Oxidation of Linoleic Acid in Emulsions: Effect of Substrate, Emulsifier, and Sugar Concentration

L. Ponginebbi, W.W. Nawar, and P. Chinachoti*

Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

ABSTRACT: Linoleic acid oxidation in oil-in-water emulsions stabilized by a nonionic surfactant (Tween-20) was studied. The emulsion composition was varied at a constant oil droplet size. Lipid oxidation was measured as a function of time in the presence of a catalyst ($FeSO_4$/ascorbic acid) by two methods: gas chromatographic determination of residual substrate and ultraviolet-visible spectrophotometric determination of conjugated dienes. Rate of oxidation was influenced by the emulsion composition (relative concentrations of substrate and emulsifier) and especially by the partition of the emulsifier between the interface and water phase. Concentrations of emulsifier exceeding the critical micelle concentration protected the fatty acid against oxidation. Excess surfactant formed micelles and mixed micelles with linoleic acid, which retarded oxidation by diluting the substrate or perhaps by replacing linoleic acid at the interface, making it less accessible to radical attack. The addition of sucrose also had a protective effect, but only up to a certain concentration, indicating the effect may involve factors other than viscosity.

Paper no. J8734 in *JAOCS 76*, 131–138 (January 1999).

KEY WORDS: Emulsion, linoleic acid, lipid oxidation, sucrose.

Lipid oxidation is one of the main deteriorative reactions that takes place during preparation and storage of many food products, and it can make them unacceptable for human consumption (1,2). Most current knowledge on the mechanisms of lipid oxidation was obtained from the study of bulk oils (3,4). The chemical composition of foods is complex. Lipid molecules of different properties and reactivities may coexist with water, proteins, carbohydrates, metals, vitamins, etc., all of which may affect oxidation. Yet, in food, great importance must be attributed to physical characteristics which can impact chemical reactions. It is clear that oxidation theories that apply to bulk oils may not be suitable for predicting reactions in more complex systems.

Because many foods are emulsified materials (e.g., milk, mayonnaise, coffee creamers, salad dressings, butter, baby foods), a better understanding of the mechanics of lipid oxidation in emulsions is crucial for the formulation, production, and storage of food products (5). For example, it was observed that in oil-in-water emulsions the more unsaturated fatty acids oxidized more slowly than the less unsaturated ones (6–8), whereas in bulk oils, the more unsaturated oils oxidized faster (9,10). The effectiveness of antioxidants at the interface relies heavily on their polarity (11–14). Oil-in-water emulsions consist of three different components: water (the dispersing phase), oil (the dispersed phase), and surface-active agent (the interface). The amount and composition of the oil phase in an emulsion are important factors that influence oxidative stability, formation of volatiles, and partition of the decomposition products between the oil and the water phase (5,15–17). Coupland *et al.* (15) proposed that the ratio of oxidizable to nonoxidizable compounds in emulsion droplets affects the rate at which lipid oxidation proceeds. They postulated that oxidation started at the interface of the oil droplets and that the concentration of the oxidizable substrate at the interface depended on its surface activity. Other factors influencing lipid oxidation in emulsions are particle size of the oil droplets (5) and packing properties of the surface-active molecules (12).

Soluble components in the water phase, such as sugars, could lead to changes that may influence lipid oxidation, including the effect on viscosity that decreases diffusion of reactants and reaction products (18–20). Carbohydrates can also bind metals and scavenge radicals, thus preventing oxidation (21–23), or they can accelerate the peroxidation process (24–26).

In the present study the effects of substrate, emulsifier, and sugar concentration on oxidation were investigated in a simple model emulsion system of linoleic acid under accelerated conditions. Free fatty acids are relatively minor components in food systems (12,27); therefore, the results of this study cannot be directly applied to all foods or to any specific food. However, results of simple model systems should contribute to a fundamental understanding of the oxidative behavior of lipids in simple model systems. More research is necessary to apply such information to more complex food systems.

MATERIALS AND METHODS

All reagents (analytical grade) were purchased from Sigma (St. Louis, MO), sucrose from Mallinckrodt Inc. (Paris, KY), and solvents (high-performance liquid chromatography grade

*To whom correspondence should be addressed
E-mail: pavinee@foodsci.umass.edu

hexane, isopropanol, and methanol) and phosphate salts from Fisher Scientific (Pittsburgh, PA). Polyoxyethylene sorbitan monolaurate (Tween-20) was obtained from Curtin Matheson Scientific, Inc. (Houston, TX). The aqueous phase of the emulsions consisted of phosphate buffer 0.05 M (pH 7.4). Stock solutions of $FeSO_4$ (0.028 μg/mL) and ascorbic acid (0.35 μg/mL) were freshly made for each experiment.

Emulsion preparation. Emulsions (100 mL) were prepared by mixing appropriate amounts of linoleic acid (LA) and Tween-20 with 50 mL of the phosphate buffer in a beaker with a magnetic stirrer (Mag-Mix, Precision Scientific Co., Chicago, IL) under nitrogen. The mixture was then emulsified by means of a blender (Waring commercial blender 700; Waring, New Hartford, CT) for 3 min. More mixing time was used as needed to reach the required oil droplet size. Since linoleic acid is very sensitive to heat and oxygen, a few droplets of liquid nitrogen were added in the blender cup, prior to blending, to saturate the environment with nitrogen gas and to lower the temperature. After emulsification, additional phosphate buffer, Tween-20, or sucrose solution was added to make up the total volume of 100 mL and to reach the desired final composition (as shown in Table 1). Mean diameter of the oil droplets was 0.15 ± 0.04 μm with unimodal distribution in the range of 0.1 to 1.0 μm (technique described below). In the experiment in which Tween-20 was varied and LA concentration remained constant, the average diameter changed within a range of 0.15–0.30 μm. No creaming or phase separation occurred throughout the experiments.

