Hiromi Yoshida[a, b]
Yuka Tomiyama[a]
Yuki Hirakawa[a]
Yoshiyuki Mizushina[a, b]

[a] Department of Nutritional Science, Kobe-Gakuin University, Kobe, Hyogo, Japan
[b] High Technology Research Center, Kobe-Gakuin University, Kobe, Hyogo, Japan

Variations in the composition of acyl lipids and triacylglycerol molecular species of pumpkin seeds (*Cucurbita* spp.) following microwave treatment

Whole pumpkin seeds (*Cucurbita* spp.) of two cultivars were exposed to microwaves for 6, 12, 20 or 30 min at a frequency of 2450 MHz. The kernels were separated from the whole seeds, and were investigated not only for the different acyl lipids and their fatty acid compositions, but also for the molecular species of triacylglycerols (TAGs). A modified argentation TLC procedure, developed to optimize the separation of the complex mixture of total TAGs, provided 11 different groups of TAGs, based on both the degree of unsaturation and the chain-length of fatty acid groups. With a few exceptions, dioleopalmitin (5.8–18.8 wt-%), dipalmitolinolein (8.1–8.8 wt-%), triolein (6.3–20.5 wt-%), palmitoleolinolein (15.0–16.1 wt-%), dioleolinolein (16.7–23.0 wt-%), dilinoleopalmitin (4.6–15.4 wt-%) and dilinoleolein (6.7–19.4 wt-%) were the main TAG components. When pumpkin seeds were microwaved for 20 min or more, significant differences ($p < 0.05$) occurred in the acyl lipids as well as their fatty acid distributions with a few exceptions. Therefore, microwave roasting caused a significant decrease ($p < 0.05$), not only in TAGs molecular species containing more than 4 double bonds, but also in the amounts of diene species present in triacylglycerols. These results contribute to the study of the functional properties of pumpkin seed products.

Keywords: Acyl lipids, $AgNO_3$-TLC, free fatty acids, microwave oven, molecular species, kernels, pumpkin seeds, triacylglycerols.

1 Introduction

Recently much attention has been focused on the utilization of food-processing byproducts and wastes, as well as on underutilized agricultural products. Obviously, such utilization could contribute to increase the exploitation of the available resources and result in the production of various new food products. At the same time, a major contribution to avoid waste disposal problems could be made. The search for less known crops, many of which are potentially valuable as human and animal foods, has been intensified to maintain a balance between population growth and agricultural productivity, particularly in the tropical and subtropical areas of the world. The value of pumpkin (*Cucurbita* spp.) seeds as a useful source of oils and proteins has been reviewed by a few workers [1, 2]. Therefore, pumpkin seeds are utilized in several countries as snacks after salting and roasting [3] for human consumption.

Correspondence: *Hiromi Yoshida,* Department of Nutritional Science, Kobe-Gakuin University, 518 Arise, Ikawadani-cho, Nishi-ku, Kobe, Hyogo 651-2180, Japan. Phone: +81-78-974-1551-3233, Fax: +81-78-974-5689; e-mail: yoshida@nutr.kobe-gakuin.ac.jp

Microwave roasting is unique and has gained acceptance in food preparation because of its convenience, efficiency, speed and low operating costs. Therefore, microwave ovens are found in the majority of homes in Japan, and today more people use microwaves for cooking or reheating than ever before. However, microwave energy effects on various food components could differ significantly from those of conventional cooking. For example, it has been speculated that reactive free radicals may be formed by exposure to microwave energy [4], especially in those applications as toasting and frying that result in abnormally high temperatures. However, these reports have been conducted on the fatty acid level of total lipids in seeds, but triacylglycerols (TAGs), the main component of the seeds, has not been investigated on the molecular level.

Until now there has been no information on how microwave energy affects the lipid components in the kernels of pumpkin seeds. The aim of the present study was to evaluate the variations in the composition of various acyl lipids and TAG molecular species in the kernels of pumpkin seeds roasted in a microwave oven and to compare the results among the two cultivars with those obtained from unroasted pumpkin seeds.

Research Paper

2 Materials and methods

2.1 Sample seeds

Commercially available pumpkin seeds (*Cucurbita* spp.) used in this work were from two Japanese cultivars, Kuriebisu and Rikyu that were grown in Japan during the summer of 2002. Cultivars were purchased from *Takii Seed* Co. (Kyoto, Japan) and selected for uniformity based on seed weights of 150–200 mg for Kuriebisu, and 170–250 mg for Rikyu. The seeds were hand-selected to eliminate those with cracked or otherwise damaged hulls. All the seeds were divided into two groups for storage in stainless steel containers at 4°C until needed.

2.2 Reagents and standards

All chemicals and solvents used were of analytical grade (*Nacalai Tesque,* Kyoto, Japan). Thin-layer chromatography (TLC) pre-coated silica-gel 60 plates (10×20 or $20 \times 20\,cm^2$, 0.25 mm layer thickness) were purchased from *Merck* (Darmstadt, Germany). The TLC standard mixture, containing 1,3- and 1,2-diacylglycerols (DAGs), free fatty acids (FFAs) and steryl esters (SEs), was from *Nacalai Tesque* (Kyoto, Japan).

Standard TAGs (trimyristin, tripalmitin, tristearin, triolein, trilinolein and trilinolenin) were obtained from *Sigma Chemical* Co. (St. Louis, MO, USA). Methyl pentadecanoate (C15:0, 100 mg, *Merck*) was dissolved in *n*-hexane (20 mL) and used as internal standard. Boron trifluoride (BF_3) in methanol (14%; *Wako Pure Chemical* Inc, Osaka, Japan) was used to prepare fatty acid methyl esters (FAMEs). As phospholipid standards a phospholipid kit (*Serdary Research Laboratory,* Ontario, Canada) was used.

