Influence of Fatty Acids on the Tocopherol Stability in Vegetable Oils During Microwave Heating

Hiromi Yoshida*, Mikiko Tatsumi and **Goro Kajimoto**
Department of Nutrition, Kobe-Gakuin University, Kobe 651-21, Japan

Effects of 0, 0.05, 0.25, 0.50 and 1.0% levels of fatty acids (caproic, caprylic, capric and lauric) or hydrocarbons (decane and dodecane) on tocopherol stability in vegetable oils during microwave heating were determined by measuring tocopherol losses and carbonyl and anisidine values. The fatty acids showed similar prooxidant activities toward tocopherols in purified vegetable oils when heated in a microwave oven. However, decane or dodecane, which had the same number of carbons as capric or lauric acid but no carboxylic group, did not show prooxidant activity. The shorter the chainlength and the higher the level of fatty acids, the greater was the reduction of tocopherols in the oils. The addition of low-molecular weight fatty acids resulted in greater acceleration in the oxidation of tocopherols in the purified oils. Therefore, it is necessary to pay attention to these free fatty acids produced in the oils when heated in a microwave oven.

KEY WORDS: Anisidine value, capric acid, caproic acid, caprylic acid, carbonyl value, lauric acid, microwave heating, purified vegetable oils, tocopherols.

Application of microwave processing in both home and institutional meal preparations has increased because of its speed and convenience (1,2) as compared to traditional cooking methods, such as broiling, roasting or frying. 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, especially in those applications that result in abnormally high temperatures, as with frying and toasting. Various chemical reactions have been known to be induced by microwave energy. More recently, the use of microwave ovens in organic syntheses has been reported to considerably reduce the reaction time of an array of classical organic reactions (3). Vegetable oils containing minor components, such as free fatty acids, monoglycerides, phospholipids, tocopherols or unknown compounds (4), have shown prooxidant activity. In a previous paper (5), we reported that when vegetable oils were exposed to microwave energy, the higher the amount of polyunsaturated fatty acids in the oils, the greater became the rate of quality deterioration of the oils. However, the reduction in the amount of tocopherols in oils was not necessarily directly related to stability indices, such as thiobarbituric acid values, carbonyl values and anisidine values. Vegetable oils often contain 0.05–0.7% free fatty acids (6–9). We found that the levels of free fatty acids increase in vegetable oils when heated in a microwave oven (10). However, the effects of fatty acids on the tocopherol stability in vegetable oils during microwave heating have not been reported.

The objectives of our current study were to determine the qualitative and quantitative effects of fatty acids on the tocopherol stability in vegetable oils as a result of microwave heating. Therefore, it is important to use tocopherol-stripped oil (purified oil) with impurities removed in order to precisely investigate the relative stability of individual tocopherols during microwave heating. In this paper, all vegetable oils were purified by column chromatography and contained no tocopherol homologues before addition of tocopherols.

EXPERIMENTAL PROCEDURES

Materials—fatty acids. Commercially available saturated fatty acids without additives were purchased from Nacalai Tesque Inc. (Kyoto, Japan). All fatty acids were of *n*-form; caproic (C6:0), caprylic (C8:0), capric (10:0) and lauric (C12:0) acids. Purity of each fatty acid was above 99.0% as determined by gas chromatography (GC).

Hydrocarbons. Decane (C10:0) and dodecane (C12:0) were purchased from Nacalai Tesque Inc. Purity of each hydrocarbon was above 98.0% as determined by GC.

Tocopherols. 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). Each tocopherol was added to purified vegetable oils at 2.5×10^{-7} mol/g oil as a *n*-hexane solution [0.25–0.26% (w/v)]. The hexane 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 internal standard for determination of tocopherol homologues.

Vegetable oils. Five oils with different degrees of unsaturation (by iodine values; I.V.) were used. Refined coconut (I.V. = 7.5), palm (I.V. = 52.3), rapeseed (I.V. = 108.5), soybean (I.V. = 132.3) and safflower (147.2) oils without additives were purchased from Nacalai Tesque Inc. The purified vegetable oils were prepared from these oils by aluminum oxide column chromatography (11) immediately prior to use. For solid samples at room temperature (coconut and palm oils), the column was heated with a ribbon heater (40°C), and the sample was simultaneously released with a flow of nitrogen gas. The changes in apparent color and occurrence of off-flavor in the oils after purification were estimatd by sensory evaluation according to the method of Honma and Fujimaki (12). Tocopherols in commercial and purified vegetable oils were determined by normal-phase HPLC with a fluorescence detector as described below. Fatty acid ethyl esters were prepared from the purified vegetable oils as outlined (13). The compositions were analyzed by a Shimadzu Model 7A-GC (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a glass column (2 m × 3 mm i.d.) packed with 10% EGSS-X supported on acid-washed Gaschrom Q (100/200 mesh) and connected with an integrator (Shimadzu C-R1B). Identification of the peaks and the other GC conditions were described previously (14).

