Microwave Roasting and Molecular Species of Triacylglycerols in Soybean Embryonic Axes

Hiromi Yoshida* and Sachiko Takagi

Department of Nutritional Science, Kobe-Gakuin University, Kobe 651-2180, Japan

ABSTRACT: Embryonic axes were separated from soybeans roasted in a microwave oven. Molecular species and fatty acid distributions of triacylglycerols (TAG) isolated from total lipids in the embryonic axis were analyzed by a combination of argentation thin-layer chromatography (TLC) and gas–liquid chromatography. A modified argentation-TLC procedure, developed to optimize the separation of the complex mixture of total TAG, provided 15 different groups of TAG, based on both the degree of unsaturation and the total length of fatty acid groups. Fatty acid methyl ester analysis was performed to determine the composition of each band. Fifteen molecular species of TAG were still found in the embryonic axes following roasting treatment. Microwave roasting for 6 min did not change the molecular species of the embryonic axis TAG (with a few exceptions), nor cause a loss of unsaturated fatty acids. However, microwave roasting for 12 min caused a significant decrease ($P < 0.05$) not only in molecular species containing more than four double bonds but also in the amount of diene and triene species present in TAG. These results suggest that no significant changes in molecular species or fatty acid distribution of TAG would occur within 6 min of microwave roasting, ensuring that a good quality product would be attained.

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KEY WORDS: $AgNO_3$–TLC, embryonic axis, microwave roasting, molecular species, soybeans, triacylglycerols.

The heating of foods in a microwave oven is caused by molecular friction of electrical dipoles under an oscillating electric field of specific frequency. Water is the most abundant dipole component in foods, but others (salt, fats, and proteins) also act as dielectric components (1,2). Microwave ovens are credited with rapid heating rates and high efficiency, especially owing to their high penetration power; however, the differential heating behavior of food components can result in severely uneven heating of certain foods rich in fats and proteins (3).

Microwave ovens have penetrated the majority of homes in Japan and today more people use microwaves for cooking and reheating than ever before. The appliance user prefers the microwave cooking procedure, which is characterized by speed and short cooking time compared to classical heating methods. In fact, microwaves are used in the food industry not only for baking, thawing, drying and warming but also for other applications, such as sterilizing or pasteurizing many types of food (4). Microwave heating can provide several advantages over conventional food-processing methods (5). 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 (2,6).

The number of household microwave ovens in use is increasing, mainly because consumers appreciate the advantages of owning such a product, such as economy, convenience, and savings (7). However, from time to time, consumers are concerned by reports (8) that objectionable compounds are produced in microwaved foods. The principle on which microwave roasting is based differs from that of conventional heating by conduction or convection, so it is technically difficult to measure and standardize the relevant parameters. The embryonic axis of soybeans is high in tocopherols and polyunsaturated fatty acids, especially linoleic and linolenic acids (9). Tocopherols 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 reaction (10). Many studies have been conducted on the general nature and fatty acid level of whole soybeans. Triacylglycerols (TAG) are the major fraction of total lipids, representing 70% of the embryonic axis and seed coat and 94% of the cotyledon (9). Hitherto, virtually no information has been reported on how microwave energy affects the fatty acid distributions of TAG, the main component of the embryonic axis. The aim of this study was to isolate TAG from the embryonic axis of soybeans roasted in a domestic microwave oven and, further, to determine changes in molecular species composition and fatty acid distribution of TAG during microwave roasting.

EXPERIMENTAL PROCEDURES

Materials. Commercially available soybeans (*Glycine max* L.) used for this study were obtained from three Japanese cultivars, Mikawajima, Okuhara, and Tsurunoko, grown during the summer of 1997. Soybeans were purchased from Takii

*To whom correspondence should be addressed at Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe 651-2180, Japan. E-mail: yoshida@nutr.kobegakuin.ac.jp

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 cleaned, and those with cracked or otherwise damaged seed coats were removed. The remaining selected beans were divided into groups and stored in stainless steel containers at 4°C until needed.

