Regional Distribution of Tocopherols and Fatty Acids Within Soybean Seeds

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ABSTRACT: Seed coat, axis, and sections of cotyledons in three soybean cultivars were analyzed by high-performance liquid chromatography for tocopherols, and by gas–liquid chromatography for acyl lipids. Tocopherols were predominantly detected in axis, followed by cotyledons and seed coat. With a few exceptions, dominant components were γ- and δ-tocopherols, with much smaller amounts of α- and β-tocopherols. However, α-tocopherol was higher ($P < 0.05$) for the Mikawajima cultivar than for Okuhara and Tsurunoko in all tissues. Triacylglycerols (TAG) were the major fraction of total lipids, representing 70% in axis and coat and 94% in cotyledons. A small difference ($P < 0.05$) occurred in fatty acid composition of TAG when comparing seed coat to the axis. The fatty acid composition of phosphatidylinositol (PI) differed ($P < 0.05$) from phosphatidylethanolamine (PE) and phosphatidylcholine (PC) in each tissue. Principally, the percentage of palmitic acid was higher, especially in axis and coat. In PE and PC, linoleic was greater, followed by palmitic, in all samples except for seed coat tissue in Mikawajima. The percentages of palmitic acid in both phospholipids were significant higher in the seed coat tissue from this cultivar than in cotyledon or axis of the other varieties. These results suggest that the differences in soybean cultivars could be appreciable, based on the distribution of tocopherols and fatty acids in each component part within soybean seeds. *JAOCS 75*, 767–774 (1998).

KEY WORDS: Acyl lipids, axis, coat, cotyledons, fatty acid distribution, phospholipids, soybeans, tocopherols, triacylglycerols.

Soybean is the world's single most important source of edible oil and vegetable protein. Soybean oil is an increasingly valuable commodity in world trade, with global production at about 17.7 million metric tons between 1993 and 1994 (1). The nutritional value of soybean is determined not only by quantity but also by the quality of its oil and protein. One quality factor, which has recently gained much attention, is the fatty acid composition of soybean oil. There is increasing evidence for a relationship between consumption of saturated fat and elevated serum cholesterol level (2), and a relationship between linolenic acid content and the oxidative stability and loss of flavor in a food system (3). However, the normal fatty acid composition of soybean oil is not often considered ideal in terms of oil functionality and oxidative stability.

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In addition to cereals, such as corn (4), wheat, barley, and oat (5), soybeans and their products (6) are relatively good sources of vitamin E (tocopherols). Tocopherols are important biological and nutritive components of our food. In addition to their function as cholesterol synthesis inhibitor (7,8), tocopherols are essential for protection against oxidative deterioration of polyunsaturated fatty acids in plants and animals. They exert their antioxidant effect by numerous biochemical and biophysical mechanisms, including scavenging active oxygen species and free radicals and through action as efficient chain terminators in lipid autoxidation reactions (9). Eight natural compounds possess vitamin E activity, namely α-, β-, γ-, and δ-tocopherols (10) and the four closely related tocotrienols (11). Generally, antioxidative and biological activities of the isomers increase in the following order: α, β, γ, and δ (12,13), but this order of antioxidant potency in vegetable oils may be influenced significantly by experimental conditions, such as temperature or light (14). Tocotrienols occur at much lower levels, and their structures differ from the tocopherols in that they have three double bonds in the side chain (15). All forms have been found in foods and fats, although δ-tocotrienol is rare and, among foods, has been reported only in palm oil. A literature search revealed that there is limited information on fatty acid distribution within soybean seed (16). In an oilseed plant, such as the soybean plant, there are two types of lipids: membrane lipids and storage lipids. Storage lipids are mainly triacylglycerols (TAG), which are localized in seed oil bodies of cotyledon tissues. Membrane lipids are mainly phospholipids, which are low in quantity and located in all cells of living tissues (17). There are few reports concerning various acyl lipids within each tissue in soybeans. Furthermore, little has been reported on the distribution of tocopherols in relation to fatty acid composition of various acyl lipids within each structural part and section of soybeans. The objective of the present research was to distinguish not only the regional distribution of tocopherols and different acyl lipids but also their fatty acid compositions in seed coat, axis, and sections of cotyledons from three soybean cultivars.