*To whom correspondence should be addressed at Department of Nutrition, Kobe-Gakuin University, Arise, Ikawadani-cho, Nishi-ku, Kobe 651-21, Japan.

METHODS

Microwave heating treatment. Equimolar mixture of α-, β-, γ-, and δ-tocopherols (2.5×10^{-7} mol/g oil) was added to purified soybean and rapeseed oils containing 0, 0.05, 0.25, 0.50 or 1.0% levels of caproic, caprylic, capric, lauric acid, decane or dodecane. Five grams of each prepared oil were placed in separate 25-mL brown glass bottles (35 mm i.d.). Sample bottles were prepared in triplicate and sealed with polyethylene film. All oil samples were simultaneously heated at a frequency of 2,450 MHz for each time period (a range for 4 to 20 min) in a microwave oven as reported previously (5). After fixed time intervals, the residual amounts of tocopherols in the oils were estimated by measuring the reductions by HPLC. The carbonyl value and *p*-anisidine value of the heated oils were measured according to standard methods (15,16). Free fatty acids in the sample oils after microwave heating were determined by alkaline titration (17) and expressed as oleic acid percentages.

High-performance liquid chromatography. A 0.5-g portion of each oil sample, 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.) connected to a Shimadzu Chromatopac C-R6B recording data processor. The chromatographic system consisted of a normal-bonded phase 250 × 4.6 mm i.d. Shimpack CLC-SIL (M) column (Shimadzu) protected by 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 fully loaded 20-μL loop. The tocopherols were monitored with a fluorescence spectrophotometer set at excitation wavelength 296 nm and emission wavelength 320 nm and quantitated by comparison to the content before microwave heating. The other HPLC conditions were described previously (18).

Fatty acid composition of free fatty acids produced by microwave heating. Microwave-heated oils were fractionated on a silicic acid column by a modification of the method of Rouser *et al.* (19). Silicic acid was Unisil (Clarkson Chemical Co., Williamsport, PA) purchased from Nacalai Tesque Inc. The columns were prepared as described previously (20), and a measured quantity (750 mg) of the heated oil was added with 5 mL of *n*-hexane. By successive elution with 200 mL of *n*-hexane (fraction I), 200 mL of *n*-hexane/diethyl ether (50:50, v/v; fraction II) and then 150 mL of diethyl ether (fraction III), the heated oils were separated into 3 fractions, and they were further purified, when necessary, with a silica gel column. Free fatty acids, eluted in fraction II, were further isolated by one-dimensional thin-layer chromatography (TLC) on a Silica Gel G plate, activated at 120°C for 2 hr immediately before use. The solvent system was *n*-hexane/diethyl ether/acetic acid (70:30:0.5, v/v/v). After developing, the free fatty acid bands were visualized with iodine vapor and then scraped into test tubes. The free fatty acids were analyzed by GC after ethylation by the method described above.

Statistical analysis of experimental data. The value at each heating time is an average of three determinations. In order to illustrate relative stability of tocopherols during microwave heating, the values before heating were normalized to 100. The analytical data of the effects of free fatty acids and hydrocarbons on tocopherol stability in the oils during microwave heating were analyzed by Duncan's Multiple Range test (21).

RESULTS AND DISCUSSION

The purified vegetable oils were colorless, tasteless and odorless, and contained no free fatty acids as determined by TLC. No tocopherol homologues were detected in the oils by HPLC as described previously (10). Their chemical characteristics were less than 0.2 meq/kg oil for peroxide value, 0.3 for carbonyl value, and 0.2 for anisidine value. Table 1 presents the fatty acid compositions of purified vegetable oils before microwave heating. The fatty acid compositions of commercial and purified vegetable oils were essentially the same. Coconut oil, the most commonly used of the lauric acid group of oils, contained the highest content of total saturated fatty acids (91.4%), mainly made up of lauric (47.8%) and myristic (19.4%). Moreover, coconut oil contains about 6% of short-chain saturated fatty acids such as caprylic (C8:0) and capric (C10:0). The low degree of unsaturation results in high oxidative stability. However, lauric acid oils are easily hydrolyzed, although the oils are oxidation-stable when used for cooking and frying (22). Palm oil has a low ratio of polyunsaturated to saturated fatty acids; it contains 55% saturates [palmitic (44.9%), myristic (4.8%), and stearic (3.7%)] and 8.5% polyunsaturates (linoleic). On the

