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Interfacial Engineering Using Mixed Protein Systems: Emulsion-Based Delivery Systems for Encapsulation and Stabilization of β-Carotene

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ABSTRACT: Emulsion-based delivery systems are needed to encapsulate, protect, and deliver lipophilic bioactive components in the food, personal care, and pharmaceutical industries. The functional performance of these systems can be controlled by engineering the composition and structure of the interfacial layer coating the lipid droplets. In this study, interfacial properties were controlled using two globular proteins with widely differing isoelectric points: lactoferrin (LF: pI ≈ 8.5) and β-lactoglobulin (BLG: pI ≈ 5). Oil-in-water emulsions were prepared with different interfacial properties: [LF]-only; [BLG]-only; [LF]-[BLG]-(laminated); [BLG]-[LF]-(laminated); and [BLG/LF]-(mixed). The influence of pH, ionic strength, and temperature on the physical stability of β-carotene-enriched emulsions was investigated. [LF]-emulsions were stable to droplet aggregation from pH 2 to 9 (0 mM NaCl), but all other emulsions aggregated at intermediate pH values. [BLG]-emulsions aggregated at high salt levels (≥50 mM NaCl), but all other emulsions were stable (0 to 300 mM NaCl). [BLG/LF]-emulsions were unstable to heating (≥60 °C), but all other emulsions were stable (30 to 90 °C). Color fading due to β-carotene degradation occurred relatively quickly in [BLG]-emulsions (37 °C) but was considerably lower in all other emulsions, which was attributed to the ability of LF to bind iron or interact with β-carotene. This study provides useful information for designing emulsion-based delivery systems to encapsulate and protect bioactive lipids, such as carotenoids.

KEYWORDS: *interfacial engineering, multilayer emulsions, β-carotene, carotenoids, degradation, electrostatic interactions, lactoferrin, β-lactoglobulin, stability*

INTRODUCTION

There is increasing interest within the food and beverage industry in the development of colloidal delivery systems to encapsulate, stabilize, and deliver bioactive lipophilic compounds, such as carotenoids, oil-soluble vitamins, flavonoids, phytosterols, and polyunsaturated fats.[1−3] Oil-in-water emulsions are particularly suitable for incorporating these components into aqueous-based food and beverage products because the bioactive components can simply be solubilized within the oil phase prior to homogenization. One of the most important factors determining the physical and chemical stability of emulsions is the nature of the interfacial layer coating the fat droplets. Traditionally, oil-in-water emulsions are prepared using a single type of emulsifier that adsorbs to the droplet surfaces during homogenization.[4] In this case, the physicochemical stability of the fat droplets can be controlled by selecting emulsifiers that form interfaces with different characteristics, e.g., polarity, thickness, charge, and chemistry.[1] There are a number of different emulsifiers that can be used by the food industry to achieve this goal, including small molecule surfactants, phospholipids, proteins, and polysaccharides.[4−6] Nevertheless, the scope for controlling interfacial properties is limited by the number of legally acceptable emulsifiers that are commercially available.[7−9]

There has therefore been considerable interest in improving the functional performances of emulsion-based delivery systems by engineering the properties of the interfacial layer coating the fat droplets using mixed emulsifier systems.[1,10−12] In this article, we focus on the modification of interfacial properties using mixed globular protein systems since the food industry often wants to use natural emulsifiers to stabilize emulsions. In principle, a variety of different interfacial structures can be formed using two different globular proteins as emulsifiers: P_1 and P_2 (Figure 1).

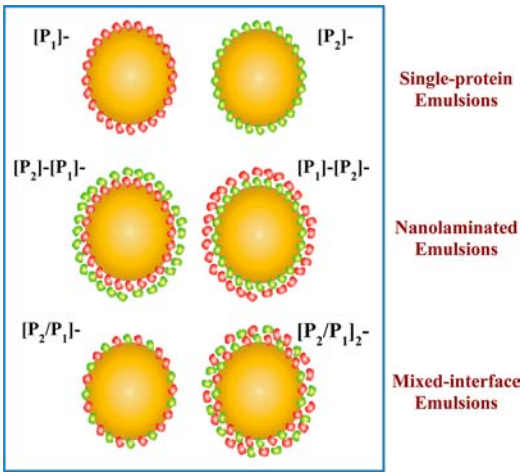

Figure 1. Variety of different interfacial structures can be formed using two different proteins, including emulsions stabilized by a single protein layer, nanolaminated protein layers, or mixed interfacial layers. See online version for colored diagram.