MATERIALS AND METHODS

Samples. Three cultivars of soybeans (*Glycine max* L.), Okuhara, Mikawajima and Tsurunoko, were used. Soybean

plants were all grown in Japan during the summer of 1996. Cultivars were purchased from Takii Seed Co. (Kyoto, Japan) and were selected for uniformity based on bean weights of 300 to 315 mg for Okuhara, 320 to 336 mg for Mikawajima, and 350 to 367 mg for Tsurunoko. The beans were hand-selected to eliminate those with cracked or otherwise damaged seed coats. All beans were cleaned and divided into groups for storage in stainless steel containers at 4°C until used.

Chemicals. All chemicals were of analytical grade (Nacalai Tesque, Kyoto, Japan) and were used without further purification. Vitamin E homologs (α, β, γ, and δ) were purchased from Eisai Co. (Tokyo, Japan). All tocopherols were D-isomers (*RRR*-), and the purity of each tocopherol was better than 98.5% when analyzed by high-performance liquid chromatography (HPLC). Precoated Silica-Gel G 60 plates (10 or 20 × 20 cm, 0.25 mm layer thickness), used for thin-layer chromatography (TLC), were purchased from Merck (Darmstadt, Germany). The TLC standard mixture, containing diacylglycerols (1,2- and 1,3-DAG), free fatty acids (FFA), TAG, and steryl esters (SE), was purchased from Nacalai Tesque. The phospholipid standards were obtained from Serdary Research Lab (London, Ontario, Canada; now Doosan Serdary Research Laboratories, Englewood Cliffs, NJ). Fatty acid methyl ester standards (F and OR mixture #3) were purchased from Applied Science (State College, PA). One hundred milligrams of methyl pentadecanoate (Merck) was dissolved in *n*-hexane and stored in a 20-mL glass volumetric flask until used as an internal standard. Boron trifluoride (14%) in methanol (Wako Pure Chemical Ind. Ltd., Osaka, Japan) was used to prepare the fatty acid methyl esters.

Seed sectioning and lipid extraction. Whole beans were first dissected into three structural parts, seed coat, cotyledons and axis, with a razor blade. The two cotyledons were then halved and designated as cotyledon I, the section closest to the axis area, and cotyledon II, the section opposite the axis area. Each pair of cotyledons was separated medially, then transversely, to produce four sections. Five hundred beans were separated and sectioned for each genotype. Each sample (cotyledon sections, seed coats, and axes) was subsequently ground with 50 to 200 mL chloroform/methanol (1:1, vol/vol) in a Waring blender at 0°C. The plant material was re-extracted three more times with 50 to 150 mL chloroform/methanol (2:1, vol/vol). The extraction solvent contained butylated hydroxytoluene (0.01%), which was added to prevent oxidative degradation of fatty acids and tocopherols during experimental procedures. The extract 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 extract was redissolved in 125 mL chloroform and shaken with 20 mL saturated aqueous solution of 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 at 35°C. The pressure was further reduced to 500 mm Hg at 35°C for 30 min, and the oil extract was flushed with nitrogen to remove residual chloroform. Extracted lipids were weighed to determine the lipid content within each soybean section and then redissolved in chloroform/methanol (2:1, vol/vol). Lipid samples were stored in a 5- or 25-mL brown glass volumetric flask under nitrogen in the dark at −25°C until used for further analysis.

HPLC. A measured quantity (50 to 200 mg) of lipids was transferred quantitatively to a 5-mL brown volumetric flask, and the solvents were evaporated under nitrogen. The residue was dissolved with the mobile phase (*n*-hexane/1,4-dioxane/ethanol 490:10:1, vol/vol/vol) used for HPLC analysis. The chromatographic system was the same as previously described (18) and operated at a flow rate of 2.0 mL/min. An aliquot (5 μL) was injected with a fully loaded 20-μL loop. Each tocopherol was detected with a fluorimetric detector (Shimadzu RF-535, Shimadzu Instruments Inc, Kyoto, Japan). The other HPLC conditions were as reported previously (18).

