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Food Chemistry 66 (1999) 345–351

Food Chemistry

www.elsevier.com/locate/foodchem

Microwave heating influences on fatty acid distributions of triacylglycerols and phospholipids in hypocotyl of soybeans (*glycine* max L.)

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Received 22 July 1998; received in revised form and accepted 5 February 1999

Abstract

Soybeans were exposed to microwave heating for 6, 12 or 20 min at a frequency of 2450 MHz. The hypocotyls were then separated from the other tissues, and the lipid components and the positional distribution of fatty acids in triacylglycerols (TAG) and phospholipids (PL) were investigated. Major lipid components were TAG and PL, while steryl esters (SE), free fatty acids (FFA), and 1,3- and 1,2-diacylglycerols (DAG) were minor ones. Following microwave heating, a significant increase ($p < 0.05$) was observed in FFA and in both forms of DAG (primarily 1,3-DAG). The greatest rate of PL losses ($p < 0.05$) was observed in phosphatidylethanolamine (PE), followed by phosphatidylcholine (PC) and phosphatidylinositol (PI). Significant differences ($p < 0.05$) in fatty acid distributions occurred (with few exceptions) when soybeans were microwaved for 12 min or more. Nevertheless, the principal characteristics for the positional distribution of fatty acids still remained after 20 min of microwave heating.

1. Introduction

Microwave energy is an important new method of heating which has become readily available commercially and is forecast to be utilized much more extensively in the future (Mudgett, 1989). Theoretically, the heating effects of microwave energy upon various food components can differ significantly from those produced by heating in a conventional oven. For example, it has been speculated that reactive free radicals can be formed by exposure to microwave energy (Lie Ken Jie & Yan-Kit, 1988), especially when a high temperature is reached, as is the case when fatty foods are cooked. Soybean seeds contain various anti-nutritional factors (Bau, Villaume, Nicolas, & Méjean, 1997), such as trypsin inhibitor, which are shown to reduce protein digestibility and to cause pancreatic hypertrophy (Kakade, Simons, Liener, & Lambert, 1972). Therefore, various heating methods, including dry roasting (Johnson, Deyoe, Hoover, & Schwenke, 1980), boiling (Collins & Beaty, 1980) and microwave roasting (Hutton & Foxcroft, 1975), have been used to improve the nutritional value of soybeans. However, careful control of processing conditions is essential to avoid damage to protein function and its nutritional value. In a microwave oven, heating is the result of the interaction of an electromagnetic field with the chemical components in the food, generating instant heat due to molecular friction (Decareau, 1985). Vetrimani, Jyothirmayi, Haridas Rao, and Ramadoss (1992) showed that microwave heating was quite effective in inactivating enzymes present in cereal bran, germ and soybeans. Microwave ovens are credited with rapid heating rates and high efficiency, because of their high penetration power (Burfoot, James, Foster, Self, Wilkins & Philips, 1990). Yoshida, Mieno, Takagi, Yamaguchi, and Kajimoto (1995) reported the effects of microwave heating on acyl lipids in whole soybeans in relation to moisture. In an oilseed plant, such as soybeans, there are two types of lipids: storage lipids and membrane lipids. PL in the hypocotyl of soybeans are the major constituents of cell membranes, and they have a high degree of unsaturation. However, tocopherols are highly concentrated in the hypocotyl compared to the cotyledons or the seed coat. Hitherto, virtually no information has been reported on how microwave energy affects, not only the lipid components in the hypocotyl, but also the fatty acid distribution of TAG and PL.

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0308-8146/99/$ - see front matter
PII: S0308-8146(99)00072-2

The objective of this study was to explore the effect of microwave treatment on the composition and positional distribution of fatty acids in the PL as well as the TAG in the hypocotyl of soybeans.

2. Materials and methods

Commercially available soybeans (*glycine* max L.) used in this research were from two Japanese cultivars: Mikawajima, and Tsurunoko and grown during the summer of 1997. The two cultivars were procured from Takii Seed Co. (Kyoto, Japan) and were selected for uniformity based on bean weights of 300–350 mg for Mikawajima, and 350–400 mg for Tsurunoko. The beans were hand-selected to eliminate those with cracked or otherwise damaged seed coats. All the beans were divided into groups for storage in stainless steel containers at 4°C until needed.