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Mixed-droplet emulsions contain two different kinds of fat droplets, with each kind being coated by a different type of globular protein.[8,13,14] These emulsions are formed by mixing an emulsion containing $[P_1]$-coated droplets with another emulsion containing $[P_2]$-coated droplets (here we use square brackets to designate a single interfacial layer). Nanolaminated emulsions consist of fat droplets coated by multiple layers of protein, with each layer consisting of a single protein type.[15−17] These emulsions are usually formed by sequential deposition of oppositely charged proteins onto fat droplet surfaces using an electrostatic layer-by-layer (LbL) method.[18] Interfacial layers with different structures can be formed using the same pair of proteins depending on which protein is used to stabilize the initial emulsion formed during homogenization, e.g., $[P_1]$-$[P_2]$-coated or $[P_2]$-$[P_1]$-coated droplets. Mixed-interface emulsions consist of fat droplets coated by one or more layers of protein, with each layer consisting of a mixture of different kinds of protein, e.g., $[P_1/P_2]$-coated.[19] These emulsions can be formed by mixing the two proteins together prior to homogenization and then homogenizing the oil and mixed protein solution together under conditions where both proteins adsorb to the fat droplet surfaces.

In the current study, we use two globular proteins to fabricate oil-in-water emulsions with different interfacial compositions and structures: β-lactoglobulin (BLG) and lactoferrin (LF). BLG is a surface-active globular protein normally isolated from bovine milk that has a molecular weight of 18.4 kDa and an isoelectric point (pI) around pH 5.[3,20] Previous studies suggest that BLG forms a thin electrically charged coating around fat droplets that opposes droplet aggregation through electrostatic and steric repulsion.[13] The strength of the electrostatic repulsion depends on pH, and it is typically strongest at pH values well away from the protein's isoelectric point (pI ≈ 5).[13] The steric repulsion is important at all pH values, but because BLG only forms a thin interfacial layer, the interaction is short-range, and so it can typically inhibit coalescence but not flocculation. LF is a surface-active globular glycoprotein that is naturally present in mammalian secretions such as milk, saliva, tears, semen, and mucous.[21] LF has a molecular weight of 80 kDa and an isoelectric point around pH 8.5.[22] Previous studies suggest that LF forms a thick electrically charged coating around fat droplets that also opposes droplet aggregation through both steric and electrostatic repulsion.[17,23] Again, the electrostatic repulsion is most important at pH values appreciably above or below the isoelectric point (pI ≈ 8.5) of LF, whereas steric repulsion operates over all pH values. The steric repulsion for [LF]-coated droplets is typically of longer range than that for [BLG]-coated droplets because of the higher molecular weight of LF and because it has hydrophilic carbohydrate groups attached to the polypeptide chain. Indeed, it has been suggested that this steric repulsion is able to inhibit both flocculation and coalescence in LF-stabilized emulsions.[17,23] LF and BLG tend to form electrostatic complexes over a fairly wide pH range due to the appreciable differences in their isoelectric points, i.e., 5 < pH < 8.5.[16,17] This characteristic enables various kinds of interfacial structures to be assembled from these two globular proteins.

The main purpose of this study was to examine the influence of interfacial composition and structure on the physical stability of emulsions to environmental stresses (pH and salt) and on the chemical degradation of an encapsulated bioactive lipid (β-carotene). β-Carotene is a carotenoid that is widely used in foods as a colorant but is also being increasingly used as a nutraceutical agent due to its pro-vitamin A activity.[24,25] β-Carotene has a conjugated polyunsaturated hydrocarbon chain that makes it particularly prone to auto-oxidation,[25−27] which leads to color fading and loss of desirable nutritional attributes.[27,28] We hypothesized that the nature of the protein interface coating the fat droplets would influence the rate of β-carotene degradation in emulsion-based delivery systems.

MATERIALS AND METHODS

Materials. Corn oil was purchased from a commercial food supplier (Mazola, ACH Food Companies, Inc., Memphis, TN) and stored at 4 °C until use. Lactoferrin powder was supplied by FrieslandCampinanin (Delhi, NY), and the manufacturer reported that it contained 97.7% protein and 0.12% ash. β-Lactoglobulin powder (BioPURE) was supplied by Davisco Foods International (Eden Prairie, MN). The manufacturer reported the composition of this powder to be 97.4% total protein, 92.5% β-lactoglobulin, and 2.4% ash. Other components present in the protein powders probably included water and carbohydrates. β-Carotene (Type I, C9750, synthetic, purity ≥93%) was purchased from the Sigma Chemical Company (St. Louis, MO). All other chemicals used in this research were purchased from Sigma-Aldrich (St Louis, MO). Double distilled water was used to make all solutions.

