Microwave Roasting and Phospholipids in Soybeans (*Glycine max.* L.) at Different Moisture Contents

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ABSTRACT: The effects of microwave roasting on phospholipids in soybeans were investigated in relation to moisture. Whole soybeans at different moistures (9.6, 38.2, and 51.9%) were roasted by exposure to microwaves at a frequency of 2,450 MHz. During microwave treatments, the lower the moisture content, the higher was the internal temperature in soybeans at the end of microwave roasting. Total lipids were extracted from the beans after microwave treatment, and the phospholipids were separated with thin-layer chromatography. Phosphatidylcholine was the principal phospholipid in the extracted lipids from all unroasted and roasted bean samples. After microwave roasting, phospholipids containing an amino group, especially phosphatidylethanolamine, decreased substantially ($P < 0.05$) in lower-moisture soybeans. However, increasing the moisture content depressed a rise in the internal temperature of soybeans and prevented a reduction in phospholipids and/or polyunsaturated fatty acids in the phospholipids. Based on the changes in the composition and fatty acid distribution of phospholipids in soybeans during microwave roasting, it is necessary to consider the moisture content in soybeans when roasting in a microwave oven.

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KEY WORDS: Fatty acid distributions, microwave roasting, moisture, phosphatidylcholine, phosphatidylethanolamine, phospholipids, soybeans.

Soybean seeds are the world's single most important source of edible oils and vegetable proteins, but they contain various antinutritional factors, such as a trypsin inhibitor. Therefore, various heating methods, including dry roasting (1), boiling (2) and microwave heating (3), have been used to improve the nutritional value of soybeans. Careful control of processing conditions is essential to prevent damage to protein functional and nutritional values.

Microwave energy is an important means of heating, which is readily commercially available, and is forecast to be utilized much more extensively in the future (4). In fact, microwaves are used in the food industry not only for thawing, drying and baking, but also for other applications, such as pasteurization and sterilization of many types of foods (5,6). Moreover, short-time microwave heating of peanuts yielded a 95% reduction of the aflatoxin content without measurable changes in the protein and lipid concentrations (7). Advantages associated with microwave energy are depth of penetration and rapid rate of heating, both of which are functions of frequency and dielectric properties of the system. Microwave heating is considered to be the interaction of polar molecules with the electric component of the electromagnetic field, heat being generated by friction as the molecules attempt to orient themselves within the oscillating field. In general, it has been assumed that microwave action in terms of microbial destruction or enzyme inactivation is due to thermal effects (8–10), although there is some evidence for "nonthermal" effects. We (11) suggested that microwave treatment is more effective for inactivating trypsin inhibitor in soybeans with higher moisture contents. Therefore, soybeans have generally been moistened before treatment in the belief that the presence of excess moisture was necessary for nutritional improvement by microwave treatment. Water is the most abundant dipole component in foods, while others (salt, fats, and proteins) also act as dielectric components (4,12). Microwave ovens are credited with rapid heating rates and high efficiency, especially because of their high penetration power (13). Phospholipids in soybeans are the major constituents of cell membranes, and they have a high degree of unsaturation. However, little has been reported on how microwave energy affects phospholipids in soybeans at different moisture contents and their individual fatty acid distributions.

The present study examines the composition and fatty acid distribution of phospholipids in soybean seeds at different moisture levels when roasted in a microwave oven.

MATERIALS AND METHODS

Samples and chemicals. Samples of soybeans (*Glycine max.* L.) were of three cultivars: Tsurunoko, Okuhara and Mikawajima. The soybeans were all grown in Japan in the summer of 1995. These soybean cultivars were purchased from Takii Seed Co. (Kyoto, Japan) and were selected for uniformity based on bean weight (i.e., between 300–315 mg for Mikawajima, 320–336 mg for Okuhara and 350–367 mg for Tsurunoko, respectively). Soaking was done to prepare beans

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with different moisture contents immediately before microwave roasting, as previously described (11). The beans were placed on a filter paper (Toyo #2) to dry at ambient temperature for 20 min. The moisture content in the beans was determined in duplicate (14). All chemicals were of analytical grade (Nacalai Tesque, Kyoto, Japan) and were used without further purification. Thin-layer chromatography (TLC) precoated Silica-Gel G 60 plates (20 × 20 cm, 0.25 mm layer thickness) were purchased from E. Merck (Darmstadt, Germany). The phospholipid standards were from a phospholipid kit (Serdary Research Laboratories, Ontario, Canada). Fatty acid methyl ester standards (F & OR mixture #3) were purchased from Applied Science (State College, PA). One-hundred mg of methyl pentadecanoate (E. Merck) was dissolved in *n*-hexane and stored in a 20-mL glass volumetric flask until required as an internal standard. BF_3 (14%) in CH_3OH (Wako Pure Chemical Ind., Ltd., Osaka, Japan) was used to prepare the fatty acid methyl esters.

