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Effects of Chitosan and Rosmarinate Esters on the Physical and Oxidative Stability of Liposomes

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Liposomes have substantial potential to deliver bioactive compounds in foods. However, the oxidative degradation and physical instability of liposomes limit their utilization. This research evaluated the ability of chitosan and rosmarinic acid and its esters to increase the physical and oxidative stability of liposomes. Particle size analysis studies showed that the physical stability of liposomes was enhanced by depositing a layer of cationic chitosan onto the negatively charged liposomes. The combination of octadecyl rosmarinate (40 μM) and chitosan coating resulted in significantly greater inhibition of lipid oxidation in the liposomes compared to chitoson or octadecyl rosmarinate alone. Increasing the concentrations of octadecyl rosmarinate to a concentration of 40 μM in the chitosan-coated liposomes decreased lipid oxidation. Only butyl rosmarinate exhibited stronger antioxidant activity than free rosmarinic acid. Eicosyl rosmarinate (20 carbons) had lower antioxidant activity than all other rosmarinic acid derivatives. These results suggest that by combining the inclusion of appropriate antioxidants such as rosmarinic acid and the deposition of a chitosan coating onto the surface of liposomes may significantly increase the oxidative and physical stability of liposomes.

KEYWORDS: Chitosan; rosmarinic acid; liposomes; lipid oxidation; antioxidant; lipid delivery system

INTRODUCTION

Liposomes are spherical, single- or multiple-layer vesicles that are spontaneously formed when phospholipids are dispersed in water. In recent years, liposomal encapsulation technologies have been extensively investigated in the food and agricultural industries as delivery systems to entrap and protect functional and unstable components such as antimicrobials, flavors, antioxidants, and bioactive ingredients. Liposomes can entrap both hydrophobic and hydrophilic compounds within their structure, protect entrapped compounds from decomposition, and release the entrapped compounds at designated targets (*1*, *2*). Commercially available phospholipid preparations, commonly referred to as lecithin, are isolated from natural sources such as chicken egg yolk and soybeans (*3*) and are composed of mixtures of a variety of individual phospholipids. In the food industry, lecithins are generally recognized as safe (GRAS) food ingredients that are biocompatible, biodegradable, and nontoxic. They are used as both emulsifiers and texture modifiers (*2*, *4*, *5*). Phosphatidylcholine (PC) is the major phospholipid found in most lecithins (*6*).

One of the problem with liposomes in practical applications is their insufficient physical and chemical stability, leading to changes in particle size distribution, turbidity, and ability to contain the encapsulated compounds. Aggregation, rupture, and coalescence of liposomes will change their size distribution. This destabilization is particularly prevalent when surface charges are reduced at low pH conditions and at high ionic strengths (*7*). The chemical stability of liposomes may also be problematic due to oxidation or hydrolysis of the fatty acids (*8*, *9*).

Many lecithins are susceptible to lipid oxidation because the phospholipids in the lecithin may contain fatty acids that are highly unsaturated. Transition metals such as iron can accelerate the oxidation of liposomes by interacting with residual lipid hydroperoxides in the phospholipids to produce free radicals that promote oxidation (*10*, *11*). In addition, the overall surface charge of liposomes manufactured from commercial lecithins is generally negative, resulting in electrostatic attraction of transition metals and thereby increasing metal−lipid interactions and further promoting oxidation (*12*). To minimize oxidative degradation of liposomes, several strategies have been reported including selecting high-quality lecithins with low levels of hydroperoxides and transition metals (*13*), using phospholipids that are high in saturated fatty acids (e.g., hydrogenated phospholipids; *14*), adding antioxidants (*15*), and modifying the liposomal surface charges (*11*, *16*, *17*).

Chitosan has been used successfully as a secondary layer on phospholipid-stabilized oil-in-water emulsion droplets to increase physical stability (*18*−*20*). Modification of liposome surfaces by coating with chitosan has been demonstrated to enhance the physical stability of liposomes against aggregation for up to 45 days (*21*). Electrostatic deposition of chitosan onto phospholipid-stabilized

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oil-in-water emulsion droplets has been shown to inhibit lipid oxidation presumably by producing a cationic interface that causes charge repulsion of iron, thus minimizing lipid–metal interactions (*22*).

The objective of this research was to determine the impact of antioxidants and/or surface charge modifications on the chemical and physical stability of liposome. Surface charge modifications were accomplished by adsorbing a layer of chitosan on the surface of the liposomes using a layer-by-layer electrostatic deposition method. Chitosan was chosen as substrate for the electrostatic deposition because it is positively charged and thus can be electrostatically bound to negatively charged surfaces. The antioxidant tested in this study was a phenolic acid compound, rosmarinic acid. The antioxidant activity of surface active rosmarinic acid esters produced with aliphatic chains of various lengths was also determined.