Emulsion Preparation. A series of oil-in-water emulsions were prepared that had different interfacial properties:

Preparation of Single-Protein Emulsions. The oil phase was prepared by dispersing crystalline β-carotene (0.25 wt %) in corn oil by sonicating for 1 min, then heating (50−60 °C) for 5 min, and then stirring at room temperature for about 1 h. After this process, the β-carotene was fully dissolved within the corn oil, i.e., a clear orange/yellow colored oil was obtained. Aqueous emulsifier solutions were prepared by dispersing either LF (0.75 wt %) or BLG (0.75 wt %) powder and 0.01% sodium azide (prevention of bacterial growth) into double distilled water and then stirring for 3 h at room temperature to ensure complete dispersion. The LF solution had to be filtered to remove a small amount of insoluble particles. Primary emulsions were prepared by homogenizing 5 wt % oil phase with 95 wt % aqueous phase at ambient temperature with the samples being covered by aluminum foil (to avoid light exposure). Coarse emulsions were formed by blending using a high shear mixer at ∼10,000 rpm with a 1 cm stator diameter (M133/1281-0, Biospec Products Inc., ESGC, Switzerland) for 2 min. These emulsions were then passed 4 times through a high-pressure microfluidizer (M-110 L processor, Microfluidics Inc., Newton, MA) at 11,000 psi (75.8 MPa).

Preparation of Nanolaminated Emulsions. Nanolaminated emulsions containing [LF]-[BLG]-coated droplets were formed by mixing 1.5% LF solution with 10% oil-in-water emulsions containing [BLG]-coated droplets in a 1:1 ratio at pH 6.5. Nanolaminated emulsions containing [BLG]-[LF]-coated droplets were formed by mixing 1.5% BLG solution with 10% oil-in-water emulsions containing [LF]-coated droplets in a 1:1 ratio at pH 6.5. At this pH, the BLG is negatively charged (pH > pI), and the LF is positively charged (pH < pI), which promotes adsorption of the globular proteins in solution to the oppositely charged droplet surfaces. Both the final nanolaminated emulsions produced using this method contained 5 wt % oil phase (β-carotene + corn oil), 0.75 wt % LF, and 0.75 wt % BLG.

Preparation of Mixed-Interface Emulsions. Mixed-interface emulsions containing [BLG/LF]-coated droplets were prepared by blending 5 wt % oil phase (β-carotene + corn oil) with 95% aqueous phase (0.75 wt % LF + 0.75 wt % BLG in solution) using a high shear mixer (M133/1281-0, Biospec Products Inc., ESGC, Switzerland) for 2 min. The coarse emulsions produced were then passed 4 times through a microfluidizer (M-110 L processor, Microfluidics Inc., Newton, MA) at 11,000 psi (75.8 MPa). After formation, all emulsions were stored overnight prior to analysis.

Color Degradation of β-Carotene Enriched Emulsions. Color fading of β-carotene emulsions was measured using a nondestructive colorimetric method described previously.[29] Samples were covered with foil to avoid light exposure and stored at 37 °C to accelerate pigment degradation. A colorimeter (ColorFlex EZ, Hunter lab) was used to

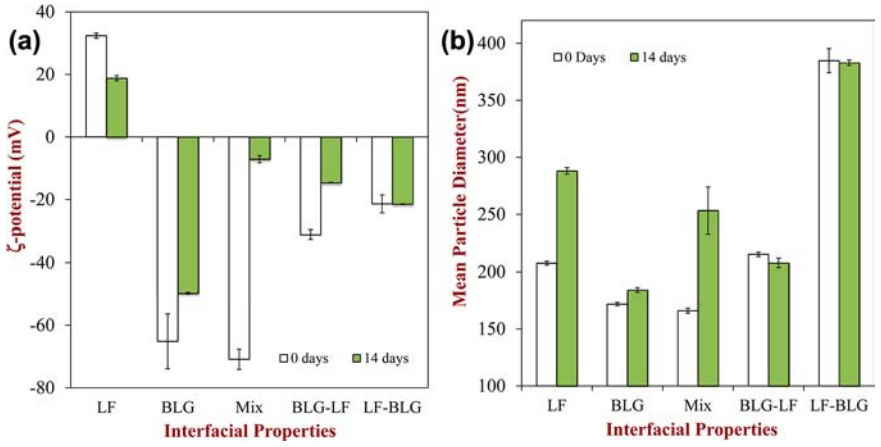

Figure 2. (a) ζ-Potential of β-carotene enriched emulsions with different interfacial properties measured after 0 or 14 days storage at 37 °C. (b) Mean particle size of β-carotene enriched emulsions with different interfacial properties measured after 0 and 14 days of storage.

measure the tristimulus color coordinates (L^*, a^*, b^*) of the emulsions during storage. The total color difference (ΔE^*) was then calculated from these values:

$$\Delta E^* = \sqrt{[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]} \quad (1)$$

where L^*, a^*, and b^* are the measured color coordinates of the emulsions at a certain incubation time, and L_0^*, a_0^*, and $b_0^*$are the initial values at zero time.

