JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Impact of Surface-Active Compounds on Physicochemical and Oxidative Properties of Edible Oil

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The physical properties of lipids can have a major influence on lipid oxidation reactions. Edible oils contain surface-active compounds and water that can form physical structures such as reverse micelles. This study used the fluorescence probe, 5-dodecanoylaminofluorescein (DAF), to study both the physical and the chemical properties of stripped corn oil containing oleic acid and phosphatidylcholine. The fluorescence intensity of DAF increased with increasing water concentration in the edible oil. The addition of oleic acid decreased DAF fluorescence due to the ability of the free fatty acid to decrease the pH of the aqueous phase of the bulk oil. Phosphatidylcholine increased DAF fluorescence due to its ability to increase DAF exposure to the aqueous phase. Oleic acid had no impact on interactions between DAF and water-soluble peroxyl radicals, while phosphatidylcholine decreased peroxyl radical degradation of DAF. These results suggest that DAF could be a useful analytical tool to study the impact of the aqueous environment of bulk oil on lipid oxidation.

KEYWORDS: Interfacial tension; stripped corn oil; phosphatidylcholine; oleic acid; lipid oxidation; reverse micelles; association colloids; 5-dodecanoylaminofluorescein

INTRODUCTION

Bulk oils contain numerous minor components that are surface-active, such as mono- and diacylglycerols, phospholipids, sterols, free fatty acids, and polar products arising from lipid oxidation, such as lipid hydroperoxides, aldehydes, ketones, and epoxides (*1*). The existence of surface-active compounds in commercial bulk oil can be observed by stripping [e.g., with alumina (Al_2O_3)] oils of their minor components, a process that results in an increase in interfacial tension as compared to the original refined oil (*1*). This increase in interfacial tension in the stripped oil is the result of the removal of surface-active molecules from the refined oil that can migrate to and concentrate at a water–oil interface. The nature and amounts of these minor components in commercial oils are important because they alter the susceptibility of oils to lipid oxidation (*2*). The ability of minor components to impact oxidative reactions is thought to originate from both their chemical reactivity (e.g., antioxidants) and physical properties (surface activity).

Surface-active molecules are soluble in both water and oil (*3*). In a system containing both water and oil, the hydrocarbon part of the surface-active molecule is responsible for its solubility in oil, while the polar headgroup has sufficient affinity for water to solubilize the nonpolar hydrocarbon chain in the aqueous phase. In a system with an air–water or oil–water interface, these molecules migrate to and concentrate at interfaces. Their hydrophilic head groups preferentially interact with the aqueous phase while the lipophilic hydrocarbon chains interact with the air or oil phase (**Figure 1A**,**B**). This situation is energetically more favorable than solubilization in either phase (*3*). In the case of surface-active molecules in food oils, air is less polar than oil (e.g., the dielectric constant of air is 1.0 as compared to approximately 3.0 for food oils; *4*), and thus, there would not be a driving force for surface-active molecules to migrate to the air−oil interface (*1*). However, commercial oils contain small amounts of water, which would provide an oil–water interface that would allow the surface-active components of the oil to self-aggregate into structures such as reverse micelles, which are energetically more favorable than solubilized surfactant monomers in the oil or water (**Figure 1C**).

The diverse combination of surface-active compounds found in commercial oils can result in the formation of a wide variety of physical structures known as association colloids. Association colloids such as reverse micelles and lamellar bilayers can increase chemical reactions by creating surfaces that increase interactions between lipid- and water-soluble compounds. This is especially true for lipid oxidation since one of the major reaction substrates is lipid hydroperoxides, which are surface-active (*5*). Surface-active lipid hydroperoxides could therefore accumulate at oil–water interfaces in commercial oils where they could be decomposed by transition metals into free radicals that further promote lipid oxidation. Metal-promoted lipid hydroperoxide decomposition is also responsible for the decomposition of fatty acids into the small molecular weight volatile compounds that are responsible for rancidity.

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10.1021/jf072071o CCC: $40.75
Published on Web 12/29/2007

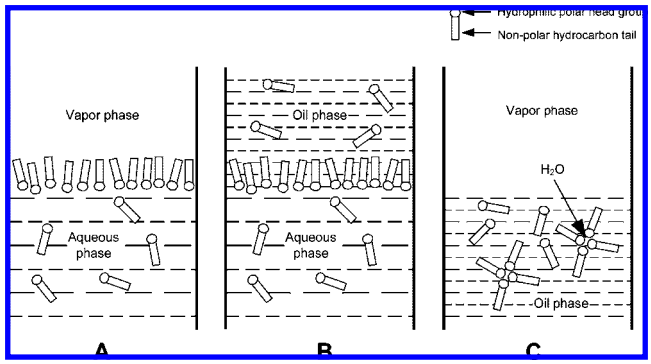

Figure 1. Adsorption of surface-active molecules at (**A**) air–water, (**B**) oil–water (modified from Shaw; *3*), and (**C**) air−oil interfaces.