2.3 Microwave roasting

A modified domestic size *Sharp* microwave oven (Model 5550; Osaka, Japan) capable of generating 0.5 kW power at 2450 MHz was used. Whole pumpkin seeds were arranged in a single layer in *Pyrex Petri* dishes (12.0 cm diameter). Three dishes, containing 100 seeds each, were placed on the turntable plate in the oven. The total weight of the 100 seeds thus treated was 106.4 ± 2.3 g. After covering the dishes, the seeds were microwaved for 6, 12, 20 or 30 min [5]. As soon as they were removed from the oven after each treatment, the internal temperature of the treated seeds was determined as previously described [6].

2.4 Lipid extraction

The roasted whole pumpkin seeds were allowed to cool to ambient temperature before homogenization for lipid extraction. After microwave roasting the kernels were peeled from the roasted whole pumpkin seeds with a razor blade. The kernels (200 seeds) were extracted using a *Maxim* homogenizer (*Nihonseiki Kaisha* Ltd. Tokyo, Japan) at high speed for 10 min (0 °C, under ice) with 150 mL of chloroform/methanol (2:1, vol/vol), fortified with 0.01% BHT, which was added to inhibit the oxidative degradation of lipids during analysis.

The homogenate was vacuum-filtered through defatted filter paper on a *Büchner* funnel, and the filter residue was rehomogenized with a second volume of the chloroform/methanol mixture. The filtrates were combined and dried in a rotary vacuum evaporator at 35 °C. The residue was dissolved in 100 mL of chloroform/methanol (2:1, vol/vol). 20 mL of aqueous potassium chloride (0.75 wt-%) was added [7], and the phases were vigorously mixed.

After phase separation, the chloroform layer was withdrawn, dried with anhydrous sodium sulfate and filtered. The organic phase was concentrated under vacuum. The extracted lipids were weighed to determine the lipid content of the kernels and then transferred to a 25-mL brown glass volumetric flask with chloroform/methanol (2:1, vol/vol) solutions [8], and stored under nitrogen in the dark at −25 °C until analyzed. Samples of unroasted pumpkin seeds were extracted by the same procedures and used as control.

2.5 Lipid class analysis and TAG composition

Using previous methods [9], the total lipid extracts were fractionated by TLC into 6 fractions. The crude lipid extracts were applied to the TLC plates as 7-cm bands (approximately 20 mg per plate) with a microsyringe (*Hamilton* Co. Reno, NV, USA). The TLC standard mixture was applied as a reference on each plate, and the plate was developed in a mixture of *n*-hexane/diethyl ether/acetic acid (80:30:1, vol/vol/vol). Bands corresponding to SEs, TAGs, FFAs, 1,3- and 1,2-DAGs and phospholipids (PLs) were scraped into test-tubes (105×16 mm; poly(tetrafluoro-ethylene)-coated screw caps). Methyl pentadecanoate solution (C15:0, 25 or 100 μg) was added as internal standard to each tube.

FAMEs were prepared from the isolated lipids by heating for 90 min at 80 °C in BF_3-methanol on an aluminium block bath [10]. After cooling, 5 mL of *n*-hexane was added. The organic layer containing the FAMEs was recovered. The solvent was removed under a stream of nitrogen and the residue quantified on a *Shimadzu Model-14A* GC

(*Shimadzu,* Kyoto, Japan) as described [9]. The detection limit was 0.05 wt-% of total fatty acids for each FAME in a FAME mixture, and results are expressed as wt-% of total FAMEs.

Samples of the extracted polar lipids, obtained as described above, were further separated by TLC into several fractions with chloroform/methanol/ acetic acid/deionized water (170:30:20:7, vol/vol/vol/vol) as the mobile phase. PL classes were detected by iodine vapor and were consistent with the authentic standards. Bands corresponding to phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl inositol (PI), and others were carefully scraped into test tubes. Then, methyl pentadecanoate (25 μg) was added to each tube, FAMEs were prepared by the same method as described above and analyzed by GC.

On the other hand, TAGs isolated by TLC were directly analyzed by GC following the method of *Matsui* et al. [11], using a *Shimadzu Model-14A* GC equipped with a hydrogen flame ionization detector [12]. TAG peaks were identified by co-chromatography with standards. Peak areas were calculated by addition of a known quantity (50 μg) of trimyristin internal standard using an electronic integrator (*Shimadzu C-R4A*).

2.6 TAG species analysis

Molecular species separation of total TAGs was carried out by silver nitrate/silica gel TLC according to the method of *De La Roche* et al. [13]. Briefly, TAG classes differing in degree of unsaturation were separated by argentation-TLC using 1.8% (vol/vol) methanol in chloroform, depending on their degree of unsaturation [14]. This system was varied according to temperature and humidity conditions. Individual bands were visualized by spraying with 2',7'-dichlorofluorescein (*Nacalai Tesque,* Kyoto, Japan; 0.1% in methanol) and viewed under ultraviolet radiation. Bands were recovered from the plates by extraction with 10% aqueous HCl in diethyl ether. The combined extracts were purified by alumina column chromatography (5.0 × 30 mm; alumina column) to remove the 2',7'-dichlorofluorescein.

The identity and purity of each band was verified by analytical silver nitrate/silica gel TLC after co-chromatography with the reference TAG mixture. Determination of relative amounts of each TAG subfraction was made by comparison of FAMEs with a known amount (25 μg) of methyl pentadecanoate as internal standard. Each subfraction was converted into FAMEs and quantified by GC as described above.

2.7 Statistical analysis

All experiments were done in triplicate and the results analyzed by one-way variance [15]. Multiple comparison tests were performed to determine any significant differences ($p < 0.05$) among treatments [16].

3 Results and discussion

3.1 Microwave roasting and lipid components

The internal temperature of the pumpkin seeds at the end of each roasting time was compared (data not shown). Briefly, the temperature of the seed sample was 25 °C before microwave roasting and was increased to 93, 117, 124 and 132 °C, at 6, 12, 20 and 30 min of microwave roasting, respectively. Profiles of the different lipids in the kernels of pumpkin seeds before and after microwave roasting were compared among the two cultivars (Tab. 1).

