, the amounts of tocopherols still remaining were >75% of the original level in cotyledon I and the axis after 20 min of roasting (Table 1). All data for cotyledon II before and after microwave roasting were omitted from Tables 1–3 and Figures 1–3 because they were essentially the same as those of cotyledon I among the three cultivars.

Lipid components within each structural part of soybeans during microwave roasting. Comparisons were made of the effects of microwave roasting on the amounts of total lipids, TAG, and phospholipids within each structural part and section of soybeans (Table 2). Longer microwave processing resulted in (i) a

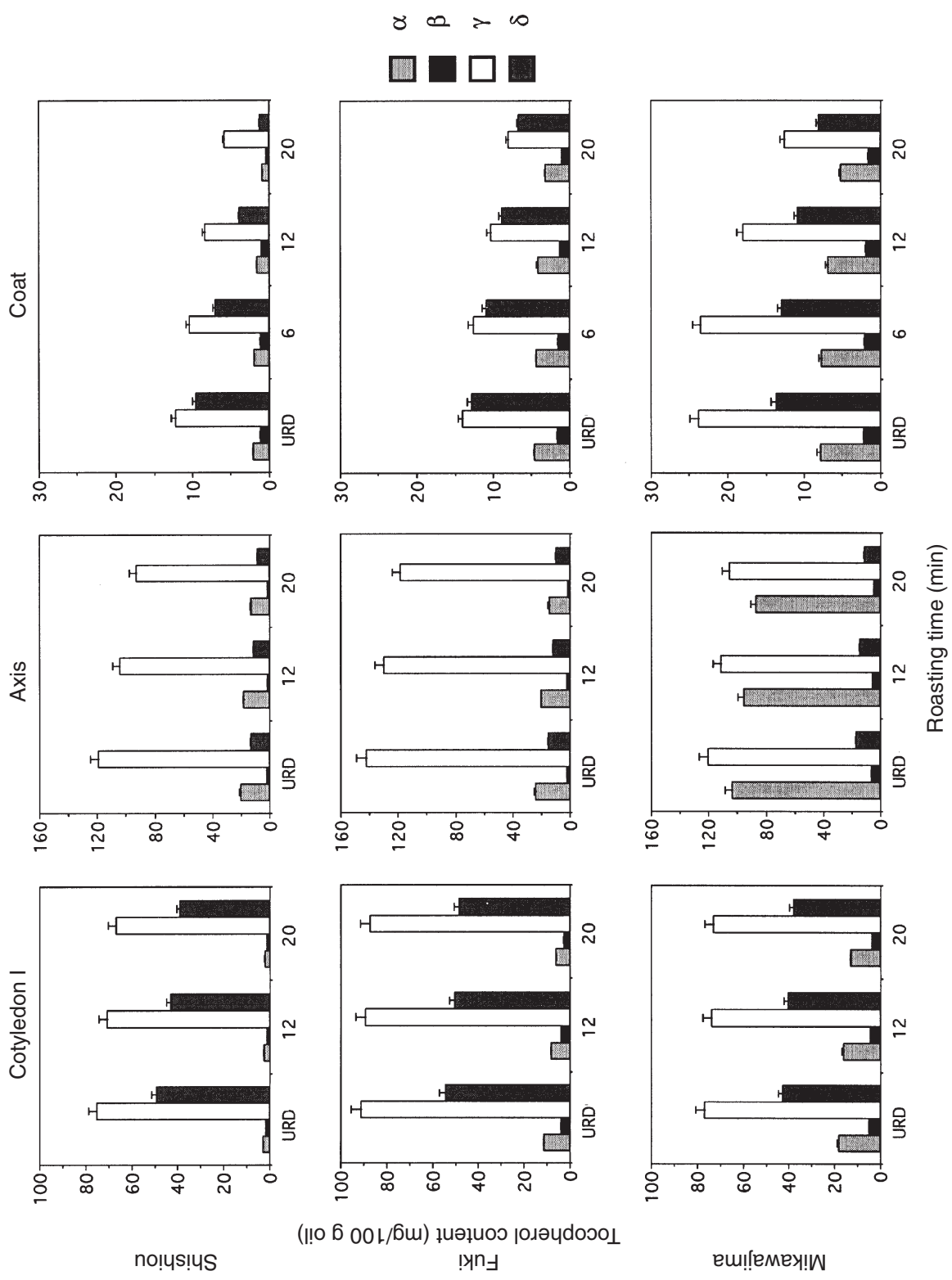

FIG. 1. Changes in distribution of individual tocopherols in the oils prepared from each structural part and section of soybeans roasted in a microwave oven. Each value represents the average of three replicates, and vertical bars represent the SD of the mean value. URD, raw.

