

Vitamin E and Oxidative Stability of Soya Bean Oil Prepared with Beans at Various Moisture Contents Roasted in a Microwave Oven

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Abstract: Whole soya beans (*Glycine max*) at various moistures (96, 382 and 519 g kg⁻¹) were roasted by exposure to microwaves at a frequency of 2450 MHz and the effects on the tocopherols of soya beans were studied in relation to chemical changes in the oils. The amounts of α -, β -, γ - and δ -tocopherols in the soya beans before microwave treatments ranged from 62 to 187, 43 to 89, 673 to 757 and 542 to 593 mg kg⁻¹ oil, respectively. Increasing moisture contents by soaking prevented, not only the reduction of tocopherols but also, the oxidative deterioration of soya bean oils during microwave roasting. The amounts of tocopherols still remained >80% of the original level in soaked soya bean oils after 20 min of roasting, and microwave roasting after soaking caused no significant differences ($P > 0.05$, with few exceptions) in the chemical changes of the oils in comparison with those before soaking. These results implied that microwave roasting after soaking would be effective in making full-fat soya flour with high vitamin E without a burnt odour and browning from raw beans.

Key words: anisidine value, carbonyl value, microwave roasting, peroxide value, soaking, soya beans, tocopherols, TBA-reactive substances (TBARS), vitamin E.

INTRODUCTION

Soya beans (*Glycine max*) have long been recognised not only as an excellent source of protein, but also as a valuable source of an edible oil for feeding both man and animals. However, they also contain various anti-nutritional factors, such as trypsin inhibitor, which are thought to reduce protein digestibility and to cause pancreatic hypertrophy (Kakade *et al* 1972). Therefore, a heating process to remove antinutritional factors is essential in the use of soya beans as a food or feed. Various methods of heating, including toasting (Gardner 1975), dry roasting (Johnson *et al* 1980), heating in boiling water (Collins and Beaty 1980) and micronisation (Hutton and Foxcroft 1975) have been used to improve the nutritional value of soya beans and have proved effective.

Microwave energy is an important new means of heating which has become readily available commercially and is forecast to be utilised much more

extensively in the future (Mudgett 1989). Advantages associated with microwave energy are its depth of penetration and rapid rate of heating. Recently, therefore, microwave cooking has become increasingly popular. Many advances have been made in the area of microwave product development. Researchers have become more and more concerned with the flavour generation and flavour changes of spices or seasoning during microwave cooking.

Soya beans and their products are relatively good sources of vitamin E (tocopherols), as well as cereal germs such as corn or wheat (Guzman and Murphy 1986; Piironen *et al* 1986). In addition to their vitamin E function, tocopherols are natural antioxidants in foods and are very important biological and nutritive components of our food. Eight natural compounds possess vitamin E activity, namely the α -, β -, γ - and δ -tocopherols (Ball and Ratcliff 1978) and the four closely related tocotrienols (Slover 1971). The latter group of compounds occur at much lower levels and their structures differ from the tocopherols in that they have three double bonds in the side chain (Carpenter 1979). All

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forms have been found in foods and fats, although δ -tocotrienol is rare and, among foods, has been reported only in palm oil. The mechanism of action of the tocopherols is not yet fully understood. Moisture content is one of the most important quality factors affecting the storage, handling, marketing and processing of grains and oilseeds. Several methods can be used to determine moisture content of oilseed, and microwave oven drying has been shown to be feasible for soya beans and sunflower, with the added benefits of saving time (Backer and Walz 1985; Sharma and Hanna 1989). Esaka *et al* (1986) suggested that microwave treatment was more effective for inactivating lipoxygenase in soya beans with higher moisture contents. Therefore, soya beans have generally been moistened before treatment in the belief that the presence of excess moisture was necessary for nutritional improvement by microwave treatment. However, little has been reported regarding on how the moisture and roasting time affect the tocopherol homologues and oxidative stability of the soya bean oil.

The present study was undertaken to distinguish between the content of vitamin E and the oxidative stability of lipids in soya beans at various moisture contents when roasted in a microwave oven.

EXPERIMENTAL

Materials

Soya beans

The samples of soya beans (*Glycine max* L) used in this study were of three cultivars: *Okuhara*, *Mikawajima* and *Tsurunoko*. The soya beans were all grown in Japan in the summer of 1994. These soya bean cultivars (purchased from Takii Seed Co, Kyoto, Japan) were selected for uniformity based on bean weight (ie 270–319 mg for *Okuhara*, 300–369 mg for *Mikawajima* and 430–499 mg for *Tsurunoko*, respectively). All the beans were cleaned and divided into groups for storage in stainless steel containers at 4°C until required. Soaking was done to prepare all soya beans with different moisture contents immediately before microwave treatments, as described (Yoshida and Kajimoto 1988). The soaked soya beans were placed on filter paper (Toyo No 2) to dry at ambient temperature for 30 min. The moisture content in the beans was determined in duplicate (AOAC 1980).

