Ability of sodium dodecyl sulfate (SDS) micelles to increase the antioxidant activity of α-tocopherol

Ability of Sodium Dodecyl Sulfate (SDS) Micelles to Increase the Antioxidant Activity of α-Tocopherol

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ABSTRACT

Once emulsifiers saturate the surface of an emulsion droplet, the excess emulsifier partitions into the aqueous phase. Aqueous phase surfactants can increase the activity of antioxidants but it is not known if this is due to chemical or physical effects. When α-tocopherol was added to emulsions after homogenization, a 70% increase in partitioning of α-tocopherol to the aqueous phase was observed when sodium dodecyl sulfate (SDS) concentrations exceeded the SDS critical micelle concentration. However, when α-tocopherol was added to the lipid prior to emulsification, excess SDS did not increase aqueous phase partitioning of α-tocopherol. Increasing α-tocopherol in the aqueous phase with SDS micelles increased the oxidative stability of oil-in-water emulsions. Experiments suggest that this was due to the ability of surfactant micelles to decrease the prooxidant activity of α-tocopherol Considering these results, surfactant micelles could play a key role in increasing the antioxidant activity of α-tocopherol.

KEYWORDS: oil-in-water emulsion, α-tocopherol, micelle, antioxidant, sodium dodecyl sulfate, anionic surfactant, lipid oxidation

1. INTRODUCTION

Emulsion systems are important physical constituents of a wide variety of food products and are responsible for many of their physico-chemical properties.[1] Lipids are important ingredients of both oil-in-water (O/W) or water-in-oil (W/O) emulsions. In either formulation, lipids are susceptible to oxidation, which may occur at any stage of food processing and storage.[2] Lipid oxidation is a critical phenomenon that determines the shelf life of lipid-based products because it may result in undesired aroma, toxicity, and co-oxidation of other compounds such as proteins, vitamins, or pigments.[3] Therefore, a major concern of the food industry is how to retard or inhibit oxidative reactions in emulsions to improve food quality. The addition of free radical scavenging antioxidants is one of main methods employed by food scientists to inhibit oxidative rancidity.[4]

To get maximum antioxidant activity, free radical scavengers should ideally partition themselves in locations where free radicals are produced. In this aspect, the polarity of antioxidants plays an important role in the determination of antioxidant activity. The antioxidant polar paradox hypothesis states that hydrophobic antioxidants are more effective in O/W emulsions than hydrophilic homologs.[5,6] This higher activity of non-polar antioxidants in O/W emulsions has been proposed to be dependent on antioxidant retention in the emulsion droplet or partitioning of the antioxidant at the droplet interface where oxidation primarily takes place.[5,7] When the lipophilic antioxidants are at the interface, free radicals are scavenged before they can cross the droplet membrane and enter the lipid phase.[2] However, recent publications highlighted that hypothetical expectations of the polar paradox do not always accurately predict an antioxidant's behavior.[8,9] For instance, in O/W emulsions it was observed that increasing an antioxidant's hydrophobicity by adding fatty acid chains to the antioxidant increases activity only to a point after which further increasing hydrophobicity actually decreases antioxidant activity. This means that hydrophobicity could critically affect the antioxidant activity in both positive and negative manners.[9] These authors suggested that these observations could be due to factors such as "reduced mobility",

68 “internalization” and “self-aggregation” of the antioxidants in the emulsion droplet. However,
69 hydrophobicity is not the only factor affecting the validity of polar paradox theory. The antioxidant
70 concentration is also significant factor because the prooxidant effect at high concentrations may
71 give false indication of the antioxidant effect.[8] When antioxidant effect of caffeic acid esterified
72 with fatty alcohols of different chain length on two fish oil enriched food systems (mayonnaise and
73 milk) was evaluated, the results showed that the optimal alkyl chain length of antioxidant esters for
74 oxidation prevention greatly depends on the matrix studied.[10] Furthermore, other factors, such as
75 chemical composition of the emulsion, play a key role in antioxidant partitioning, thus affecting
76 antioxidant activity.[11]
77 The inclusion of emulsifier in food formulation helps to physically stabilize the emulsion
78 system. However, once the emulsifier saturates the emulsion droplet surface, the excess surfactant
79 partitions to the aqueous phase. If aqueous phase surfactant concentrations are high enough,
80 micelles form which could promote the solubilization of lipophilic antioxidant out of the emulsion
81 droplet and into the aqueous phase. This solubilization depends on type and concentration of the
82 surfactant, as well as molecular polarity, size and geometry of the antioxidant, affecting
83 incorporation of antioxidant into the surfactant micelles.[11] It was found that adding Tween 20 to an
84 O/W emulsion increased partitioning of tocopherol homologs into aqueous phase with partitioning
85 decreasing with increasing number of methyl group on the tocopherol (e.g. δ-tocopherol > α-
86 tocopherol).[12] This work also found that the antioxidant activity of tocopherols was enhanced when
87 the surfactant partitioned the tocopherol into the aqueous phase. Unfortunately, in these Tween 20-
88 stabilized emulsions, all the emulsions had Tween 20 concentrations above its critical micelle
89 concentration (CMC) meaning that the Tween 20 would exist both at the emulsion droplet interface
90 and as micelles in the aqueous phase. Therefore, it is was not known whether the increased activity
91 of tocopherols was due to surfactant micelle solubilization of the antioxidants into the aqueous

phase, Tween 20 chemically inhibiting oxidation or Tween 20 chemically interacting with tocopherols to increase antioxidant activity.

Surfactant micelles orient themselves in a way that non-polar tails are located away from water and polar head is in contact with water. This structure allows non-polar and amphiphilic antioxidants to be solubilized in the hydrophobic core. The micelles in the system are capable of transferring components involved in lipid oxidation, including antioxidants and prooxidants, which may result in increased or decreased oxidative activity. In the presence of surfactant micelles, the radicals in lipid droplets can start the oxidation of neighboring droplets, which may result in decreased oxidative stability.[13] On the other hand, micelles can also solubilize fatty acid hydroperoxides[14] and metal ions[15] out of the lipid droplets, thus removing prooxidants from oil phase to decrease lipid oxidation rates.