2.1. Reagents

All chemicals and solvents were of analytical grade (Nacalai Tesque, Kyoto, Japan), and were used without further purification. Precoated Silica-Gel G 60 plates (20× 20 cm, 0.25 mm layer thickness), used for thin-layer chromatography (TLC), were purchased from Merck (Darmstadt, Germany). Lipase was from porcine pancreas, and was used after purification with acetone and then diethyl ether. Phospholipase A_2 was from bee venom, *Apis mellifera*. Both enzymes (lipase and phospholipase A_2) were obtained from Sigma Chemical Co. (St. Louis, MO). The PL standards were made from a PL kit obtained from Serdary Research Lab, Ontario, Canada (now Doosan Serdary Laboratories, Englewood Cliffs, NJ). Fatty acid methyl ester standards (F&OR mixture No. 3) were purchased from Applied Science (State College, PA, USA). One-hundred milligrams of methyl pentadecanoate (Merck) were dissolved in *n*-hexane and stored in a 20-ml glass volumetric flask 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. Heating of soybeans

Whole soybeans were placed in a single layer in Pyrex™ Petri dishes (12 cm i.d.), covered, and then placed on the rotatory plate of the microwave oven (Sharp, Model R-5550, Osaka, Japan). These were then heated for 6, 12 or 20 min, [times based on previous results (Yoshida, Miene, et al., 1995a)]. The heated soybeans were allowed to cool to ambient temperature prior to lipid extraction.

2.3. Seed sectioning and lipid extraction

After microwave heating of the soybeans, the hypocotyl was separated from the other tissues (coat and cotyledons) by using a razor blade. One thousand hypocotyls were crushed with 50 ml of chloroform-methanol (2:1, v/v) in a Waring blender (at 0°C), and the lipids were extracted by vigorous shaking of triplicate samples, in the method as described by Yoshida et al. (1995a). The solvents contained butylated hydroxytoluene (0.01%), which was added to inhibit the oxidative degradation of lipids during experimental procedures. The combined extracts, dried in a rotatory vacuum apparatus at 35°C, were dissolved in 100 ml of chloroform-methanol (2:1, v/v). The solution was washed with a 20 ml aqueous solution of potassium chloride (0.75%) according to the Folch procedure (Folch, Lees, & Stanley, 1957). The chloroform layer was removed and the aqueous salt phase was further re-extracted twice with 20 ml chloroform. The combined chloroform extracts were dried using anhydrous sodium sulphate. The lipid extracts were filtered through a lipid-free filter paper and the solvents were removed in vacuo at temperatures below 35°C. Extracted lipids were weighed to determine the lipid content of the hypocotyl and then kept 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 procedures, lipids were extracted from the hypocotyl of raw soybeans for use as a control.

2.4. Lipid class analysis

According to previous methods (Yoshida, Shigezaki, Takagi & Kajimoto, 1995), the total lipid was fractionated by TLC into the following six fractions. Bands corresponding to SE, TAG, FFA, 1,3-DAG, 1,2-DAG, and polar lipids were scraped separately into test-tubes (10.5cm×16 mm) with poly (tetrafluoroethylene)-coated screw caps. Methyl pentadecanoate (25 or 100 μg) was added as an internal standard to each tube. Part of the polar lipids were further separated on TLC into several fractions developed by a mixture of chloroform–methanol–acetic acid–deionized water (85:15:10:3.5, v/v/v/v). Individual polar lipids were identified by comparison with R_f-values of a standard PL also chromatographed. TLC plates were partially covered with a glass plate and exposed to iodine vapour. Unexposed bands corresponding to PE, PC, PI, phosphatidylserine (PS), and others were quantitatively scraped into test tubes (10.5 cm×16 mm) fitted with poly (tetrafluoroethylene)-coated screw caps, and methyl pentadecanoate (25 μg) was added as an internal standard to each tube.

Fatty acid methyl esters were prepared from the isolated lipids by heating the samples at 80°C for 1 h in BF_3-methanol (Morrison & Smith, 1964). After cooling, 5 ml of *n*-hexane was added and mixed by Vortex mixer. The fatty acid methyl esters were recovered from the upper phase after washing with deionised water, then separated and quantitated by a Shimadzu Model GC-14A

(Shimadzu, Kyoto, Japan) according to the method of Yoshida, et al. (1995b).

2.5. Enzymic hydrolysis of lipids

The procedures to examine the in vitro TAG hydrolysis were as described previously (Yoshida & Alexander, 1983). Basically, the purified TAG (20 mg) was hydrolyzed with 10 mg pancreatic lipase in 3 ml of 0.25 M Tris buffer pH 7.5, with 0.1 ml of 0.01 M $CaCl_2$ and 0.25 ml deoxylcholate (0.1%) in a 10 ml test tube. After approximately 60% of the TAG was hydrolyzed (3.0 min), adding 0.5 ml of 6 N HCl and 1 ml ethanol stopped the reaction. Thereafter, the lipids were extracted three more times with 2 ml *n*-hexane. The reaction products were separated by TLC as already indicated (Yoshida, Shibahara, & Kajimoto, 1975). The free fatty acids and *sn*-2-monoacylglycerols bands, representing, respectively, the 1,3-positions and 2-position of TAG, were scraped off the plate and transmethylated. The procedure was checked by comparing the fatty acid composition of the original TAG and those remaining after the partial hydrolysis.