Reagents

All solvents were of analytical grade (Nacalai tesque, Kyoto, Japan) and used without further purification. Vitamin E homologues (α , β , γ and δ) were purchased from Eisai Co (Tokyo, Japan). All the tocopherols were of the D-form (RRR-) and the purity of each tocopherol was $> 985 \text{ g kg}^{-1}$ by HPLC. Thin-layer chromatog-

raphy (TLC) pre-coated silica-gel 60 plates (20 × 20 cm, 0.25 mm layer thickness) were purchased from Merck (Darmstadt, Germany). BF_3 in methanol (140 g litre⁻¹) (Wako Pure Chemical Ind Ltd, Osaka, Japan) was used to prepare the fatty acid methyl esters.

Methods

Roasting of soya beans

A modified domestic size microwave oven (Sharp Model R-5550, Osaka, Japan), capable of generating 0.5 kW power at 2450 MHz, was used. Whole soya beans with different moisture contents (96, 382 and 519 g kg⁻¹) were placed as a single layer in a Pyrex petri dish of diameter 8.0 cm and subjected to microwave treatments at 2450 MHz for various periods (4–20 min) after covering the dish. The internal temperatures of soya beans immediately after each treatment were determined with a chromel-alumel thermocouple as previously described (Yoshida and Kajimoto 1989). Each petri dish contained *c* 25–30 g (78–85 beans) soya beans; three dishes were treated once at each of the different exposures to provide sufficient sample material for analyses and testing. The samples after treatments were allowed to cool to ambient temperature and immediately packed in a polyethylene bag and used for various analyses.

The moisture content of a sample roasted in the oven was expressed as an apparent moisture and was calculated by dividing weight of moisture loss after each roasted time by the weight of the sample before roasting.

Lipid extraction

The roasted soya beans (50 beans) were ground with 100 ml chloroform/methanol (2:1, v/v) at 0°C in an electric blender and the lipids were further extracted three times with 150 ml of chloroform/methanol (once at 1:1, v/v and then twice at 2:1, v/v) in the blender. These solvents contained butylated hydroxytoluene (100 mg litre⁻¹), which was added to inhibit the oxidative degradation of tocopherols and lipids during extraction. The combined extracts were dried in a rotary vacuum evaporator at 35°C, and residues were dissolved in chloroform/methanol (2:1, v/v). The solution was washed with water (Folch *et al* 1957) and dried over anhydrous Na_2SO_4 . Solvents were removed from filtrates with a rotary evaporator under reduced pressure at 35°C to obtain total lipids. The extracted lipids were redissolved in chloroform/methanol (2:1, v/v) and stored in a 25 ml brown glass volumetric flask under nitrogen in the dark at -25°C until required for further analysis. Lipids were also extracted from raw beans for a control by the same method as described before.

HPLC

A 0.2 g portion of sample, before and after microwave roasting, was transferred quantitatively to a 5 ml brown volumetric flask and the solvents removed under nitrogen gas. The residue was dissolved with mobile phase (for HPLC as described below). The chromatographic system consisted of a normal-bonded phase Shim-pack CLC-SIL (M) column (5 μm , 250 cm \times 4.6 mm id, Shimadzu) protected by a 1 cm guard column (Shim-pack G-SIL). The mobile phase was a mixture of *n*-hexane/1,4-dioxane ethanol (490:10:1, v/v/v) at 2.0 ml min⁻¹. An aliquot (6 μl) was injected with a fully loaded 20 μl loop. The tocopherols were monitored with a fluorescence spectrophotometer (Shimadzu RF-535, Shimadzu Instruments Inc, Kyoto, Japan) set at excitation wavelength 295 nm and emission wavelength 320 nm, quantitated by comparison with peak areas of internal standard (2,3,5,7,8-pentamethyl-6-hydroxy chroman, Eisai Co, Tokyo, Japan). The other HPLC conditions were as reported previously (Yoshida and Kajimoto 1989).

Analysis of lipids

Official methods (AOCS 1990) were used for determination of peroxide (method Cd 8-53) and 2-thiobarbituric acid reactive substances (TBARS: method Cd 19-90). The TBARS was expressed as mg malonaldehyde equivalent kg⁻¹ oil. The *p*-anisidine value and carbonyl value of the roasted soya bean oils were measured according to standard methods (IUPAC (1987) in JOCS (1986), respectively). As an index colour development (Fritsch 1981), the absorbance at 420 nm of a 50 g kg⁻¹ solution of lipids in chloroform was determined with a Shimadzu spectrophotometer UV 200S.

Lipid class analysis

The total lipids were fractionated by TLC into the following two fractions: triacylglycerols (TAG) and polar lipids (PL). The crude lipid extracts were applied on TLC plates as 7 cm bands (*c* 20 mg per plate) with a microsyringe (Hamilton Co, Reno, NV, USA). The plates were developed in *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v) after applying standard mixture as previously described (Yoshida *et al* 1995a) and then were covered with other glass plates, leaving the reference zone exposed to be visualised by exposure to iodine vapour. Bands corresponding to TAG and PL were scraped separately into test tubes (10.5 cm \times 16 mm) with Teflon-coated screw caps, respectively. Methyl pentadecanoate (15:0) was added as internal standard to the total lipids and to each fraction at *c* 10% level (w/w esters).