Many publications in the past few decades have revealed the importance of physical phenomena that govern the structure formation in food systems on the chemistry of lipid oxidation, especially in oil-in-water emulsions (*6–21*). In oil-in-water emulsions, lipid oxidation chemistry is very dependent on the physical properties of the water–lipid interface of oil-in-water emulsions droplets since the emulsion droplet interface can influence the ability of aqueous phase transition metals to interact with lipid hydroperoxides in the emulsion droplet. For instance, positively charged and thick emulsion interfaces can decrease iron and lipid hydroperoxide interactions, thus inhibiting lipid oxidation (*12, 13*). Another example of how physical properties influence lipid oxidation is the "antioxidant polar paradox" (*14–21*). While there are many factors involved in the effectiveness of antioxidants in complex heterogeneous systems (*22*), the physical location of the antioxidants is extremely important since an antioxidant must be near the site of oxidative reactions for it to be effective. The antioxidant polar paradox states that nonpolar antioxidants are effective in oil-in-water emulsions because they are retained in the emulsion droplet while polar antioxidant are effective in bulk oils because they accumulate at oil−air (*22*) or oil−water interfaces (*1*).

Phospholipids are capable of inhibiting lipid oxidation in bulk oils (*23*). The ability of phospholipids to decrease lipid oxidation rates has been postulated to be due to metal-chelating and free radical-scavenging (via nitrogen groups) activity. In addition, phospholipids inhibit lipid oxidation in bulk oils in a synergistic manner in the presence of tocopherols (*24–34*). Koga and Terao (*33, 34*) published the to date most detailed studies in this area and suggested that phospholipids enhance the radical-scavenging activity of tocopherols via a physical mechanism that increases the accessibility of tocopherols toward chain-initiating radicals in an aqueous microenvironment where lipid oxidation reactions are prevalent. Free fatty acids are known to promote lipid oxidation in bulk oils. The prooxidant activity of free fatty acids has been postulated to be due to their ability to form complexes with transition metals and increase metal solubility (*35*) and to promote the acid-catalyzed decomposition of lipid hydroperoxides (*36*). Free fatty acids are surface-active with a low HLB number of ≈1.0 (*37*), suggesting that they could form reverse micelles in bulk oils.

The development of highly specialized probes has made fluorescence spectroscopy an extremely powerful tool to study the biochemistry and biophysics of food systems. These fluorescence probes are easily detected at low concentrations (*38*), and certain probes respond to changes in environment, pH, and oxidative stress (*38–45*). Among the fluorescence probes, 5-dodecanoylaminofluorescein (DAF) has the potential to be used as a surface-active compound that can be used to study the physical and chemical properties of food systems. DAF has a fluorescent polar headgroup attached to a hydrocarbon chain (**Figure 2**). The amphiphilic nature of DAF suggests that it would concentrate at the oil–water interface of association colloids where it can be used to measure physical and chemical properties. In addition, the polar headgroup contains fluorescein that can be used to detect the presence of free radicals (*46, 47*), thus making it a useful tool for evaluating oxidative reactions in association colloids in bulk oils.

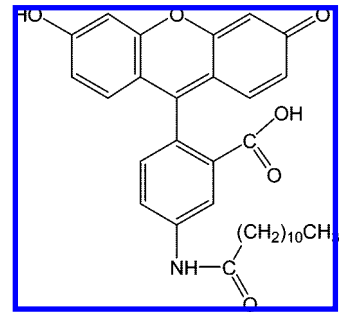

Figure 2. Chemical structure of DAF.

The objective of this study was to utilize the unique properties of DAF to study how surface-active compounds (free fatty acids and phospholipids) influence the chemical and physical properties of the microenvironments of bulk oil in relation to lipid oxidation chemistry.

MATERIALS AND METHODS

Materials. Commercially available corn oil (Mazola) was obtained from a local retailer. DAF was purchased from Molecular Probes (Eugene, OR) and was used without any further purification. 2,2′-Azobis(2,4-dimethylvaleronitrile), AMVN, was purchased from Cayman Chemical (Ann Arbor, MI). Alumina (Al_2O_3, type WN-3: neutral), 2,2′-azobis(2-amidinopropyl)dihydrochloride (AAPH), hexadecane, oleic acid, and phosphatidylcholine dioleoyl were purchased from Sigma-Aldrich Co. (St. Louis, MO). All other reagents were analytical grade or purer and were obtained from Sigma-Aldrich Co. or Fisher Scientific (Pittsburgh, PA).