The positional distribution of fatty acids in each of the PE, PC, and PI samples isolated by preparative TLC was determined with phospholipase A_2 hydrolysis (Yoshida & Kajimoto, 1978). Briefly, 3–7 mg of each PL was suspended in 0.5 ml of 0.2 M Tris buffer (pH 7.5) containing 0.01 M of $CaCl_2$ in a 20-ml Erlenmeyer flask. To this suspension, 5 mg of phospholipase A_2 and 10 ml of diethyl ether were added. The reaction mixture was incubated for 5 h at 28°C under a nitrogen atmosphere. The hydrolysis was almost complete (>98%) in this period, as judged from a preliminary experiment using a standard phospholipid (L-3-phosphatidylcholine 1-palmitoyl-2-oleoyl). After hydrolysis of the PL, the lysophospholipid and free fatty acid were separately scraped into test tubes from the plate, and the constituent fatty acids were analyzed by GC after transesterification as described above.

2.6. Data and statistical analysis

All experiments were repeated twice at each point, before and after microwave treatment, to improve the reliability of the results. Statistical evalution of the data was conducted by the Statistical Analysis System (SAS, 1990) with the general linear model (GLM) analysis of variance. Significant differences among treatment means were determined by using Duncan's multiple range test, at a level of $p < 0.05$ (Duncan, 1955).

3. Results and discussion

A relative percentage of the hypocotyl was 1.8% of whole soybean seeds, and there were no significant differences ($p > 0.05$) in the ratio, not only between the two cultivars, but also following microwave heating. A dark brownish colour and burnt smell became apparent at 12 min. The colour of the lipids extracted from the hypocotyl changed gradually from light-yellow at 6 min of heating to brown at 12 min, and finally deep-brown at 20 min (data not shown). Profiles of the various acyl lipid classes (namely SE, TAG, FFA, 1,3- and 1,2-DAG, polar lipids) in the hypocotyl before and after microwave heating were compared (Table 1). There were significant differences ($p < 0.05$) in lipid components between the two cultivars before microwave heating. In general, the amounts of the individual lipids were higher in Tsurunoko than in Mikawajima. However, the major fractions were TAG and polar lipids (mainly PL), representing 65–72% and 22–28%, respectively, following microwave heating. With increased microwave heating time, an appreciable change (with an exception of SE) was observed and more pronounced differences ($p < 0.05$) were observed after 12 min heating. Conversely, the amounts of FFA, 1,3-DAG and 1,2-DAG, as degradation products, increased 2.3–6.0, 5.4–7.3, and 2.7–9.8 fold, respectively, after 20 min heating; they

Table 1
Changes in lipid components of the hypocotyl of soybeans heated in a microwave oven (at a frequency of 2450 MHz)[b]

Cultivar	Heating time (min)	Total lipids[a]	Steryl esters[a]	Triacyl glycerols[a]	Free fatty acids[a]	1,3-Diacylglycerols[a]	1,2-Diacylglycerols[a]	Polar lipids[a]
Mikawajima	Unheated	824a	24.6a (3.0)[c]	565a (68.6)	1.6a (0.2)	3.3a (0.4)	1.6a (0.2)	228a (27.6)
	6	797a	24.4a (3.1)	549a (68.8)	3.8b (0.5)	5.4b (0.7)	3.1b (0.4)	212b (26.5)
	12	727b	24.5a (3.4)	487b (67.0)	9.3c (1.3)	15.0c (2.1)	5.7c (0.8)	186c (25.4)
	20	649c	24.5a (3.8)	424c (65.3)	9.7c (1.5)	24.0d (3.7)	15.6d (2.4)	151d (23.3)
Tsurunoko	Unheated	925a	27.6a (3.0)	663a (71.6)	4.6a (0.5)	4.6a (0.5)	3.7a (0.4)	222a (24.0)
	6	891a	27.6a (3.1)	641a (71.9)	5.2b (0.6)	5.2b (0.6)	6.0b (0.7)	206b (23.1)
	12	813b	26.3ab (3.2)	569b (69.9)	6.4c (0.8)	18.4c (2.3)	7.9c (1.0)	186c (22.8)
	20	715c	25.2ab (3.5	489c (68.4)	10.8d (1.5)	25.0d (3.5)	10.0d (1.4)	155d (21.7)

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

[b] Each value is an average of two determinations and expressed as mg lipid per 1000 hypocotyls.

