Effects of Emulsifiers on the Oxidative Stability of Soybean Oil TAG in Emulsions

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ABSTRACT: The effects of two types of commercial emulsifiers, sucrose FA esters and polyglycerol FA esters, on the oxidation of soybean oil TAG-in-water emulsions were studied. Both emulsifiers influenced the oxidative stability of soybean oil TAG in the emulsion, but they had little effect on the oxidation of TAG in bulk phase. When the TAG were dispersed with sucrose esters having the same FA composition, the oxidative stability increased as their hydrophilic-lipophilic balance (HLB) increased. On the other hand, when the HLB was the same, the oxidative stability increased with increasing acyl chain length of the FA esterified on sucrose ester. However, the effect of the polyglycerol ester could not be explained by the relationship with HLB or the acyl composition. When the stability of TAG in emulsion was compared under the same concentrations of TAG, emulsifier, and oxidation inducer, the TAG dispersed with sucrose esters were oxidatively less stable than with polyglycerol esters. Analysis of the emulsion droplet size suggested that the lower oxidative stability of TAG dispersed with sucrose esters was partly due to their relatively smaller droplet sizes.

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KEY WORDS: Droplet size, emulsifier, emulsion, HLB, oxidative stability.

Lipid oxidation has been extensively studied in bulk fats and oils, and there is a fairly good understanding of the mechanisms and the factors that affect oxidation in such systems (1,2). On the other hand, lipid oxidation is still not well understood in systems in which the fat is dispersed as emulsion droplets, although a large number of foods exist partially or entirely in the form of emulsions. Food emulsions are complex, multicomponent, heterogeneous systems in which different molecular species interact with each other. The various molecules in an emulsion system become distributed according to their polarity and surface activity between different phases, which include the oil phase, the water phase, and the interfacial region. Lipid oxidation in such systems is an interfacial phenomenon that is greatly influenced by the nature of the interface. However, understanding of the factors that affect lipid oxidation in an emulsion system is still fairly poor, and this is an active research area (2).

In oil-in-water emulsions, oil droplets are surrounded by a membrane of emulsifier molecules that prevents them from coalescing. In addition to enhancing the physical stability of emulsion droplets, these membranes can also protect lipids in the droplet interior from oxidation by acting as a barrier to the attack of oxidation inducers such as metals and free radicals (3). Therefore, it is interesting to determine the effects of emulsifier on the oxidative stability of lipids in an emulsion. In this study, we evaluate the oxidative stability of soybean oil TAG dispersed with different types of sucrose esters or polyglycerol esters in emulsion. Since both esters are food emulsifiers that are widely used in Japan and other countries, the present study will provide useful information for protecting against lipid oxidation in food emulsion systems.

MATERIALS AND METHODS

Sample lipids. Soybean oil was obtained from Kanto Chemical Co. (Tokyo, Japan). The oil was passed through a column packed with a 1:1 mixture (w/w) of activated carbon/Celite 545 to which *n*-hexane was added to remove tocopherols (4). The recovered oil was refined on a silicic acid column by eluting with a diethyl ether/*n*-hexane solution just before use. The fraction eluted with diethyl ether/*n*-hexane (10:90 and 20:80, vol/vol) was used as the oil sample for oxidation. The purified oil sample contained no tocopherol, as determined by HPLC (5), and gave only a single spot corresponding to TAG on a thin-layer chromatogram with normal-phase silica plates (Merck, Darmstadt, Germany) developed with diethyl ether/*n*-hexane/acetic acid (40:60:1, by vol). The PV of each sample was less than 1.0 as determined by the Official AOCS Method (6). The FA composition of the soybean oil TAG was determined by GC after conversion of fatty acyl groups in TAG to their methyl esters by heating in a sealed tube at 90–100°C for 1 h with 7% BF_3/MeOH (wt/vol) under nitrogen. GC analysis was performed on a Shimadzu GC-14B instrument (Shimadzu Seisakusho, Kyoto, Japan) equipped with an FID and a capillary column [Omegawax 320 (30 m × 0.32 mm i.d.); Supelco, Bellefonte, PA]. The FA composition of soybean oil TAG was as follows (acid, mol%): 16:0, 10.4; 18:0, 3.4; 18:1n-7, 1.6; 18:1n-9, 25.6; 18:2n-6, 54.2; 18:3n-3, 3.8.