Microwave roasting. A modified domestic-size microwave oven (Sharp Model R-5, 550; Osaka, Japan), capable of generating 0.5 kW power at 2,450 MHz, was used. Whole soybeans with different moisture contents were placed in a single layer in a Pyrex Petri dish (12.0 cm diameter) and then roasted separately by microwaves for 2.0, 4.0, 6.0, 8.0, 12.0, 16.0 or 20.0 min after covering the dish. The internal temperature of beans immediately after each treatment were determined with a chromel-alumel thermocouple as previously described (11). Each dish contained *ca.* 60 g (185 seeds) of soybeans; dishes were separately prepared at each of the different exposures to provide sample material for analyses and testing. After microwave roasting, the soybeans were allowed to cool to ambient temperature before lipid extraction.

Lipid extraction. The soybeans (100 seeds) treated by microwaves were ground with 200 mL of chloroform/methanol (1:1, vol/vol) in an electric blender at 0°C in a blender jar placed in an ice bath. The lipids were further extracted three times with 150 mL of chloroform/methanol (2:1, vol/vol) in the blender. The extraction solvent contained butylated hydroxytoluene (0.01%) to inhibit lipid oxidation during extraction. The mixture was filtered through lipid-free filter paper, and solvent was removed from the filtrate by reduced pressure at 35°C with a rotary evaporator. The extract was redissolved in chloroform (125 mL) and shaken with saturated aqueous solution of sodium chloride (20 mL). The chloroform layer was removed, and the aqueous salt phase was re-extracted twice with 20 mL chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate under reduced pressure at 35°C. The pressure was further reduced to 500 mm Hg at 35°C for 30 min, and the oily extract was flushed with nitrogen to remove residual chloroform. Extracted lipids were redissolved in chloroform/methanol (2:1, vol/vol) and stored in a 25-mL brown glass volumetric flask under nitrogen in the dark at −25°C until required for further analysis. Lipids were also extracted from raw beans by this method for a control.

Lipid analysis. As an index of color (15), the absorbance at 420 nm of a 5.0% wt/vol solution of total lipids in chloroform was measured with a Shimadzu spectrophotometer UV 2500PC (Kyoto, Japan). Total lipids were fractionated by TLC into the following two fractions: triacylglycerols (TAG) and polar lipids. Crude lipid extracts were applied to TLC plates as 7-cm bands (*ca.* 20 mg/plate) with a micro syringe (Hamilton Co., Reno, NV). TLC standard mixture was applied as a reference on one side of each plate, and the plates were developed in *n*-hexane/diethyl ether/acetic acid (60:40:1, vol/vol/vol) as previously described (16). The plates were covered with another glass plate, leaving the reference exposed for visualization by exposure to iodine vapor. Bands corresponding to TAG and polar lipids were scraped into test tubes (10.5 cm × 16 mm) with a Teflon-coated screw-cap, respectively, and methyl pentadecanoate (50 or 100 μg) was added as an internal standard to the total lipids and to each fraction. Fatty acid methyl esters, prepared by transesterification (17), were analyzed by gas–liquid chromatography (GLC) as described previously (18). After recording on a Shimadzu C-R4A integrating system, component peaks were identified by comparing retention times with those of standards (F & OR mixtures #3), and were quantitated with methyl pentadecanoate as an internal standard. Peak areas were computed, and percentages of fatty acid methyl esters were obtained as weight percentage by direct internal normalization as described earlier (16).

Chromatographic separation of phospholipids. Part of the polar lipid extracts, obtained as described above, was further separated by TLC into the following five phospholipid fractions: phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI). The polar lipid extracts were applied on precoated Silica Gel G 60 plates as 12-cm bands (*ca.* 15 mg per plate) with a micro syringe. The TLC phospholipid standards were applied as reference on one side of each plate, and the plates were developed in acetone/acetic acid/water (100:2:1, vol/vol/vol) as the first solvent for removal of glycolipids from the phospholipids. The solvent front was allowed to run for 18 cm, and the plate was nitrogen-dried in the hood for 10 min before rechromatography. The chromatogram was developed in the same direction with chloroform/methanol/acetic acid/water (170:30:20:7, by vol) as the second solvent until the front had run for 18 cm. After development, each band was located by exposure to iodine vapor, and phospholipid classes were identified not only by comparison with R_f-values of standard phospholipids similarly chromatographed, but also by the specific spray reagents: Dragendorff reagent for choline lipids (19), 0.25% ninhydrine in acetone for amino-containing lipids, and molybdate reagent for phospholipids (20). Each band, corresponding to PA, PE, PC, PS, and PI, respectively, was quantitatively scraped into test tubes (10.5 cm × 16 mm) capped with a Teflon-coated screw cap, and methyl pentadecanoate (25 μg) was added to each tube as before. Fatty acid methyl esters, prepared by transesterification (17), were analyzed by GLC according to the method described above.