Lipid analysis. The total crude lipid was fractionated by TLC into six fractions: SE, TAG, FFA, 1,3-DAG, 1,2-DAG, and polar lipids. Samples were applied to 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 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 described (19). Following 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 SE, TAG, FFA, 1,3-DAG, 1,2-DAG, and polar lipids were scraped separately into test tubes (10.5 cm × 16 mm) with poly(tetrafluoroethylene)-coated screw caps. Methyl pentadecanoate (50 or 100 μg) was added as an internal standard to each tube. Samples of the polar lipid extracts, obtained as described above, were further separated by TLC into several fractions with chloroform/methanol/acetic acid/water (170:30:20:7, by vol) as the mobile phase (20). Phospholipid classes were detected by iodine vapor and were consistent with the authentic standards. Bands corresponding to phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), and others were quantitatively scraped into separate screw-cap test tubes (10.5 cm × 16 mm), and methyl pentadecanoate (25 μg) was added to each tube. Fatty acid methyl esters, prepared by transesterification (21), were analyzed by gas chromatography (GC) as described previously (22). A Shimadzu C-R4A integrating system was used to integrate peaks, which were identified by comparing their retention times with those of standards. Fatty acids were quantitated by reference to the internal standard. Data were expressed as percentages of fatty acid methyl esters and were obtained as weight percentages by direct internal normalization. No correction factors were necessary because response factors of the predominate fatty acids were similar.

Statistical evaluation. 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 (23). Least

significant difference (LSD) values were computed at the 5% level (24).

RESULTS AND DISCUSSION

Structural parts and sections of soybean. Typical relative percentages of soybean component parts (cv. Tsurunoko) are shown in Figure 1. Each value is an average of three determinations and expressed as individual weight percentages per 200 beans. Each cotyledon accounted for about 45.9 to 46.4% of seed mass. The seed coat (5.4 to 6.3%) and axis (1.8 to 1.9%) accounted for the remaining mass of the whole soybeans (data not shown). No significant differences ($P > 0.05$) in these percentages were observed among the three cultivars.

Tocopherol distributions within each structural part and section of soybean. As shown in Figure 2, the amount of total tocopherols per 100 g oil was highest in the axis, followed by cotyledons and seed coat. Tocopoherol contents varied significantly ($P < 0.05$), not only between the three cultivars but also within each structural part and section. The greatest amount of total tocopherols ($P < 0.05$) was observed in Tsurunoko, followed by Okuhara and Mikawajima. This was also true for the axis, cotyledons, and seed coat. There were no significant differences ($P > 0.05$) in the distribution of tocopherol between cotyledon halves among the three cultivars. Total tocopherols and the distribution of the homologs in plant foods vary by species, variety, stage of maturity, harvesting conditions, processing procedures, storage time, and conditions and moisture content during storage (25).

Figure 3 shows the distribution of individual tocopherols within each structural part and section of soybeans. The types and amounts of individual tocopherols depended on the cultivars and/or tissues. However, except for the axis and seed coat in Mikawajima, dominant components were γ- and δ-tocopherols, with much smaller amounts of α- and β-tocopherols in the four tissues. Okuhara and Tsurunoko had similar tocopherol distributions in all tissues. The amounts of α-, β-, γ-, and δ-tocopherols in cotyledons I and II ranged from 6.2 to 16.6, 4.3 to 4.8, 64.2 to 80.0, and 32.5 to 71.8 mg per 100 g oil, respectively. The tocopherol level may be associated with the level of unsaturated fatty acids in plant tissues. Typically, there is a positive relation between unsaturation and amount of antioxidants in the oil (26).

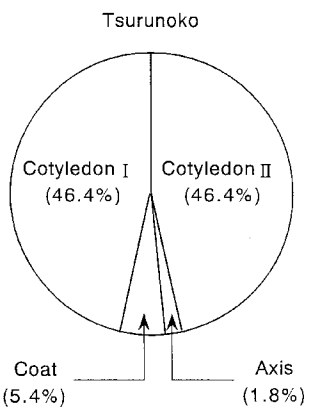

FIG. 1. Typical relative percentage of each structural part and section of cotyledons in whole soybeans. Each value is an average of three determinations and is expressed as a percentage of the corresponding total weight.

