Tocopherol Distribution and Oxidative Stability of Oils Prepared from the Hypocotyl of Soybeans Roasted in a Microwave Oven

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ABSTRACT: Whole soybeans (*Glycin max* L.) were roasted by exposure to microwaves at a frequency of 2,450 MHz, and their hypocotyls were separated from other tissues (seed coat and cotyledons). The quality characteristics and composition in the hypocotyl oils were studied in relation to their tocopherol distributions and were evaluated as compared to an unroasted oil sample. Only minor increases ($P < 0.05$) in chemical and physical changes of the oils, such as carbonyl value, anisidine value and color development, occurred with increased roasting time. Significant decreases ($P < 0.05$) were observed in the amounts of phospholipids in the oils after microwave roasting. Nevertheless, compared to the original level, more than 80% tocopherols still remained after 20 min of roasting. These results suggest that the exposure of soybeans to microwaves for 6 to 8 min caused no significant loss or changes in the content of tocopherols and polyunsaturated fatty acids in the hypocotyls. Therefore, a domestic microwave oven would be useful as a simple and quick means for preparing hypocotyl oil of good quality.

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KEY WORDS: Anisidine value, carbonyl value, hypocotyl, microwave roasting, oxidative stability, peroxide value, soybeans, TBARS (thiobarbituric acid-reactive substances), vitamin E homologs.

Microwave ovens are found in the majority of homes in Japan, and today more people use microwaves for cooking and reheating than ever before. 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. However, the differential heating behavior of food components can result in severely uneven heating of certain foods rich in fats and proteins (1). Consumers are concerned by reports that noxious compounds are produced in microwaved food (2).

Little investigation has been conducted concerning the effects of microwave roasting on the oxidative stability of the oils in relation to tocopherol distribution in whole soybeans. We demonstrated the effects of microwave energy on the oxidative stability of the oils (3) or the fatty acid distribution (4) in whole soybeans. The distribution of lipoxygenase activity in whole soybeans is remarkable in the hypocotyl, followed by the cotyledons, and the seed coat (5). Therefore, Snyder and Kwon (6) reported that the hypocotyl is the source of beany off-flavors, and some processors of soymilk have tried to remove the hypocotyl to avoid off-flavors in soymilk. Microwaves were also applied to inactivate soybean lipoxygenase to prevent the formation of undesirable off-flavors (7). This work showed the interrelationship treatment time, enzyme inactivation, and protein extractability. However, no research has been reported on how microwave roasting affects not only the distribution of tocopherols but also the oxidative stability of the lipids, especially within each structural part (seed coat, cotyledons, and hypocotyl) of soybeans. We reported that the hypocotyl of soybeans is high in tocopherols and polyunsaturated fatty acids, especially linoleic and linolenic acids (8). For the purpose of effectively using the hypocotyl, this study was undertaken to investigate the interrelation between the oxidative stability of lipids and the content of tocopherols in the hypocotyl of soybeans when roasted in a domestic microwave oven.

MATERIALS AND METHODS

Samples. Commercially available soybeans (*Glycine max* L.) used for this study were from three Japanese cultivars, Mikawajima, Okuhara, and Tsurunoko, and grown during the summer of l997. Cultivars were purchased from Takii Seed Co. (Kyoto, Japan) and were selected for uniformity based on bean weights of 300 to 369 mg for Mikawajima, 320 to 369 mg for Okuhara, and 360 to 429 mg for Tsurunoko. The beans were hand-selected to eliminate those with cracked or otherwise damaged seed coats. All beans divided into groups were sealed up polyethylene bags and stored in stainless steel containers at 4°C until needed.

Reagents. All chemicals were of analytical grade (Nacalai Tesque, Kyoto, Japan) and were used without further purification. Vitamin E homologs (β, γ, and δ) were purchased from Eisai Co (Tokyo, Japan). All tocopherols were of the D-form (*RRR*-), and the purity of each tocopherol was better than 98.5% when determined by high-performance liquid chro-

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matography (HPLC) as described later. Thin-layer chromatography (TLC) pre-coated silica-gel 60 plates (20 × 20 cm, 0.25 mm layer thickness) were purchased from Merck (Darmstadt, Germany). Fifty mg of methyl pentadecanoate (Merck) was dissolved in *n*-hexane and stored in a 10-mL glass volumetric flask until needed 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.

