Relationship Between Oxidative Stability of Vitamin E and Production of Fatty Acids in Oils During Microwave Heating

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Effects of microwave heating on the oxidative stability of *d*-tocopherols were studied in relation to the production of fatty acids in oils. During microwave heating, the stability of tocopherols decreased in the order $\delta > \beta > \gamma > \alpha$. This order did not depend on the types of ethyl esters of fatty acids or oils present. But, the shorter the chainlength and the lower the degree of unsaturation of the fatty acid ethyl esters, the greater was the reduction in amount of individual tocopherols. A similar tendency was observed when tocopherol-stripped vegetable oils, with equimolar mixtures of tocopherols added, were treated under the same conditions. The reduction in tocopherols became greater with increasing levels of free fatty acids.

KEY WORDS: Coconut oil, fatty acid ethyl esters, fatty acids, microwave energy, palm oil, safflower oil, tocopherol isomers, vitamin E.

Tocopherols are fat-soluble substances widely distributed in nature. In addition to their vitamin E function, they are natural antioxidants in foods and are important for stability of vegetable oils. Eight natural compounds possess vitamin E activity, namely α-, β-, γ-, δ-tocopherols (1), and four closely related tocotrienols (2). The latter compounds occur at much lower levels and their structures differ from tocopherols in that they have three double bonds in the side chain (3). All these forms have been found in foods and fats, although δ-tocotrienol is rare and, among foods, has been reported only in palm oil. The antioxidant aspect of tocopherol chemistry has been studied extensively (4–7), especially with regard to the relative antioxidant activities of α-, γ- and δ-tocopherols, the forms commonly found in vegetable oils. α-Tocopherol contributes more biologically active vitamin E potency to diets than any other tocopherol isomer; however, α-tocopherol is unstable in many food products during storage (8). This is an important problem in the shelf-life of fried foods. When they are reheated in a microwave oven after frying, further degradation of tocopherols may occur. Little is known about how microwave reheating affects stability of tocopherols in oils or fried foods.

The application of microwave processing in both home and institutional meal preparation has increased because of its speed and convenience as compared to conventional cooking methods (9–11). Microwave ovens are considered among the most energy-efficient types of oven, and the most rapid method for reheating food items (12). The heating effect of microwave energy on various food components may differ significantly from that of conventional cooking. For example, it has been speculated that reactive free radicals may be formed by exposure to microwave energy, especially in those applications that result in abnormally high temperatures, as with frying and toasting. More recently, various chemical reactions have been reported to be induced by microwave energy (13). When some vegetable oils were exposed to microwave energy, the reduction in the amount of tocopherols in the oils was not necessarily in direct relation to the index for their other chemical properties (14). Therefore, it is important to use tocopherol-stripped oils when investigating the oxidative stability of individual tocopherols during microwave heating. In our study, tocopherol-stripped vegetable oils were prepared by column chromatography, and each tocopherol was added to the oils at 2.5×10^{-7} mol/g oil immediately before use.

The objectives of our study were to evaluate relative stability of vitamin E in a model system during microwave heating, and to establish if different lipid substrates influence the stability.

MATERIALS AND METHODS

Materials. The α-, β-, γ-, and δ-tocopherols were purchased from Eisai Co. (Tokyo, Japan). All tocopherols were of the *d*-form, and purity of each tocopherol was above 98.5% as determined by high-performance liquid chromatography (HPLC). The tocopherols were added as ethanol solutions to refined substrate, *i.e.*, specific fatty acid ethyl esters or tocopherol-stripped vegetable oils. The ethanol was removed by evaporation under a stream of nitrogen before microwave heating. The 2,2,5,7,8-pentamethyl-6-hydroxy chroman (Eisai Co.) was used as an internal standard for determination of tocopherol isomers.

Commercially available fatty acid ethyl esters without additives were purchased from Wako Pure Chemicals Ind. Ltd. (Osaka, Japan). The saturated fatty acid esters were ethyl laurate, ethyl myristate, ethyl palmitate and ethyl stearate; the unsaturated fatty acid esters were ethyl oleate and ethyl linoleate. All fatty acid ethyl esters were further refined by column chromatography on silicic acid before use. The column for solid oil samples (ethyl stearate, coconut and palm oils) was heated with a ribbon heater (40°C), and the sample was simultaneously released with a flow of nitrogen gas.