The major lipid component, TAGs, still represented >90 wt-% of the total lipids after 30 min of roasting, while FFAs and PLs were also present in minor proportions. However, SEs, 1,3- and 1,2-DAGs were designated as "others" in Tab. 1 because these lipids were detected in very small amounts. FFA level was higher in Kuriebisu than in Rikyu. The presence of FFAs in oil samples may be due to the partial enzymatic hydrolysis of reserve TAGs during storage of the seeds [17].

As microwave roasting proceeded, an appreciable change in FFAs, 1,3- and 1,2-DAGs was observed at 12 min, and even more pronounced differences ($p < 0.05$) were observed at 30 min. Conversely, the amount of PLs gradually decreased by 13.4–21.4 mg for 12 min, 26.7–42.7 mg for 20 min, and 39.0–66.3 mg for 30 min, respectively. *Cossignani* et al. [18] reported that there was a significant decrease in the TAG fraction and increased DAG and monoacylglycerol fractions in olive oil following microwave treatment. Although *Abou-Gharbia* et al. [19] observed that TAGs of sesame seeds were gradually hydrolyzed randomly by microwaves to produce DAGs and FFAs, the present work showed that there was an increase in "others" fractions, primarily with increased FFAs and browning substances (data not shown).

3.2 Effect of microwave roasting on fatty acid compositions

Fatty acid distributions (expressed in terms of FAME as wt-% of total fatty acids) of TAGs, FFAs and PLs isolated by TLC from the oils separated from the kernels of pumpkin seeds roasted for different time periods in a microwave oven were compared among the two cultivars (Figs. 1–3), respectively. The principal fatty acids for each

Tab. 1. Lipid components in oils prepared from the kernels of pumpkin seeds roasted at different times in a microwave oven (at a frequency of 2450 MHz)[†].

Cultivar	Roasting time [min]	Total lipids	Triacylglycerols	Free fatty acids	Phospholipids	Others
		[mg/200 seeds]				
Kuriebisu	Unroasted	12431.0^{a}	11610.6^{a} (93.4)	435.0^{a} (3.5)	186.5^{a} (1.5)	198.9^{a} (1.6)
	6	12065.2^{b}	11231.2^{b} (93.0)	453.4^{b} (3.8)	175.3^{b} (1.5)	205.3^{ab} (1.7)
	12	11784.6^{c}	10890.8^{c} (92.4)	505.2^{c} (4.3)	165.1^{c} (1.4)	223.5^{c} (1.9)
	20	11374.4^{d}	10417.3^{d} (91.5)	575.3^{d} (5.1)	143.8^{d} (1.3)	238.0^{de} (2.1)
	30	11013.9^{e}	10019.9^{e} (91.0)	631.9^{e} (5.7)	120.2^{e} (1.1)	241.9^{e} (2.2)
Rikyu	Unroasted	7429.3^{f}	6887.0^{f} (92.7)	215.4^{f} (2.9)	111.4^{f} (1.5)	215.5^{a} (2.9)
	6	7349.6^{g}	6802.8^{g} (92.6)	223.0^{g} (3.0)	104.0^{g} (1.4)	219.8^{ab} (3.0)
	12	7261.2^{h}	6691.2^{h} (92.2)	234.2^{h} (3.2)	98.0^{h} (1.3)	237.8^{cd} (3.3)
	20	7137.7^{i}	6537.4^{i} (91.6)	269.3^{i} (3.7)	84.7^{i} (1.2)	246.3^{d} (3.5)
	30	6959.5^{j}	6308.1^{j} (90.6)	313.1^{j} (4.6)	72.4^{j} (1.0)	265.9^{e} (3.8)

[†] Each value is the average of 3 determinations and expressed as mg lipid in 200 kernels. Values in the same column with different indices are significantly different from those for unroasted seeds ($p < 0.05$). Values in parentheses are relative content of the individual lipids within total lipids. Others contain steryl esters, diacylglycerols, browning substances and unknown.

