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Molecular species of triacylglycerols in the seed coats of soybeans (*glycine max* L.) following microwave treatment

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Abstract

Soybeans (*glycine max*) were exposed to microwaves for 6 or 12 min at a frequency of 2450 MHz. The seed coats were then stripped from the soybeans and roasted in a microwave oven. Molecular species and fatty acid distributions of triacylglycerols (TAG), isolated from total lipids in the seed coats were analysed by a combination of argentation thin-layer chromatography (TLC) and gas chromatography (GC). Based on their different degrees of unsaturation and total chain-length of fatty acid groups, 15 molecular species of TAG were still detectable in the seed coats after the roasting treatment. However, roasting caused a significant decrease ($P < 0.05$), not only in molecular species containing more than four double bonds, but also in the amounts of diene and triene species present in a TAG. These results indicate that microwaves could ($P < 0.05$) affect TAG in the seed coats more significantly than those in the other structural parts of soybeans.

1. Introduction

The heating of foods in a microwave oven is caused by molecular friction of electrical dipoles in an oscillating electric field of specific frequency. Water is the most abundant dipole component in foods, but others (salts, fats, and proteins) also act as dielectric components (Decareau & Peterson, 1985; Mudgett, 1986). Numerous investigations have been carried out to evaluate the effects of microwave on food constituents (Finot, 1995). In fact, microwave are used in the food industry not only for baking, thawing, drying and warming but also for other applications, such as sterilising or pasteurising many types of foods (Kudra, van de Voort, Raghavan & Ramaswamy, 1991; Rosenberg & Bögl, 1987). 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 (Hoffman & Zabik, 1985; Mudgett, 1986). The number of household microwave ovens in use is increasing, mainly because consumers appreciate the advantages, such as economy, convenience, and savings (Nelson, Senter & Fobus, 1985). However, from time to time consumers are concerned by reports (Lubec, Wolf & Bartosch, 1989) that objectionable compounds are produced in microwaved foods. Many studies have been conducted on the general nature and fatty acid level of whole soybeans. Takagi, Ienaga, Tsuchiya and Yoshida (1999) demonstrated the effects of microwave energy on the distribution of tocopherols and acyl lipids within each structural part and section of a soybean. Takagi et al. showed that microwave heating effects more significant differences ($P < 0.05$) in the tocopherols and acyl lipids in the seed coats than those of the embryonic axes or cotyledons. Triacylglycerols (TAG) are the major fraction of total lipids, representing 70% of the embryonic axes and seed coats and 90% of the cotyledons (Yoshida, Takagi, Ienaga & Tsuchiya, 1998). Hitherto, little investigation has been conducted concerning on how microwave energy affects the surface lipids of soybeans, especially molecular species of TAG, the main components of the seed coats.

The objectives of the current study were to isolate TAG from the seed coats of soybeans roasted in a domestic microwave oven and to evaluate changes in molecular species composition and fatty acid distribution of TAG during microwave roasting and, further, to compare the results with those obtained using unroasted seed coats.

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2. Materials and methods

Commercially available soybeans (*glycine max* L.) used in this study were from three Japanese cultivars: Mikawajima, Okuhara, and Tsurunoko, were grown during the summer of 1998. The three cultivars were purchased from Takii Seed Co. (Kyoto, Japan) and were selected for uniformity, based on bean weights of 280–349 mg for Mikawajima, 290–379 mg for Okuhara, and 360–429 mg for Tsurunoko. The beans were hand-selected to eliminate those with cracked or otherwise damaged seed coats. The beans divided into groups were sealed in polyethylene bags and stored in stainless steel containers at 4°C until needed.

2.1. Reagent and standards

All chemicals and solvents used were of analytical-grade (Nacalai Tesque, Kyoto, Japan), and were used without further purification. Precoated Silica-Gel 60 plates (20×20 cm, 0.25 mm layer thickness) used for thin-layer chromatography (TLC) were purchased from Merck (Darmstadt, Germany). Standard TAG (trimyristin, tripalmitin, tristearin, triolein, trilinolein and trilinolenin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). One hundred miligrams of methyl pentadecanoate (Merck) were 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.

