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Influence of Environmental Conditions on the Stability of Oil in Water Emulsions Containing Droplets Stabilized by Lecithin−Chitosan Membranes

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Oil-in-water emulsions containing cationic droplets stabilized by lecithin−chitosan membranes were produced using a two-stage process. A primary emulsion containing anionic lecithin-coated droplets was prepared by homogenizing oil and emulsifier solution using a high-pressure valve homogenizer (5 wt % corn oil, 1 wt % lecithin, 100 mM acetic acid, pH 3.0). A secondary emulsion containing cationic lecithin−chitosan-coated droplets was formed by diluting the primary emulsion with an aqueous chitosan solution (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, and 0.036 wt % chitosan). The stabilities of the primary and secondary emulsions with the same oil concentration to thermal processing, freeze−thaw cycling, high calcium chloride concentrations, and lipid oxidation were determined. The results showed that the secondary emulsions had better stability to droplet aggregation during thermal processing (30−90 °C for 30 min), freeze−thaw cycling (−10 °C for 22 h/30 °C for 2 h), and high calcium chloride contents (≤500 mM $CaCl_2$) and exhibited less lipid oxidation (peroxide formation) than primary emulsions. The interfacial engineering technology used in this study could lead to the creation of food emulsions with improved stability to environmental stresses.

KEYWORDS: Emulsion; chitosan; lecithin; stabilit; ζ-potential

INTRODUCTION

Traditionally, oil-in-water emulsions are produced in the food industry by homogenizing an oil phase and an aqueous phase together in the presence of an emulsifier (*1*−*3*). An emulsifier is a surface active ingredient that adsorbs to the surface of freshly formed droplets during homogenization (*3*−*9*). Emulsifiers usually decrease the average size of the droplets in emulsions produced by homogenization because they reduce the interfacial tension, thereby facilitating droplet disruption (*7*). Emulsifiers also improve the stability of emulsions to droplet aggregation by generating repulsive forces between the droplets and/or by forming interfacial membranes around the droplets that are resistant to rupture (*1*, *2*). In general, a good emulsifier should rapidly adsorb to the surface of the oil droplets formed during homogenization, it should lower the interfacial tension appreciably, and it should protect the droplets against aggregation during emulsion processing, storage, and utilization (*2*, *7*, *10*). A wide variety of different kinds of synthetic and natural emulsifiers can be legally used in food emulsions, including small-molecule surfactants, phospholipids, proteins, and polysaccharides (*3*, *8*, *9*). These emulsifiers vary considerably in their ability to form and stabilize emulsions, with each type having its own particular advantages and disadvantages. For example, some emulsifiers are highly effective at generating small emulsion droplets during homogenization but are less effective at providing long-term stability against droplet aggregation and vice versa (*10*).

Oil-in-water (O/W) emulsions with improved stability can be created by combining the beneficial attributes of different kinds of emulsifiers to form multilayered membranes around the oil droplets (*11*−*14*). In the present study, we utilize a protocol recently developed to create O/W emulsions containing small cationic droplets coated with lecithin−chitosan membranes (*14*). A *primary* emulsion containing small anionic droplets coated with a lecithin membrane is produced by homogenizing oil and water together in the presence of lecithin, a low molecular weight emulsifier that rapidly adsorbs to the surface of oil droplets during homogenization. A *secondary* emulsion containing droplets coated with a lecithin−chitosan membrane is then produced by adding chitosan to the primary emulsion. The electrical charge on the droplets increased from highly negative (−49 mV) to highly positive (+54 mV) as the chitosan concentration was increased, which indicated that chitosan adsorbed to the droplet surfaces (*14*). The mean particle diameter of the emulsions increased dramatically and the emulsions became unstable to creaming when the chitosan concentration exceeded a certain level, which was attributed to charge neutralization and bridging flocculation effects. Nevertheless, relatively stable secondary emulsions could be prepared by adding enough chitosan to ensure that the droplets had a high positive charge (and were thus capable of generating strong electrostatic repulsive forces) and then breaking down any flocs formed by the application of disruptive energy, for example, blending, homogenization, or sonication (*14*). We postulate that

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a two-layer membrane consisting of an inner layer of lecithin and an outer layer of chitosan surrounds the droplets in the secondary emulsions. Nevertheless, the precise structural organization of the molecules within the membrane and the physicochemical characteristics (e.g., thickness and rheology) of the membrane still need to be established.