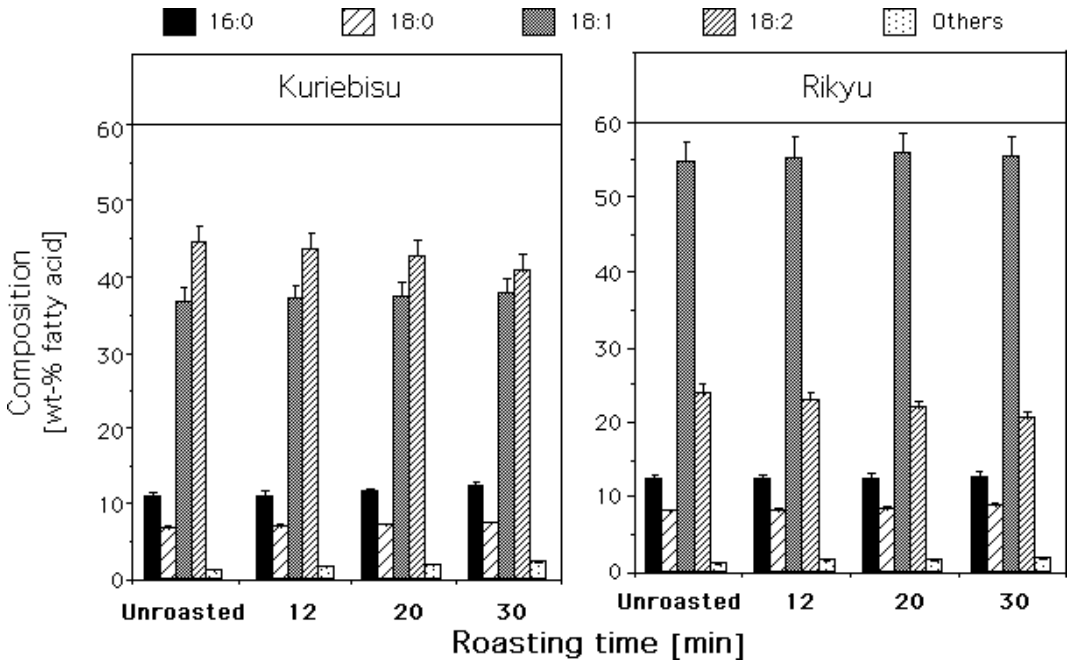

Fig. 1. Fatty acid distributions of triacylglycerols in the kernels of pumpkin seeds roasted for different time periods in a microwave oven at a frequency of 2450 MHz. Each value represents the average of 3 replicates, and vertical bars show the mean error of the replicates. Others contain C14:0, C16:1, C18:3 or C20:0.

genotype were: oleic, linoleic, palmitic, and stearic. However, significant differences ($p < 0.05$) occurred in the fatty acid composition among these lipids when comparing Kuriebisu and Rikyu. The percentage of oleic acid was lower ($p < 0.05$) in the Kuriebisu than in the Rikyu, and the value was compensated by an increase ($p < 0.05$) in linoleic acid (Figs. 1–3). These trends were more pronounced in the PLs than in the TAGs or FFAs. Low percentages were detected for myristic, palmitoleic, linolenic and arachidic acids, and they are shown as "others" in Figs. 1–3.

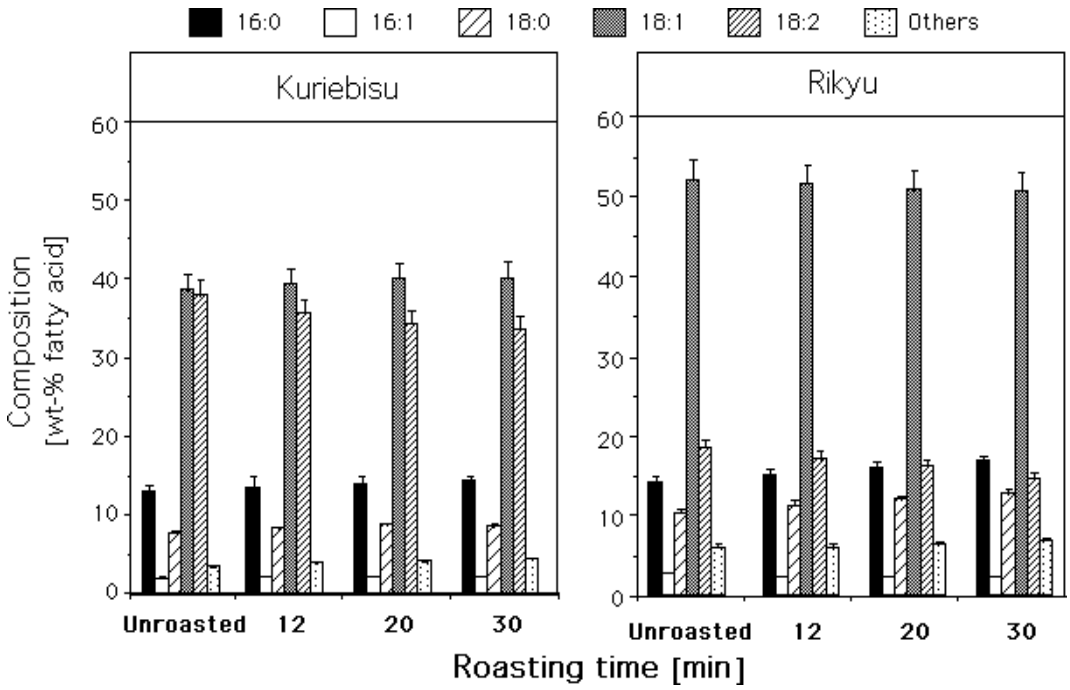

Fig. 2. Fatty acid distributions of free fatty acids in the kernels of pumpkin seeds roasted at different times in a microwave oven at a frequency of 2450 MHz. Each value represents the average of 3 replicates, and vertical bars show the mean error of the replicates. Others contain C14:0, C16:1, C18:3 or C20:0.