Influence of Environmental Stresses on Emulsion Physicochemical Stability. After preparation, emulsions were subjected to a range of environmental stresses. (1) pH: emulsions were adjusted to a particular pH value (pH 3 to 9) using either 1 M HCl or 1 M NaOH solutions, stirred for 10 min, and then stored overnight. (2) Ionic strength: a series of systems with different salt concentrations was prepared by mixing 50 mL aliquots of emulsions with 50 mL aliquots of sodium chloride solutions (0 to 600 mM) at pH 6.5. The samples were stirred for 10 min and then stored overnight. (3) Temperature: glass test tubes containing 5% oil-in-water emulsions were placed in a water bath set at a particular temperature (from 30 to 90 °C) for 30 min, then cooled to room temperature, and then stored overnight.

Particle Characterization. Particle size distributions were determined using dynamic light scattering (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, U.K.). Emulsion samples were diluted in pH-adjusted double distilled water at a ratio of 1:200 (v/v) and then placed in a capillary test tube that was loaded into the instrument. Particle sizes were reported as the Z-average mean diameter calculated from the measured particle size distribution. The ζ-potential of the particles in the emulsions was determined using a particle electrophoresis instrument (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK). Emulsions were diluted to a droplet concentration of approximately 0.001 wt % using pH-adjusted water to avoid multiple scattering effects. After loading the samples into the instrument, they were equilibrated for about 120 s before particle charge data was collected over 20 continuous readings.

Statistical Analysis. All experiments were carried out three times on newly prepared samples, with two to three measurements being made per sample. Results are reported as averages and standard deviations of these measurements.

RESULTS AND DISCUSSION

Formulation of Emulsions with Different Interfacial Properties. The electrical charges on fat droplets coated by different kinds of interfacial protein layers were measured (Figure 2a). At pH 6.5, the ζ-potential was initially +32 mV for the [LF]-coated droplets and −65 mV for the [BLG]-coated droplets, indicating that these two globular proteins had opposite electrical charges under these conditions. The electrical charges on the [BLG]-[LF]- and [LF]-[BLG]-laminated droplets were intermediate between that of the [BLG]- and [LF]-coated droplets, suggesting that their interfaces contained a mixture of proteins. Interestingly, the charge did not seem to be dominated by the protein that was believed to form the outer layer at the interface. The mixed-interface emulsions initially had a high negative charge that was similar to that on the [BLG]-emulsions. This may have occurred due to preferential adsorption of the smaller BLG molecules to the droplet surfaces during homogenization or because the BLG molecules tended to be located in the outer layers. After 14 days of storage, we noticed an appreciable decrease in the negative charge on the mixed-interface emulsions, which suggests that there was some change in the interfacial composition or structure over time. This may have occurred because LF molecules originally present in the aqueous phase adsorbed to the oil droplet surfaces, which could have been through a competitive adsorption or coadsorption process. In addition, there may have been some rearrangement in the structural organization of the LF and BLG molecules at the droplet surfaces over time, which changed their electrical characteristics

The mean particle diameter (d_{43}) of emulsions with different interfacial characteristics was measured after 0 and 14 days storage at ambient temperature (Figure 2b). The initial particle size was relatively small (d_{43} < 250 nm) in most of the emulsions, suggesting that they were relatively stable to droplet aggregation. However, the particles in the [BLG]-[LF]-emulsion (d = 385 nm) were appreciably larger than those in the [LF]-emulsion (d = 208 nm), which suggests that some droplet aggregation occurred during the preparation of these emulsions. One possible reason is that the BLG molecules did not completely saturate the surfaces of the [LF]-coated droplets, and so some bridging flocculation occurred in this system between positive patches on one droplet and negative patches on another droplet. After 14 days of storage, the droplet size either remained fairly similar to the initial values ([BLG]-, [BLG]-[LF]-, and [LF]-[BLG]-coated emulsions) or increased appreciably ([LF]- or mixed interface emulsions), suggesting that some droplet aggregation occurred during storage in these later systems.