The production of emulsions containing cationic droplets surrounded by multilayers may have a number of important applications in the food industry. For example, positively charged droplets are less susceptible to destabilization by multivalent cations, such as calcium (*15*, *16*). Lipids in cationic droplets are much less susceptible to iron-catalyzed oxidation because of the electrostatic repulsion between the droplet surface and cationic iron ions (*17*, *18*). Droplets coated with a relatively thick interfacial layer of emulsifier may also have better stability to aggregation (*11*−*13*) and to lipid oxidation (*19*) than those coated with thinner layers. The objective of this study was to compare the stability of the primary and secondary emulsions produced using the procedure described above to thermal processing, freeze−thaw cycling, high calcium chloride concentrations, and lipid oxidation. Our ultimate goal is to produce emulsions with improved stability and physicochemical characteristics that can be produced economically using food grade ingredients.

MATERIALS AND METHODS

Materials. Powdered chitosan (medium molecular weight; deacetylation, 81%; viscosity of 1 wt % solution in 1 wt % acetic acid, 286 Cps; moisture, 4.6 wt %; ash, 0.5 wt %) was obtained from Aldrich Chemical Co. (St. Louis, MO). Chitosan is the partially deacetylated form of chitin and has a chemical structure that consists of 2-acetamido-2-deoxy-β-D-glucose monomers attached via a β1−4 linkage (*14*). Chitosan has a positive charge in acidic solutions due to the presence of protonated amino groups along its backbone that have pK_a values between 6.3 and 7.0 (*14*). Powdered lecithin (Ultralec P; acetone insolubles, 97.5%; acid value, 27.9 mg/g; peroxide value, 0.9 mequiv/kg; moisture, 0.77 wt %) was donated from ADM-Lecithin (Decatur, IL). Analytical grade sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium azide (NaN_3), sucrose, and sorbitol were purchased from the Sigma Chemical Co. (St. Louis, MO). Calcium chloride (fine granulated, $CaCl_2$) was purchased from Fisher Science (Chicago, IL). Distilled and deionized water was used for the preparation of all solutions.

Solution Preparation. A stock buffer solution was prepared by dispersing 100 mM acetic acid in water (containing 0.02 wt % sodium azide as an antimicrobial agent) and then adjusting the pH to 3.0 using 1 M HCl. A chitosan solution was prepared by dispersing 0.2 wt % powdered chitosan into stock buffer solution. An emulsifier solution was prepared by dispersing 1.0 wt % lecithin powder into stock buffer solution. The emulsifier solution was sonicated for 30 s at a frequency of 20 kHz, amplitude of 40%, and duty cycle of 0.5 s (model 500, Sonic Disembrator, Fisher Scientific, Pittsburgh, PA) to disperse the lecithin. The pH of the solution was adjusted back to 3.0 using HCl, and then the solution was stirred for ∼1 h to ensure complete dissolution of the lecithin.

Emulsion Preparation. A stock primary emulsion was prepared by homogenizing 5 wt % corn oil with 95 wt % aqueous emulsifier solution in a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Basel, Switzerland) followed by one pass at 5000 psi through a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA). This stock emulsion was diluted with either buffer solution or an aqueous chitosan solution to form a primary emulsion (0 wt % chitosan) and a secondary emulsion (0.036 wt % chitosan) with the same oil concentration (1 wt % corn oil, 0.2 wt % lecithin, 100 mM acetic acid, pH 3.0). Any flocs formed in the secondary emulsion were disrupted by passing it once through a high-pressure valve homogenizer at a pressure of 4000 psi as described previously (*14*).

Emulsion Environmental Stresses. We compared the influence of various kinds of environmental stresses on the mean particle diameter and ζ-potential of primary and secondary emulsions with the same oil concentration (1 wt %).