MATERIALS AND METHODS

Materials. Soy lecithin (UltralecP) was kindly provided by Archer Daniels Midland (Decatur, IL). Sodium acetate trihydrate (99.1%) and glacial acetic acid ($C_2H_4O_2$) were purchased from Fisher Scientific (Fair Lawn, NJ). The chitosan used in this study were donated by Primex, Reykjavik, Iceland, and had an average molecular weight of 205.3 ± 2.0 kDa, a degree of deacetylation of 91.8%, and a viscosity of 45 cP in 1% acetic acid solution, according to the certificate of analysis supplied by the manufacturer. Sodium azide (NaN_3, 99.5%) was purchased from Sigma-Aldich (St. Louis, MO). All other chemicals were of reagent grade. Double-distilled and deionized water was used for the preparation of all solutions.

Synthesis of Rosmarinate Esters. The chemoenzymatic esterification of rosmarinic acid to obtain rosmarinic esters was carried out following the procedure described by Lecomte et al. (*23*). Briefly, the chemical esterification of rosmarinic acid (56 μmol) was carried out in sealed brown flasks each containing 5 mL of alcohol (methanol, 123.44 mmol; *n*-butanol, 54.64 mmol; *n*-octanol, 31.905 mmol; *n*-dodecanol, 22.46 mmol; *n*-hexadecanol, 16.95 mmol; *n*-octadecanol, 15.09 mmol or *n*-eicosanol, 13.6 mmol). The reaction mixtures were stirred (orbital shaker, 250 rpm, 55–70 °C) prior to the addition of the catalyst; the strongly acidic sulfonic resin Amberlite IR-120H (5% w/w, total weight of both substrates) that had been previously dried at 110 °C for 48 h. The water generated during the reaction was removed by adding 3 Å, 4–8 mesh molecular sieves (40 mg/mL, Aldrich, St. Louis, MO) to the medium. Samples (20 μL) were regularly withdrawn from the reaction medium and then mixed with 980 μL of methanol, filtered (0.45 μm syringe filter Millex-FH, Millipore Corp., Bedford, MA), and finally analyzed by reverse phase HPLC with UV detection at 328 nm (*23*). After complete (4–21 days) conversion of rosmarinic acid into the corresponding ester, the latter was purified in a two-step procedure. First, a liquid–liquid extraction using hexane and acetonitrile was performed to remove the excess alcohol. Then, the remaining traces of the alcohol and rosmarinic acid were eliminated by flash chromatography on a CombiFlash Companion system (Teledyne Isco Inc., Lincoln, NE). Separation was carried out on a silica column using an elution gradient of hexane and ether (20–100% in 35 min). The yield of purified esters, obtained as pale yellow to yellow amorphous powders, was calculated from calibration curves previously established with pure compounds. Pure esters and rosmarinic acid were then fully characterized by ESI-MS, ^{1}H NMR, and ^{13}C NMR as previously described by Lecomte et al. (*23*).

Preparation of Liposomes. Multilayer liposomes were prepared according to the method described by Laye et al. (*21*) with slight modifications. A lecithin stock solution (1%, w/v) was freshly prepared in 0.1 M acetate buffer (pH 3.0 ± 0.1). The lecithin solution was mixed for 2 min with a hand-held high-speed blender at maximum speed (Bio homogenizer, model M133.1281-0, Biospec Product Inc., Bartlesville, OK). Chitosan stock solution (1%, w/v) was prepared with the same buffer and stirred overnight followed by filtration through Miracloth (Calbiochem, USA) and a hydrophobic PTFE 5.0 μm Millipore filter (Millex-LS; Danvers, MA). Stock solutions were stored at 5 °C and used within 24 h. In experiments using rosmarinic acid esters, the esters were first dissolved in methanol and then mixed with the lecithin stock solution followed by mixing with a hand-held high-speed blender as described above. Samples without antioxidants contained an equal amount of methanol as the antioxidant-treated samples.

Liposomes were prepared by passing the lecithin stock solution three times through a high-pressure homogenizer at 9000 psi (model 110 L, Microfluidizer, Microfluidics, Newton, MA). To produce chitosan-coated liposomes, the homogenized liposomes were added to an equal volume of chitosan solution under constant stirring (700 rpm for 2 min) to obtain final concentrations of 0.5% (w/v) lecithin and 0.2% (w/v) chitosan. To decrease bridging flocculation of the liposomes by chitosan, the chitosan-coated liposome solutions were passed three times through a high-pressure homogenizer at 9000 psi. To inhibit microbial growth during the study, all liposome solutions were mixed with 0.04% (w/v) NaN_3 and stirred for 2 min. To conduct stability studies, 1 mL of coated or uncoated liposome solutions was transferred to 10 mL headspace vials, sealed with poly-(tetrafluoroethylene) butyl rubber septa, and stored at 50 °C in the dark. In experiments with ethylenediaminetetraacetic acid (EDTA), EDTA (100 μM) was added after liposome preparation.

Liposomal Charge and Size Measurements. The electrical charge and size of liposomes were measured by dynamic light scattering measurements (Zetasizer Nano-ZS, model ZEN3600, Malvern Instruments, Worchester, U.K.) and expressed as ζ-potential and *z*-average mean diameter, respectively. Samples were diluted approximately 10-fold with the same buffer, mixed, and immediately transferred into plastic cuvettes for size determination or capillary cells for ζ-potential determination (DTS1060, Malvern Instruments).

