Oxidative Stability of Polyunsaturated Fatty Acids and Soybean Oil in an Aqueous Solution with Emulsifiers

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ABSTRACT: The oxidative stability of polyunsaturated fatty acids (PUFA) and soybean oil homogenized with emulsifiers was investigated. Model emulsions were prepared from PUFA, including linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and from soybean oil emulsified with different emulsifiers: three Tween emulsifiers (Tween 20, Tween 60, Tween 80) and two sucrose esters (S-1170 and S-1570) were used. The results showed that the emulsions prepared from LA and the various emulsifiers, oxidized at 40°C, were unstable. However, the corresponding AA, EPA, and DHA emulsions were stable, indicating that PUFA with a higher degree of unsaturation were more stable with emulsifiers than without the emulsifiers. In the soybean oil-in-water model system, the oxidation of soybean oil with various emulsifiers was less than without the emulsifiers.

Paper no. J8924 in *JAOCS 76*, 201–204 (February 1999).

KEY WORDS: Arachidonic acid, autoxidation, docosahexaenoic acid, eicosapentaenoic acid, emulsifier, emulsion, linoleic acid, oxidative stability, peroxide formation, polyunsaturated fatty acid, soybean oil.

Because of their great abundance in foodstuffs and relatively high susceptibility to autooxidation, polyunsaturated fatty acids (PUFA) are among the most important precursors of undesirable flavor compounds. This fact has stimulated a number of studies on oxidation stability of PUFA and lipid-containing foods. The oxidative stability of unsaturated lipids generally decreases with increasing degree of unsaturation (1,2). However, the oxidative stability of PUFA in the aqueous phase increases with increasing degree of unsaturation (3). In addition, emulsifiers have been reported to affect lipid peroxidation (4). Miyashita *et al.* (5) have reported that the oxidative stability of linoleic acid (LA) with Tween 20 was slightly less than that of LA, while the stability of docosahexaenoic acid (DHA) with Tween 20 was markedly increased. Thus, apparently, the characteristic of oxidative stability of PUFA in an aqueous phase would be affected by the nature of the emulsifiers used. Numerous emulsifiers, varying in their structure and properties, are at present available to the food industry. The effect of Tween 20 on LA and DHA in an aqueous solution has been reported. However, the effects of other emulsifiers on the oxidation of PUFA in an aqueous solution and on edible oils have not been understood. Hence, this investigation presents a systematic study of the interactive effects of various emulsifiers on the oxidation of PUFA in aqueous phases, and of soybean oil-in-water emulsion.

MATERIALS AND METHODS

Materials. Linoleic (18:2n-6, LA), arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3, EPA), and docosahexaenoic (22:6n-3, DHA) acids were obtained from the Aldrich Chemical Co. (Milwaukee, WI). The three Tween emulsifiers (Tween 20, Tween 60, and Tween 80) were purchased from Merck (Darmstadt, Germany), and the two sucrose esters (S-1170 and S-1570) were a gift from the Gemfont Co. (Taipei, Taiwan, ROC).

Oxidative procedure. Fatty acid (0.28045 g) was dispersed in 50 mL phosphate buffer (0.05 M, pH 7.4) that contained 0.02805 g (10%, relative to fatty acid) or 0.00280 g (1%, relative to fatty acid) of emulsifier during homogenization. Three Tween emulsifiers (Tween 20, Tween 60, and Tween 80) and two sucrose ester emulsifiers (S-1170 and S-1570) were used as individual emulsifiers for preparing the substrate emulsions. The substrate emulsion solution (2.5 mL) was pipetted into a test tube (20 mL, 2.4 cm i.d.) that contained 2.5 mL of 0.2 M phosphate buffer (pH 7.0) and was incubated at 40°C. For the control test, 0.28045 g fatty acid without emulsifiers and 50 mL phosphate buffer (0.02 M, pH 7.4) was homogenized, and an aliquot (2.5 mL) of homogenized solution was mixed with 2.5 mL phosphate buffer (0.2 M, pH 7.0). The degree of oxidation was measured according to the thiocyanate method (6) by sequentially adding ethanol (4.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), substrate emulsion (0.1 mL), and ferrous chloride (2.0 mM in 3.5% HCl) solution (0.1 mL). After the mixture was stirred for 3 min, the peroxide value (PV) was determined by reading the absorbance at 500 nm. All tests were run in triplicate, and the analyses of all samples were run in duplicate. Averages were then calculated.