The aim of the study was to investigate α-tocopherol partitioning in the presence of sodium dodecyl sulfate (SDS)-stabilized stripped soybean oil-in-water emulsion in the absence and presence of SDS micelles. SDS was chosen because it has a higher critical micelle concentration than Tween 20 and thus emulsions could be prepared with aqueous phase surfactant in both monomer and micelle forms. Subsequently, oxidation studies were carried out to understand how the micelles affected the antioxidant activity of α-tocopherol in the O/W emulsions when the α-tocopherol was added prior to or after homogenization of the lipid.

2. MATERIALS AND METHODS

Refined soybean oil was purchased from a local retail store in (Hadley, MA). Iso-octane, 2-propanol, methanol, 1-butanol, *n*-hexane, hydrochloric acid, and sodium phosphate dibasic were supplied by Fisher Scientific (Fair Lawn, NJ). Ethylenediaminetetraacetic acid (EDTA) was purchased from Chempure Ultra (Houston, TX). Silicic acid (100–200 mesh), activated charcoal (100–400 mesh), sodium dodecyl sulfate (SDS), sodium phosphate monobasic, barium chloride

dihydrate, ammonium thiocyanate, iron (II) sulfate heptahydrate, cumene hydroperoxide, hexanal, and (±)-α-tocopherol were supplied by Sigma-Aldrich (St. Louis, MO). Solvents were HPLC grade, and all other chemicals were analytical grade. Double distilled and deionized water was used throughout the study. Glassware was incubated in 2 M HCl overnight to remove metals, followed by rinsing with double-distilled water before use.

2.1 Preparation of stripped soybean oil

Stripped soybean oil was prepared according to the method of Boon et al.[16] and was used in all experiments. Briefly, silicic acid (100 g) was washed three times with a total of 3 L of distilled water and activated at 110 °C for 20 h. The activated silicic acid (22.5 g) and activated charcoal (5.63 g) were suspended in 100 and 70 mL of *n*-hexane, respectively. A chromatographic column (3.0 cm internal diameter × 35 cm height) was then packed sequentially with silicic acid, followed by activated charcoal and then another layer of silicic acid. Thirty grams of soybean oil were dissolved in 30 mL of *n*-hexane and passed through the column by eluting with 270 mL of *n*-hexane. In order to retard lipid oxidation during stripping, the collected soybean oil was held in an ice bath and covered with aluminum foil throughout the process. After complete elution, *n*-hexane was removed with a vacuum rotary evaporator (Model RE 111, Buchi, Flawil, Switzerland) at 37 °C and traces of the remaining solvent were evaporated under a nitrogen stream. The stripped soybean oil was flushed with nitrogen and stored at −80 °C until use. Removal of tocopherols in stripped soybean oil was verified by HPLC.[17]

2.2 Emulsion preparation

Stripped soybean oil (0.5 %, wt) and the surfactant SDS (0.05%, wt; final emulsion concentration 1.73 mM) in 10 mM phosphate buffer solution (pH 7.0), were used for the formulation of the control group of O/W emulsions. To minimize oxidation in the antioxidant

partitioning studies, 200 μM of EDTA was included in the phosphate buffer solution. In the first step, a coarse emulsion was made by blending with a hand-held two-speed homogenizer (Model M133/1281-0, BioSpec Products Inc., Bartlesville, OK) at the high-speed setting for 2 min. After that, three passes at a pressure of 9 Kbar were carried out using a microfluidizer (Model M-110L Microfluidics, Newton, MA) to further reduce the particle size of the O/W emulsions. During this process, ice was used to cover the homogenizer chamber and coil to maintain the emulsion temperature at ≤ 25 °C.

SDS was added to 20 g of control emulsion to obtain a final aqueous phase emulsifier concentration of 2.0 to 15 mM; followed by mixing with an electronic stirrer (Model 2008, Thermo Fisher Scientific Inc., Raleigh, NC) at 500 rpm for 30 min at room temperature and in the dark. An α-tocopherol stock solution in methanol was added directly to the emulsion or to the oil prior to emulsification. For the determination of antioxidant partitioning, α-tocopherol in methanol was added to the O/W emulsions at final concentration of 140 μM to optimize HPLC detection. For the oxidation studies, α-tocopherol was added to the lipid or directly into the emulsions at a final concentration of 30 μM to produce lag phases of ≤ 10 days.

2.3 Physical characteristics of emulsions

Particle size distributions and ζ-potential of the final emulsions were measured using a dynamic light scattering instrument (Zetasizer Nano-ZS Malvern Instruments Ltd., Worcestershire, UK). Samples for particle size distribution measurements were diluted into 10 mM phosphate buffer solution (pH 7.0) at an emulsion:buffer ratio of 1:50 to prevent the multiple scattering effects. The ζ-potential of all samples was measured by laser Doppler electrophoresis (Zetamaster, Malvern, Worcestershire, UK). Prior to measurement, samples were diluted with 5 mM phosphate buffer at pH 7.0. Five measurements were taken per sample injected, and each sample was measured in duplicate for a total of ten ζ-potential readings per sample.

2.4 Determination of α-tocopherol partitioning and α-tocopherol loss in emulsions

The concentration of α-tocopherol was determined in the aqueous phase of the O/W emulsion by high-performance liquid chromatography (HPLC), according the method described by Panya et al.[17] Briefly, the emulsions containing different concentrations of SDS plus α-tocopherol were centrifuged at 162102 *x g* (46,000 rpm) for 1 h at 4 °C using a PTI F65L-6x13.5 rotor with a Sorvall WX Ultra 80 high-speed centrifuge (Thermo Fisher Scientific Inc., Asheville, NC) to cream the emulsion droplets. After centrifugation, 1 mL of the aqueous phase was collected and α-tocopherol was extracted with 2 mL of iso-octane:2-propanol solution (3:1, v/v). The mixed solution was centrifuged at 4000 rpm for 5 min (CL10 centrifuge, Thermo Fisher Scientific Inc., Waltham, MA) and 1 mL of upper solvent phase was dried using a vacuum centrifuge. α-Tocopherol was redissolved in 200 μL of methanol, filtered with a PTFE membrane (Acrodisc CR 13 mm syringe filter with 0.45 μm PTFE Membrane, Pall Life Sciences, Westborough, MA) and injected into a Shimadzu 10A VP HPLC (Shimadzu, Columbia, MD), which was coupled to a C18 reversed phase column (150 mm x 4.6 mm i.d., particle size 5 μm, Beckman Coulter Inc., Brea, CA), a C18 security guard column (4 mm x 3 mm i.d., particle size 5 μm; Phenomenex, Torrance, CA) and a fluorescence detector (Waters 474 Scanning Fluorescence Detector, Waters, Milford, MA). The separation was carried out with an isocratic elution using methanol as mobile phase, at a flow rate of 1 mL/min. α-Tocopherol detection was performed using an excitation wavelength of 290 nm and emission wavelength of 330 nm. The concentration of α-tocopherol was calculated using an external standard calibration curve.