Measurements of Lipid Hydroperoxides. Lipid hydroperoxide formation in liposome solutions was determined according to an adapted method as described by Alamed et al. (*24*). Liposome solutions (0.3 mL) were mixed with 5 mL of isooctane/2-propanol (3:1 v/v) and vortexed (10 s, three times). After centrifugation at 1000*g* for 2 min, 200 μL of the organic solvent phase was mixed with 2.8 mL of methanol/1-butanol (2:1). Hydroperoxide detection was started by the addition of 15 μL of 3.94 M ammonium thiocyanate and 15 μL of ferrous iron solution (prepared by adding equal amounts of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). After 20 min of incubation at room temperature, the absorbance was measured at 510 nm using a UV–vis spectrophotometer (Genesys 20, Thermo Spectronic). Hydroperoxide concentrations were determined using a standard curve prepared from hydrogen peroxide.

Measurement of Hexanal. Headspace hexanal was determined according to the method described by Pignoli et al. (*25*) with some modification using a Shimadzu GC-2014 gas chromatograph (GC) equipped with an AOC-5000 autoinjector (Shimadzu, Tokyo, Japan). A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) stable flex SPME fiber (Supelco, Bellefonte, PA) was inserted through the septum into the vial and exposed to the sample headspace for 15 min at 55 °C. The SPME fiber was desorbed at 250 °C for 3 min in the GC detector at a split ratio of 1:7. The chromatographic separation of volatile aldehydes was performed on a fused-silica capillary column (30 m × 0.32 mm i.d. × 1 μm) coated with 100% poly(dimethylsiloxane) (Equity-1, Supelco). The temperatures of the oven, injector, and flame ionization detector were 65, 250, and 250 °C, respectively. Sample run time was 10 min. Concentrations were determined using a standard curve made from hexanal.

Determination of Antioxidant Partitioning. For the determination of the physical location of rosmarinic acid and its esters in the liposome suspensions, liposome solutions were centrifuged at 146550*g* (40000 rpm) for 1 h at 4 °C using a Sorvall A-1256 rotor with a high-speed centrifuge (Sorvall Ultra 80, Waltham, MA). The supernatant was carefully collected with a pipet, and the amounts of rosmarinic acid esters in the supernatants were determined at 333 nm using a UV–vis scanning spectrophotometer (UV-2010PC) with a quartz cell. The concentrations of rosmarinic acid esters were calculated using a standard curve made from rosmarinic acid dissolved in methanol.

Statistics. All analyses were performed on triplicate samples. Oxidation lag phases were defined as the first data point significantly greater than the 0 time value. In all cases, comparisons of the means were performed using Duncan's multiple-range tests. A significance level of $p < 0.05$ was defined as being statistically different. All calculations were performed using SPSS17 (http://www.spss.com; SPSS Inc., Chicago, IL).

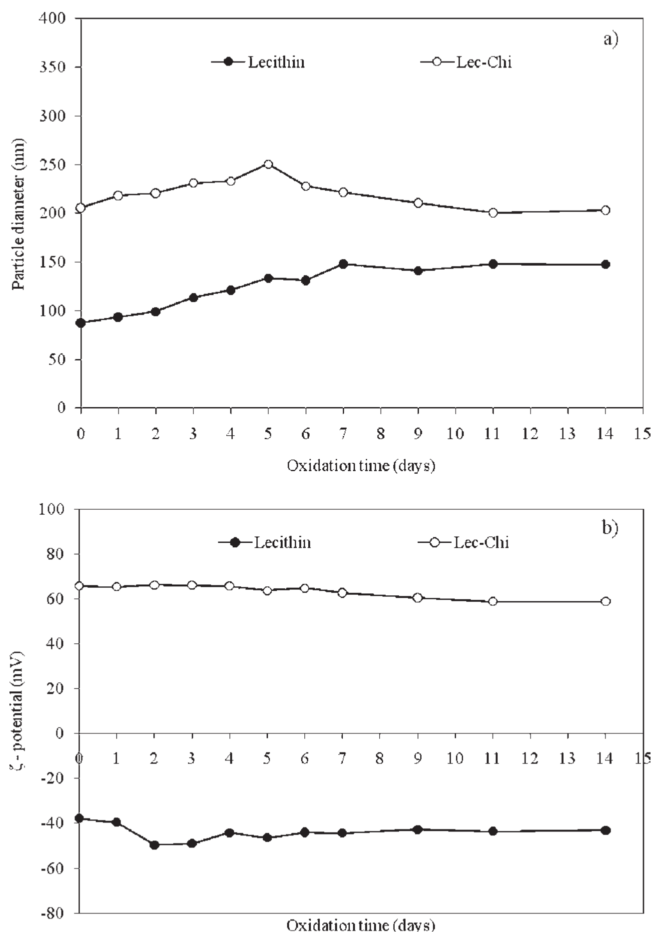

Figure 1. Physical stability of liposomes (pH 3.0 and 55 °C) during storage as determined by particle diameter (**a**) and droplet charge (**b**).