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The soybean oil-in-water emulsions (100 mL) were made with 20 g of oil, made up with deionized water in a 250-mL Erlenmeyer flask and emulsified with 0.2 g (1%, relative to soybean oil) or 2.0 g (10%, relative to soybean oil) of emulsifier by a homogenizer (Ystral, Germany). For the control test, 20 g of oil and 80 mL of deionized water was homogenized. An aliquot of 2.0 g emulsion solution was then weighed and placed in an open 50-mL beaker. Each treatment was placed in an oven at 60°C. The PV of each treatment was determined by the Official Method Cd 8-53 (7) at intervals during storage. All tests were run in triplicate, and the analyses of all samples were run in duplicate. Averages were then calculated.

Statistical analyses. Statistical analysis involved use of the Statistical Analysis System (8) software package. Analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

The effects of different emulsifiers at 1 and 10% (relative to LA) on the oxidative stability of LA, determined by the thiocyanate method, are plotted in Figure 1. No significant difference ($P > 0.05$) in the oxidation stability of LA was found between LA with any emulsifier (1%) and LA without emulsifier (Fig. 1A). However, the oxidative stability of LA with a high amount of emulsifier (10%) was less than that of LA without emulsifier (Fig. 1B). This observation implies that 10% of emulsifier, used in the model system, enhanced the oxidation of LA. This result is in agreement with the studies of Miyashita *et al.* (5), who reported that the oxidative stability of LA with Tween 20 was slightly less than that of LA without Tween 20.

As shown in Figure 2, the oxidation of AA with any emulsifier at 1% (Fig. 2A) and 10% (Fig. 2B) was slightly more stable than that of AA without emulsifier. In general, emulsifier at 1 and 10% at 12 h testing shows that the formation of peroxide in AA with Tween 20, Tween 60, and Tween 80 was less than that of AA with S-1170 and S-1570, indicating that oxidation of AA with Tween 20, Tween 60, and Tween 80 was more stable in the early stages than that of S-1170 and S-1570. Apparently, each emulsifier used in the AA model system was able to reduce AA oxidation.

The formation of peroxides in EPA with each emulsifier at 1 and 10% was less than that of EPA without emulsifiers (Figs. 3A and 3B). A rapid increase in the amount of peroxides was observed in the oxidation of EPA, at 12 and 8 h of testing (Figs. 3A and 3B) without emulsifier. However, EPA treated with any emulsifier showed a gradual increase in PV formation, indicating that the oxidation of EPA with emulsifiers was more stable than that of EPA without emulsifiers. Of all emulsifiers, the oxidative stability of EPA with 1 and 10% Tween 20 was less than that of EPA with other emulsifiers.

The data of DHA peroxidation at 40°C after the addition of emulsifier at 1 and 10% are plotted in Figure 4. Figure 4A shows that, in the early stages, the oxidation of DHA with or without emulsifier (1%) was accompanied by a slow increase of PV at 12 h of testing. A rapid increase in the amount of peroxides was observed during the oxidation of DHA after 12 h of testing. A significant difference ($P < 0.05$) was found between DHA without emulsifier and DHA with emulsifier. Figure 4B shows that, in the early stages, the oxidation of DHA with or without emulsifier was accompanied by an increase of PV at 6 h of testing. A rapid increase in the amount of peroxides was observed during the oxidation of DHA after 6 h of testing. A significant difference ($P < 0.05$) was found between DHA without emulsifier and DHA with emulsifier. These results (Figs. 4A and 4B) indicate that the oxidative stability of DHA can be improved by adding emulsifiers.