Emulsifier. In the present study, we used nonionic emulsifiers, sucrose FA esters and polyglycerol FA esters (Table 1), which are the most commonly used food emulsifiers in Japan. Both emulsifiers were kindly donated by Mitsubishi Food Co. (Tokyo, Japan). The polyglycerol ester contained free polyglycerol in the amounts of 12.8 wt%, HS10 (Mitsubishi catalog number); 8.2 wt%, S28D; 11.2 wt%, DS10; 15.9 wt%,

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TABLE 1
HLB, FA Composition, and Percentage of Esterification of Emulsifier[a]

Emulsifier	HLB	FA esterified (wt%) 12:0	14:0	16:0	18:0	Percent esterification
Sugar ester						
S570	5.0	—	—	30.0	70.0	27.5
S1170	11.0	—	—	30.0	70.0	18.8
S1670	16.0	—	—	30.0	70.0	15.0
P1670	16.0	—	—	80.0	20.0	15.0
L1695	16.0	95.0	—	—	—	15.0
Polyglycerol ester						
HS10	8.1	—	4.0	43.0	52.0	28.6
S28D	8.0	—	0.5	28.8	70.0	29.5
DS10	8.7	—	4.0	43.0	52.0	28.0
SWA20D	8.7	—	0.5	28.8	70.0	24.3
L-7D	13.2	99.6	—	—	—	15.7

[a]HLB, hydrophilic-lipophilic balance. Emulsifiers supplied by Mitsubishi Food Co. (Tokyo, Japan).

SWA20D; and 57.2 wt%, L-7D. The free polyglycerol content was determined by an HPLC technique using a combination of a reversed-phase column (YMC-AM312; YMC, Kyoto, Japan) and a gel permeation column (Asahipak GS310Q; Asahi Kasei Co., Tokyo, Japan). The HPLC analysis was done isocratically using a mixture of methanol/water (30:70, vol/vol) as a mobile phase at a constant flow rate of 0.7 mL/min. Peaks were monitored by refractive index detector. The percent esterification in Table 1 represents the ratio of the number of esterified FA to that of hydroxyl groups available for esterification in each emulsifier. In the case of the polyglycerine ester, this ratio was calculated by considering the amount of free polyglycerol. The amount of FA esterified was estimated by determining the saponification value.

Aqueous oxidation. An aliquot of soybean oil TAG was mixed homogeneously with an emulsifier in chloroform. After removing the chloroform by gently sweeping with nitrogen, 0.05 M phosphate buffer (pH 7.4 at 37°C) was added to the mixture. The emulsion was formed by sonicating the mixture of TAG and emulsifier in the buffer, and oxidation was initiated by adding an aqueous solution of 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH; Wako Pure Chemical Industry, Osaka, Japan).

Analysis of aqueous oxidation. Oxidative stability was evaluated by analyzing oxygen consumption. For continuous monitoring of oxygen uptake by lipids in solution, a model 5300 biological oxygen monitor (Yellow Springs Instrument, Yellow Springs, OH) was used. As soon as the AAPH solution had been added to the substrate solution, the reaction vessel was charged with 3 mL of the reaction solution and the concentration of dissolved oxygen in the solution was measured. Final concentrations were substrate (1.0 or 3.0 wt%), emulsifier (0.1 or 1.0 wt%), and AAPH (1.0 or 3.0 mM).

Three or more aliquots of each sample were subjected to oxidation. For each determination there was a slight difference in the oxidation rate, but the order of the oxidative stability of different emulsion samples used in the present study was unchanged, and there was no siginificant difference in the oxidation rate between these samples.

Autoxidation of soybean oil TAG in bulk phase. Soybean oil TAG (9.9 g) was mixed with an emulsifier (0.1 g) in chloroform. After removing the chloroform by gently sweeping with nitrogen, the whole lipid sample in a flat-bottomed glass tube was autoxidized by incubation in the dark at 37°C. Samples for determination of PV were taken from the oxidized samples at selected time intervals. Peroxide values were determined by the AOCS Official Method (6).