2.2. Microwave roasting and lipid extraction

A modifed domestic size Sharp microwave oven (Model 5550, Osaka, Japan), capable of generating 0.5 kW power at 2450 MHz, was used. Whole soybeans were placed in a single layer (not in piles) in Pyrex Petri dishes (12.0 cm diameter), and then put on the turntable of the microwave oven. The internal dimensions of the cavity were 31.0×31.6×22.4 cm, and beans were roasted for 6 or 12 min; times were based on previous results (Yoshida & Takagi, 1996): roasting for 6–10 min was optimal to prepare full-fat soyflour without a burnt odour. As soon as they were taken out of the oven, the internal temperature of the treated soybeans was determined with a chromel–alumel thermocouple, as previously described (Yoshida & Kajimoto, 1988). The roasted soybeans were allowed to cool to ambient temperature prior to lipid extraction. After microwave roasting of soybeans, the seed coats were stripped from the other tissues (cotyledons and embryonic axes) with a razor blade. The seed coats (3000), separated from other tissues, were crushed in a Waring blender (0°C) with 300 ml of chloroform-methanol (2:1,v/v) containing butylated hydroxytoluene (0.01%), which was added to inhibit the oxidative degradation of lipids during experimental procedures. After filtration through lipid-free paper, the residue was further extracted, three times, with 150 ml of choloroform-methanol (twice at 2:1 and then once at 1:1, v/v) in the blender. The filtrates were combined, most of solvent was removed by means of a rotary vacuum apparatus at 35°C and, finally, the residue was taken to near dryness under nitrogen. The residue was dissolved in 100 ml of choloroform-methanol (2:1, v/v). The solutions were washed with a 20 ml aqueous solution of potassium chloride (0.75%) according to the Folch procedure (Folch, Lees & Sloane-Stanley, 1957). The upper layer was removed by aspiration and the rest was washed twice with 20 ml methanol–saline (1:1, v/v). Finally, the bottom layer was dried using 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 seed coats and then stored in a mixture of chloroform–methanol (2:1, v/v) in 5-ml brown glass volumetric flasks under nitrogen in the dark at −25°C. Using the same conditions, lipids were extracted from the seed coats of raw soybeans for use as a control.

2.3. Lipid class analysis and TAG composition

According to previous methods (Yoshida, Shigezaki, Takagi & Kajimoto, 1995), the total lipids were fractionated by TLC into the two fractions, TAG and polar lipids (PL). The plates were developed in *n*-hexane–diethyl ether–formic acid (60:40:1, v/v/v) after applying a standard mixture alongside each plate. The phospholipid fraction was isolated from total lipids by multiple-development TLC. Neutral lipids were removed by developing with the solvent mentioned above, and glycolipids were further removed by developing with acetone-acetic acid-deionized water (100:2:1, v/v/v). Bands corresponding to the TAG, PL, and phospholipids were scraped separately into tubes (105×16 mm) fitted with Teflon-line screwcaps. Methyl pentadecanoate (15:0; 25 or 100 μg) was added as an internal standard to the total lipids and to each fraction at ca. 10% level (w/w ester). After transesterification, using the method of Morrison and Smith (1964), fatty acid methyl esters were analysed by a Shimadzu GC-14A gas chromatograph (Shimadzu Instrument Inc. Kyoto, Japan) and were quantitated by means with a Shimadzu C-R4A electronic integrator (Yoshida & Takagi, 1996). The other gas chromatographic conditions were as described previously (Yoshida et al., 1995).

TAG, isolated by TLC, were analysed by GC with the method of Matsui, Watanabe and Ikekawa (1973), using a Shimadzu Model-14A GC equipped with a hydrogen flame ionisation detector as previously described (Yoshida, 1984). 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).

2.4. 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, Weber and Alexander (1971). Briely, TAG classes differing in unsaturation were separated by argentation TLC using 0.8–5.0% methanol in chloroform, depending on their degree of unsaturation (Blank, Verdino & Privett, 1965). For quantitation of species containing the trienoic acid (linolenic acid), plates were streaked with 10–15 mg TAG and developed with 5% methanol in chloroform. Remaining species were separated by streaking 8–10 mg TAG on the plates and developing the plates with 0.8–1.5% methanol in chloroform. This system was varied according to temperature and humidity conditions. Individual bands were visualised by spraying with 0.1% 2′,7′-dichlorofluorescein in methanol and viewed under UV light. 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. The purity and identity of each band were checked by analytical silver nitrate-silica gel TLC after co-chromatographing with the reference TAG mixture, applied alongside.

