Microwave Roasting and Positional Distribution of Fatty Acids of Phospholipids in Soybeans (*Glycine max* L.)

Hiromi Yoshida* and Sachiko Takagi

Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan

ABSTRACT: Whole soybeans were exposed to microwave roasting for 6, 12, and 20 min at a frequency of 2,450 MHz and were studied not only for phospholipid composition but also for positional distribution of the fatty acids. During microwave roasting, the greatest rate of phospholipid losses ($P < 0.05$) was observed in phosphatidylethanolamine (PE), followed by phosphatidylcholine (PC) and phosphatidylinositol (PI), respectively. Therefore, the effects of microwave roasting on the composition and positional distribution of the fatty acids are likely clearer in PE than in PC or PI. However, the principal characteristics for the positional distribution of fatty acids are still retained during microwave roasting: unsaturated fatty acids, especially linoleic, are predominantly concentrated in the 2-position, and saturated fatty acids, especially palmitic, primarily occupy the 1-position after 12 or 20 min of roasting. The results suggest that unsaturated fatty acids located in the 2-position are significantly protected from microwave roasting.
JAOCS 74, 915–921 (1997)

KEY WORDS: Cultivar, fatty acids, microwave roasting, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phospholipids, positional distribution, soybeans.

Now, microwave cooking is the most versatile method worldwide. It is energy-efficient and reduces cooking time compared to conventional heating. The appliance user prefers the microwave cooking procedure, which is characterized by speed and short cooking time, compared to classical cooking methods. In fact, microwaves are used in the food industry not only for warming, drying, thawing, and baking but also for other applications, such as for pasteurizing and sterilizing many types of foods (1). Microwave heating can provide several advantages over conventional food processing methods (2). Microwave ovens are easy to use (3). The food is exposed to high temperatures for a shorter period of time; this may mean that fewer heat-sensitive nutrients are lost, thus improving the nutritive value of electronically cooked products, although this point is being debated (4,5).

The number of domestic microwave ovens is increasing, mainly because consumers appreciate the advantages, such as convenience, economy, and time savings (6,7). Yoshida and Kajimoto (8,9) found that microwave heating was not only effective for trypsin inhibitor inactivation in whole soybeans, but also for making high vitamin E, full-fat soy flour from raw beans. Esaka *et al.* (10) demonstrated that the microwave treatment was more effective for inactivating lipoxygenase in beans with higher moisture contents. However, from time to time, consumers are concerned by reports that noxious compounds are produced in microwaved food (11). Yoshida *et al.* (12) reported that triacylglycerols in soaked soybeans were already hydrolyzed into diacylglycerols and free fatty acids during soaking and were further hydrolyzed by microwaves.

The principle of microwave heating is different from that of conventional heating by conduction or convection, so it is technically difficult to measure and standardize the influential parameters. A recent study (13) has shown that, at the molecular level, intensive membrane degradation occurs in lipid bodies isolated from soybeans. Phospholipase D was shown to convert PE and PC to phosphatidic acid fairly rapidly at 30°C. But heat treatment with live steam or microwave treatment, apparently through moisture removal, enzymic inactivation, or morphological changes in the lipid bodies present in the flakes, results in more complete extraction of phosphatides (14). Microwave ovens are credited with rapid heating rates and high efficiency, because of their high penetration power (15). 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 roasting affects the composition and positional distribution of fatty acids of phospholipids in soybeans. The current study was undertaken to provide information about the effect of microwave treatment on the composition and positional distribution of fatty acids in the three major phospholipids of whole soybeans.

MATERIALS AND METHODS

Materials and chemicals. Commercially available soybeans (*Glycine max* L.) used in this work were of three cultivars: Okuhara, Mikawajima, and Tsurunoko. The soybeans were all grown in Japan in the summer of 1996. These soybean cultivars were purchased from Takii Seed Co. (Kyoto, Japan) and were selected for uniformity based on bean weight (between 250–299 mg for Okuhara, 270–319 mg for Mikawa-