It is important to stress that the stability of the laminated emulsions to droplet aggregation was very sensitive to the method used to prepare them. We found that less aggregation occurred when [LF]-emulsions were mixed with BLG solution (or [BLG]-emulsions were mixed with LF solution) at pH 8 and

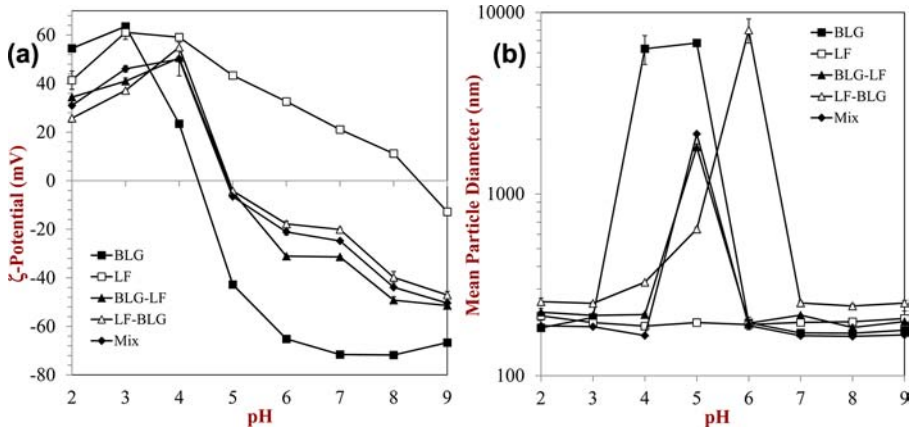

Figure 3. (a) Influnece of pH on ζ-potential in β-carotene enriched emulsions with different interfacial properties (5% corn oil and 0.25% β-carotene). (b) Influence of pH on droplet aggregation in β-carotene enriched emulsions with different interfacial properties (5% corn oil and 0.25% β-carotene).

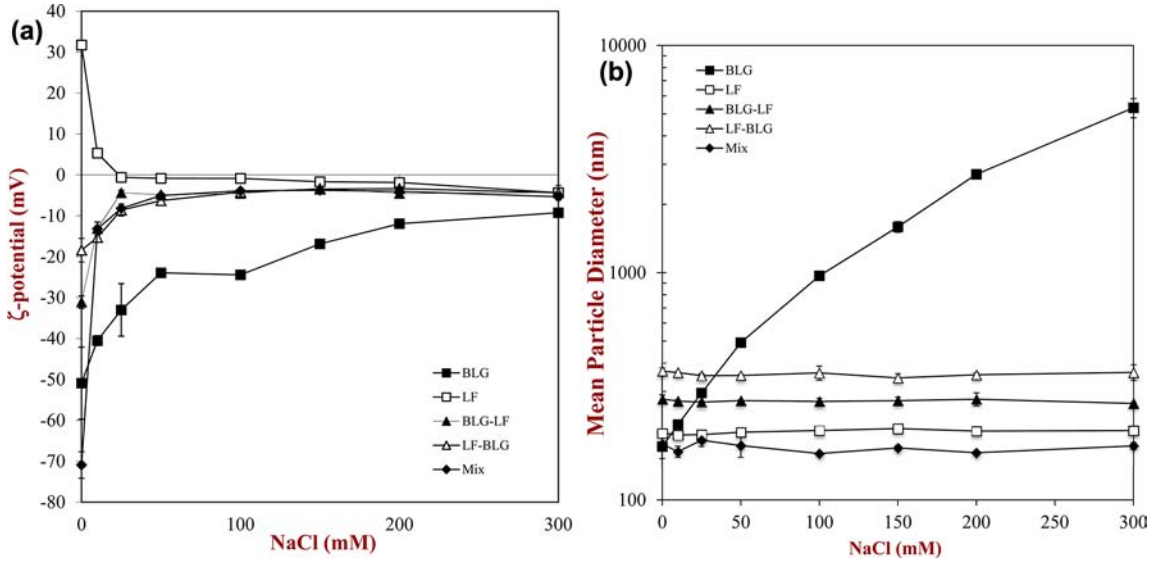

Figure 4. (a) Influence of ionic strength on the ζ-potential of β-carotene enriched emulsions with different interfacial properties (5% corn oil and 0.25% β-carotene). (b) Influence of ionic strength on droplet aggregation of β-carotene enriched emulsions with different interfacial properties (5% corn oil and 0.25% β-carotene).

then adjusted to pH 6.5 than if they were directly mixed at pH 6.5. A similar finding was reported for the formation of multilayer emulsions from protein-stabilized droplets and oppositely charged polysaccharides.[30]