The fatty acid methyl esters prepared by transesterification (Morrison and Smith 1964) were analysed by a Shimadzu Model 14A-gas chromatograph (GC) as described previously (Yoshida and Kajimoto 1994).

After recording on a Shimadzu C-R4A integrating system, component peaks were identified by comparison of their retention times with those standards (F & OR mixtures No 3) and quantified by reference to the internal standard. The other GC conditions were as reported previously (Yoshida and Kajimoto 1981). Peak areas were computed and proportions of the fatty acid methyl esters were obtained as weight percent of total methyl esters by direct internal normalisation. No correction factors were used because the response factors of the main fatty acids were close to unity.

Statistical analysis of experimental data

Each reported value is the mean of three determinations, and the data were subjected to analysis of variance with a randomised complete block design to partition the effects of different parameters (Steel and Torrie 1980). Duncan's multiple range test (Duncan 1955) was applied to determine significance of differences between means.

RESULTS AND DISCUSSION

Proximate analysis using AOAC (1980) procedures showed that the moisture contents of the soya beans were 96 g kg⁻¹ before soaking, 382 g kg⁻¹ after 1 h soaking and 519 g kg⁻¹ after 5 h soaking. Effects of microwave roasting (*cv* *Tsurunoko*) were compared on the basis of the internal temperature of whole soya beans at the various moisture contents (Fig 1). Each point shows an average at the end of each roasting time. A linear relationship occurred between the internal temperature and exposure time in the first 4 min of roasting. The temperature of all experimental groups of soya beans increased most during the first 2–4 min of microwave treatment. Thereafter, the temperature of soya

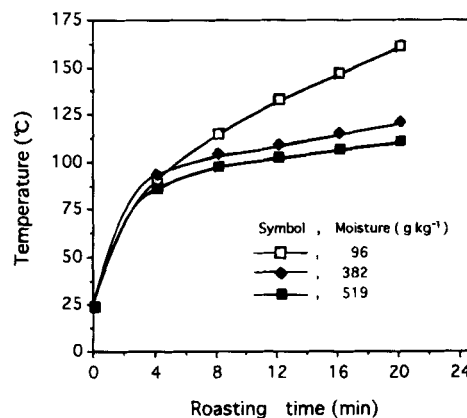


Fig 1. Relationship between roasting time and internal temperature of soya beans at various moisture contents roasted in a microwave oven (2450 MHz). All data points represent the means of measurements from three replicates; the standard errors are within the size of the symbols.

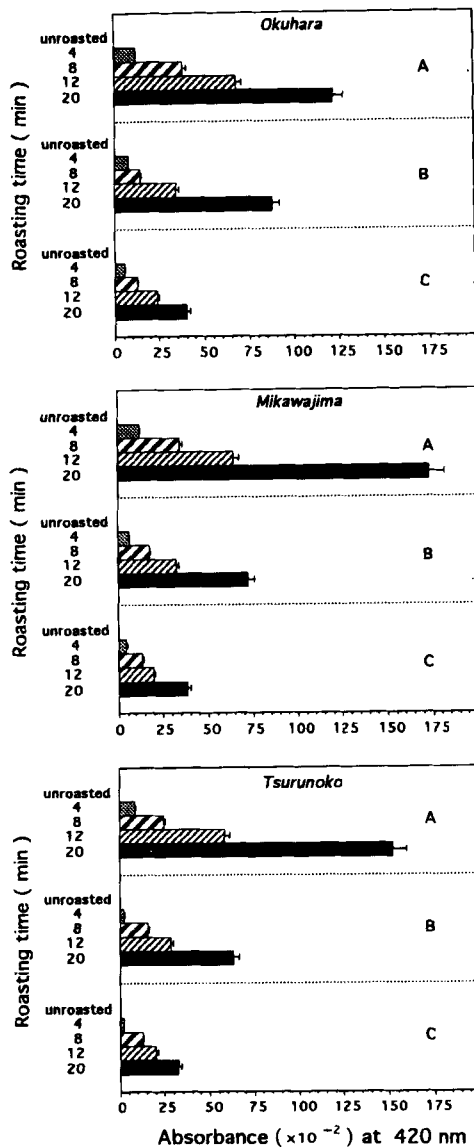


Fig 2. Changes in the colour (absorbance at 420 nm) of the oils prepared from soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg⁻¹; (B) moisture 382 g kg⁻¹; (C) moisture 519 g kg⁻¹. Each value represents the average of three replicates and vertical bars represent standard error of the replicates.

beans of lower moisture contents (96 g kg⁻¹) increased more rapidly than that for the other two experimental groups: namely, the final temperature of soya beans soaked for 5 h was below 110°C whereas that of those not soaked was more than 160°C. A dark brownish colour, indicating caramelisation of the sugar released during microwave roasting and burnt odour became apparently at 12 min in soya beans that were not soaked, but only occurred slightly at 20 min in soya beans of 382 and 519 g kg⁻¹ moisture.