TABLE 2
Lipid Contents in the Oils Prepared from Each Structural Part and Sections Roasted in a Microwave Oven[a]

Tissue	Cultivar	Roasting time (min)	Total lipids (mg)	TAG (mg)	TAG (%)	Phospholipids (mg)	Phospholipids (%)
	Shishiou	Unroasted	7448.9^{c,d}	6747.0^{b,c}	(90.6)	512.8^{c}	(6.9)
		6	7527.2^{c,d}	6675.9^{b,c}	(88.6)	478.1^{d}	(6.4)
		12	7631.2^{c,d}	6359.9^{c,d}	(83.3)	443.2^{e}	(5.8)
		20	7766.8^{c,d}	5960.2^{d}	(76.8)	352.3^{h}	(4.5)
Cotyledon I	Fuki	Unroasted	7616.3^{c,d}	6928.4^{b}	(91.0)	541.2^{b}	(7.1)
		6	7696.7^{c,d}	6866.2^{b}	(89.3)	511.5^{c}	(6.6)
		12	7823.1^{d}	6524.3^{c}	(83.4)	446.1^{e}	(5.7)
		20	7991.2^{d}	6125.5^{d}	(76.7)	370.9^{g}	(4.6)
	Mikawajima	Unroasted	6120.3^{b}	5526.7^{e}	(90.3)	439.8^{e}	(7.2)
		6	6188.6^{b}	5484.3^{e}	(88.6)	405.4^{f}	(6.6)
		12	6283.6^{b}	5214.4^{f}	(82.9)	374.5^{g}	(6.0)
		20	6402.5^{b}	4868.1^{g}	(76.0)	325.5^{i}	(5.1)
	Shishiou	Unroasted	452.8^{b}	320.5^{b}	(70.7)	120.7^{b}	(26.7)
		6	426.7^{c}	308.7^{b}	(72.4)	108.4^{c}	(25.4)
		12	400.3^{d}	286.7^{c}	(71.6)	94.8^{d}	(23.7)
		20	369.5^{e}	262.3^{d}	(71.0)	76.3^{g}	(20.6)
Axis	Fuki	Unroasted	371.8^{e}	268.3^{d}	(72.2)	92.9^{d}	(25.0)
		6	345.5^{f}	250.0^{e}	(72.3)	81.4^{f}	(23.6)
		12	320.6^{g}	230.1^{f}	(71.7)	69.5^{h}	(21.7)
		20	293.2^{h}	207.7^{g}	(70.9)	59.3^{i}	(20.2)
	Mikawajima	Unroasted	449.9^{b}	322.4^{b}	(71.7)	115.2^{b}	(25.6)
		6	424.1^{c}	308.1^{b}	(72.6)	103.7^{c}	(24.5)
		12	398.0^{d}	279.7^{c}	(70.3)	89.2^{e}	(22.4)
		20	362.5^{e}	258.1^{d}	(71.2)	71.8^{h}	(19.8)
	Shishiou	Unroasted	312.5^{b}	221.3^{b}	(70.9)	54.2^{b}	(17.3)
		6	289.0^{c}	194.7^{c}	(67.4)	47.1^{c}	(16.3)
		12	260.7^{d}	155.8^{e}	(59.7)	39.8^{e}	(15.3)
		20	230.3^{e}	113.3^{h}	(49.2)	28.6^{g}	(12.4)
Coat	Fuki	Unroasted	239.6^{e}	168.0^{d}	(70.1)	42.4^{d}	(17.7)
		6	217.8^{f}	147.5^{f}	(67.8)	36.9^{e}	(16.9)
		12	190.7^{g}	117.7^{h}	(61.7)	31.4^{f}	(16.5)
		20	160.5^{h}	88.9^{j}	(55.4)	24.4^{i}	(15.2)
	Mikawajima	Unroasted	212.5^{f}	152.5^{e}	(71.8)	36.6^{e}	(17.2)
		6	193.5^{g}	127.3^{g}	(65.8)	32.6^{f}	(16.8)
		12	169.0^{h}	102.6^{i}	(60.7)	26.8^{h}	(15.9)
		20	139.7^{i}	70.7^{k}	(50.6)	20.1^{j}	(14.4)