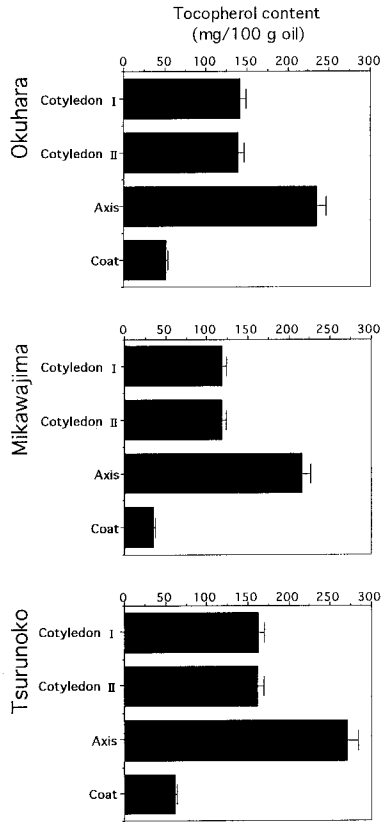

FIG. 2. Total tocopherol distributions in the oils prepared from each structural part and section of soybeans. Each value represents the average of three replicates, and vertical bars represent standard errors of the replicates.

Lipid components within each structural part and section of soybean. Profiles of TAG, 1,3- and 1,2-DAG, SE, and polar lipids were compared in the oils obtained from cotyledons, axis, and seed coat (Table 1). The major fraction in all cultivars was TAG, composing 70% in axis and coat, and 94% in cotyledons. Wilson and Kwanyuen (27) reported that, at maturity, the oil content of soybean seeds was approximately 20% (w/w), and 90% of this was TAG. The other acyl lipids, such as 1,3-DAG, 1,2-DAG, SE, and FFA are minor components and are designated as "others" in Table 1. On a percentage basis, the greatest amount of polar lipids was found in the axis (25.0 to 25.6%), followed by seed coat (17.2 to 18.3%) and cotyledons (5.7 to 7.3%).

Fatty acid composition of TAG within each structural part and section of soybean. Fatty acid distributions (expressed in terms of the esters by weight) of TAG in the cultivars Mikawajima and Tsurunoko were compared in cotyledons I and II, axis, and seed coat (Fig. 4). The fatty acid composi-