Thermal Processing. Emulsion samples (5 mL) were transferred into glass test tubes (internal diameter = 16 mm, height = 160 mm), which were then stored in a water bath for 30 min at a fixed temperature ranging from 30 to 90 °C. The emulsion samples were then placed immediately into a 30 °C water bath, where they were stored prior to analysis.

Freeze−Thaw Cycling Stability. Sucrose (10 wt %), sorbitol (10 wt %), sucrose (5 wt %) + sorbitol (5 wt %), and water (10 wt %) (no additive) were mixed with primary and secondary emulsions. Emulsion samples (5 mL) were transferred into plastic test tubes (internal diameter = 15 mm, height = 150 mm), which were frozen by placing them in a −10 °C freezer for 22 h and then thawed by placing them in a water bath at 30 °C for 2 h. This freeze−thaw cycle was repeated six times, and its influence on emulsion properties was measured after each cycle.

$CaCl_2$. Emulsions containing different $CaCl_2$ concentrations (0−1000 mM) were prepared by adding powdered calcium chloride to primary and secondary emulsions. After the $CaCl_2$ was dissolved, the pH of the emulsions was adjusted back to 3.0 using HCl or NaOH. Emulsion samples (5 mL) were then transferred into glass test tubes (internal diameter = 16 mm, height = 160 mm) and stored at room temperature prior to analysis.

Lipid Oxidation. Oxidation stability at room temperature was evaluated by measuring lipid peroxides using a modified method of Shentha and Decker (*20*). Emulsion samples (0.3 mL) were added to 1.5 mL of isooctane−2-propanol followed by vortexing three times for 10 s each and centrifuging for 2 min at 2000*g*. Next, the organic phase (0.2 mL total volume containing 0.015−0.2 mL of lipid extract) was added to 2.8 mL of methanol/butanol (2:1 v/v), followed by 15 μL of thiocyanate solution (3.94 M) and 15 μL of ferrous iron (0.072 M acid solution). The solution was vortexed, and then the absorbance at 510 nm was measured after 20 min. Lipid peroxide concentrations were determined using a cumene hydroperoxide standard curve (*20*).

Particle Size Measurements. Concentrated emulsions were diluted to a droplet concentration of ∼0.005 wt % using buffer solution (pH 3) prior to analysis to avoid multiple scattering effects. The particle size distribution of the emulsions was then measured using a laser light scattering instrument (Horiba LA-900, Irvine, CA). This instrument measures the angular dependence of the intensity of laser light (λ = 632.8 nm) scattered by a dilute emulsion and then finds the particle size distribution that gives the best agreement between theoretical predictions and experimental measurements. A refractive index ratio of 1.08 was used in the calculations of the particle size distribution. Particle size measurements are reported as weight-average mean diameters, d_{43} ($= \Sigma n_i d_i^4 / \Sigma n_i d_i^3$, where n_i is the number of particles with diameter d_i). Mean particle diameters were calculated as the average of measurements made on at least two samples, with standard deviations being less than 5% for nonaggregated droplets and 20% for aggregated droplets. It should be noted that the theory used to calculate the particle size distribution assumes that the particles are spherical and homogeneous, and therefore the data obtained on emulsions that contained flocs should be treated with caution because they are nonspherical and nonhomogeneous.

ζ-Potential Measurements. Concentrated emulsions were diluted to a droplet concentration of ∼0.005 wt % using buffer solution (pH 3) prior to analysis. Emulsions were then injected into the measurement chamber of a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcs., U.K.), and the ζ-potential was determined by measuring the direction and velocity that the droplets moved in the applied electric field. The ζ-potential measurements are reported as the average and standard deviation of measurements made on at least two samples, with 10 instrument readings taken per sample.