TABLE 1

Fatty Acid Composition of Tocopherol-Stripped Vegetable Oils Before Microwave Heating[a]

Type of oil	Fatty acid (wt%)										PUFA[b]
	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	22:1	
Coconut	5.6	5.5	47.8	19.4	9.9	3.2	7.1	1.5	—[c]	—	1.5 (8.6)[d]
Palm	—	—	1.6	4.8	44.9	3.7	36.0	8.5	0.5	—	9.0 (45.0)
Rapeseed	—	—	—	—	3.9	1.5	58.7	23.4	12.3	0.2	35.7 (94.6)
Soybean	—	—	—	—	10.6	3.6	22.7	54.8	8.3	—	63.1 (85.8)
Safflower	—	—	—	—	6.3	2.2	12.6	78.2	0.7	—	78.9 (91.5)

[a]Each value is an average of 3 determinations.

[b]PUFA, polyunsaturated fatty acids (linoleic plus linolenic).

[c]—, Not detected.

[d]Values in parentheses are for total unsaturated fatty acids.

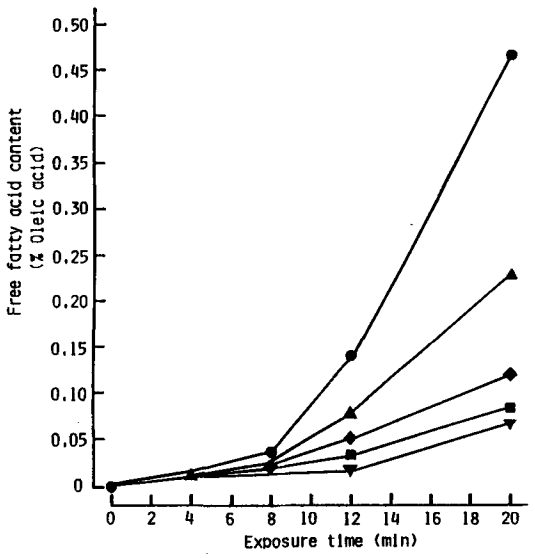

FIG. 1. Effect of exposure time on free fatty acid formation in purified vegetable oils during microwave heating (frequency 2,450 MHz). ▼, safflower oil; ■, soybean oil; ◆, rapeseed oil; ▲, palm oil; ●, coconut oil.

contrary, the other three oils have a low ratio of saturated-to-unsaturated fatty acids and contain high levels of unsaturated fatty acids (85–94%); rapeseed oil contains oleic (58.7%), linoleic (23.4%) and linolenic (12.3%); soybean oil contains linoleic (54.8%), oleic (22.7%) and linolenic (8.3%); and safflower oil contains linoleic (78.2%) and oleic (12.6%).

Figure 1 shows the effect of exposure time on free fatty acid formation in purified vegetable oils during microwave heating. An appreciable change in fatty acids was observed after heating, and the change depended on the differences in the types of oils. The free fatty acid levels in all oils increased slowly in the first 8 min of heating and rapidly thereafter, in the following order: safflower, soybean, rapeseed, palm and coconut. 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, there were substantially greater differences ($p<0.01$) with time for coconut and palm oils, which contain shorter fatty acyl chains such as caprylic, capric, lauric and myristic acids (Table 1). For instance, the free fatty acids detected at 12 min of heating were 0.14% for coconut oil, 0.07% for palm oil and less than 0.05% for the other three oils. As expected, the levels of free fatty acids increased with longer heating and attained greater than 3-fold increases from 12 min to 20 min of heating; 0.47% for coconut oil, 0.23% for palm oil, and 0.06–0.12% for the other three. Coconut oil produced the highest level of free fatty acids, followed by palm, rapeseed, soybean and safflower oils.

The compositions of free fatty acids produced in purified vegetable oils after microwave heating are presented in Table 2. However, data for the fatty acid compositions at 4 min of heating were omitted from Table 2 because there were no significant detections ($p>0.05$) of free fatty acids in comparison with the purified oils before microwave heating. The level of the short- (C8:0 and C10:0) or medium- (C12:0, C14:0 and C16:0) chain saturated fatty acids increased somewhat ($p<0.05$), both by longer exposure to microwave energy and by increasing levels of free fatty acids (Fig. 1). During microwave heating, there were no significant differences ($p>0.05$) in the free fatty acid compositions for the unsaturated oils, namely rapeseed, soybean and safflower oils.