Energy absorption during microwave treatments is attributable to two principal processes—elevation of bean temperature and vaporisation of water. The results

were also supported by an increase of the absorbance at 420 nm (Fig 2). The changes in the temperature and colour of soya beans were similar for all three soya bean cultivars. The higher the moisture content of the soya beans (382 or 519 g kg⁻¹), the lower the internal temperature of the soya beans over the same roasting periods after 4 min exposure time. Therefore, increasing bean moisture content markedly inhibited the formation of browning substances. Although bean weight differed significantly ($P < 0.05$) among the cultivars used in this study, the initial internal moisture levels differed by only 80–98 g kg⁻¹. Therefore, no significant differences ($P > 0.05$) in the internal temperature at each moisture content were observed among the three soya bean cultivars during microwave treatments (data not shown).

The effects of microwave roasting were compared on the total and individual tocopherol contents in the soya beans by the differences in moisture content (Fig 3). Tocopherol contents varied significantly ($P < 0.05$) between soya bean cultivars before microwave treatments. The possibility that sample variations, year and place of seed production contributed to these differences cannot be excluded. The variation in vitamin E contents of soya bean cultivars may be due to genetic differences or to the geographical origin of the beans, as has been noted for corn (Weber 1984). However, regardless of soya bean cultivar, dominant components were γ - and δ -tocopherols, with much smaller amounts of α - and β -tocopherols in the whole soya beans before and after microwave roasting. The rate of loss of tocopherol gradually increased during microwave roasting. However, the longer the soaking period, the smaller ($P < 0.05$) the rate of the total and individual tocopherol losses in the beans. Microwave treatments for 12–20 min still retained over 88% of the original tocopherols (with few exceptions) among the three cultivars. Therefore, increasing bean moisture content inhibited the reduction of tocopherols during microwave roasting. This occurred because the internal temperature of higher moisture whole beans (318 and 519 g kg⁻¹) was lower than that of unsoaked beans (Fig 1).

The characteristics and qualities of the oils prepared from soya beans at the various moisture contents are shown in Figs 4–7, respectively. The peroxide value serves as an indicator of the extent of primary oxidation products in the oils, while the carbonyl value, anisidine value or TBARS value reflects the degree of secondary oxidation products (Augustin and Berry 1983). Figure 4 shows the changing patterns of peroxide value in the oils during microwave roasting. An appreciable change ($P < 0.05$) was observed between the oils from unsoaked and soaked soya bean, and the change depended on the differences in the moisture content. The peroxide value before roasting was below 3.0 meq kg⁻¹ in the oil process unsoaked soya bean, but an appreciable increase was observed in the oils