RESULTS AND DISCUSSION

Influence of Thermal Processing on Emulsion Stability. The purpose of these experiments was to examine the influence of thermal processing on the stability of emulsions containing

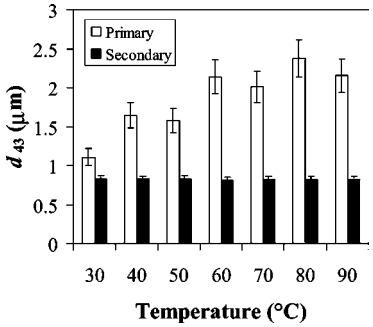

Figure 1. Dependence of mean particle diameter on isothermal storage temperature (30–90 °C for 30 min) for primary and secondary emulsions.

droplets coated by a lecithin membrane (primary emulsion) as compared with those coated by a lecithin−chitosan membrane (secondary emulsion). Primary and secondary emulsions with the same oil concentration (1 wt %) were held at temperatures ranging from 30 to 90 °C for 30 min, then cooled to room temperature, and stored for 1 week. There was no significant effect of heating on the ζ-potential of the negatively charged droplets in the primary emulsion (−43 ± 3 mV) or on the positively charged droplets in the secondary emulsion (50 ± 3 mV). Nevertheless, thermal processing did have a significant impact on the stability of some of the emulsions to droplet aggregation (**Figure 1**). There was no significant change in the mean diameter of the particles in the secondary emulsion with holding temperature (0.82 ± 0.04 μm), but there was a significant increase in the size of the particles in the primary emulsion, with d_{43} increasing from 1.1 ± 0.2 μm in the emulsions stored at 30 °C to 2.2 ± 0.4 μm in the emulsions stored at 90 °C. These results clearly indicated that the thermal stability of lecithin-stabilized emulsions could be improved by coating them with a chitosan layer. The lecithin−chitosan-coated droplets may have been more stable to aggregation during heating for a number of reasons. First, dehydration of the hydrophilic headgroups of the lecithin molecules at elevated temperatures may have changed their optimum spontaneous curvature, leading to droplet coalescence (*21*−*24*). This type of coalescence would have been prevented when the droplets were coated with chitosan because the biopolymer layer would have prevented the lecithin layers on different droplets from coming into close contact. Second, the electrostatic repulsion between the droplets in the secondary emulsion would have been greater than those in the primary emulsion due to the higher magnitude of the ζ-potential in the former (*21*, *25*).

Influence of Freeze—Thaw Cycling on Emulsion Stability. The purpose of these experiments was to examine the influence of freeze−thaw cycling on the stability of primary and secondary emulsions. Preliminary experiments indicated that all of the emulsions were unstable to freeze−thaw cycling in the absence of cryoprotectants. For this reason, we also examined the freeze−thaw stability of primary and secondary emulsions containing cryoprotectants commonly used in the food industry (10 wt % sucrose; 10 wt % sorbitol; 5 wt % sucrose + 5 wt % sorbitol). Emulsions were subjected to six freeze−thaw cycles consisting of 22 h at −10 °C in a freezer followed by 2 h at 30 °C in a water bath. As mentioned earlier, all of the emulsions were unstable to freeze−thaw cycling in the absence of cryoprotectants, there being a >10-fold increase in mean particle diameter after the first cycle in both primary and secondary emulsions (**Figure 2**). A number of physicochemical mechanisms may be responsible for the extensive droplet aggregation observed in the absence of cryoprotectants. First, when the emulsions were placed in the freezer, some of the water

