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Production and Characterization of Oil-in-Water Emulsions Containing Droplets Stabilized by β-Lactoglobulin−Pectin Membranes

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Oil-in-water emulsions containing droplets stabilized by β-lactoglobulin (β-Lg)−pectin membranes were produced using a two-stage process. A primary emulsion containing small droplets ($d_{32} \approx 0.3$ μm) was prepared by homogenizing 10 wt % corn oil with 90 wt % aqueous solution (1 wt % β-Lg, 5 mM imidazole/acetate buffer, pH 3.0) using a high-pressure valve homogenizer. The primary emulsion was then diluted with pectin solutions to produce secondary emulsions with a range of pectin concentrations (5 wt % corn oil, 0.45 wt % β-Lg, 5 mM imidazole/acetate buffer, 0−0.22 wt % pectin, pH 3.0). The electrical charge on the droplets in the secondary emulsions decreased from +33 ± 3 to −19 ± 1 mV as the pectin concentration was increased from 0 to 0.22 wt %, which indicated that pectin adsorbed to the droplet surfaces. The mean particle diameter of the secondary emulsions was small ($d_{32} < 1$ μm) at relatively low pectin concentrations (<0.04 wt %), but increased dramatically at higher pectin concentrations (e.g., $d_{32} \approx 13$ μm at 0.1 wt % pectin), which was attributed to charge neutralization and bridging flocculation effects. Emulsions with relatively small mean particle diameters ($d_{32} \approx 1.2$ μm at 0.1 wt % pectin) could be produced by disrupting flocs formed in secondary emulsions containing highly negatively charged droplets, for example, by sonication, blending, or homogenization. The particles in these emulsions probably consisted of small flocs containing a number of protein-coated droplets bound together by pectin molecules. These emulsions had good stability to further particle aggregation up to relatively high ionic strengths (≤500 mM NaCl) and low pH (pH 3). The interfacial engineering technology used in this study could lead to the creation of food emulsions with improved physicochemical properties or stability.

KEYWORDS: Emulsion; β-lactoglobulin; pectin; stability; aggregation; ζ-potential

INTRODUCTION

Oil-in-water emulsions that consist of small lipid droplets dispersed in an aqueous medium form the basis of many kinds of foods, for example, milk, cream, beverages, dressings, dips, sauces, batters, and desserts (*1*−*3*). Emulsions are thermodynamically unstable systems because of the unfavorable contact between oil and water phases, and because the oil and water phases have different densities, they will always break down over time (*2*, *4*−*6*). Emulsion breakdown is usually retarded by using emulsifiers, which are surface-active ingredients that adsorb to the surface of freshly formed lipid droplets during homogenization (*2*−*4*, *7*). Once adsorbed, they facilitate further droplet disruption by lowering the interfacial tension, thereby reducing the size of the droplets produced during homogenization. Emulsifiers also reduce the tendency for droplets to aggregate by forming protective membranes and/or by generating repulsive forces between the droplets. A good emulsifier should rapidly adsorb to the surface of the lipid droplets formed during homogenization, rapidly lower the interfacial tension by a significant amount, and protect the droplets against aggregation during emulsion processing, storage, and utilization (*2*, *4*, *7*).

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, as well as in their cost, ease of utilization, ingredient compatibility, and environmental sensitivity. Each type of emulsifier has its own particular advantages and disadvantages. For example, some emulsifiers are effective at generating small emulsion droplets during homogenization but are poor at providing long-term stability against droplet aggregation and vice versa (*2*, *10*). Consequently, there is no single emulsifier that is ideal for use in every food product. A number of studies have shown it is possible to improve emulsion stability by combining the beneficial attributes of different types of emulsifiers by forming emulsions containing droplets stabilized by multilayered membranes (*11*−*15*). These emulsions can be formed using a two-step procedure. First, a

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primary emulsion is formed using an ionic emulsifier that facilitates the formation of small droplets during homogenization. Second, a secondary emulsion is formed by adding an oppositely charged polymer that adsorbs to the droplet surfaces. The resulting droplets are coated by a two-layer interfacial membrane that may provide improved emulsion stability and physicochemical properties.