Statistical analysis of experimental data. Each reported value is the mean of three determinations, and the data were subjected to one-way analysis of variance with a randomized complete block design to partition the effects of different parameters (21). Significant differences among treatment means were separated by using Duncan's multiple range test, at a level of $P < 0.05$ (22).

RESULTS AND DISCUSSION

Internal temperature of soybeans. The moisture contents of soybeans were 9.6% in unsoaked beans, 38.2% after 1 h soaking, and 51.9% after 5 h soaking, respectively. Effects of microwave roasting (*cv.* Okuhara) were compared on the internal temperature of whole soybeans at different moisture contents (Fig. 1). A linear relationship occurred between the exposure time and internal temperature in the first 4 min of roasting. Thereafter, the temperature of soybeans of lower moisture content (9.6%) increased more rapidly than that for the other two experimental groups. However, an increase in soaking time gradually decreased the final temperature of soybeans after sufficient roasting: namely, the final temperature of soybeans soaked for 5 h was below 110°C, whereas the final temperature of samples not soaked was more than 160°C. The higher the moisture content of soybeans (38.2 or 51.9%), the lower the internal temperature of soybeans over the same roasting period after 4-min exposure time. A dark brownish color and burnt odor occurred after 12 min of heating in soybeans that were not soaked, but was only slight after 20 min in soybeans of 38.2 and 51.9% moisture. The results were also supported by an increase of the absorbance at 420 nm (*cv*. Tsurunoko) shown in Figure 2. However, there were no significant differences ($P > 0.05$) in the temperature and color of soybeans during microwave roasting among the three soybean cultivars (data not shown).

Lipid component and fatty acid composition. The dominant components were TAG, with much smaller amounts of polar lipids and other compounds (data not shown). Longer soaking and microwave processing resulted in greater amounts of total lipids extracted, as previously described (23). This also reflected an increase of the polar lipid fraction due to browning substances formed during microwave roasting (23). Therefore, increasing the bean moisture content markedly inhibited the formation of browning substances. TAG was the predominant component (76–88%) extracted in all three cultivars at all different moisture contents before and after microwave roasting. Profiles of the fatty acid composition of total lipids and polar lipids in unsoaked soybean (9.6%) cultivars before and after microwave roasting were compared (data not shown). The fatty acid composition of TAG was essentially the same as that of the total lipids. Linoleic (47.8–64.3%), oleic (4.0–22.5%), palmitic (9.8–20.1%), linolenic (8.2–12.6%), and stearic (2.4–3.8%) acids were the principal fatty acids in all soybeans. A small difference ($P < 0.05$) occurred in fatty acid composition between total lipids and polar lipids before microwave roasting. There were also differences ($P < 0.05$) in linoleic, oleic, and palmitic acid content between total lipids and polar lipids; polar lipids were higher (61.0–64.3%) in linoleic and (17.3–20.1%) in palmitic and lower (4.0–6.3%) in oleic than those in total lipids. The fatty acid distribution of phospholipids in soybeans at different moistures was compared after roasting in a microwave oven (Table 1). A small difference ($P < 0.05$) occurred in fatty acid composition between unsoaked soybeans (9.6%) and soaked soybeans for 5 h (51.9%) after 12 min of microwave roasting: in unsoaked soybeans, the percentages of linoleic and linolenic acids gradually decreased ($P < 0.05$), and these values were compensated by relative increases ($P < 0.05$) in the percentage of saturated fatty acids, such as palmitic and stearic acids. This may reflect differences in the decomposi-