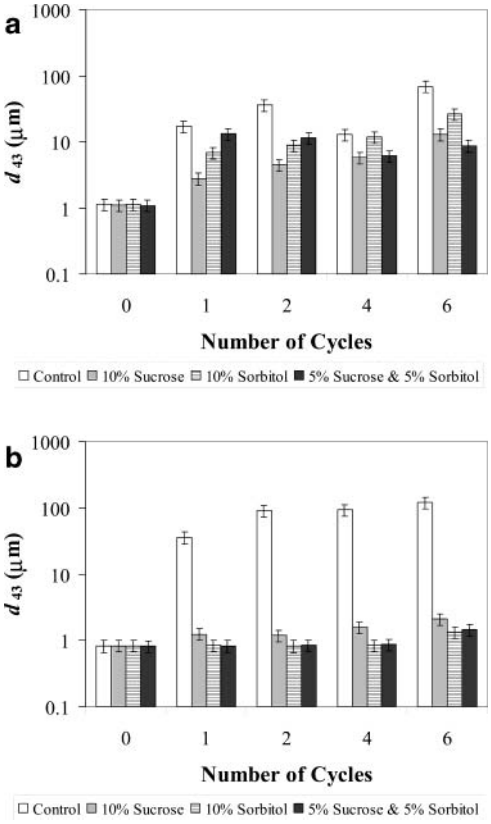

Figure 2. Dependence of mean particle diameter of (a) primary emulsions and (b) secondary emulsions on number of freeze–thaw cycles (–10 °C for 22 h/30 °C for 2 h) in the absence and presence of cryoprotectants.

crystallized, which caused the droplets to come into closer proximity because they were confined to the nonfrozen regions remaining in the aqueous phase (*26*). As more and more water crystallized the droplets would have been forced closer together (*26*), and there may not have been sufficient free water present to fully hydrate the droplet surfaces (*27*−*29*), thus favoring droplet−droplet interactions. Second, ice crystallization leads to an increase in the ionic strength of the freeze-concentrated nonfrozen aqueous phase surrounding the emulsion droplets (*28*), and previous studies have shown that both primary and secondary emulsions are unstable to aggregation at high ionic strength (*14*). Third, it is possible that ice crystals formed during freezing may have penetrated into the oil droplets and disrupted their interfacial membranes, thus making them more prone to coalescence. Fourth, cooling may have caused some of the fat in the emulsion droplets to crystallize, which may have promoted partial coalescence due to penetration of a fat crystal from one droplet through the membrane of another droplet (*30*, *31*).

In general, the presence of cryoprotectants improved the stability of emulsions to droplet aggregation during freeze−thaw cycling, as well as highlighting differences between the stability of primary and secondary emulsions (**Figure 2**). For the primary emulsion, the addition of 10% sucrose provided the best protection against droplet aggregation during freeze−thaw cycling (**Figure 2a**). Nevertheless, there was still a significant increase in mean particle diameter after the first cycle (d_{43} increasing from 1.1 ± 0.2 to 2.8 ± 0.5 μm) and extensive droplet aggregation after six cycles (d_{43} increasing to 13 ± 2 μm). These results showed that droplet aggregation could be retarded, but not completely prevented, by the addition of cryoprotectants to emulsions stabilized by lecithin only. By contrast, the presence of cryoprotectants in the secondary emulsions during freeze−thaw cycling greatly improved the

stability to droplet aggregation (**Figure 2b**). For example, after one cycle there was no significant change in the mean particle diameter in the secondary emulsion containing 10% sorbitol (d_{43} going from 0.83 ± 0.04 to 0.84 ± 0.04 μm), and even after six cycles d_{43} increased to only 1.3 ± 0.2 μm. In the secondary emulsions, the effectiveness of the cryoprotectants at preventing droplet growth during freeze−thaw cycling increased in the following order: 5 wt % sucrose & 5 wt % sorbitol ≈ 10 wt % sorbitol > 10 wt % sucrose. The origin of the observed differences in the effectiveness of the various cryoprotectants at stabilizing the emulsions against freeze−thaw cycling is currently not known. It is likely to depend on the precise physicochemical mechanism(s) by which the cryoprotectants exhibit their protective effects.