Methods. *Isolation of Corn Oil Triacylglycerols.* Commercial corn oil was stripped of its minor polar components using column chromatography with alumina [Al_2O_3; modified from Wang et al. (*48*)]. Corn oil (150 g) was mixed with hexane and dry alumina (dried at 250 °C for 2 h prior to use) at a ratio of 1:1:1 by weight. The mixture was stirred for 30 min at room temperature and then poured through a column (2.5 cm i.d. and 60 cm long) containing 150 g of dry alumina, and isolated triacylglycerols were eluted with 200 mL of hexane. Sample was collected, and the hexane was evaporated with a rotary evaporator at 45 °C under vacuum. Corn oil was stripped in several batches, and each lot was mixed together to ensure homogeneity. Stripped oil was placed in 50 mL glass vials, flushed with nitrogen, sealed with Teflon PTFE covers, and stored at −80 °C until use. The water content of the stripped corn oil was determined by a Karl Fischer Coulometer (*49*), tocopherols and tocotrienols were determined by high-performance liquid chromatography (*50*), and lipid hydroperoxides were determined by the thiocyanate method (*51*).

Measurement of Interfacial Tension. The ability of the surface-active fluorescence probe, DAF, to accumulate at a lipid−water interface was evaluated by interfacial tensiometry using a drop shape analyzer (DSA10, Kruss USA, Charlotte, NC) (*52, 53*). DAF was dissolved in stripped corn oil at 0–200 μM. The mixture was loaded in a syringe and ejected to form a droplet at the inverted tip of a hypodermic needle that was submerged in double-distilled water. The tip of the needle was positioned on an optical bench between a light source and a high-speed charge couple device (CCD) camera. The CCD camera was connected to a video frame-grabber board to record the image onto the hard drive of a computer at a speed of one frame per second. The shape of pendant drops was determined through numerical analysis of

the entire drop shape. The interfacial tension was calculated from the drop shape using the Young−Laplace equation of capillarity (*54*). The methodology requires accurate determination of solution densities, which were measured using a digital density meter (DMA 35N, Anton Paar USA, Ashland, VA). All interfacial tension measurements were carried out in triplicate at room temperature.

Fluorescence Probe Partitioning Behavior. The DAF partitioning behavior was determined in a hexadecane−water system. Hexadecane was used as a nonoxidizable solvent to minimize DAF oxidation. DAF was added to hexadecane using a methanol carrier. The methanol was then evaporated from the hexadecane with nitrogen at room temperature. DAF in hexadecane (2 mL) at different concentrations (0–30 μM) was mixed with 25 mM phosphate buffer pH 7.0 (2 mL) in a test tube (Fisherbrand 13 mm × 100 mm) and allowed to equilibrate at room temperature (22–23 °C) in the dark for 4 days. Preliminary experiments showed that the partitioning of DAF into the aqueous phase increased during the first 4 days and then became constant; therefore, partitioning data were collected after 4 days. DAF concentrations in the aqueous phase after equilibration were determined using a PTI spectrofluorometer (Photon Technology International, Birmingham, NJ), and the data were acquired by Felix32 software. The excitation wavelength was set to 494 nm, and the fluorescence emission was recorded at 515 nm. DAF concentrations in the aqueous phase were determined from a standard curve of DAF in 25 mM phosphate buffer at pH 7.0.

Impact of pH on Fluorescence of DAF. The influence of pH on DAF fluorescence was determined using a PTI spectrofluorometer (Photon Technology International), and the data were acquired by Felix32 software. DAF (0.5 μM) was dissolved in 25 mM phosphate buffer over a pH range of 4.6–11.7. The excitation wavelength was 494 nm, and the emission was recorded over the range of 500–650 nm.

Influence of Surface-Active Compounds and Water on Fluorescence of DAF in Bulk Oil. DAF (20 μM) was added to stripped corn oil using a methanol carrier with methanol being removed by evaporation under nitrogen at room temperature. Stock solutions of the remaining lipids (oleic acid or phosphatidylcholine dioleoyl) were dissolved in stripped corn oil and then diluted into the DAF/stripped corn oil solution to produce the desired final concentrations. Phosphate buffer (25 mM) was added into the oil by vortexing for 30 s at speed 5 (Fisher Vortex Genie2, Fisher Scientific) and sonication (Ultrasonic Cleaner Bath model 250, E/MC, Division of RAI Research, Long Island, New York) for 2 min. Samples were then stirred at room temperature for 30 min prior to the measurements of steady-state fluorescence spectra (*43*). The spectra were scanned by the PTI spectrofluorometer (Photon Technology International). The excitation wavelength was 494 nm (as determined by absorbance maximum of DAF), and the emission wavelength was recorded in the range of 500–650 nm.

