Participation of Free Fatty Acids in the Oxidation of Purified Soybean Oil During Microwave Heating

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Effects of 0 to 1.0% levels of caprylic, capric, lauric, myristic, palmitic or stearic acid on the oxidative stability of purified soybean oil were investigated under microwave heating conditions. A prooxidative effect of the fatty acids introduced into the systems was established. The extent of this effect depended on the acyl chain and levels of added fatty acids. During microwave heating, the oxidative rate of purified soybean oil by the fatty acids was rapid compared to the addition of their corresponding hydrocarbons; the shorter the chainlength and the higher the levels of fatty acids, the more accelerated was the thermal oxidation in the oil. The results are explained on the basis of the catalytic effect of the carboxylic group on the formation of free radicals by the decomposition of hydroperoxides. Therefore, particular attention should be paid to the free fatty acid content, which affects the oxidative stability of purified soybean oil.

KEY WORDS: Anisidine value, carbonyl value, fatty acids, microwave heating, oxidative stability, peroxide value, purified soybean oil.

The minor components isolated from some vegetable oils contain free fatty acids, monoglycerides, phospholipids, thermally oxidized compounds, tocopherols or other unknown compounds (1-3). Minor components, such as monoglycerides and oxidized triglycerides, have shown prooxidant activity toward vegetable oils (3,4). Soybean oils often contain 0.05-0.7% free fatty acids (5-8), those levels can increase further by hydrolysis of oils during processing, storage, frying or cooking.

In a previous paper (9) we reported that when vegetable oils were exposed to microwave energy, the higher the amount of polyunsaturated fatty acids in the oils, the greater was the rate of quality deterioration of the oils. However, tocopherols in unsaturated vegetable oils are more stable than those in saturated vegetable oils (10,11). We found that the levels of free fatty acids increase in purified vegetable oils when heated in a microwave oven, and that the reduction in tocopherols became greater with increased free fatty acid levels (11). On the other hand, the effect of fatty acids alone on the oxidative stability of purified vegetable oils during microwave heating has not been studied systematically.

The present work was undertaken to determine the qualitative and quantitative effects of fatty acids on the oxidative stability of purified soybean oil as a result of microwave heating. Therefore, it is important to use purified soybean oil without minor impurities, such as described above, to investigate the oxidative stability of vegetable oils during microwave heating. In this paper, soybean oil was purified by column chromatography and contained no minor impurities before addition of free fatty acids.

EXPERIMENTAL PROCEDURES

Soybean oil. Refined soybean oil [iodine value (IV) = 132.0] without additives was purchased from Nacalai Tesque Inc. (Kyoto, Japan). The sample (200 g) was further purified by passage over aluminum oxide (100 g), which had been activated at 200°C for 3 h immediately before use. The alumina column (45 × 3 cm i.d.) and collection vessels were wrapped in aluminum foil, and the oil was drawn through the column by suction without solvent. This procedure for purification was repeated twice to thoroughly remove tocopherol homologues. After rechromatography, gas chromatographic analysis gave the following fatty acid composition (wt%): palmitic acid, 10.6%; stearic acid, 3.6%; oleic acid, 22.7%; linoleic acid, 54.8%; and linolenic acid, 8.3%.

Fatty acids. Commercially available saturated fatty acids [caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0) and stearic (18:0)] without additives were purchased from Nacalai Tesque Inc. All the fatty acids were of the *n*-form, and their purities were above 99.0% as determined by gas chromatography (GC).

Hydrocarbons. Hydrocarbons which had the same number of carbons as the fatty acids were purchased from Nacalai Tesque Inc. The purity of each hydrocarbon was above 99.0% as determined by GC.

Microwave heating procedure. Purified soybean oil containing 0, 0.05, 0.25, 0.50 or 1.0% levels of caprylic, capric, lauric, myristic, palmitic or stearic acid was prepared. Each 5.0-g prepared sample was placed in a 25-mL brown glass bottle and 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 (9). The temperatures of the oils immediately after heating for each time period were determined with a chromel-alumel thermocouple. The occurrence of off-flavor was estimated by sensory evalution according to the method of Homma and Fujimaki (12). Purified soybean oil samples containing hydrocarbons at the same levels as fatty acids were also treated under the same conditions. A control sample was heated with no added fatty acids.

Gas chromatography. Methyl ester derivatives of fatty acids were prepared from purified soybean oil as outlined earlier (13). Analysis of fatty acid esters was carried out with 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 (100/120 mesh) and connected with an integrator (Shimadzu C-R6B). Identification of the peaks and the other GC conditions were as described previously (14).