The loss of total α-tocopherol in emulsions was carried out after hexanal determination. Briefly, 0.5 mL of O/W emulsion sample was extracted by mixing with 1 mL iso-octane:2-propanol solution (3:1, v/v). After extraction, the sample was dried using a vacuum centrifuge and

resuspended in 200 μL of methanol. After filtering with PTFE membrane, the samples were injected to HPLC and measured with the same method as described above.

2.5 Evaluation of lipid oxidation

For the lipid oxidation studies, 1 mL of the emulsion treatments were transferred into 10 mL GC headspace vials, capped with aluminum caps with PTFE/Silicone (tetrafluoroethylene) septa and stored at 37 °C in dark. The samples were analyzed periodically for lipid hydroperoxides, headspace hexanal and α-tocopherol concentrations. Lipid hydroperoxide formation was determined using a modified method described by Shantha and Decker.[18] Hydroperoxide analysis was performed after headspace hexanal determination was complete. Emulsion samples (0.3 mL) were mixed with 1.5 mL of isooctane:2-propanol solution (3:1, v/v) and vortexed (10 s, three times). The mixed solution was centrifuged at 3,400 *x g* for 10 min (Centrific TM Centrifuge, Thermo Fisher Scientific Inc., Fairlawn, NJ). The upper organic layer (0.2 mL) was mixed with 2.8 mL of a methanol:1-butanol solution (2:1, v/v), followed by the addition of 15 μL of 3.94 M ammonium thiocyanate and 15 μL of Fe^{2+} solution. The clear Fe^{2+} solution was prepared freshly from equal amounts of 0.132 M $BaCl_2$ (in 0.4 M HCl) and supernatant of 0.144 M $FeSO_4$ in double-distilled water obtained after centrifugation at 3000 rpm for 5 min (CL10 centrifuge, Thermo Fisher Scientific Inc., Waltham, MA). The solution was vortexed and, after 20 min of incubation at room temperature in the dark, the absorbance of the samples was measured at 510 nm using an UV−Vis spectrophotometer (Genesys 20, Thermo Fisher Scientific Inc., Waltham, MA). Samples with high absorbance values (> 1.2) were diluted with methanol/1-butanol (2:1, v/v) before measurements. Hydroperoxide concentrations were determined using a standard curve prepared from cumene hydroperoxide.

Headspace hexanal was measured according to the method described by Panya et al.[19], using a gas chromatography instrument coupled to a flame ionization detector (GC-FID, Model GC-2014,

Shimadzu Co., Tokyo, Japan) and equipped with an autosampler (Model AOC-5000, Shimadzu Co., Tokyo, Japan). Emulsions in the headspace vials were heated at 55 °C for 10 min in the autosampler heating block followed by insertion of a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) stable flex solid phase microextraction (SPME) fiber (Supelco Co., Bellefonte, PA) for 2 min to adsorb volatile compounds. The desorption was carried out at 250 °C for 3 min in the injection port of the GC which was operated in the split mode at a 1:7 ratio. Helium was used as carrier gas, at a constant flow of 1 mL/min. Volatile compounds were separated on a fused-silica capillary Equity-1 Supelco column (30 m x 0.32 mm i.d. x 1 μm), coated with 100% polydimethylsiloxane (PDMS) at 65 °C for 10 min. The detector temperature was set at 250 °C. Hexanal concentrations were determined from peak areas using a hexanal standard curve made by adding hexanal to the control emulsion.

2.6 Statistical analysis

All data shown represent the mean values ± standard deviation of triplicate measurements and were repeated twice. The data obtained were analyzed by one-way analysis of variance (ANOVA), using SPSS version 21 (SPSS Inc., Chicago, IL). The differences between mean values were compared using Tukey's HSD test with a level of significance of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physical characteristics of emulsions

The droplet size of the emulsions used in this study averaged 174.1 ± 1 nm. For the duration of the experiments, all samples had no visible creaming or changes in particle size (data not shown). The SDS-stabilized emulsion had a zeta potential of - 95.9 mV ± 1.6. The addition of SDS in O/W emulsion further decreased the surface charge (data not shown) up to -103.7 ± 1.52 for 15 mM SDS.

3.2 Impact of increasing surfactant concentrations on α-tocopherol location

The percentage of α-tocopherol in aqueous phase of the 0.5% stripped soybean oil-in-water emulsion was determined in the presence of increasing concentrations of SDS. The purpose of this was to study SDS concentrations where SDS would exist as individual molecules when their concentration was below their critical micelle concentration (CMC) and as surfactant micelles at concentrations above the CMC.

α-Tocopherol has low water solubility which explains why less than 3% of the added α-tocopherol was in the aqueous phase in the absence of added SDS (Figure 1). When α-tocopherol was added after emulsification, low concentrations of SDS (2-4 mM) slightly increased aqueous phase α-tocopherol concentrations (< 8% of added α-tocopherol). However, when SDS concentrations were above 4 mM, a dramatic increase in aqueous phase α-tocopherol was observed with up to 70% of the α-tocopherol partitioning into the aqueous phase at an SDS concentrations of 5.75 mM suggesting that SDS micelles were solubilizing α-tocopherol out of the emulsion droplet. However, this solubilization of α-tocopherol into the aqueous phase by SDS occurred below the reported CMC of SDS (8.3 mM in pure water).[20] This suggests that SDS and α-tocopherol formed co-micelles which had a lower CMC than pure SDS. When α-tocopherol was added to the oil prior to emulsification, increasing SDS concentrations did not have a considerable impact on aqueous phase α-tocopherol concentrations. Higher levels of SDS were added to ensure that SDS micelles were formed but even in the presence of 15 mM SDS, no significant increase in aqueous phase α-tocopherol was observed (Figure 1).