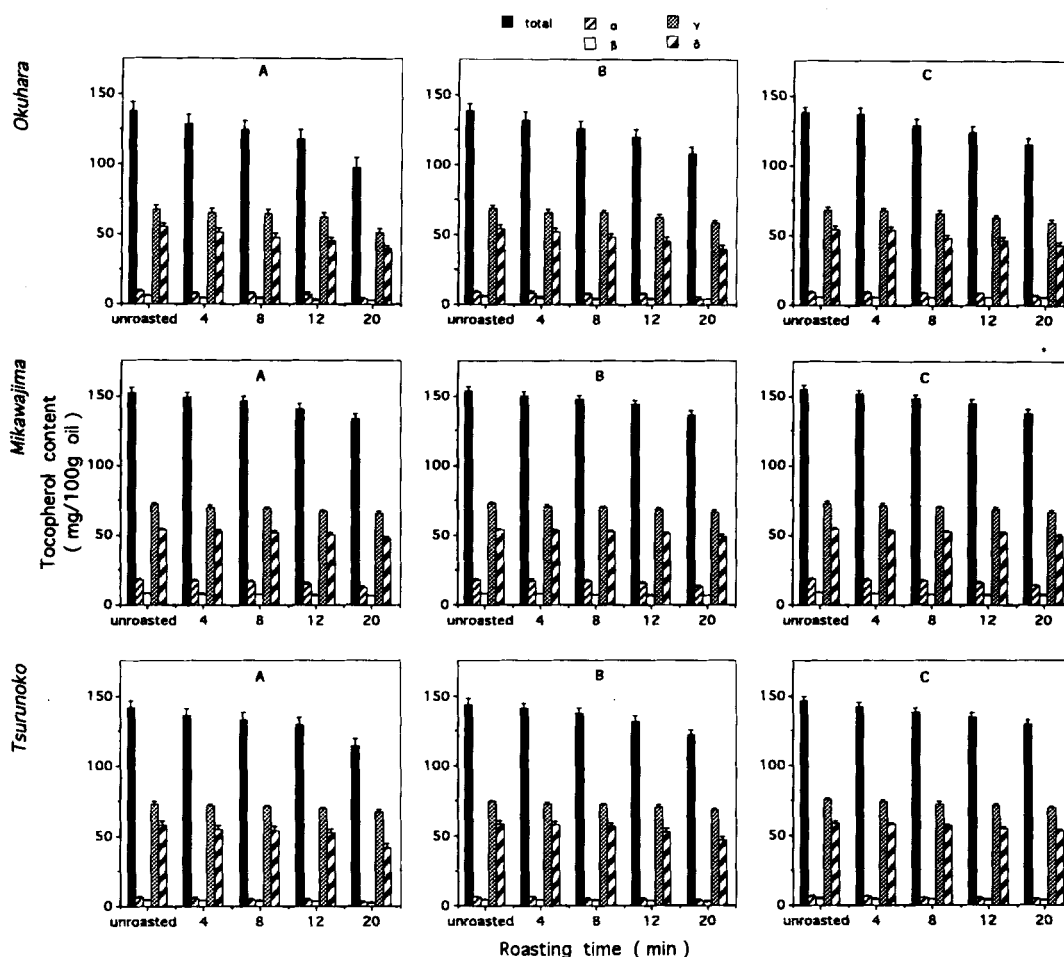


Fig 3. Changes in total and individual tocopherol contents in the oils prepared from soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg^{-1} ; (B) moisture 382 g kg^{-1} ; (C) moisture 519 g kg^{-1} . Each value represents the average of three replicates and vertical bars represent standard error of the replicates.

from soaked beans and resulted in $>7.0 \text{ meq kg}^{-1}$ immediately after 5 h soaking. The results implied the occurrence of action of lipoxygenase during soaking (Whitaker 1991). However, Esaka *et al* (1986) and Kermasha *et al* (1993) reported that microwave roasting was effective for inactivation of soya bean lipoxygenase. As microwave roasting progressed, the peroxide value gradually increased at a similar rate in unsoaked soya bean oil, whereas the value substantially decreased in soaked soya bean oils. These trends became more pronounced ($P < 0.05$) with longer soaking and roasting time. Therefore, the higher the moisture content and the longer the roasting period, the faster was the rate of degradation of hydroperoxides. The results indicated that microwave energy is not only effective for inactivation of the lipoxygenase, but also destroys the hydroperoxides formed during soaking.

In general, the peroxide values do not represent the absolute state of oxidation of an oil because hydroperoxides are unstable to heat, resulting in rapid transformation to secondary products (Gray 1978). Hitherto, little is known about how microwave energy affects the

oxidative stability of soaked soya bean oils when roasted in a microwave oven. Figures 5, 6 and 7 illustrated the effect of microwave energy on the TBARS value, carbonyl value and anisidine value of unsoaked and soaked soya bean oils, respectively. An exception with unsoaked soya bean oil (96 g kg^{-1}) was the changing patterns which were in the reverse order compared with those of the peroxide values. In the initial stage of roasting, the TBARS value and carbonyl value became more pronounced ($P < 0.05$) in the unsoaked soya bean oil than those in the soaked soya bean oils, and further increased significantly ($P < 0.05$) with longer roasting (Figs 5 and 6). On the other hand, the anisidine value increased slowly in the first 12 min of roasting and rapidly thereafter (Fig 7). With microwave exposure longer than 12 min, these trends became more pronounced ($P < 0.05$) in the unsoaked soya bean oils than those in the soaked soya bean oils. These observations are consistent with the appearance of a dark brownish colour and burnt odour which became apparent at 12 min in soya bean with increasing roasting times (Fig 2).

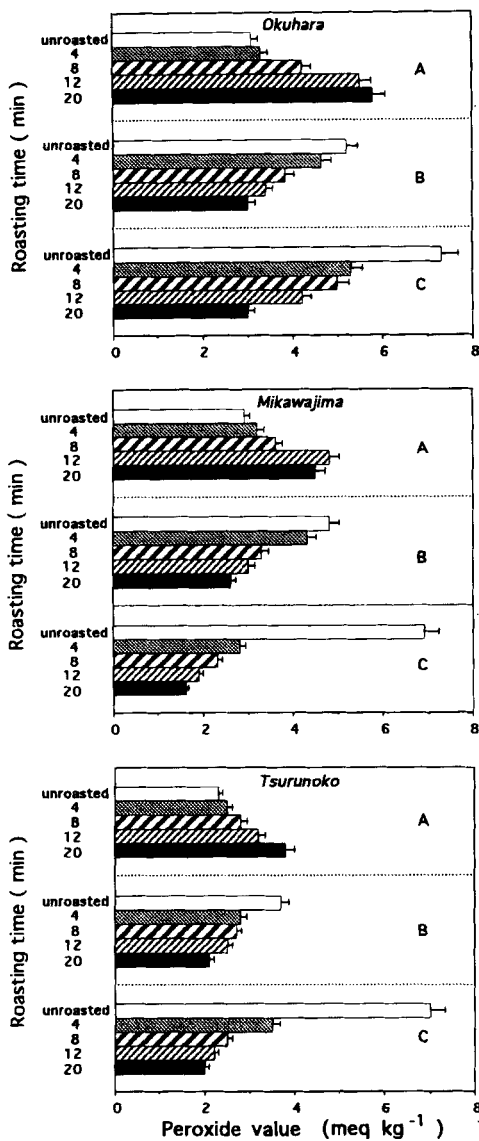


Fig 4. Changes in peroxide value of the oils prepared from soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg⁻¹; (B) moisture 382 g kg⁻¹; (C) moisture 519 g kg⁻¹. Each value represents the average of three replicates and vertical bars represent standard error of the replicates.