Droplet size measurement. Emulsion droplet size and size distribution were determined with a Beckman Coulter LS130 particle size analyzer (laser diffraction technique) (Beckman Co., Tokyo, Japan). An emulsion was diluted 10 times with water before it was transferred into the chamber of the instrument. All particle size measurements were carried out 10 min after emulsification.

RESULTS AND DISCUSSION

Although the emulsifiers used in the present study had no effect on the oxidative stability of soybean oil TAG in bulk phase (Fig. 1), the stability of the TAG in emulsion was strongly influenced by emulsifier (Figs. 2–4). Figure 2 shows the oxidative stability of soybean oil TAG (1.0 wt%) in emulsions dispersed with sucrose esters (0.1 wt%) in the presence of 1.0 (A) or 3.0 mM (B) AAPH. When oxidative stabilities of TAG dispersed with sucrose esters having the same FA composition (S570, S1170, S1670) were compared, the stabilities increased with increasing HLB. On the other hand, when the HLB of the sucrose esters were the same [S1670 and L1695 (A); S1670, P1670, and L1695 (B)], the oxidative stability increased with increasing acyl chain length of the esterified FA. The same effect of acyl chain length was also found in the oxidation of soybean oil TAG (3.0 wt%) dispersed with sucrose ester (1.0 wt%) (Fig. 3).

Polyglycerol esters also affected the oxidative stability of soybean oil TAG in emulsion (Fig. 4); however, the degree of this effect on oxidative stability was less than that of sucrose

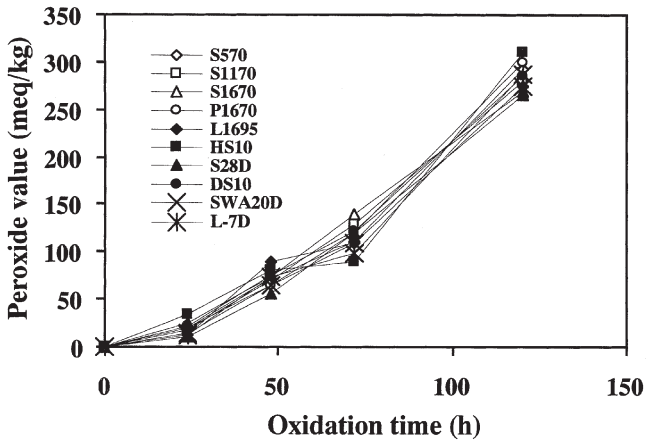

FIG. 1. Oxidative stability of soybean oil TAG in bulk phase. All emulsifiers supplied by Mitsubishi Food Co. (Tokyo, Japan). Sugar ester emulsifiers: S570, S1170, S1670, P1670, and L1695. Polyglycerol ester emulsifiers: HS10, S28D, DS10, SWA20D, and L-7D.