Evaluation of effects of fatty acids and hydrocarbons on purified soybean oil. Acid value was determined by the AOCS (15) titration method on samples every 4 min, and results were expressed as mg KOH to neutralize 1 g of oil. After fixed time intervals, the carbonyl and *p*-anisidine values of the heated oils were measured according to JOCS methods 2•4•22-73 and 2•4•26-81 (16), respectively. For peroxide and iodine values, AOCS Official Methods (17) were used.

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 randomized

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complete block design to partition the effects of different parameters (18). Duncan's multiple range test (19) was applied to determine significance of differences between means.

RESULTS AND DISCUSSION

The purified soybean oil was colorless, odorless and tasteless. The analyses showed the product to be devoid of tocopherols and scored < 0.3 meq/kg oil for peroxide value, 0.4 for carbonyl value, none for anisidine value and acid value. As expected, temperatures of the oils at the end of microwave heating increased with an increase in microwave time: *ca.* 120°C after heating of 4 min, 160°C after 8 min, 180°C after 12 min, and *ca.* 205°C at 20 min. In general, the frying conditions for fried foods, such as potato chips or french fries (20,21), are about 160–180°C for 3–5 min and correspond to the 8–12 min heating in this study. There were no significant differences ($P > 0.02$) in temperatures among the oils containing added fatty acids or hydrocarbons. However, there were significant differences ($P < 0.05$) in sensory evaluation among the oils with or without fatty acids. Thus, off-flavor occurred in the oils with added fatty acids after heating of 8 min and developed in the control oil or the oils containing added hydrocarbons at 16 min.

Figure 1 shows the increase in acid value of purified soybean oils after addition of fatty acids at different concentrations before microwave heating. The higher the levels and the smaller the molecular weight of added fatty acids, the greater was the acid value of prepared sample oils. Figure 2 shows the acid value changes during microwave heating of purified soybean oil with different concentrations (0.05–1.0 wt%) of fatty acids added. Although all acidic components were titrated by the acid value method, it was assumed that only fatty acids were measured. Fatty acid content is a dynamic value because at the same time that acids are being produced, they have sufficient vapor pressure at frying temperatures to evaporate from the surface (22). Unexpectedly, the acid value increased significantly ($P < 0.05$) due to production of fatty acids by microwave energy. Many authors indicate that fatty acids catalyze the further hydrolysis of triglycerides (23). However, the slopes of the control oil and the oils containing added fatty acids were not significantly different ($P > 0.05$) from each other. The results suggest that covering the vessel with polyethylene film while microwave heating prevented evaporation of added and/or produced fatty acids. The increase in acid value for the control oil is in agreement with the results obtained previously (11). Data generated with lauric or palmitic acid (data not shown) demonstrated similar patterns to those shown in Figure 2.

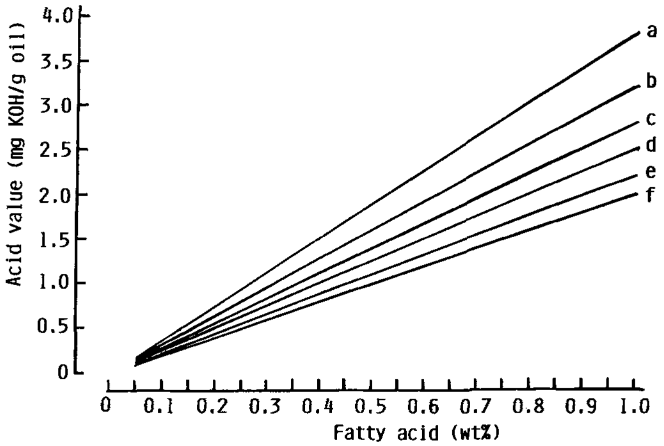

FIG. 1. Linear regression of acid value changes in purified soybean oil containing added fatty acids at different levels: a, $C_{8:0}$; b, $C_{10:0}$, c, $C_{12:0}$; d, $C_{14:0}$; e, $C_{16:0}$; f, $C_{18:0}$.