We (10) found that the shorter the chainlength and the lower the degree of unsaturation of the vegetable oils, the greater was the loss of individual tocopherols. Also, the loss was significantly greater ($p<0.05$) with increasing levels of free fatty acids in the oils. Therefore, we are proposing that the tocopherol destruction during microwave heating is caused not only by thermal oxidative de-

TABLE 2

Composition of Free Fatty Acids Produced in the Vegetable Oils After Microwave Heating[a]

Type of oil	Exposure time (min)	Fatty acid (wt%)									
		8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	PUFA[b]
Coconut	8	1.8	2.5	44.2	17.1	18.3	6.8	9.0	0.3	—[c]	0.3
	12	3.2	4.4	46.3	18.9	16.3	3.2	7.5	0.2	—	0.2
	20	4.5	4.7	46.7	19.5	15.6	3.1	5.7	0.2	—	0.2
Palm	8	—	—	0.6	2.4	62.8	9.8	23.5	0.9	—	0.9
	12	—	—	0.7	3.1	65.7	8.4	21.3	0.8	—	0.8
	20	—	—	2.8	5.5	66.5	6.7	18.2	0.3	—	0.3
Rapeseed	8	—	—	—	—	4.2	1.7	58.5	22.4	13.2	35.6
	12	—	—	—	0.2	5.9	1.8	58.2	21.9	12.0	33.9
	20	—	—	—	0.6	6.8	2.5	57.3	20.8	12.0	32.8
Soybean	8	—	—	—	0.1	12.0	3.2	24.5	52.8	7.4	60.2
	12	—	—	—	0.3	13.2	3.5	24.3	51.5	7.2	58.7
	20	—	—	—	0.3	14.6	4.3	23.5	50.3	7.0	57.3
Safflower	8	—	—	—	0.6	9.4	3.1	11.4	73.5	2.0	75.5
	12	—	—	—	0.6	9.3	3.0	11.6	73.5	2.0	75.5
	20	—	—	0.5	0.7	9.2	3.3	11.2	73.5	1.6	75.1

[a]Each value is an average of 3 determinations.
[b]PUFA, polyunsaturated fatty acids (linoleic plus linolenic).
[c]—, Not detected.

terioration but also by the nonvolatile compounds such as free fatty acids accumulating in the oil.

To clarify the effects of free fatty acids on the destruction of tocopherols during microwave heating, 0.05 to 1.0% of the fatty acids (caproic, caprylic, capric and lauric) were added to purified soybean and rapeseed oils containing equimolar mixture of α-, β-, γ-, and δ-tocopherols (2.5×10^{-7} mol/g oil). Soybean, palm, low-erucic acid rapeseed and sunflower oils accounted for 60% of the world production of edible vegetable oils in 1988 (23). In Japan, soybean oil is the major edible oil, followed by rapeseed oil, and they are used in margarines, shortenings and cooking oils (24). In addition, these oils are used in many frozen foods or packaged dry mixes. Therefore, we chose soybean and rapeseed oils to investigate the effects of free fatty acids on the destruction of tocopherols during microwave heating. Figure 2 shows the effects of caproic acid at different concentrations on the oxidative stability of tocopherols in purified soybean oil during microwave heating. Figure 3 also shows the results when capric acid was added to purified rapeseed oil at the same concentrations from 0.05 to 1.0%. Judging from the decreases in the remaining amounts of tocopherol homologues as shown in Figures 2 and 3, the highest oxidative rate was seen in α-, followed by γ-, β-, and δ-tocopherols. The loss of tocopherols increased at a faster rate at the higher levels of added fatty acids. This order did not depend on the kind of purified oils or the types of the added fatty acids. However, the longer the acyl chain of fatty acids, the less was the reduction of individual tocopherols during microwave heating at each exposure time. After 8 min of heating, there were significant differences ($p<0.01$) in the reduction of tocopherols, not only between the types of tocopherols but also among the concentrations of fatty acids. Data generated with caprylic or lauric acid (omitted from this paper) demonstrated similar patterns to those obtained with caproic or capric acid.