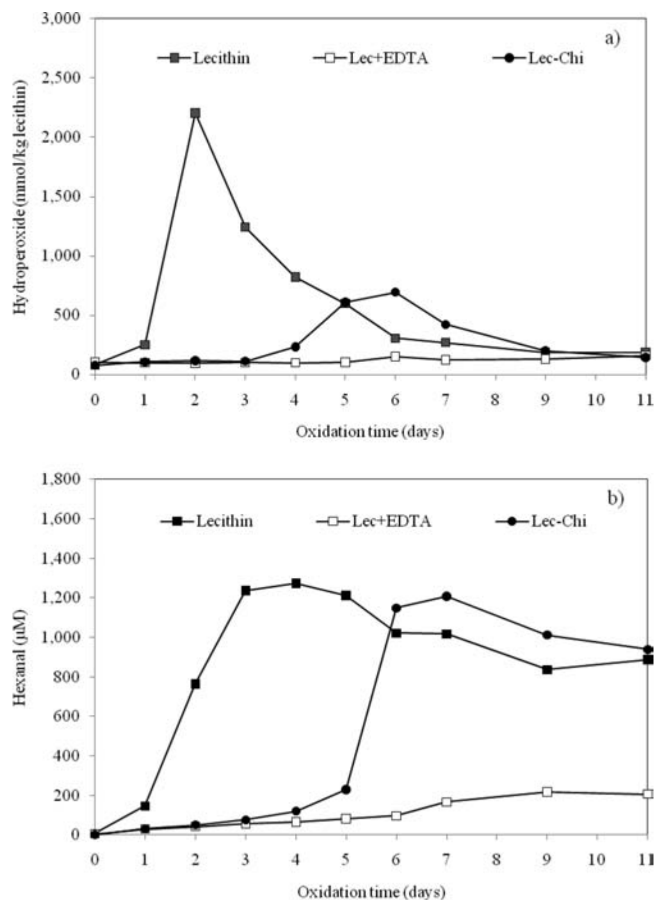

Figure 2. Oxidative stability of uncoated (lecithin with and without EDTA) and chitosan coated (lec-chi) liposomes during storage (pH 3.0 and 55 °C) as determined by formation of hydroperoxides (**a**) and hexanal (**b**).

RESULTS AND DISCUSSION

Liposomes are susceptible to oxidative degradation, limiting their utilization in foods. Therefore, this study was conducted to find methods to reduce lipid oxidation in liposomes using antioxidant technologies including electrostatic deposition of chitosan onto the surface of liposomes and use of surface active antioxidants. Whereas electrostatic deposition of chitosan onto the surface of liposomes did alter the physical properties of the liposomes (see discussion below), none of the antioxidants tested had any impact on either liposome size or charge (data not shown).

Effect of Chitosan and EDTA on Liposomal Stability. A secondary chitosan layer coated on liposomes led to higher physical and chemical stability of liposomes during incubation at pH 3.0 and 55 °C. **Figure 1a** shows that immediately after preparation (0 h), chitosan-coated liposomes were significantly larger in diameter (205.1 nm) than uncoated liposomes (87.8 nm). The increase in liposome size in the presence of chitosan could be due to the thicker interface of the coated liposomes as well as bridging flocculation by the chitosan polymer. Electrostatic deposition of chitosan onto the liposomes increased the charge of liposomes from −37.8 mV for the uncoated liposomes to +66.3 mV for the coated liposomes (**Figure 1b**). Change in charge is due to the electrostatic deposition of the positively charged chitosan onto the negatively charged phospholipid. The observed increase in size and charge of the liposomes after coating was in agreement with other studies (*18*, *21*, *26*). During storage, the particle diameter of the coated liposomes increased only slightly (approximately 1.02-fold), whereas the uncoated liposomes increased approximately 1.5-fold by the end of the storage study (although none of the liposome systems exhibited sedimentation over the course of the oxidation studies). The higher physical stability of the coated liposome could be due to an increased charge density and a thicker outer layer, which could decrease liposome coalescence and aggregation during storage.

Lipid oxidation was significantly inhibited by coating the liposomes with chitosan as indicated by lipid hydroperoxides and hexanal determination (**Figure 2**, panels **a** and **b**, respectively). Without coating, lipid oxidation of liposomes occurred rapidly, with the lag phase of both lipid hydroperoxide and hexanal formation being ≤1 day. Coating with chitosan extended the lag phase to 3 and 4 days for lipid hydroperoxide and hexanal formation, respectively. Lipid oxidation was strongly inhibited by EDTA, indicating that transition metals were major prooxidants in the liposome system. The fact that transition metals were important prooxidants suggests that inhibition of lipid oxidation by the chitosan coating was due to the formation of a cationic layer on the surface of the liposomes that can electrostatically repel transition metals away from the lipid−water interface (*22*, *27*, *28*).