*To whom correspondence should be addressed.
E-mail: yoshida@nutr. kobegakuin.ac.jp

jima, and 350–429 mg for Tsurunoko, respectively). The soybeans were hand-selected to eliminate those with cracked or otherwise damaged seed coats. All chemicals were of analytical grade (Nacalai Tesque, Kyoto, Japan) and used without further purification. Precoated Silica-Gel 60 plates (10 × 20 or 20 × 20 cm, 0.25-mm layer thickness) for thin-layer chromatography (TLC) were purchased from Merck (Darmstadt, Germany). Phospholipid and glycolipid standards were obtained from Serdary Research Lab (London, Ontario, Canada). Phospholipase A_2 was from bee venom, *Apis mellifera,* and obtained from Sigma Chemical Co. (St. Louis, MO). Fatty acid methyl ester standards (F & OR mixture #3) were purchased from Applied Science (State College, PA). One-hundred milligrams of pentadecanoic acid (Merck) was dissolved in *n*-hexane and stored in a 20-mL glass volumetric flask until required as 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 microwave oven (Sharp Model R-5,550, Osaka, Japan), capable of generating 0.5 kW power, was used. Soybeans were placed in a single layer in a Pyrex petri dish (12.0 cm diameter) and then roasted separately by microwaves for 6.0, 12.0, and 20.0 min after covering the dish. Internal temperatures of beans immediately after each treatment were determined as described previously (9). Each petri dish contained approximately 60 g (185 seeds) of soybeans; dishes were separately prepared at each of the different exposure levels 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 chloroform/methanol (1:1, vol/vol) in a Waring blender at 0°C under ice, and the lipids were extracted three more times with 150 mL 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 the solvent was removed from the filtrate by reduced pressure at 35°C with a rotary evaporator.

The extracts were redissolved in 125 mL chloroform and shaken with 20 mL saturated sodium chloride. 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 below 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 weighed to determine the lipid content of the beans and then kept in chloroform/methanol (2:l, vol/vol) solutions 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. Total lipids were fractionated by TLC into two fractions: polar lipids and phospholipids. Neutral lipids were removed from the lipid mixture by developing first with the solvent of *n*-hexane/diethyl ether/acetic acid (70:40:1, vol/vol/vol); glycolipids were subsequently removed from the lipid mixture by developing with the solvent acetone/acetic acid/water (100:2:1, vol/vol/vol). Part of each phospholipid extract, obtained as described previously, was further isolated by TLC into four fractions: PE, PC, PI, and the others. The phospholipid extracts were applied on precoated Silica Gel G 60 plates as 15-cm bands (approximately 15 mg/plate) with a microsyringe. The TLC phospholipid standards were applied as a reference on one side of each plate, and the plates were developed with chloroform/methanol/acetic acid/water (170:30:20: 7, by vol) to 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 specific spray reagents: Dragendorff reagent for choline lipids (16), 0.25% ninhydrine in acetone for amino-containing lipids, and molybdate reagent for phospholipids (17). Bands that corresponded to PE, PC, PI, and the others were quantitatively scraped into test tubes (10.5 cm × 16 mm) with poly(tetrafluoroethylene)-coated screw caps, and pentadecanoic acid (25 µg) was added as an internal standard to each tube. Fatty acids were converted to methyl esters by heating in BF_3-methanol (18). The recovered fatty acid methyl esters were analyzed by gas–liquid chromatography (GC) according to the method of Yoshida *et al.* (19). After recording on a Shimadzu C-R4A integrating system, component peaks were identified by comparing retention times with those of standards (F & OR mixture #3) and were quantitated with methyl pentadecanoate. Peak areas were computed, and percentages of fatty acid methyl esters were obtained as weight percentages by direct internal normalization. No correction factors were necessary because response factors of the main fatty acids are close to unity.

Enzymic hydrolysis of phospholipids. The positional distribution of fatty acids in each of the PE, PC, and PI samples isolated by preparative TLC was determined with phospholipase A_2 hydrolysis on a silica gel plate by a modification of the method of Dutta *et al.* (20). A phospholipase A_2 solution (2.5 mg in 0.25 mL water) was applied to a 0.5-mm Silica Gel G plate. Each phospholipid in ethanol/methanol (95:5, vol/vol) was applied evenly over the enzyme band, and the plate was placed immediately in a chamber, saturated with ether vapor, for 40 min at ambient temperature. After hydrolysis of the phospholipids, the plate was developed to the top with chloroform/methanol/14 M ammonium hydroxide (85:15:2, vol/vol/vol). The phospholipid bands were detected by the method described previously. The lysophospholipid and free fatty acid were separately scraped into test tubes from the plate, and the constituent fatty acids were analyzed by GC after transesterification as described previously.