The effect of various fatty acids on the destruction of tocopherols was also determined at constant level (0.25% fatty acid/g oil). This level was selected because commonly used vegetable oils (6–9) contain 0.05–0.7% free fatty acids (as oleic). Figure 4 shows the effect of fatty acids (C8:0–C12:0) at this level on the oxidative stability of tocopherols in purified rapeseed oil. When different fatty acids were added to purified rapeseed oil at 0.25% level, the reduction of tocopherols increased slowly in the first 4 min of heating and rapidly thereafter, in the following order: lauric, capric and caprylic acids, and δ-, β-, γ-, and α-tocopherols. After 8 min of heating, there were substantial differences ($p<0.01$) with time by the addition of fatty acids. For α-tocopherol as a typical example, caprylic acid caused reductions of 32, 55 and 82%; capric acid caused reductions of 20, 40 and 63%; and lauric acid caused reductions of 10, 25 and 52%, after heating of 8 min, 12 min and 20 min, respectively. With microwave heating longer than 4 min, these trends became also more clear for the other three tocopherol homologues.

Finally, the effect of hydrocarbons was investigated at different concentrations on the oxidative stability of tocopherols in purified vegetable oils during microwave heating. Decane and dodecane, which have the same number of carbons as capric and lauric acids, were used in this study. Figure 5 shows the effect of decane (C10:0) at different concentrations (0.05–1.0%) on the oxidative

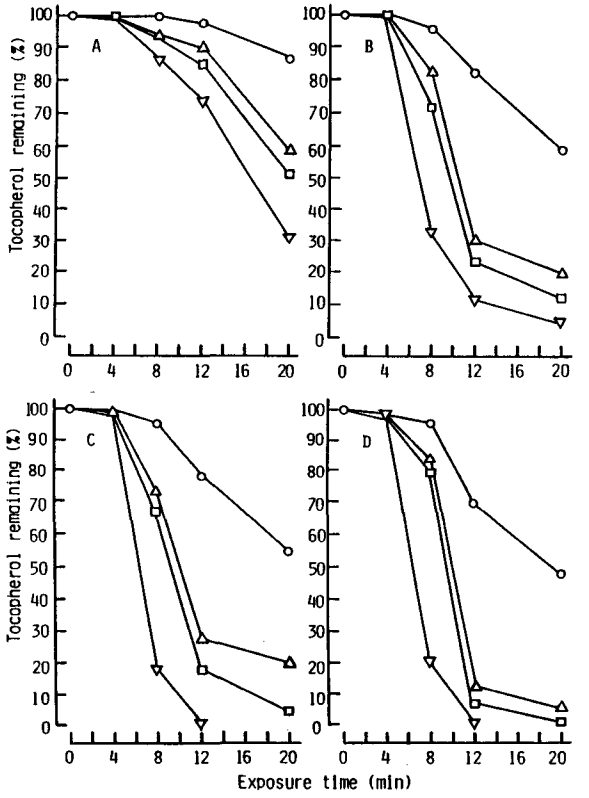

FIG. 2. Effect of caproic acid (C6:0) at different concentrations on oxidative stability of tocopherols in purified soybean oil during microwave heating (frequency 2,450 MHz). A, 0.05%; B, 0.25%; C, 0.50%; D, 1.0%. ▽, α-tocopherol; □, γ-tocopherol; △, β-tocopherol; ○, δ-tocopherol.

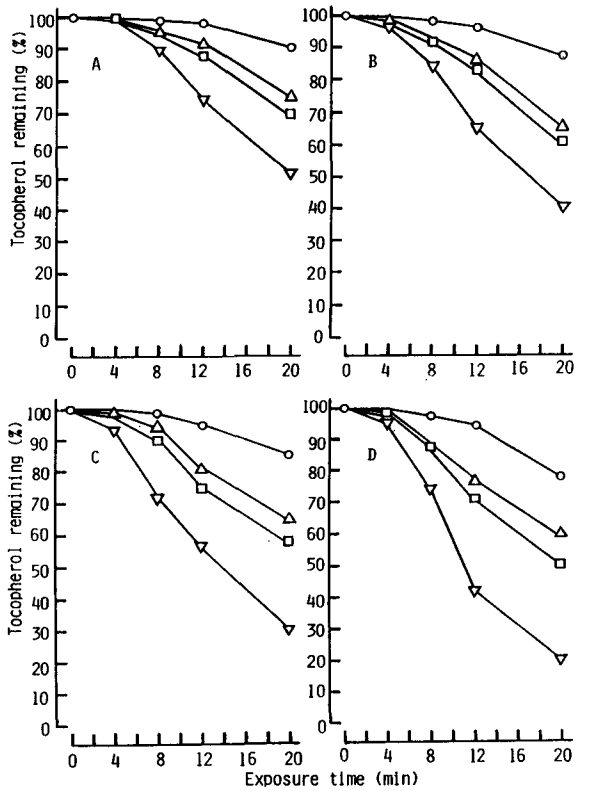

FIG. 3. Effect of capric acid (C10:0) at different concentrations on oxidative stability of tocopherols in purified rapeseed oil during microwave heating (frequency 2,450 MHz). A, 0.05%; B, 0.25%; C, 0.50%; D, 1.0%. ▽, α-tocopherol; □, γ-tocopherol; △, β-tocopherol; ○, δ-tocopherol.