Reagents and standards. All chemicals were of analytical grade (Nacalai Tesque, Kyoto, Japan), and were used without further purification. Thin-layer chromatography (TLC) precoated silica-gel 60 plates (20 × 20 cm, 0.05-mm layer thickness) were purchased from Merck (Darmstadt, Germany). Standard TAG (trimyristin, tripalmitin, tristearin, triolein, trilinolein, and trilinolenin) were procured from Sigma Chemical Co. (St. Louis, MO). One hundred milligrams of methyl pentadecanoate (Merck) was dissolved in *n*-hexane (20 mL) and used as an internal standard. Boron trifluoride (14%) in methanol (Wako Pure Chemical Ind., Osaka, Japan) was used to prepare the fatty acid methyl esters.

Microwave roasting and lipid extraction. Whole soybeans were placed in a single layer in Pyrex petri dishes (12.0 cm diameter), then placed on the turntable of the microwave oven (Sharp model R-5550; Osaka, Japan). After covering the dishes, the contents were then roasted for 6, 12, or 20 min [times based on previous results (11)]. As soon as they were taken out of the oven, the internal temperature of the treated soybeans was detemined with a chromel-alumel thermocouple as previously described (12). The roasted soybeans were allowed to cool to ambient temperature prior to lipid extraction. After microwave roasting of soybeans, the embryonic axis was separated from the other tissues (seed coat and cotyledons) with a razor blade. Two thousand embryonic axes were processed in a Waring blender (0°C) and lipids were extracted three times, each with 50 mL of chloroform/methanol (2:1, vol/vol) containing butylated hydroxytoluene (0.01%). The mixture was then filtered through lipid-free paper and the solvent was removed from the combined extracts by means of a rotary vacuum apparatus at 35°C. The residue was dissolved in 100 mL of chloroform/methanol (2:1, vol/vol). The solutions were washed with 20 mL of an aqueous solution of potassium chloride (0.75%) according to Folch *et al.* (13). The chloroform layer was removed, and the aqueous salt phase was extracted twice further with 20 mL chloroform. The combined chloroform extracts were dried over anhydrous Na_2SO_4. The lipid extracts were filtered through lipid-free filter paper, and the solvent was removed *in vacuo* at temperatures below 35°C. Extracted lipids were weighed to determine the lipid content of the embryonic axis and then stored in a mixture of chloroform/methanol (2:1, vol/vol) in 5-mL brown glass volumetric flasks under nitrogen in the dark at −25°C. By using the same procedures, lipids were extracted from the embryonic axes of raw soybeans for use as a control.

Triacylglycerol composition. TAG were isolated from the other lipid classes by TLC. The crude lipid extracts were applied onto TLC plates as 7-cm bands (*ca.* 10 mg/plate) with a microsyringe (Hamilton Co., Reno, NV). The plates were developed in *n*-hexane/diethyl ether (80:20, vol/vol) after which triolein was applied and the plates covered with other glass plates, leaving the reference zone exposed to be visualized by exposure to iodine vapor. TAG, isolated by TLC, were analyzed by gas chromatography (GC) with the method of Matsui *et al.* (14), using a Shimadzu Model-14A gas chromatograph (Kyoto, Japan) equipped with a hydrogen flame-ionization detector as previously described (15). TAG peaks were identified by co-chromatography with known standards. Peak areas were calculated by addition of a known weight (100 μg) of trimyristin as an internal standard using an electronic integrator (Shimadzu C-R4A).

TAG species analysis. Molecular species analysis of total TAG was performed by silver nitrate-silica gel TLC according to the method of De La Roche *et al.* (16). TAG molecular species were separated by argentation TLC using 0.8 to 5.0% methanol in chloroform, depending upon the degree of species unsaturation (17). For quantitation of species containing the trienoic acid (linolenic acid), plates were streaked with 10 to 15 mg TAG and developed with 5.0% methanol in chloroform. Remaining species were separated by streaking 8 to10 mg TAG on the plates and developing with 0.8 to 1.5% methanol in chloroform. This system was varied according to temperature and humidity conditions. Individual bands were visualized by spraying with 0.1% 2′,7′-dichlorofluorescein (Nacalai Tesque, Kyoto, Japan) in methanol and viewed under ultraviolet radiation. Bands were recovered from the plate by extraction with 10% methanol in diethyl ether, followed by acidification of the absorbent with 10% aqueous HCl in a separatory funnel and extraction with diethyl ether. Determination of relative amounts of each TAG subfraction was carried out by comparison of fatty acid methyl esters with a known amount (50 μg) of methyl pentadecanoate as an internal standard. The subfraction was converted into fatty acid methyl esters by heating it with boron trifluoride (14%) in methanol, and quantitated by GC as previously described (18).