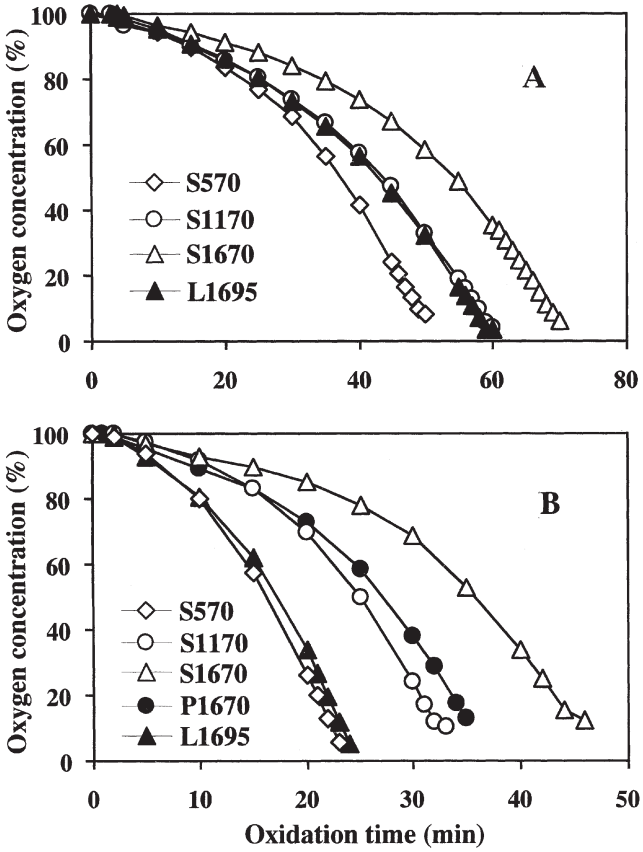

FIG. 2. Oxidative stability of soybean oil TAG dispersed with sucrose esters in emulsion. The concentrations of TAG and emulsifier were 1.0 and 0.1 wt%, respectively. Oxidation was induced by 1.0 (A) and 3.0 mM (B) of 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH). For manufacturer see Figure 1.

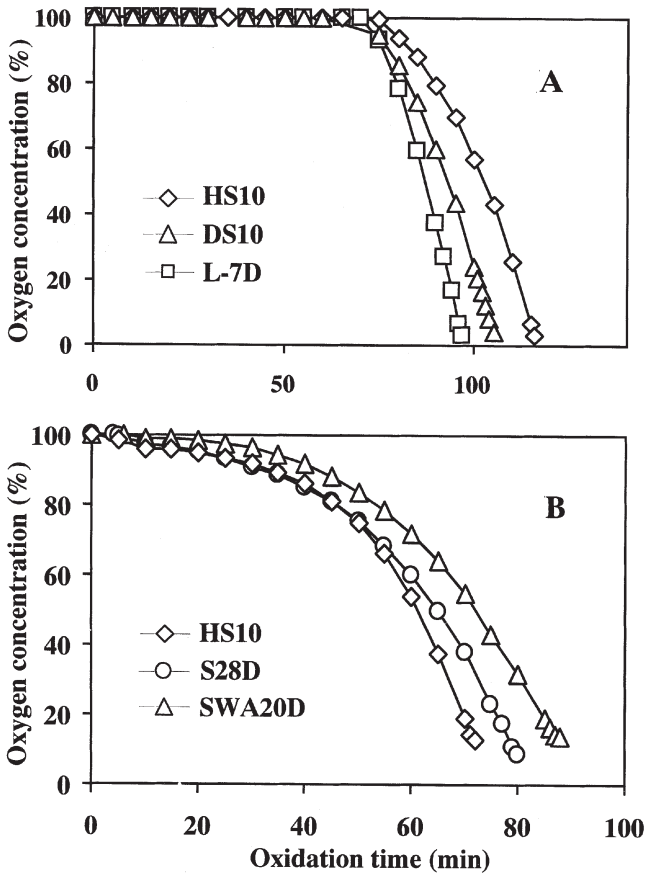

FIG. 4. Oxidative stability of soybean oil TAG dispersed with polyglycerol esters in emulsion. The concentrations of TAG and emulsifier were 1.0 and 0.1 wt%, respectively. Oxidation was induced by 1.0 (A) and 3.0 mM (B) of AAPH. For abbreviation see Figure 2. For manufacturer see Figure 1.

ester (Figs. 1 and 2). In addition, the difference in the oxidative stability of TAG dispersed with polyglycerol ester could not be explained by the relationship with HLB or the acyl composition. Since polyglycerols are mixtures of those having different degrees of polymerization, they have a broad chain-length distribution. Therefore, the number of hydroxyl groups for esterification per one polyglycerol molecule also varies widely, whereas sucrose has only eight hydroxyl groups available for esterification. Furthermore, as described in the Materials and Methods section, the polyglycerol ester contained unreacted polyglycerol. More studies are required to explain the relationship between the nature of the polyglycerol ester and its effect on the oxidative stability of soybean oil TAG.