Oxidation experiment. A given volume of the freshly prepared catalyst was added to the prepared 100 mL emulsion to obtain a final concentration of 1 μm $FeSO_4$ and 20 μm ascorbic acid. The emulsions were incubated (37°C) in 250 mL conical flasks with shaking (100 rpm) in air. Aliquots of 0.5 mL were periodically taken for analysis. Each experiment was performed twice and the analyses duplicated. The results are reported as average ± standard deviation. Paired *t*-tests were performed to assess significant differences (95% confidence level) among treatments (28).

Emulsion characterization. Particle size distribution was assessed by Static Light Scattering (Horiba LA-700; Horiba Institute Inc., Irvine, CA) following the methods of Coupland *et al.* (15). The mean diameter was reported as the volume-surface diameter ($d_{3,2}$) according to the following formula:

$$d_{(3,2)} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2 \quad [1]$$

where n_i = number of droplets with diameter d_i.

The particle size was confirmed using transmission electron microscopy (TEM CM10; Phillips, Endhoven, The Netherlands) after staining the emulsions with 1% uranyl acetate solution for 30 s. Photographs were taken from randomly selected fields and used to calculate the average particle size. All measurements were averages of two duplicate emulsion preparations.

Viscosity. Kinematic viscosity of the sucrose solutions at 37°C was measured with a Cannon-Fenske type capillary viscometer (Fisher Scientific).

Linoleic acid extraction from emulsion. Internal standard (0.5 mL) (heptadecanoic acid ~1.5 mg/mL in hexane) was added to a 0.5 mL sample, followed by two drops of 6 N HCl to facilitate separation of the organic and aqueous phase.

Preliminary tests were done to investigate the stability of peroxides under the acidic conditions of the experiment. Oxidation was monitored in LA extracted from the emulsions with and without the use of 6 N HCl. No decomposition of hydroperoxides was observed in the emulsions where 6 N HCl was used. Therefore, the use of 6 N HCl should not have influenced the results.

For extraction, 10 mL of the extraction solvent (isopropanol/hexane, 1:2) was added and the samples thoroughly mixed and centrifuged for 15 min at 4.5×10^7 *g* (centrifuge Model 86B; United Electric Controls Co., Watertown, MA). The top layer, containing the fatty acid, was methylated and evaluated by gas chromatography (GC) or analyzed for conjugated dienes.

TABLE 1
Emulsion Composition in Four Different Experiments[a]

Experiments	LA/Tween-20 (% wt/vol:% wt/vol)	Sucrose (%w/w)
1A. Effect of LA concentration	1.00:0.100	0
(10:1 LA/Tween-20 ratio)	0.50:0.050	
	0.10:0.001	
	0.03:0.003	
1B. Effect of LA concentration	1.00:0.05	0
(0.05% wt/vol Tween-20)	0.50:0.05	
	0.10:0.05	
	0.03:0.05	
2. Effect of Tween-20 concentration	0.50:0.01	0
(0.50% wt/vol LA)	0.50:0.05	
	0.50:0.10	
3. Effect of sucrose concentration	0.50:0.05	0,14,22,31
(0.50% wt/vol LA, 0.05% wt/vol Tween-20)		

[a]The aqueous phase (with or without sucrose) contained water, 0.05 M phosphate buffer (pH 7.4), and catalysts $FeSO_4$ (1 μm) and ascorbic acid (20 μM). LA, linoleic acid; Tween-20, polyoxyethylene sorbitan monolaurate.

Residual substrate determination. Fatty acid (0.5–1 mg) was methylated with 2 mL of 14% BF_3 in methanol for 10 min at 80°C. At the end of the reaction, 0.5 mL hexane and 1–2 mL saturated NaCl/water solution were added and the sample was centrifuged (International Chemical Centrifuge, International Equipment Co., Boston, MA). The top hexane layer was analyzed by GC (Varian Model 3700 gas chromatograph; Varian Instrumental Division, Palo Alto, CA) with a Supelcowax™ fused-silica capillary column (30 m, 0.20 mm i.d., 0.2 μm film thickness, Supelco Inc., Bellefonte, PA) and an SP2470 Integrator (Spectra Physics, San Jose, CA). Helium gas head pressure was fixed at 50 mL/min and the injection port at 250°C in the splitless mode. The flame-ionization detector temperature was 300°C, and the oven temperature was programmed from 150 to 220°C at a rate of 3°C/min. LA methyl ester was quantified by comparing the area of each peak to that of the internal standard. The percentage residual substrate was plotted against time (see Fig. 7). All curves exhibited a sigmoidal pattern and were analyzed using a modified Fermi's equation (29) as follows:

$$y = \frac{1}{1 + \exp\ \frac{x - t}{a}} \qquad [2]$$

where x is time, y is the residual substrate, t is time at the inflection point, and a is a constant (in hours) representing the steepness of the decay of the substrate. A paired t-test was performed on the a and t parameters obtained (28).

