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Spray-Dried Multilayered Emulsions as a Delivery Method for ω-3 Fatty acids into Food Systems

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Emulsion can be produced with electrostatic layer-by-layer deposition technologies to have cationic, thick multilayer interfacial membranes that are effective at inhibiting the oxidation of ω-3 fatty acids. This study investigated the stability of spray-dried multilayer emulsion upon reconstitution into an aqueous system. The primary (lecithin) and multilayered secondary emulsions (lecithin and chitosan) were spray-dried with corn syrup solids (1−20 wt %). The lecithin−chitosan multilayer interfacial membrane remained intact on the emulsion droplets upon reconstitution into an aqueous system. Reconstituted secondary (lecithin−chitosan) emulsions were more oxidatively stable than reconstituted primary (lecithin) emulsions. A minimum of 5 wt % corn syrup solids was needed to microencapsulate the secondary emulsion droplets. Maximum oxidative stability of both the powder and the reconstituted secondary emulsions was observed in samples with 5% and 20% corn syrup solids. Addition of EDTA (25 μM) inhibited oxidation of reconstituted primary and secondary emulsions. These studies suggest that a microencapsulated multilayered emulsion system could be used as a delivery system for ω-3 fatty acids in functional foods.

KEYWORDS: Fish oil; ω-3 fatty acids; microencapsulation; lipid oxidation; chitosan; oil-in-water emulsion; antioxidants

INTRODUCTION

Humans cannot synthesize ω-3 polyunsaturated fatty acids, making these fatty acids an essential part of the diet. α-Linolenic acid (ALA, 18:3 ω-3) is considered the essential ω-3 fatty acid because it can potentially be synthesized into other ω-3 fatty acids within the body. Unfortunately, humans do not efficiently convert ALA to the long-chain fatty acids, EPA (eicosapentaenoic acid, C20:5 ω-3) and DHA (docosahexaenoic acid, C22:6 ω-3), therefore requiring humans to consume these fatty acids through the diet to obtain nutritionally significant concentrations of these bioactive lipids. EPA and DHA are widely recognized for their beneficial health attributes. These long-chain ω-3 fatty acids are beneficial for brain and retinal development, cardiovascular disease, and decreasing inflammatory responses in humans (*1*−*5*). Even though there is evidence showing the health benefit of consuming ω-3 fatty acids, the average dietary intake in most consumers is not high enough to produce maximal health benefits (*6*). Main dietary sources of EPA and DHA are seafood as well as refined marine and algal oils. Addition of these oils into foods is very difficult for food processors due to the oil's instability to oxidative rancidity.

Interfacial engineering of oil-in-water emulsion droplets can be used to inhibit lipid oxidation by decreasing the interactions between the ω-3 oil and prooxidative species in the aqueous phase. For example, research has shown that a thick interfacial membrane may act as a barrier that decreases the ability of aqueous phase iron to decompose lipid hydroperoxides, which in turn can oxidize fatty acids in the interior of the oil droplet (*7*−*9*). Further, engineering the interface of oil-in-water emulsion droplets with a cationic charge can inhibit lipid oxidation by electrostatically repelling prooxidative metals away from the lipids in the emulsion droplet core (*7*, *10*−*12*).

Research has shown that electrostatic layer-by-layer deposition techniques can be used to produce oil-in-water emulsion droplets where cationic chitosan is absorbed onto anionic lecithin at the droplet interface (*7*, *8*, *13*). This layer-by-layer technique can be used to inhibit the oxidation of ω-3 fatty acids in oil-in-water emulsions presumably because it produces emulsion droplet interfacial membranes that are both cationic and thick. Klinkesorn and co-workers (*7*, *14*) showed that tuna oil-in-water emulsions stabilized with lecithin−chitosan membranes could be microencapsulated with corn syrup solids by either freeze or spray drying. This microencapsulated tuna oil was found to be more oxidatively stable than bulk tuna oil when stored at 37 °C (*15*). While layer-by-layer deposition technologies inhibit the oxidation of oil-in-water emulsions microencapsulated in corn syrup solids, it is unknown what will happen when the powders are added to a liquid food and the carbohydrate matrix dissipates, leaving the emulsion droplets exposed to the aqueous phase of the food.

The objective of this research was to evaluate the oxidative stability of spray-dried menhaden oil-in-water emulsions that

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are coated with a lecithin−chitosan multilayer system. The oil (core) to carbohydrate (wall) ratio could affect the oxidative stability of the microencapsulated emulsion during storage because the amount of wall material can influence the amount of lipid exposed to the environment (*16*, *17*). If the oil can effectively be microencapsulated with less carbohydrate, less spray-dried ω-3 oil powder will need to be added to functional foods to obtain the desired amount of fortification. Therefore, the oxidative stability of spray-dried powders prepared with different amounts of corn syrup solids was evaluated. An additional objective was to determine if the lecithin−chitosan multilayer system remained intact and inhibited lipid oxidation after reconstitution of the spray-dried powder into an aqueous system.

MATERIALS AND METHODS

Materials. Powdered chitosan (medium molecular weight, deacetylation 75−85) was acquired from Aldrich Chemical Co. (St. Louis, MO). Powdered lecithin (Ultralec P, moisture 1 wt %) was dontated by ADM-Lecithin (Decatur, IL). Corn syrup solids (CSS; Maltrin M250) were donated from Grain Processing Corp. (dextrose equivalent, 24; moisture 6.0%, ash 0.5%). Menhaden oil (Omega Protein Corp., Reedville, VA; eicosapentaenoic acid, 10−17%; docosahexenoic acid, 7−12%) was stored at −80 °C upon arrival and thawed in cold tap water 15 min prior to use. Disodiumethylenediaminetetraacetic acid (EDTA) was purchased from Sigma Chemical Co. (St. Louis, MO). Reagent grade chemicals were used otherwise. For preparation of solutions, distilled and deionized water was used.