Effect of Rosemarinic Acid Ester on the Oxidative Stability of Liposomes. Initial studies were conducted to determine if rosmarinic acid esters could increase the oxidative stability of uncoated and chitosan-coated liposomes. Octadecyl rosmarinate was chosen for these initial studies because phenolic esters with 18-carbon chains have been found to inhibit lipid oxidation in oil-in-water emulsions (*29*). As shown in **Figure 3**, octadecyl rosmarinate (40 μM) did not increase the oxidative stability of uncoated

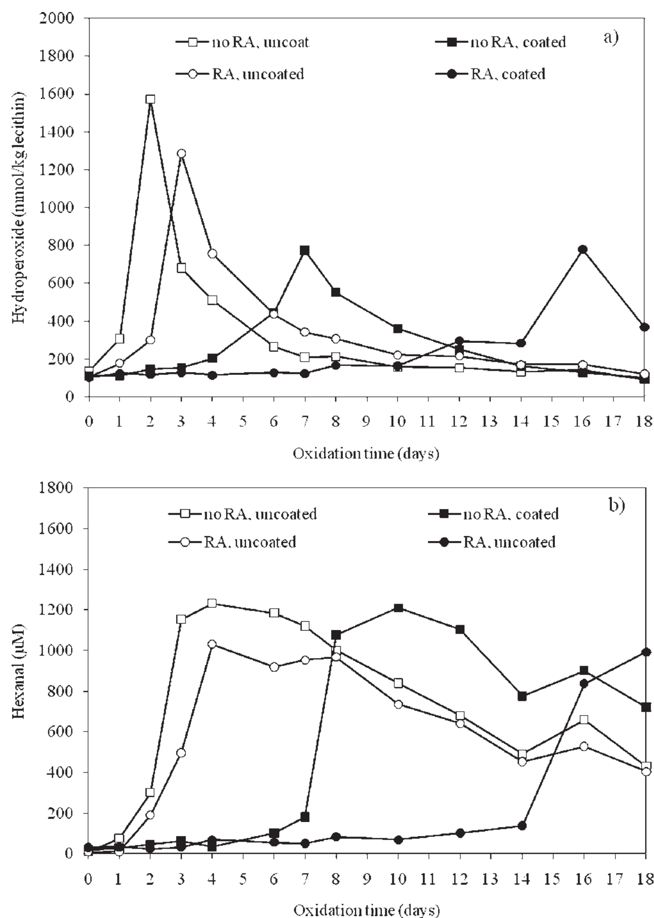

Figure 3. Oxidative stability of uncoated and chitosan-coated liposomes in the presence and absence of 40 μM octadecyl rosmarinic acid ester (RA) during storage at 55 °C and pH 3.0. Lipid oxidation was monitored by measuring hydroperoxide (**a**) and hexanal (**b**).

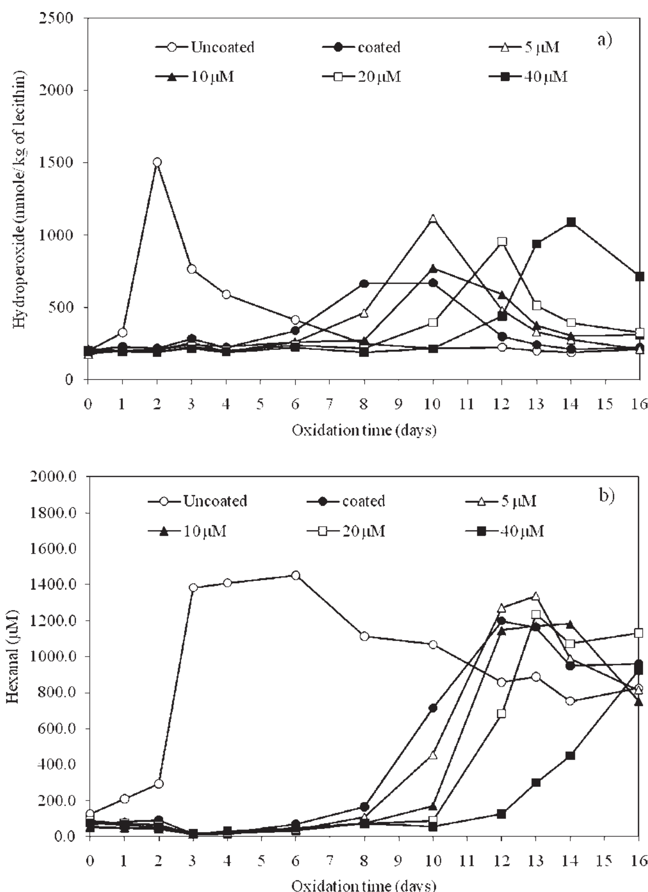

Figure 4. Influence of various concentrations of octadecyl rosmarinic acid ester on oxidative stability of chitosan-coated liposomes during storage at 55 °C and pH 3.0. Lipid oxidation was monitored by measuring hydroperoxide (**a**) and hexanal (**b**).

liposomes as determined by both lipid hydroperoxide and hexanal formation. However, the combination of octadecyl rosmarinate and chitosan coating very effectively inhibited oxidation, with the lag phase of lipid hydroperoxide and hexanal formation increasing to 10 and 14 days, respectively, compared to a lag phase of 4 days for hydroperoxides and 7 days for hexanal for the chitosan coating alone. The synergistic antioxidant activity of octadecyl rosmarinate and chitosan coating could be due to the ability of chitosan to decrease the reactivity of transition metals by providing a cationic barrier that decreases metal−lipid hydroperoxide interactions. By decreasing metal−lipid hydroperoxide interactions, fewer free radicals would be generated, thus sparing the octadecyl rosmarinate from rapid degradation as seen in the uncoated liposomes. Thus, in the chitosan-coated liposomes, the octadecyl rosmarinate concentrations would remain higher for a longer period of time and thus be more effective at inhibiting free radical promoted lipid oxidation.