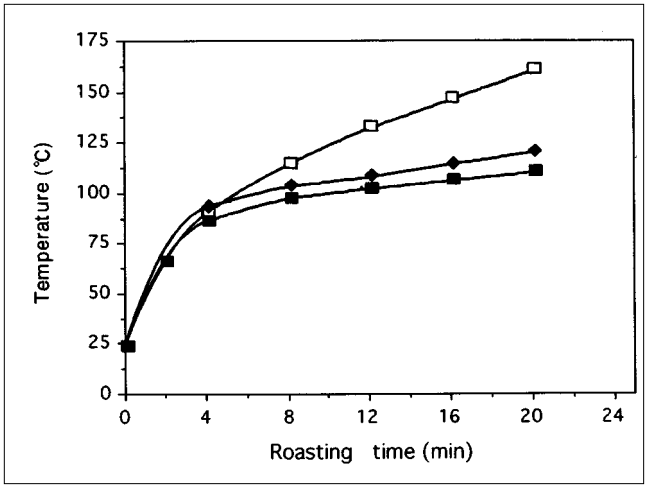

FIG. 1. Relationship between roasting time and internal temperature of soybeans at different moisture contents after microwave roasting (at a frequency of 2,450 MHz). □, 9.6% moisture; ◆, 38.2% moisture; ■, 51.9% moisture. Data points are means of three measurements from three replicates; standard errors are within the size of the symbols.

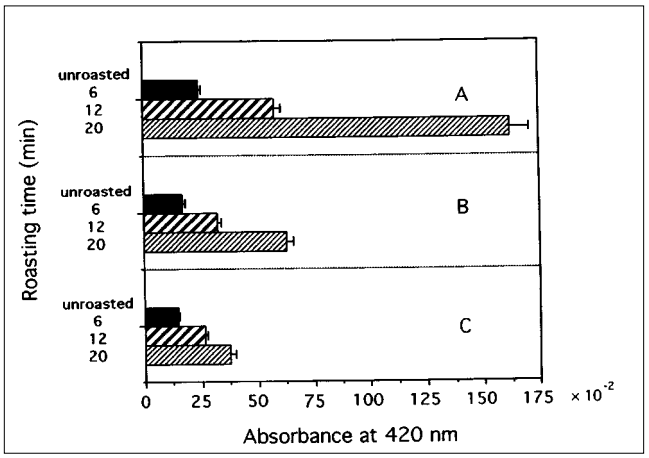

FIG. 2. Changes in absorbance at 420 nm (color) of lipids prepared from soybeans at different moisture contents after microwave roasting (at a frequency of 2,450 MHz). A, 9.6% moisture; B, 38.2% moisture; C, 51.9% moisture. Averages of three replicates. Vertical bars represent standard error of replicates.

TABLE 1
Changes in Fatty Acid Composition of Phospholipids in Soybeans at Different Moisture Contents During Microwave Roasting (at a frequency of 2,450 MHz)[a,b]

Cultivar	Moisture (%)	Roasting time (min)	Fatty acid (wt%) 14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
		Unroasted	0.1[a]	18.2	0.4[b]	3.6[c]	4.9[c]	62.5[b,c]	9.4[d]	0.9[d]
	9.6	6	0.2[a]	21.2[b]	0.7[d]	3.8[c]	5.1[c]	59.3[a,b]	9.0[d]	0.7[c]
		12	0.2[a]	22.6[b]	1.0[e]	3.9[c,d]	5.2[c]	58.2[a,b]	8.6[c]	0.3[a,b]
Tsurunoko		20	0.2[a]	23.6[c]	1.1[e]	3.9[c,d]	5.6[c,d]	57.5[a]	7.8[b,c]	0.3[a,b]
		Unroasted	0.1[a]	19.1[a,b]	0.6[d]	3.7[c]	5.4[c,d]	61.8[b,c]	8.9[c,d]	0.4[a,b]
		6	0.1[a]	20.7[a,b]	0.7[d]	3.8[c]	5.5[c,d]	60.3[b]	8.4[c]	0.5[b]
	51.9	12	0.1[a]	21.6[b]	0.4[b]	4.0[d]	5.6[c,d]	59.4[a,b]	8.4[c]	0.5[b]
		20	0.2[a]	22.5[b,c]	0.4[b]	4.1[d]	5.6[c,d]	58.3[a,b]	8.3[c]	0.6[b,c]
		Unroasted	0.1[a]	18.8[a]	0.2[a]	4.0[d]	4.4[a]	63.1[c]	9.1[d]	0.3[a,b]
	9.6	12	0.2[a]	21.7[a,b]	0.2[a]	4.2[d]	4.8[a]	59.6[a,b]	8.8[c,d]	0.5[b]
		20	0.5[b]	22.8[b,c]	0.3[a]	4.1[d]	5.8[c,d]	57.4[a]	8.6[c]	0.5[b]
Okuhara		Unroasted	0.1[a]	17.7[a]	0.3[a]	3.5[c]	4.0[a]	63.3[c]	10.7[f]	0.4[a,b]
	51.9	12	0.3[a]	20.5[a,b]	0.3[a]	3.5[c]	4.6[a,b]	60.5[b]	9.8[e]	0.5[b]
		20	0.2[a]	21.8[b]	0.3[a]	4.3[d]	4.6[a,b]	60.0[b]	8.5[c]	0.3[a,b]
		Unroasted	0.1[a]	22.0[b,c]	0.7[d]	2.4[a]	6.1[e]	59.8[a,b]	8.6[c]	0.3[a]
	9.6	12	0.1[a]	25.0[d]	0.8[d]	3.1[c]	6.8[f]	56.2[a]	7.6[b]	0.4[a,b]
Mikawajima		20	0.2[a]	25.8[d]	0.8[d]	3.6[c]	7.0[f]	55.1[a]	7.0[a]	0.5[b]
		Unroasted	0.1[a]	22.0[b,c]	0.7[d]	2.4[a]	6.3[e]	60.0[b]	8.3[c]	0.2[a]
	51.9	12	0.1[a]	22.5[b,c]	0.8[d]	2.7[a,b]	6.4[e]	59.8[a,b]	8.2[c]	0.3[a,b]
		20	0.1[a]	24.1[d]	0.5[c]	2.8[a,b]	6.5[e,f]	57.5[a]	8.3[c]	0.2[d]