Predominant components were TAG (852–927 g kg⁻¹), with much smaller amounts of PL (68–95 g kg⁻¹), and others were minor lipid components (less than 18 g kg⁻¹) in all soya bean cultivars before and after microwave roasting as earlier described (Yoshida *et al* 1995b). Typical profiles (*c Tsurunoko*) of fatty acid composition (expressed in terms of the esters by weight) of major lipid classes in all soya beans before and after microwave roasting were compared (Fig 8). Fatty acid compositions in the first 4 min of roasted and soaked soya beans (382 g kg⁻¹) for 1 h were omitted because they were essentially the same as before roasting and soaking. Linoleic (48.8–62.4%), oleic (5.2–26.3%), palmitic (10.3–20.5%), linolenic (6.5–13.6%)

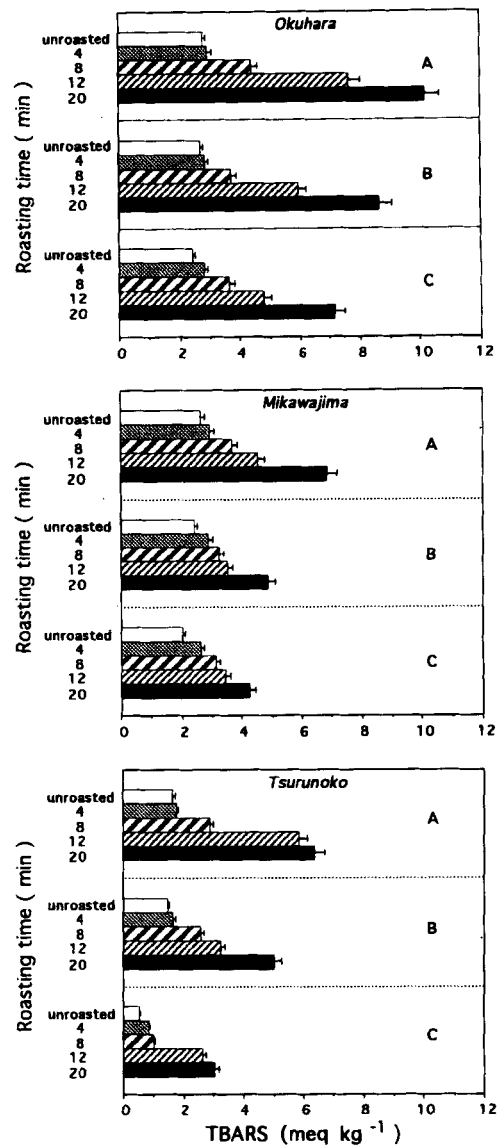


Fig 5. Changes in TBARS value of the oils prepared from soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg⁻¹; (B) moisture 382 g kg⁻¹; (C) moisture 519 g kg⁻¹. Each value represents the average of three replicates and vertical bars represent standard error of the replicates.

and stearic (2.1–3.8%) were principal fatty acids in all soya beans. A small difference ($P < 0.05$) occurred in fatty acid composition between total lipids and PL and between TAG and PL of bean lipids before microwave roasting. There were also differences ($P < 0.05$) in linoleic, oleic and palmitic acids between total lipids or TAG and PL; PL was higher (61.8–62.4%) in linoleic, (16.3–18.6%) in palmitic and lower (5.2–5.6%) in oleic than those in total lipids or TAG. After microwave roasting, no differences ($P > 0.05$) were observed in fatty acid compositions between total lipids and TAG in unsoaked and soya beans soaked for 1 h (382 g kg⁻¹). However, in unsoaked soya beans (96 g kg⁻¹ moisture), the percentages of linoleic and linolenic acids in TAG