The impact of increasing concentrations of the octadecyl rosmarinate on the oxidative stability of the chitosan-coated liposomes was also determined. The lag phases of lipid hydroperoxide formation in chitosan-coated liposomes were 10, 10, and 12 days for 10, 20, and 40 μM octadecyl rosmarinate, respectively (**Figure 4a**). Hexanal formation showed a similar trend with increasing octadecyl rosmarinate concentrations increasing the lag phase in chitosan-coated liposomes to 8, 8, and 10 days for 10, 20, and 40 μM, respectively (**Figure 4b**). Addition of 5 μM octadecyl rosmarinate did not inhibit either lipid hydroperoxide or hexanal formation in chitosan-coated liposomes.

Effect of Rosmarinic Acid Esters Hydrocarbon Length on the Oxidative Stability of Coated Liposomes. The effects of the hydrocarbon chain length of rosmarinic acid esters on the ability of the rosmarinate esters to inhibit lipid oxidation in the coated liposomes were determined at a molar antioxidant concentration of 40 μM. Panels **a** and **b** of **Figure 5** show a nonlinear relationship between the chain length and antioxidative activity of rosmarinate esters. For lipid hydroperoxide formation, the lag phases were 18, 21, 18, 18, and 14 days in chitosan-coated liposomes for rosmarinic acid, butyl rosmarinate, dodecyl rosmarinate, octadecyl rosmarinate, and eicosyl rosmarinate, respectively. A similar trend was observed for hexanal formation with lag phases in the chitosan-coated liposomes of 17, 21, 18, 16, and 10 days, respectively. Overall, butyl rosmarinate was the most effective, whereas eicosyl rosmarinate was the least effective. Addition of dodecyl or octadecyl hydrocarbons onto the rosmarinic acid did not improve antioxidant activity compared to rosmarinic acid alone.

This nonlinear relationship between antioxidant polarity and antioxidant activity has also been reported in other esters of antioxidants in various systems. Takahashi et al. (*30*) showed nonlinear effects of length of fatty acid ester side chains of L-ascorbic acid on their antioxidative activity in liposomal membranes. In addition, fatty acid esters of gallic acid exhibited a nonlinear relationship between chain length and antioxidative activity in SDS, partially hydrolyzed lecithin, and Brij 58 stabilized oil-in-water emulsions (*31*). Various explanations have been proposed to explain this phenomenon. In the case of gallate esters, their lower antioxidative activity with increasing

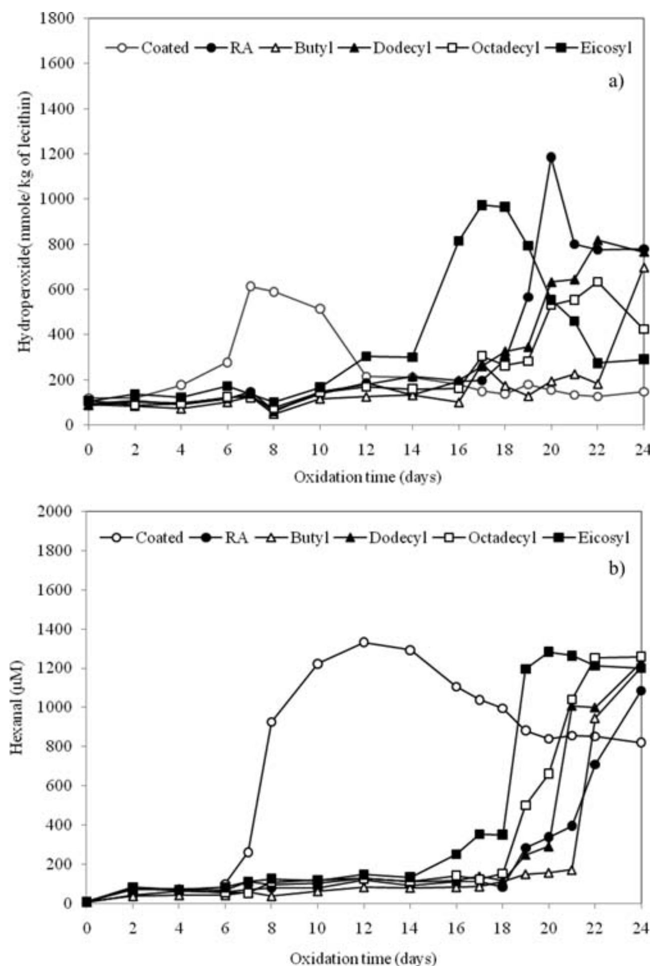

Figure 5. Influence of different aliphatic side chains on rosmarinic acid esters on oxidative stability of chitosan-coated liposomes during storage (pH 3.0 and 55 °C). Lipid oxidation was monitored by measuring hydroperoxide (**a**) and hexanal (**b**).