Influence of Environmental Stresses on Emulsion Stability. Emulsion-based delivery systems containing β-carotene may be incorporated into food and beverage products that have different pH values and ionic strengths. In addition, ingested delivery systems must pass through the gastrointestinal tract where they are exposed to variations in pH and ionic strength in the mouth, stomach, and small intestine. We therefore examined the influence of pH and ionic strength on the electrical characteristics and stability of the β-carotene enriched emulsions. Emulsion samples were incubated at different pH and ionic strength values for 24 h to ensure that they had reached a steady state, and then their physicochemical properties were measured.

pH. The ζ-potential of [BLG]-emulsions went from highly positive to highly negative as the pH was increased from 2 to 9, with a point of zero charge around pH 4.5 (Figure 3a). This result can be attributed to the fact that the pH moved from below to above the isoelectric point of the BLG molecules adsorbed to the droplet surfaces.[30−32] The charge on the droplets in the [LF]-emulsions went from highly positive to slightly negative as the pH was increased from 2 to 9, with a point of zero charge around pH 8.5, which can be attributed to the appreciably higher isoelectric point of LF compared to BLG.[17,23] The ζ-potential of all the emulsions containing both BLG and LF also went from positive to negative with increasing pH, but the point of zero charge was around pH 5, i.e., slightly higher than that of [BLG]-coated droplets. Surprisingly, the mixed-interface, [LF]-[BLG]-nanolaminated, and [BLG]-[LF]-nanolaminated emulsions all had fairly similar charge−pH profiles (Figure 3a). These results suggest that the droplets were coated by a mixture of both LF and BLG molecules and that the BLG molecules dominated the overall electrical characteristics of the interfaces. This may have occurred because there were more BLG than LF molecules present at the droplet surfaces or because the BLG molecules arranged themselves so that they were preferentially located within the outer protein layer.

The mean particle diameter of the [BLG]-emulsions was relatively small at low pH values ($\leq$3) and high pH values ($\geq$6)

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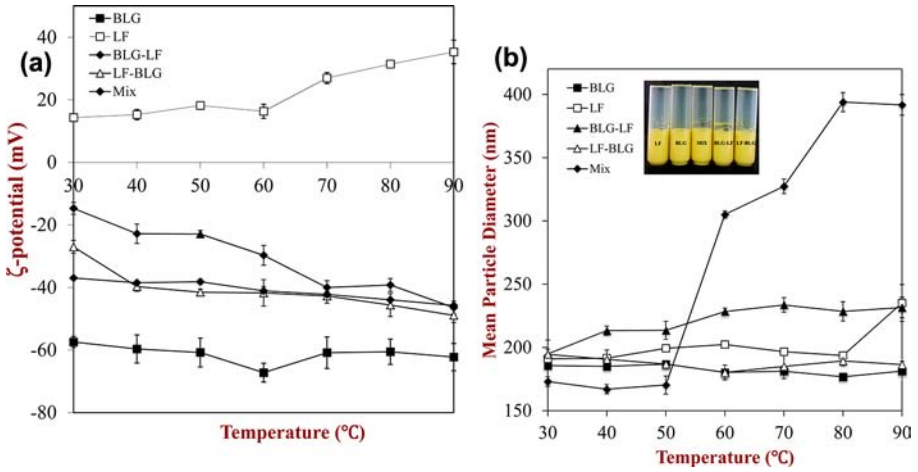

Figure 5. (a) Influence of holding temperature (for 30 min) on the ζ-potential of β-carotene enriched emulsions with different interfacial properties (5% corn oil and 0.25% β-carotene). (b) Influence of holding temperature (for 30 min) on droplet aggregation of β-carotene enriched emulsions with different interfacial properties (5% corn oil and 0.25% β-carotene).

(Figure 3b), which can be attributed to strong electrostatic repulsion between [BLG]-coated droplets in these pH ranges.[31] However, the [BLG]-emulsions were unstable to droplet aggregation at intermediate pH values (pH 4 and 5), which can be attributed to the fact that the protein molecules were near their isoelectric point, and so there was little charge on them. Under these conditions, the van der Waals attraction is sufficiently strong to overcome the weak electrostatic repulsion between the droplets, thereby leading to aggregation.[5] The size of the particles in the [LF]-emulsions remained relatively low across the entire pH range studied, indicating that the [LF]-coated droplets were stable to droplet aggregation. As discussed previously, steric repulsion plays an important role in determining the aggregation stability of [LF]-coated droplets due to the fact that LF molecules have a relatively high molecular weight and contain hydrophilic carbohydrate side chains that protrude into the aqueous phase.[16,17,23]