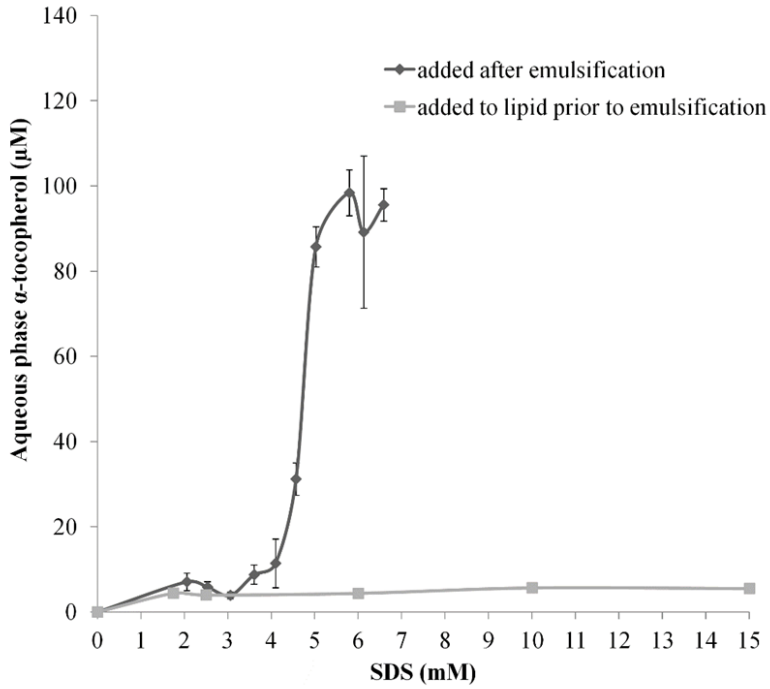


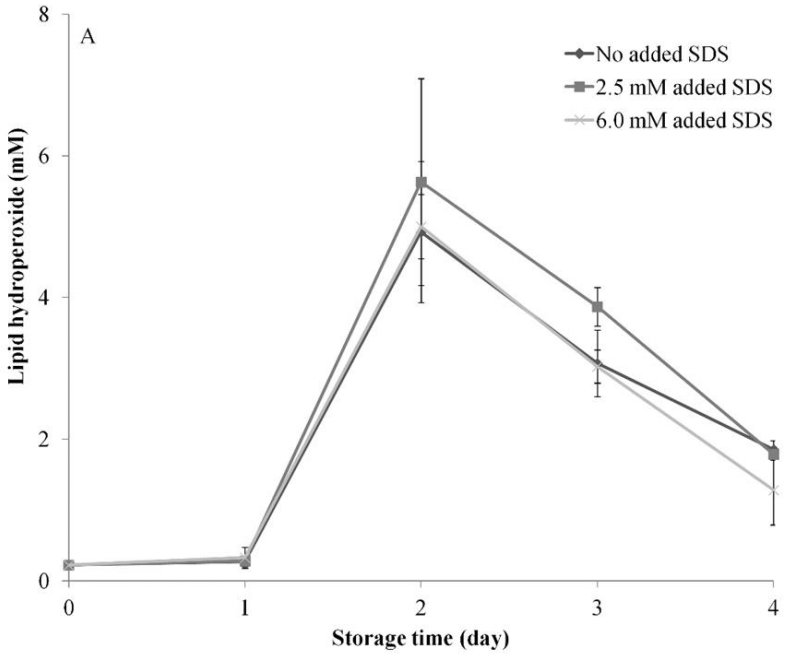
Figure 1. Effect of SDS addition (from 2 to 15 mM) on the partitioning of α-tocopherol (140 μM) into the aqueous phase of 0.5% stripped soybean oil-in-water emulsions when the α-tocopherol was added after emulsification or to the lipid prior to emulsification. Data points and error bars represent means (n = 3) ± standard deviations.

Other investigators have found that surfactants could increase the concentrations of antioxidant in the aqueous phase. It was found that excess Tween 20 at concentrations as low as 0.8 mM increased aqueous phase concentrations of eicosyl rosmarinate over 7.5-fold.[17] Kiralan et al.[12] found similar results with Tween 20 and α, γ and δ tocopherols. In both of these papers, Tween 20 was used as the surfactant. The CMC of Tween 20 has been reported to range from 0.01 to 0.4 mM[21] meaning that all the emulsions in these studies likely had Tween 20 micelles. Surfactant solubilization of antioxidants was also observed where solubilization of propyl gallate out of emulsion droplets into the continuous phase was increased 2.3-fold after increasing Brij concentrations from 0.3 to 2.8%.[11] The partitioning of the lipophilic spin probe, 4-phenyl-2,2,5,5-

tetramethyl-3-imidazoline-1-oxyl nitroxide, when added to tetradecane-in-water emulsions stabilized with SDS was also studied.[22] They found that approximately 1.5 fold more spin probe could be solubilized into the aqueous phase by addition of 35 and 70 mM SDS. To our knowledge, this study is the first to show that surfactants primarily increased aqueous phase α-tocopherol concentrations at concentrations above the CMC and that surfactants were not able to increase α-tocopherol concentrations in the aqueous phase when added to the oil prior to emulsion formation.