Methods. *Preparation of Microencapsulated Emulsions.* A pH 3.0, 100 mM stock buffer solution was prepared with 2 mM sodium acetate and 98 mM acetic acid in water. The stock buffer solution was used to prepare an emulsifier solution containing 2.2 wt % lecithin. To ensure proper dispersion of the lecithin, the emulsifier solution was sonicated for 1 min at a frequency of 20 kHz, amplitude of 70%, and duty cycle of 0.5 s (model 500, sonic dismembrator, Fisher Scientific, Pittsburgh, PA). The pH of the opaque lecithin emulsifier solution was adjusted to 3.0 and stirred overnight to complete dispersion of the lecithin. The stock buffer solution was also used to prepare a chitosan solution containing 1.5 wt % chitosan. This solution was allowed to stir overnight to ensure hydration of the chitosan.

A primary menhaden oil-in-water emulsion was prepared by emulsifying 10% menhaden oil with 90% lecithin emulsifier solution (10 wt % oil, 2 wt % lecithin) using a high-speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), followed by 4 passes at 3000 psi in a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA). In between passes, the emulsion was captured in a beaker and submerged within an ice/water mixture to keep the emulsion as cool as possible. After homogenization, the primary emulsion was divided into two aliquots to which buffer (primary emulsion) or the chitosan solution (secondary emulsion) was added at a ratio of 1:1. The secondary emulsion was passed through a two-stage high-pressure valve homogenizer for two passes at 3000 psi to decrease bridging flocculation. The final emulsions had 5 wt % menhaden oil, 1 wt % lecithin, and 0.2 wt % chitosan. Prior to spray drying, corn syrup solids were added directly to the emulsions at concentrations ranging from 1 to 20 wt %. In some samples, EDTA (25 μM) was directly added to the emulsions. Emulsions were then stored overnight at 4 °C followed by drying with a centrifugal atomizer on a Niro spray dryer at a feed rate of 2.2 L/h at an inlet temperature of 180 °C (Nerco-Niro, Nicolas & Research Engineering Corp., Copenhagen, Denmark). Powders were reconstituted in pH 3, 100 mM (98 mM acetic acid and 2 mM sodium acetate) buffer at a concentration of 2.8 or 20 mg menhaden oil/mL buffer. The powder was stirred into buffer for 30 min to ensure proper dispersion. Reconstituted powder was place into 10 mL capped vials and stored at 37 °C in the dark.

Characterization of Microencapsulated and Reconstituted Emulsions. Emulsion droplet size was measured with a static light scattering particle sizer (Malvern Mastersizer model 3.01, Malvern Instruments, Worcestershire, U.K.). Samples were diluted with acetic acid/acetate buffer (pH 3.0) within the stirring chamber of the instrument to a concentration less than 0.02 wt % oil to prevent multiple scattering effects. Samples were continuously stirred to ensure a homogeneous sample. Dispersibility of spray-dried powders was tested within the stirring chamber of the laser light scattering unit (Malvern Mastersizer model 3.01, Malvern Instruments, Worcestershire, U.K.). The mean obscuration, or the fraction of light lost from the laser beam, was monitored over time after the powder is placed into the stirring cell (*18*). All powders (1−20 wt % CSS) were added at the same lipid concentration, equivalent to 0.005% oil. Surface charge of the emulsion droplets was determined by measuring the zeta potential (ZEM 5003, Zetamaster, Malvern Instruments, Worcs., UK; (*8*)).

Electron micrographs were obtained by affixing samples to 95% ethanol cleaned aluminum mounting stubs with conductive carbon tape. Edges of the tape and sample were painted with colloidal graphite to ensure conductivity. The samples were then dried in a vacuum to remove any volatile substances. Samples were then sputter coated with a Polaron E5100 Magnetron Cool Sputtering coater unit (2.2 kV for 2 min in the presence of low pressure argon). A JEOL JSM-5400 scanning electron microscope was used to observe the powdered samples.

Lipid oxidation in the samples was monitored by measuring lipid hydroperoxides, headspace propanal, and thiobarbituric acid reactive substances (TBARS) during storage in the dark at 37 °C. Microencapsulated, spray-dried samples were stored at 33% relative humidity by placing in a desiccator containing a saturated magnesium chloride solution. Lipid oxidation products in reconstituted emulsions were measured directly while microencapsulated, spray-dried samples were dissolved in the stock buffer solution at a concentration of 5% oil immediately prior to analysis. Lipid hydroperoxide was determined by an adapted method of Shantha and Decker (*19*). Samples were vortexed (three times at 10 s each) with 1.50 mL of an isooctanol + isopropanol solution (3:1 v:v). The samples were then centrifuged for 2 min at 3400*g* (Centrific TM Centrifuge, Fisher Scientific, Fairlawn, NJ). The upper organic layer was extracted and mixed with 2.80 mL of a methanol + butanol solution (2:1 v:v) and 0.03 mL of a 15 μM thiocyanate solution. After 20 min, the absorbance was measured at 510 nm.