[a]Each value is the average of three determinations and expressed as mg per 200 beans for cotyledon or 500 beans for others.
[b–j]Values in the same column with different indices are significantly different from those values in raw beans ($P < 0.05$).

greater amount of total lipids being extracted from the cotyledons but (ii) a smaller amount of those being extracted from the axis or the coat. These results may reflect differences in the amounts of chemical components, such as protein or sugars, within each tissue of soybeans during microwave roasting. Collins and Beaty (21) indicated that heat caused some protein denaturation, possibly leading to improved lipid extractability.

The major components in all cultivars were TAG, still representing 70% in the axis and the coat, and 90% in cotyledon I before microwave roasting (Table 2). Before microwave roasting, the greatest percentage of phospholipids was observed for the axis (25.0 to 26.7%), followed by the coat (17.3 to 17.7%) and the cotyledon I (6.9 to 7.2%). In an oilseed plant such as the soybean, there are two different types of lipids: membrane lipids and storage lipids. Storage lipids are mainly TAG, and they are high in quantity and localized in oil bodies of the cotyledon tissues.

Membrane lipids, are composed mainly of phospholipids, and they are low in quantity and located in all cells of living tissues (22). As microwave roasting proceeded, the rate of phospholipid losses became more pronounced ($P < 0.05$) than did that of TAG in each tissue of the three cultivars, with a few exceptions of the coat. The greatest percentage of loss was observed for the coat (42.5–47.2%), followed by the axis (36.2–37.7%) and cotyledon I (26.0–31.5%). TAG was still retained at more than 88% in the cotyledon and 77% in the axis, after roasting for 20 min, whereas that in the coat decreased further to less than 53% of the original value. These results suggested that microwave energy caused more significant ($P < 0.05$) effects on the acyl lipids in the coat than those in the cotyledons or the axis.

TABLE 3
Phospholipid Contents in the Oils Prepared from Each Structural Part and Section of Soybeans Roasted in a Microwave Oven[a]

Tissue	Cultivar	Roasting time (min)	PE (mg)	PC (mg)	PI (mg)	Others[b] (mg)
	Shishiou	Unroasted	124.6^{d}	255.4^{c,d}	89.2^{d}	43.6^{d}
		6	102.8^{f}	239.8^{d}	86.7^{e}	48.8^{e}
		12	66.2^{i}	218.6^{e}	85.3^{e,f}	73.1^{h}
		20	15.3^{e}	170.4^{g}	82.0^{f}	84.6^{j}
Cotyledon I	Fuki	Unroasted	138.5^{c}	264.1^{c}	92.1^{d}	46.5^{e}
		6	115.4^{e}	248.9^{d}	92.0^{d}	55.2^{f}
		12	67.8^{g}	213.3^{e}	90.1^{d}	74.9^{i}
		20	18.9^{k}	175.2^{g}	85.6^{e,f}	91.2^{k}
	Mikawajima	Unroasted	90.2^{g}	214.2^{e}	97.6^{c}	37.8^{c}
		6	72.5^{h}	195.6^{f}	94.7^{c}	42.6^{d}
		12	43.8^{j}	179.8^{g}	90.2^{d}	60.7^{g}
		20	9.2^{m}	158.3^{h}	87.6^{d}	70.4^{h}
	Shishiou	Unroasted	27.5^{c}	60.4^{c}	19.1^{c}	13.7^{e}
		6	21.8^{d}	54.2^{d}	16.8^{d}	15.6^{f}
		12	15.5^{f}	47.5^{f}	14.2^{f}	17.6^{g}
		20	2.0^{i}	40.5^{g}	12.2^{g}	21.6^{h}
Axis	Fuki	Unroasted	21.9^{d}	46.2^{f}	15.0^{e}	9.8^{c}
		6	18.5^{e}	38.6^{h}	14.0^{f}	10.3^{c}
		12	12.3^{g}	31.3^{j}	13.8^{f}	12.1^{d}
		20	1.6^{j}	27.7^{k}	12.5^{g}	17.5^{g}
	Mikawajima	Unroasted	29.1^{c}	53.0^{d}	18.9^{e}	14.2^{e}
		6	22.1^{d}	50.6^{d}	16.2^{d}	14.8^{f}
		12	16.3^{f}	41.2^{g}	15.0^{e}	16.7^{g}
		20	2.6^{h}	34.3^{i}	13.6^{f}	21.3^{h}
	Shishiou	Unroasted	12.2^{c}	25.3^{c}	10.4^{c}	6.3^{d}
		6	9.0^{d}	24.0^{c}	8.0^{d}	6.1^{d}
		12	5.2^{g}	20.3^{d}	7.3^{e}	7.0^{e}
		20	ND	14.0^{g}	7.0^{f}	7.6^{f}
Coat	Fuki	Unroasted	12.3^{c}	18.6^{e}	6.0^{g}	5.5^{c}
		6	9.8^{d}	15.9^{f}	5.2^{h}	6.0^{d}
		12	5.1^{g}	14.5^{g}	4.8^{i}	7.0^{e}
		20	ND	10.2^{i}	4.4^{j}	9.8^{h}
	Mikawajima	Unroasted	6.7^{f}	16.2^{f}	5.3^{h}	8.4^{g}
		6	4.8^{h}	13.3^{h}	5.0^{h}	9.5^{h}
		12	2.0^{i}	9.3^{j}	4.8^{i}	10.7^{i}
		20	ND	3.3^{k}	4.5^{j}	12.3^{j}