[a]Each value is an average of three determinations. Values in the same column with different lower-case letters (a–f) are significantly different from unroasted seeds ($P < 0.05$).
[b]Others: containing 16:2, 17:0, 20:0, and 22:0.

tion of individual phospholipids in soybeans at different moistures (Fig. 3). The fatty acid distribution in soaked soybeans (38.2%) for 1 h was omitted because it was essentially the same as that for 5 h soaking.

Composition of phospholipid fractions. To clarify the effects of microwave roasting on phospholipids in soybeans at different moisture contents, phospholipids were isolated from polar lipids by TLC, and then fractionated into the PA, PE, PC, PS and PI fractions, which were further separated on TLC plates in the presence of authentic standards. Figure 4 shows the effects of microwave roasting on phospholipids in soybeans at different moisture contents. An increase in the moisture content of soybeans before microwave treatment resulted in a significant ($P < 0.05$) reduction in phospholipids. Furthermore, phospholipids were significantly ($P < 0.05$) reduced when the soybean seeds were subjected to microwave roasting. However, the reduction during microwave roasting was inhibited by increasing the moisture content. 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 extraction. A recent study (24) has shown that, at the molecular level, intensive membrane degradation occurs in lipid bodies isolated from soybeans. Heat treatment by microwave roasting, apparently through moisture removal (Fig. 1), enzyme inactivation (10, 25) or morphological changes in the lipid bodies present in the soybeans, results in more complete extraction of phosphatides. Figure 3 represents the changing profiles of PA, PE, PC, PS, and PI in soybeans at different moisture contents before and after microwave roasting. Before microwave roasting, PC, PE, and PI were the major phospholipids in all cultivars, and the concentrations were 181–230 mg, 77–120 mg, and 78–110 mg per 100 seeds, respectively. Low percentages were recorded for other phospholipids, such as PA (8–15 mg) and PS (5–9 mg). Unidentified phospholipids were also detected in all soybeans, but they were not determined because their levels did not appear to change as much as did the five major phospholipids (less than 1 mg). Effects of microwave roasting on phospholipids depended on moisture content and internal temperature in the soybeans during microwave roasting. An increase in moisture content of the soybeans depressed the internal temperature and inhibited a reduction ($P < 0.05$) in the phospholipid content. Longer microwave roasting time produced the greatest phospholipid losses ($P < 0.05$) in PE, followed by PC and PI. The trends became more pronounced with decreasing moisture (9.6%). However, a slight increase in PA was observed, and PS was almost constant during microwave roasting. This increase indicated that PA was probably formed from other phospholipids.

Fatty acid distribution of the individual phospholipids. Figure 5 shows a typical fatty acid distribution of individual phospholipids in soybeans (*cv.* Tsurunoko) at different moisture contents during microwave roasting. In all soybean cultivars (data not shown), the fatty acid composition of PI differed from those of other phospholipids. Namely, the percentage of palmitic acid was higher. However, the fatty acids in the greatest percentages in the major five phospholipids were