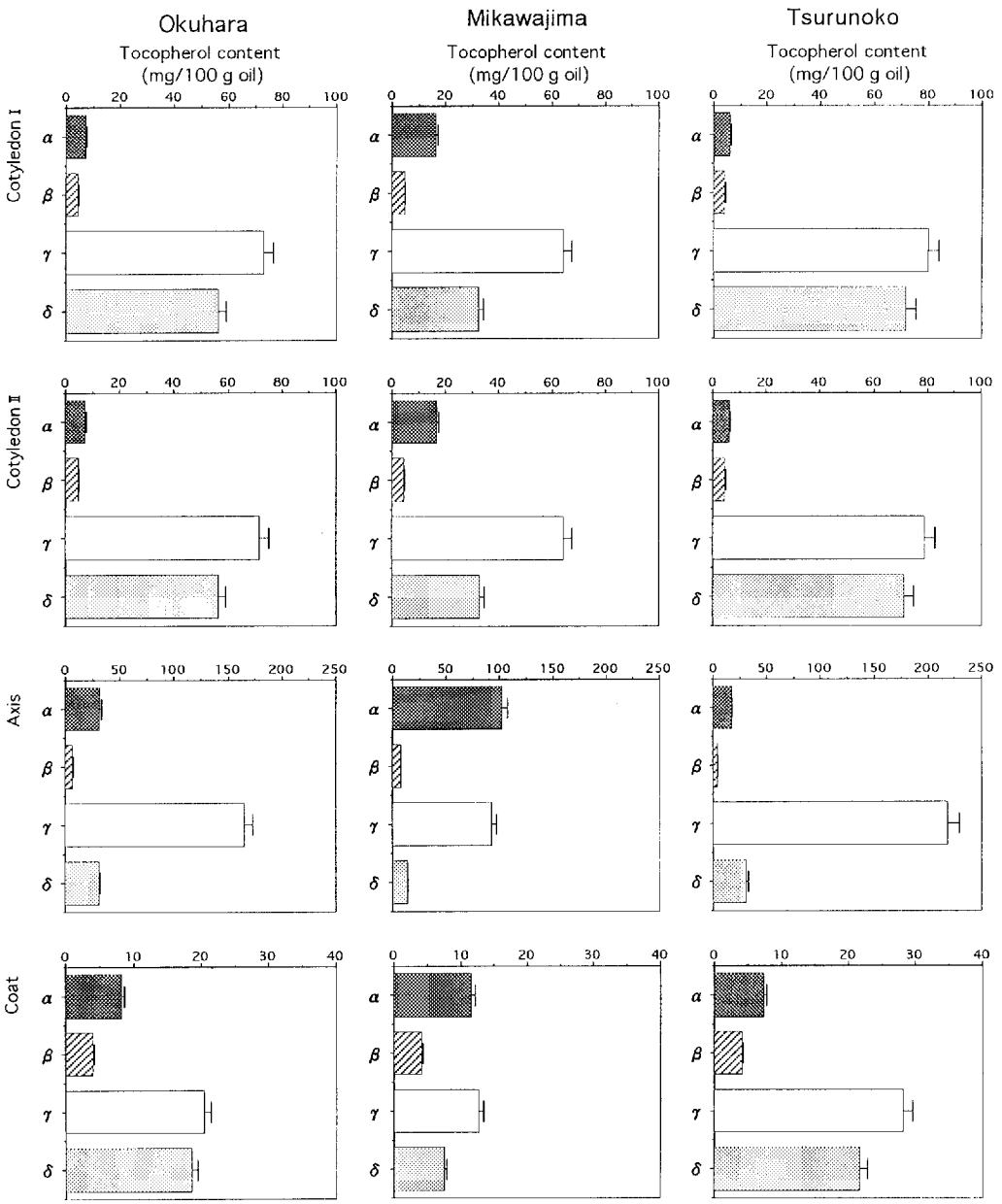

FIG. 3. The content of individual tocopherols in the oils prepared from each structural part and section of soybeans. Each value represents the average of three replicates, and vertical bars represent standard errors of the replicates.

tion was essentially the same not only in the two cultivars but also among the individual tissue types. The principal fatty acids for each genotype and for each tissue were: linoleic, oleic, palmitic, linolenic, and stearic. However, a small difference ($P < 0.05$) occurred in fatty acid composition when comparing cotyledons and seed coat to the axis. The data showed that the percentage composition of linolenic acid was higher than that of oleic acid in the axis, while the reverse was true in the cotyledons and seed coat. The pattern of fatty acid composition in TAG for Okuhara is not shown in Figure 4 because it was essentially the same as that found in Tsurunoko.

Fatty acid composition and distribution in phospholipids. Figure 5 shows the fatty acid profiles in the total phospholipid fractions from cotyledons I and II, axis, and seed coat for cultivars Okuhara and Mikawajima. The axis and seed coat contained higher concentrations of linolenic acid than the cotyledons in both cultivars. The data for Tsurunoko are not shown in Figure 5 because they were essentially the same as those for Okuhara. To determine the fatty acid composition of individual phospholipids within each structural part and section of soybean, major phospholipids were isolated from polar lipids by TLC and then fractionated into PE, PC, PI, and other phospholipids. These were further separated on TLC plates and identified by comigration with authentic standards. Table

TABLE 1
Lipid Components in the Oils Obtained from Each Structural Part and Section of Soybeans[a]

Cultivar	Tissue	Total lipids	Triacylglycerols	Polar lipids	Others[b]
Okuhara	Cotyledon I	5682.6^{d}	5324.9^{d} (93.7)	327.5^{d} (5.8)	30.2^{c,d} (0.5)
	Cotyledon II	5657.6^{d}	5306.8^{d} (93.8)	322.5^{d} (5.7)	28.3^{d} (0.5)
	Axis	173.7^{f}	124.5^{f} (71.7)	43.6^{e,f} (25.1)	5.6^{g} (3.2)
	Coat	71.3^{h}	50.1^{i} (70.3)	12.3^{h} (17.2)	8.9^{f} (12.5)
Mikawajima	Cotyledon I	5767.6^{d}	5398.5^{d} (93.6)	334.5^{d} (5.8)	34.6^{c} (0.6)
	Cotyledon II	5740.5^{d}	5373.5^{d} (93.6)	332.6^{d} (5.8)	34.4^{c} (0.6)
	Axis	161.9^{f}	114.5^{g} (70.7)	41.4^{f} (25.6)	6.0^{g} (3.7)
	Coat	70.1^{h}	48.5^{i} (69.2)	12.8^{h} (18.3)	8.8^{f} (12.5)
Tsurunoko	Cotyledon I	7472.7^{c}	6895.1^{c} (92.3)	544.8^{c} (7.3)	32.8^{c,d} (0.4)
	Cotyledon II	7456.5^{c}	6881.3^{c} (92.3)	543.1^{c} (7.3)	32.1^{c,d} (0.4)
	Axis	198.4^{e}	140.5^{e} (70.8)	49.6^{e} (25.0)	8.3^{f} (4.2)
	Coat	113.6^{g}	78.6^{h} (69.2)	19.7^{g} (17.3)	15.3^{e} (13.5)