[a]Each value is the average of three determinations and experessed as mg per 200 beans for cotyledon or 500 beans for others.
[b]Contains diphosphatidylglycerol, PA, and phosphatidylglycerol.
[c–m]Values in the same column with different indices are significantly different from those values in raw beans ($P < 0.05$).
ND, not detected.

FA composition and distribution in phospholipids during microwave roasting. Figure 2 shows a typical FA distribution of phospholipids within each structural part of soybeans during microwave roasting. Linoleic (39.0 to 62.0%), palmitic (18.2 to 29.1%), linolenic (5.2 to 18.0%), oleic (3.5 to 14.5%), and stearic (2.3 to 6.2%) acids were the principal FA in each tissue of the three cultivars. These trends were the same as those of TAG, as previously described (23). In general, with increased roasting times, the changing patterns of FA composition became more pronounced ($P < 0.05$) in the coat, followed by the cotyledon I and then the axis. FA compositions of TAG were omitted from this study because they were already described in a previous report (23). To determine the FA composition of individual phospholipids within each structural part of soybeans, major phospholipids were isolated by TLC and then identified by comigration with authentic standards. Table 3 represents the profiles of PE, PC, PI, and others within each structural part of soybeans. (Diphosphatidylglycerol, PA, and phosphatidylglycerol are shown as "others" in Table 3).

Regardless of the soybean cultivar, PC, PE, and PI were the major phospholipids in all tissues, and the highest concentration was observed for PC (43.9 to 50.0%), followed by PE (18.3 to 29.0%) and PI (14.2 to 22.2%). Longer microwave processing produced the greatest phospholipid losses ($P < 0.05$) in PE, followed by PC and PI. The reduction in phospholipids after microwave treatment may be due to the decomposition of phospholipids and/or formation of a complex with protein or carbohydrate, which may prevent solvent