3.3 Evaluation of lipid oxidation in O/W emulsions with and without surfactant micelles

The impact of added SDS on lipid oxidation was determined by monitoring lipid hydroperoxides and headspace hexanal concentrations. Added SDS at 2.5 mM would not have micelles while 6.0 mM added SDS would. In the absence of added α-tocopherol, the control emulsion and emulsions with added SDS (2.5 and 6.0 mM) had same lag phase for lipid hydroperoxide formation ($p > 0.05$) (Figure 2A). Hexanal formation was slightly greater in the presence of the added SDS (Figure 2B). Overall, this shows that the SDS and SDS micelles did not have a major impact on lipid oxidation rate when there is no α-tocopherol in the environment. Previous research with salmon oil has shown that aqueous phase SDS only slightly increased oxidation rates presumably by binding iron and increasing its activity.[23]



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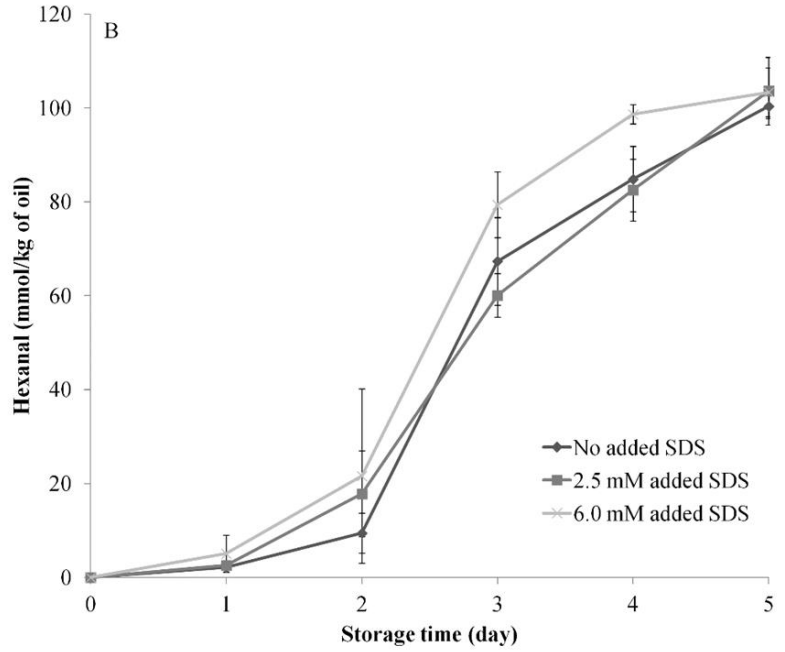
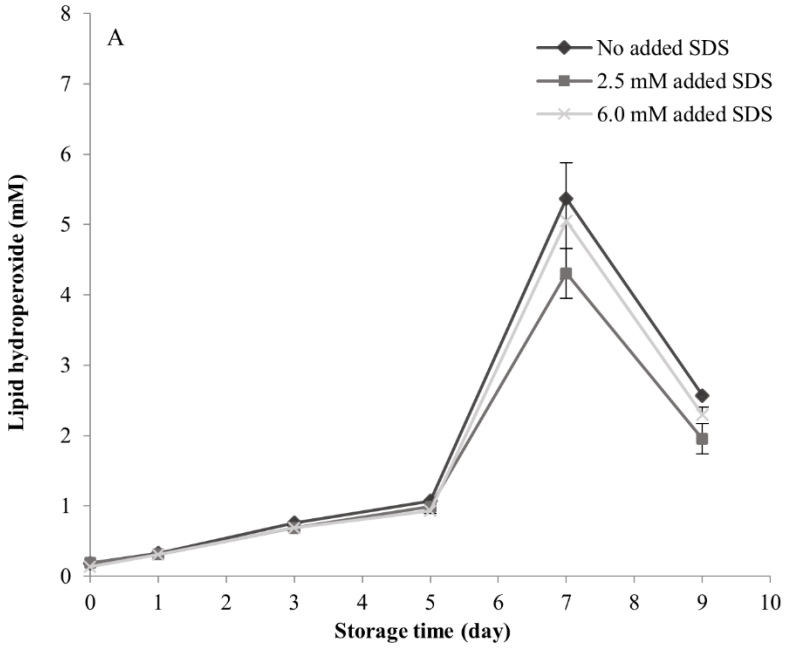


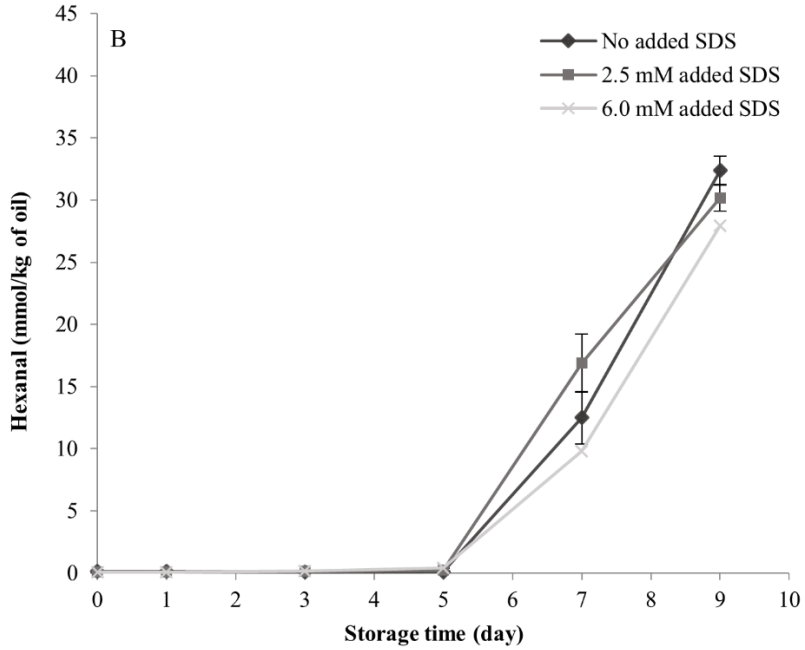
294

295 **Figure 2.** Lipid hydroperoxide (A) and hexanal (B) formation in 0.5% stripped soybean oil-in-water
296 emulsions without α-tocopherol and in the presence of 0, 2.5 or 6.0 mM added SDS during storage
297 at 37 °C. Data points and error bars represent means (n = 3) ± standard deviations.

298

In emulsions where α-tocopherol was added to the lipid phase prior to homogenization, the addition of SDS to the emulsion had no effect on lag phase of lipid hydroperoxide or hexanal formation ($p > 0.05$) (Figure 3), presumably because there were no changes in α-tocopherol location (Figure 1) and excess SDS by itself did not impact oxidation rates (Figure 2). In emulsions where α-tocopherol (30 μM) was added to the aqueous phase after homogenization, the addition of 2.5 mM SDS slightly decreased the lipid hydroperoxide formation and increased the hexanal lag phase 2 days longer than the control ($p < 0.05$) (Figure 4). This small increase in oxidative stability could be due to the ability of 2.5 mM SDS to solubilize a small amount of α-tocopherol (< 8% of added α-tocopherol). When 6.0 mM SDS was added, the lag phase of lipid hydroperoxide and hexanal formation were both 6 days longer than the control ($p < 0.05$) (Figure 4), indicating a strong antioxidant effect by the combination of α-tocopherol and 6 mM SDS.