Refined coconut, palm and safflower oils without additives were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Tocopherol-stripped vegetable oils were prepared by column chromatography on aluminum oxide with a modification of the method described previously (15). The column was heated for coconut and palm oils, and the sample oil was simultaneously released by the method described above. The fatty acid ethyl esters were prepared from the tocopherol-stripped vegetable oils as outlined (16) and then analyzed by gas chromatography (GC). A Shimadzu Model 7A-GC (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a glass column (200 cm × 3 mm I.D.) packed with 10% EGSS-X supported on acid-washed Gaschrom Q (100/200 mesh) was used. Identification of the peaks and the other GC conditions were described previously (17).

Microwave heating treatment. Each ethyl ester (5.0 g), containing equimolar mixtures of α-, β-, γ-, and δ-tocopherols (2.5×10^{-7} mol/g ethyl ester of fatty acid or

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oil), was placed in separate, small, brown glass bottles (25 mL, 35 mm I.D.) individually sealed with polyethylene film, and all oil samples were simultaneously heated at a frequency of 2,450 MHz in a microwave oven as reported previously (18). After fixed time intervals, the residual amounts of tocopherols in the esters were estimated by HPLC. The carbonyl value, *p*-anisidine value and thiobarbituric acid (TBA) number of the heated samples were measured according to JOCS official methods (19), IUPAC (20), and Tarladgis *et al.* (21), respectively. For peroxide values, AOCS official methods (22) were used. Free fatty acids (FFA) in the sample oils were determined by alkaline titration according to AOAC methods (23), and were expressed as oleic acid percentages.

High-performance liquid chromatography (HPLC). A 0.5-g portion of each sample containing equimolar tocopherol mixtures, before and after microwave heating, was placed in a 5-mL brown glass volumetric flask and diluted with the mobile phase as described below. The tocopherol analyses were performed in a Shimadzu Model LC-6A HPLC (Shimadzu Instruments Inc., Kyoto, Japan) connected to a Shimadzu Chromatopac C-R6B recording data processor. The chromatographic system consisted of a normal-bonded phase 250 cm × 4.6 mm I.D. Shim-pack CLC-NH_2 column (Shimadzu) and a 1-cm amino guard column (Shim-pack G). The mobile phase was *n*-hexane/1,4-dioxane/ethanol (490:10:1, v/v/v) at 2.0 mL/min. Five-microliter samples were injected with a 20-μL loop. Tocopherols were monitored with a fluorescence spectrophotometer set at excitation wavelength 296 nm and emission wavelength 320 nm and quantitated by comparison to content before microwave heating. The other HPLC conditions were described previously (18).

Column-regeneration. At the end of each day, methanol was pumped throughout the HPLC column for 60 min. This removed the more polar oil constituents that were adsorbed on the column during the analysis. After this washing step, 30 min of pumping the above mobile phase throughout the column was adequate for re-equilibration before injection of the next sample.

Statistical analysis of experimental data. The oils in separate brown bottles were simultaneously heated in the microwave oven for each exposure time. Three samples of each oil prepared for each exposure time were subjected to each chemical determination, respectively. Each value was an average of three determinations, and the values before heating for tocopherols were normalized to 100. Free fatty acids were expressed as oleic acid percentages. The analytical data of the effect of microwave heating on the oxidative stability of vitamin E and production of fatty acids were analyzed by Duncan's Multiple Range Test (24).

RESULTS AND DISCUSSION

The column chromatography removed hydroperoxides and other trace compounds from fatty acid esters, and also trace amounts of impurities such as fatty acids, phospholipids, hydroperoxides and polar compounds from vegetable oils. Each refined ethyl ester showed a purity exceeding 99.0% in GC analyses. The purified vegetable oils were colorless, tasteless and odorless. The analyses showed the product to be devoid of tocopherols (Table 1) and to contain less than 0.3 meq/kg oil for peroxide value, 0.2 for TBA value, 0.5 for carbonyl value (data not shown). Table 2 shows the results of the fatty acid compositions obtained from tocopherol-stripped oils. The fatty acid compositions of commercial and purified vegetable oils were essentially the same.

The relative stability of individual tocopherols during microwave heating was first compared in a model system in the presence of fatty acid ethyl esters of different chainlengths. Figure 1 shows the tocopherol stability in the three esters of saturated fatty acids with various acyl chainlengths. Tocopherols exposed to microwave heating were significantly ($p < 0.05$) destroyed in the presence of the three fatty acid esters. The tocopherols decreased at a similar rate in each ethyl ester and then substantially after 12 min of heating. From an inspection of the curves, tocopherol stability in saturated fatty acid ethyl esters decreased in the order δ-, β-, γ-, and α-tocopherols, and the rates of tocopherol reduction for β- and γ-tocopherols had a similar pattern. The α-tocopherol levels in the three saturated fatty acid ethyl esters dropped drastically in the initial stage after microwave heating, but the other three tocopherol isomers (γ-, β-, and δ-) were still retained (80%) after 12 min of heating and, thereafter, decreased markedly at 20 min of heating.