All of the emulsions containing a mixture of BLG and LF were unstable to droplet aggregation at intermediate pH values (Figure 3b). This effect can be partly attributed to the relatively low magnitude of the electrical charge on the droplets in this pH range (Figure 3a), and so the electrostatic repulsion between them would be relatively weak. However, the fact that the [LF]-coated droplets were stable but the [LF]-[BLG]-nanolaminated droplets were not suggests that LF was unable to provide strong steric repulsion when used in combination with BLG. This may have occurred because LF did not form a uniform outer layer around the [BLG]-coated droplets. Instead, a mixed-interfacial layer may have formed or there may have been some uncovered patches on the droplet surfaces.

Ionic Strength. The influence of salt addition on the electrical characteristics and aggregation stability of the various emulsion-based delivery systems was measured at pH 6.5 (Figure 4). The magnitude of the electrical charge on all of the emulsions decreased with increasing salt concentration (Figure 4a), which can be mainly attributed to electrostatic screening effects.[5] Interestingly, we observed charge reversal in the [LF]-coated droplets, with the charge going from highly positive to slightly negative with increasing NaCl concentration. This suggests that there may have been some binding of anionic chloride ions (Cl^-) to positively charged groups on the surfaces of the LF molecules.

The [BLG]-emulsions were unstable to salt addition, with the mean particle diameter increasing with increasing NaCl concentration (Figure 4b). Droplet aggregation was attributed to progressive screening of the electrostatic repulsion between the [BLG]-coated droplets as the salt concentration increased. All of the other emulsions appeared to be stable to salt addition as indicated by the fact that there was no change in mean particle diameter with increasing salt concentration. This effect may have been partly due to the steric repulsion generated by the presence of LF molecules at the droplet surfaces.[16]

Thermal Treatment. The effect of thermal treatment on the stability of β-carotene enriched emulsions was determined at pH 6.5 (no salt) by holding samples at temperatures ranging from 30 to 90 °C for 30 min. There were some appreciable changes in the electrical characteristics (ζ-potential) of the droplets in some of the emulsions with increasing holding temperature (Figure 5a), which suggests that there were alterations in the composition and/or structural organization of the droplet interfaces in these systems. For example, the positive charge on the [LF]-coated droplets increased appreciably when the holding temperature was increased from 60 to 70 °C, which may have been due to thermal denaturation of this globular protein and subsequent alterations in the organization of the cationic groups in the interface. There was also an appreciable increase in the negative charge on the droplets in both laminated emulsions ([LF]-[BLG]- and [BLG]-[LF]-) with increasing holding temperature. A possible explanation for this effect is that there was a reorganization of the LF and BLG molecules at the droplet surfaces due to thermal treatment, e.g., BLG molecules may have preferentially moved to the outer surfaces, or LF molecules may have desorbed from the droplet surfaces. Further studies are needed to identify the physicochemical basis of these changes in droplet charge with heating.

All single ([LF]- or [BLG]-) and laminated ([LF]-[BLG]- or [BLG]-[LF]-) emulsions exhibited good stability to droplet aggregation at all holding temperatures as evidenced by their relatively small particle sizes and ability to flow when the test tubes were inverted (Figure 5b). However, there was an appreciable increase in the mean particle size of the mixed-interface emulsions held at ≥50 °C, suggesting that some droplet aggregation occurred within these systems. Even so, the mixed-interface emulsions still remained fluid when they were inverted,

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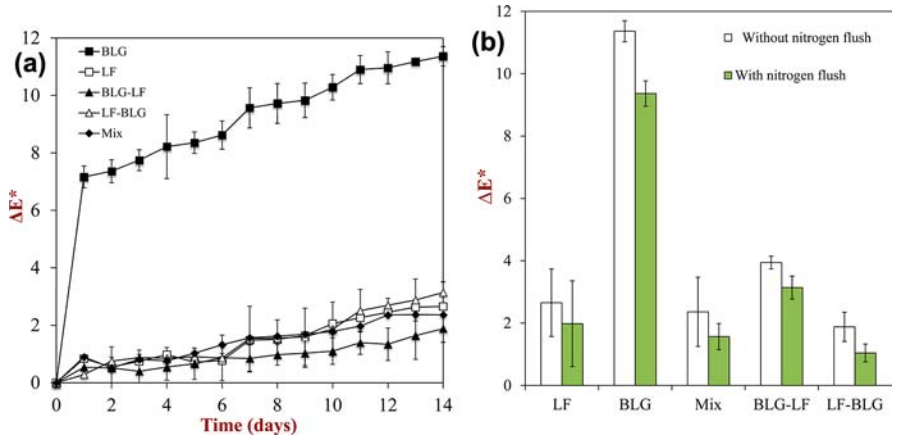