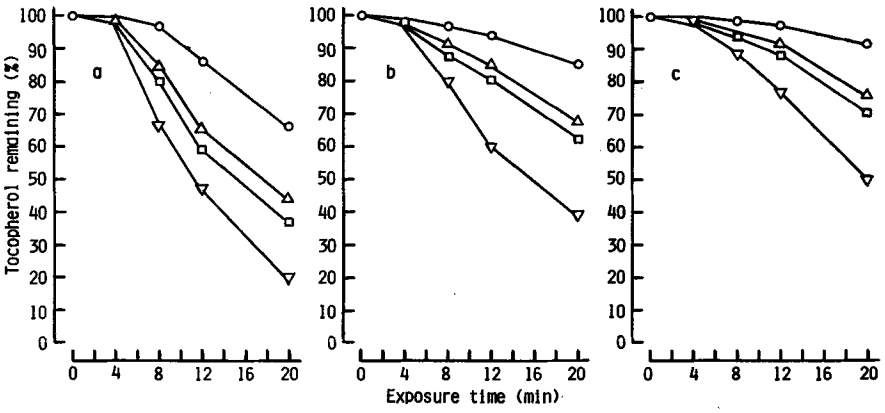

FIG. 4. Effect of different fatty acids at 0.25% level on oxidative stability of tocopherols in purified rapeseed oil during microwave heating (frequency 2,450 MHz). a, caprylic acid; b, capric acid; c, lauric acid. ∇, α-tocopherol; □, γ-tocopherol; △, β-tocopherol; ○, δ-tocopherol.

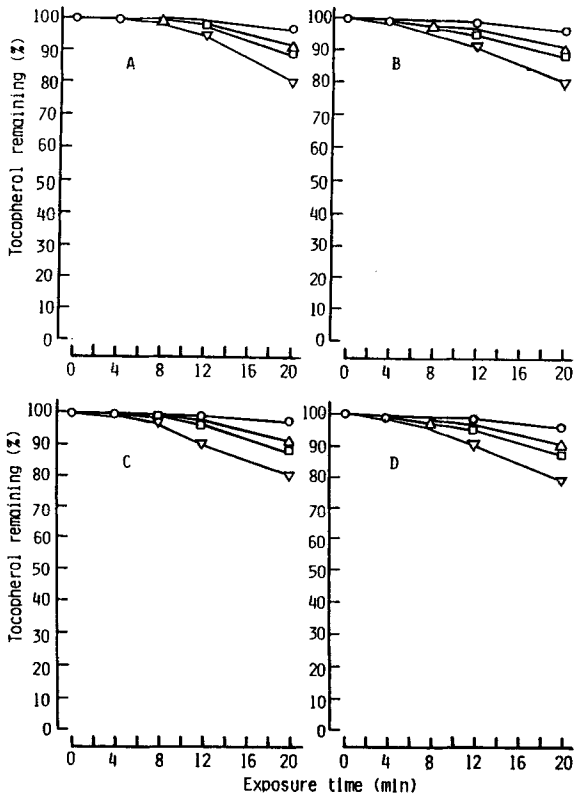

FIG. 5. Effect of decane (C10:0) at different concentrations on oxidative stability of tocopherols in purified soybean oil during microwave heating (frequency 2,450 MHz). A, 0.05%; B, 0.25%; C, 0.50%; D, 1.0%. ∇, α-tocopherol; □, γ-tocopherol; △, β-tocopherol; ○, δ-tocopherol.

stability of tocopherols in purified soybean oil during microwave heating. After 8 min of heating, a small loss of tocopherols was observed. Eighty percent of the original level for all tocopherols was still retained after 20 min of heating. But there were significant differences ($p<0.05$) between the types of tocopherols after 12 min of heating. Decane, which has the same number of carbons as capric acid but no carboxylic group, did not act as a prooxidant. Data for dodecane were omitted from Figure 5 because they showed similar patterns to those for decane. Mistry and Min (4) reported that the carboxylic groups of free fatty acids may produce prooxidant activity in purified soybean oil. Miyashita and Takagi (25) also suggested that the addition of stearic acid accelerated the oxidative rate of soybean oil. These investigations were carried out under autoxidative conditions at 50–55°C. However, little is known about how free fatty acids affect the oxidative stability of soybean oil when treated in a microwave oven.