Statistical analysis. Statistical evaluation of data was conducted by the Statistical Analysis System (21) with general linear model (GLM) analysis of variance. Least significant difference (LSD) values were computed at the 5% level.

TABLE 1
Changes in Lipid Components of Soybeans Roasted in a Microwave Oven (at frequency of 2,450 MHz)[a]

Cultivar	Roasting time (min)	Total lipids	Polar lipids	Phospholipids
	Unroasted	4608.0^{b}	419.3^{b}	331.2^{d,e}
Okuhara	6	4702.4^{b}	432.6^{b,c}	301.7^{e}
	12	4820.2^{b,c}	457.2^{c}	263.6^{f}
	20	5152.3^{c}	565.4^{e,f}	215.2^{g}
	Unroasted	5454.0^{d}	494.5^{d}	388.2^{c}
Mikawajima	6	5590.3^{d,e}	508.7^{d,e}	350.1^{d}
	12	5731.8^{d,e}	538.8^{e}	316.3^{e}
	20	6120.3^{e,f}	656.9^{g}	269.0^{f}
	Unroasted	5871.0^{e}	534.3^{e}	410.7^{b}
Tsurunoko	6	5965.2^{e}	548.8^{e}	379.2^{e}
	12	6115.2^{e,f}	587.4^{f}	328.5^{e}
	20	6355.2^{f}	680.9^{g}	278.4^{f}

[a]Each value is an average of three determinations and expressed as mg per 100 beans.
[b–g]Values in the same column with different superscript letters are significantly different from those for unroasted beans ($P < 0.05$).

RESULTS AND DISCUSSION

Microwave roasting and lipid components. Proximate analyses with AOAC (22) methods showed that the moisture content of soybeans before microwave roasting was 9.5–9.8%. 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 as reported previously (9). Therefore, the roasting times were selected at 6, 12, and 20 min in this study. The temperature of the soybean samples was 25°C before roasting and increased from 97°C to 162°C, at 6 and 20 min after microwave roasting, respectively (data not shown). A dark brownish color and burnt odor became apparent at 12 min (127°C). The amount of lipid components changed during microwave treatment (Table 1).

Longer microwave processing resulted in a greater amount of total lipids being extracted from the beans. Collins and Beaty (23) indicated that heat caused some protein denaturation, possibly leading to improved lipid extractability. Their lipid extraction was probably performed after roasting rather than before roasting. Polar lipids increased slowly in the first 12 min of microwave roasting and rapidly thereafter, and attained about a 1.3-fold increase after 20 min of roasting. On the other hand, phospholipids gradually decreased, and at 20 min, over 30% of the pre-microwave roasting phospholipids was lost. When soybeans were roasted in a microwave oven, Hafez *et al.* (24,25) observed not only an increase in browning substances but also a decrease in phospholipids. The increase in browning substances may be attributed to the increase of polar lipids. Such increases in the polar lipid fraction were expected because lipid extraction was carried out after roasting.

Major phospholipids and fatty acid composition. To clarify the effects of microwave roasting on phospholipids, further separation of the phospholipid fraction into three fractions (PE, PC, and PI) was carried out on TLC in the presence of authentic standards. Table 2 shows the changing profiles of the PE, PC, and PI in the soybeans before and after mi-

TABLE 2
Changes in the Major Phospholipids of Soybeans Roasted in a Microwave Oven (at frequency of 2,450 MHz)[a]

Cultivar	Roasting time (min)	Phosphatidyl-ethanolamine	Phosphatidyl-choline	Phosphatidyl-inositol
	Unroasted	82.6^{c}	162.8^{d,e}	79.8^{d,e}
Okuhara	6	63.4^{e}	153.8^{e}	73.3^{e}
	12	32.1^{h}	135.2^{f}	68.8^{f}
	20	9.8^{i}	108.4^{g}	57.6^{g}
	Unroasted	89.1^{b}	195.7^{b,c}	93.6^{b}
Mikawajima	6	68.5^{d}	172.9^{d}	86.1^{c,d}
	12	42.9^{f}	155.3^{e}	76.6^{e}
	20	8.7^{j}	132.5^{f}	68.4^{f}
	Unroasted	93.2^{b}	205.0^{b}	96.1^{b}
Tsurunoko	6	72.5^{d}	191.9^{c}	87.9^{c}
	12	38.9^{g}	159.6^{e}	83.6^{c,d}
	20	9.8^{i}	132.3^{d}	73.6^{e}

[a]Each value is an average of three determinations and expressed as mg per 100 beans.
[b–j]Values in the same column with different superscript letters are significantly different from those for unroasted beans ($P < 0.05$).