In this study a cationic protein emulsifier [β-lactoglobulin (β-Lg) at pH 3] that rapidly adsorbs to the surface of lipid droplets during homogenization will be used to produce a primary emulsion with small droplet sizes, and then an anionic polysaccharide (pectin) will be added to the system to produce secondary emulsions containing droplets coated with an emulsifier−biopolymer membrane. Under certain environmental conditions (protein-to-pectin ratio, pH, ionic strength) the emulsions are likely to become unstable to flocculation due to charge neutralization and bridging flocculation effects (*15*). However, when sufficient pectin is added to the emulsions, we expect that the net droplet charge will switch from positive to negative, and kinetically stable emulsions containing anionic droplets could be produced, as has been shown for other types of emulsion systems (*13*, *14*). One of the objectives of this study is to identify a cost-effective process that utilizes food ingredients for producing stable emulsions containing multilayered droplets and to test the influence of solution conditions (pH, ionic strength) on emulsion stability. It should be mentioned that a number of studies have examined the influence of polysaccharides on the stability of protein-stabilized emulsions (*15*−*17*). Nevertheless, most of these studies have focused on either the utilization of covalent protein−polysaccharide complexes to improve emulsion stability or the ability of polysaccharides to promote instability in protein-stabilized emulsions, rather than on the utilization of protein−polysaccharide interfacial interactions to develop emulsions with improved stability.

Pectin was selected as a potential stabilizer of food emulsions because it is already widely utilized in the food industry as an ingredient (*18*, *19*). It is a purified carbohydrate product obtained by aqueous extraction of edible plant material (usually from citrus fruits or apples) and has a chemical structure that consists of a mixture of methyl esterified galacturonan, galactan, and araban residues varying in proportion for pectins from different sources. Pectin has a negative charge in mildly acidic solutions due to the presence of ionized carboxylic groups along its backbone that have pK_a values of ∼3.5 (*19*). Its functional characteristics in aqueous solutions are largely determined by its degree of esterification and degree of polymerization.

MATERIALS AND METHODS

Materials. Analytical grade sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium azide (NaN_3) were purchased from the Sigma Chemical Co. (St. Louis, MO). Powdered β-Lg was obtained from Davisco Foods International (lot JE 001-1-922, Le Sueur, MN). As stated by the manufacturer, the total protein content of the powder was 93.2% (with β-Lg making up 95%) and the moisture content was 4.3%. Distilled and deionized water was used for the preparation of all solutions. Pectin (extracted from citrus) was purchased from Sigma-Aldrich Co. (St. Louis, MO). As stated by the manufacturer, the degree of esterification of the pectin was 59%. Corn oil was purchased from a local supermarket and used without further purification.

Solution Preparation. An aqueous emulsifier solution containing 0.5 wt % protein was prepared by dispersing powdered β-Lg into 5 mM imidazole/acetate buffer (pH 3.0) containing 0.04 wt % NaN_3 (as an antimicrobial agent). Solutions containing 0.5 wt % pectin were prepared by dispersing weighed amounts of the powdered material into 5 mM imidazole/acetate buffer (pH 3.0). Each solution was then stirred for at least 2 h to ensure complete dissolution of the powders, and the pH was readjusted to 3.0 if necessary.

Emulsion Preparation. A primary emulsion was prepared by homogenizing 10 wt % corn oil with 90 wt % aqueous emulsifier solution with a high-speed blender (M133/1281-0, Biospec Products, Inc.) followed by five passes through a two-stage high-pressure valve homogenizer: 7500 psi first stage, 750 psi second stage (Lab 1000, APV-Gaulin, Wilmington, MA). This emulsion was diluted with aqueous pectin solutions to form secondary emulsions with varying compositions: 5 wt % corn oil, 0.45 wt % β-Lg, and 0−0.22 wt % pectin (pH 3.0). The emulsions were then stored at room temperature for 24 h before being analyzed. All experiments were carried out in duplicate or triplicate using freshly prepared emulsions, and the results are reported as the mean and standard deviation.