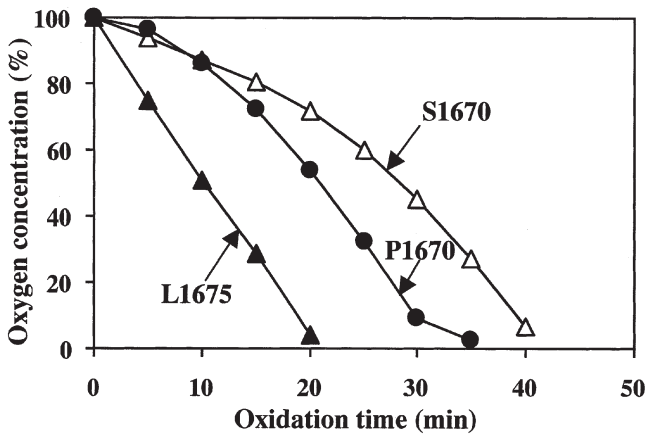

FIG. 3. Oxidative stability of soybean oil TAG dispersed with sucrose esters in emulsion. The concentrations of TAG and emulsifier were 3.0 and 1.0 wt%, respectively. Oxidation was induced by 1.0 mM of AAPH. For abbreviation see Figure 2. For manufacturer see Figure 1.

When the oxidative stability of TAG dispersed with sucrose esters was compared with that of polyglycerol esters in the same concentrations of substrate, emulsifier, and AAPH (Figs. 2 and 4), the stability with sucrose esters was always less than with polyglycerol esters. As shown in Figure 5, the average droplet sizes of emulsions dispersed with sucrose esters were smaller than those with polyglycerol esters. When the concentrations of TAG and emulsifier were the same, the area of interface increased with decreasing droplet size. The possibility of reaction of an oxidation inducer such as AAPH with TAG at the interface increases with increasing interfacial area; therefore, the lower oxidative stability of sucrose esters would be partly due to the smaller droplet size of sucrose esters.

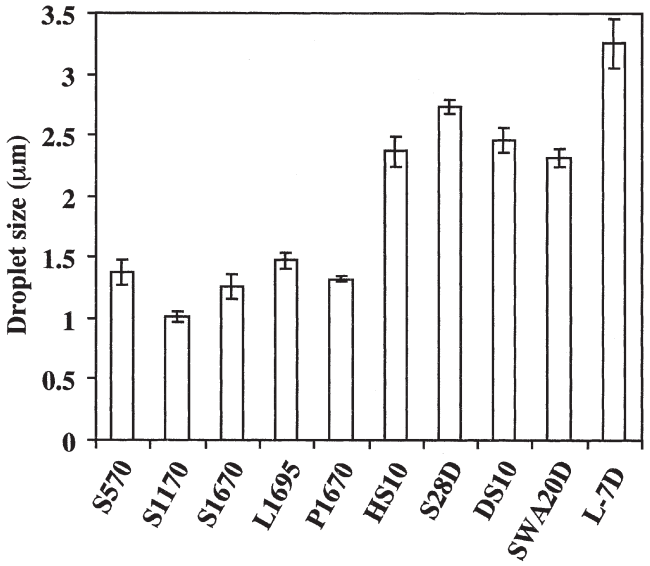

FIG. 5. Average droplet size of emulsions prepared by mixing soybean oil TAG (1.0 wt%) and emulsifier (0.1 wt%). Error bars represent SD ($n = 3$).

On the other hand, there was little difference in droplet sizes among emulsions dispersed with different kinds of sucrose esters or among different kinds of polyglycerol esters (Fig. 5). This result suggests that the different effect of sucrose esters or polyglycerol esters on the oxidative stability of soybean oil TAG had little relation to the emulsion droplet size but rather was related to the nature of interface. In oil-in-water emulsions, oil droplets are dispersed in the continuous water phase. It is known that lipid oxidation in the emulsion is an interfacial phenomenon that is greatly influenced by the nature of the interface of the droplets (2). Since the oxidation of lipids in the emulsion proceeds from the interface, the nature of the interface affects lipid oxidation processes in many ways (3). For example, lipid oxidation in an emulsion can be controlled by altering the electrical charge of the interface or the packing of the emulsifier molecules in the droplet membrane (3). Since sucrose ester and polyglycerol ester are nonionic, the difference in effects of emulsifier on the oxidative stability of TAG found in the present study would be due to the different effectiveness of the packing of the emulsifier. Therefore, the results in Figure 2 suggest that a higher HLB or a longer acyl chain of sugar ester would cause tighter packing at the oil-water interface and the membrane would become a more efficient barrier against diffusion of lipid oxidation initiators such as AAPH.

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