For α-tocopherol, 8 min of heating caused reductions of 15, 20 and 40%, 12 min caused reductions of 30, 40 and 60%, and 20 min caused reductions of 63, 75 and 100%, in fatty acid ethyl palmitate, myristate and laurate, respectively. Tocopherols were much more stable during microwave heating in the longer fatty acyl chain esters than they were in the shorter fatty acyl chain esters. However, the reduction of individual tocopherols was somewhat less than that previously reported (25). All fatty acid ethyl esters used were purified beforehand by silicic acid or aluminum oxide column chromatography, to remove free fatty acids and peroxides. Therefore, no effect of the latter compounds on the reduction in tocopherols during microwave heating was expected. We previously reported that the greater the degree of unsaturation of the samples, the more clearly were the changing patterns for the chemical properties such as carbonyl, anisidine and TBA values (14). From these model system results (Figs. 1 and 2), the reduction of individual tocopherols was somewhat greater with medium-chain saturated fatty acid esters such as ethyl laurate or ethyl myristate than with long-chain saturated or unsaturated fatty acid esters, such as ethyl palmitate and ethyl stearate or ethyl oleate and ethyl linoleate, respectively.

The relative stability of vitamin E in vegetable oils heated in a microwave oven was also evaluated. In general, vegetable oils contain not only tocopherols or tocotrienols (26,27), but also trace amounts of impurities such as free fatty acids (28) or polar compounds (29). Therefore, it was important to use tocopherol-stripped oils with those impurities removed in order to precisely investigate the relative stability of individual tocopherols during microwave heating. For this purpose, all vegetable oils were purified by the methods described above and contained no tocopherol isomers before the addition of tocopherols (Table 1). Figure 3 shows changes in tocopherol stability in the vegetable oils with different degrees of unsaturation, namely coconut, palm and safflower oils, after microwave heating. Judging from the remaining amounts of tocopherols, the highest rate of loss

TABLE 1

Tocopherol and Tocotrienol Contents of Vegetable Oils Before and After Aluminum Oxide Column Chromatography[a]

Vegetable oil	Chromatography	Tocopherols (T) and tocotrienols (T3), mg/100 g oil								
		α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3	Total
Coconut	Before	1.25	3.29	—	—	0.56	1.03	0.77	—	6.90
	After	—[c]	—	—	—	—	—	—	—	—
Palm	Before	6.69	5.51	—	—	5.49	7.56	3.06	1.77	30.08
	After	Tr[b]	—	—	—	—	Tr	—	—	Tr
Safflower	Before	43.72	—	0.83	—	2.62	—	0.35	—	47.52
	After	Tr	—	—	—	—	—	—	—	—

[a]Each value is an average of three determinations.

[b]Tr, trace.

[c]—, Not detected.

TABLE 2

Fatty Acid Composition of Vegetable Oils Refined by Chromatography on a Aluminum Oxide Column[a]

Fatty acid[b]	Vegetable oil		
	Coconut	Palm	Safflower
8:0	5.6	—[c]	—
10:0	5.5	—	—
12:0	47.8	1.8	—
14:0	19.4	5.1	—
16:0	9.9	44.9	6.3
18:0	3.2	3.7	2.2
18:1	7.1	36.0	12.6
18:2	1.5	8.5	78.2
18:3	—	—	0.7
Categorical summary			
Saturated	91.4	55.5	8.5
Unsaturated	8.6	44.5	91.5

[a]Each value is an average of three determinations and is given as weight percentage of total fatty acid ethyl esters.

[b]In this notation for fatty acids, the first number indicates chainlength, the number after the colon indicates the number of methylene-interrupted double bonds.

[c]—, Not detected.