Roasting and sectioning of soybeans. Whole soybeans were placed in a single layer in Pyrex petri dishes (12.0 cm diameter), covered, and put on the rotating plate of a microwave oven (model R-5550; Sharp, Osaka, Japan). The internal dimensions of the cavity were 31.0 × 31.6 × 22.4 cm, and the beans were roasted for 6, 8, 12, or 20 min in the turntable mode based on previous results (3): roasting for 6–10 min was optimal to prepare full-fat soyflour without a burnt odor. The roasted soybeans were allowed to cool to ambient temperature prior to separating the hypocotyl from the other tissues (coat and cotyledons) with a razor blade.

Lipid extraction. Two thousand hypocotyls were crushed with 50 mL of chloroform/methanol (2:1, vol/vol) containing butylated hydroxytoluene (0.0l%) in a Waring blender (done at 0°C), and the lipids were extracted by vigorous shaking of triplicate samples, as described by Yoshida and Takagi (4). The combined extracts, dried in a rotary vacuum apparatus at 35°C, were dissolved in 100 mL of chloroform/methanol (2:1, vol/vol). The solution was washed with 20 mL aqueous solution of potassium chloride (0.75%) according to Folch *et al.* (9). The chloroform layer was removed, and the aqueous salt phase was further reextracted twice with 20 mL chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered, and the solvent removed *in vacuo* at temperatures below 35°C. Extracted lipids were weighed to determine the lipid content of the hypocotyl and then kept in chloroform/methanol (2:1, vol/vol) in 5-mL brown glass volumetric flasks under nitrogen in the dark at –25°C. Using the same procedures, lipids were extracted from the hypocotyl of raw soybeans for use as a control.

HPLC. A 100-mg portion of lipids, before and after microwave roasting, was transferred quantitatively to a 5-mL brown volumetric flask, and the solvents were removed under nitrogen gas at ambient temperature in a draft chamber. The residue was dissolved in the mobile phase for the HPLC, as described below. The chromatographic system consisted of a normal-bonded phase Shim-pack CLC-SIL (M) column (5 µm, 250 × 4.6 mm i.d.; Shimadzu, Kyoto, Japan) protected by a 10-mm guard column (Shim-pack G-SIL). A mixture of *n*-hexane/1,4-dioxane/ethanol (490:10:1, vol/vol/vol) was used as the mobile phase at a flow rate of 2.0 mL/min. An aliquot (5 µL) was injected with a fully loaded 20-µL loop. The tocoph-erols were monitored with a fluorescence detector (Shimadzu RF-535) and were quantitated as described previously (3).

Analysis of lipids. AOCS Official Methods (10) were used for determination of peroxide value (Method Cd 8-53) and 2-thiobarbituric acid reactive substances (TBARS; method Cd 19-90). The TBARS were expressed as mg malonaldehyde equivalent per kg oil. The *p*-anisidine value and carbonyl value of the oils were measured according to IUPAC and JOCS (Japanese Oil Chemists' Society) standard methods (11,12). As an index of color development (13), the absorbance at 420 nm of a 5.0% (wt/vol) solution of total lipid in chloroform was determined with a Shimadzu spectrophotometer UV-2500 PC.

Lipid class analysis. The total lipid was fractionated by TLC into two fractions, triacylglycerols (TAG) and polar lipids (PL). The plates were developed in *n*-hexane/diethyl ether/formic acid (60:40:1, vol/vol/vol) after applying a standard mixture as described previously (14). The phospholipid fraction was isolated from total lipids by multiple-development TLC. Neutral lipids were removed by developing with the solvents just mentioned, and glycolipids were further removed by developing with acetone/acetic acid/ water (100:2:l, vol/vol/vol). Bands corresponding to the TAG, PL, and phospholipids were scraped separately into a tube (105 × 16 mm) fitted with a Teflon-lined screwcap. Methyl pentadecanoate (15:0) was added as an internal standard to the total lipids and to each fraction at *ca.* 10% (w/w esters). After transesterification using the method of Morrison and Smith (15), fatty acid methyl esters were analyzed with a Shimadzu GC-14A gas chromatograph and were quantitated by means with a Shimadzu C-R4A electronic integrator (16). The other gas chromatographic conditions were as described previously (14).