[c] Values in parentheses are relative content of the individual lipids in total lipids.

were detected as very low levels (1.6–4.6 mg per 1000 hypocotyl) in the hypocotyl before microwave heating. The results suggested that TAG was gradually degraded by microwaves to produce FFA and DAGs (Yoshida, Mieno, et al., 1995). No trace of monoacylglycerol was detectable on the TLC-plate before or after microwave heating.

Fig. 1 shows the fatty acid distribution (expressed in terms of the esters by weight) of the TAG before and after microwave heating. The major fatty acids in the TAG were linoleic, linolenic, palmitic, and oleic acids, whereas stearic, and several fatty acids (palmitoleic, arachidic and behenic, shown as "others" in Fig. 1) were minor fatty acid components of TAG. The minor fatty acids have not been considered, as no significant differences ($p > 0.05$) were found in the hypocotyls from either the Mikawajima or Tsurunoko cultivar. However, there was a significant difference ($p < 0.05$) in the percentages of linoleic and linolenic acids between the two cultivars. The hypocotyl separated from Tsurunoko was higher in linolenic and lower in linoleic than that of Mikawajima. The increase in the percentage of linolenic acid was mainly at the expense of linoleic acid while other fatty acid levels remained unchanged. Following microwave heating, small significant differences ($p < 0.05$) were observed in the essential patterns of the fatty acid composition and positional distribution in the residual TAG. However, saturated fatty acids, especially palmitic and stearic, were mostly located in the 1,3-positions, whereas unsaturated ones, particularly linoleic, primarily occupied the 2-position of the TAG molecules. Therefore, linolenic and oleic acids were almost evenly distributed in the 1-, 2- or 3-position. Other laboratories (Brockerhoff & Yurkowski, 1966; Fatemi & Hammond, 1977; Reske, Siebrecht, & Hazebroek, 1997) have reported similar results.

Table 2 presents the changing patterns of the PL fractions in the hypocotyl before and after microwave heating. The original amounts of the individual PL before microwave heating were approximately 51, 100, 36, 5, and 17 mg per 1000 hypocotyl for PE, PC, PI, PS, and others, respectively. However, no lysophospholipids were detected by two dimensional-TLC in the PL fractions after microwave heating. The proportion of PE, PC, and PI in the hypocotyl differed from that typically reported in whole soybean PL (Yoshida & Takagi, 1997), possibly because of varietal tissue. There were no significant differences ($p > 0.05$) in the original levels of each PL between the two cultivars. The greatest rate of PL losses ($p < 0.05$) was observed for PE, followed by PS, PC, PI, and others. The trends became more pronounced with longer heating times (after 12 min). The reduction of PL following microwave treatment may be due to the decomposition of PL and/or formation of a complex with protein or carbohydrate. In Maillard reaction, PLs are particularly reactive as they contain