Headspace propanal was measured using an adapted method of Mancuso and co-workers (*11*). Reconstituted emulsions (1 mL, 2.8 mg menhaden oil/mL buffer) were placed into 10 mL glass vials and sealed with aluminum seals containing poly-(tetrafluoroethylene) (PTFE)/butyl rubber septa. Prior to measurement, samples were heated to 45 °C within a Hewelett-Packard 19395A autosampler for 15 min. The autosampler injected 1 mL of headspace into a Shimatzu 17A gas chromatograph (Avondale, PA). Headspace and gas chromatograph conditions were as follows: sample temperature, 45 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; and injection, 1.0 min. Propanal was separated isothermally at 70 °C on a HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 μm film thickness). The splitless injector temperature was 180 °C, and the eluted compounds were detected with a flame ionization detector at 200 °C. Concentrations were determined from peak areas using a standard curve made from authentic propanal.

TBARS were measured using an adapted method of McDonald and Hultin (*20*). The thiobarbituric acid (TBA) solution was prepared with 15 g of trichloroacetic acid, 0.375 g of TBA, 1.76 mL of 12 N HCl, and 82.9 mL of water. TBA solution (100 mL) was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol. Powdered emulsion was reconstituted with pH 3, 100 mM (98 mM acetic acid and 2 mM) at a concentration of 0.1 wt % menhaden oil. For storage studies using reconstituted emulsions, samples were diluted with acetate buffer to obtain 0.1 wt % menhaden oil. The mixture of 1 mL of sample and 2 mL of TBA/butylated hydroxytolulene/ethanol solution was vortexed and heated in a boiling water bath for 15 min. The samples were cooled to room temperature and centrifuged at 3400*g* for 30 min. Absorbance of the supernatant was measured at 532 nm. TBARS concentrations were determined based upon a calibration curve prepared with 1,1,3,3-tetraethoxypropane.

Statistics. Experiments were preformed in duplicate, and all samples were analyzed in triplicate. Differences between treatments were analyzed using the Student's *t* test (*21*).

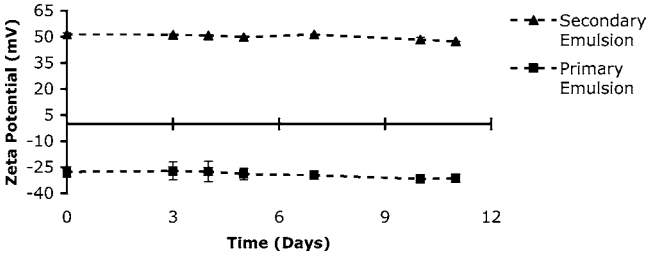

Figure 1. Zeta potential of primary and secondary emulsion droplets in stored (37 °C) reconstituted spray-dried menhaden oil-in-water emulsions containing 20% corn syrup solids.

RESULTS AND DISCUSSION

Characteristics of Reconstituted, Microencapsulated Emulsions. Previous work showed that lecithin−chitosan-stabilized dried tuna oil-in-water emulsions are more oxidatively stable than bulk tuna oil (*15*). However, for these encapsulated ω-3 fatty acids to be used effectively for food fortification, they should quickly disperse into the liquid phase of food products. In addition, an optimal microencapsulated ω-3 fatty acids delivery system would have antioxidative emulsion droplet characteristics (e.g., cationic emulsion droplet interfacial membrane) that would be retained after the microencapsulated emulsion is dispersed into an aqueous-based food. Therefore, initial experiments determined the physical characteristics and oxidative stability of spray-dried primary (lecithin) and secondary (lecithin−chitosan) menhaden oil-in-water emulsions encapsulated with 20% CSS after reconstitution into a liquid system.

To determine the ability of the primary and secondary emulsion droplet interfacial membranes to remain intact upon reconstitution of the emulsions encapsulated with 20% CSS into water, zeta potential measurements were recorded during storage at 37 °C (**Figure 1**). Reconstituted emulsion droplets stabilized with lecithin (primary emulsion) had an initial zeta potential of −29.5 mV, which did not change during 11 days of storage. Emulsion coated with the multilayer system of lecithin and chitosan (secondary emulsion) had an initial zeta potential of +50.9 mV, which again did not change during storage. The negative emulsion droplet charge for the primary emulsion indicates that anionic lecithin remained absorbed to the emulsion droplet surface after reconstitution and during storage. Similarly, cationic chitosan remained adhered to the anionic lecithin layer after reconstitution and during storage as can been seen by the positive zeta potential of the emulsion droplet.

The oxidative stability of the reconstituted microencapsulated primary and secondary emulsion powders (20% CSS) was determined by measuring lipid hydroperoxides (**Figure 2a**) and headspace propanal (**Figure 2b**). The spray-dried emulsions were reconstituted to obtain a concentration of 2.8 mg oil/mL buffer. This concentration was chosen to mimic the level of ω-3 fatty acids that would be added to a single serving of a beverage (200 mg of ω-3 fatty acids/240 mL). During storage at 37 °C, lipid hydroperoxides were detected in the primary emulsion after 3 days of storage with a maximum hydroperoxide concentration being observed at 4 days after which hydroperoxide concentrations decreased (**Figure 2a**). In the secondary emulsion, lipid hydroperoxides were much lower than in the primary emulsion, increasing gradually until they reached a maximum of 342.3 mmol/kg oil after 10 days of storage. Similar trends in oxidative stability between the two reconstituted emulsions were observed when headspace propanal was used to monitor lipid oxidation. Propanal was detected in the primary