Figure 6. (a) Total color change (ΔE^*) of β-carotene enriched emulsions with different interfacial properties during storage at 37 °C. (b) Total color change (ΔE^*) of β-carotene enriched emulsions with different interfacial properties after storage at 37 °C for 14 days with or without nitrogen flushing.

indicating that droplet aggregation did not cause gelation. These studies show that the initial interfacial composition and structure does have an influence on the thermal stability of the protein-coated lipid droplets. Previous studies have reported that [LF]-coated lipid droplets aggregated when heated above around 50 °C,[16,23,33] which was attributed to unfolding of the adsorbed globular proteins leading to an increase in hydrophobic attraction between the droplets. However, we found that the [LF]-coated lipid droplets were relatively stable to droplet aggregation at elevated temperatures in the current study (Figure 5b). This apparent discrepancy may be due to differences in the amount of LF available to cover the droplet surfaces in the different studies. Previous studies have shown that the stability of fat droplets to aggregation is better at high LF-to-fat droplet ratios[16,34] than at low ratios.[16,23,33] Presumably, at higher protein-to-fat ratios the droplets are more stable to aggregation because the LF forms a uniform and thick interfacial layer that generates a strong steric repulsion.

Influence of Interfacial Properties on β-Carotene Degradation. β-Carotene is a polyunsaturated molecule that is highly susceptible to chemical degradation and therefore tends to lose its orange-red color during storage.[25] We therefore measured color fading of the β-carotene emulsions using a nondestructive colorimetric method described previously.[29] There was a rapid change in the total color difference of the [BLG]-emulsions during the first day of storage, followed by a more gradual increase afterward (Figure 6a). However, there was only a slight increase in ΔE^* for the other emulsions over time. These results suggested that the presence of LF within the emulsion-based delivery systems was able to retard the chemical degradation of encapsulated β-carotene. This could be due to the ability of lactoferrin molecules to strongly bind iron ions (Fe^{2+} or Fe^{3+})[35,36] since transition metals are known to catalyze oxidation reactions.[25] The nature of the interfacial coatings ([LF]-, [LF]-[BLG]-, [BLG]-[LF]-, or [LF/BLG]-) did not appear to have a major impact on the rate of β-carotene degradation, again highlighting the importance of having some LF present in the systems. We also found that ΔE^* was somewhat less for samples that were routinely flushed with nitrogen during storage to lower the oxygen concentration (Figure 6b), which highlights the role that oxygen plays in color fading.

Development of Bioactive Delivery Systems. The results of this study have important implications for the development of effective delivery systems for bioactive lipophilic components. The stability of protein-coated emulsions to droplet aggregation under different pH and ionic strength conditions depended on interfacial properties. Lactoferrin-coated droplets were stable to droplet aggregation when the pH was varied from 2 to 9, whereas those containing [BLG]-, [BLG]-[LF]-, [LF]-[BLG]-, or [LF/BLG]-coatings were all unstable to droplet aggregation at intermediate pH values. [LF]-Coated droplets also had good stability to the presence of NaCl (even though the droplet charge was close to zero) and to elevated temperatures. These results suggest that [LF]-coated droplets are stabilized primarily by steric repulsion, rather than electrostatic repulsion. The chemical degradation of β-carotene occurred relatively quickly in emulsions containing [BLG]-coated oil droplets but was much slower in all emulsions containing LF. Metal ions are known to play an important role in promoting the chemical degradation of carotenoids, and therefore, the ability of lactoferrin to chelate metal ions would account for LF's ability to delay β-carotene degradation. Alternatively, the LF may have been able to bind to β-carotene and protect it from degradation. Overall, this study suggests that droplets coated by LF alone may be the most suitable for forming physically and chemically stable delivery systems for β-carotene. However, LF is relatively expensive, and cationic droplets may cause some problems in commercial products (such as astringent mouthfeel and precipitation with anionic components), and therefore, using LF in combination with BLG may be desirable for certain applications. Finally, we should mention that a recent study in our laboratory found that the bioaccessibility of β-carotene measured using an in vitro method was relatively low for [LF]-coated oil droplets.[37] Consequently, it is important to ensure that carotenoids are both protected during storage and released after ingestion.

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Notes

The authors declare no competing financial interest.

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