Floc Disruption Conditions for Secondary Emulsions. To disrupt flocs formed during the preparation of the secondary emulsions, some of the samples were treated by sonication, blending, or homogenization. Sonication was carried out using a programmable high-intensity ultrasonic generator with a titanium alloy horn (model 500, Sonic Disembrator, Fisher Scientific, Pittsburgh, PA). Blending was carried out using a high-speed blender (M133/1281-0, Biospec Products, Inc.). Homogenization was carried out using a two-stage high-pressure valve homogenizer, with the pressure of the second valve set at 10% of the first valve (Lab 1000, APV-Gaulin).

Particle Size Measurements. Emulsions were diluted to a droplet concentration of ∼0.005 wt % using buffer solution immediately before measurement to avoid multiple scattering effects. The particle size distribution of the emulsions was measured using a laser light scattering instrument (LS230, Coulter Corp.). 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. The particle diameter was calculated from three separate injections of the same sample.

ζ-Potential Measurements. Emulsions were diluted to a droplet concentration of ∼0.005 wt % using buffer solution immediately before measurement to avoid multiple scattering effects. The diluted emulsions were then injected into the measurement chamber of a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcs., U.K.). The ζ-potential was determined by measuring the direction and velocity of droplet movement in a well-defined electric field. The ζ-potential was calculated from three separate injections of the same sample, with five readings made per injection.

RESULTS AND DISCUSSIONS

Influence of Pectin Concentration on Droplet Characteristics of Secondary Emulsions. The electrical charge and mean particle diameter of secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 5 mM imidazole/acetate buffer, pH 3.0) containing different pectin concentrations (0−0.22 wt %) were measured 24 h after preparation (**Figures 1** and **2**).

In the absence of pectin, the electrical charge on the emulsion droplets was ∼33 ± 3 mV, which was due to the β-Lg being below its isoelectric point (IEP = 5.2) at pH 3 and therefore having a net positive charge (*20*). The electrical charge on the droplets changed from positive to negative as the pectin concentration in the emulsions was increased (**Figure 1**). There was no net charge on the droplets when the pectin concentration was ∼0.04 wt %, which corresponded to a pectin to β-Lg mass ratio (R) of ∼0.1 g/g. The negative charge on the droplets reached a constant value (≈−19 ± 1 mV) when the pectin concentration exceeded ∼0.1 wt % ($R \approx 0.22$ g/g). These measurements indicated that negatively charged pectin adsorbed to the surface of the positively charged β-Lg stabilized emulsion droplets. The negatively charged pectin molecules continued

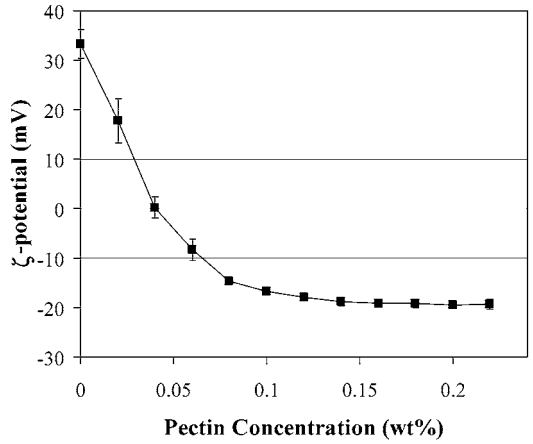

Figure 1. Dependence of electrical charge of emulsion droplets (ζ-potential) on pectin concentration for primary and secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 5 mM imidazole/acetate buffer, pH 3.0) after dilution.