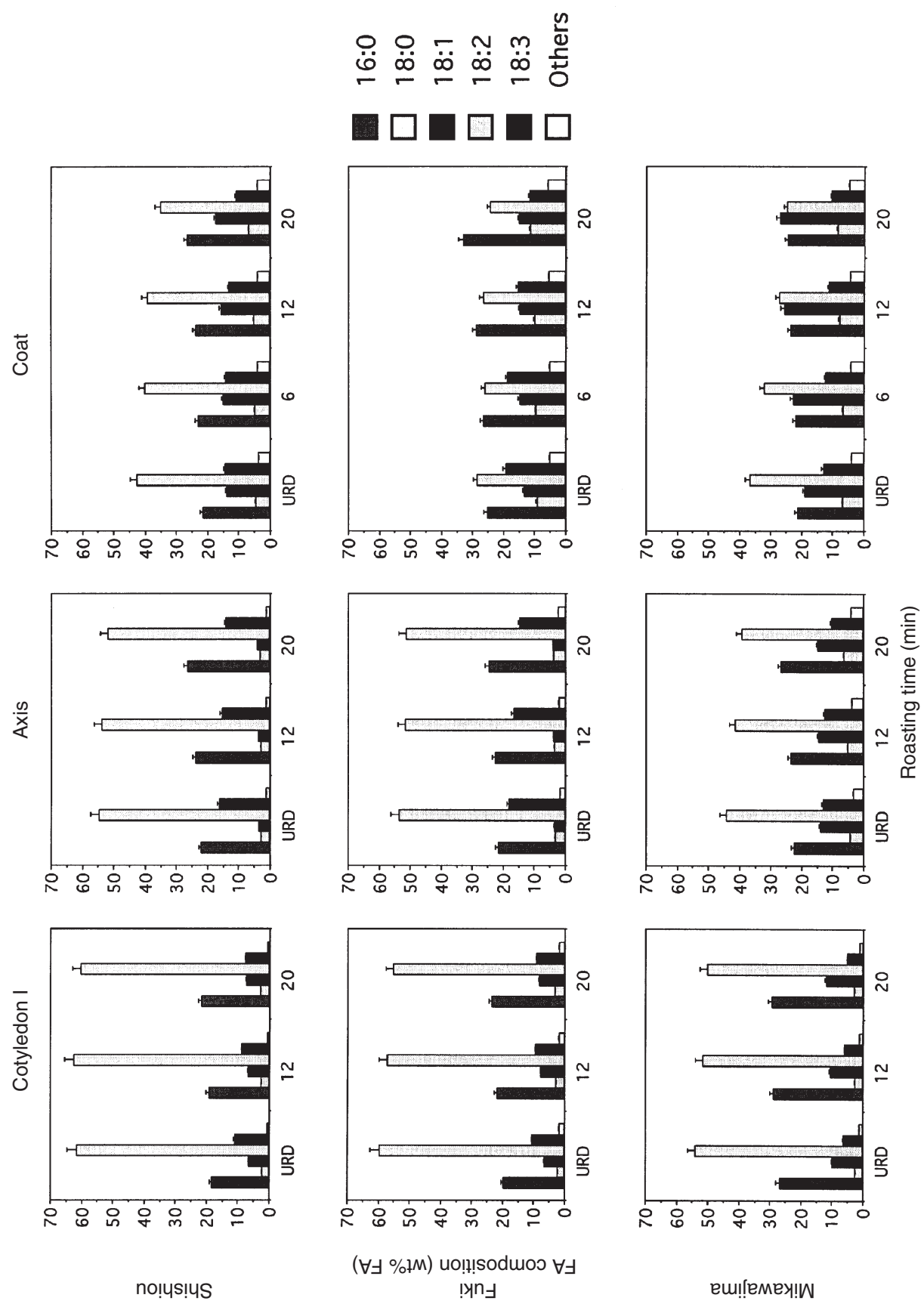

FIG. 2. FA composition of phospholipids in the oils prepared from each structural part and section of soybeans roasted in a microwave oven. Each value represents the average of three replicates, and vertical bars represent the SD of the mean value. "Others" contained 16:1, 16:2, 20:0, and 22:0. For abbreviation see Figure 1.