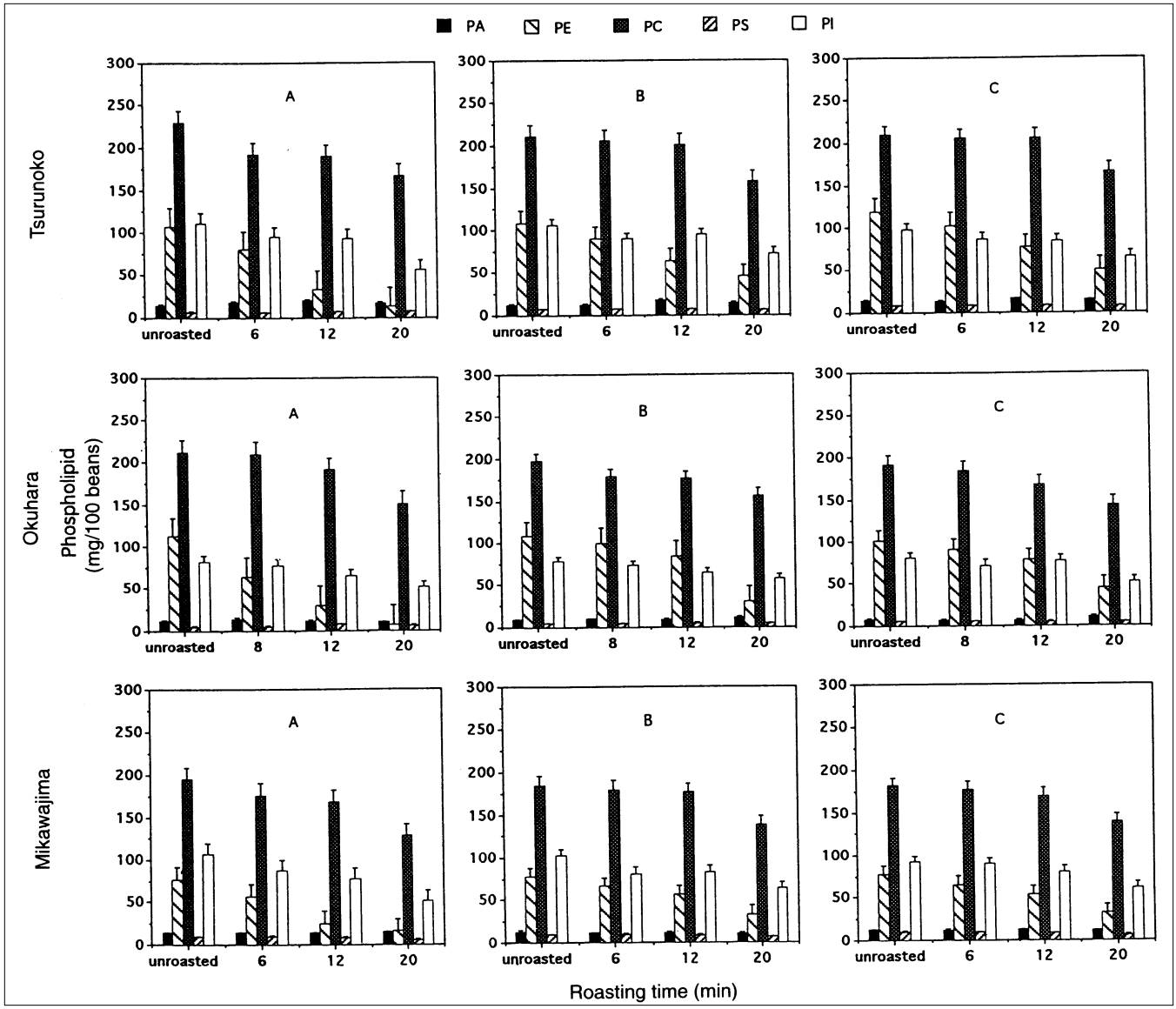

FIG. 3. Distributions of phospholipid classes in soybeans at different moisture contents after microwave roasting (at a frequency of 2,450 MHz). PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol. A, 9.6% moisture; B, 38.2% moisture; C, 51.9% moisture. Averages of three replicates. Vertical bars represent standard error of replicates.

linoleic, followed by palmitic, oleic, and linolenic. In general, with longer microwave roasting, decreases ($P < 0.05$) occurred in percentages of polyunsaturated fatty acids, and increases in saturated fatty acids compared to the values before soaking and microwave roasting. These trends became more pronounced ($P < 0.05$) in PE, followed by PS and PA, than PI. Furthermore, the PE ($P < 0.05$) content in lower-moisture soybeans decreased considerably during microwave roasting, compared to PI (Fig. 4). The results suggested that the free amino group of PE or PS could contribute to the formation of browning substances. Hafez *et al.* (26) observed that an increase in microwave roasting time was accompanied by an increase in the browning substances in soybeans. In the Maillard reaction (27), phospholipids can be particularly reactive if they contain both polyunsaturated fatty acids and amines. Recent work (28,29) with model systems has shown that, when Maillard reactions are carried out in the presence of lipids, especially phospholipids, the quantities of Maillard reaction products are changed and a number of compounds are produced that arise from the interaction of lipid degradation products as a result of the Maillard reaction. In conclusion, an increase in moisture content depressed a rise in the internal temperature of soybeans and prevented a greater reduction in phospholipids and/or polyunsaturated fatty acid content, especially the PE that contains an amino group. Therefore, it is necessary to carefully consider the moisture content in soybeans before microwave roasting. Further research is necessary in this area for a better understanding of the brow-