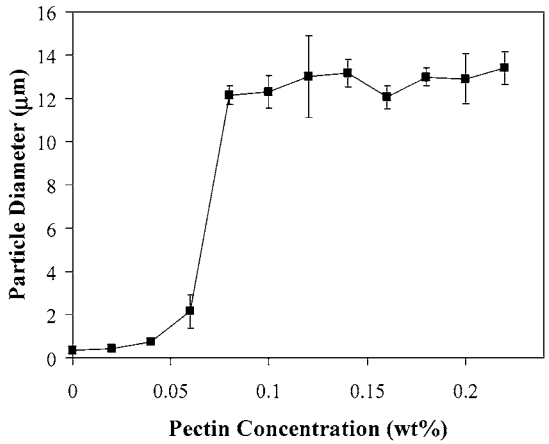

Figure 2. Dependence of mean particle diameter on pectin concentration for primary and secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 5 mM imidazole/acetate buffer, pH 3.0) after dilution.

to adsorb to the surfaces of the emulsion droplets, even though the droplets had no net charge or were negatively charged, up to a certain saturation level (**Figure 1**). The ability of charged polyelectrolytes to adsorb to the surface of oppositely charged colloidal particles and cause charge reversal is well established in the literature (*21*−*25*).

There was a relatively small but significant increase (from $d_{32} = 0.36 \pm 0.04$ to 2.2 ± 0.8 μm) in the mean particle diameter of the secondary emulsions when the pectin concentration was increased from 0 to 0.06 wt % (**Figure 2**), which corresponded to the region where charge neutralization occurred (**Figure 1**). At relatively high pectin concentrations (0.08−0.2 wt %) there was a large increase in mean particle diameter ($d_{32} \approx 13$ μm), which was attributed to extensive droplet aggregation (**Figure 2**). One might have expected the emulsions containing highly negatively charged particles (e.g., at 0.2 wt % pectin) to be stable to droplet aggregation because of the strong electrostatic repulsion between the droplets (*2*). It therefore seems likely that pectin molecules adsorbed to the surface of more than one emulsion droplet during the formation of the secondary emulsions, thus acting as polymeric bridges that held the droplets together (*15*−*17*).

Influence of Mechanical Agitation Conditions. In previous studies with lecithin−chitosan emulsions it has been shown that the flocs formed in secondary emulsions could be disrupted by application of mechanical energy (*14*). The aim of this series of experiments was therefore to determine whether we could use mechanical agitation to disrupt the flocs formed in secondary emulsions containing β-Lg−pectin-stabilized droplets. We compared the effectiveness of a high-speed blender, a high-pressure valve homogenizer, and a sonicator for breaking down the flocs. These experiments were carried out using secondary emulsions consisting of 5 wt % corn oil, 0.45 wt % β-Lg, and 0.2 wt % pectin (5 mM imidazole/acetate buffer, pH 3.0), that is, at a pectin concentration sufficiently high to produce strongly negatively charged droplets (**Figure 1**). The secondary emulsions were treated with mechanical agitation and then stored for 24 h before their particle diameter was measured by laser diffraction (**Table 1**). All three mechanical devices could be used to reduce the mean particle diameter to values much smaller that that of the untreated secondary emulsion ($d \approx 13$ μm). For the sonicator, there was a large decrease in mean particle diameter after 30 s of sonication of the emulsions, followed by little or no change at longer sonication times (**Table 1**). As would be expected, the initial reduction in mean particle diameter was greater at higher sonication power levels. For the high-speed blender, there was a large decrease in mean particle diameter after 1 min of blending but little change at longer blending times (**Table 1**). For the high-pressure valve homogenizer, the mean particle diameter decreased with increasing number of passes through the homogenizer and with increasing homogenization pressure. It should be noted that these homogenization pressures were always below those used to create the primary emulsion (7500 psi), so that little disruption of the individual droplets would be expected.