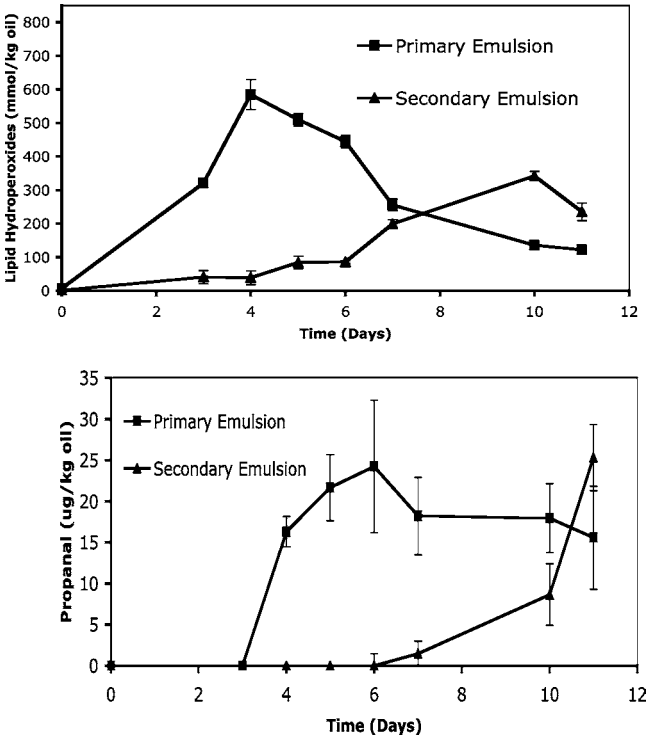

Figure 2. Development of (**a**) lipid hydroperoxides and (**b**) propanal between primary and secondary reconstituted, spray-dried menhaden oil-in-water emulsions containing 20% corn syrup solids stored at 37 °C.

emulsion after 4 days of storage and in the secondary emulsion after 7 days of storage (**Figure 2b**). The observation that the reconstituted microencapsulated lecithin−chitosan menhaden oil-in-water emulsion droplets are cationic and more oxidatively stable than the reconstituted microencapsulated lecithin emulsions suggests that the multilayer system is able to maintain a thick cationic layer around the emulsion droplet that can inhibit lipid oxidation by repulsion of iron and/or formation of a thick physical barrier that decreases lipid−prooxidant metal interactions.

Effect of Corn Syrup Solid Concentrations on the Stability of Microencapsulated Multilayered Emulsions. Recent work has shown that 20 wt % CSS is effective for the microencapsulation of spray-dried lecithin−chitosan stabilized menhaden oil-in-water emulsion (*15*). However, it would be more economical if a higher concentration of menhaden oil could be successfully microencapsulated into a spray-dried powder. To determine the amount of corn syrup solids necessary to successfully microencapsulate lecithin−chitosan menhaden oil-in-water emulsion, corn syrup solids were varied from 1% to 20%. Scanning electron microscopy (SEM) images show that spray-dried powders containing 5, 10, and 20 wt % CSS (**Figure 3a**−**c**) had discrete, individual particles, while emulsions spray-dried with 1 or 2 wt % CSS (**Figure 3d**,**e**) had particles that had extreme clumping and coalescence. This observation suggests that the 1 and 2 wt % CSS emulsions were not fully microencapsulated, and thus particles fused during the drying process.

The rate at which a powder disperses into liquid is an important quality for food powders. To measure dispersibility, spray-dried powders with varying concentrations of CSS were placed in the stirring cell of a laser diffraction unit at a constant oil concentration, and percent obscuration was monitored over time. Droplet obscuration increases as the powder disperses into solution and thus is a good measurement of the physical