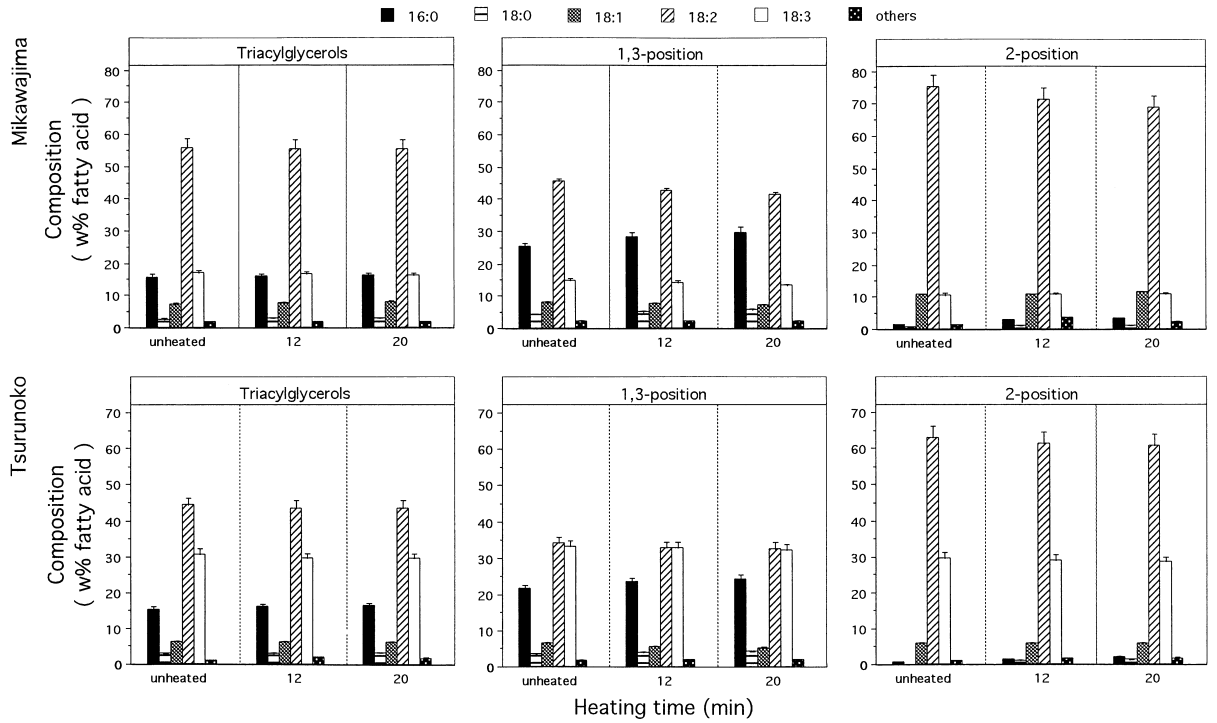

Fig. 1. Changes in composition and positional distribution of fatty acids of triacylglycerols obtained from the hypocotyl of soybeans heated in a microwave oven (at a frequency of 2450 MHz). Each value represents the average of three replicates, and vertical bars represent the standard errors of the replicates.

Table 2
Changes in the major phospholipids of the hypocotyl of soybeans heated in a microwave oven (at a frequency of 2450 MHz)[b]

Cultivar	Heating time (min)	Phosphatidylethanolamine[a]	Phosphatidylcholine[a]	Phosphatidylinositol[a]	Phosphatidylserine[a]	Others[a,c]
Mikawajima	Unheated	50.9a (24.2)[d]	103a (48.7)	35.8a (17.0)	5.3a (2.5)	16.0a (7.6)
	6	42.1b (22.2)	93.4b (49.2)	33.7b (17.8)	4.3b (2.3)	16.3a (8.5)
	12	28.3c (18.6)	76.8c (50.8)	28.3c (18.6)	3.3c (2.1)	15.8a (10.3)
	20	10.5d (9.4)	59.6d (53.6)	24.5d (22.0)	2.2d (2.0)	14.4b (13.0)
Tsurunoko	Unheated	51.8a (24.8)	98.6a (47.1)	35.7a (17.1)	5.2a (2.5)	17.9a (8.6)
	6	43.6b (23.4)	88.3b (47.3)	32.8b (17.6)	4.2b (2.2)	17.8b (9.5)
	12	29.5c (19.3)	73.6c (48.1)	28.5c (18.6)	3.0c (2.0)	18.4a (12.0)
	20	11.3d (9.8)	61.5d (53.8)	23.7d (20.7)	2.6d (2.3)	15.3b (13.4)

[a] Values within the same column and soybean cultivars with different superscript letters are significantly different from those for unheated soybeans ($p < 0.05$).
[b] Each value is an average of two determinations and expressed as mg lipid per 1000 hypocotyls.
[c] Containing diphosphatidylglycerol, phosphatidic acid, and phosphatidylglycerol.
[d] Values in parentheses are relative content of the individual lipids in phospholipids.