Influence of Surface-Active Compounds on Peroxyl Radical-Scavenging by DAF in Bulk Oil. DAF (20 μM), oleic acid (1 mmol/kg lipid), and/or phosphatidylcholine dioleoyl (1 mmol/kg lipid) were added to stripped corn oil as described above. In experiments with the water-soluble radical initiator, 10 μL of 400 mM AAPH in 25 mM phosphate buffer (pH 7.0) was added to 1 mL of stripped corn oil in a test tube (Fisherbrand 13 mm × 100 mm) and then dispersed into the solution by vortexing for 30 s at speed 5 (Fisher Vortex Genie2, Fisher Scientific) and sonication (Ultrasonic Cleaner Bath model 250, E/MC, Division of RAI Research) for 2 min. In a sample with the lipid-soluble radical initiator, 10 μL of 400 mM AMVN in ethanol was placed in a test tube (Fisherbrand 13 mm × 100 mm). The ethanol was evaporated with nitrogen at room temperature, and then, 1 mL of stripped corn oil was added. Phosphate buffer (10 μL of 25 mM, pH 7.0) was added into the lipid mixture and dispersed as described above. Decomposition of both AAPH and AMVN into peroxyl radicals was highly temperature-dependent. Therefore, all samples and solutions used in these experiments were kept at <4 °C during sample preparation. To initiate peroxyl radical generation, samples were placed in a 65 °C water bath, and the fluorescence of DAF was determined periodically using the PTI spectrofluorometer. The excitation wavelength was 494 nm, and the fluorescence emission at 515 nm was recorded for 1 h.

Statistical Analysis. All experiments were performed in duplicate or triplicate and repeated twice. Data are expressed as mean ± standard deviations (SDs). Statistical analysis was performed using one-way analysis of variance. Mean separations were determined using Duncan's multiple range tests with a level of significance of $p < 0.05$ (*55*).

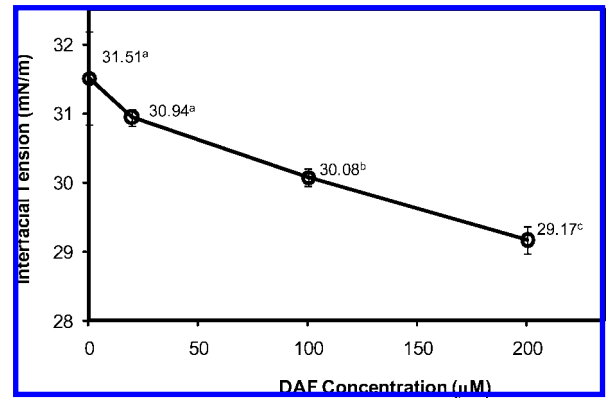

Figure 3. Influence of DAF on interfacial tension of stripped corn oil after 20 min of equilibration at room temperature. Data points represent means ($n = 3$) ± SDs.

RESULTS AND DISCUSSION

To study the effects of minor surface-active components on the chemical and physical properties of oil, corn oil was stripped of surface-active compounds using alumina. Tocopherols were used to evaluate the effectiveness of the stripping process. The original corn oil contained 241 ppm α-tocopherol, 10 ppm α-tocotrienol, and 546 ppm γ-tocopherol. The other tocopherol and tocotrienol homologues were not detected by this method, which had a detection limit of 1 ppm. After stripping, all tocopherol and tocotrienol homologues were lower than the detection limit. The removal of surface-active compounds from commercial corn oil was confirmed by interfacial tension measurements, which showed that the stripped corn oil (31.51 ± 0.68 mN/m) had a higher interfacial tension (less surface-active agents that can decrease the interfacial tension) than the original refined corn oil (20.13 ± 0.09 mN/m). While the stripping process was effective at removing surface-active compounds, it increased the formation of lipid oxidation products. The original corn oil contained 0.47 ± 0.01 mmol lipid hydroperoxide/kg oil as compared to 0.98 ± 0.02 mmol/kg for stripped corn oil. This increase in lipid hydroperoxides is likely due to the increased oxidative stress caused by the stripping process and the removal of endogenous antioxidants such as the tocopherols. Both the original corn oil and the stripped corn oil had no significant difference in water content, which was in the range of 230–240 ppm.