Conjugated dienes. The method used is a modification of AOAC method 28.044a (30). The fatty acid, extracted as described above, was diluted with hexane to a concentration of 0.015 mg/mL. Approximately 3 mL of sample was analyzed for UV absorbance at 235 nm by a Lambda-3 UV/VIS Spectrophotometer (Perkin-Elmer Co., Oak Brook, IL) against a blank of hexane. A calibration curve was obtained by measuring the absorbance (y) of a series of solutions containing 0–0.03 mg conjugated LA/mL hexane (x) resulting in the linear regression equation

$$x = y/74.306\ (R^2 = 0.989) \qquad [3]$$

The results were expressed as mg conjugated LA/mg initial LA.

RESULTS AND DISCUSSION

Effect of LA concentration. In Experiment 1A, four emulsions containing different concentrations of LA but all having the same LA/Tween-20 ratio, i.e., 10:1, were prepared (Table 1). Since all samples were prepared from the same starting emulsion, they all had the same oil droplet size. This prevented any effect due to the change in exposed surface area. Residual LA and conjugated dienes were monitored at 8, 24, 48, and 72 h of incubation (Figs. 1 and 2, respectively). Although some

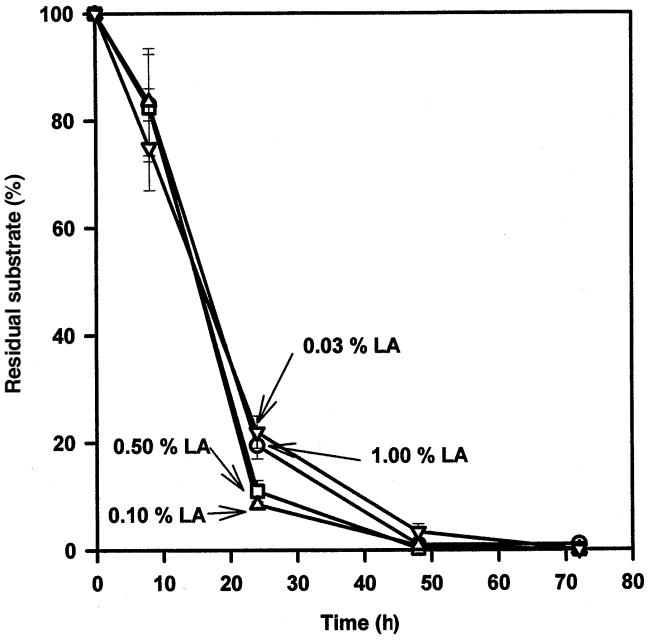

FIG. 1. Substrate disappearance over time for emulsions of increasing linoleic acid (LA) concentration. Ratio oil-to-emulsifier = 10:1. Storage at 37°C, in air, shaking at 100 rpm. Data points are the average of two experiments with duplicate measurements.

difference was observed at 24 h, differences in LA concentrations within a time period did not have a marked effect on oxidation rate (Fig. 1). Conjugated diene data (Fig. 2) suggested some effect of LA concentration. However, a significant difference ($P = 0.05$) was found only at 24 h where the level of conjugated dienes was in the following order: 0.03% > 0.1%

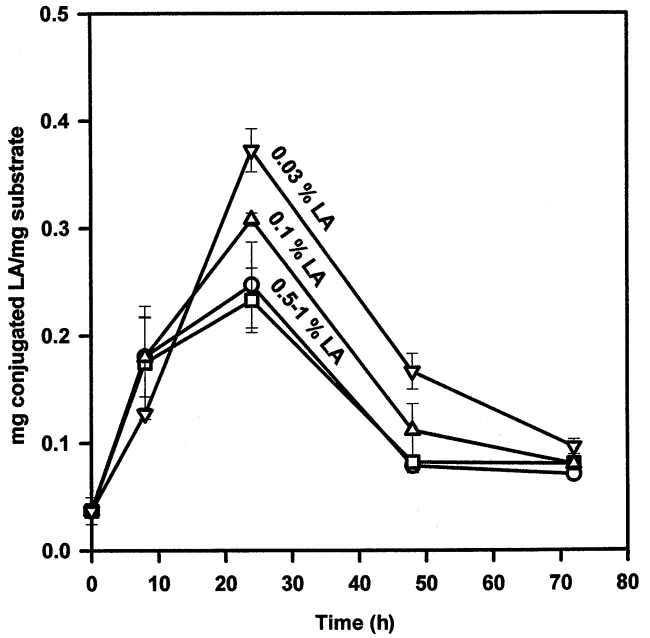

FIG. 2. Conjugated diene determination for emulsions of increasing LA concentration. Ratio oil-to-emulsifier = 10:1. Storage at 37°C, in air, shaking at 100 rpm. Data points are the average of two experiments with duplicate measurements. See Figure 1 for abbreviation.

> 0.5% = 1.0%. Statistical analysis did not show any significant difference at 48 h. The decrease in conjugated dienes with time indicates extensive oxidation, possibly due to the high temperature, presence of catalysts, and the large exposed surface. In the emulsions containing 1.0, 0.5, and 0.1% LA, Tween-20 exceeded its critical micelle concentration of 3.5×10^{-5} M (31). The critical micelle concentration is defined as the concentration at which the emulsifier starts to aggregate (32). The emulsified lipid system contained different phases, i.e., water, lipid, interface, and Tween-20 micelles. In addition, LA, which is itself surface active, could form micelles and mixed micelles with the emulsifier (12). Also, small amounts of LA could be present in solution. By assuming a monolayer coverage and an average particle size of 0.15 μm (12), it was possible to estimate the amounts of emulsifier in the aqueous phase and at the interface (Table 2). When the emulsion was diluted to 0.03% LA, the amount of emulsifier was below the critical micelle concentration. In this case, no micelles or mixed micelles were formed in the water phase. This increased the probability of direct oxidation of the substrate droplets. More discussion on this point is given in later sections.