Table 1
Weights of seed coat and lipid components in oils prepared from the seed coat of soybeans roasted in a domestic microwave oven (at a frequency of 2450 MHz)[a,b]

Cultivar	Roasting (min)	Seed coat[c] (g/3000 beans)	Total lipids[c]	Triacylgylcerols[c]	Phospholipids[c]	Others[cd]
Okuhara	Unroasted	58.4570a	636.9d	446.7c (70.1)	112.8c (17.7)	77.4b (12.2)
	6	56.8323bc	574.5e	389.5e (67.7)	97.5d (17.0)	87.9c (15.3)
	12	54.9897cd	497.7g	307.2f (61.7)	81.9f (16.5)	108.6e (21.8)
Mikawajima	Unroasted	57.4297bc	570.6e	409.5d (71.8)	98.4d (17.2)	62.7a (11.0)
	6	56.0337bc	527.5f	351.8e (66.7)	86.7e (16.4)	89.0c (16.9)
	12	54.1176cd	452.3h	279.7g (61.8)	70.5g (15.6)	102.1d (22.6)
Tsurunoko	Unroasted	61.0817a	878.1a	621.9a (70.8)	152.4a (17.4)	103.8d (11.8)
	6	59.2638ab	807.6b	561.6b (69.5)	131.7b (16.3)	114.3e (14.2)
	12	57.7952bc	703.4c	463.2c (65.9)	109.5c (15.6)	130.7h (18.5)

[a] Each value is an average of two determinations. Seed coat was separated from 3000 beans and their lipids are expressed as mg lipid per 3000 seed coats.
[b] Values in parentheses are relative content of the individual lipids total lipids.
[c] Values within the same column and soybean cultivars with different letters are significantly different from those for unroasted soybeans ($P < 0.05$).

Table 2
Fatty acid compositions of triacylglycerols in oils prepared from the seed coat of soybeans roasted in a domestic microwave oven (at a frequency of 2450 MHz)[a]

Cultivar	Roasting time (min)	Fatty acid (wt%)[b]							
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others[c]
Okuhara	Unroasted	0.2a	12.3a	1.5b	2.7c	21.0b	36.2b	24.5b	1.6b
	6	0.2a	12.8a	1.5b	2.8c	21.6b	36.7b	22.8c	1.6b
	12	0.3a	13.5b	1.6c	2.9d	24.6c	34.5c	20.9c	1.7c
Mikawajima	Unroasted	0.2a	16.0c	1.3a	3.0e	18.5a	38.8a	20.7c	1.5b
	6	0.2a	16.5c	1.3a	3.2e	20.1b	38.0a	19.2d	1.5b
	12	0.3a	18.0d	1.4a	3.5f	22.0b	35.2b	18.0e	1.6b
Tsurunoko	Unroasted	0.2a	13.4b	1.5b	2.3a	20.8b	34.3c	26.2a	1.3a
	6	0.2a	14.6b	1.5b	2.3a	21.6b	33.5c	25.0a	1.3a
	12	0.3a	15.8c	1.6c	2.5b	23.0c	32.7c	22.6c	1.5b

[a] Each value is an average of two determinations.
[b] Values within the same column and soybean cultivars with different letters are significantly different from those for unroasted soybeans ($P < 0.05$).

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 (Morrison & Smith, 1964), and quantitated by GC as previously described (Yoshida, 1994).

2.5. Statistical analysis

All experiments were repeated twice at each point, before and after microwave treatment, to improve the reliability of the results. The data were subjected to analysis of variance with a randomised complete block design to partition the effects of different parameters (Steel & Torrie, 1980). Duncan's multiple range test was performed to determine any significant differences ($P < 0.05$) among treatments (Duncan, 1955).

3. Results and discussion

Effects of microwave roasting (cv. Mikawajima) were compared on the basis of the internal temperature of the soybeans at the end of each roasting time (data not shown). The temperature of the bean sample was 25°C before microwave roasting and increased from 98 to 162°C, at 6 and 12 min after microwave roasting, respectively. Effects of microwave roasting on total lipids and major acyl lipids were compared among the three cultivars (Table 1). Before microwave treatment, dominant components were TAG (ca. 70%), with much smaller amounts of phospholipids (ca. 17%). Minor