The smallest droplet sizes obtained by each technique were $d = 3.0$ μm for sonication, $d = 3.8$ μm for blending, and $d = 2.7$ μm for homogenization. These mean particle diameters are

Table 1. Influence of Mechanical Agitation Conditions on Mean Particle Diameter (in Micrometers) of Secondary Emulsions (5 wt % Corn Oil, 0.45 wt % β-Lg, 0.20 wt % Pectin, 5 mM Imidazole/Acetate Buffer, pH 3.0) Stabilized by β-Lg–Pectin Membranes

	Sonication		
	amplitude 20%	amplitude 30%	amplitude 40%
0 s	12.9	12.9	12.9
30 s	4.3	2.8	2.6
60 s	3.3	2.9	3.2
120 s	3.3	3.0	3.2
180 s	3.2	3.2	3.1

	Blending (High Speed)
0 min	12.9
1 min	4.7
2 min	4.3
3 min	4.1
4 min	3.8
5 min	4.3

	Homogenization				
	1000 psi	2000 psi	3000 psi	4000 psi	5000 psi
0 passes	12.9	12.9	12.9	12.9	12.9
1 pass	11.8	11.9	12.0	9.9	9.6
2 passes	8.7	6.2	8.4	5.0	4.7
3 passes	5.6	4.0	5.3	3.9	3.9
4 passes	4.2	2.8	4.8	2.8	2.8
5 passes	3.1	2.9	2.7	3.0	2.9

[a] Emulsions were diluted to ∼0.005 wt % corn oil prior to measurement. The mean particle diameter in the primary emulsion was 0.32 ± 0.04 μm. Standard deviations were always <15% of the mean particle diameters reported in this table.

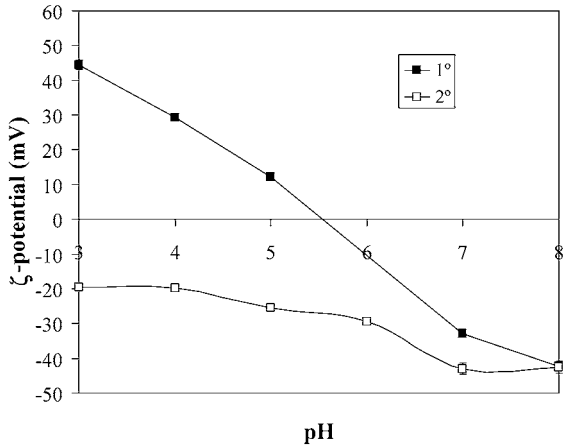

Figure 3. Dependence of electrical charge of emulsion droplets (ζ-potential) on pH for primary and secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 0.2 wt % pectin, 5 mM imidazole/acetate buffer, pH 3.0) after dilution to 0.005 wt % corn oil.

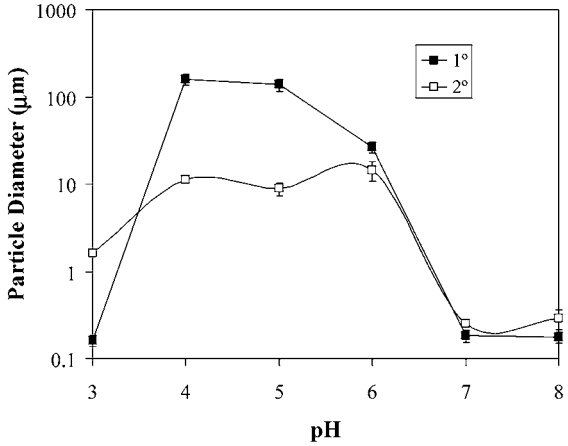

Figure 4. Dependence of mean particle diameter on pH for primary and secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 0.2 wt % pectin, 5 mM imidazole/acetate buffer, pH 3.0) after dilution to 0.005 wt % corn oil.