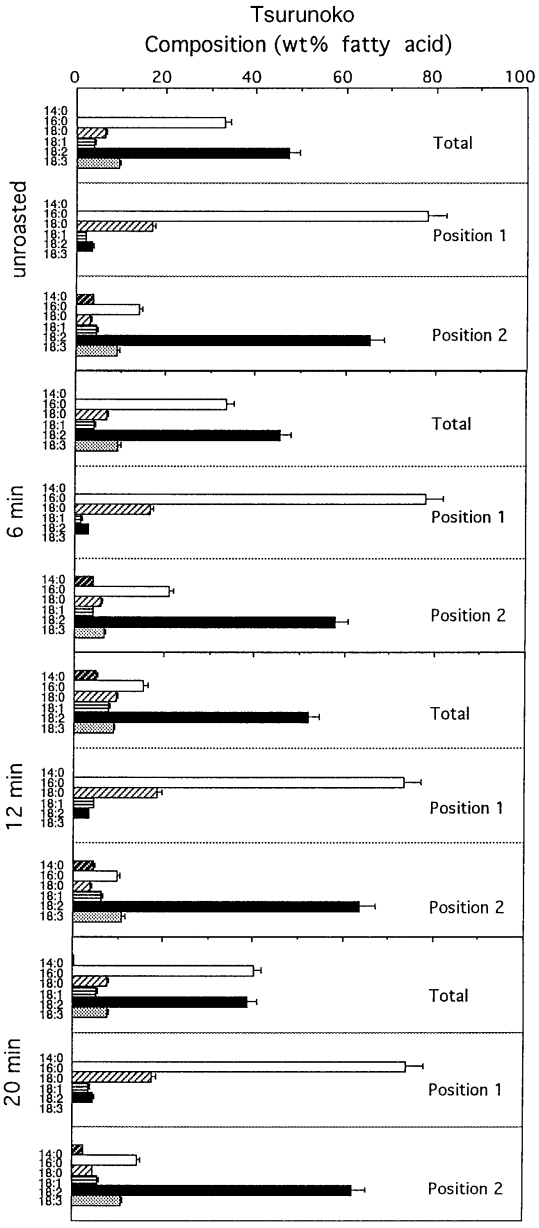

FIG. 1. Changes in composition and positional distribution of fatty acids of phosphatidylinositol of soybeans (cv. Tsurunoko) roasted in a microwave oven (at a frequency of 2,450 MHz). Each value represents the average of three replicates, and vertical bars represent the standard error of the replicates.