In Experiment 1B, four emulsions containing different concentrations of LA but all having the same Tween-20 concentration, i.e., 0.05% (wt/vol), were prepared (Table 1, 1B). The emulsions were prepared by dilution with aqueous solutions of the emulsifier. Therefore, the oil droplet size was identical among the four samples. At 1% LA the substrate decreased at a significantly faster rate than at other concentrations (Fig. 3). At 48 h some oxidation had taken place in the emulsions containing 0.5, 0.1, and 0.03% LA, but no significant difference in oxidation was observed among the three concentrations. Only at 72 h did the substrate decrease more rapidly with increasing LA concentrations. Conjugated diene levels followed the same trend. The 1.0% LA emulsions showed a significantly more rapid increase in conjugated dienes than the 0.5, 0.1, and 0.03% LA emulsions (Fig. 4). After 48 h, conjugated dienes increased with increased percentage of LA up to 0.5% LA and then decreased.

Experiments 1A and 1B both involved a change in LA concentration; 1A kept a constant LA/Tween-20 ratio while 1B kept a constant Tween-20 concentration (LA/Tween ratio changed as LA concentration was varied). In the case of experiment 1A, oxidation increased with decreased LA concentration. In the case of 1B the opposite was observed. This difference may be related to the partition of Tween-20 between the oil-water interface and the aqueous phase. Table 2 (last two columns) presents the amount of Tween-20 in each of the fractions. Since a monolayer coverage was assumed, the amount of Tween-20 at the interface/unit weight should be constant at constant particle size. On the other hand, the amount of Tween-20 in water varied significantly. In the case of experiment 1A, estimated amounts of Tween-20 in water increased with increased LA concentration, but in the case of experiment 1B it decreased with increased LA concentration (Table 2). In both cases, oxidation was found to increase con-

TABLE 2
Tween-20 Molar Concentration, LA Particle Dimension, and Estimated Tween-20 Distribution Between Interface and Aqueous Phase for Samples Made in Experiments 1A, 1B, and 2[a]

Experiment	Composition	Initial Tween-20 concentration (mol/100 mL)	Particle size (μm)	No. of LA particles (/100 mL)	Tween-20 on the Total area (cm^2/100 mL)	Tween-20 on the surface (mol/100 mL)	Tween-20 in water (mol/100 mL)
1A. Effect of LA concentration (10:1 LA/Tween-20 ratio)	1.0% LA 0.1% Tween-20	8.2×10^{-4}	0.15	6.5×10^{14}	4.6×10^{5}	3.8×10^{-5}	4.4×10^{-5} [b]
	0.50% LA 0.05% Tween-20	4.1×10^{-4}	0.15	3.2×10^{14}	2.3×10^{5}	1.9×10^{-5}	2.2×10^{-5} [b]
	0.10% LA 0.01% Tween-20	8.2×10^{-5}	0.15	6.5×10^{13}	4.6×10^{4}	3.8×10^{-6}	4.4×10^{-6} [b]
	0.030% LA 0.003% Tween-20	2.5×10^{-5}	0.15	1.9×10^{13}	1.3×10^{4}	1.1×10^{-6}	1.4×10^{-6}
1B. Effect of LA concentration (0.05% wt/vol Tween-20)	1% LA 0.05% Tween-20	4.1×10^{-4}	0.15	6.5×10^{14}	4.6×10^{5}	3.8×10^{-5}	3.0×10^{-6}
	0.50% LA 0.05% Tween-20	4.1×10^{-4}	0.15	3.2×10^{14}	2.3×10^{5}	1.9×10^{-5}	2.2×10^{-5} [b]
	0.1% LA 0.05% Tween-20	4.1×10^{-4}	0.15	6.5×10^{13}	4.6×10^{4}	3.8×10^{-6}	3.7×10^{-5} [b]
	0.03% LA 0.05% Tween-20	4.1×10^{-4}	0.15	1.9×10^{13}	1.3×10^{4}	1.1×10^{-6}	4.0×10^{-5} [b]
2. Effect of Tween-20 concentration (0.50% wt/vol LA)	0.5% LA 0.01% Tween-20	8.2×10^{-5}	0.30	4.2×10^{13}	1.2×10^{5}	9.7×10^{-6}	7.3×10^{-5}
	0.5% LA 0.05% Tween-20	4.1×10^{-4}	0.20	1.4×10^{14}	1.8×10^{5}	1.5×10^{-5}	2.6×10^{-5}
	0.5% LA 0.1% Tween-20	8.2×10^{-4}	0.15	3.2×10^{14}	2.3×10^{5}	1.9×10^{-5}	6.3×10^{-5}

[a]See Table 1.
[b]Larger than the critical micelle concentration of 3.5×10^{-5} M (31). For abbreviations see Table 1.