[a]Each value is an average of three determinations and expressed as mg lipid per 200 tissues. Values in parentheses are relative contents of the individual lipids in total lipids.
[b]Contains steryl esters, free fatty acids, and 1,3- and 1,2-diacylglycerols.
[c–i]Values in the same column with different superscript letters are significantly different from those among the three cultivars ($P < 0.05$).

2 represents the profiles of PE, PC, PI, and others within each structural part and section of soybean. Phosphatidic acid, diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylserine are shown as "others" in Table 2. Regardless of the soybean cultivar, PC, PE and PI were major phospholipids in all tissues, and the highest concentration was observed for PC (45.0 to 49.3%), followed by PE (18.0 to 26.0%) and PI (12.5 to 21.7%). The proportion of PE, PC, and PI in this study differed from that typically reported in soybean phospholipids (28), possibly because of varietal differences. However, similar results have been reported by others (29,30). These data also showed higher PE concentration in Tsurunoko than in the other cultivars.

Figure 6 shows a typical fatty acid distribution of PE, PC, and PI within each structural part and section of soybean (Okuhara and Mikawajima). Each phospholipid class had a distinct fatty acid composition, depending on the tissue examined (coat tissue). However, in all soybean cultivars, the fatty acid distribution in PI differed from those of PE and PC in each tissue. The percentage of palmitic acid was higher, especially in axis and seed coat. In PE and PC, linoleic concentration was greater, followed by palmitic in all samples except for seed coat

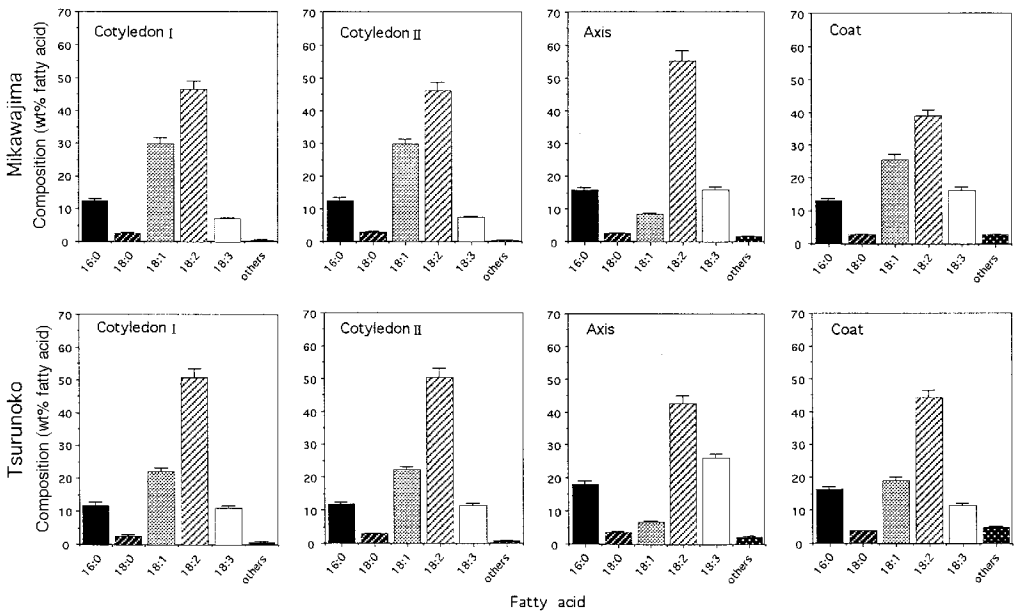

FIG. 4. Fatty acid distributions of triacylglycerols in the oils prepared from each structural part and section of soybeans. Averages of three replicates. Vertical bars represent standard errors of the replicates. "Others" contained 16:1, 16:2, 17:0, 20:0, and 22:0.