Statistical analysis. All experiments were replicated three times at each point of treatment to improve the reliability of the results. The data were subjected to analysis of variance with a randomized complete block design to partition the effects of different parameters (19). Duncan's multiple range test was performed to determine any significant differences ($P < 0.05$) among treatments (20).

RESULTS AND DISCUSSION

Microwave roasting and lipid components. Effects of microwave roasting (cv. Okuhara) were compared on the basis of the internal temperature of the embryonic axis at the end of each roasting time (data not shown). The temperature of the embryonic axis sample was 25°C before roasting and increased to 98 and 165°C, at 6 and 20 min of microwave roasting, respectively. Effects of microwave roasting on total lipids and major acyl lipids were compared among the three cultivars (Table 1). Dominant components were TAG, with much smaller amounts of phospholipids. The other acyl lipids such

TABLE 1
Weights of Embryonic Axes and Lipid Components in Oils Prepared from the Soybeans Roasted in a Domestic Microwave Oven[a]

Cultivar	Roasting time (min)	Embryonic axis (g/1,000 beans)	Total lipids	Triacylglycerols	Phospholipids	Others[b]
Okuhara	Unroasted	7.0461[c]	1726.3[d]	1260.8[c] (73.0)	393.7[d] (22.8)	71.8[d] (4.2)
	6	6.8347[c,d]	1624.6[e]	1174.6[d] (72.3)	354.2[e] (21.8)	95.8[f] (5.9)
	12	6.4120[d,e]	1430.5[f,g]	1024.7[f] (71.6)	293.3[g] (20.5)	113.1[j] (7.9)
	20	6.1090[e]	1403.5[g]	965.7[g] (68.8)	269.5[h] (19.0)	101.2[g] (7.2)
Mikawajima	Unroasted	6.7726[c,d]	1683.5[d,e]	1221.3[d] (72.5)	395.6[d] (23.5)	66.6[c] (4.0)
	6	6.5152[d]	1604.8[e]	1149.7[e] (71.6)	356.8[e] (22.3)	98.3[f] (6.1)
	12	6.0750[e]	1461.2[f,g]	1045.7[f] (71.6)	312.7[f] (21.4)	102.8[g] (7.0)
	20	5.7703[f]	1387.9[g]	959.4[g] (69.1)	281.8[g,h] (20.3)	146.7[j] (10.6)
Tsurunoko	Unroasted	7.1158[c]	1823.3[c]	1287.2[c] (70.7)	450.4[c] (24.7)	85.7[e] (4.7)
	6	6.8098[c,d]	1736.0[c,d]	1215.7[d] (70.0)	413.2[d] (23.8)	107.1[h] (6.2)
	12	6.4185[d,e]	1562.6[e,f]	1097.7[e] (70.2)	353.5[e] (22.6)	111.4[h] (7.1)
	20	6.1196	1502.1[f]	997.6[f,g] (66.4)	323.0[f] (21.5)	181.5[k] (12.1)

[a]Each value is an average of three determinations. Embryonic axes were obtained from 1,000 beans. Lipids are expressed as mg/2,000 embryonic axes. Values in parentheses are relative contents (%) of the individual lipids.
[b]Contains steryl esters, free fatty acids, 1,3- and 1,2-diacylglycerols, glycolipids, and browning substances.
[c–k]Values in the same column with different superscript letters are significantly different from those for unroasted seeds among the three cultivars ($P < 0.05$).

as steryl esters, 1,3- and 1,2-diacylglycerols, glycolipids, and free fatty acids were minor components and have been designated as "others" in Table 1. These results are in agreement with the findings of other researchers (21,22). "Others" also contained browning substances, which were produced in the embryonic axis during microwave roasting. With the progress of microwave roasting, the amounts of TAG as well as total lipids gradually decreased by 71.5 to 86.2 mg for 6 min, 175.6 to 236.3 mg for 12 min, and 261.9 to 295.1 mg for 20 min among the three cultivars. Cossignani *et al.* (23) reported that there were significant decreases in the TAG fraction and increases in the diacylglycerol and monoacylglycerol fractions of olive oils following microwave treatment. However, no trace of monoacylglycerol was detected on the soybean TLC plate. In general, these losses were lower ($P < 0.05$) for the Mikawajima cultivar than for Okuhara or Tsurunoko. But the percentage of the losses was not so much as that of phospholipids in the three cultivars. This would reflect differences in the composition of their fatty acids, especially the content of linolenic acid in TAG, among the three cultivars (Table 2).