Physical Location of the Surface-Active Fluorescence Probe in Stripped Corn Oil. The fluorescence probe, DAF, has a structure similar to other surface-active compounds in that it contains a polar headgroup and a hydrocarbon chain (**Figure 2**). To determine if DAF was surface-active, it was added to the stripped corn oil, and the interfacial tension was measured at the water–oil interface using a drop shape analyzer. All measurements showed that the interfacial tension decreased during the first 20 min and then reached a plateau. Therefore, all surface tension values were reported at 20 min. DAF at 0−200 μM significantly decreased the interfacial tension ($p < 0.05$) from 31.51 to 29.17 mN/m, indicating that DAF was accumulating at the water–oil interface (**Figure 3**).

Interfacial tension data indicate that DAF can accumulate at an oil–water interface. However, DAF would also be expected to partition into water. To determine the partitioning behavior of DAF, it was added to hexadecane (0–30 μM) with the hexadecane being layered on top of the water. The system was

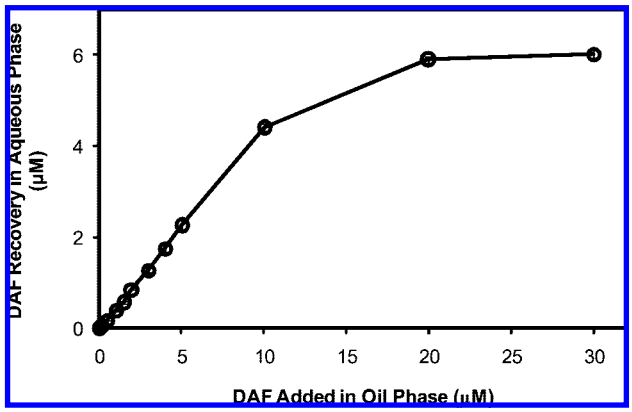

Figure 4. Ability of DAF to partition into the aqueous phase of the hexadecane−water bilayer at room temperature after 4 days of equilibration. Data points represent means ($n = 2$) ± SDs (some error bars may lie within the data points).

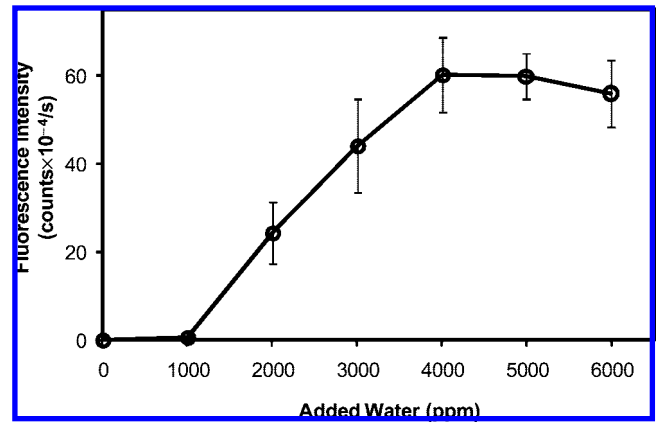

Figure 5. Influence of the addition of water (as 25 mM phosphate buffer, pH 7.0) on the fluorescence intensity of 20 μM DAF in stripped corn oil (λ_{EX} 494 and λ_{EM} 515 nm). Data points represent means ($n = 3$) ± SDs.

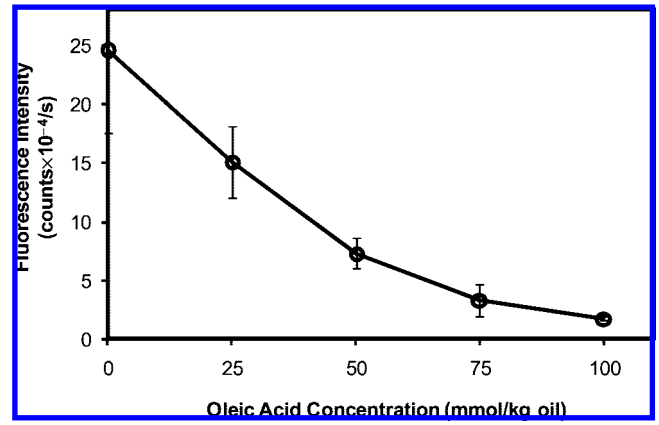

Figure 6. Influence of oleic acid on fluorescence intensity (λ_{EX} 494 and λ_{EM} 515 nm) of 20 μM DAF in stripped corn oil containing 2000 ppm added water (as 25 mM phosphate buffer, pH 7.0). Data points represent means ($n = 3$) ± SDs.

then incubated at room temperature (22–23 °C) in the dark for 4 days until equilibration was reached (as determined by the preliminary experiments). Because these experiments take several days, hexadecane instead of stripped corn oil was used since the hexadecane does not oxidize, a process that would cause the degradation of DAF. DAF concentrations in the aqueous phase increased linearly when 0.2−10 μM DAF was added to the hexadecane (**Figure 4**). Over this concentration range, 33–45% of the DAF partitioned into the aqueous phase. Aqueous phase concentrations reached a maximum when DAF concentrations in hexadecane were ≥20 μM. These results indicate that DAF will partition into the aqueous phase.