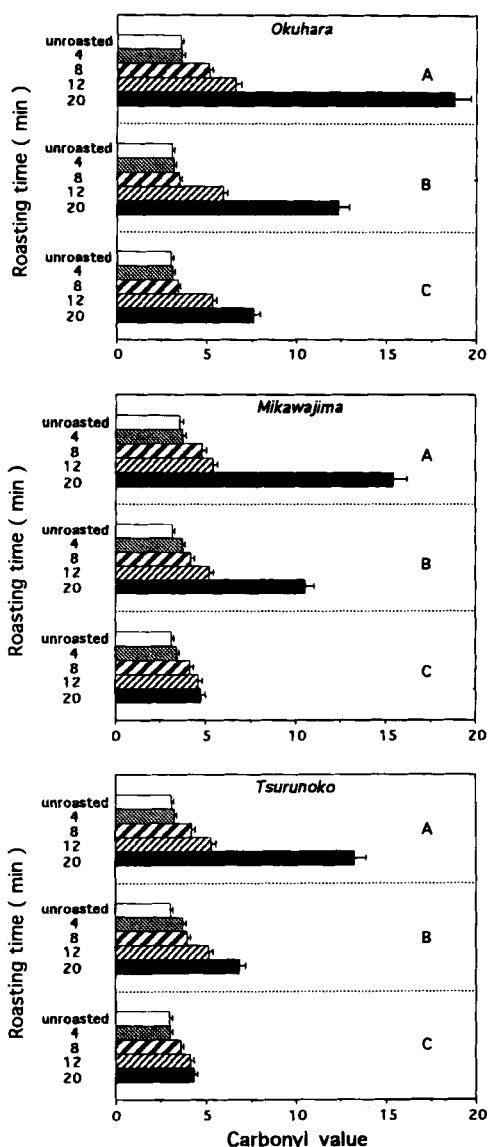


Fig 6. Changes in carbonyl value of the oils prepared from soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg^{-1} ; (B) moisture 382 g kg^{-1} ; (C) moisture 519 g kg^{-1} . Each value represents the average of three replicates and vertical bars represent standard error of the replicates.

decreased gradually ($P < 0.05$) and these values were compensated by increases ($P < 0.05$) in palmitic and oleic acids. Differences ($P < 0.05$) occurred in the changing patterns of fatty acid composition of PL in soya beans at the different moistures during microwave roasting. The lower the moisture content and the longer the roasting time, the greater were the proportions of palmitic and the lower were those of linoleic or linolenic acid. This may reflect differences in decomposition of individual phospholipids in soya beans at the different moistures as described previously (Yoshida *et al* 1995b). Tomioka and Morioka (1992) reported that the oxidative deterioration of phospholipids in fish meat proceeded with heating, and effects of microwave heating were much larger than with other heating techniques.

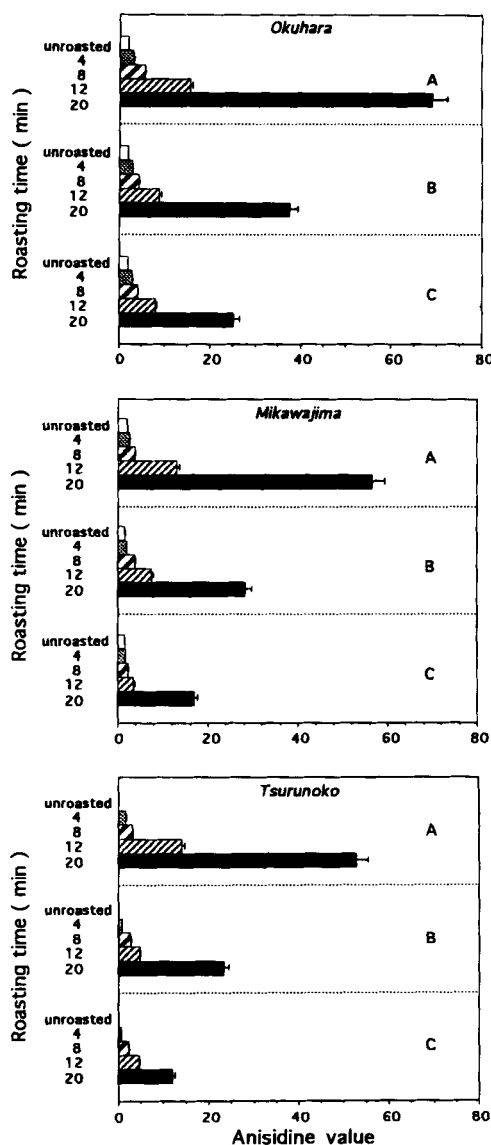


Fig 7. Changes in anisidine value of the oils prepared from soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg^{-1} ; (B) moisture 382 g kg^{-1} ; (C) moisture 519 g kg^{-1} . Each value represents the average of three replicates and vertical bars represent standard error of the replicates.