Major TAG content and total fatty acid composition. Embryonic axis contained even carbon-numbered TAG for C_{44} to C_{56} before microwave roasting. Dominant components consisted of C_{52} and C_{54} TAG in the three cultivars. Minor com-

TABLE 2
Fatty Acid Compositions of Triacylglycerols in Oils Prepared from the Embryonic Axes of Soybeans Roasted in a Domestic Microwave Oven[a]

Cultivar	Roasting time (min)	Fatty acid (wt%)							
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	Other[b]
Okuhara	Unroasted	0.3[c]	13.5[c]	0.4[c]	2.4[c]	8.3[e]	45.5[d]	29.2[d]	0.4[c]
	6	0.2[c]	14.0[c]	0.4[c]	2.6[c]	8.5[e]	45.0[d]	29.0[d]	0.3[c]
	12	0.5[d]	14.5[c]	0.4[c]	2.9[c]	8.6[e]	44.3[d]	28.3[e]	0.5[d]
	20	0.7[e]	15.2[d]	0.4[c]	3.0[d]	10.4[f]	43.2[d]	26.5[f]	0.6[e]
Mikawajima	Unroasted	0.2[c]	14.6[c]	0.3[c]	2.7[c]	7.5[d]	50.3[c]	24.0[g]	0.4[c]
	6	0.2[c]	14.8[c]	0.3[c]	2.7[c]	7.5[d]	50.2[c]	23.8[g]	0.5[d]
	12	0.3[c]	15.0[d]	0.3[c]	2.8[d]	7.8[d]	50.0[c]	23.2[g]	0.6[e]
	20	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	0.2[c]	14.5[c]	0.3[c]	2.8[d]	6.0[c]	45.0[d]	31.0[c]	0.2[c]
	6	0.3[c]	14.7[c]	0.5[c]	3.0[d]	6.2[c]	45.0[d]	30.0[c]	0.3[c]
	12	0.4[c]	15.4[d]	0.7[d]	3.2[e]	6.5[c]	43.9[d]	29.6[d]	0.3[c]
	20	0.5[d]	16.8[f]	0.7[d]	3.5[e]	6.8[c]	43.4[d]	27.8[e]	0.5[d]

[a]Each value is an average of three determinations.
[b]Other minor fatty acids include: 16:2, 17:0, 20:0, and 22:0.
[c–h]Values in the same column with different superscript letters are significantly different from those for unroasted seeds among the three cultivars ($P < 0.05$).

ponents (<5.0 mg) such as C_{44} and C_{56} TAG were omitted from Figure 1. With continuous microwave roasting, the amounts of C_{54} TAG decreased substantially, as did C_{52} TAG (though to a lesser degree). A significantly greater loss ($P < 0.05$) was observed for Okuhara and Tsurunoko cultivars than for Mikawajima. These results would depend on differences in the amounts of TAG composed of linoleic and linolenic acids. This is supported by the fact that TAG composed of one diene (D) and two triene (T) moieties (DT_2) were detected to be more than 1.5-fold greater in the Okuhara and Tsurunoko than Mikawajima cultivars before microwave roasting (Fig. 2). On the other hand, the amount of C_{50} TAG did not change ($P > 0.05$) in any of the three cultivars during microwave roasting because this TAG is predominantly composed of saturated fatty acids such as palmitic and stearic acids. The data for 12 min of microwave roasting were omitted from Figure 1 because they were essentially the same as those at 20 min of roasting. Fatty acid compositions (expressed in terms of the esters by weight) of TAG during microwave roasting were compared among the cultivars (Table 2). A small difference ($P < 0.05$) occurred in fatty composition of TAG between Mikawajima and Okuhara or Tsurunok. Mikawajima was higher (49.8 to 50.3%) in linoleic and lower (22.6 to 24.0%) in linolenic than those of Okuhara or Tsurunoko. No significant differences ($P > 0.05$) were observed in the fatty acid composition of TAG when roasted for 6 min in a domestic microwave oven. However, the longer the roasting time, the greater the percentages of palmitic, stearic and oleic acids, and the lower those of linoleic and linolenic acids.