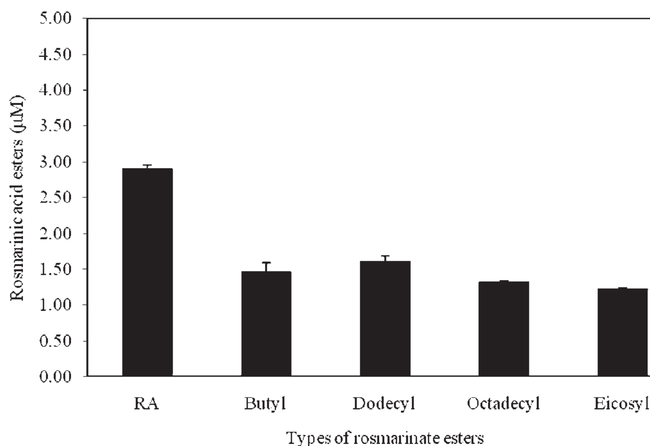

Figure 6. Concentration of rosemarinic acid and its esters in the aqueous phase of chitosan-coated liposomes.

number and length of hydrocarbon chain may be explained by reduction of the intramembrane and intermembrane mobilities of antioxidant esters due to an increase in hydrophobic interaction with the surfactant or phospholipid membrane (*30*, *31*). Results suggested that rosmarinic acid required at least four carbons on the side chain to obtain enough hydrophobicity to penetrate into the palisade layer of the liposomal membrane. Interestingly, the same was true for gallate esters in partially hydrolyzed lecithin-stabilized oil-in-water emulsions as reported by Stockman et al. (*31*). Even though there were differences in the studied systems (emulsions vs liposomes), both systems had phospholipids in the interfacial region bordering the aqueous phase. The observed decrease in the antioxidative activity of eicosyl rosmarinate has also been reported for chlorogenic acid esterified to long-chain hydrocarbons (≥C18) in oil-in-water emulsions (*29*).

The antioxidant polar paradox hypothesis states that nonpolar antioxidants are more effective in lipid dispersion because they are more highly retained in the lipid phase, where oxidation is most prevalent (*32*, *33*). To determine if there was a correlation between antioxidant location and antioxidant activity in the chitosan-coated liposomes, the concentration of the rosmarinic acid derivatives in the aqueous phase of the liposome system was determined (**Figure 6**). Overall, partitioning into the liposomes increased as the hydrocarbon chain length on the rosmarinic acid increased from 0 to 20 carbons. Rosmarinic acid showed a high affinity for the lipid phase in 10% o/w emulsions, where 83% of the antioxidant partitioned into the oil phase compared to 30% of gallic acid in the oil phase (*34*). Although statistically significant differences could be seen between the different rosmarinic acid esters, it should be noted that these differences were very small (< 2 μM). Such small differences in aqueous phase antioxidant concentrations suggest that the overall partitioning of the antioxidants into the liposomes is not responsible for differences in antioxidant activity. However, it is possible that the size of the hydrocarbon chain could affect the orientation and depth of the antioxidant in the lipid bilayer, which could affect its ability to scavenge free radicals (*31*).

LITERATURE CITED

(1) Benech, R. O.; Kheadr, E. E.; Lacroix, C.; Fliss, I. Inhibition of *Listeria innocua* in cheddar cheese by addition of nisin Z in liposomes or by in stu production in mixed culture. *Appl. Environ. Microbiol.* **2002**, *68*, 3683–3690.
(2) Shehata, T.; Ogawara, K.-I.; Higaki, K.; Kimura, T. Prolongation of residence time of liposome by surface-modification with mixture of hydrophilic polymers. *Int. J. Phram.* **2008**, *359*, 279–279.
(3) Nara, E.; Miyashita, K.; Ota, T. Oxidative stability of liposomes prepared from soybean PC, chicken egg PC, and salmon egg PC. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1736–1739.
(4) Gomez, A.; Korczak, J. Tea constituents (*Camellia sinensis* L.) as antioxidants in lipid systems. *Trends Food Sci. Technol.* **2005**, *16*, 351–358.
(5) U.S. Food and Drug Administration. Food ingredients and packaging: Generally Recognized as Safe (GRAS); http://www.fda.gov/Food/FoodIngredientsPackaging/default.htm (accessed March 1, **2010**).
(6) Taylor, T. M.; Davidson, P. M.; Bruce, D. B.; Weiss, J. Liposomal nanocapsules in food science and agriculture. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 1–19.
(7) Gregoriadis, G. Drug entrapment in liposomes. *FEBS Lett.* **1973**, *36*, 292–296.
(8) Gift, M.; Crommelin, D. J. A. Chemical stability of liposomes: implications for their physical stability. *Chem. Phys. Lipids* **1993**, *65*, 3–18.
(9) Thompson, A. K.; Haisman, D.; Singh, H. Physical stability of liposomes prepared from milk fat globule membrane and soya phospholipids. *J. Agric. Food Chem.* **2006**, *54*, 6390–6397.
(10) Mozuraityte, R.; Rustad, T.; Storro, I. The role of iron in peroxidation of polyunsaturated fatty acids in liposomes. *J. Agric. Food Chem.* **2008**, *56*, 537–543.
(11) Tampo, Y.; Yonaha, M. Effects of membrane charges and hydroperoxides on Fe(II)-supported lipid peroxidation in liposomes. *Lipids* **1996**, *31*, 1029–1038.