Statistical analysis. All experiments were repeated in duplicate at each point before and after microwave roasting. Statistical evalution of the data was conducted using a Statistical Analysis System (17) with a general linear model analysis of variance. Significant differences among treatment means were determined using Duncan's multiple range test at a level of $P < 0.05$ (18).

RESULTS AND DISCUSSION

Microwave roasting and color development. Proximate analyses with AOAC (19) methods showed that the moisture content of soybeans was 8.0–8.3% before microwave roasting. The hypocotyl (1.8 to 1.9%) accounted for the remaining mass of the whole soybeans. No significant differences ($P > 0.05$) in these percentages were observed among the three cultivars. Effects of microwave roasting (cv. Tsurunoko) were compared on the basis of the internal temperature of the hypocotyl at the end of each roasting time (data not shown). The temperature of the hypocotyl samples was 25°C before roasting and increased from 98 to 165°C, at 6 and 20 min after microwave roasting, respectively. A dark brownish color and burnt smell became apparent at 12 min (125°C). Therefore, the optimal roasting time should be not more than 12 min when roasted in a domestic microwave oven. Although bean weights differed significantly ($P < 0.05$) among the three cultivars, there were no significant differences ($P > 0.05$) in the internal temperature at the end of each exposure. The color of

the lipids extracted from the hypocotyl changed gradually from light-yellow at 6–8 min of roasting to brown at 12 min, and finally deep-brown at 20 min. The results were also supported by an increase of the absorbance in the oils as shown in Figure 1. There were significant differences ($P < 0.05$) in the color of total lipids among the three cultivars after 12 min of microwave roasting. The color development was mostly higher in Okuhara and Tsurunoko cultivars than in Mikawajima. This may reflect differences in the composition of their fatty acids, especially with respect to the content of linolenic acid (Tables 1 and 2). All data at 8 min of microwave roasting are omitted from Figures and Tables because the data were essentially the same as those found at 6 min.

Tocopherol distributions. The effects of microwave roasting were compared for total and individual tocopherol contents in the hypocotyl based on the cultivars (Fig. 2). Tocopherol contents varied significantly ($P < 0.05$) among the three cultivars before microwave treatments, and the greatest amount of total tocopherols was observed in Tsurunoko, fol-

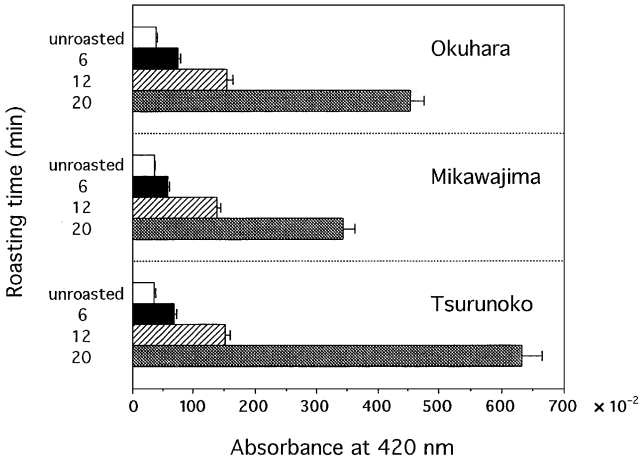

FIG. 1. Changes in absorbance (color) of the oils prepared from the hypocotyl of soybeans roasted in a domestic microwave oven (at a frequency of 2,450 MHz). Each value represents the average of two replicate and horizontal bars represent standard error of the replicates.