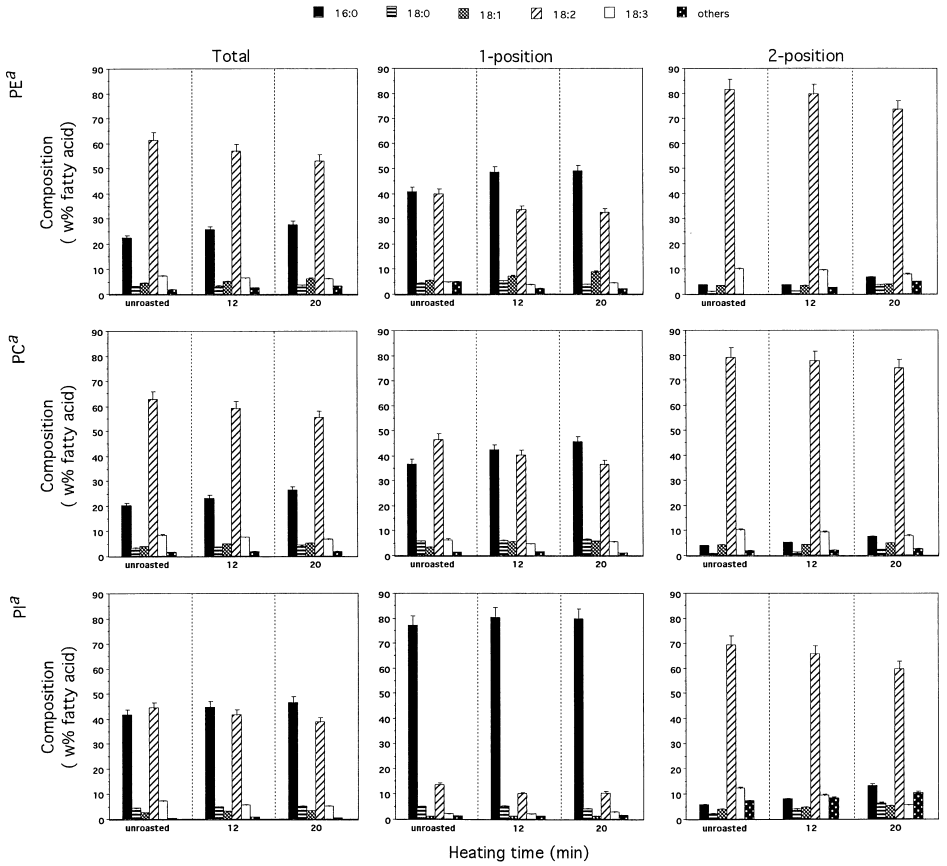

Fig. 2. Changes in composition and positional distribution of fatty acids of major phospholipids obtained from the hypocotyl of soybeans (cv. Mikawajima) heated in a microwave oven (at a frequency of 2450 MHz). Each value represents the average of three replicates, and vertical bars represent the standard errors of the replicates. [a]Abbreviations, see text.

both polyunsaturated fatty acids and amines (Pokorny, 1981). However, the hypocotyl was higher in tocopherols than other tissues such as the cotyledons or the seed coat (Yoshida, Takagi, Ienaga, & Tsuchiya, 1998). The amino group of PE or PS can apparently facilitate hydrogen or electron donation to tocopherols in the hypocotyl (Hudson & Ghavami, 1984). At elevated heating, some of the naturally occurring classes of PL, in particular PE, greatly enchance the activity of primary antioxidants in edible oils, while PI is without synergistic activity. Therefore, there were no significant differences ($p > 0.05$) in the essential profiles of these PL_S between the two cultivars resulting from microwave heating.

The changing profiles of composition and positional distribution of fatty acids of PE, PC, and PI in the hypocotyl were compared before and after microwave heating (Figs. 2 and 3, respectively). The major fatty acids in the three PL were linoleic, palmitic, and linolenic acids (Figs. 2 and 3). However, PI was unique in that it had the highest saturated fatty acid content among the three PL. The three PL of Tsurunoko (Fig. 3) were higher in linolenic and lower in linoleic than those of Mikawajima (Fig. 2). The increase in linolenic acid was mainly at the expense of linoleic and palmitic or oleic acids while other fatty acid levels remained almost unchanged. Before microwave heating, the concentration of saturated acids in the 1-position of PI was markedly higher than that of PE or PC, probably due to differences in their biosynthetic pathways (Vogel & Browse, 1996). The changing patterns in fatty acid distributions for PS were omitted from this study because no reliable fatty acid analysis could be done for their positional distributions. The percentage of linoleic acid showed significant decreases ($p < 0.05$) in the three PL after 12 min of heating (with a few exceptions). The trends became more pronounced in Mikawajima than those in Tsurunoko. In the composition and positional distribution of fatty acids, the percentage of linoleic acid was more significant decreases ($p < 0.05$) in the PL (Figs. 2 and 3) than that in TAG (Fig. 1), and for Mikawajima than for Tsurunoko. These results may be due to the high penetration power of microwaves, and the differences in the content of tocopherol homologues present in the hypocotyl (Yoshida et al., 1998). α-Tocopherol in the hypocotyl was higher for Mikawajima than were for Tsurunoko, whereas γ- and δ-tocopherols were much lower for the former than the latter. α-Tocopherol is reported to have antioxidant activity at low concentrations but prooxidant activity at high concentrations, and γ-tocopherol, on the other hand, has antioxidant activity at higher concentrations than α-tocopherol (Cillard & Cillard, 1980; Koskas, Cillard, & Cillard, 1984). Therefore, unsaturated fatty acids, especially linoleic acid, were predominantly distributed in the 2-position, and saturated fatty acids, particularly palmitic, were highly concentrated in the 1-position after 20 min of microwave heating.