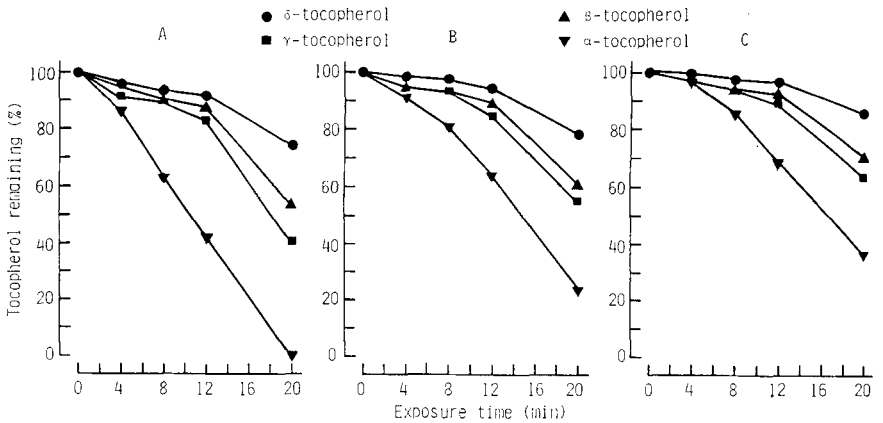

FIG. 1. Relationship between exposure time during microwave heating (frequency 2,450 MHz) and reduction of individual tocopherols in the ethyl esters of saturated fatty acids. A, ethyl laurate; B, ethyl myristate; C, ethyl palmitate.

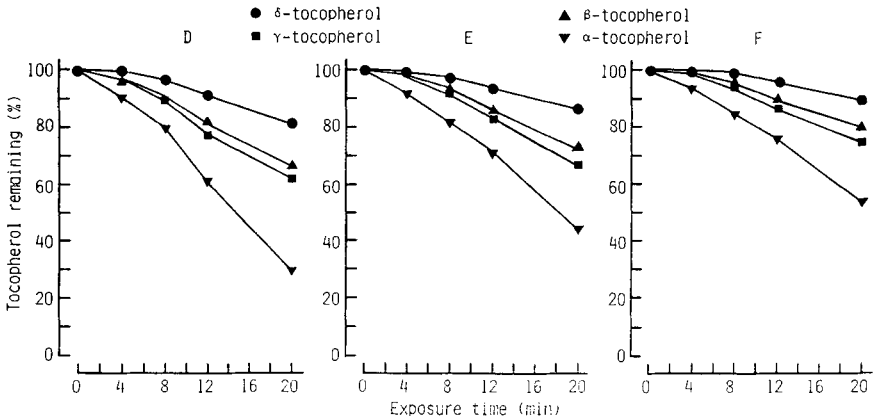

FIG. 2. Relationship between exposure time during microwave heating (frequency 2,450 MHz) and reduction of individual tocopherols in the different ethyl esters of unsaturated fatty acids. D, ethyl stearate; E, ethyl oleate; F, ethyl linoleate.

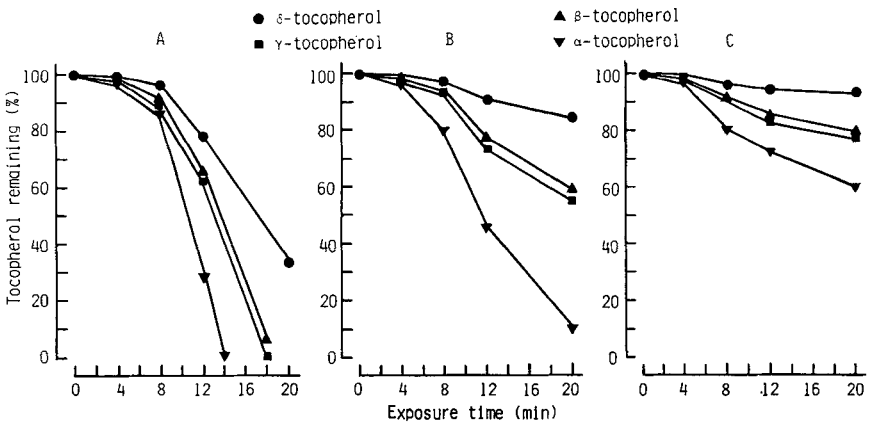

FIG. 3. Relationship between exposure time during microwave heating (frequency 2,450 MHz) and reduction of individual tocopherols in tocopherol-stripped vegetable oils. A, coconut; B, palm; C, safflower.

was shown by α-tocopherol, followed by γ-, β- and δ-tocopherols, in coconut, palm and safflower oils.