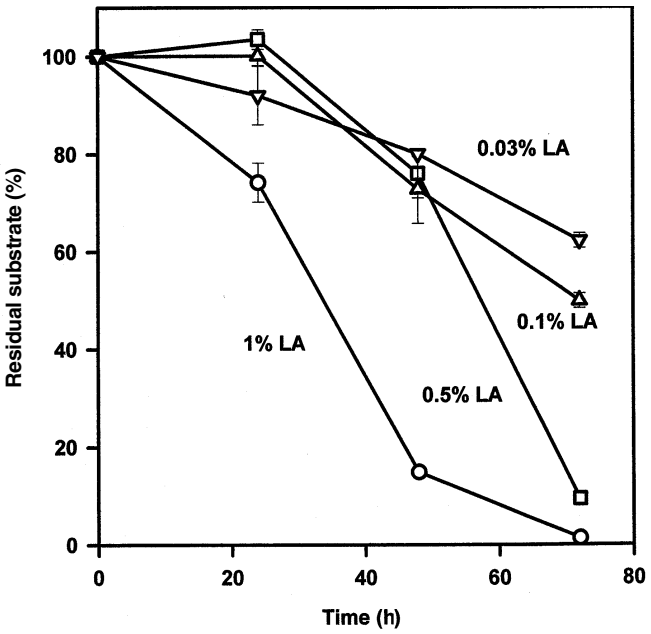

FIG. 3. Effect of LA concentration (% wt/vol) on LA oxidation as measured by substrate disappearance [gas chromatography (GC)] at 37°C. Constant Tween-20 concentration (0.05%, wt/vol), increasing LA concentration. Data points are the average of two experiments with duplicate measurements. For abbreviation see Figure 1.

sistently with decreasing concentration of Tween-20 in water. Thus, it could be understood that the presence of Tween-20 in the aqueous phase helped inhibit the oxidation of LA in the emulsion droplets. Tween-20 in the aqueous phase may have competed with LA for oxidation as it, too, was oxidizable and readily exposed to the catalysts in solution. Additionally, it is possible that some LA was incorporated into micelles of Tween-20 in the aqueous phase and, therefore, protected from oxidation. However, this interaction only occurred when the aqueous Tween-20 concentration exceeded the critical micelle concentration.

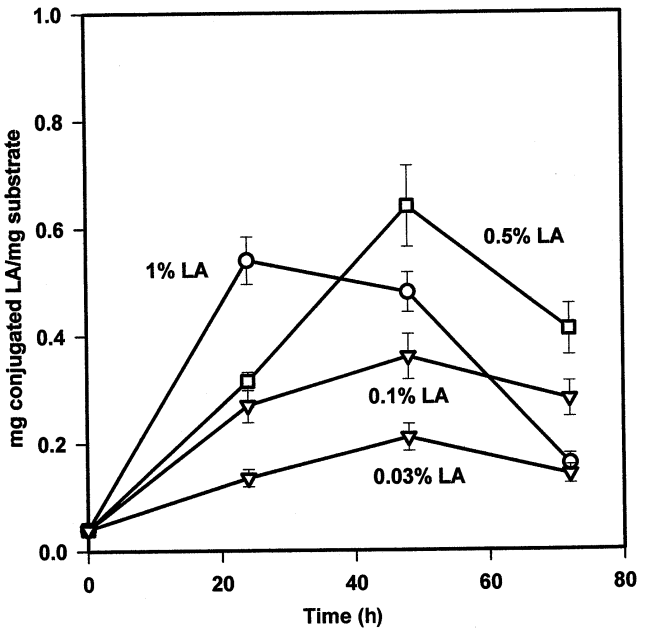

FIG. 4. Effect of LA concentration (% wt/vol) on LA oxidation based on conjugated diene measurement (absorbance at 235 nm) at 37°C. Constant Tween-20 concentration (0.05%, wt/vol), increasing LA concentration. Data points are the average of two experiments with duplicate measurements. For abbreviation see Figure 1.

Effect of emulsifier concentration. In this experiment, three emulsions containing different concentrations of Tween-20 but all having the same LA concentration, i.e., 0.5% wt/vol, were prepared (Tables 1 and 2). The ratio of LA to emulsifier was varied (50:1, 10:1, 5:1). The mean diameter of the oil droplets (normally distributed) decreased significantly with increasing amounts of emulsifier (from 0.30 ± 0.04 to 0.15 ± 0.03 μm). Such a decrease in oil droplet size, and consequent increase in area/volume ratio, was expected to accelerate the oxidation rate. Yet no differences were noticed (Fig. 5). As explained earlier, a higher concentration of Tween-20 in the water phase would be expected to exert protection by competing for oxidation. However, this effect may have been counteracted by the increase in surface area. Evidently, the excess Tween-20 in the surrounding medium had an additional protective effect.

The presence of Tween-20 in the water phase may also retard oxidation of LA by displacement of interfacial LA, providing a compact noncharged layer separating the negatively charged substrate and the positively charged iron in the water (33), and/or by formation of mixed micelles with linoleic acid.

Effect of sucrose concentration. In this experiment, four emulsions containing different concentrations of sucrose but the same LA and Tween-20 concentration, i.e., 0.5 and 0.05% wt/vol, respectively, were prepared (Tables 1 and 3). All samples with added sucrose significantly retarded oxidation, compared with the control (Figs. 6–7). However, no significant difference in oxidation rate was found among the three sucrose-containing samples. Statistical analysis of Fermi's parameters, *t* and *a*, was performed (Table 3). Both *t* and *a* were statistically lower in the control sample than in the samples containing 14, 22, and 31% sucrose, indicating faster oxidation in the control.