TABLE 1
Fatty Acid Composition of Triacylglycerols in the Oils Prepared from the Hypocotyl of Soybeans Roasted in a Domestic Microwave Oven[a,b]

Cultivar	Roasting time (min)	Total fatty acid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
Okuhara	Unroasted	100^{c}	0.3^{c}	13.9^{c}	0.4^{c}	2.9^{d}	8.3^{e}	44.5^{d}	29.2^{d}	0.5^{c}
	6	98.21^{c}	0.3^{c}	14.3^{c}	0.4^{c}	2.9^{d}	8.5^{e}	44.0^{d}	28.8^{d}	0.8^{e}
	12	93.34^{d}	0.6^{d}	14.5^{c}	0.4^{c}	3.0^{d}	8.8^{e}	43.6^{d}	28.3^{e}	0.8^{e}
	20	85.30^{e}	0.8^{d}	15.2^{d}	0.4^{c}	3.0^{d}	10.4^{f}	43.2^{d}	26.3^{f}	0.7^{e}
Mikawajima	Unroasted	100^{c}	0.2^{c}	14.6^{c}	0.3^{c}	2.7^{c}	7.5^{d}	50.3^{c}	24.0^{f}	0.4^{c}
	6	97.62^{c}	0.2^{c}	14.8^{d}	0.3^{c}	2.7^{c}	7.5^{d}	50.2^{c}	23.8^{g}	0.5^{c}
	12	92.94^{d}	0.3^{c}	15.0^{d}	0.3^{c}	2.8^{d}	7.8^{d}	50.0^{c}	23.2^{g}	0.6^{d}
	20	84.18^{e}	0.4^{c}	15.2^{d}	0.3^{c}	3.0^{d}	8.2^{e}	49.8^{c}	22.6^{h}	0.5^{c}
Tsurunoko	Unroasted	100^{c}	0.2^{c}	14.2^{c}	0.5^{c}	2.6^{c}	6.0^{c}	44.3^{d}	32.0^{c}	0.2^{c}
	6	97.08^{c}	0.3^{c}	14.3^{c}	0.5^{c}	2.6^{c}	6.2^{c}	44.2^{d}	31.5^{c}	0.4^{c}
	12	91.37^{d}	0.4^{c}	15.0^{d}	0.6^{d}	2.7^{c}	6.3^{c}	43.9^{d}	30.5^{c}	0.6^{d}
	20	81.05^{e}	0.5^{c}	15.4^{d}	0.7^{d}	2.7^{c}	6.5^{c}	43.6^{d}	30.0^{d}	0.6^{c}

[a]Each value is an average of two determinations. The content of total fatty acids in each sample was calculated relative to the unroasted sample (100%). The content of each individual fatty acid is given as a percentage of the corresponding total.
[b]"Others" contains 16:2, 17:0, 20:0, and 22:0.
[c,d,e,f,g,h]Values in the same column with different superscript letters are significantly different from those for unroasted seeds among the three cultivars ($P < 0.05$).

TABLE 2
Fatty Acid Distribution of Phospholipids in the Oils Prepared from the Hypocotyl of Soybeans Roasted in a Domestic Microwave Oven[a,b]