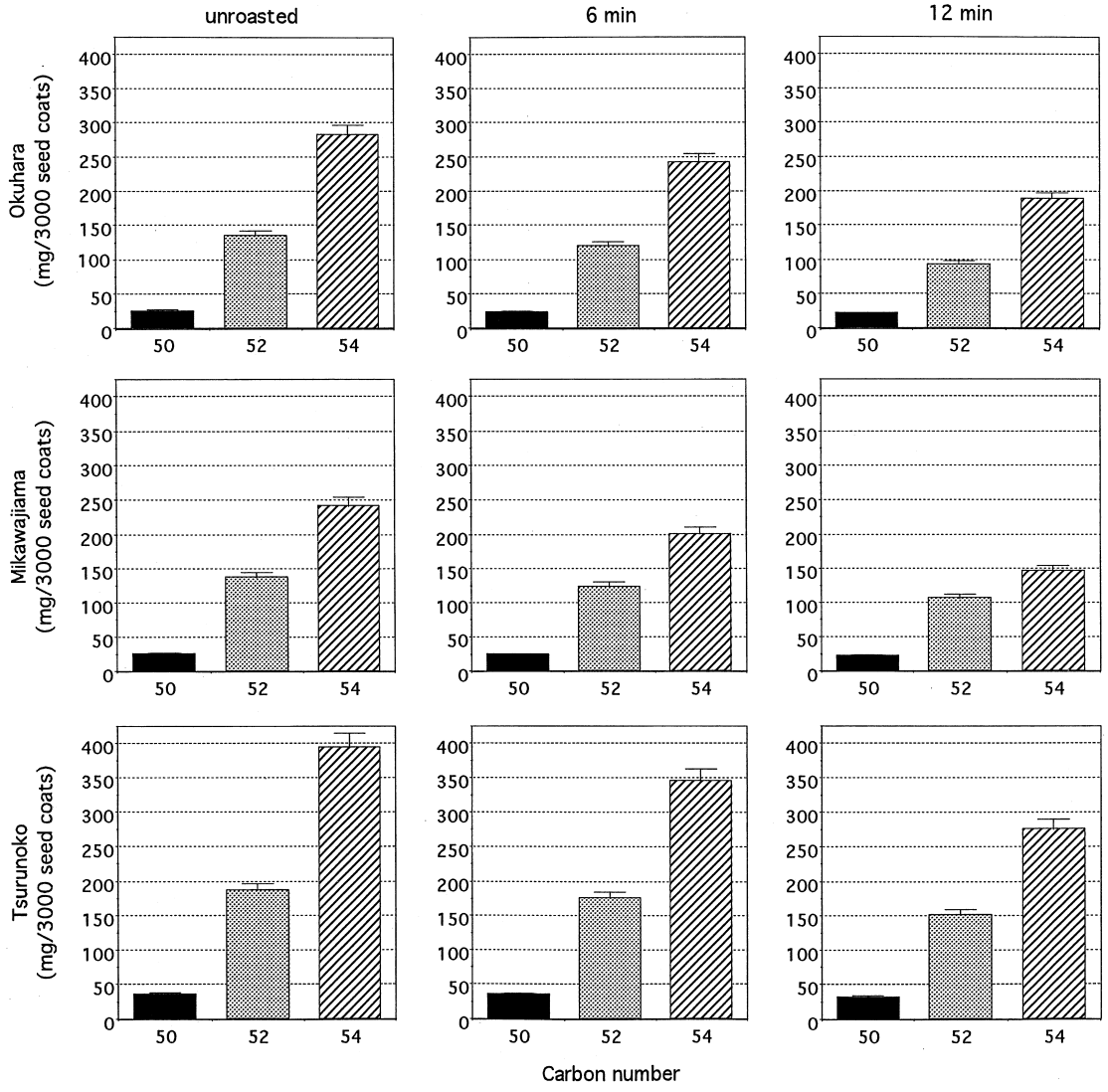

Fig. 1. Changes in the triacylglycerol content of seed coat within soybeans roasted in a domestic microwave oven. Carbon number shows length of total acyl chain present in a triacylglycerol. Vertical bars represent standard error of the replicates.