A number of researchers (18–20) hypothesized that sugar retarded oxidation by increasing viscosity. Such an increase may reduce mobility of the reactants and reaction products, prevent coalescence of the LA droplets, and retard creaming. This was not the case in this study. More inhibition occurred when the sucrose content increased from 0 to 14%. However, the corresponding increase in viscosity was small (Fig. 8). In addition, 22–31% sucrose resulted in a more significant viscosity increase than 14% sucrose, with no additional effect on oxidation. Therefore, viscosity may not be the major factor involved here. Other inhibitory mechanisms may be involved, e.g., quenching metals, scavenging radicals, and hydroperoxides (22,23,34–36). It is possible that at 14% sucrose

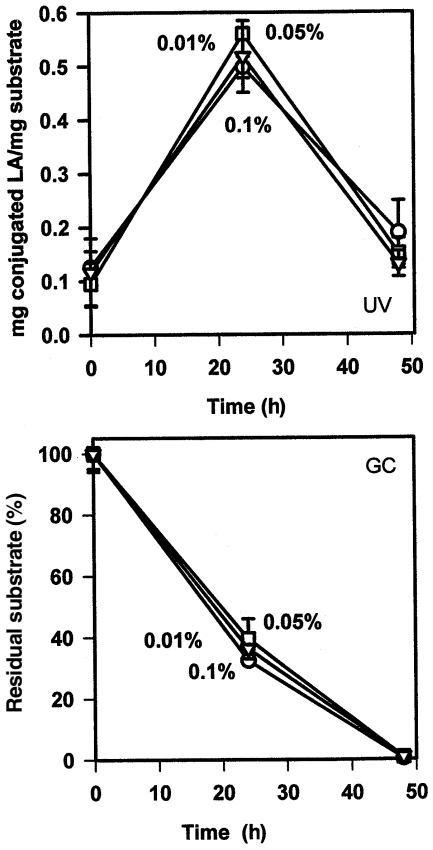

FIG. 5. Effect of Tween-20 concentration on LA oxidation (Table 1, Experiment 2) at 37°C as quantified by conjugated diene measurement at 235 nm [ultraviolet (UV)] and substrate disappearance (GC). Data points are the average of two experiments with duplicate measurements. See Figures 1 and 3 for abbreviations.

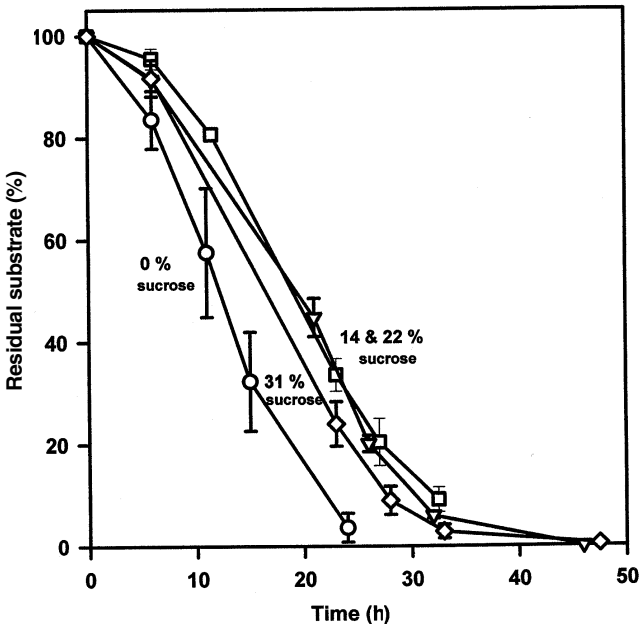

FIG. 6. Effect of sucrose concentration on LA oxidation measured by substrate disappearance (GC) at 37°C. LA, 0.5% wt/vol; Tween-20, 0.05% wt/vol. Data points are the average of three to four experiments with duplicate measurements. See Figures 1 and 3 for abbreviations.

the inhibitory effect other than viscosity had already reached the maximum level.

ACKNOWLEDGMENTS

Contribution of Project MAS 000709 of the Agriculture Experiment Station, University of Massachusetts, Amherst, is gratefully acknowledged. The authors thank J.N. Coupland, E. Decker, and D.J. McClements for their help and advice.

REFERENCES

1. Fritsch, C.W., Lipid Oxidation—The Other Dimension, *INFORM 5*:423–436 (1994).
2. Labuza, T.P., Kinetics of Lipid Oxidation in Foods, *CRC Crit. Rev. Food Technol. 2*:355–405 (1971).
3. Nawar, W.W., Lipids, in *Food Chemistry*, edited by O. Fennema, 3rd edn., Marcel Dekker, Inc., New York, 1996.
4. Frankel, E.N., Review. Recent Advances in Lipid Oxidation, *J. Sci. Food Agric. 54*:495–511 (1991).
5. Coupland, J.N., and D.J. McClements, Lipid Oxidation in Food Emulsions, *Trends Food Sci. Technol. 7*:83–91 (1996).
6. Miyashita, K., E. Nara, and T. Ota, Oxidative Stability of Polyunsaturated Fatty Acids in Aqueous Solution, *Biosci. Biotechnol. Biochem. 57*:1638–1640 (1993).
7. Bruna, E., E. Petit, M. Beljean-Leymarie, S. Huynh, and A. Nouvelot, Specific Susceptibility of Docosahexaenoic Acid and Eicosapentaenoic Acid to Peroxidation in Aqueous Solution, *Lipids 24*:970–975 (1989).
8. Miyashita, K., E. Nara, and T. Ota, Comparative Study on the Oxidative Stability of Phosphatidylcholines from Salmon Egg and Soybean in Aqueous Solution, *Biosci. Biotechnol. Biochem. 58*:1772–1775 (1994).
9. Cho, S., K. Miyashita, T. Miyazawa, K. Fujimoto, and T. Kaneda, Autoxidation of Ethyl Eicosapentaenoate and Docosahexaenoate, *J. Am. Oil Chem. Soc. 64*:876–879 (1987).