In addition to transparency, colour is an important physical characteristic of oil. With the progress of microwave roasting, the colour of the oils in unsoaked beans changes gradually from yellow-brown at 2–8 min of roasting to brown at 12 min of roasting and finally deep-brown at 20 min roasting. However, formation of browning substances was markedly inhibited by increasing moisture content (Fig 2). The predominance of γ - and δ -tocopherols in soya bean oils has a significance in relation to the nutritional value of the beans and to the stability of the oils to oxidative damage. Tocopherols are commonly used as antioxidants in vegetable oils and current evidence suggests that γ - or δ -tocopherol is a better antioxidant than α -tocopherol (Khafizov *et al* 1975). It seems likely that γ - and δ -

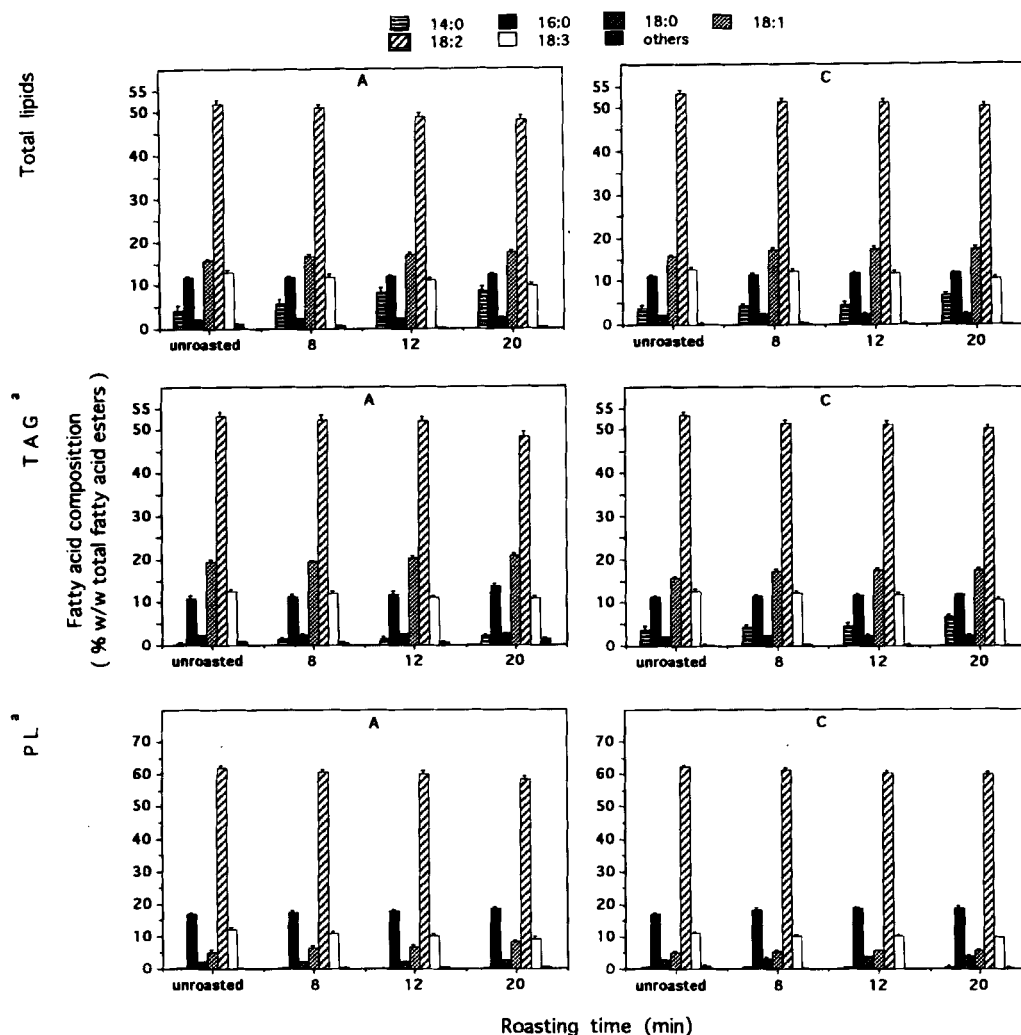


Fig 8. Fatty acid distributions of total lipids, triacylglycerols and polar lipids of soya beans at various moisture contents roasted in a microwave oven (2450 MHz). (A) Moisture 96 g kg^{-1} ; (B) moisture 382 g kg^{-1} ; (C) moisture 519 g kg^{-1} . Each value represents the average of three replicates and vertical bars represent standard error of the replicates. Others contained 16:1, 20:0 and 22:0.
^a For abbreviations see text.

Tocopherols are also effective antioxidants in soya beans during microwave treatments.

CONCLUSIONS

When soya beans at various moistures were roasted in a domestic microwave oven, the lower the moisture content (unsoaked soya beans) the higher was the internal temperature in the beans at the end of roasting. Therefore, formation of browning substances was considerably accelerated after 12 min roasting. However, increasing moisture content inhibited, not only the formation of browning substances but also appreciably prevented, the reduction of tocopherols and the oxidative deterioration of soya bean oils from microwave roasting. These results suggested that microwave roasting of raw beans by soaked for 1 h or 5 h might be an effective means for producing full-fat soya flour with high vitamin E. On the other hand, the peroxide value

of the oil in the soya beans slightly increased immediately after the soaking procedure. However, it is unlikely to have any harmful effect because the value was very small ($<7.0 \text{ meq kg}^{-1}$) immediately after 5 h soaking. Nonetheless, further studies are necessary to investigate the possible action of the endogenous lipoxygenase in soya beans during soaking.

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