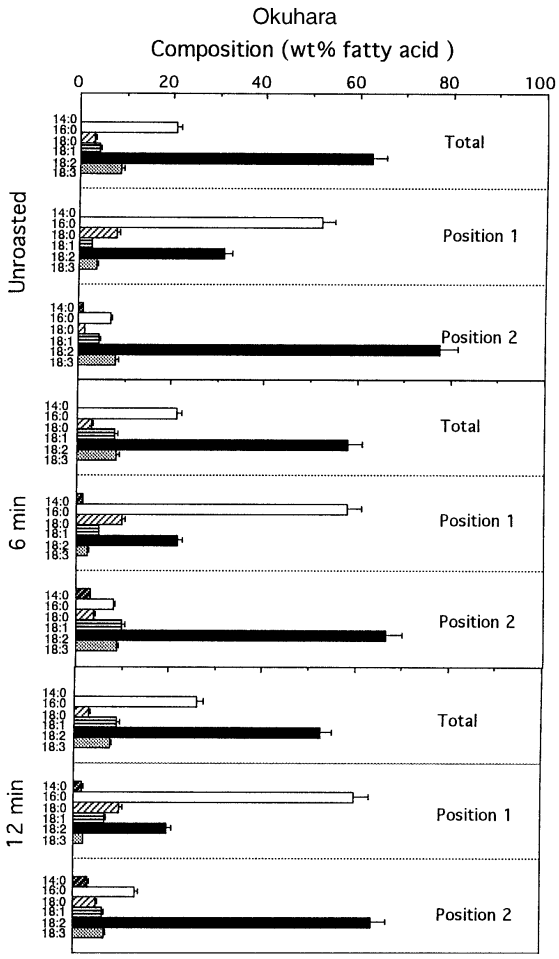

FIG. 2. Changes in composition and positional distribution of fatty acids of phosphatidylethanolamine of soybeans (cv. Okuhara) roasted in a microwave oven (at a frequency of 2,450 MHz). Each value represents the average of three replicates, and vertical bars represent the standard error of the replicates.

crowave roasting. The original amounts of the individual phospholipids before microwave roasting ranged from 82.6–93.2, 162.8–205.0, and 79.8–96.1 mg/100 beans for PE, PC, and PI, respectively. There were significant differences ($P < 0.05$) in the original amounts of each phospholipid among the three cultivars. The proportion of PE, PC, and PI in this study differed from that typically reported in soybean phospholipids (26), possibly because of varietal variation.

The greatest rate of phospholipid losses ($P < 0.05$) was observed for PE, followed by PC and PI, respectively. The trends became more pronounced with longer roasting; the amount of PE at 20 min was less than 12% of that before microwave roasting. However, PI was still over 72% in all three cultivars after 20 min of roasting. It was anticipated that the composition and positional distribution of fatty acids in the PI (Fig. 1) would differ from that in PE or PC (Fig. 2 or 3). There were no significant differences ($P > 0.05$) in the changing patterns of these phospholipids among the three cultivars during microwave roasting. The amino group of PE can apparently facilitate hydrogen or electron donation to tocopherols in the soybeans (27). At elevated temperatures, some of the naturally occurring classes of phospholipids, in particular PE, greatly enhance the activity of primary antioxidants in edible oils, but PI is without synergistic activity. Furthermore, phospholipids are reported to cause a browning phenomenon during heating (28).

As shown in Table 3, a small difference ($P < 0.05$) occurred in fatty acid composition (expressed in terms of esters by weight) of phospholipids among the three cultivars before microwave roasting. There were significant differences ($P < 0.05$) in palmitic, oleic, linoleic, and linolenic acids be-

tween Mikawajima and Okuhara or Tsurunoko. Mikawajima was higher in palmitic and in oleic, and lower in linoleic and in linolenic than those of Okuhara or Tsurunoko. Low percentages were detected for arachidic and behenic acids, and they were shown as "others" in Table 3. During microwave roasting, no differences ($P > 0.05$) were observed in changing patterns of the fatty acid compositions among the three cultivars. Fatty acids are attached to the 1- and 2-positions of the glycerol moiety of phospholipids in a pattern that is characteristic of the particular class of phospholipid of the source from which the lipid is derived, and of the history of the organism.