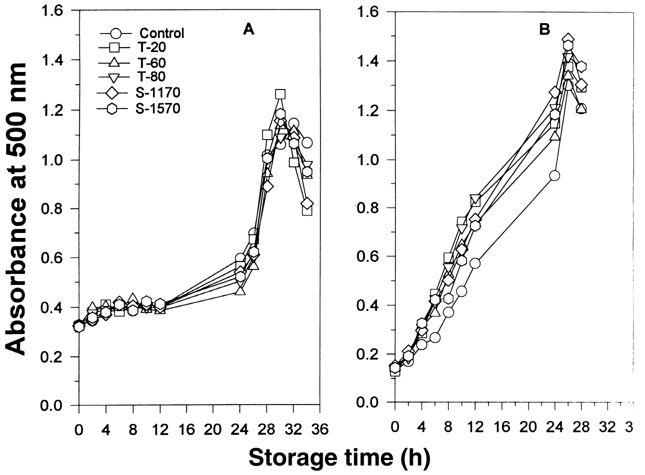

FIG. 1. Effects of different emulsifiers at 1% (A) and 10% (B) on the oxidative stability of linoleic acid. Total peroxide formation is expressed as absorbance at 500 nm. Emulsifiers: Tween 20 (T-20), Tween 60 (T-60), Tween 80 (T-80); sucrose esters: S-1170 and S-1570.

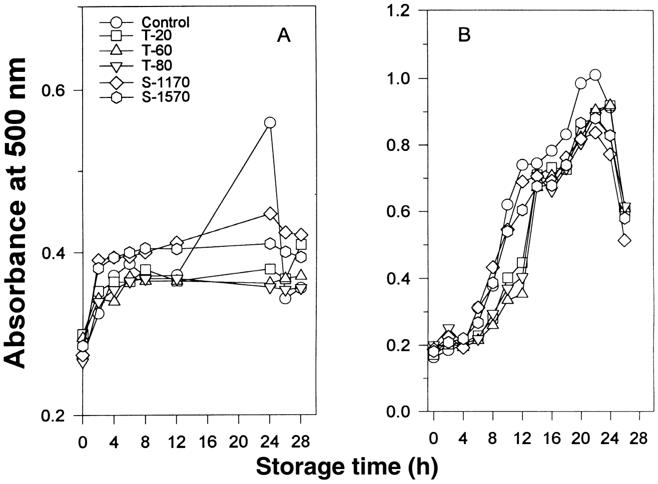

FIG. 2. Effects of different emulsifiers at 1% (A) and 10% (B) on the oxidative stability of arachidonic acid. Total peroxide formation is expressed as absorbance at 500 nm. See Figure 1 for abbreviations.

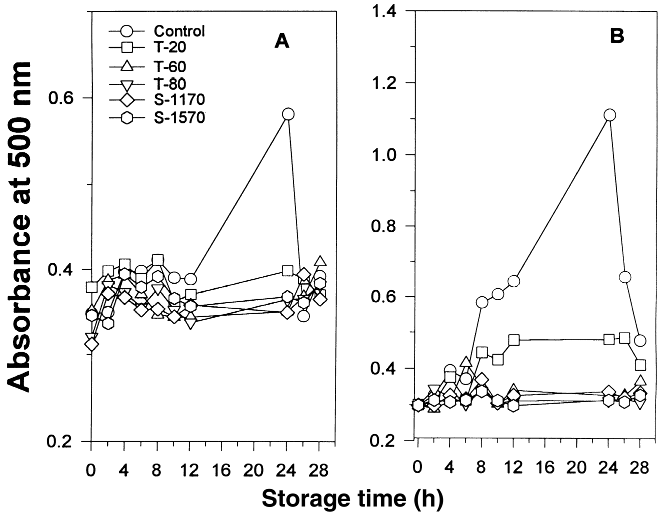

FIG. 3. Effects of different emulsifiers at 1% (A) and 10% (B) on the oxidative stability of eicosapentaenoic acid. Total peroxide formation is expressed as absorbance at 500 nm. See Figure 1 for abbreviations.

Some literature (4) suggests that PUFA as substrates may not completely reflect lipid peroxidation, due to their unique physical properties in aqueous micelles. Hence, it is necessary to evaluate the effect of emulsifiers on the oxidation of substrates other than PUFA.