appreciably greater than that of the primary emulsion ($d \approx 0.3$ μm) and would be considered too large to provide good stability in many food emulsions (*2*, *4*). We therefore examined the possibility of using a combination of homogenization and sonication for effectively disrupting the flocs. A secondary emulsion (5% oil, 0.45 wt % β-Lg, and 0.2 wt % pectin) was homogenized at 2000 psi for five passes, and then a portion of it (10 g) was sonicated for 30 s at a frequency of 20 kHz, an amplitude of 40%, and a duty cycle of 0.5 s. This procedure enabled us to produce secondary emulsions containing particles with a mean diameter of ~1.5 μm, which was still considerably larger than that in the primary emulsion. It therefore seemed that the interactions between the negatively charged pectin molecules and the positively charged droplets were so strong that the flocs could not easily be disrupted. Nevertheless, this part of the study did show that emulsions containing relatively small particles could be produced by using combined homogenization and sonication treatments.

Influence of Solution Conditions on Emulsion Stability. The purpose of these experiments was to identify the influence of pH and ionic strength on the stability of primary (β-Lg membranes) and secondary (β-Lg−pectin membranes) emulsions. A secondary emulsion (5 wt % corn oil, 0.45 wt % β-Lg, 0.2 wt % pectin, 5 mM imidazole/acetate buffer, pH 3.0) was prepared containing highly negatively charged droplets using the procedure described above. Flocs in this emulsion were then disrupted by passing it through a high-pressure valve homogenizer (five passes at 2000 psi) followed by sonication (30 s at 40% power). Primary and secondary emulsions were then prepared with different pH values (*3*−*8*) and ionic strengths (0−500 mM NaCl) as described earlier. The influence of pH on the ζ-potential and mean particle diameter of primary and secondary emulsions was then measured after they had been stored at room temperature for 24 h (**Figures 3** and **4**).

The ζ-potential of the β-Lg-stabilized droplets in the primary emulsions went from being highly positive to highly negative as the pH was increased from 3 to 8, which is due to the change in the electrical charge of the protein molecules as they move from below to above their isoelectric point (*27*). The ζ-potential of the β-Lg−pectin-stabilized droplets in the secondary emulsions was negative at all pH values but became increasingly negative as the pH increased from 3 to 8 (**Figure 3**). To understand the pH dependence of the electrical charge on the droplets in the secondary emulsions, it is necessary to understand the pH dependence of the electrical charge of the pectin and protein molecules. Pectin is uncharged at low pH (<1.5) but becomes increasingly negatively charged as the pH approaches and exceeds the pK_a value (≈3.5) of the carboxyl groups (*19*). The net electrical charge on β-Lg molecules is positive at low pH but becomes negative once the pH exceeds the protein's isoelectric point (IEP ≈ 5.2) (*20*). At pH 3, the net negative charge observed on the droplets in the secondary emulsions (**Figure 4**) is therefore due to the negative charge on the pectin molecules outweighing the positive charge on the protein molecules. As the pH is increased, the droplets become more negatively charged because of the increase in negative charge of the pectin and reduction of positive charge/increase of negative charge of the proteins. Even so, one might have expected the decrease in ζ-potential with increasing pH in the secondary emulsions to have been larger than that actually observed because there was a much greater decrease in ζ-potential with increasing pH in the primary emulsions (where the β-Lg was losing its positive charge) and because the negative charge on the pectin molecules was increasing. This suggests that when the pH was increased, either some of the negatively charged pectin molecules desorbed from the surface of the emulsion droplets or the thickness of the adsorbed layer increased so that the electrical charge at the shear-plane decreased (*28*).

It is also interesting to note that at pH 8 the droplets in the primary and secondary emulsions had fairly similar negative charges, which suggests that the pectin was completely displaced from the droplet surfaces so that the ζ-potential was solely due to the adsorbed proteins. This is not surprising because there would be a relatively strong electrostatic repulsion between the negatively charged protein and negatively charged polysaccharide at this pH.