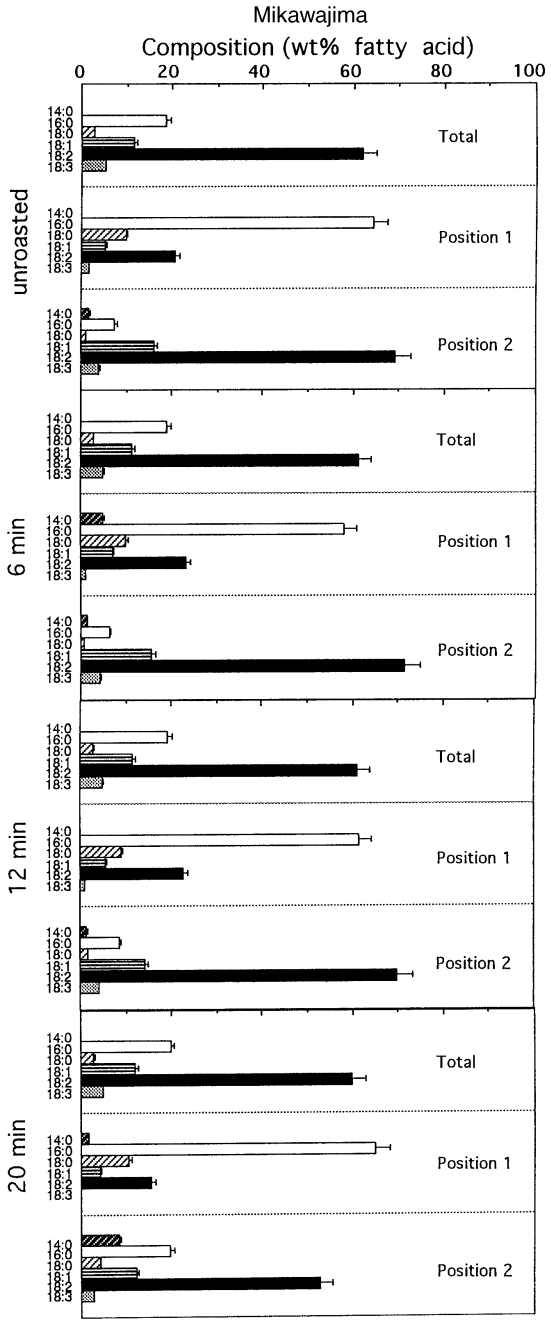

FIG. 3. Changes in composition and positional distribution of fatty acids of phosphatidylcholine of soybeans (cv. Mikawajima) roasted in a microwave oven (at a frequency of 2,450 MHz). Each value represents the average of three replicates, and vertical bars represent the standard error of the replicates.

Composition and positional distribution of fatty acids in the major phospholipids. To study the effects of microwave roasting on composition and positional distribution of fatty acids in the major phospholipids, the PE, PC, and PI isolated by preparative TLC, as described previously, were hydrolyzed with phospholipase A_2. The representative changing profiles of composition and positional distribution of fatty acids of PE, PC, and PI in soybeans were compared before and after microwave roasting (Figs. 1–3). The positional distribution of fatty acids in PE, PC, and PI was observed in all phospholipids of the three cultivars before microwave roasting: saturated fatty acids were mainly located in the 1-position, and unsaturated fatty acids predominantly occupied the 2-position. Before microwave roasting, however, the concentration of saturated acids in the 1-position of PI was markedly higher than that of PE or PC, probably due to differences in their biosynthetic pathways. Similar distributions are reported by Miyazawa and Fujino (29) and by Nakagawa (30) for pea or soybean seeds.

As shown in Table 2, the PE content was markedly reduced from 82.6–93.2 mg in unroasted beans to 8.7–9.8 mg after 20 min of roasting. The data for PE at 20 min were omitted from Figure 2 because the sample was too small for enzymic hydrolysis to determine the positional distribution of fatty acids. Roasting for 6 min caused no significant changes ($P > 0.05$) in the positional distribution of fatty acids in the major phospholipids. After 12 min of microwave roasting, the percentage of palmitic and stearic acids was higher in the 1-position, and that of linoleic and linolenic acids was less in the 2-position than before roasting. The trends became more pronounced ($P < 0.05$) in the PE with longer roasting. Therefore, the effects of microwave roasting on the composition and positional distribution of fatty acids in soybeans are clearer in PE than in PC or PI. However, the principal characteristics for the positional distribution of fatty acids in these phospholipids were still retained among the three cultivars during microwave roasting. Unsaturated fatty acids, especially linoleic acid, were predominantly distributed in the 2-position, and saturated fatty acids, especially palmitic, were highly concentrated in the 1-position after 20 min of microwave roasting. The results suggest that unsaturated fatty acids, located in the 2-position, are significantly protected from microwave roasting.

The formation of browning substances was considerably accelerated after 12 min roasting when whole soybeans were roasted in a domestic microwave oven. Of the relative stability of the phospholipids during microwave roasting, the greatest rate of phospholipid losses was observed in PE, followed by PC and PI, respectively. However, the principal characteristics for the positional distribution of fatty acids in these phospholipids were retained during microwave roasting: unsaturated fatty acids predominantly occupied the 2-position, and saturated fatty acids were highly concentrated in the 1-

TABLE 3
Fatty Acid Composition of Phospholipids of Soybeans Roasted in a Microwave Oven (at frequency of 2,450 MHz)[a]