TABLE 3
Modified Fermi's Equation Parameters Obtained from the Average of 3–4 Repetitions[a]

Fermi's equation parameters	0% sucrose (wt/wt) $r^2 = 1.000$	14% sucrose (wt/wt) $r^2 = 1.000$	22% sucrose (wt/wt) $r^2 = 0.993$	31% sucrose (wt/wt) $r^2 = 0.980$
t (time at inflection, h)[b]	13.87 ± 2.55[c]	19.24 ± 0.80[d]	24.41 ± 3.58[d]	18.78 ± 1.85[d]
a (steepness, h)[b]	3.54 ± 0.19[c]	4.53 ± 0.88[d]	5.16 ± 0.44[d]	4.44 ± 0.15[d]

[a]See Reference 29.

[b] $y = \frac{1}{1 + \exp\frac{x - t}{a}}$, where *x* is time; *y*, residual substrate; *t*, time at the inflection point; *a*, constant (h) representing the steepness of the decay of the substrate.

[c,d]Superscripts represent results that are statistically significant ($P = 0.05$) according to a paired *t*-test.

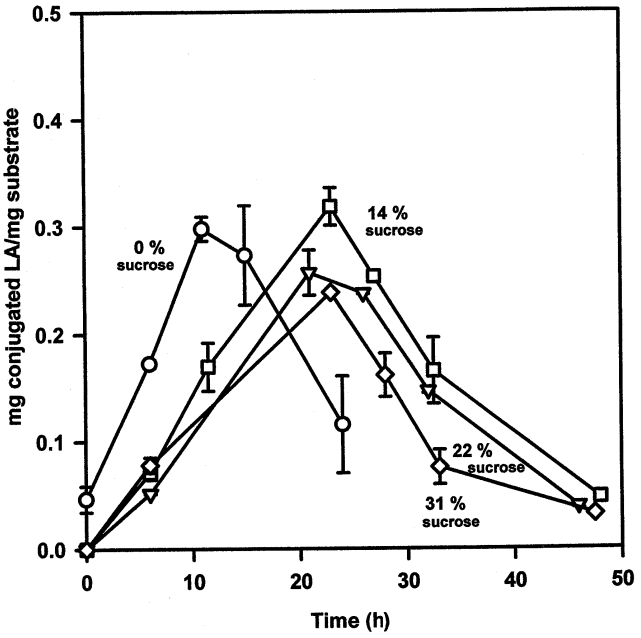

FIG. 7. Effect of sucrose concentration on LA oxidation measured by conjugated diene formation (UV absorbance at 234 nm) at 37°C. LA 0.5% wt/vol; Tween-20, 0.05% wt/vol. Data points are the average of three to four experiments with duplicate measurements. See Figures 1 and 5 for abbreviations.

10. Rosas Romero, A.J., and I.D. Morton, A Kinetic Study of the Competitive Oxidation of Oleic Acid–Linoleic Acid Mixtures, *J. Sci. Food Agric. 26*:1353–1356 (1975).
11. Frankel, E.N., S. Huang, J. Kanner, and B. German, Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs. Emulsions, *J. Agric. Food Chem. 42*:1054–1059 (1994).

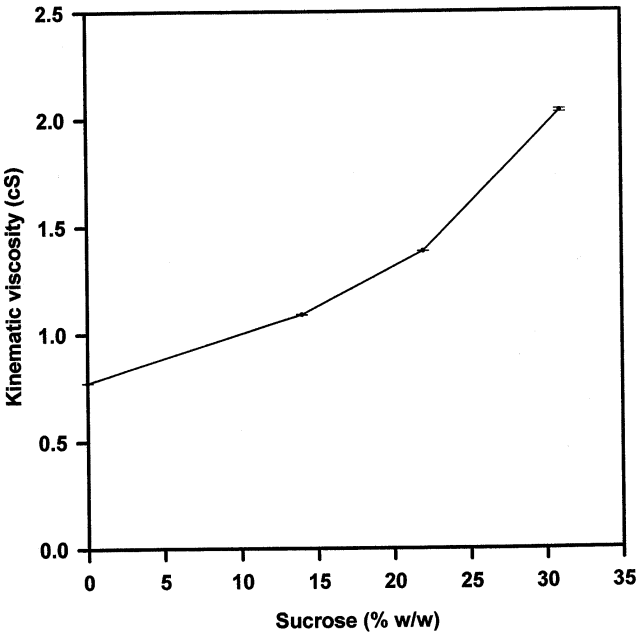

FIG. 8. Kinematic viscosity of sucrose solutions at 37°C measured by capillary viscometry. Data are averages of triplicate measurements (<5% error).