Behavior of DAF in Stripped Oil with Added Water. DAF has an absorbance maximum at 494 nm in 25 mM phosphate buffer at pH 7.0. Upon excitation at 494 nm, DAF in phosphate buffer shows a maximum emission at 515 nm (data not shown). DAF exhibits weak fluorescence in lipids as compared to water. At a DAF concentration of 0.5 μM, no detectable fluorescence was observed in stripped corn oil with no added water. When DAF concentrations were increased to >20 μM in stripped corn oil, fluorescence was detected at 1533 counts/s as compared to 281555 counts/s in phosphate buffer at pH 7.0 containing 0.5 μM DAF. The addition of water (1000–6000 ppm, as 25 mM phosphate buffer, pH 7.0) to stripped corn oil containing 0.5 μM DAF resulted in an increase in fluorescence intensity (**Figure 5**). This increase in fluorescence is likely due to the partitioning of DAF into the aqueous phase as well as localization of the fluorescent polar fluorescein headgroup in the water phase of the oil–water system. In addition to the increased fluorescence intensity of DAF upon addition of phosphate buffer to the stripped corn oil, its emission spectra gradually shifted from 515 to 518 nm. Such red shifts or Stokes' shift in fluorescence probes are often due to a change in solvent polarity (*38, 39, 41–45*) as would be expected if the probe was partitioning at the lipid−oil interface. The increased red shift with an increase in water concentrations could be due to the increase in the lipid−water interfacial area, which would allow more DAF to reside at the lipid−water interface, thus increasing the wavelength of maximum emission.

Impact of Oleic Acid on DAF Fluorescence in Bulk Oil. Free fatty acids are endogenous to commercially available edible oils at concentrations ranging from 1.0 to 140 mmol free fatty acid/kg oil (*1*). Free fatty acids are known to promote the oxidation of bulk oils (*23*). The surface activity of free fatty acids (*5*) could alter the physiochemical properties of association colloids in bulk oils. Oleic acid was chosen as a model free fatty acid to study the impact of free fatty acids on fluorescent properties of DAF because it is liquid at room temperature as compared to long chain saturated fatty acids and because it is more oxidatively stable than polyunsaturated fatty acids (e.g., methyl linolenate) and thus would have less impact on lipid oxidation. The addition of oleic acid (0–100 mmol/kg oil) to the stripped corn oil containing 20 μM DAF and 2000 ppm added water significantly decreased ($p < 0.05$) the fluorescence emission intensity of DAF (**Figure 6**). This loss of fluorescence upon addition of oleic acid could be due to the ability of oleic acid to change the physical location of DAF (e.g., decreasing DAF concentration in the aqueous phase or the oil–water interface). However, oleic acid could also be influencing DAF fluorescence by altering the pH of the aqueous phase. The fluorescence intensity of many fluorescence probes is dependent on pH (*38–40*). To determine if pH influenced fluorescence, DAF (0.5 μM) was dissolved in 25 mM phosphate buffer over the pH range of 4.6–11.7 (**Figure 7**). Increasing the pH from 4.6 to 5.0 did not significantly increase ($p > 0.05$) the fluorescence intensity of DAF, which was in the range of 1536–4088 counts/s. Further increasing the pH significantly increased ($p < 0.05$) the fluorescence intensity until a maximum was reached at pH 10.0–11.0. To determine if oleic could decrease the pH of the phosphate buffer added to the stripped corn oil, an increase in the concentration of oleic acid was added to the phosphate buffer at the same buffer:oleic acid ratio used in

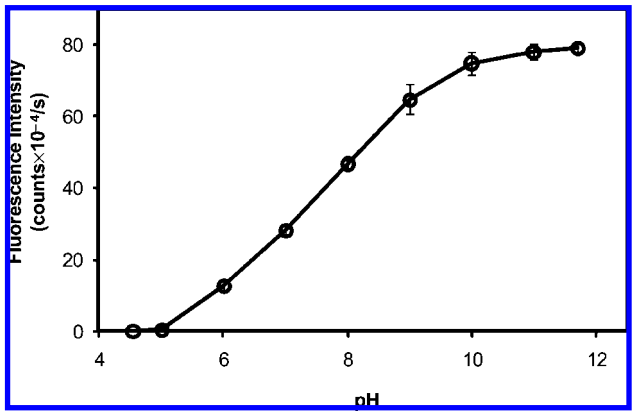

Figure 7. Influence of pH on fluorescence intensity (λ_{EX} 494 and λ_{EM} 515 nm) of 0.5 μM DAF in 25 mM phosphate buffer. Data points represent means ($n = 3$) ± SDs.