Cultivar	Roasting time (min)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
	Unroasted	1.2[b]	17.2[b]	0.2[b]	4.2[c]	4.0[b]	62.9[b]	9.9[b]	0.4[b]
Okuhara	6	1.2[b]	18.2[b]	0.2[b]	4.3[a]	4.2[b]	61.8[b]	9.5[b]	0.6[c]
	12	2.3[d]	18.8[b]	0.1[b]	4.4[c]	4.6[b]	59.4[b,c]	8.9[b]	1.5[d]
	20	2.8[d]	19.4[b]	0.1[b]	4.9[c]	5.7[b]	57.8[c]	8.2[c]	1.1[d]
	Unroasted	1.5[b]	23.5[d]	0.2[b]	3.1[b]	10.8[d]	54.5[c]	4.5[d]	1.9[d]
Mikawajima	6	1.7[c]	23.7[d]	0.2[b]	3.3[b]	11.5[d]	53.5[c]	4.3[d]	1.8[d]
	12	2.4[d]	24.0[d]	0.2[b]	3.3[b]	11.8[d]	53.4[c]	4.3[d]	0.6[c]
	20	2.4[d]	24.7[d]	0.1[b]	3.5[b]	12.3[d]	52.1[c]	4.2[d]	0.6[c]
	Unroasted	1.5[b]	19.3[b]	0.2[b]	4.4[c]	5.3[c]	59.9[b]	8.6[c]	0.8[c]
Tsurunoko	6	1.5[b]	19.4[b]	0.2[b]	4.6[c]	5.4[c]	59.5[b]	8.5[c]	0.9[c,d]
	12	1.5[b]	19.6[b]	0.2[b]	4.8[c]	5.6[c]	58.1[b,c]	8.3[c]	1.9[d]
	20	1.7[c]	20.2[c]	0.1[b]	4.9[c]	5.8[c]	57.2[c]	8.3[c]	1.8[d]

[a]Each value is an average of three determinations.
[b–d]Values in the same column with different superscript letters are significantly different from those for unroasted beans ($P < 0.05$).

position after 20 min of microwave roasting. Unsaturated fatty acids distributed in the 2-position were significantly protected from microwave roasting. Nonetheless, further studies are necessary to demonstrate how microwave energy plays a role in the degradation of PE in soybeans in relation to the molecular species of phospholipids.