Distribution of TAG species. Fifteen different molecular species were detected in the embryonic axis of untreated soybeans (Fig. 2). Major TAG species consisted of SMD (where

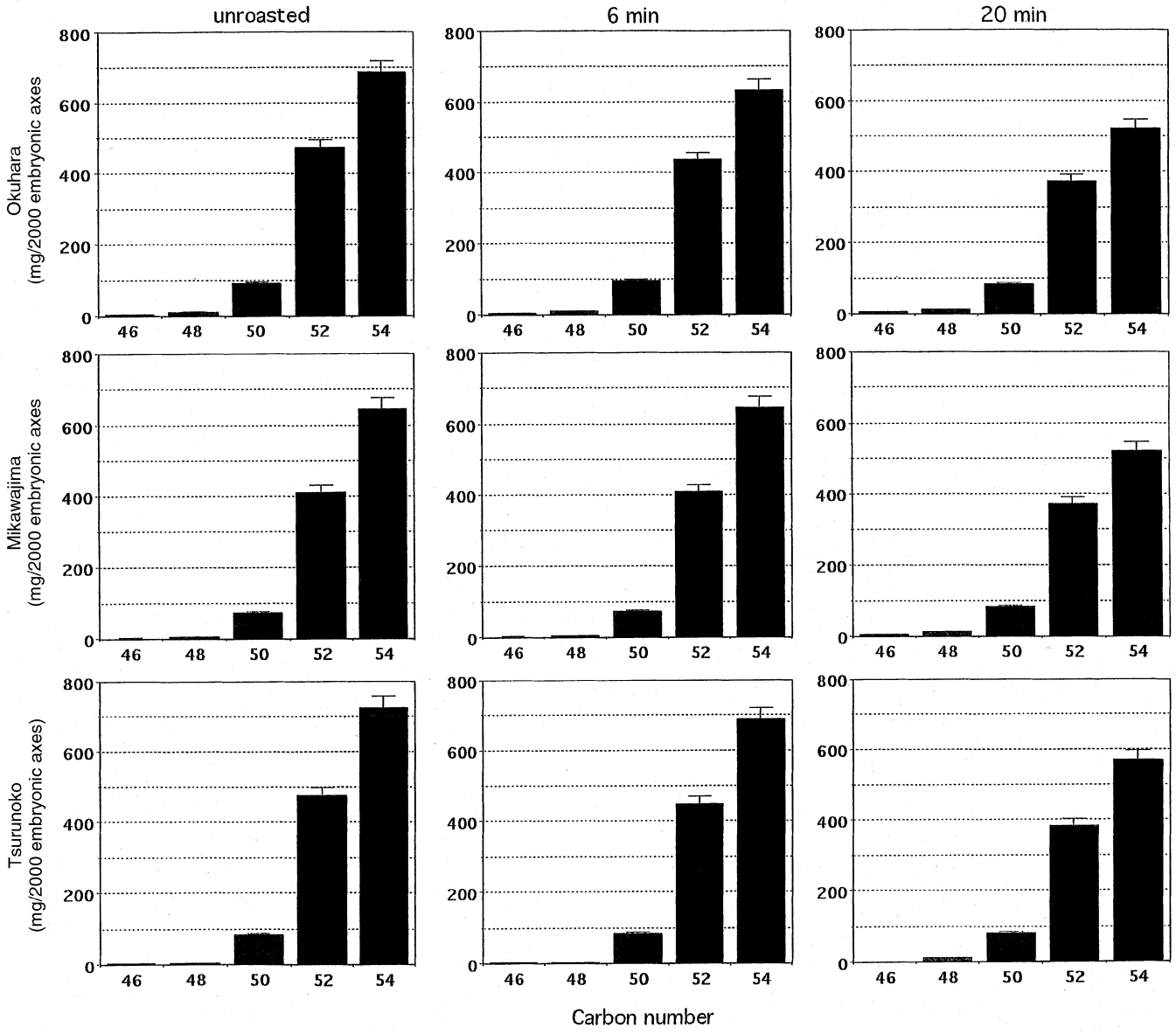

FIG. 1. Changes in the triacylglycerol content of embryonic axes within soybeans roasted in a domestic microwave oven. Carbon number shows length of total acyl chains present in a triacylglycerol. Vertical bars represent standard errors of the replicates.