Cultivar	Roasting time (min)	Total fatty acid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
Okuhara	Unroasted	100^{c}	0.3^{c}	19.4^{c}	0.3^{c}	2.8^{c}	3.4^{e}	52.6^{d}	21.0^{d}	0.2^{c}
	6	93.02^{d}	0.4^{c}	19.6^{c}	0.3^{c}	3.0^{d}	3.5^{e}	51.9^{d}	20.8^{d}	0.5^{c}
	12	86.00^{e}	0.7^{d}	19.8^{c}	0.3^{c}	3.2^{d}	3.6^{f}	51.5^{d}	20.5^{d}	0.4^{c}
	20	74.65^{f}	0.7^{d}	20.2^{d}	0.3^{c}	3.2^{d}	3.8^{g}	51.1^{d}	20.2^{d}	0.5^{c}
Mikawajima	Unroasted	100^{c}	0.6^{d}	20.2^{d}	0.5^{c}	2.9^{c}	3.7^{f}	56.8^{c}	14.7^{e}	0.6^{d}
	6	92.13^{d}	0.6^{d}	20.6^{d}	0.5^{c}	2.9^{c}	3.8^{g}	56.2^{c}	14.6^{e}	0.8^{d}
	12	87.30^{e}	0.7^{d}	21.6^{d}	0.5^{c}	3.0^{d}	3.8^{g}	55.4^{c}	14.4^{e}	0.7^{d}
	20	74.02^{f}	1.2^{e}	21.8^{e}	0.5^{c}	3.2^{d}	4.0^{h}	54.3^{c}	14.2^{e}	0.8^{d}
Tsurunoko	Unroasted	100^{c}	0.2^{c}	19.2^{c}	0.3^{c}	2.8^{c}	2.7^{c}	49.4^{e}	25.5^{c}	0.2^{c}
	6	93.71^{d}	0.2^{c}	19.4^{c}	0.3^{c}	2.8^{c}	2.7^{c}	49.3^{e}	25.0^{c}	0.3^{c}
	12	84.32^{d}	0.3^{c}	19.8^{c}	0.3^{c}	2.9^{c}	2.8^{c}	49.2^{e}	24.3^{c}	0.4^{c}
	20	72.48^{f}	0.3^{c}	20.6^{d}	0.3^{c}	2.9^{c}	2.9^{d}	48.5^{e}	24.0^{c}	0.5^{c}

[a]Each value is an average of two determinations. The content of total fatty acids in each sample was calculated relative to the unroasted sample (100%). The content of each individual fatty acid is given as a percentage of the corresponding total.
[b]"Others" contains 16:2, 17:0, 20:0, and 22:0.
[c,d,e,f,g,h]Values in the same column with different superscript letters are significantly different from those for unroasted seeds among the three cultivars ($P < 0.05$).

lowed by Mikawajima and Okuhara. As microwave roasting progressed, the tocopherol concentration gradually decreased at a similar rate in all cultivars, and the percentages of the losses increased significantly ($P < 0.05$) after a 12-min exposure time. Figure 2 also shows the individual tocopherol distributions within the hypocotyl before and after microwave roasting. A similar profile in each tocopherol distribution was observed in Okuhara and Tsurunoko: γ-tocopherol was dominant component. On the other hand, the distribution of tocopherol homologs in Mikawajima differed significantly ($P < 0.05$) from those for the other two cultivars: α-and γ-tocopherols were main components. However, β-tocopherol occurred at very low levels in the three cultivars. Almonor *et al.* (20) suggested that the antioxidant capacity of γ-tocopherol appeared to be directly associated with changes in oil quality that were mediated more by genetic than by enviromental influences on the concentration of linolenic acid. The percentages of tocopherol losses gradually increased with longer microwave processing. After 20 min of roasting, however, the amount of tocopherol homologs was still over 80% of the original levels (with few exceptions) among the three cultivars.

Oxidative stability and lipid components. The characteristics and qualities of the oils prepared from the hypocotyl of soybeans roasted in the range of 6 to 20 min are shown in Figure 3. The peroxide value serves as an indicator of the extent of primary oxidation products in the oils, while the carbonyl value, anisidine value or TBARS value reflects the degree of secondary oxidation products (21). There were only minor significant increases ($P < 0.05$) in parameters such as peroxide, carbonyl, anisidine, and TBARS values, in the hypocotyl oils prepared until 6 min. Thereafter, these values increased with longer roasting periods, especially the anisidine and TBARS values. The results imply that carbonyl, anisidine, and TBARS values would be more useful than peroxide value when evaluting by chemical means the quality of hypocotyl oils after roasting. According to the results shown in Figure 1, the color development of the oils increased markedly with roasting time up to 12 min. The color formation in the hypocotyl oil could be attributed to both nonenzymatic browning and phospholipid degradation during roasting, since phospholipids are reported to cause browning during roasting (22). The amount of lipid components changed during microwave treatment (Table 3); however, the major fractions were TAG and phospholipids, comprising 68.2 to 72.4% and 20.0 to 26.4% of the total lipids, respectively, following microwave roasting. With increased microwave roasting time, an appreciable loss was more apparently observed in phospholipids than in total lipids (mainly TAG). Hafez *et al.* (23) reported not only an increase in browning substances but also a decrease in phospholipids during microwave heating. The increase in browning substances may be attributed to the increase of PL (data not shown). Therefore, the percentage of the phospholipid losses was more pronounced ($P < 0.05$) than that for total lipids or TAG during microwave roasting (Table 3).