components such as steryl esters, 1,3- and 1,2-diacylglycerols, free fatty acids, and glycolipids, are designated as "others" in Table 1 because no reliable fatty acid analysis could be done in this paper. "Others" also contained browning substances, which were substantially produced in the seed coats during microwave roasting. These results are in agreement with the findings of other researchers (Bilyk, Piazza, Bistline & Haas, 1991; Pham, Casa, Gregorio & Kwon, 1998). With the progress of microwave roasting, the amounts of TAG, as well as total lipids, gradually decreased by 57.2 to 60.3 mg for 6 min, and 130 to 159 mg for 12 min among the three cultivars, respectively. Cossignani, Simonetti, Neri and Damiani, (1998) 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, we could not detect any trace monoacylglycerol on the TLC plate among the three cultivars after microwave roasting. In general, these losses were lower ($P < 0.05$) for the Mikawajima cultivar than for Okuhara or Tsurunoko. The trends were essentially the same as those for phospholipids. This would reflect differences in the composition of their fatty acids, especially the content of linolenic acid in TAG before microwave roasting, among the three cultivars (Table 2). The percentage composition of linoleic acid was higher for the Mikawajima cultivar than for Okuhara or Tsurunoko, while that of linolenic acid was lower for the former cultivar than for the latter cultivars.