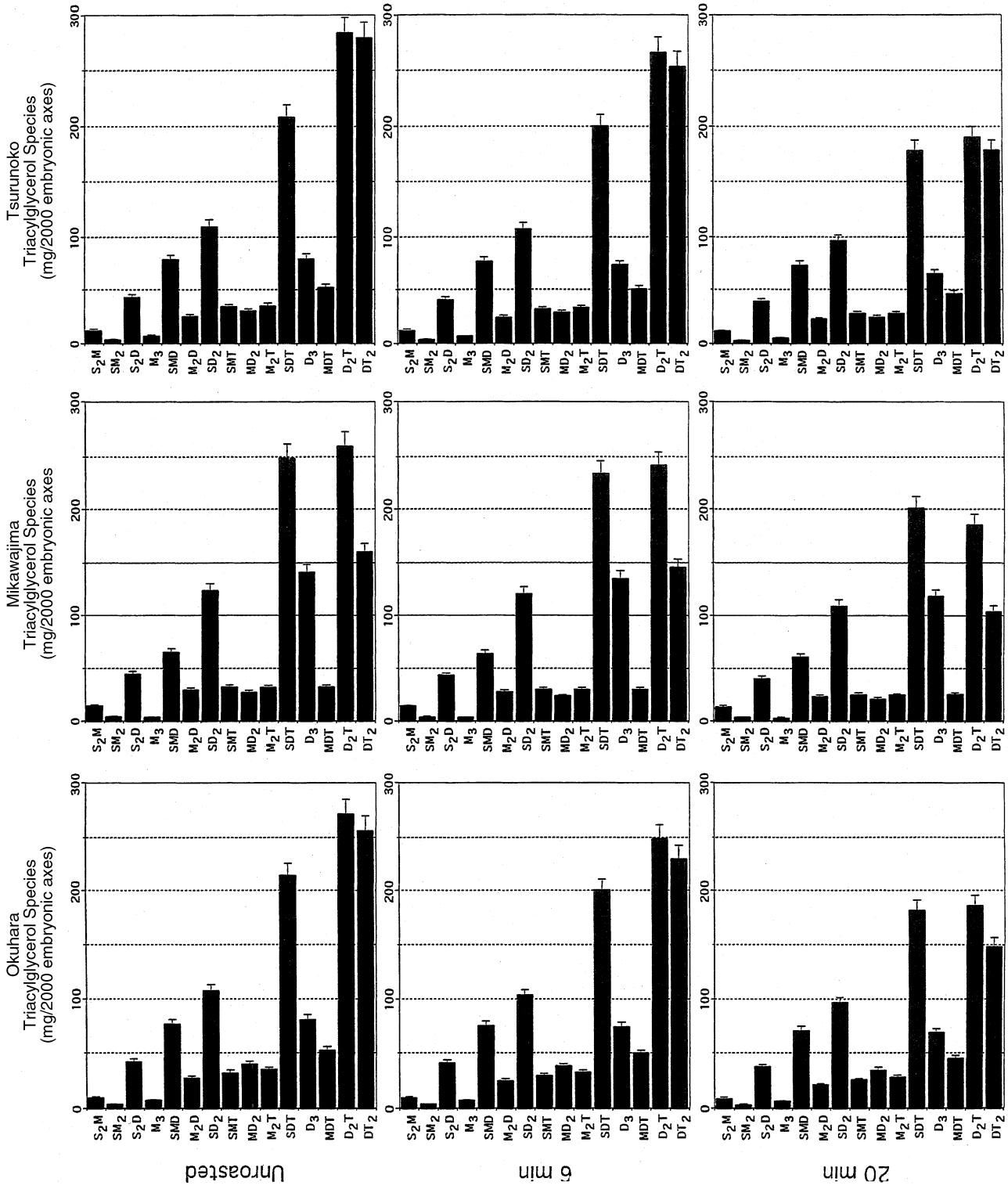

FIG. 2. Changes in the molecular species of triacylglycerols of embryonic axes within soybeans roasted in a domestic microwave oven. Saturated fatty acids (S) consisting of myristic (14:0), palmitic (16:0) and stearic (18:0) acids. Unsaturated fatty acids, oleic (18:1), linoleic (18:2) and linolenic (18:3), are denoted as monoene (M), diene (D) and triene (T), respectively. Horizontal bars represent standard errors of the replicates.