12. Huang, S., A. Hopia, K. Schwarz, E.N. Frankel, and J.B. German, Antioxidant Activity of α-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs. Oil-in-Water Emulsions, *Ibid. 44*:444–452 (1996).
13. Cillard, J., and P. Cillard, Behavior of α-, γ- and δ-Tocopherols with Linoleic Acid in Aqueous Media, *J. Am. Oil Chem. Soc. 57*:39–42 (1980).
14. Bisby, R.H., and S. Ahmed, Transverse Distribution of α-Tocopherol in Bilayer Membranes Studied by Fluorescence Quenching, *Free Radical Biol. Med. 6*:231–239 (1989).
15. Coupland, J.N., Z. Zhu, H. Wan, D.J. McClements, W.W. Nawar, and P. Chinachoti, Droplet Composition Affects Rate of Oxidation of Emulsified Ethyl Linoleate, *J. Am. Oil Chem. Soc. 73*:795–801 (1996).
16. Roozen, J.P., E.N. Frankel, and J.E. Kinsella, Enzymic and Autoxidation of Lipids in Low Fat Foods: Model of Linoleic Acid in Emulsified Hexadecane, *Food Chem. 50*:33–38 (1994).
17. Roozen, J.P., E.N. Frankel, and J.E. Kinsella, Enzymic and Autoxidation of Lipids in Low Fat Foods: Model of Linoleic Acid in Emulsified Triolein and Vegetable Oils, *Ibid. 50*:39–43 (1994).
18. Hsieh, Y.P., and N.D. Harris, Oxidation of Ascorbic Acid in Copper-Catalyzed Sucrose Solutions, *J. Food Sci. 52*: 1384–1386 (1987).
19. Sims, R.J., J.A. Fioriti, and J. Trumbetas, Effect of Sugars and Sugar Alcohols on Autoxidation of Safflower Oil in Emulsion, *J. Am. Oil Chem. Soc. 56*:742–745 (1979).
20. Joslyn, M.A., and H. Supplee, Solubility of Oxygen in Solutions of Various Sugars, *Ibid. 14*:209–215 (1949).
21. Sagone, A.L., J. Greenwald, E.H. Kraut, J. Bianchine, and D. Singh, Glucose: A Role as a Free Radical Scavenger in Biological Systems, *J. Lab. Clin. Med. 101*:97–103 (1983).
22. Yamauchi, R., Y. Aoki, T. Sugiura, K. Kato, and U. Yoshimitsu, Effect of Sugars and Sugar Analogs on Autoxidation of Methyl Linoleate and Safflower Oil, *Agric. Biol. Chem. 46*:2997–3002 (1982).
23. Yamaguchi, N., and A. Yamada, Studies on Antioxidative Activity of Brown Sugar, *Nippon Shokuhin Kogyo Gakkaishi 28*:303–308 (1981).
24. Yamauchi, R., Y. Tatsumi, M. Asano, K. Kato, and U. Yoshimitsu, Effect of Metal, Salts and Fructose on the Autoxidation of Methyl Linoleate in Emulsions, *Agric. Biol. Chem. 52*:849–840 (1988).
25. Mabrouk, A.F., Kinetics of Methyl Linoleate Emulsions Autoxidation in the Presence of Polyhydroxyl Compounds, *J. Am. Oil Chem. Soc. 41*:331–334 (1964).
26. Mabrouk, A.F., and L.R. Dugan, Kinetic Investigation into Glucose-Fructose and Sucrose Activated Autoxidation of Methyl Linoleate Emulsions, *Ibid. 38*:692–695 (1961).
27. Hopia, A.I., S. Huang, K. Schwarz, J.B. German, and E.N. Frankel, Effect of Different Lipid Systems on Antioxidant Activity of Rosemary Constituents Carnosol and Carnosic Acid With and Without α-Tocopherol, *J. Agric. Food Chem. 44*: 2030–2036 (1996).
28. Damon, R.A., and W.R. Harvey, One-Way Classification of Data, in *Experimental Design, ANOVA and Regression,* edited by R.A. Damon, Harper & Row, New York, 1987, pp. 12–37.
29. Peleg, M., Mapping the Stiffness–Temperature–Moisture Relationship of Solid Biomaterials at and Around Their Glass Transition, *Rheol. Acta 32*:575–580 (1993).
30. *Official Methods of Analysis of the AOAC*, Method 28.044a, edited by W. Horowitz, American Organization of Analytical Chemists, Washington, D.C., 1970, p. 452.
31. Courtaudon, J.L., E. Dickinson, and D.G. Dalgleish, Competitive Adsorption of β-Casein and Nonionic Surfactants in Oil-in-Water Emulsions, *J. Colloid Interface Sci. 145*:390–395 (1991).

32. Hiemenz, P.C., Colloidal Structures in Surfactant Solutions, *Principles of Colloid and Surface Chemistry*, edited by P.C. Hiemenz, 2nd edn., Marcel Dekker, New York, 1986, Chapter 8, pp. 427–481.
33. Yoshida, Y., and E. Niki, Oxidation of Methyl Linoleate in Aqueous Dispersion Induced by Copper and Iron, *Arch. Biochem. Biophys. 295*:107–114 (1992).
34. Asada, K., Oxygen Toxicity, *Seikagaku 48*:226–257 (1976).
35. Hendry, D.C., and G.A. Russel, Solvent Effects in the Reaction of Free Radicals and Atoms. Effect of Solvent Polarity on the Reactions of Peroxy Radicals, *J. Am. Chem. Soc. 86*: 2368–2371 (1964).
36. Joslyn, M.A., and L. Miller, Effect of Sugars on Oxidation of Ascorbic Acid. I. Kinetics of Auto-Oxidation of Ascorbic Acid, *Food Res. 14*:325–339 (1949).

[Received December 8, 1997; accepted October 28, 1998]