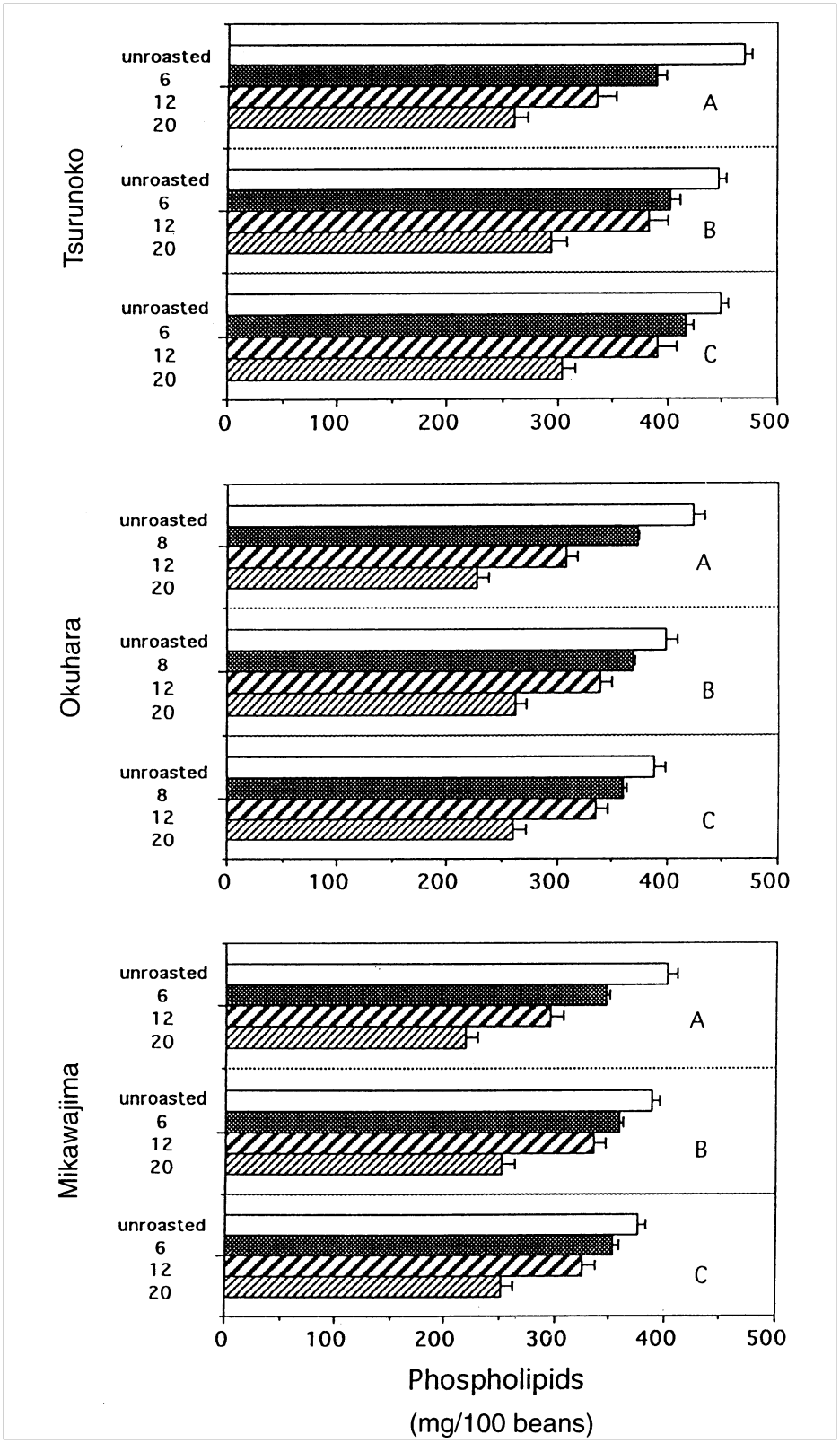

FIG. 4. Changes in the amount of phospholipids in soybeans at different moisture contents after microwave roasting (at a frequency of 2,450 MHz). A, 9.6% moisture; B, 38.2% moisture; C, 51.9% moisture. Averages of three replicates. Vertical bars represent standard error of replicates.