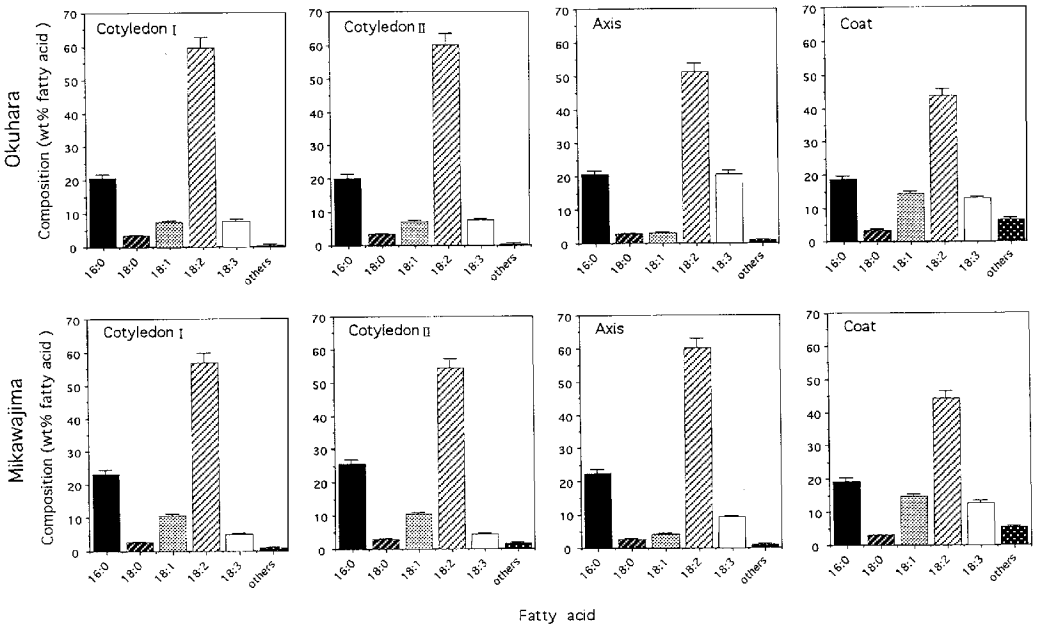

FIG. 5. Fatty acid distributions of phospholipids in the oils prepared from each structural part and section of soybeans. Averages of three replicates. Vertical bars represent standard errors of the replicates. "Others" contained 16:1, 16:2, 17:0, 20:0, and 22:0.

tissue of Mikawajima. The percentage of palmitic acid in both phospholipids was significantly higher in the seed coat tissue from this cultivar than in cotyledon or axis of the other varieties.

In the cotyledon tissue, fatty acids are found in lipid storage bodies, whereas growing tissues such as the axis contain almost no storage lipids. Thus, fatty acids in the axis are found in membranes. Because membrane lipids are involved in fundamental cell processes, they are highly conserved in terms of both quantity and quality (31). The low oleic acid content (Figs. 4 and 5), accompanied by slightly higher amounts of linolenic acid in axis, is consistent with the fact that the axis is the most active tissue of soybean seeds. The food or feed value of soybean axes is unknown. However, Synder and Kwon (32) reported that the embryo axis is the source of beany off-flavors, and some processors of soy milk have tried to remove the germ to avoid off-flavors in soy milk. Our observation of high levels of polyunsaturated fatty acids in the soybean axis supports this practice. These results indicate that differences in soybean varieties can be predicted by determining the regional distribution of tocopherols and fatty acids within soybean component parts. Therefore, further studies are necessary to demonstrate what roles tocopherols play in relation to various acyl lipids within each structural part and section of soybean seeds.

TABLE 2
Phospholipid Contents in the Oils Obtained from Each Structural Part and Section of Soybeans[a]