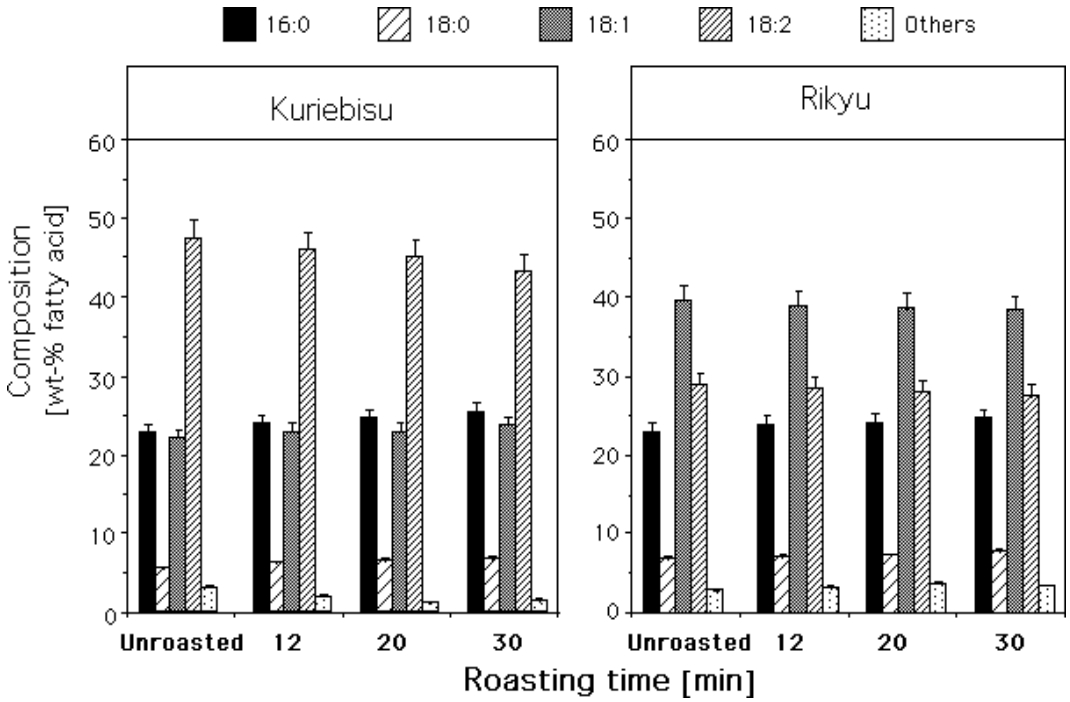

Fig. 3. Fatty acid distributions of phospholipids in the kernels of pumpkin seeds roasted at different times in a microwave oven at a frequency of 2450 MHz. Each value represents the average of 3 replicates, and vertical bars show the mean error of the replicates. Others contain C14:0, C16:1, C18:3 or C20:0.

Longer microwave processing caused higher roasting temperatures that resulted in a lower percentage of linoleic acid and higher percentages of oleic, palmitic and stearic acids in PLs (Fig. 3) as well as TAGs (Fig. 1) or FFAs (Fig. 2). With increase of microwave roasting time, the changes in these fatty acid compositions became more pronounced ($p < 0.05$) in the PLs, followed by the FFAs and then TAGs with a few exceptions. In generally, the changing profiles in these fatty acid distributions were more appreciable in Kuriebisi than in Rikyu because of the differences in the content of linoleic acid.

3.3 Effect of microwave roasting on phospholipids and their fatty acid distributions

To clarify the effects of microwave roasting on PLs, further separation of the PL fraction into 3 fractions (PE, PC and PI) was done by TLC in the presence of authentic samples. Tab. 2 exhibits the changes in the PL fractions of the pumpkin kernels before and after microwave roasting. During microwave roasting, dominant components were PC (>50 wt-%), followed by PI and then PE among the two cultivars. The greatest rate of PL losses ($p < 0.05$) was observed for PE, followed by PC or PI. The trends became more pronounced with longer roasting time. However, these losses were not so much as observed earlier after roasting sesame seeds or soybeans [20, 21]. The hull of pumpkin seeds may somewhat protect the lipid components in the kernels from microwave energy (unpublished work).

The changing profiles of composition and distribution of fatty acids of PE, PC and PI in the pumpkin kernels were compared among the two cultivars before and after microwave roasting as shown in Fig. 4. The major fatty acids in the 3 PLs were linoleic, oleic, palmitic and stearic acids. However, the percentage of palmitic acid was higher ($p < 0.05$) in the PE and PC than the value observed

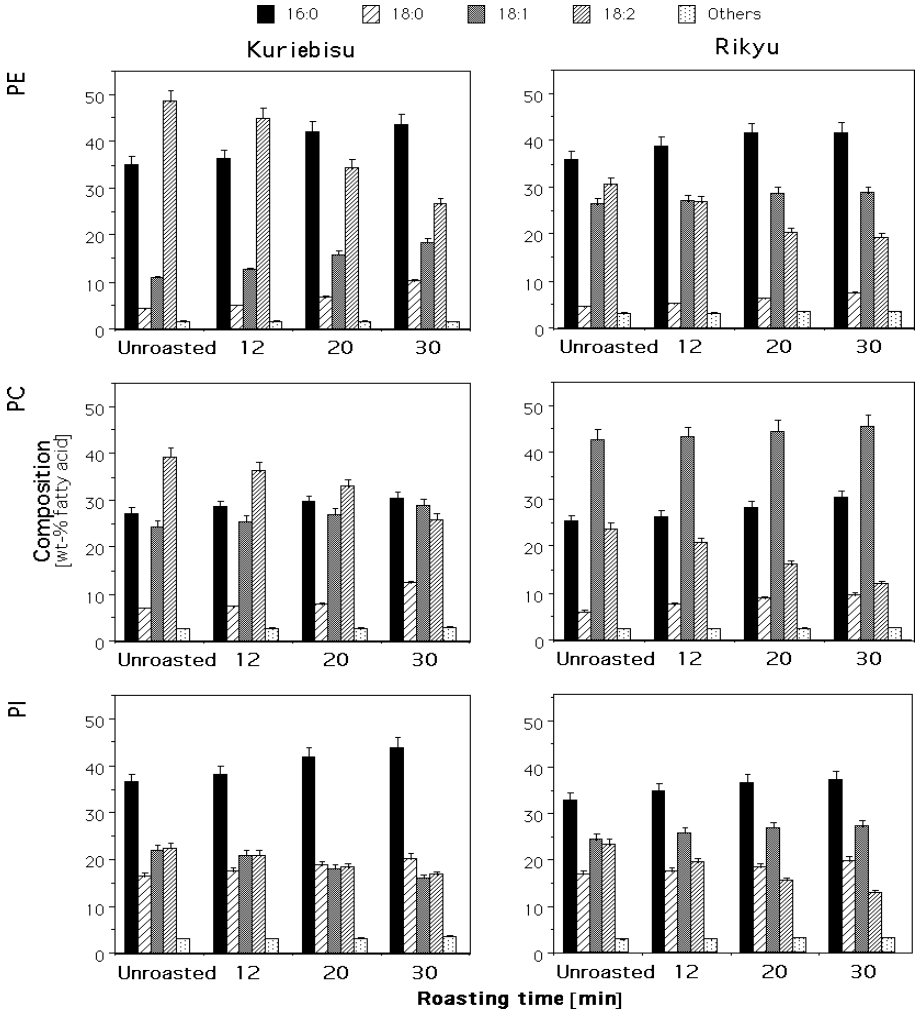

Fig. 4. Fatty acid distributions of major phospholipids in the kernels of pumpkin seeds roasted at different times in a microwave oven at a frequency of 2450 MHz. Each value represents the average of 3 replicates, and vertical bars show the mean error of the replicates. Others contain C14:0, C16:1, C18:3 or C20:0.