The effect of different emulsifiers at 1 and 10% (relative to soybean oil) on oxidative stability of soybean oil during storage at 60°C was evaluated by determining PV (Fig. 5). Figure 5A shows the effect of 1% emulsifier on the oxidation of soybean oil. In all treatments, a rise in PV was observed, which indicated oxidation of soybean oil-in-water emulsion. In the early stages, at 4 d of testing, the peroxidation of soybean oil with or without emulsifier was accompanied by a gradual increase in PV. The control (without emulsifier), which reached a maximum PV of 134.17 meq/kg after 6 d of testing, was oxidized to the highest extent. Samples with emulsifiers showed gradual increases in PV, which reached 68.34, 61.68, 79.05, 47.68, and 45.48 meq/kg for T-20, T-60, T-80, S-1170 and S-1570, respectively, indicating that they exhibited greater oxidative stability than the control during oxidation. Figure 5B shows that the control, which reached a maximal PV of 187.00 meq/kg after 5 d of testing, was oxidized to the highest extent. A significant difference ($P < 0.05$) in the formation of PV was found between soybean oil with emulsifier and soybean oil without emulsifier. According to the data presented in Figures 5A and 5B, the oxidative stability of soybean oil-in-water emulsion can be improved by adding emulsifiers.

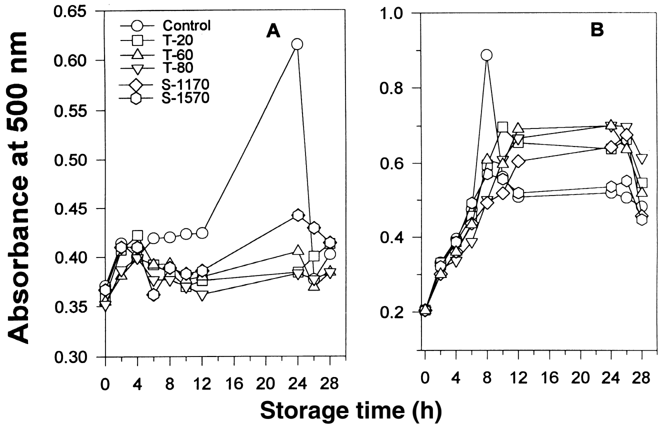

FIG. 4. Effects of different emulsifiers at 1% (A) and 10% (B) on the oxidative stability of docosahexaenoic acid. Total peroxide formation is expressed as absorbance at 500 nm. See Figure 1 for abbreviations.

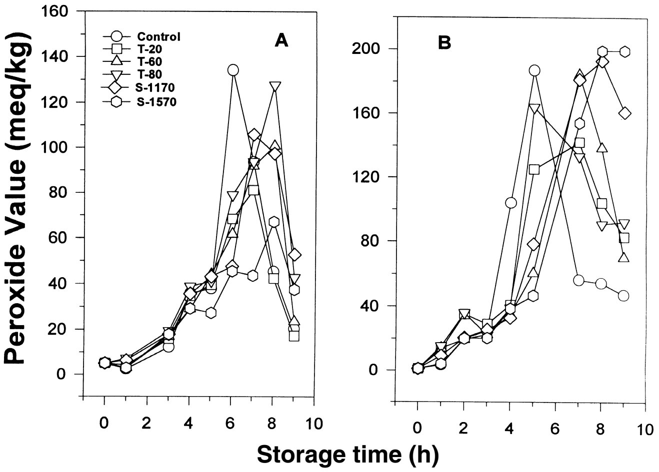

FIG. 5. Effects of different emulsifiers at 1% (A) and 10% (B) on the oxidative stability of soybean oil during storage at 60°C as measured by peroxide value. See Figure 1 for abbreviations.

In the present study, LA with emulsifiers in an aqueous solution was most susceptible to oxidation. On the other hand, AA, EPA, and DHA with addition of emulsifiers at 1 or 10% were stable. In other words, the oxidative stability of PUFA with emulsifier increased with an increasing degree of unsaturation. Miyashita *et al.* (3) noted that DHA and EPA take on a more tightly packed conformation in an emulsion solution; hence, the micelles composed of emulsifiers and AA, EPA, or DHA took on a more stable conformation against the attack by free radicals and/or oxygen. Besides, the characteristic oxidative stability of PUFA in an aqueous phase can be strongly correlated with the nature of the emulsifier and with the behavior of the complex formed by the PUFA and the emulsifer. This may in part explain why emulsifiers stabilize the oxidation of AA, EPA, and DHA. However, more information is needed on the effect of emulsifiers with respect to LA to better explain their role in prooxidant activity.

ACKNOWLEDGMENT

This research work was partially supported by the National Science Council, Republic of China, under Grant NSC 87-2314-B-041-002.

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[Received June 29, 1998; accepted November 7, 1998]