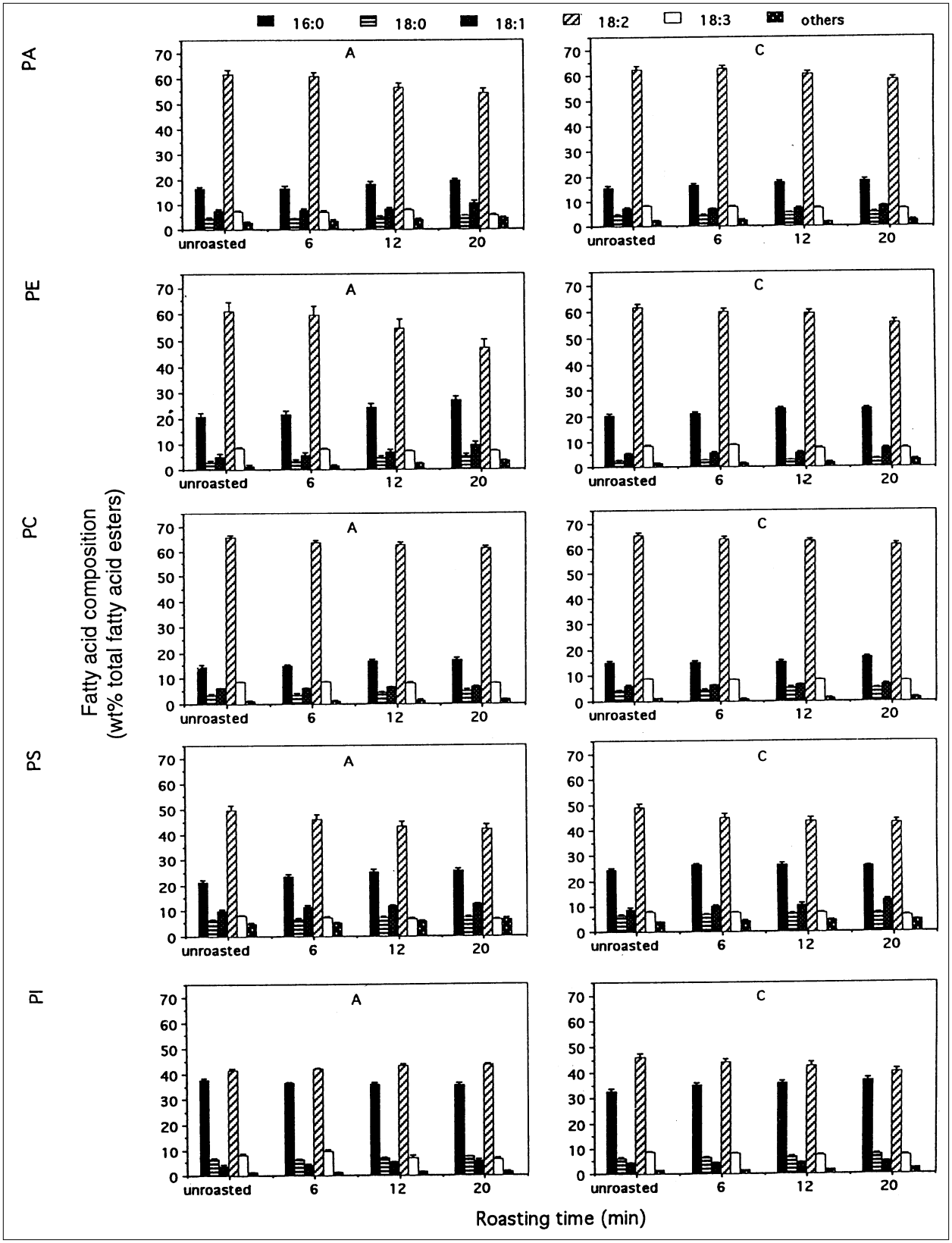

FIG. 5. Fatty acid distributions of the individual phospholipids in soybeans at different moisture contents after microwave roasting (at a frequency of 2,450 MHz). A, 9.6% moisture; C, 51.9% moisture. Averages of three replicates. Vertical bars represent standard error of replicates. Others: contained 16:1, 17:0, 20 and 22:0. See Figure 3 for abbreviations.

ing reaction kinetics during microwave roasting and the interaction of lipid degradation products with the Maillard reaction.

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