in other plant seeds [22, 23]. Following microwave roasting, the percentage of linoleic acid depicted significant decreases ($p < 0.05$) in the 3 PLs after 20 min of microwave roasting (with a few exceptions), whereas the inverse trend was found in the percentage of palmitic, stearic and oleic acids. These trends were more pronounced ($p < 0.05$) in the PE and PC than in the PI, TAGs (Fig. 1) and FFAs (Fig. 2). These profiles would reflect the differences in fatty acid compositions among the two cultivars. The data for 6 min of roasting period were omitted from Fig. 4 because they were essentially the same as those before microwave roasting. These results may be due to the high penetration power of microwaves.

3.4 Effect of microwave roasting on distribution of triacylglycerol molecular species

Pumpkin kernels contained even numbered carbon TAGs for C_{44}–C_{56} before microwave roasting (Fig. 5). Dominant components consisted of C_{52} and C_{54} TAGs, with much smaller amounts of C_{50} and C_{56} TAGs. Minor amounts (<6.8 mg) of C_{44}, C_{46} and C_{48} TAGs were omitted from this study because the samples were too small to obtain reliable results. With increased microwave roasting time, a significantly greater loss ($p < 0.05$) was observed for C_{54} TAGs compared to the other TAGs. The amount of C_{54} TAGs decreased after 30 min substantially by 3900 mg

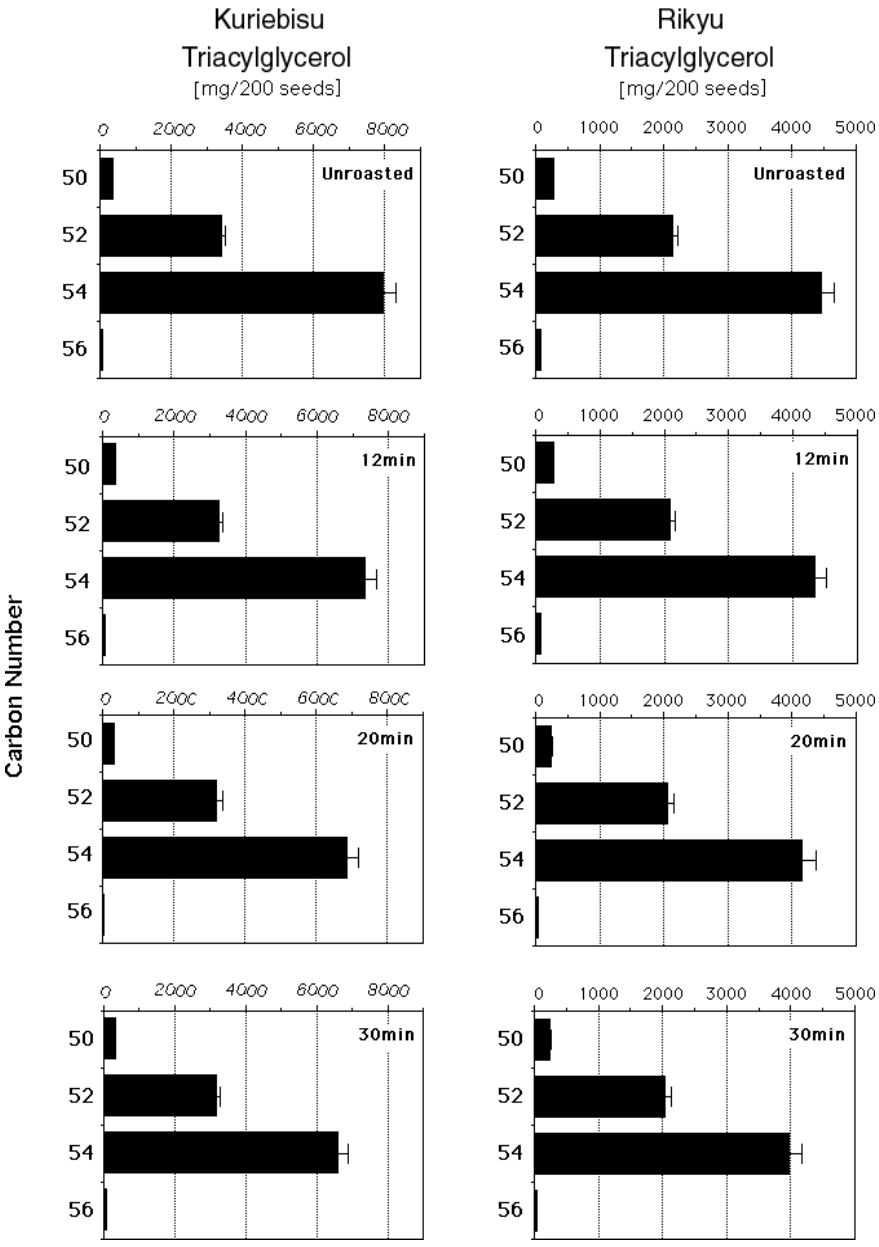

Fig. 5. Changes in the triacylglycerol content in the kernels of pumpkin seeds roasted at different times in a microwave oven at a frequency of 2450 MHz. Carbon numbers show the length of the total acyl chain in a triacylglycerol. Each value shows the average of 3 replicates, and horizontal bars represent the mean errors of the replicates.

Tab. 2. Major phospholipid contents in the oils obtained from the kernels of pumpkin seeds roasted at different times in a microwave oven (at a frequency of 2450 MHz)[†].