(12) Fukuzawa, K.; Tadokoro, T.; Kishikawa, K.; Mukai, K.; Gebicki, J. M. Site-specific induction of lipid peroxidation by iron in charged micelles. *Arch. Biochem. Biophys.* **1988**, *260*, 146–152.
(13) Miyashita, K.; Nara, E.; Ota, T. Comparative study on the oxidative stability of phosphatidylcholines from salmon egg and soybean in an aqueous solution. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1772–1775.
(14) Samuni, A.; Lipman, A.; Barenholz, Y. Damage to liposomal lipids: protection by antioxidants and cholesterol-mediated dehydration. *Chem. Phys. Lipids* **2000**, *105*, 121–134.
(15) Nacka, F.; Cansell, M.; Entressangles, B. In vitro behavior of marine lipid-based liposomes. Influence of pH, temperature, bile salts, and phospholipase A_2. *Lipids* **2001**, *36*, 35–42.
(16) Kogure, K.; Fukuzawa, K.; Kawano, H.; Terada, H. Spermine accelerates iron-induced lipid peroxidation in mitochondria by modification of membrane surface charges. *Free Radical Biol. Med.* **1993**, *14*, 501–507.
(17) Yoshida, K.; Terao, J.; Suzuki, T.; Takama, K. Inhibitory effect of phosphatidylserine on iron-dependent lipid peroxidation. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 1077–1081.
(18) Chen, R. H.; Win, H. P.; Fang, H. J. Vesicle size distribution, stability, and rheological properties of liposomes coated with water-soluble chitosans of different molecular weights and concentrations. *J. Liposome Res.* **2001**, *11*, 211–228.
(19) Ogawa, S.; Decker, E. A.; McClements, D. J. Production and characterization of o/w emulsions containing cationic droplets stabilized by lecithin−chitosan membranes. *J. Agric. Food Chem.* **2003**, *51*, 2806–2812.
(20) Aoki, T.; Decker, E. A.; McClements, D. J. Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. *Food Hydrocolloids* **2005**, *19*, 209–220.
(21) Laye, C.; McClements, D. J.; Weiss, J. Formation of biopolymer-coated liposomes by electrostatic deposition of chitosan. *J. Food Sci.* **2008**, *37*, N7–N15.
(22) Shaw, L. A.; McClements, D. J.; Decker, E. A. Spray dried multilayered emulsions as a delivery method for omega-3 fatty acids into food systems. *J. Agric. Food Chem.* **2007**, *55*, 3112–3119.
(23) Lecomte, J.; López Giraldo, L. J.; Laguerre, M.; Baréa, B.; Villeneuve, P. Synthesis, characterization and free radical scavenging proteries of rosmarinic acid fatty esters. *J. Am. Oil. Chem. Soc.* **2010**. doi:10.1007/s11746-010-1543-8.
(24) Alamed, J.; Chaiyasit, W.; McClements, D. J.; Decker, E. A. Relationships between free radical scavenging and antioxidant activity in foods. *J. Agric. Food Chem.* **2009**, *57*, 2969–2976.
(25) Pignoli, G.; Bou, R.; Rodriguez-Estrada, M. T.; Decker, E. A. Suitability of saturated aldehydes as lipid oxidation markers in washed turkey meat. *Meat Sci.* **2009**, *83*, 412–416.
(26) Henriksen, I.; Smistad, G.; Karlsen, J. Interactions between liposomes and chitosan. *Int. J. Pharm.* **1994**, *101*, 227–236.
(27) Mei, L.; McClements, D. J.; Decker, E. A. Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsion droplets. *J. Agric. Food Chem.* **1999**, *47*, 2267–2273.
(28) Kulmyrzaev, A.; Silvestre, M. P. C.; McClements, D. J. Rheology and stability of whey protein stabilized emulsions with high $CaCl_2$ concentrations. *Food Res. Int.* **2000**, *33*, 21–25.
(29) Laguerre, M.; López Giraldo, L. J.; Lecomte, J.; Figueroa-Espinoza, M. C.; Baréa, B.; Weiss, J.; Decker, E. A.; Villeneuve, P. Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. *J. Agric. Food Chem.* **2009**, *57*, 11335–11342.
(30) Takahashi, M.; Komuro, E.; Niki, E.; Tanaka, K. Action of fatty acid esters of L-ascorbic acid as antioxidants in phosphatidylcholine liposomal membranes. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 679–684.
(31) Stockman, H.; Schwarz, K.; Huynh-Ba, T. The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. *JAOCS* **2000**, *77*, 535–542.
(32) Porter, L. P. Paradoxical behavior of antioxidants in food and biological systems. *Toxicol. Ind. Health* **1993**, *9*, 1–2.
(33) Frankel, E. N.; Huang, S.-W.; Kanner, J.; German, J. B. Interfacial phenomena in the evaluation of antioxidants: bulk oils vs. emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054–1059.
(34) Huang, S.-W.; Frankel, E. N.; Aeschbach, R.; German, J. B. Partition of selected antioxidants in corn-oil−water model systems. *J. Agric. Food Chem.* **1997**, *45*, 1991–1994.

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