In the primary emulsions, the mean particle diameter was relatively small at low (pH 3) and high (pH ≥7) pH values (**Figure 4**), presumably because the electrostatic repulsion between the droplets was sufficiently strong to prevent droplet aggregation (*2*). On the other hand, at intermediate pH values (pH 4−6), there was evidence of extensive droplet aggregation, which can be attributed to the fact that the relatively weak electrostatic repulsion between the droplets was insufficient to overcome the attractive van der Waals and hydrophobic interactions (*2*). In the secondary emulsions, the mean particle diameter at pH 3 was appreciably greater than in the primary

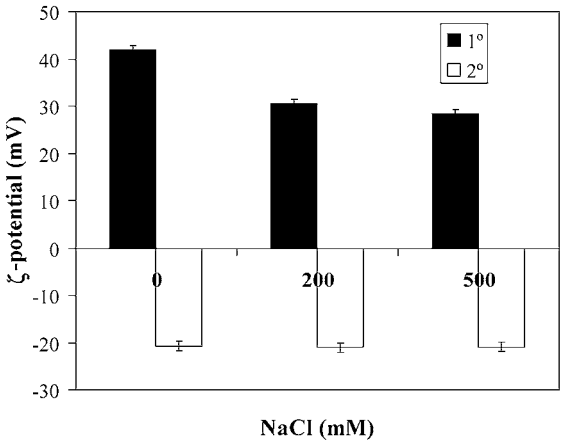

Figure 5. Dependence of electrical charge of emulsion droplets (ζ-potential) on NaCl concentration for primary and secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 0.2 wt % pectin, 5 mM imidazole/acetate buffer, pH 3.0) after dilution to 0.005 wt % corn oil.

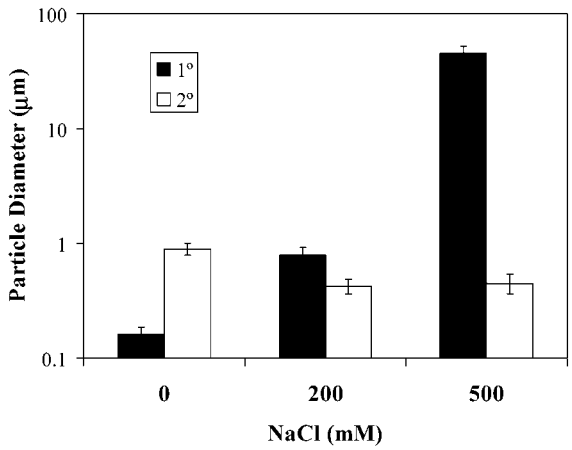

Figure 6. Dependence of mean particle diameter on NaCl concentration for primary and secondary emulsions (5 wt % corn oil, 0.45 wt % β-Lg, 0.2 wt % pectin, 5 mM imidazole/acetate buffer, pH 3.0) after dilution to 0.005 wt % corn oil.

emulsions (**Figure 4**), because it was not possible to disrupt all of the flocs by application of mechanical agitation (see earlier). At pH values from 4 to 6 the secondary emulsions were highly unstable to droplet aggregation ($d = 9-15$ μm). This was surprising because the magnitude of the negative charge on the particles increased with increasing pH (**Figure 4**), which would have been expected to increase the electrostatic repulsion between the particles, thereby reducing flocculation (*2*). A possible explanation for this observation is that the interaction between the pectin molecules and the droplet surfaces became weaker as the pH was increased due to the decrease in positive charge of the adsorbed protein layer. Consequently, it may have become easier for part of a pectin molecule to detach itself from one droplet and bind to another droplet, promoting bridging flocculation (*15*−*17*). At high pH (pH 7 and 8), the mean particle diameter of the secondary emulsions was similar to that of the primary emulsions, which suggests that the individual droplets had been released from the flocs in the secondary emulsions. As mentioned previously, it seems likely that the pectin molecules were desorbed from the surface of the droplets in the secondary emulsion at high pH due to electrostatic repulsion between the negatively charged proteins and negatively charged polysaccharides. Hence, emulsion stability at high pH was similar for primary and secondary emulsions.