Cultivar	Roasting time [min]	Phosphatidyl-ethanolamine	Phosphatidyl-choline	Phosphatidyl-inositol
Kuriebisu	Unroasted	35.4[a] (19.0)	102.8[a] (55.1)	48.3[a] (25.9)
	6	32.8[b] (18.7)	96.0[b] (54.8)	46.5[a] (26.5)
	12	30.6[c] (18.5)	90.7[c] (54.9)	43.8[b] (26.5)
	20	27.0[d] (18.8)	75.3[d] (52.2)	41.5[c] (28.9)
	30	15.3[i] (12.7)	70.9[e] (59.0)	34.0[d] (28.3)
Rikyu	Unroasted	25.1[e] (22.6)	59.2[f] (53.1)	27.1[e] (24.3)
	6	23.0[f] (22.1)	54.2[g] (52.1)	26.8[e] (25.8)
	12	20.3[g] (20.8)	50.8[h] (51.8)	26.9[e] (27.4)
	20	14.7[h] (17.4)	43.9[i] (51.8)	26.1[e] (30.8)
	30	10.0[j] (13.8)	37.7[j] (52.1)	24.7[f] (34.1)

[†] Each value is the average of 3 determinations and expressed as mg lipid per 200 kernels. Values in parentheses are relative contents in % of the individual lipids in total lipids. Values in the same column with different indices are significantly different from those for unroasted pumpkin seeds ($p < 0.05$).

for Rikyu and 6500 mg for Kuriebisu, respectively. These results would depend on differences in the amounts of TAGs composed of oleic and linoleic acids.

Fig. 6 shows the typical changing patterns in the TAG molecular species isolated from the kernels of pumpkin seeds microwaved for different time periods. Eleven different molecular species were detected in the oils extracted from the kernels of unroasted pumpkin seeds. The three-letter designation does not suggest fatty acyl positional isomers in the TAGs (P, palmitic; St, stearic; O, oleic; L, linoleic). Major TAG species were POO or StOO (SM_2), PPL, PStL or StStL (S_2D), OOO (M_3), POL or StOL (SMD), and OOL (M_2D) among the two cultivars, and further StLL or PLL (SD_2) and OLL (MD_2) for Kuriebisu. These results would reflect the differences in the composition and distribution of fatty acids composed of TAGs (Fig. 1). The other species (S_3, S_2M, D_3 or D_2T) were detected as minor components (Fig. 6). With increased microwave roasting time, an appreciable loss ($p < 0.05$) was apparent in the TAG molecular species having more than 4 double bonds. These trends became more pronounced ($p < 0.05$)

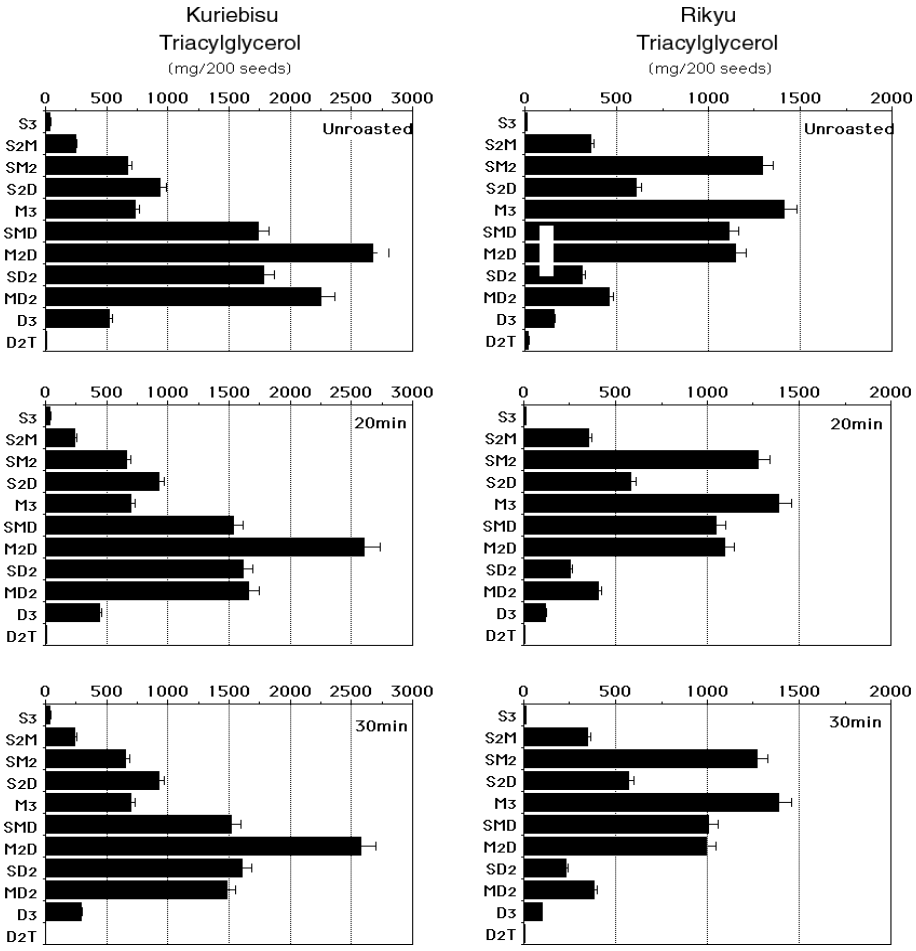

Fig. 6. Changes in the molecular species of triacylglycerols in the kernels of pumpkin seeds roasted at different times in a microwave oven at a frequency of 2450 MHz. Saturated fatty acids (S) consist of myristic (C14:0), palmitic (C16:0) and stearic (C20:0) acids. Unsaturated fatty acids, oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids are denoted as monoene (M), diene (D) and triene (T), respectively. Each value shows the average of 3 replicates, and horizontal bars depict the mean errors of the replicates.

after 20 min of roasting. The rate of fatty acid breakdown is related to the number of double bonds in the carbon chain of the molecule. As the number of double bonds increases, the rate of oxidation increases. The ratios of the rates of oxidation of oleate to linoleate to linolenate are reported to be 1:10:20 [24].