The food or feed value of soybean hypocotyl is unknown. However, Snyder and Kwon (1987) reported

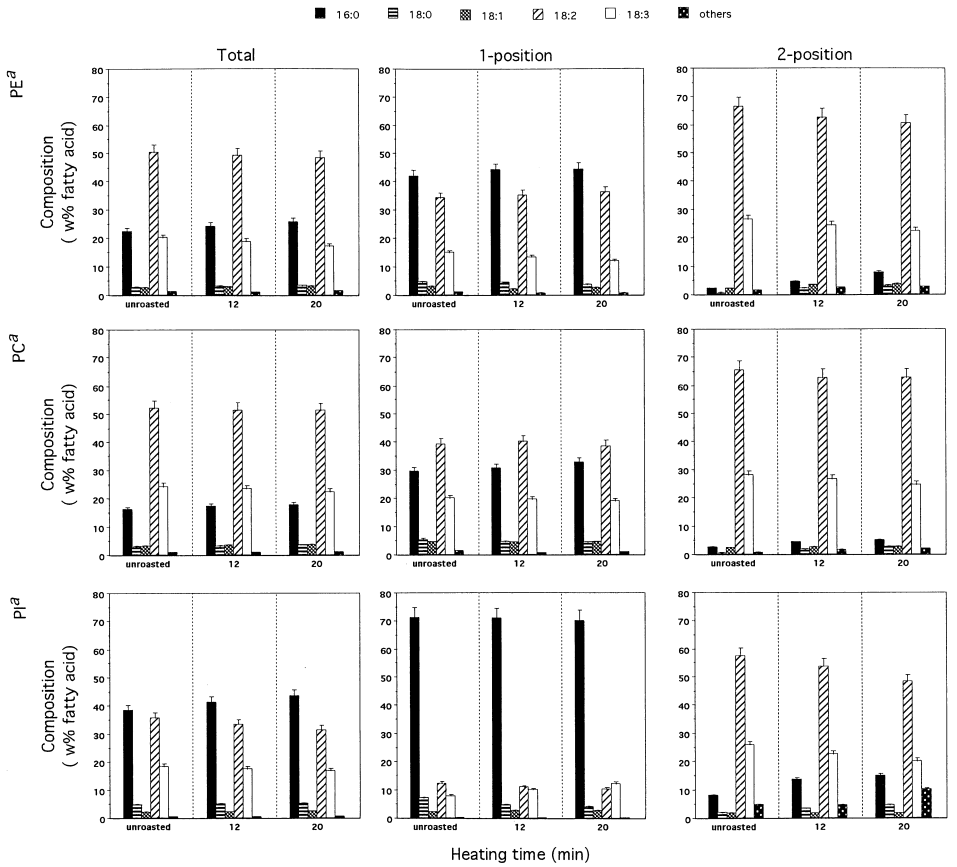

Fig. 3. Changes in composition and positional distribution of fatty acids of major phospholipids obtained from the hypocotyl of soybeans (cv. Tsurunoko) heated in a microwave oven (at a frequency of 2450 MHz). Each value represents the average of three replicates, and vertical bars represent the standard errors of the replicates. [a]Abbreviations, see text.

that the embryo hypocotyl is the source of beany off-flavours, and some processors of soya milk have tried to remove the germ to avoid off-flavours in soya milk. Our observation of high levels of polyunsaturated fatty acids in the hypocotyl supports this practice. Additional studies are being carried out to elucidate a relationship between the content of tocopherols and oxidative stability of lipids in the hypocotyl.

Acknowledgements

The authors express their gratitude to Professor Dr Bruce J Holub at the Department of Human Biology and Nutritional Sciences, University of Guelph, Canada, for editing the manuscript.

References

Bau, H. M., Villaume, C., Nicolas, J. P., & Méjean, L. (1997). Effect of germination on chemical composition, biochemical constitutents and antinutritional factors of soya bean (*glycine* max) seeds. *Journal of the Science of Food and Agriculture, 73*, 1–9.

Brockerhoff, H., & Yurkowski, M. (1966). Stereospecific analyses of several vegetable fats. *Journal of Lipid Research, 7*, 62–64.

Burfoot, D., James, S. J., Foster, A. M., Self, K. P., Wilkins, T. J., & Philips, I. (1990). Temperature uniformity after reheating in microwave ovens. In R. W. Field, & J. A. Howell (Ed.), In *Processing Engineering in the Food Industry*, Vol. 2, (pp. 1–14). New York: Elsevier Applied Science.

Cillard, J., & Cillard, P. (1980). Behavior of alpha, gamma, and delta tocopherols with linoleic acid in aqueous media. *Journal of the American Oil Chemists' Society, 57*, 39–42.

Collins, J. L., & Beaty, B. F. (1980). Heat inactivation of trypsin inhibitor in fresh green soybeans and physiological responses of rats fed the beans. *Journal of Food Science, 45*, 542–546.