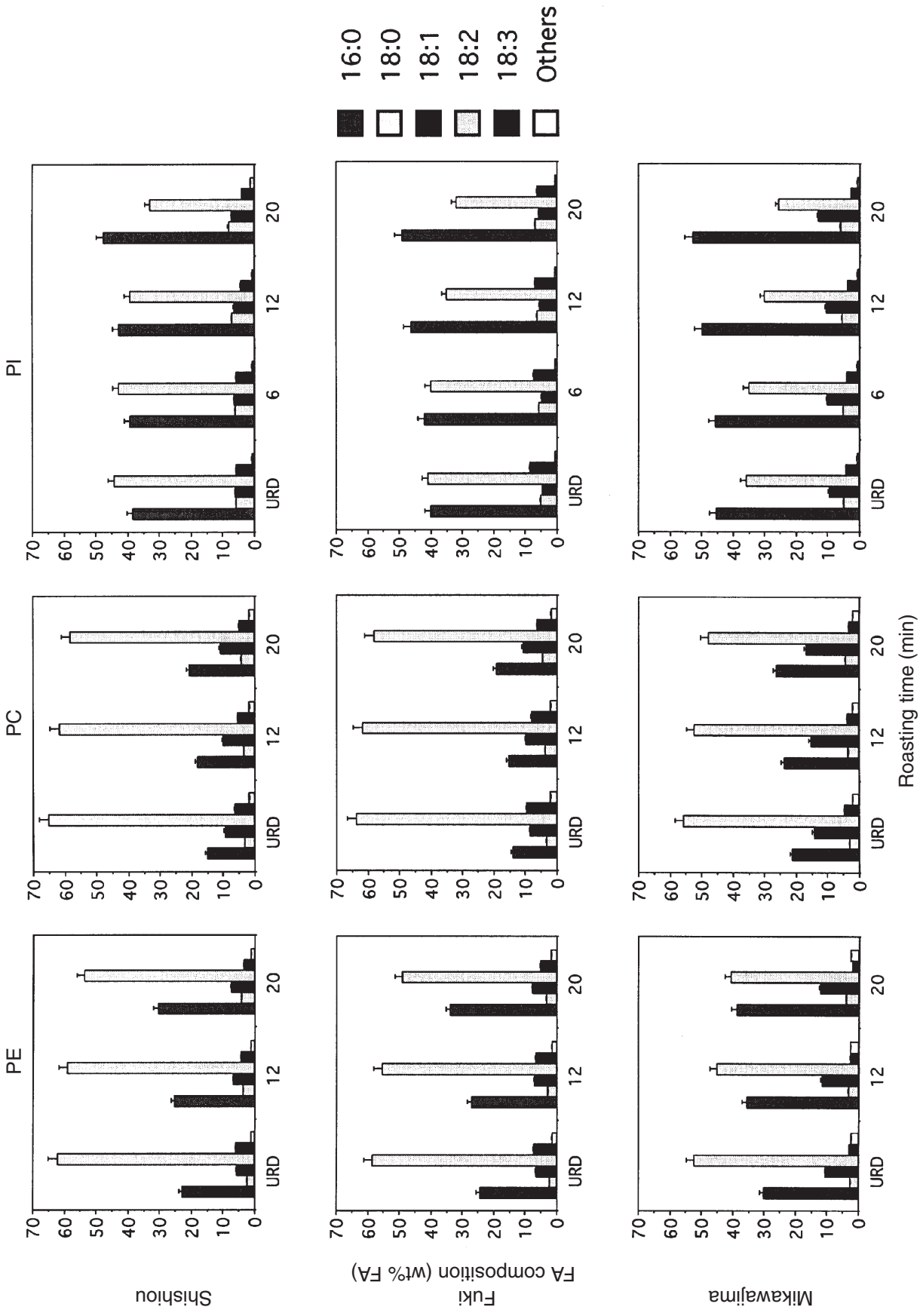

FIG. 3. FA distributions of major phospholipids of the oils prepared from cotyledon I of soybeans roasted in a microwave oven. Each value represents the average of three replicates, and vertical bars represent the SD of the mean value. "Others" contained 16:1, 16:2, 20:0, and 22:0. For abbreviation see Figure 1.