4 Conclusions

When pumpkin seeds were microwaved for 20 min or more, significant differences ($p < 0.05$) occurred in the acyl lipids as well as in their fatty acid distributions with a few exceptions. These trends became more pronounced ($p < 0.05$) in Kuriebisu than in Rikyu because of the differences in the compositions and distributions of fatty acids composed of the acyl lipids. Therefore, microwave roasting caused a significant decrease ($p < 0.05$), not only in TAGs molecular species containing more than 4 double bonds, but also in the amounts of diene species present in TAGs. These results suggest that no significant changes in molecular species or fatty acid distribution of TAGs would occur within 12 min of microwave roasting, ensuring that a good quality product would be attained. Pumpkin kernels flours have great potential for addition to food systems, not only as nutrient supplements but also as a functional agent. These results could contribute to study the functional properties of pumpkin seed products.

Acknowledgement

This work was partly financially supported by a research grant for Health Science of Kobe-Gakuin University.

References

[1] *M. Tu, W. D. Eustace, C. W. Deyoe:* Nutritive value of buffalo gourd seed protein. Cereal Chem. **55** (1978) 766–772.

[2] *T. A. El-Adawy, K. M. Taha:* Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. J. Agric. Food Chem. **49** (2001) 1253–1259.

[3] *A. S. Al-Khalifa:* Physicochemical characteristics, fatty acid composition and lipoxygenase activity of crude pumpkin and melon seed oils. J. Agric. Food Chem. **44** (1996) 964–966.

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

[5] *H. Yoshida, S, Takagi:* Effects of seed roasting temperature and time on the quality characteristics of sesame (*Sesamum indicum*) oil. J. Sci. Food Agric. **75** (1997) 19–26.

[6] *H. Yoshida, G. Kajimoto:* Effects of microwave treatment on the trypsin inhibitor and molecular species of triglycerides in soybeans. J. Food Sci. **53** (1988) 1756–1760.

[7] *J. Folch, M. Lees, G. H. Sloane-Stanley:* A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226** (1957) 497–509.

[8] *S. Takagi, H. Yoshida:* Microwave heating influences on fatty acid distributions of triacylglycerols and phospholipids in hypocotyl of soybeans. Food Chem. **66** (1999) 345–351.

[9] *H. Yoshida, J. Shigezaki, S. Takagi, G. Kajimoto:* Variations in the composition of various acyl lipids, tocopherols and lignans in sesame seed oils roasted in a microwave oven. J. Sci Food Agric. **68** (1995) 407–415.

[10] *Association of Official Analytical Chemists:* Official Methods of Analysis, 14th Ed. Assoc. Off. Anal. Chem, Washington DC (USA) 1980.

[11] *M. Matsui, T. Watanabe, N. Ikekawa:* Effects of α-tocopherol deficiency on carp-III. Analytical method for triglyceride composition analysis of fish lipid. Nippon Suisan Gakkaishi **39** (1973) 367–373.

[12] *H. Yoshida:* Molecular species and fatty acid distributions of triacylglycerols from germinating soybean cotyledons. Lipids **19** (1984) 936–941.

[13] *I. A. De La Roche, E. J. Weber, D. E. Alexander:* The selective utilization of diglyceride species into maize triglycerides. Lipids **6** (1971) 537–540.

[14] *M. L. Blank, B. Verdino, O. S. Privett:* Determination of triglyceride structure *via* silver nitrate-TLC. J. Am. Oil Chem. Soc. **42** (1965) 87–90.

[15] *R. C. D. Steel, J. H. Torric*: Analysis of variance I: The one-way classification. In: Principles and procedures of statistics. 2nd Ed. Eds. *R. C. D. Steel, J. H. Torrie*, McGraw-Hill, New York (USA) 1980, pp. 137–171.

[16] *R. J. Baker:* Multiple comparison tests. Canadian J. Plant Sci. **60** (1980) 325–327.

[17] *M. H. Aboul-Nasr, B. R. Ramadan, R. A. El-Dengawy:* Chemical composition of pumpkin seeds. Assiut J. Agric. Sci. **28** (1997) 168–172.

[18] *L. Cossignani, M. S. Simonetti, A. Neri, P. Daminani:* Changes in olive oil composition due to microwave heating. J. Am. Oil Chem. Soc. **75** (1998) 931–937.

[19] *H. A. Abou-Gharbia, A. Adel, Y. Shehata, F. Ahahidi:* Effect of processing on oxidative stability and lipid classes of sesame oil. Food Res. Int. **33** (2000) 331–340.

[20] *G. C. Yen, S. L. Shyu, T. C. Lin:* Studies on improving the processing of sesame oil. I. Optimum processing conditions. Food Sci. **13** (1986) 198–211.

[21] *H. Yoshida, S. Takagi:* Microwave roasting and positional distribution of fatty acids of phospholipids in soybeans (*Glycine max* L.). J. Am. Oil Chem. Soc. **74** (1997) 915–921.

[22] *M. Noda, T. Obata:* Fatty acid distribution in perilla seed lipids (Lipids of leaves and seeds part VII). Nippon Nogeikagaku Kaishi **49** (1975) 251–256.

[23] *H. Yoshida, J. Shigezaki, S. Takagi, G. Kajimoto:* Variations in the composition of various acyl lipids, tocopherols and lignans in sesame seed oils roasted in a microwave oven. J. Sci. Food Agric. **68** (1995) 407–415.

[24] *S. H. Fatemi, E. G. Hammond:* Analysis of oleate, linoleate and linolenate hydroperoxides in oxidized ester mixtures. Lipids **15** (1980) 379–385.

[Received: August 27, 2003; accepted: November 18, 2003]