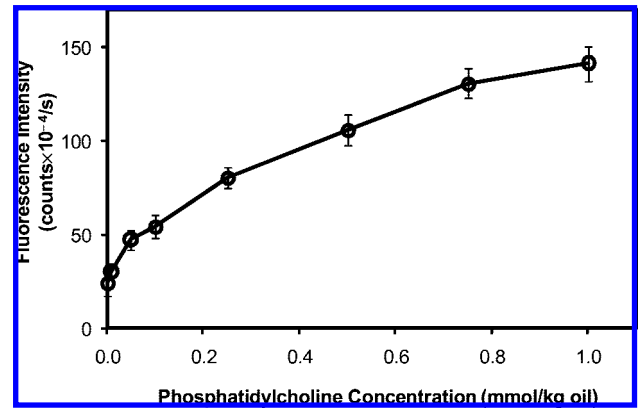

Figure 8. Influence of phosphatidylcholine dioleyl on fluorescence intensity (λ_{EX} 494 and λ_{EM} 515 nm) of 20 μM DAF in stripped corn oil containing 2000 ppm added water (as 25 mM phosphate buffer, pH 7.0). Data points represent means ($n = 3$) ± SDs.

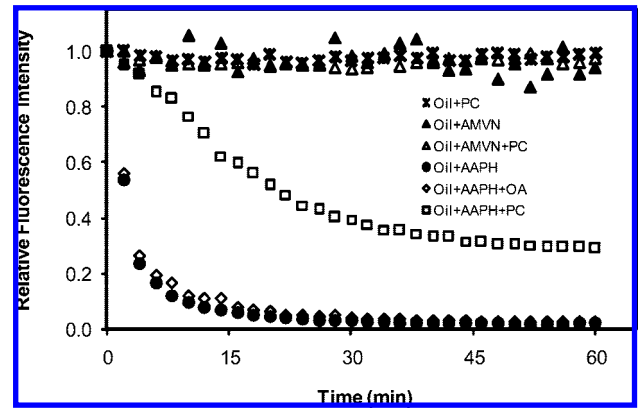

Figure 9. Influence of oleic acid and phosphatidylcholine dioleyl (1 mmol/kg lipid) on the degradation of 20 μM DAF as determined by relative fluorescence intensity (λ_{EX} 494 and λ_{EM} 515 nm) in stripped corn oil containing 1% 25 mM phosphate buffer (pH 7.0) in the presence of peroxyl radicals generated by 4 mM AMVN or AAPH at 65 °C for 1 h. The fluorescence intensity is given relative to zero time values. Data points represent means ($n = 3$) ± SDs.

the bulk oil experiments. Upon addition of oleic acid, the pH of the buffer decreased (data not shown), suggesting that the loss of DAF fluorescence in the stripped corn oil was due to a decrease in the pH of the aqueous phase.

These data suggest that in bulk oils containing water, oleic acid accumulates at the oil–water interface or solubilizes in the aqueous phase where it can alter pH. Because oleic acid is virtually insoluble in water, it is more likely that it is accumulating at the oil–water interface. This could help explain why free fatty acids are often found to act as a prooxidant in bulk oils since both oleic acid and lipid hydroperoxides would accumulate at the oil–water interface with oleic acid promoting acid-catalyzed hydroperoxide decomposition (*36*) and attracting prooxidant metals that decompose hydroperoxides (*9*).