A number of mechanisms have been proposed to account for the ability of cryoprotectants to improve the stability of emulsions to aggregation during freeze−thaw cycling. First, cryoprotectants increase the osmolyte concentration in the aqueous phase, thereby reducing its crystallization temperature, limiting the total amount of ice crystals formed and increasing the volume of nonfrozen aqueous phase available to the oil droplets (*28*). Second, cryoprotectants form hydrogen bonds with emulsifiers adsorbed to droplet surfaces, thereby reducing the tendency for interactions to occur between droplet surfaces when the free water content is reduced by ice crystallization (*29*). Third, the presence of high concentrations of cryoprotectants in the aqueous phase may alter the thermal transition temperatures of certain types of emulsifier, for example, phospholipids and proteins (*26*, *32*). The fact that the secondary emulsions were more stable to freeze−thaw cycling than the primary emulsions may also have been due to a number of different mechanisms. First, the interfacial layer in the secondary emulsions is thicker than that in the primary emulsion, so there will be a greater short-range steric repulsion between the droplets that prevents them from coming close enough to coalesce (*21*, *25*). Second, it may be more difficult for fat or ice crystals to rupture relatively thick lecithin−chitosan membranes than relatively thin lecithin membranes, thereby making the droplets in the secondary emulsion more stable to droplet coalescence or partial coalescence than those in the primary emulsion (*33*, *34*). Third, previous studies have shown that droplets coated with lecithin−chitosan membranes are more stable to aggregation at high salt concentrations than droplets coated with lecithin membranes (*14*). Hence, the increase in ionic strength in the freeze-concentrated unfrozen aqueous phase during freezing may have had less of a destabilizing effect on the secondary emulsion than on the primary emulsion.

Influence of Calcium Ions on Emulsion Stability. The purpose of these experiments was to examine the influence of high calcium chloride concentrations on the stability of primary and secondary emulsions. Emulsions were prepared containing different $CaCl_2$ concentrations (0−1000 mM), and then their pH was adjusted to 3.0. The mean particle diameter and electrical charge on the emulsions was measured after 1 week of storage at room temperature. The ζ-potential of the lecithin-stabilized droplets in the primary emulsions remained negative at all $CaCl_2$ concentrations (**Figure 3**). Nevertheless, the ζ-potential of the primary emulsions did become increasingly less negative as the $CaCl_2$ concentration was increased, which can be attributed to electrostatic screening and ion binding effects (*35*). The ζ-potential of the chitosan−lecithin-stabilized droplets in the secondary emulsions remained positive at all $CaCl_2$ concentrations. There was also a much smaller reduction in the magnitude of the ζ-potential with increasing $CaCl_2$ concentration in the primary emulsions than in the secondary emulsions. The most likely reason for this difference is that the multivalent Ca^{2+} ions are counterions for the anionic droplets in the primary emulsion, whereas the monovalent Cl^- ions are counterions for the cationic droplets in the secondary emulsion. Multivalent counterions are much more effective at electrostatic screening and binding, thereby causing a larger reduction in ζ-potential (*21*, *25*).

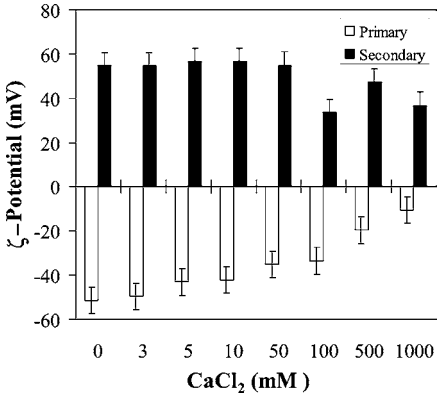

Figure 3. Dependence of electrical charge of emulsion droplets (ζ-potential) on $CaCl_2$ concentration for primary and secondary emulsions stored in the presence of calcium chloride for 1 week.

The primary emulsions were unstable to droplet aggregation above 3 mM $CaCl_2$ (Figure 4), presumably because electrostatic screening and ion binding effects reduced the electrostatic repulsion between the oil droplets (*35*). In addition, calcium chloride may have changed the optimum curvature of the interfacial membrane, making the droplets more prone to coalescence (*21*). The secondary emulsions were stable to droplet aggregation at ≤500 mM but became strongly aggregated at higher concentrations (1 M). In the case of secondary emulsions, the droplet aggregation observed at high salt concentrations may have been due to screening of the electrostatic repulsion between the droplets (*1*, *2*, *25*). Alternatively, it may have been because the structure or thickness of the interfacial membrane was altered at high salt concentrations (*4*). For example, the electrostatic repulsion between the charged groups on the chitosan would have been screened at high salt concentrations, which may have reduced the thickness of the interfacial layer and thus the steric repulsion between the droplets. Overall, these results show that emulsions that are relatively stable to high $CaCl_2$ concentrations can be produced by coating lecithin-stabilized droplets by chitosan.