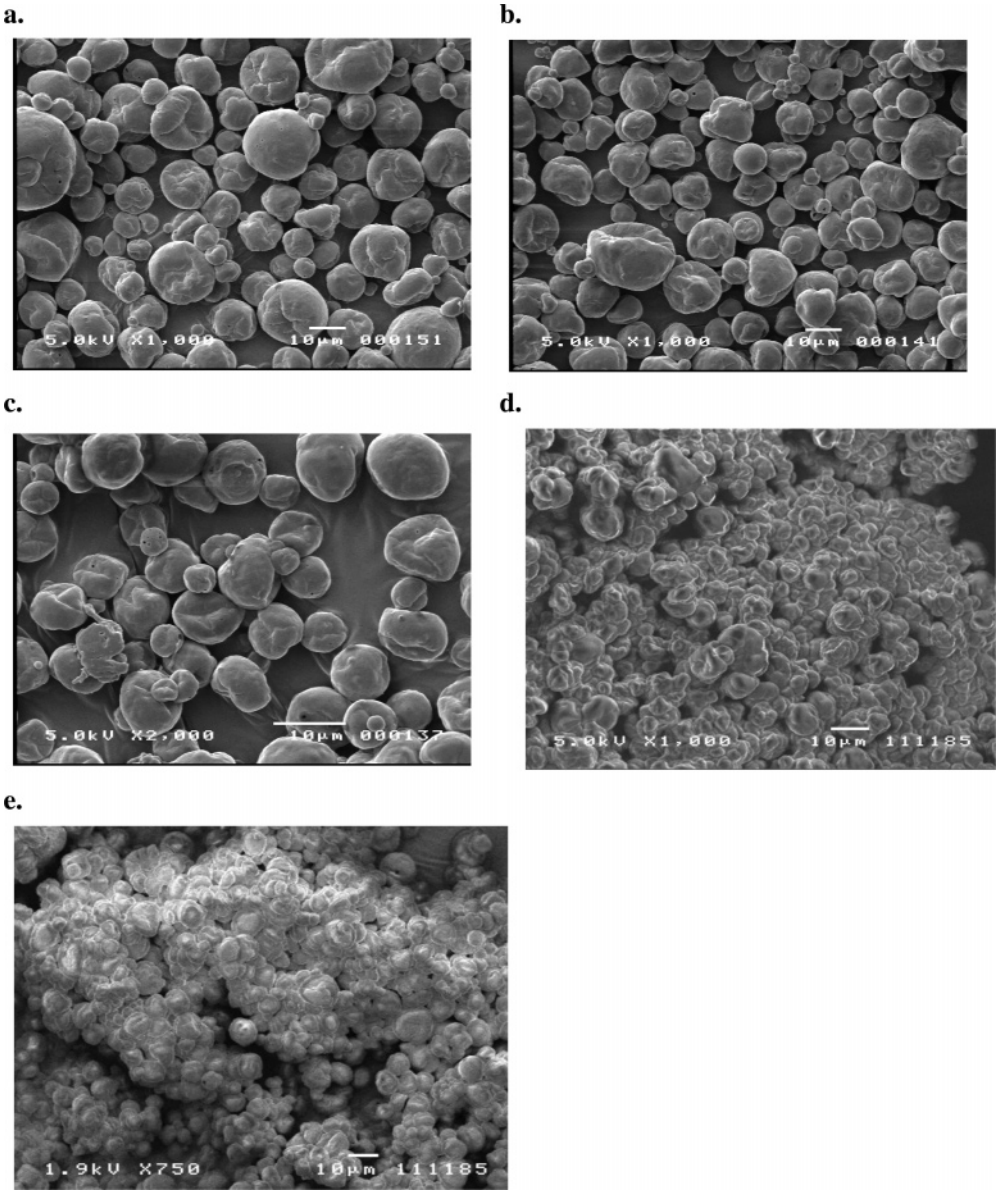

Figure 3. SEM pictures of fresh spray-dried powders, **a**–**e** (20, 10, 5, 2, and 1 wt % corn syrup solids, respectively).

properties of spray-dried powders (*15*, *22*). All powders had maximum obscuration 3 min after addition to the stirring cell of the particle sizer (**Figure 4**). Samples containing 10 and 20 wt % CSS exhibited the maximum obscuration, reaching 21−23%. Emulsions spray-dried with 5 wt % CSS had intermediate obscuration, while the lowest obscuration was observed in samples with 1 and 2 wt % CSS. The low obscuration values observed in the powders with 1 and 2 wt % CSS indicate that the powders did not efficiently disperse into the aqueous phase. This is likely due to the clumping and coalescence of the dried particles that were observed in the electron micrographs (**Figure 3d**,**e**).

The mean particle diameter of spray-dried emulsion droplets was measured during storage of the powders at 37 °C and 33% RH after reconstitution into buffer at pH 3 to a concentration of 2% oil (**Figure 5**). The powders with the lowest amount of CSS (1−5%) had the highest initial particle sizes (7.0−10.2 μm) as compared to powders made with 10% or 20% CSS (0.8−1.9 μm). The particle size of the emulsion droplets increased in size during storage of all of the reconstituted powders. However, this increase in particle size was most dramatic for powders prepared with 1−5% CSS. These data suggest that the powders with lower amounts of CSS not only had more coalescence after spray drying as evidenced by an increase in initial particle size but also were more susceptible to coalescence during storage.

Oxidative stability of the microencapsulated emulsion powder was monitored by measuring lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) over time at storage of 37 °C and 33% RH (**Figure 6a** and **b**). Because of the large number of samples used in subsequent experiments, TBARS was used as a marker of a secondary oxidation product instead of headspace propanal. While propanal is a more specific marker of lipid oxidation than TBARS, the experimental protocol for headspace propanal is too time-consuming to measure a large number of samples over a short time period. The spray-dried powders prepared with 1 and 2 wt % CSS powders oxidized very rapidly with increases in lipid hydroperoxides and TBARS being detected after 4 and 11 days, respectively. Powder prepared with 10% CSS was the next most unstable with lipid hydroperoxides and TBARS increasing over a time frame similar to that of the 1% and 2% CSS samples. However, the 10% CSS samples generally had lower amounts of lipid hydroperoxides and TBARS than samples with 1% and 2% CSS. The most

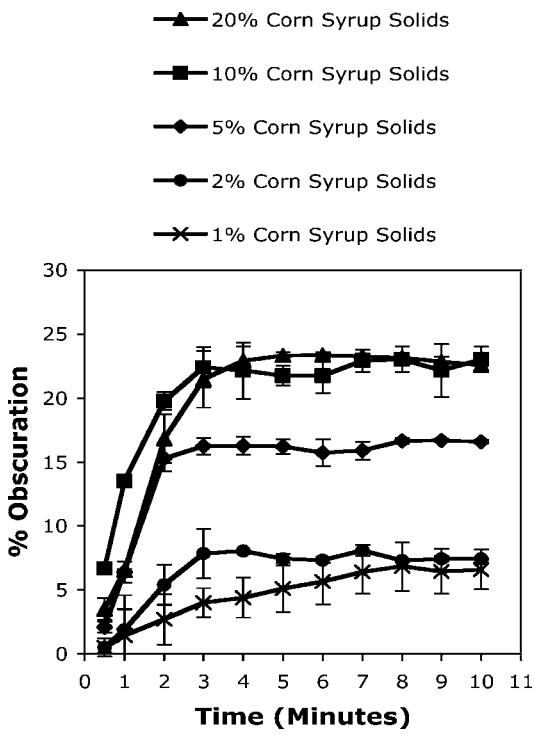

Figure 4. Influence of stirring time on the obscuration of spray-dried menhaden oil powders, containing various amounts of corn syrup solids (20–1 wt %) at day 0.