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312 **Figure 3.** Lipid hydroperoxide (A) and hexanal (B) formation in 0.5% stripped soybean oil-in-water

313 emulsions in the presence of 0, 2.5 or 6.0 mM added SDS when α-tocopherol (30 μM) was added to

314 the lipid prior to homogenization during storage at 37 °C. Data points and error bars represent

315 means (n = 3) ± standard deviations.

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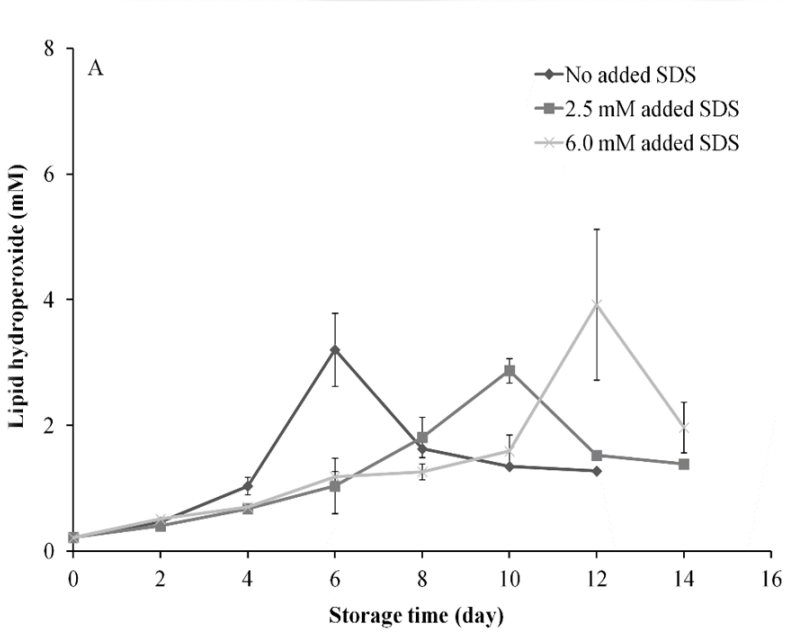

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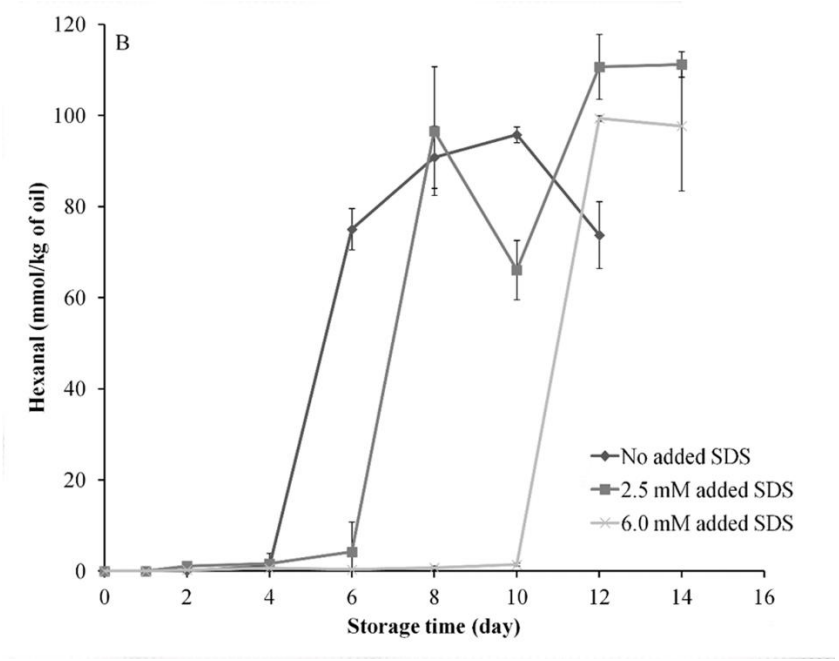


Figure 4. Lipid hydroperoxide (A) and hexanal (B) formation in 0.5% stripped soybean oil-in-water emulsions in the presence of 0, 2.5 or 6.0 mM added SDS when α-tocopherol (30 μM) was added after homogenization during storage at 37 °C. Data points and error bars represent means (n = 3) ± standard deviations.

Other investigators have found that surfactant micelles could improve the oxidative stability of emulsions. It was found that hexanal lag phase increased from 4 to 10 days in the presence of 0% and 2.50% Tween 20, respectively, in stripped soybean oil-in-water emulsions containing eicosyl rosmarinate.[17] The formation of Tween 20-eicosyl rosmarinate co-micelles allowed this highly nonpolar antioxidant to inhibit lipid oxidation whereas 20-carbon antioxidant esters were previously reported to be unable to inhibit lipid oxidation in the absence of excess surfactants.[9] Furthermore, the presence of Brij 700 surfactant micelles in salmon oil-in-water emulsions enhanced antioxidant activity of tert-butylhydroquinone.[11]

α-Tocopherol works as an antioxidant by scavenging free radicals and thus being degraded prior to the oxidation of fatty acids. This was observed in this study as total α-tocopherol

concentrations decreased prior to formation of lipid hydroperoxides and hexanal in both the emulsions with 2.5 and 6.0 added SDS (Figure 5). α-Tocopherol degradation rates were also similar in the presence of 2.5 and 6.0 mM of added SDS when α-tocopherol was added after emulsification ($p > 0.05$) (Figure 5). This suggests that the increased oxidative stability of the emulsions with 6.0 mM SDS was not due to the ability of the surfactant micelles to preserve α-tocopherol in a manner where its antioxidant protection would last longer, thus decreasing the formation of fatty acid oxidation products.