Figure 3 shows the effect of fatty acids on peroxide accumulation in the oils during microwave heating. Although the addition of each fatty acid accelerated the oxidative deterioration of the oils because tocopherol homologues were removed (24), there were no appreciable differences ($P > 0.05$) between the chainlength of added fatty acids. However, the longer the heating period and the greater the levels of added fatty acids, the faster was the rate of formation of hydroperoxides and their degradation. Data generated with lauric or palmitic acid showed similar patterns to those in Figure 3, whereas hydrocarbons, which have the same number of carbons as these fatty acids but no carboxylic group, did not act as a prooxidant (data not shown). In general, 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. However, hydroperoxides are unstable on heating, resulting in rapid transformation to secondary products, which can contribute to off-flavors. Figure 4 shows the effect of fatty acids on carbonyl values in the oils during microwave heating. The prooxidant activity of added fatty acids on the purified soybean oil was observed in the initial stage of heating; the activity was stronger for shorter-chain fatty acids than for longer chains. Carbonyl value of the oils increased somewhat ($P < 0.05$), both by longer exposure to microwave energy and by increasing levels of added fatty acids. The results suggest that the oxidative deterioration of purified soybean oil was accelerated by the carboxylic group of saturated fatty acids, but such fatty acids were not oxidized when heated in a microwave oven.

Figure 5 shows the effect of fatty acids on anisidine values in the oil during microwave heating. No significant difference ($P > 0.05$) for the values was observed until 8 min of heating, and more pronounced differences ($P < 0.05$) were observed after 12 min of heating. With an increase in levels of added fatty acids, these trends also became more distinct for shorter chainlength than for longer chainlength. Mistry and Min (25) indicated that the carboxylic group of free fatty acids may produce prooxidant activity in purified soybean oil. Handel and Guerrieri (26) also reported an effect of free fatty acids on the quality of vegetable oils. These investigations were carried out with only C_{18} fatty acids of varying degrees of unsaturation, under autoxidative conditions for 5 d at 55°C in the former demonstrations and at frying conditions for 24 h at 200°C in the latter experiments. However, little is known about how free fatty acids with different length of the acyl chain affect the oxidative stability of soybean oil when treated in a microwave oven. Moreover, the level of added free fatty acids to oil samples (wt%; 0.5, 1.0 or 5.0) was greater than that detected in commercially available vegetable oils, and the concentrations corresponded to 1.0, 2.0 or 10.0 acid value (Fig. 1).

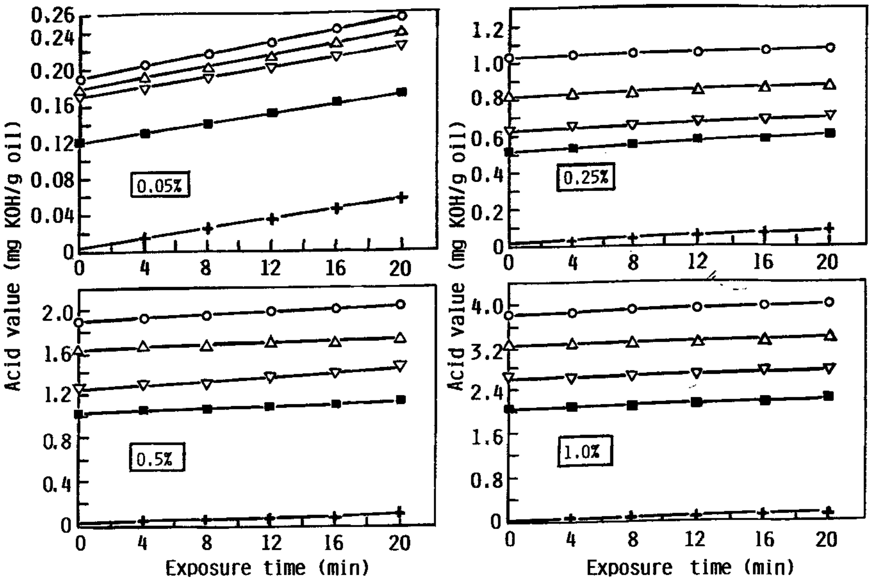

FIG. 2. Acid value changes during microwave heating (frequency 2,450 MHz) of purified soybean oil with 0, 0.05, 0.25, 0.5 and 1.0% fatty acids added: +, control; ○, $C_{8:0}$; △, $C_{10:0}$; ▽, $C_{14:0}$; ■, $C_{18:0}$.