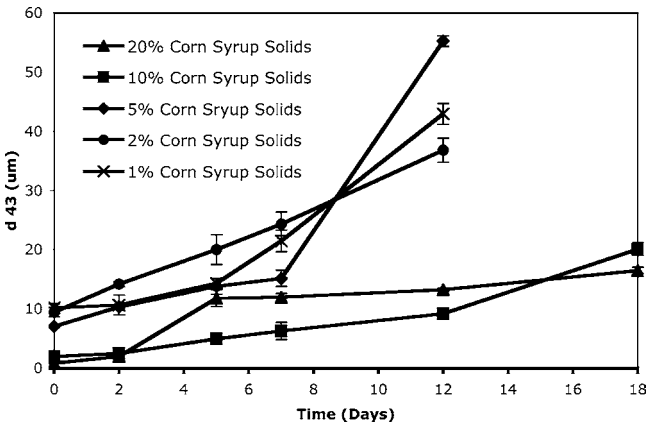

Figure 5. Particle size of spray-dried menhaden oil-in-water emulsion powder, stored at 37 °C at 33% RH.

oxidatively stable powder contained 5% CSS as its lipid hydroperoxides, and TBARS was the lowest of all samples after 13 days of storage.

As mention earlier, for an encapsulated ω-3 powder to be used successfully for the fortification of food, it must be stable in the powder as well as once it is dissolved into a food product. Therefore, in the next series of experiments, spray-dried powders stored for 0, 6, or 11 days were reconstituted into buffer, and oxidative stability of the dispersed emulsions was determined during storage. Freshly spray-dried emulsions (day 0) containing 1−20% CSS had no significant ($p \leq 0.05$) differences in lipid hydroperoxides or TBARS (**Figure 6a** and **b**). Therefore, it was not surprising that the oxidative stability of these dried emulsions upon reconstitution into an aqueous phase was similar with the exception of the sample with 2% CSS, which had higher lipid hydroperoxides and TBARS (**Figure 7a** and **b**). After the powders were stored for 6 days, lipid hydroperoxides in the powders were increased in samples containing 1%, 2%, and

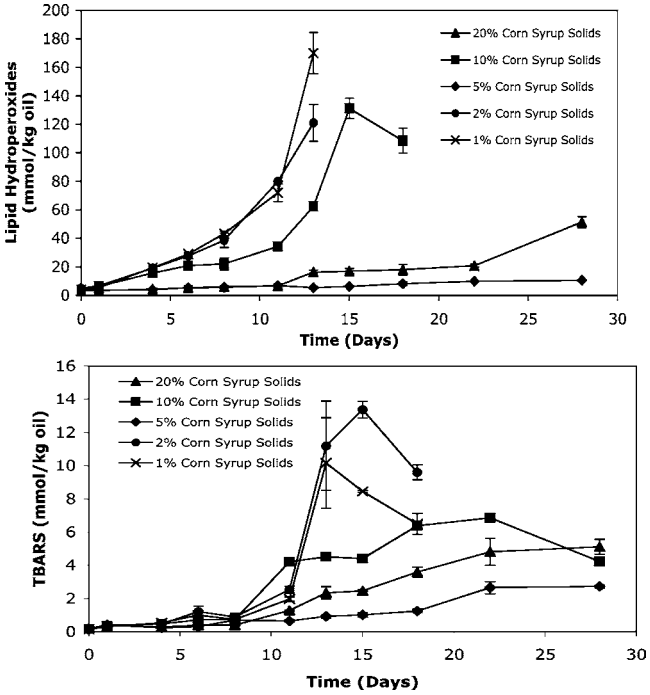

Figure 6. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in spray-dried menhaden oil powders containing various amounts of corn syrup solid (20–1 wt %) during storage at 37 °C and 33% RH.

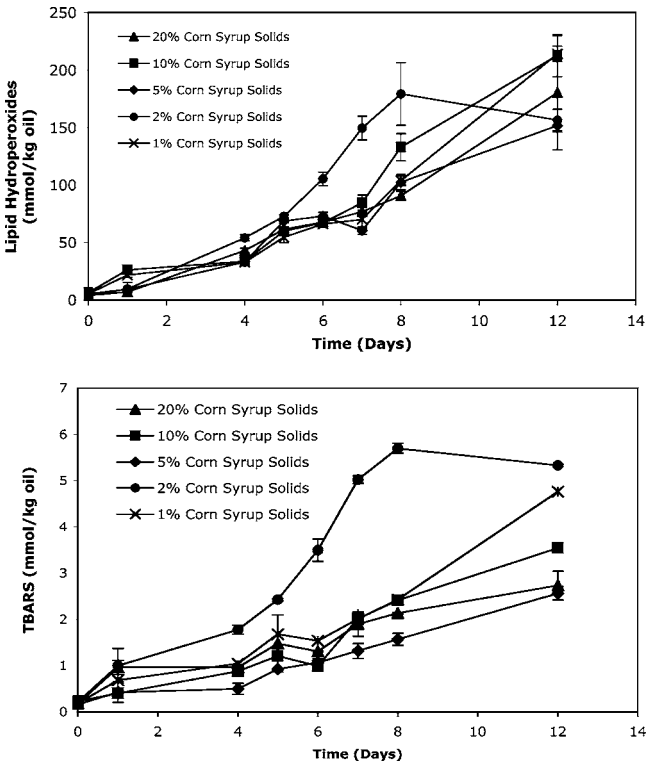

Figure 7. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in spray-dried menhaden oil "day 0" reconstituted powders containing various amounts of corn syrup solid (20–1 wt %) during storage at 37 °C.