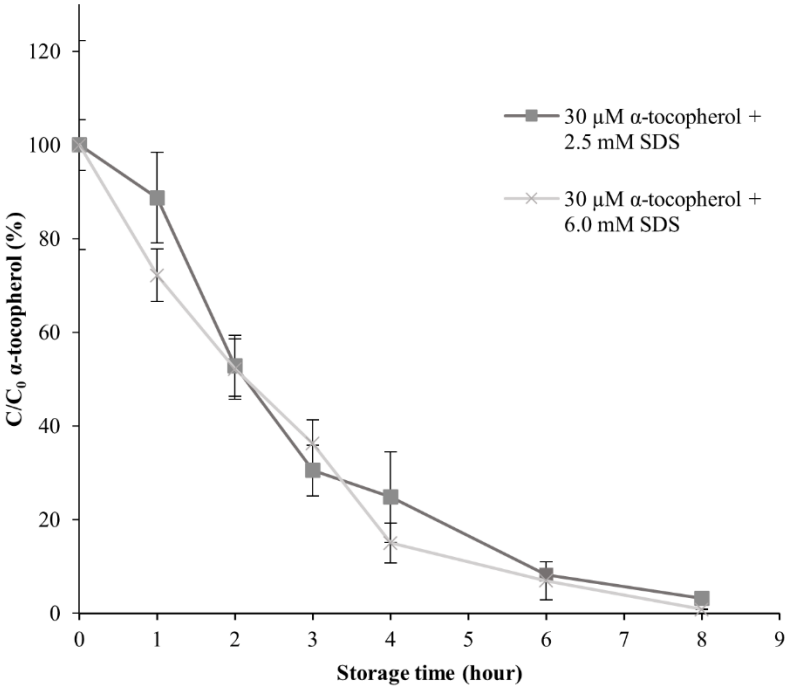
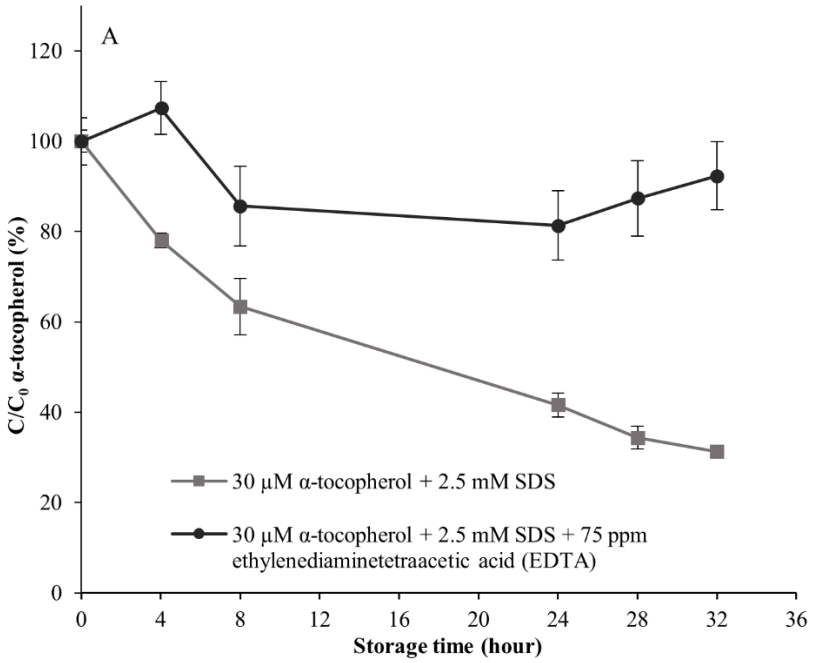


Figure 5. Changes in α-tocopherol (30 µM) (concentration/concentration at time 0; C/C_0, %) in 0.5 % stripped soybean oil-in-water emulsions containing either 2.5 mM or 6.0 mM added sodium dodecyl sulfate (SDS) during the storage at 37 °C in the dark. Data points and error bars represent means (n = 3) ± standard deviations.

348 It is possible that α-tocopherol was degrading differently in the two emulsions. In addition to
349 the degradation by interactions with free radicals, α-tocopherol can donate electrons to transition
350 metals which causes the conversion of tocopherols to tocopherol quinones.[24] This is because when
351 tocopherols reduce metals this increases their prooxidant activity as reduced metals are more
352 effective at degrading lipid hydroperoxides to free radicals. To determine if this could occur,
353 emulsions were made with non-oxidizable medium chain triacylglycerols and α-tocopherol
354 degradation was monitored in the presence of 2.5 mM (Figure 6A) and 6.0 mM (Figure 6B) of
355 added SDS when α-tocopherol was added after emulsification. Medium chain triacylglycerols were
356 used instead of stripped soybean oil to avoid free radicals from oxidizing unsaturated fatty acids
357 from degrading the α-tocopherol. In this model, total α-tocopherol decreased faster in the presence
358 of 2.5 than 6.0 mM added SDS. In addition, EDTA was able to inhibit the α-tocopherol degradation
359 in the presence of 2.5 mM SDS ($p < 0.05$) (Figure 6A) but had little effect on α-tocopherol in the
360 presence of 6.0 mM SDS ($p > 0.05$) (Figure 6B). Metals such as iron, have low solubility at pH 7.0
361 and it has been shown that under these conditions, the metals associate with emulsion droplets since
362 they are not soluble in the aqueous phase.[25] This suggests that metals were responsible for loss of α-
363 tocopherol at the low SDS concentration where the tocopherol would be more highly associated
364 with the emulsion droplets. EDTA inhibited metal promoted α-tocopherol degradation by either
365 decreasing iron reactivity or by binding iron and partitioning it away from the emulsion droplet. In
366 the presence of 6.0 mM SDS micelles, α-tocopherol would partition away from the emulsion
367 droplets where it was not able to interact with metals associated with the emulsion droplets and thus
368 its degradation was slower. Since SDS solubilized α-tocopherol was not interacting with metals,
369 EDTA had not any effect on its degradation (Figure 6B). In terms of the lipid oxidation results, in
370 the absence of SDS micelles (2.5 mM SDS), α-tocopherol would both reduce metals and scavenge
371 free radicals. In the presence of SDS micelles (6 mM SDS), α-tocopherol's ability to reduce metals
372 would be less of if it did reduce metals they would not be near the lipid hydroperoxides in the

emulsion droplet and thus it's prooxidant activity would decrease resulting in a net increase in antioxidant activity. These results suggest that α-tocopherol has both prooxidant and antioxidant activities in oil-in-water emulsions. This has previously been observed in bulk oils where higher α-tocopherol concentrations can exhibit decreased antioxidant effectiveness due to prooxidant activity.[26] Therefore, altering the physical location of α-tocopherol could alter this prooxidant/antioxidant balance towards less prooxidant activity, thus increasing the ability of α-tocopherol to inhibit lipid oxidation.