Fatty acid distribution of TAG and phospholipids. Table 1 represents the fatty acid composition (expressed in terms of

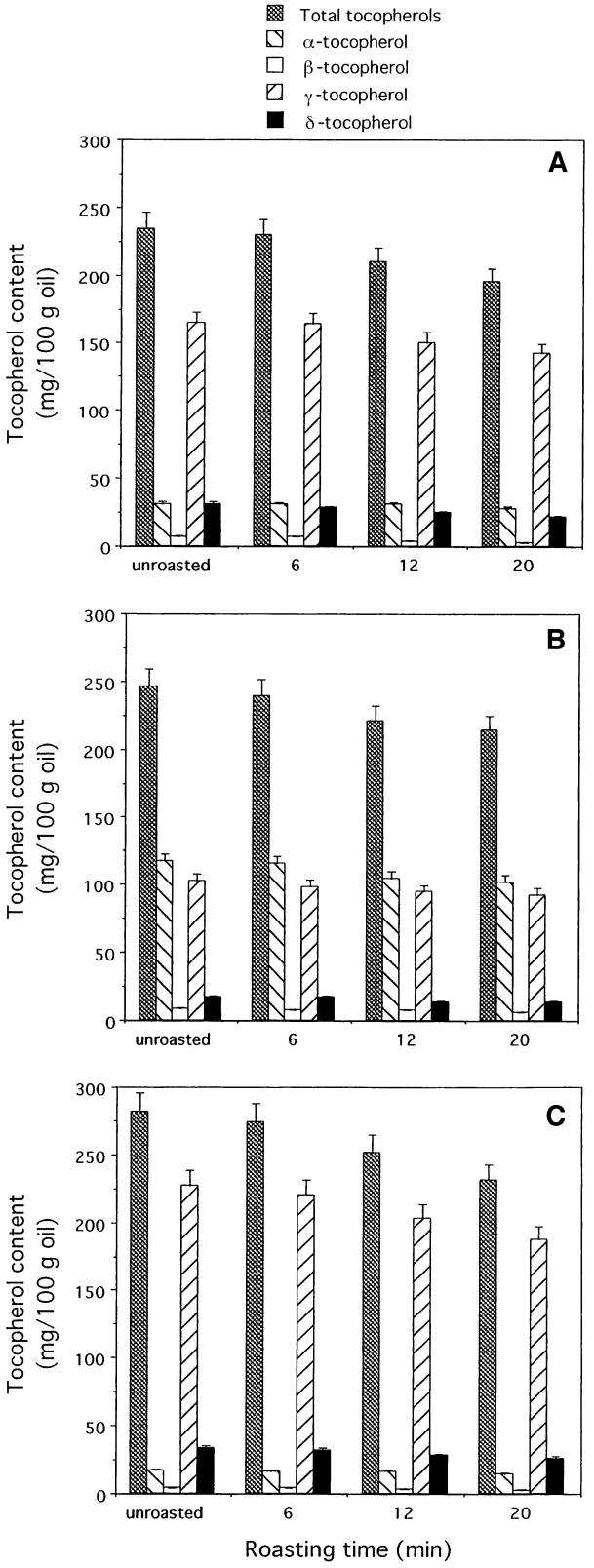

FIG. 2. Changes in total and individual tocopherols in the oils prepared from the hypocotyl of soybeans roasted in a domestic microwave oven (at a frequency of 2,450 MHz). Each value represents the average of two replicate and vertical bars represent standard error of the replicates. (A) Okuhara; (B) Mikawajima; (C) Tsurunoko.