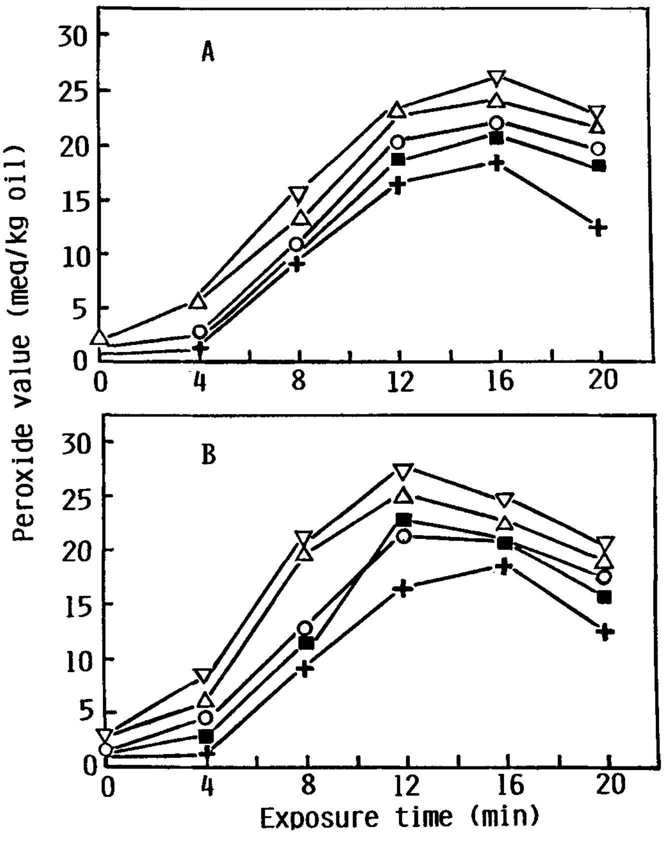

FIG. 3. Effect of fatty acids on peroxide values of purified soybean oil during microwave heating (frequency 2,450 MHz): A, 0.05%; B, 0.50%; +, control; ○, $C_{8:0}$; △, $C_{10:0}$; ▽, $C_{14:0}$; ■, $C_{18:0}$.

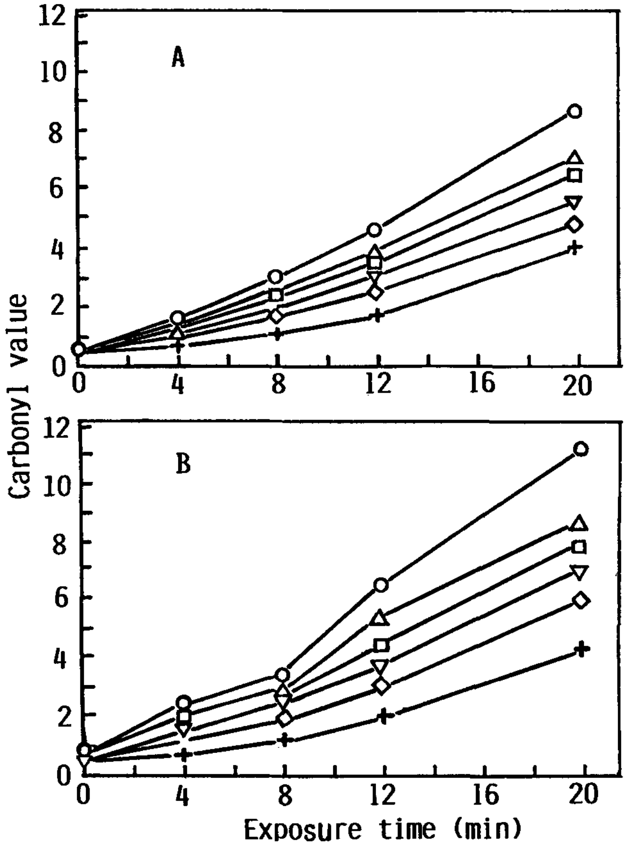

FIG. 4. Effect of fatty acids on carbonyl values of purified soybean oil during microwave heating (frequency 2,450 MHz): A, 0.05%; B, 0.50%; +, control; ○, $C_{8:0}$; △, $C_{10:0}$; □, $C_{12:0}$; ▽, $C_{14:0}$; ◇, $C_{16:0}$.