Decareau, R. V. (1985). *Microwaves in the food processing industry* (pp. 28–32). London, UK: Academic Press.

Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics, 11*, 1–42.

Fatemi, S., & Hammond, E. G. (1977). Glyceride structure variation in soybean varieties. I. Stereospecific analysis. *Lipids, 12*, 1032–1036.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A Simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Hudson, B. J. F., & Ghavami, M. (1984). Phospholipids as antioxidant synergist for tocopherols in the autoxidation of edible oils. *Food Science & Technology, 17*, 161–166.

Hutton, K., & Foxcroft, P. D. (1975). Effect of processing temperature on some indices of nutritional significance for micronized soya beans. *Processing of the Nutrition Society, 34*, 49A–50A.

Johnson, L. A., Deyoe, C. W., Hoover, W. J., & Schwenke, J. R. (1980). Inactivation of trypsin inhibitors in aqueous soybean extracts by direct steam infusion. *Cereal Chemistry, 57*, 376–379.

Kakade, M. L., Simons, N. R., Liener, I. E., & Lambert, J. W. (1972). Biochemical and nutritional assessments of different varieties of soybeans. *Journal of Agriculture and Food Chemistry, 20*, 86–90.

Koskas, J. P., Cillard, J., & Cillard, P. (1984). Autoxidation of linoleic acid and behavior of its hydroperoxides with and without tocopherols. *Journal of the American Oil Chemists' Society, 61*, 1466–1469.

Lie Ken Jie, M. S. F., & Yan-Kit, C. (1988). The use of a microwave oven in the chemical transformation of long chain fatty acid esters. *Lipids, 23*, 367–369.

Morrison, W. R., & Smith, J. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research, 5*, 600–608.

Mudgett, R. E. (1989). Microwave food processing. *Food Technology, 43* (1), 117–126.

Pokorny, J. (1981). Browning lipid-protein interactions. *Progress in Food Nutrition and Science, 5*, 421–428.

Reske, J., Siebrecht, J., & Hazebroek, J. (1997). Triacylglycerol composition and structure in genetically modified sunflower and soybean oils. *Journal of the American Oil Chemists' Society, 74*, 989–998.

SAS Institute Inc., (1990). *SAS User's guide: basic*, (5th ed.). Cary, NC.

Snyder, H. E., & Kwon, T. W. (1987). Morphology and composition. In *Soybean utilization* (pp. 31–32). New York: Van Nostrand reinhold Co.

Vetrimani, R., Jyothirmayi, N., Haridas Rao, P., & Ramadoss, C. S. (1992). Inactivation of lipase and lipoxygenase in cereal bran, germ and soybean by microwave treatment. *Food Science & Technology, 25*, 532–535.

Vogel, G., & Browse, J. (1996). Choline phosphotransferase and diacylglycerol acyltransferase. *Plant Physiology, 110*, 923–931.

Yoshida, H., & Alexander, J. C. (1983). Enzymatic hydrolysis in vitro of thermally oxidized sunflower oil. *Lipids, 18*, 611–616.

Yoshida, H., & Kajimoto, G. (1978). Fatty acid distribution in glycolipids and phospholipids in cotyledons of germinating soybeans. *Agricultural and Biological Chemistry, 42*, 1323–1330.

Yoshida, H., Mieno, A., Takagi, S., Yamaguchi, M., & Kajimoto, G. (1995a). Microwave roasting effects on acyl lipids in soybeans (*glycine* max. L.) at different moisture contents. *Journal of Food Science, 60*, 801–805.

Yoshida, H., Shibahara, A., & Kajimoto, G. (1975). Toxic character of rancid oil. X Influence of tocopherol on lipid component and triglyceride composition of rat tissues fed thermally oxidized oil. *Journal of Japan Oil Chemists Society (Yukagaku), 24*, 575–581.

Yoshida, H., Shigezaki, J., Takagi, S., & Kajimoto, G. (1995b). Variations in the composition of various acyl lipids, tocopherols and lignans in sesame seed oils roasted in a microwave oven. *Journal of the Science of Food and Agriculture, 68*, 407–415.

Yoshida, H., & Takagi, S. (1997). Microwave roasting and positional distribution of fatty acids of phospholipids in soybeans (*glycine* max L.). *Journal of the American Oil Chemists' Society, 74*, 915–921.

Yoshida, H., Takagi, S., Ienaga, H., & Tsuchiya, C. (1998). Regional distribution of tocopherols and fatty acids within soybean seeds. *Journal of the American Oil Chemists' Society, 75*, 767–774 .