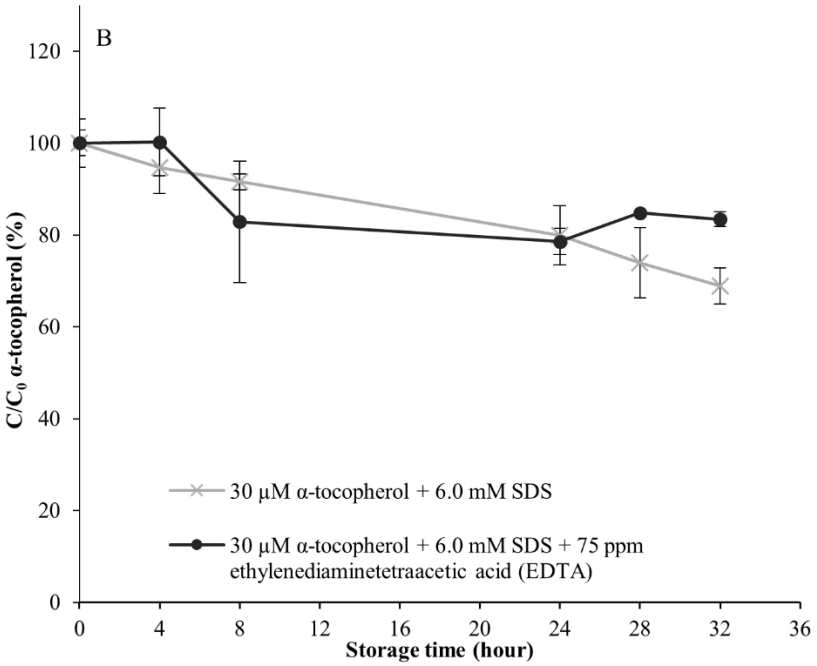


Figure 6. Changes in α-tocopherol (30 µM) (concentration/concentration at time 0; C/C_0, %) in 0.5 % medium chain triglyceride-in-water emulsions containing (A) 2.5 mM added sodium dodecyl sulfate (SDS) with and without 75 ppm of EDTA or (B) 6.0 mM added SDS with and without 75 ppm of EDTA. Emulsions were stored at 37 °C in the dark. Data points and error bars represent means (n = 3) ± standard deviations.

While others have also shown that aqueous phase surfactants improve the efficacy of antioxidants, this research highlights that this phenomenon is primarily dependent on the presence of the surfactant micelles. Surfactants could potentially be used to improve the oxidative stability of many foods containing oil-in-water emulsions since most food oils contain naturally occurring tocopherols. However, many surfactants produce off-flavors (e.g. soapy) which may limit this antioxidant approach. This could be potentially overcome by using surfactants with low CMCs (e.g. Tweens) since they could produce micelles at very low concentrations where they might not impact flavor. This research also highlights the importance of how antioxidants are added to food

emulsions since α-tocopherol added to the lipid prior to emulsification was not able to partition into surfactant micelles in the aqueous phase. Thus, the combination of adding antioxidants after emulsification and using excess surfactant could provide a new technique to increase antioxidant efficacy and thus the shelf-life and sustainability of foods.

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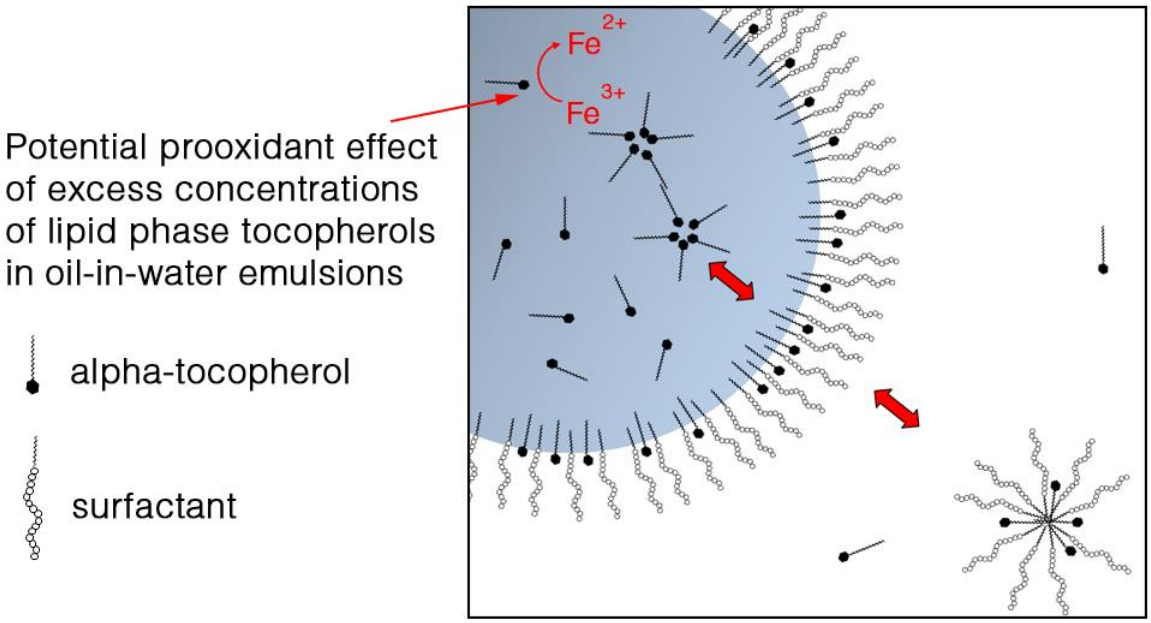

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