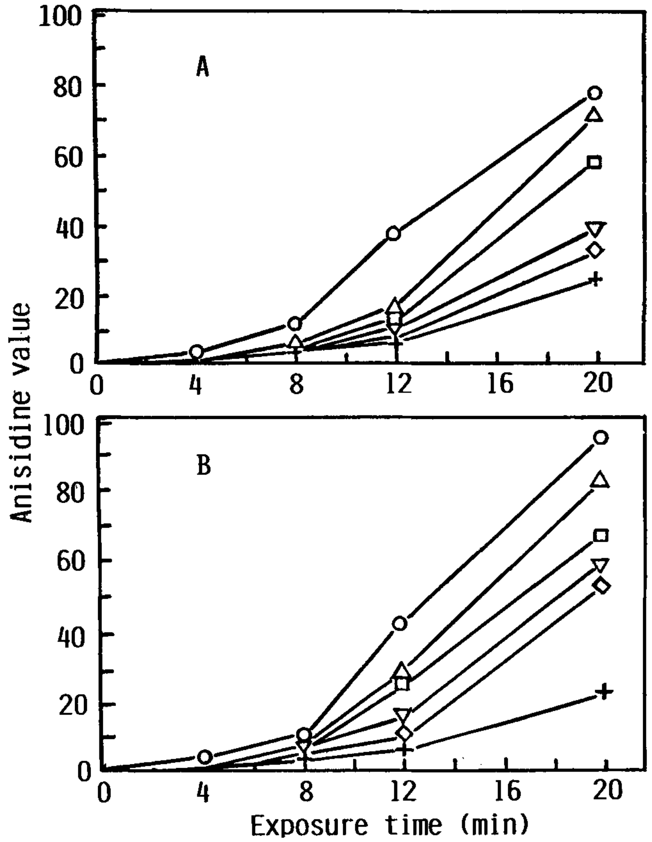

FIG. 5. Effect of fatty acids on anisidine values of purified soybean oil during microwave heating (frequency 2,450 MHz): A, 0.05%; B, 0.50%; +, control; ○, $C_{8:0}$; △, $C_{10:0}$; □, $C_{12:0}$; ▽, $C_{14:0}$; ◇, $C_{16:0}$.

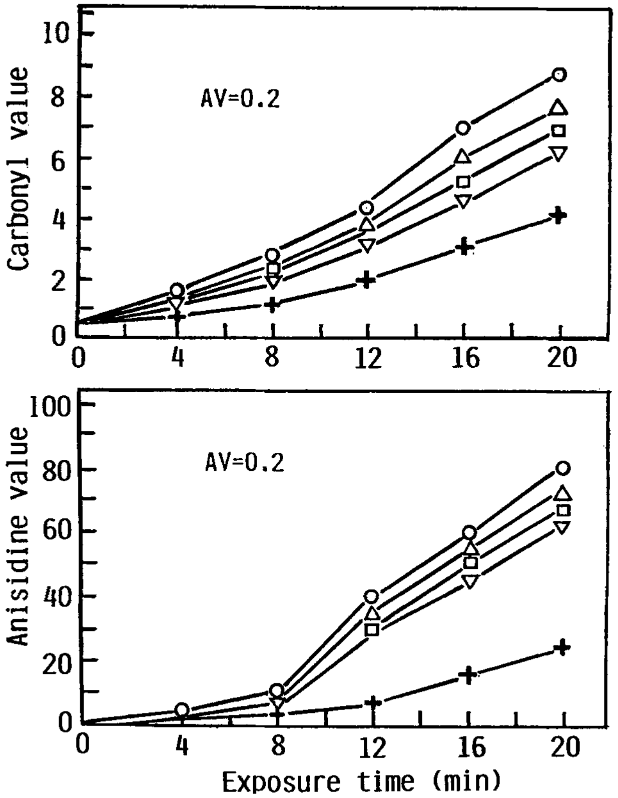

FIG. 6. Effect of fatty acids at the same mole% on carbonyl and anisidine values of purified soybean oil during microwave heating (frequency 2,450 MHz): +, control; ○, $C_{8:0}$; △, $C_{10:0}$; □, $C_{12:0}$; ▽, $C_{14:0}$; AV, acid value.

In Japan, the acid value for commercially available soybean oil is regulated to be below 0.15 for salad oil and below 0.20 for purified oil according to the Japanese Agriculture and Forestry Standard (JAS). In a previous paper (27) we reported that low-molecular weight fatty acids accelerated the oxidation of tocopherols in purified vegetable oils when heated in a microwave oven. Therefore, to clarify the effects of medium-chain fatty acids (caprylic, capric, lauric or myristic) on the oxidative stability of purified soybean oil during microwave heating, each fatty acid was added to the oil at 3.56×10^{-6} mol/g oil. This level corresponded to 0.20 acid value and to a concentration above 0.05 wt% (Fig. 1). Figure 6 shows the effect of fatty acids at the same levels (mole%) on carbonyl and anisidine values of purified soybean oil during microwave heating. After heating for 8 min, the oxidative rate of soybean oil increased, resulting in substantially greater differences ($P < 0.05$) with the shorter acyl chain of fatty acids. The results are explained on the basis of the catalytic effect of the carboxylic group on the formation of free radicals by the decomposition of hydroperoxides. Therefore, it is necessary to pay attention to the type and content of free fatty acids in oils, although many factors (minor components) present in oils, such as phospholipids, tocopherols, fatty acids and trace heavy metals, also affect the oxidative stability of oils (28–30).

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