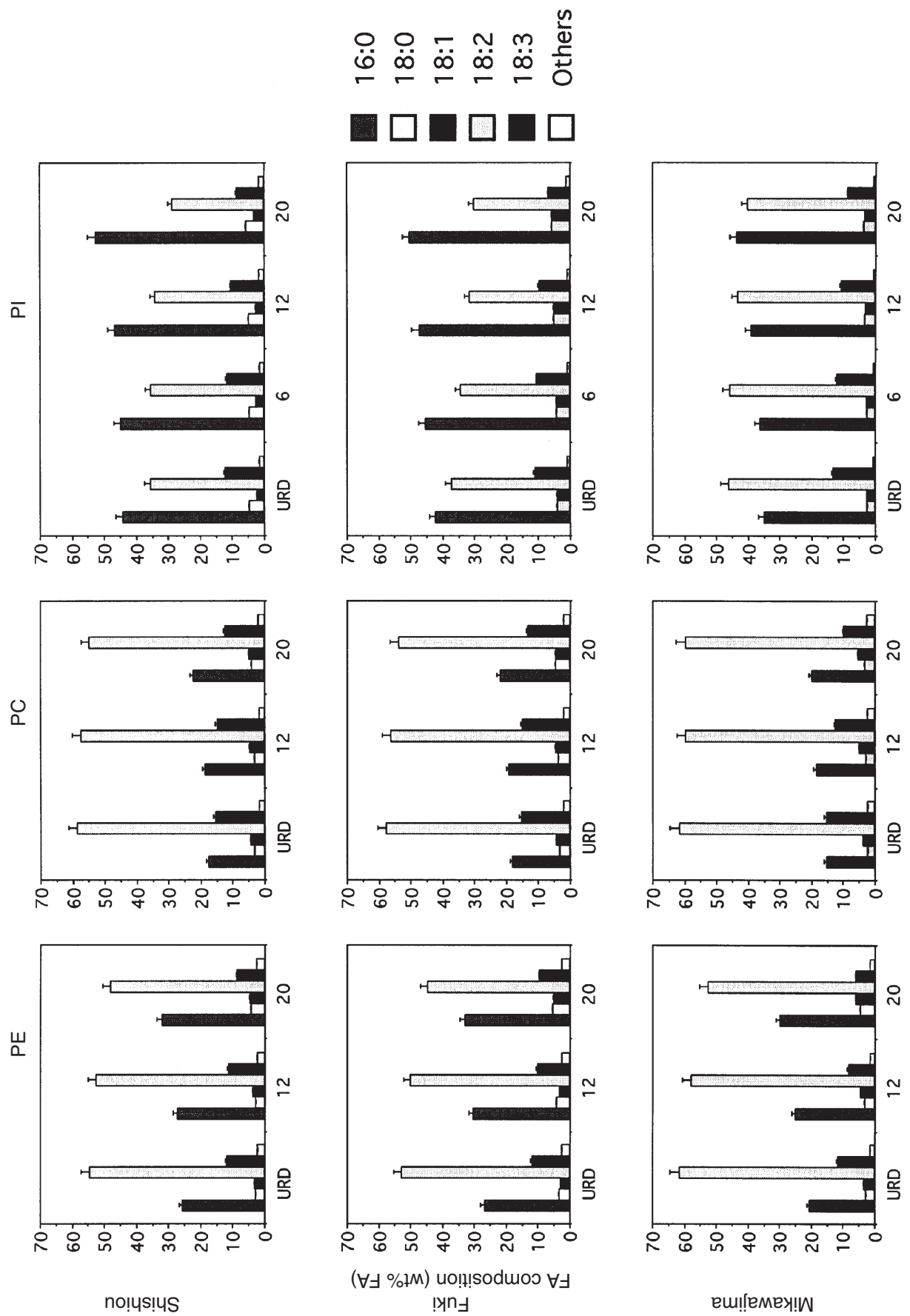

FIG. 4. FA distributions of major phospholipids of the oils prepared from axis of soybeans roasted in a microwave oven. Each value represents the average of three replicates, and vertical bars represent the SD of the mean value. "Others" contained 16:1, 16:2, 20:0, and 22:0. For abbreviation see Figure 1.

extraction. Simpson and Nakamura (24) reported that, at the molecular level, intensive membrane degradation occurs in lipid bodies isolated from soybeans.

FA distribution of the individual phospholipids. Figure 3 depicts a typical FA distribution of individual phospholipids in cotyledon I. In all soybean cultivars, the FA composition of PI differed from those of other phospholipids. The percentage of palmitic acid was higher, and that of linoleic acid was lower. In general, with longer microwave roasting, decreases ($P < 0.05$) occurred in percentages of PUFA and increases occurred in percentages of saturated FA, compared to the values for raw soybeans. These trends became more pronounced ($P < 0.05$) in PE, followed by PI then PC. The results suggested that the free amino group of PE could contribute to the formation of browning substances. In the Maillard reaction (25), phospholipids can be particularly reactive if they contain both PUFA and amines. The trends became more pronounced in the axis (Fig. 4) than in the cotyledon I (Fig. 3). The percentage of TAG was lower ($P < 0.05$) in the axis than in the cotyledon I, and the value was compensated by an increase ($P < 0.05$) in phospholipids (Table 2). Therefore, the data for FA distributions of three phospholipids in the coat were omitted from this study because the samples were too small to obtain reliable results. The data for cotyledon I and the axis at 6 min of roasting were deleted from Figures 2, 3, and 4 because they were essentially the same as those for raw seeds. These results indicate that microwaves created more significant differences ($P < 0.05$) in the distribution of tocopherols and phospholipids in the coat than in the axis or the cotyledons. Thus, the tocopherols and phospholipids change with microwave roasting, according to tissue.

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[Received August 12, 2002; accepted April 9, 2003]