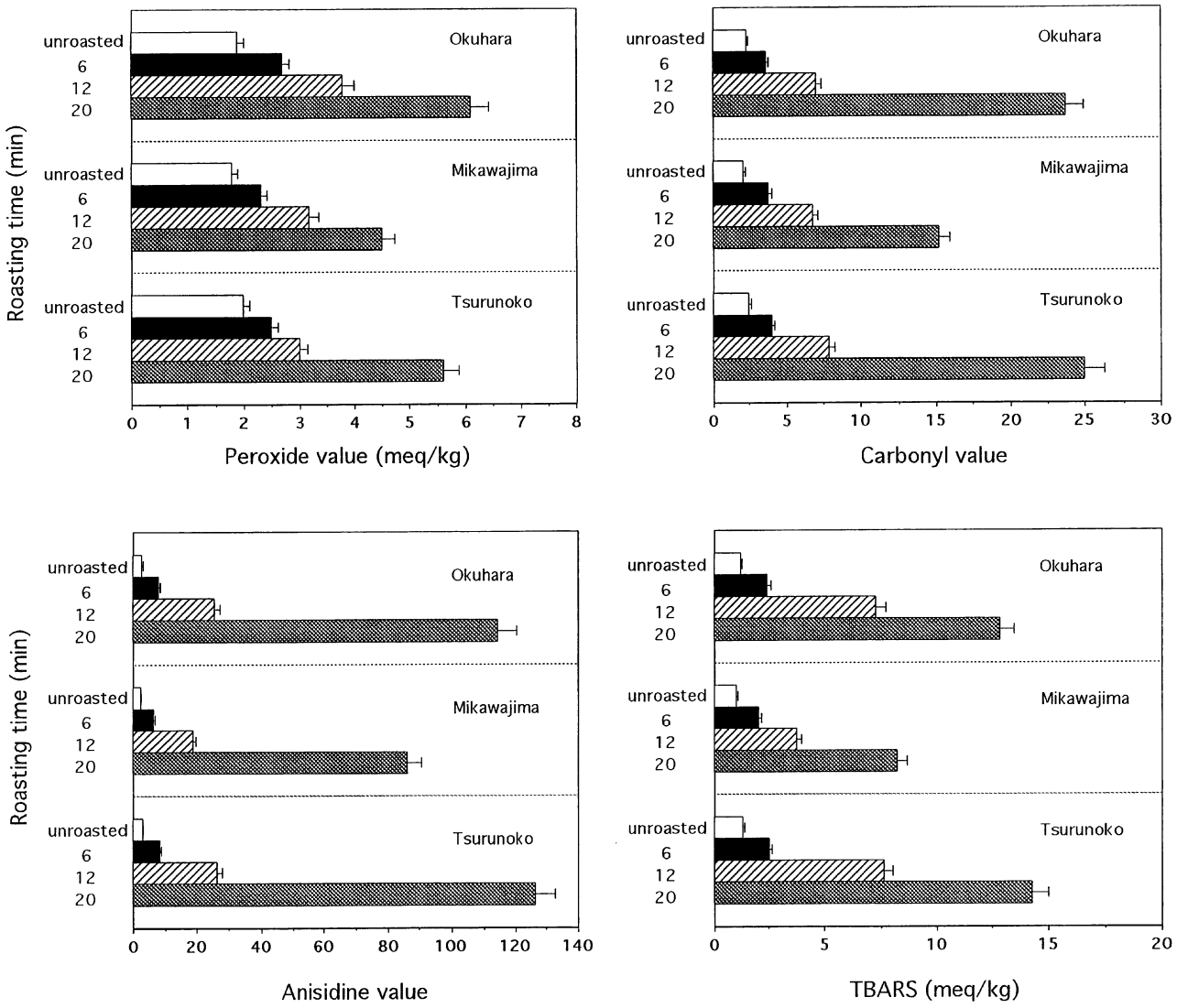

FIG. 3. Changes in selected quality parameters of the oils prepared from the hypocotyl of soybeans roasted in a domestic microwave oven (at a frequency of 2,450 MHz). Each value represents the average of two replicate and horizontal bars represent standard error of the replicates.