TABLE 3
Content of Fatty Acids in the Triacylglycerols Isolated from Embryonic Axes of Soybeans Roasted in a Domestic Microwave Oven[a]

	Cultivar	Fatty acid[b]	Roasting time (min)			
			Unroasted	6	12	20
	Okuhara	S	180.70^{e,f}	172.78^{f}	161.81^{g,h}	157.30^{h}
		M	123.13^{d}	117.70^{d}	109.50^{f}	104.59^{f,g}
		D	583.73^{d}	543.15^{f}	475.65^{h}	448.77^{i}
		T	373.19^{d}	341.48^{f}	277.54^{h}	255.07^{i}
Experimental[c]	Mikawajima	S	198.04^{d}	189.65^{d,e}	180.17^{e,f}	169.49^{f,g}
		M	105.06^{f}	99.28^{g}	92.96^{h}	86.36^{i}
		D	609.29^{d}	574.80^{e}	521.12^{f}	480.16^{g,h}
		T	308.93^{g}	285.92^{h}	251.46^{i}	223.48^{j}
	Tsurunoko	S	182.93^{e}	176.41^{e}	166.08^{f,g}	161.48^{g,h}
		M	120.42^{d}	115.87^{e}	108.77^{f}	104.08^{f,g}
		D	592.51^{d}	559.23^{e,f}	502.37^{g}	454.61^{h,i}
		T	391.33^{d}	364.17^{e}	320.70^{g}	277.44^{h}

[a]Each value is an average of three determinations and expressed as mg lipid per 2,000 embryonic axes.
[b]Saturated fatty acids (S) consisting of myristic (14:0), palmitic (16:0) and stearic (18:)) acids. Unsaturated fatty acids, oleic (18:1), linoleic (18:2) and linolenic (18:3), are denoted as monoene (M), diene (D) and triene (T), respectively.
[c]Values obtained by gas chromatography in comparison with a known amount of methyl pentadecanoate as an internal standard using triacylglycerols isolated from embryonic axes.
[d–j]Values in the same column with different superscript letters are significantly different from those for unroasted seeds among the three cultivars ($P < 0.05$).

S represents saturated fatty acid and M is a monoene), SD_2, SDT, D_3, D_2T, and DT_2. The other species were minor components (less than *ca.* 50 mg). These patterns were very similar between the Okuhara and Tsurunoko cultivars. The Mikawajima cultivar, however, was higher in SD_2, SDT, and D_3, and lower in D_2T and DT_2 than those of the two other cultivars. With increased microwave roasting time, an appreciable loss ($P < 0.05$) was more apparent in the molecular species containing more than four double bonds, with few exceptions (e.g., M_2T and MDT). The changing patterns for 12 min of roasting are not shown in Figure 2 because they were essentially the same as those found in 20 min. TAG with an unsaturated fatty acid linked at the *sn*-2 position of the glycerol moiety are more stable toward thermal oxidation than those with the same acid at the *sn*-1 or *sn*-3 positions (24). TAG stereospecific analysis (intrapositional fatty acid percentage compositions, respectively for the *sn*-1, *sn*-2, and *sn*-3 positions) was omitted from this study, however, because no reliable fatty acid analysis could be done for their positional distributions.

Table 3 represents the content of fatty acids in the TAG isolated from soybean embryonic axes before and after microwave roasting, expressed as mg per 2,000 embryonic axes according to their degree unsaturation. The amounts of fatty acids (S, M, D, and T) were obtained by GC in comparison with a known amount of methyl pentadecanoate as an internal standard using TAG isolated from the embryonic axis. There were no qualitative or quantitative differences ($P > 0.05$) in the distribution between the experimental and calculated (data not shown) values. There were significant decreases ($P < 0.05$) not only in the several molecular species containing more than four double bonds (Fig. 2) but also in the amount of diene and triene of TAG (Table 2) in the embryonic axes of soybeans when roasted in a domestic microwave oven.

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