Coconut oil was found to be most susceptible to producing free fatty acids, followed by palm and safflower oils (Fig. 4). In coconut oil, α-tocopherol was not detected after 14 min of heating, γ-tocopherol after 18 min, and β-tocopherol was only 5% of initial levels after 18 min. But, 32% of the δ-tocopherol still remained at 20 min of heating. As shown in Figure 3, the rates of individual tocopherol reductions decreased markedly with increased unsaturation of the oils, in the order of coconut, palm and safflower oils. When vegetable oils were heated by microwave (25), the chemical indices such as TBA, carbonyl and anisidine values as indicators for the thermal oxidative deteriorations were not necessarily consistent with the changes in the reduction of individual tocopherols. Pongracz (30) suggested that the heat stability of tocopherols is higher in fats with higher iodine values. Pokorny *et al.* (31) reported changes in the tocopherol content of soybean and sunflower oils treated under deep-frying conditions and showed that tocopherol destruction proceeded according to the kinetics of a first-order reaction; the rate of tocopherol reduction was increased by the addition of acids, such as citric, palmitic and phosphoric.

To clarify the relationship between the tocopherol reduction and production of free fatty acids during microwave heating, the fatty acids in the oils were first analyzed. Figure 4 shows the increases in the fatty acid levels produced by microwave heating. An appreciable change of fatty acids was observed after heating and the change depended on the type of oil. The free fatty acid levels in all oils increased slowly during the first 8 min of heating and rapidly thereafter, in the order of unsaturated fatty acids < saturated fatty acids < vegetable oils. There were no significant differences ($p > 0.05$) in the fatty acid levels for all oils until 8 min of heating. After 12 min of heating, however, there were somewhat higher levels of fatty acids with time, for the oils containing shorter fatty acyl chains such as lauric and myristic acids (Table 2). These observations are consistent with the substantial decreases in individual tocopherols that became apparent at 12 min

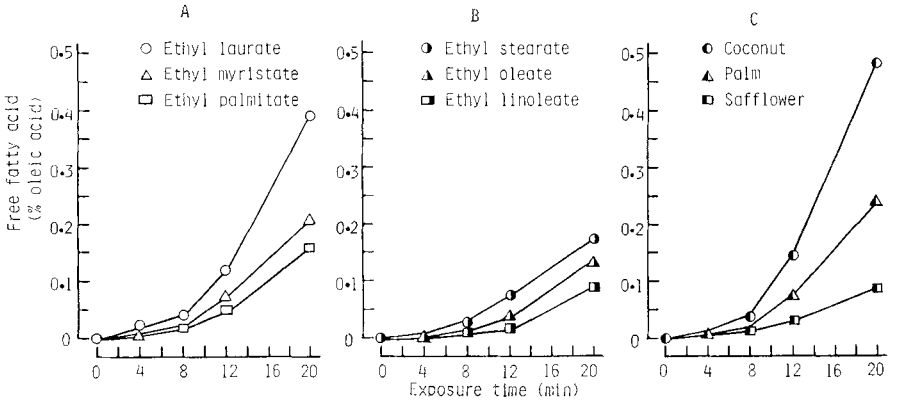

FIG. 4. Effect of exposure time during microwave heating (frequency 2,450 MHz) on the production of fatty acids in the ethyl esters or tocopherol-stripped vegetable oils. A, ethyl esters of saturated fatty acids; B, different esters of unsaturated fatty acids; C, tocopherol-stripped vegetable oil.

in the microwave-heated oils (Fig. 1). These results indicate that tocopherols in unsaturated oils are much more stable than those in saturated oils under microwave heating conditions.

Coconut oil is the most widely used of the lauric acid oils. As shown in Table 2, this oil contained the highest level of saturated fatty acids (91.4%), including lauric (47.8%) and myristic (19.4%). The low degree of unsaturation imparts high oxidative stability. However, lauric acid oils are easily hydrolyzed, although the oils are stable to oxidation when used for cooking and frying (32). Palm oil has a very low ratio of polyunsaturated to saturated fatty acids; it contains 56% saturates [palmitic (44.9%), myristic (5.1%), and stearic (3.7%)] and 8.5% polyunsaturates (linoleic). Safflower oil has a high level of unsaturated fatty acids (91.5%), containing linoleic (78.2%) and oleic (12.5%). When vegetable oils were heated with microwave energy, the higher amount of polyunsaturated fatty acids in the oils, the greater became their carbonyl, anisidine and TBA values as indicators of oxidative deterioration. The amount of tocopherols decreased substantially in olive and palm oils, whereas in corn and soybean oils 90% of the original tocopherol content was retained after microwave heating (14).

We propose that the hydroperoxide formed in highly unsaturated oils during microwave heating decomposes rapidly before reacting with tocopherols. The fatty acids are nonvolatile compounds and accumulate in the oils during heating (Fig. 4). Further research is being conducted, which examines the effect of fatty acid level on the oxidative stability of tocopherols in the oils during microwave heating.

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