Influence of Lipid Oxidation on Emulsion Stability. The purpose of these experiments was to compare the stabilities of primary and secondary emulsions to lipid oxidation. Oxidation stability of the emulsions was evaluated by measuring the evolution of lipid peroxides formed during storage at room temperature. Peroxide concentration increased throughout the oxidation period in both emulsions (**Figure 5**). The increases in the peroxide concentration with time were similar in the primary and secondary emulsions during the first 3 weeks of storage, but oxidation proceeded significantly more rapidly in the primary emulsion at longer times (except for week 8, which may have been due to the breakdown of peroxides into secondary products). Previous studies have shown that binding of Fe^{2+} or Fe^{3+} ions to the surface of negatively charged droplets accelerates lipid oxidation by bringing the catalyst and substrate into close proximity (*36*). Although our results indicated that the oxidation rate was slower in cationic lecithin−chitosan-coated droplets than in anionic lecithin-coated droplets, the

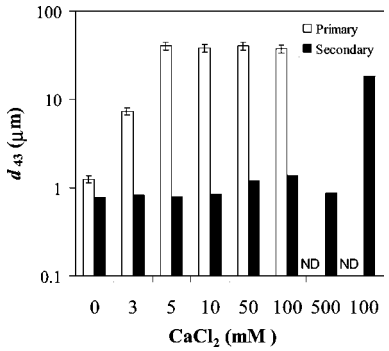

Figure 4. Dependence of mean particle diameter on $CaCl_2$ concentration for primary and secondary emulsions stored in the presence of calcium chloride for 1 week. In some emulsions the droplet aggregation was so extensive that the particle diameter could not be determined (ND) by the laser diffraction technique.

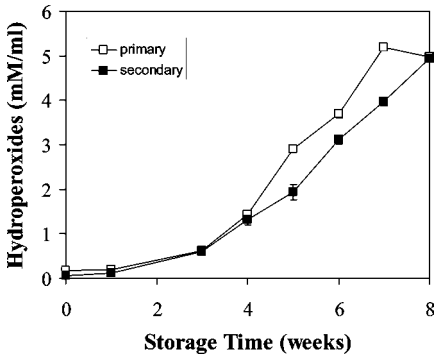

Figure 5. Progression of lipid oxidation in emulsions as determined by measuring the increase in hydroperoxides with time in primary and secondary emulsions stored at room temperature.

magnitude of the effect was much smaller than seen in previous studies that have compared lipid oxidation in emulsions containing cationic droplets with those containing anionic droplets (*36*, *37*). It is possible that endogenous Fe^{2+} or Fe^{3+} ions bound to the lecithin layer in the primary emulsion through electrostatic attraction between the negatively charged lecithin-coated droplets and the positively charged iron ions. The secondary emulsion was formed by adding chitosan to the primary emulsion, so it is possible that iron was trapped between the lecithin and chitosan layers in the secondary emulsion. Hence, the iron would have been in close contact with the oil substrate in both systems, leading to a fairly similar rate of lipid oxidation. It is clear that a more detailed study is needed using a variety of different analytical methods to follow lipid oxidation and the location of iron in the system. In addition, it would be interesting to examine the effect of adding exogenous iron to the emulsions either before or after the addition of the chitosan to form the secondary layer.

Conclusions. This study has shown that the stability of lecithin-coated emulsion droplets to aggregation induced by thermal processing, freeze−thaw cycling, and high calcium ion contents and to iron-catalyzed lipid oxidation can be improved by coating the droplets with chitosan. The ability to form emulsions containing droplets stabilized by multiple interfacial layers comprising different types of emulsifiers, rather than a single interfacial layer comprising one type of emulsifier, may lead to the development of food products with improved stability to environmental stresses. The method used to prepare these emulsions is simple and cost-effective and may therefore be of practical use to the food industry.

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