TABLE 3
Lipid Contents in the Oils Prepared from the Hypocotyl of Soybeans Roasted in a Domestic Microwave Oven[a]

		(mg/1,000 Hypocotyl)		
Cultivar	Roasting time (min)	Total lipids	Triacylglycerols	Phospholipids
Okuhara	Unroasted	848.9^{c}	586.2^{c}	223.9^{b}
	6	823.0^{c,d}	575.5^{c,d}	208.3^{c}
	12	786.2^{d}	546.7^{d}	192.6^{d}
	20	729.3^{e}	498.2^{e}	167.3^{e}
Mikawajima	Unroasted	839.9^{c}	603.5^{c}	191.3^{d}
	6	814.4^{c}	589.3^{c}	176.1^{e}
	12	780.7^{d}	560.7^{d}	167.0^{e}
	20	724.9^{e}	508.1^{e}	145.2^{f}
Tsurunoko	Unroasted	937.6^{b}	661.9^{b}	232.2^{b}
	6	912.8^{b}	642.2^{b}	217.6^{c}
	12	865.4^{c}	605.3^{c}	195.8^{d}
	20	786.0^{d}	536.4^{d}	168.3^{e}

[a]Each value is an average of two determinations and expressed as mg lipid per 1,000 seeds.
[b,c,d,e,f]Values in the same column with different superscript letters are significantly different from those of unroasted seeds among the three cultivars ($P < 0.05$).

the esters by weight) of TAG in the oils prepared from the hypocotyl before and after microwave roasting. The fatty acid compositions of total lipids were omitted from Table 1 because TAG made up greater than 70% in the hypocotyl oils (Table 3), and their fatty acid compositions were essentially the same as the fatty acid profiles of total lipids (data not shown). Phospholipids were minor components of the hypocotyl (Table 3), and their fatty acid distributions were not the same as those of TAG (Tables 1 and 2). There was a small difference ($P < 0.05$) in fatty acid composition among the three cultivars and between TAG and phospholipids before microwave roasting. Significant differences ($P < 0.05$) occurred in fatty acid compositions of TAG between Mikawajima and Okuhara or Tsurunoko. Mikawajima was higher (49.8 to 50.3%) in linoleic and lower (22.6 to 24.0%) in linolenic than were Okuhara or Tsurunoko, and similar results were observed in phospholipids; the former was higher (54.3 to 56.8%) in linoleic and lower (14.2 to 14.7%) in linolenic than those the latter cultivars (Table 2). There was little change in fatty acid composition of TAG and phospholipids of the hypocotyl oils when roasted for 6 min in a domestic microwave oven. But the longer the roasting time, the greater were the relative percentages of palmitic, stearic and oleic acids, and the less were those of linoleic and linolenic acids. However, these trends were not so pronounced as the results obtained from whole soybeans (3). Tocopherols are predominantly detected in the hypocotyl, followed by the cotyledons and the seed coat (8). The primary components were γ-tocopherol in Okuhara and Tsurunoko, but α- and γ-tocopherols in Mikawajima (Fig 2). Although γ-tocopherol has a lower vitamin E value in biological systems (24) than α-tocopherol, it is a more potent antioxidant in oils. Probably, the polyunsaturated fatty acids in the hypocotyl may be protected by γ-tocopherol from microwave roasting.

These results indicate that the exposure of soybeans to microwaves for 6 to 8 min caused no significant loss or changes in the content of vitamin E and polyunsaturated fatty acids in the hypocotyls. This information may be that the use of short-term exposure to microwaves to retard seed deterioration is technically feasible and should be examined for economic feasibilty at oilseed processing mills. Further studies are necessary to demonstrate how microwave energy plays a role in inactivating enzymes present in the hypocotyl and thus leads to the stabilization of the oil.

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