Figure 6 shows the effect of caprylic acid (C8:0) at different concentrations (0–1.0%) on the carbonyl values and anisidine values of purified soybean oil during microwave heating. The prooxidant activity of caprylic acid on the thermal oxidation of the oil was observed at a concentration of 0.05%. After 8 min of heating, both carbonyl and anisidine values became more pronounced ($p<0.05$) than those of the control, and further increased with longer microwave heating as well as with increasing levels of the fatty acid. This indicated that the fatty acid in the oil acted as a prooxidant. Although the data for other chainlengths of fatty acids were not shown in Figure 6, shorter chainlength resulted in greater acceleration in the oxidation of the soybean oil. This suggested that the carboxylic group of the fatty acid is responsible for the prooxidant activity in soybean oil. Figure 7 shows the antioxidant effect of tocopherols (equimolar mixture of α, β, γ, and δ) on purified soybean oil containing caprylic acid (0–1.0%) when heated in a microwave oven. The tocopherols inhibited significantly ($p<0.05$) the thermal oxidation and retarded the deterioration of the soybean oil during microwave heating. There was significant prolongation ($p<0.05$) for 4 to 8 min in the induction period. After 20 min of heating, the carbonyl and anisidine values dropped by 10–25% and 27–40%, respectively, in comparison with those before the addition of tocopherols (Fig. 6). From the above results (Figs. 6 and 7), it is thought that the added tocopherols suppress the prooxidant effect of free fatty acids on the purified oils.

In conclusion, the addition of low-molecular weight fatty acids resulted in acceleration in the oxidation of to-

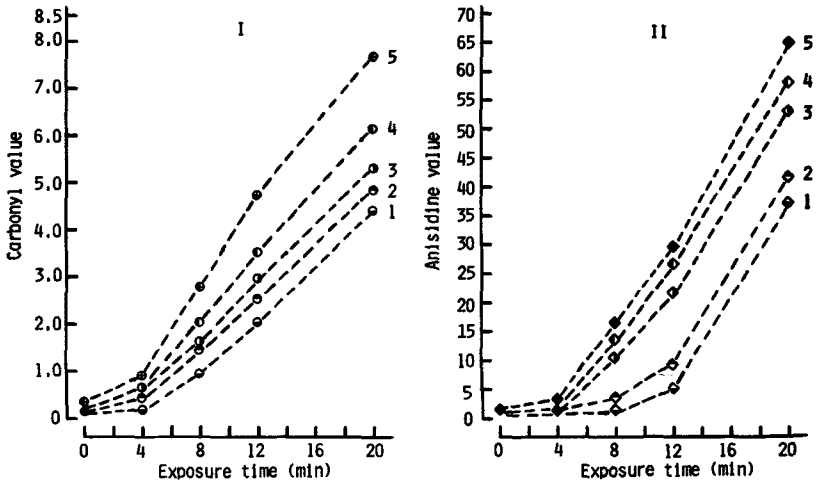

FIG. 6. Effect of caprylic acid (C8:0) at different concentrations on (I) carbonyl values and (II) anisidine values of purified soybean oil during microwave heating (frequency 2,450 MHz). 1 = ◒ & ◆, none; 2 = ◒ & ◆, 0.05%; 3 = ◑ & ◆, 0.25%; 4 = ◐ & ◆, 0.5%; 5 = ⊕ & ◆, 1.0%.