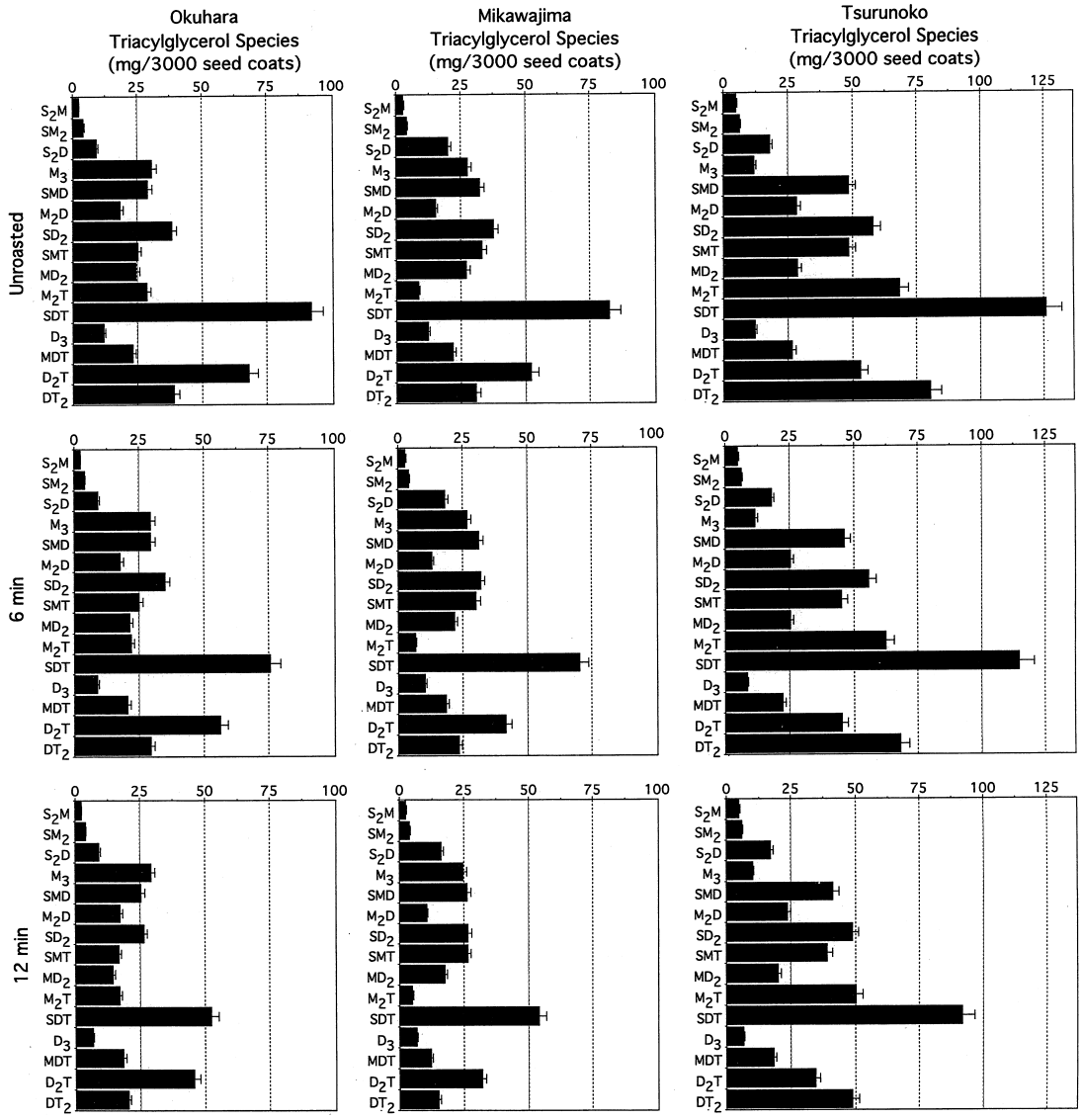

Fig. 2. Changes in the molecular species of triacylglycerols of seed coat within soybeans roasted in a domestic microwave oven. Saturated fatty acids (S) consist of myristic (14:0), palmitic (16:0) and stearic (18:0) acids. Unsaturated fatty acids, oleic (18:1), linoleic (18:2) and linolenic (18:3),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

Seed coats contained even carbon-numbered TAG for C_{46} to C_{56} before microwave roasting. Dominant components consisted of C_{52} and C_{54} TAG in the three cultivars, and minor components (<4.0 mg) such as C_{46}, C_{48} and C_{56} TAG are omitted from Fig. 1, which shows changes in the TAG levels. With increased microwave roasting time, 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) was detected to be more than 1.2–2.6 fold greater (with a few exceptions) 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 until 12 min of roasting because this TAG is predominantly composed of saturated fatty acids such as palmitic and stearic acids. 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 acid composition of TAG between Mikawajima and Okuhara or Tsurunoko. Mikawajima was higher (35.2 to 38.8%) in linoleic and lower (18.0 to 20.7%) in linolenic than those of Okuhara or Tsurunoko. With a few exceptions (linolenic acid), 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.

Fifteen different molecular species were detected in the seed coats of untreated soybeans (Fig. 2). With a few exceptions among the three cultivars, major TAG species consisted of SMD (where S represents saturated fatty acid and M is a monoene), SD_2, SMT, MD_2, SDT, D_2T, and DT_2. The other species were minor components (less than ca. 26 mg). The Mikawajima cultivar was 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. The trends became more pronounced differences ($P<0.05$) with longer roasting. TAG with an unsaturated fatty acid linked at the *sn*-2-position of glycerol moiety are more stable toward thermal oxidation than those with the same acid at the *sn*-1- or *sn*-3-positions (Wada & Koizumi, 1983). TAG stereospecific analysis (interpositional fatty acid percentage compositions, 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 the seed coats of soybeans before and after microwave roasting, expressed as mg per 3000 seed coats according to their degree unsaturation. The amounts of fatty acis (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 seed coats. 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 seed coats of soybeans when roasted in a domestic microwave oven. In conclusion, tocopherol contents are markedly lower ($P<0.05$) in the seed coats than those in the cotyledons or the embryonic axes (Yoshida, Takagi et al., 1998). Therefore, the present study showed that microwaves may affect TAG in the seed coats more significatly than those in the other structural part of soybeans. Further studies are necessary to demonstrate a relationship between the content of antioxidants and oxidative stability of molecular species of TAG in the seed coats during microwave roasting.

Table 3
Content of fatty acids in the triacylglycerols isolated from the seed coat of soybeans roasted in a domestic microwave oven (at a frequency of 2450 MHz)[a,b]

	Cultivar	Fatty acid[c]	Roasting time (min)		
			Unroasted	6	12
Experimental[d]	Okuhara	S	71.1e	64.6f	49.8g
		M	100.1c	92.4d	81.0e
		D	170.2c	145.7e	112.6g
		T	105.3c	86.5d	63.9f
	Mikawajima	S	78.8d	70.2e	58.5g
		M	85.6d	77.8e	66.1f
		D	158.2d	132.3f	102.6h
		T	86.9d	71.5e	53.5g
	Tsurunoko	S	111.5a	105.1b	90.9c
		M	133.6a	122.9b	105.1c
		D	215.3a	191.4b	156.3d
		T	161.3a	142.3b	110.9c

[a] Values within the same column and soybean cultivars with different letters are significantly different from those for unroasted soybeans ($P<0.05$).

[b] Each value is an average of two determinations and expressed as mg lipid per 3000 seed coats.

[c] 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.

[d] Values obtained by gas chromatography in comparison with a known amount of methyl pentadacanoate as an internal standard using triacylglycerols isolated from seed coat.

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