Cultivar	Tissue	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylinositol	Others[b]
Okuhara	Cotyledon I	72.6^{d} (24.1)	148.4^{d} (49.3)	52.1^{e} (17.3)	28.0^{c} (9.3)
	Cotyledon II	72.3^{d} (24.3)	145.6^{d} (49.0)	51.9^{e} (17.4)	27.6^{c} (9.3)
	Axis	11.3^{f} (27.3)	19.5^{f} (47.1)	6.3^{f} (15.2)	4.3^{f} (15.2)
	Coat	2.8^{h,i} (23.3)	6.2^{h,i} (51.7)	1.5^{i} (12.5)	1.5^{h} (12.5)
Mikawajima	Cotyledon I	57.2^{e} (20.6)	135.5^{e} (49.0)	59.2^{d} (21.4)	24.8^{d} (9.0)
	Cotyledon II	56.8^{e} (20.9)	132.8^{e} (48.9)	58.3^{d} (21.5)	23.8^{d} (8.8)
	Axis	8.4^{g} (25.0)	15.2^{g} (45.2)	5.8^{g} (17.3)	4.2^{f} (12.5)
	Coat	2.3^{i} (18.0)	5.8^{i} (45.3)	1.7^{i} (13.3)	3.0^{g} (23.4)
	Cotyledon I	104.7^{c} (27.5)	175.4^{c} (46.0)	72.3^{c} (19.0)	28.6^{c} (7.5)
Tsurunoko	Cotyledon II	104.2^{c} (27.6)	173.7^{c} (45.9)	71.9^{c} (19.0)	28.4^{c} (7.5)
	Axis	8.9^{g} (22.8)	18.5^{f} (47.3)	6.7^{f} (17.1)	5.0^{e} (12.8)
	Coat	3.1^{h} (19.4)	7.6^{h} (47.5)	2.3^{h} (14.4)	3.0^{g} (18.8)

[a]Each value is an average of three determinations and expressed as mg lipid per 200 tissues. Values in parentheses are relative contents of the individual lipids in phospholipids.
[b]Contains diphosphatidylglycerol, phosphatidic acid, phosphatidylglycerol, and phosphatidylserine.
[c–i]Values in the same column with different superscript letters are significantly different from those among the three cultivars ($P < 0.05$).

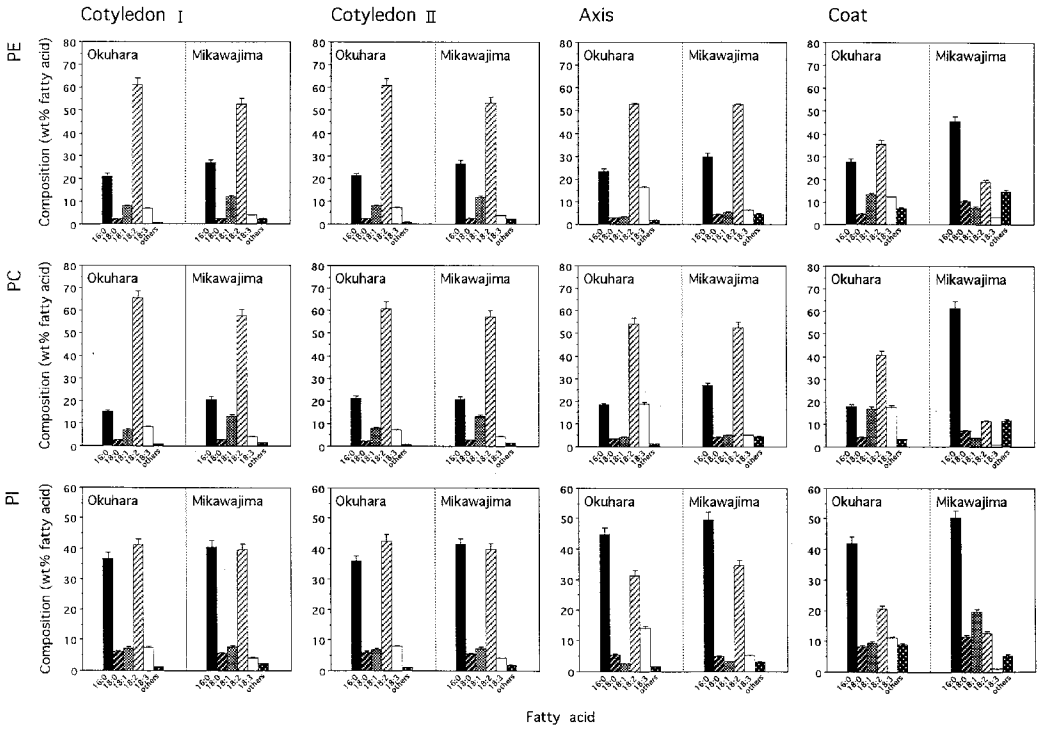

FIG. 6. Fatty acid distributions of major phospholipids in the oils prepared from each structural part and section of soybeans. Averages of three replicates. Vertical bars represent standard errors of the replicates. "Others" contained 16:1, 16:2, 17:0, 20:0, and 22:0. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol.

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