REFERENCES

1. Rosenberg, U., and W. Bögl, Microwave Pasteurization, Sterilization, Blanching and Pest Control in the Food Industry, *Food Technol. 41*:92–99 (1987).
2. Giese, J.H., Special Report: Advances in Microwave Food Processing, *Ibid. 46*:118–123 (1992).
3. Lin, Y.E., and R.C. Anantheswaran, Studies on Popping of Popcorn in a Microwave Oven, *J. Food Sci. 53*:1746–1749 (1988).
4. Hoffman, C.J., and M.E. Zabik, Effects of Microwave Cooking/Reheating on Nutrients and Food Systems: A Review of Recent Studies, *J. Am. Diet. Assoc. 85*:922–926 (1985).
5. Mudgett, R.E., Microwave Food Processing, *Food Technol. 43*:117–126 (1989).
6. Mudgett, R.E., Electrical Properties of Foods in Microwave Processing, *Ibid. 36*:109–115 (1982).
7. Nelson, S.O., S.D. Senter, and W.R. Fobus, Jr., Dielectric and Steam Heating Treatments for Quality Maintenance in Stored-Pecans, *J. Microwave Power 20*:71–74 (1985).
8. Yoshida, H., and G. Kajimoto, Effects of Microwave Treatment on the Trypsin Inhibitor and Molecular Species of Triglycerides in Soybeans, *J. Food Sci. 53*:1756–1760 (1988).
9. Yoshida, H., and G. Kajimoto, Effects of Microwave Energy on the Tocopherols of Soybean Seeds, *Ibid. 54*:1596–1600 (1989).
10. Esaka, M., K. Suzuki, and K. Kubota, Inactivation of Lipoxygenase and Trypsin Inhibitor in Soybeans on Microwave Irradiation, *Agric. Biol. Chem. 50*:2395–2396 (1986).
11. Lubec, G., G. Wolf, and S. Bartosch, Amino Acid Isomerization and Microwave Exposure, *Lancet 11*:1392–1993 (1989).
12. Yoshida, H., A. Mieno, S. Takagi, M. Yamaguchi, and G. Kajimoto, Microwave Roasting Effects on Acyl Lipids in Soybeans (*Glycine max* L.) at Different Moisture Contents, *J. Food Sci. 60*:801–805 (1995).
13. Simpson, T.D., and L.K. Nakamura, Phospholipid Degradation in Membranes of Isolated Soybean Lipid Bodies, *J. Am. Oil Chem. Soc. 66*:1093–1096 (1989).
14. List, G.R., T.L. Mounts, A.C. Lanser, and R.K. Holloway, Effect of Moisture, Microwave Heating, and Live Steam Treatment on Phospholipase D Activity in Soybeans and Soy Flakes, *Ibid. 67*:867–871 (1990).
15. Burfoot, D., S.J. James, A.M. Foster, K.P. Self, T.J. Wilkins, and I. Philips, Temperature Uniformity After Reheating in Microwave Ovens, in *Processing Engineering in the Food Industry,* Vol. 2, edited by R.W. Field and J.A. Howell, Elsevier Applied Science, New York, 1990, pp. 1–14.
16. Wagner, H., L. Hörhammer, and P. Woff, Thin-Layer Chromatography of Phosphatides, *Biochem Z. 334*:175–184 (1961).
17. Vaskovsky, V.E., E.Y. Kostetsky, and I.M. Vasendin, A Universal Reagent for Phospholipid Analysis, *J. Chromatogr. 114*:129–141 (1975).
18. Morrison, W.R., and J.M. Smith, Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride–Methanol, *J. Lipid Res. 5*:600–608 (1964).
19. Yoshida, H., I. Kondo, and G. Kajimoto, Participation of Free Fatty Acids in the Oxidation of Purified Soybean Oil During Microwave Heating, *J. Am. Oil Chem. Soc. 69*:1136–1140 (1992).
20. Dutta, J., A. Kr. Das, and A. Biswas, Enzymatic Reactions on Thin-Layer Chromatographic Plates, II. Phospholipase A_2 Hydrolysis of Phosphatidylcholine and Separation of the Products on a Single Plate, *J. Chromatogr. 173*:379–387 (1979).
21. SAS, *SAS User's* (Version 5 Edition), SAS Institute Inc., Cary, 1985.
22. AOAC, *Official Methods of Analysis*, 13th edn. Association of Official Analytical Chemists, 1980, Washington, D.C.
23. Collins, J.L., and B.F. Beaty, Heat Inactivation of Trypsin Inhibitor in Fresh Green Soybeans and Physiological Responses of Rats Fed the Beans, *J. Food Sci. 45*:542–546 (1980).
24. Hafez, Y.S., A. Mohamed, F.M. Hewedy, and G. Singh, Effects of Microwave Heating on Solubility, Digestibility and Metabolism of Soy Protein, *Ibid. 50*:415–417 (1985).
25. Hafez, Y.S., A. Mohammed, P.A. Perera, G. Singh, and A.S. Hussein, Effects of Microwave Heating and Gamma Irradiation on Phytate and Phospholipid Contents of Soybean (*Glycine max* L.), *Ibid. 54*:958–962 (1989).
26. Dornbos, Jr., D.L., R.E. Mullen, and E.G. Hammond, Phospholipids of Environmentally Stressed Soybean Seeds, *J. Am. Oil Chem. Soc. 66*:1371– 1373 (1989).
27. Hudson, B.J.F., and M. Ghavami, Phospholipids as Antioxidant Synergist for Tocopherols in the Autoxidation of Edible Oils, *Lebensm. Wiss. Technol. 17*:161–166 (1984).

28. Husain, S.R., J. Terao, and S. Matsushita, Effect of Browning Reaction Products of Phospholipids on Autoxidation of Methyl Linoleate, *J. Am. Oil Chem. Soc. 63*:1457–1460 (1986).
29. Miyazawa, T., and Y. Fujino, Phospholipids in Pea Seed, *Nippon Nogeikagaku Kaishi, 50*:169–174 (1976).
30. Nakagawa, Y., Application of Paired-Ion High-Performance Liquid Chromatography to the Separation of Molecular Species of Phosphatidylinositol, *Lipids 28*:1033–1035 (1993).

[Received December 2, 1996; accepted April 2, 1997]