10% CSS, while TBARS remained low in all samples (**Figure 6a** and **b**). Upon reconstitution of the 6 day old powder, samples containing 1% and 2% CSS had the most rapid oxidation with formation of lipid hydroperoxides (**Figure 8a**) and TBARS (**Figure 8b**) increasing after 5 days of storage. Reconstituted 6 day old powder with 10% CSS had levels of lipid hydroperoxides similar to those of samples with 5% and 20% CSS but

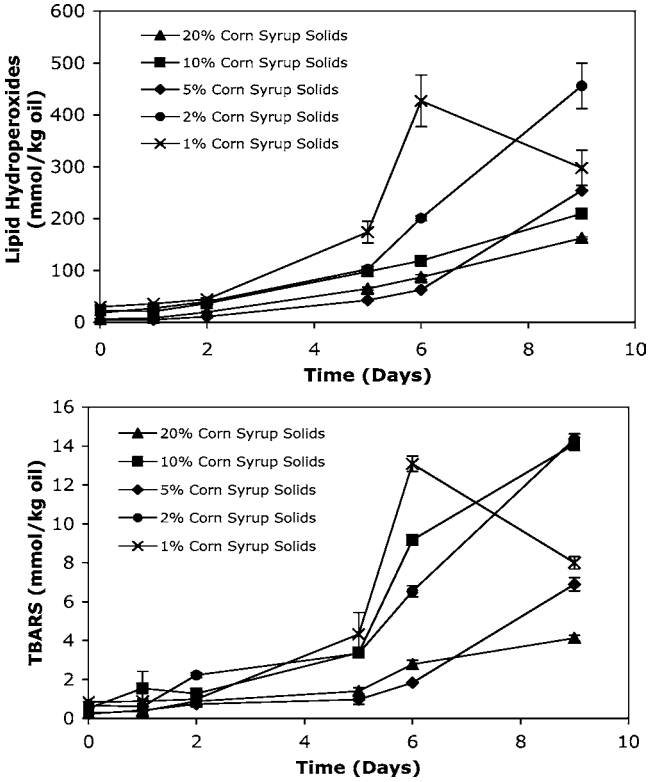

Figure 8. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in spray-dried menhaden oil "day 6" reconstituted powders containing various amounts of corn syrup solid (20–1 wt %) during storage at 37 °C.

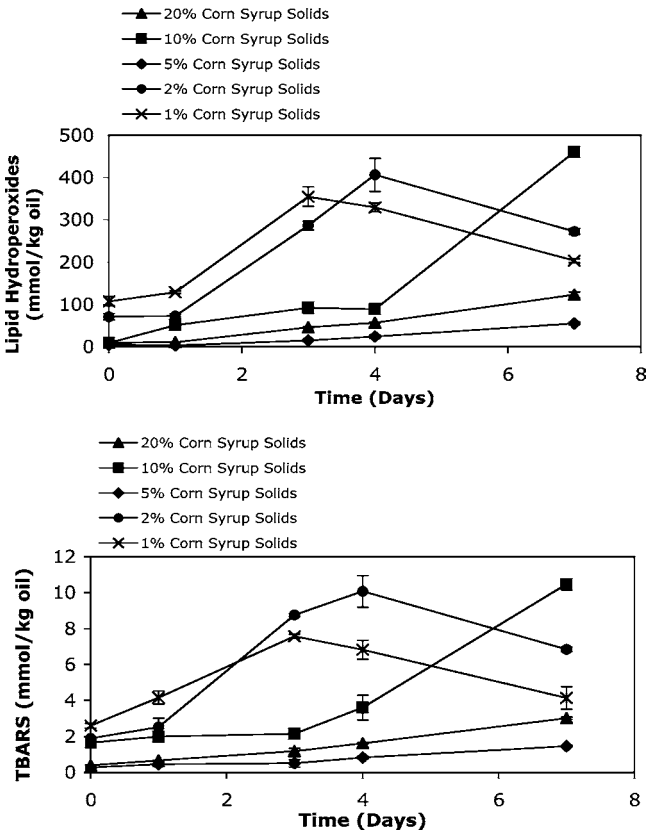

Figure 9. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in spray-dried menhaden oil "day 11" reconstituted powders containing various amounts of corn syrup solid (20–1 wt %) during storage at 37 °C.

had TBARS formation rates similar to those of samples with 1% and 2% CSS. After 11 days of storage, lipid hydroperoxide concentrations in the powders were in the order of 1% CSS = 2% CSS > 10% CSS > 5% CSS = 20% CSS, while TBARS was in the order of 10% CSS > 1% CSS = 2% CSS > 5% CSS = 20% CSS (**Figure 9a** and **b**). Upon reconstitution of the 11 day old powders, lipid hydroperoxides and TBARS increased in samples with 1% and 2% CSS after 1 day of storage. In samples with 10% CSS, lipid hydroperoxides and TBARS increased rapidly after 4 and 3 days of storage, respectively, while samples with 5% and 20% CSS had low lipid hydroperoxides and TBARS concentrations throughout storage.

Overall, as the levels of lipid hydroperoxides and TBARS increased in the microencapsulated emulsion powders (**Figure 6**), the oxidation stability of the emulsion droplets upon reconstitution into an aqueous phase decreased. This is not surprising because the presence of preformed lipid hydroperoxides in the stored powders would provide a substrate that could be decomposed into prooxidative free radicals by transition metals upon reconstitution of the powder into water.