Impact of Phosphatidylcholine Dioleoyl on DAF Fluorescence in Bulk Oil. Phospholipids are found in refined edible oils at concentrations ≤0.03 mmol phosphorus/kg oil (*1*). The ability of phospholipid to inhibit lipid oxidation in bulk oils has been attributed to their ability to scavenge free radicals and alter the microenvironment of oil–water interfaces (*23, 33, 34*). Phosphatidylcholine dioleoyl was chosen as a model phospholipid because phosphatidylcholines are common in food oils and the presence of the oleic acid provides a low melting point with good oxidative stability. The addition of phosphatidylcholine dioleoyl to the model stripped corn oil system containing 20 μM DAF and 2000 ppm phosphate buffer (pH 7.0) over the range of 0–1 mmol/kg oil significantly increased ($p < 0.05$) the fluorescence emission intensity (**Figure 8**). The addition of phosphatidylcholine dioleoyl (0–1 mmol/kg oil) to the bulk oil also shifted the emission spectra peak from 518 to 522 nm (data not shown). As with oleic acid, phosphatidylcholine could increase the DAF fluorescence intensity and emission spectra by altering the physical location of DAF (*38, 41, 42, 44*) or by changing the pH. However, unlike oleic acid, phosphatidylcholine did not change the pH when added to phosphate buffer at the same buffer:phosphatidylcholine ratio used in the bulk oil experiments (data not shown). Therefore, the ability of phosphatidylcholine to increase the fluorescence intensity and cause a red shift in the emission spectra suggests that the phosphatidylcholine is promoting an increase in the concentration and/or altering the physical location of DAF at the oil–water interface. Phosphatidylcholine has been reported to increase the accessibility of tocopherol at the oil–water interface in bulk oil (*33, 34*).

Impact of Surface-Active Compounds on the Ability of DAF to Scavenge Free Radicals. The ability of compounds to interact with free radicals can be evaluated by measuring their ability to protect oxidatively sensitive fluorescence probes in the presence of lipid- (AMVN) or water-soluble (AAPH) peroxyl radical generating systems. DAF contains fluorescein, the same fluorescent moiety used to measure antioxidant activity in the oxygen radical absorbance capacity (ORAC) assay (*46, 47*). Thus, DAF could be used to study the impact of the physical and chemical properties of bulk oils on free radical scavenging. Without free radical initiators, DAF in stripped corn oil containing 1% of 25 mM phosphate buffer was stable over the time frame used in these experiments as determined by fluorescence intensity (λ_{EX} 494 and λ_{EM} 515 nm). No differences ($p > 0.05$) were observed in the probe stability upon addition of oleic acid or phosphatidylcholine dioleoyl into the bulk oil−phosphate buffer system.

The addition of the lipid-soluble peroxyl radical initiator (AMVN) did not ($p > 0.05$) change the fluorescence intensity of DAF in the absence or presence of oleic acid or phosphatidylcholine dioleoyl (1 mmol/kg lipid) in the bulk oil−phosphate buffer system (**Figure 9**). Because DAF fluorescence requires presence at the oil–water interface or in the aqueous phase, the inability of AMVN to degrade DAF indicates that lipid-soluble free radicals could not gain access to DAF at these locations. In contrast to lipid-soluble free radical generator, water-soluble peroxyl radicals generated from AAPH significantly ($p < 0.05$)

decreased DAF fluorescence. No differences ($p > 0.05$) were observed in DAF degradation by AAPH between the control and the added oleic acid samples (**Figure 9**). This suggests that oleic acid does not alter the ability of the aqueous peroxyl radicals to attack DAF. The addition of phosphatidylcholine dioleoyl to the bulk oil significantly decreased ($p < 0.05$) the degradation of DAF by aqueous peroxyl radicals (**Figure 9**). The ability of phosphatidylcholine to increase the exposure of DAF to the aqueous phase (as determined by increase in fluorescence intensity **Figure 8**) while not increasing the susceptibility of DAF to degradation by water-soluble peroxyl radicals suggests that phosphatidylcholine concentrates DAF at the oil–water interface in a manner where the DAF is either not accessible to the water-soluble free radicals or the phosphatidylcholine is scavenging the radicals and thus protecting DAF.

CONCLUSIONS

Association colloids appear to be important in the oxidative stability of bulk oils. The surface-active fluorescence probe, DAF, is a useful tool for the characterization of the physical and chemical properties of bulk oils. DAF can be used to measure changes in pH as observed upon the addition of oleic acid to bulk oil. DAF can also be used to observe how surface-active compounds such as phosphatidylcholine can increase the exposure of surface-active compounds to the aqueous phase of association colloids. Finally, the ability of the fluorescein moiety on DAF to detect free radicals can be used to determine how minor components in oil impact oxidative reactions. Overall, these results suggest that DAF could be a useful analytical tool to study the impact of the aqueous environment of bulk oil on lipid oxidation.

ACKNOWLEDGMENT

We thank Dr. Zhimin Xu for the analysis of tocopherols and tocotrienols in corn oil.

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Received for review July 10, 2007. Accepted November 28, 2007. This research was supported in part by Grant 2007-02650 from the NRI competitive grants program of the U.S. Department of Agriculture.

JF072071O