The influence of NaCl concentration (0, 200, or 500 mM) on the ζ-potential and mean particle diameter of primary and secondary emulsions at pH 3 was measured during storage at room temperature for 24 h (**Figures 5** and **6**). As expected, the ζ-potential of the droplets in the primary emulsion decreased with increasing NaCl concentration (**Figure 5**) due to electrostatic screening effects (*28*). Surprisingly, the ζ-potential (−21 ± 1 mV) of the droplets in the secondary emulsions was relatively insensitive to NaCl concentration (**Figure 5**). It is possible that either the thickness of the adsorbed layer or the amount of pectin adsorbed to the droplets changed with NaCl concentration to compensate for the expected reduction in ζ-potential due to electrostatic screening effects. This type of charge regulation has been reported in other types of colloidal system (*28*).

The observed dependence of the ζ-potential on NaCl concentration may help to explain the influence of NaCl on the mean particle diameters (**Figure 6**). The mean particle diameter of the primary emulsions increased with increasing NaCl concentration, which can be attributed to droplet aggregation promoted by the reduction in droplet ζ-potential and electrostatic screening effects (*29*, *30*). On the other hand, there was a significant decrease in the mean diameter of the particles in the secondary emulsion with increasing NaCl concentration from 0 to 500 mM (**Figure 6**). One would have expected that the particles would have been more aggregated at higher salt concentrations due to electrostatic screening effects (*2*, *28*). There are a number of possible physicochemical phenomena that might explain this effect. The addition of NaCl may have decreased the strength of the electrostatic attraction between the positively charged droplets and the negatively charged pectin (*31*, *32*). Hence, the pectin adsorbed less strongly, causing an increase in the thickness of the interfacial membrane. The resulting β-Lg−pectin membrane formed may have been sufficiently thick to generate a strong steric repulsion between the particles, which prevented extensive droplet aggregation (*2*). Alternatively, the β-Lg−pectin membrane formed may have reduced the strength of the van der Waals attractions between the droplets, thus decreasing the driving force for particle aggregation (*2*). This result indicates that secondary emulsions may have improved stability to high salt concentrations compared with primary emulsions.

CONCLUSIONS

This study has shown that emulsions containing multilayered lipid droplets can be prepared using a relatively simple method. Initially, a primary emulsion containing small droplets was produced by homogenization of oil, water, and a cationic protein emulsifier (β-Lg at pH 3). A secondary emulsion containing anionic lipid droplets coated with a β-Lg−pectin membrane was then produced by mixing an anionic polysaccharide (pectin at pH 3) with the primary emulsion and applying mechanical agitation to help disrupt any flocs formed. The driving force for polysaccharide adsorption was presumably the electrostatic attraction between the positively charged droplets and negatively charged pectin. The resulting secondary emulsions contained relatively small particles ($d < 2$ μm) that consisted of a number of protein-stabilized oil droplets held together by pectin bridges. These emulsions had relatively good stability to particle aggregation at high salt concentration (<500 mM) under acidic conditions (pH 3). Recent studies have shown that the release of nonpolar flavors from oil-in-water emulsions during mastication can be controlled by encapsulating the oil droplets within biopolymer particles (*33*). This approach can be used to create low-fat food products with similar flavor release characteristics

to high-fat food products (*33*). The method used to prepare the secondary emulsions in our study may therefore prove to be useful for producing this type of low-fat product. In future studies, we intend to examine methods of reducing the amount of bridging flocculation occurring during the formation of the secondary emulsions, so as to produce final emulsions containing individual droplets coated by a protein−polysaccharide membrane. We also intend to examine methods of producing emulsions that are stable over a wide range of pH and salt concentrations by utilizing different biopolymer combinations.

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