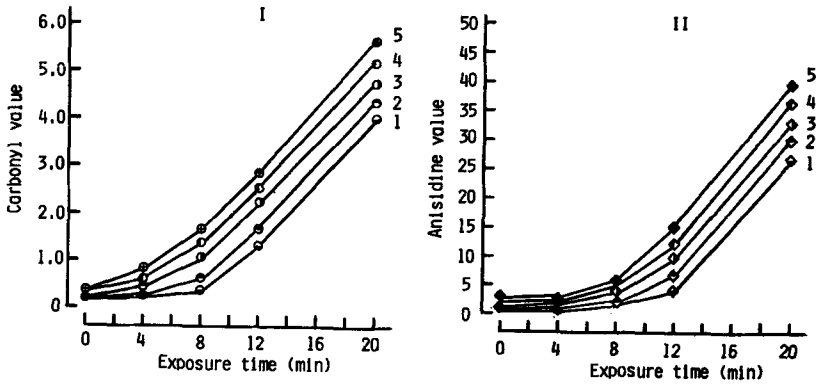

FIG. 7. Antioxidant effect of tocopherols on (I) carbonyl values and (II) anisidine values of caprylic acid-added soybean oil during microwave heating (frequency 2,450 MHz). 1 = ◒ & ◆, Toc; 2 = ◒ & ◆, 0.05% C8:0 + Toc; 3 = ◑ & ◆, 0.25% C8:0 + Toc; 4 = ◐ & ◆, 0.5% C8:0 + Toc; 5 = ⊕ & ◆, 1.0% C8:0 + Toc. Toc = Equimolar mixture of α-, β-, γ-, and δ-tocopherols (2.5×10^{-7} mol/g oil).

copherols in the purified vegetable oils. Therefore, it is necessary to pay attention to tocopherol losses and to the free fatty acids produced in the oils when heated in a microwave oven.

ACKNOWLEDGMENT

We thank Dr. B.J. Holub, University of Guelph, Ontario, for editing the manuscript.

REFERENCES

1. Cremer, M.L., and R.K. Richman, *J. Food Sci. 52*:846 (1987).
2. Lin, Y.E., and R.C. Anantheswaran, *Ibid. 53*:1746 (1988).
3. Giguere, R.J., T.L. Bray, S.M. Duncan and G. Majetich, *Tetrahedron Lett. 27*:4945 (1986).
4. Mistry, B.S., and D.B. Min, *J. Food Sci. 52*:786 (1987).
5. Yoshida, H., N. Hirooka and G. Kajimoto, *Ibid. 55*:1412 (1990).
6. Pryde, E.H., in *Handbook of Soy Oil Processing and Utilization*, edited by D.R. Erickson, E.H. Pryde, O.L. Brekke, T.L. Mounts and R.A. Falb, American Soybean Association, St. Louis, MO, and American Oil Chemists' Society, Champaign, IL, 1980, p. 13.
7. Dobarganes, M.C., and M.C. Perez-Camino, *Fat Sci. Technol. 89*:216 (1987).
8. Dobarganes, M.C., and M.C. Perez-Camino, *J. Am. Oil Chem. Soc. 65*:101 (1988).
9. Warner, K., E.N. Frankel and T.L. Mounts, *Ibid. 66*:558 (1989).
10. Yoshida, H., M. Tatsumi and G. Kajimoto, *Ibid. 68*:566 (1991).
11. Yoshida, H., A. Shibahara and G. Kajimoto, *J. Jpn. Oil Chem. Soc.* (YUKAGAKU) *24*:575 (1975).
12. Honma, S., and M. Fujimaki, *Agric. Biol. Chem. 46*:301 (1982).
13. Morrison, W.R., and L.M. Smith, *J. Lipid Res. 5*:600 (1964).
14. Yoshida, H., and G. Kajimoto, *Agric. Biol. Chem. 44*:183 (1980).
15. JOCS (ed.) *Kijun Yushui Bunseki Shikenho*, 2•4•22-73, The Japan Oil Chemists' Society, Tokyo, Japan, 1986.
16. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, IUPAC, II.D. 26, App. Chem. Div., Commission on Oils, Fats and Derivatives, 6th edn., edited by C. Paqout, Pergamon Press, New York, NY, 1979.
17. *Official Methods of Analysis*, Association of Official Analytical Chemists, 13th edn., Washington, DC, 1980, p. 41, Method No.

28.029.
18. Yoshida, H., and G. Kajimoto, *J. Food Sci. 54*:1596 (1989).
19. Rouser, G., G. Kritchevsky, G. Simon and G.L. Nelson, *Lipids 2*:37 (1967).
20. Yoshida, H., and J.C. Alexander, *Ibid. 18*:611 (1983).
21. Duncan, D.B., *Val. J. Sci. 2*:171 (1951).
22. Young, F.V.K., *J. Am. Oil Chem. Soc. 60*:374 (1983).
23. Yoshitomi, K., *J. Jpn. Oil Chem. Soc.* (YUKAGAKU) *38*:449 (1989).
24. Machida, T., in *Oil & Fat in Nutrition and Disease*, edited by I. Hara, Saiwai Shobo Co., Ltd. Tokyo, Japan, 1990, p. 397.
25. Miyashita, K., and T. Takagi, *J. Am. Oil Chem. Soc. 63*:1380 (1986).

[Received May 29, 1991; accepted November 14, 1991]