Increasing the concentrations of CSS increased the microencapsulation of the emulsions as can be seen by the electron micrographs (**Figure 3**). At CSS concentrations above 5%, the powders had similar visual properties. However, the powder with 5% CSS was less physically stable, as can be seen by a higher particle size and lower percent obscuration than microencapsulated powders with 10% or 20% CSS. The oxidative stability of spray-dried lipids has been associated with the amount of exposed or non-encapsulated lipids in the powders. Therefore, it was surprising to see that the emulsions microencapsulated with 10% CSS were less oxidatively stable than emulsions microencapsulated with 5% CSS. Reducing sugars have been reported to be prooxidative possibly through their ability to reduce and activate transition metals (*23*). Therefore, it is possible that the CSS in the spray-dried powders have both protective and destabilizing activities with lower amounts of CSS being less prooxidative by having less interactions with transition metals, while high concentrations of CSS are protective by more effectively encapsulating the emulsified lipid. Thus, the powders with 5% CSS were more oxidatively stable than powders with 10% CSS due to lower reducing sugar concentrations, while powders with 10% CSS were less oxidatively stable than powders with 20% CSS due to less efficient microencapsulation.

Influence of EDTA on the Oxidative Stability of Reconstituted Microencapsulated Powders. Transition metals and in particular iron are a major prooxidant in oil-in-water emulsions (*11*, *12*). EDTA is one of the most effective metal chelators available for use in food emulsions to inhibit lipid oxidation (*11*, *12*, *24*). Therefore, the oxidative stability of the reconstituted microencapsulated emulsions could be improved with addition of EDTA to the aqueous system. To test this, emulsions were microencapsulated with 20% CSS because microencapsulation with 20% CSS gave both good oxidative and physical stability. EDTA (25 μM) significantly ($p \leq 0.05$) inhibited the formation of both lipid hydroperoxides (**Figure 10a**) and headspace propanal (**Figure 10b**) in microencapsulated primary (lecithin) and secondary (lecithin + chitosan) emulsions reconstituted into buffer (pH 7.0). In previous studies, EDTA has shown to be an effective metal chelator that can physically remove prooxidative transition metals from the surface and interior of emulsion droplets (*25*, *26*). Also, EDTA can bind metals and inhibit redox cycling, thus decreasing the overall activity of the metal (*27*). Inhibition of lipid oxidation in the reconstituted primary and secondary dried emulsion suggests

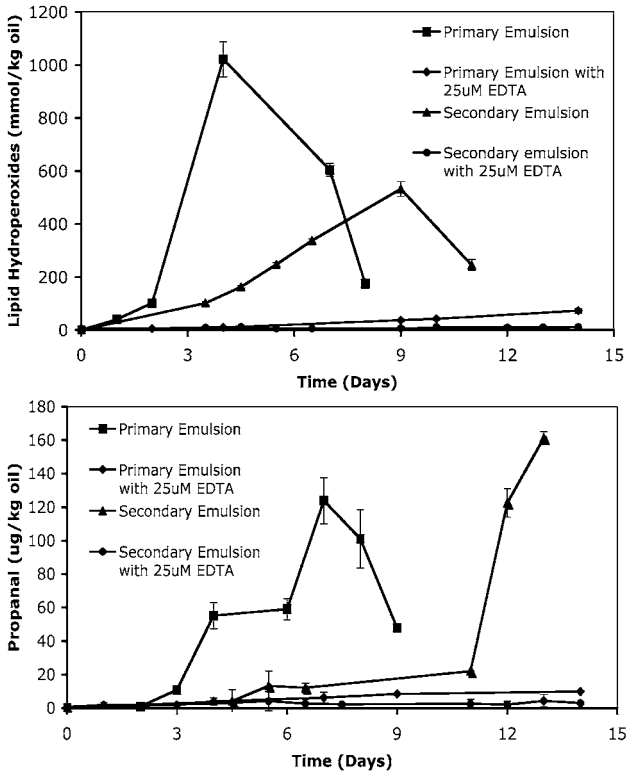

Figure 10. Development of (**a**) lipid hydroperoxides and (**b**) propanal between primary, secondary, and primary and secondary, both with 25 μM EDTA, reconstituted, spray-dried menhaden oil-in-water emulsions stored at 37 °C.

that the main cause of lipid oxidation within the system is due to prooxidant metals. Increased oxidative stability of the secondary emulsion in the presence of EDTA suggests that either the cationic, thick multilayer emulsifier system is not able to completely inhibit iron−lipid interactions or EDTA is increasing the partitioning of transition metals out of the lipid core of the emulsion droplet, thus inhibiting lipid oxidation in the lipid phase.

Conclusions. In conclusion, this study has shown that the interfacial properties of microencapsulated lecithin−chitosan multilayer emulsion droplets remain intact upon reconstitution into an aqueous system. The reconstituted secondary (lecithin−chitosan) emulsion was more oxidatively stable than the reconstituted primary (lecithin) emulsion. Use of high amounts of corn syrup solids (10% or 20%) to microencapsulate multilayer emulsions resulted in more physically stable powders than when CSS was added at 1−5%. Maximum oxidative stability was observed at 5% and 20% CSS, suggesting that CSS could have both prooxidative and antioxidative properties in the microencapsulated powders. EDTA (25 μM) increased the oxidative stability of the menhaden oil in both reconstituted primary and secondary emulsion. Overall, these data suggest that microencapsulation of menhaden oil-in-water emulsions stabilized with a multilayer system consisting of lecithin and chitosan could be an effective technology to produce an ω-3 fatty acid delivery system for use in functional foods.

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