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Stabilization of Soybean Oil Bodies Using Protective Pectin Coatings Formed by Electrostatic Deposition

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Soybeans contain oil bodies that are naturally coated by a layer of phospholipids and proteins. In nature, this coating protects the oil bodies from environmental stresses and could be utilized by food manufacturers for the same purpose. However, natural oil bodies are physically unstable to aggregation because of the relatively weak electrostatic repulsion between them, which limits their application in many foods. In this study, oil bodies were extracted from soybean using an aqueous extraction method and then coated by a pectin layer using electrostatic deposition. The influence of NaCl (0–500 mM), pH (2–8), and freeze–thaw cycling (−20 °C, 22 h/40 °C, 2 h) on the properties and stability of the oil bodies coated by the pectin layer was analyzed using ζ-potential, particle size, and creaming stability measurements. These results suggest that pectin-coated oil bodies have similar or improved stability compared to uncoated oil bodies and may provide a new way of creating functional soy products for use in the food and other industries.

KEYWORDS: Soybean; oil; emulsion; oil bodies; oleosin; pectin

INTRODUCTION

Oil bodies are lipid storage vesicles that are naturally found in many plant seeds (*1*). Oil bodies consist of a lipid core that is coated by a layer of oleosin proteins and phospholipids (*2–5*). The oleosin proteins provide the oil bodies with physical and chemical stability against environmental stresses, such as moisture and temperature fluctuations and the presence of oxidative reagents (*3, 5, 6*). These oil bodies and oleosins have recently gained attention due to potential applications in foods, cosmetics, and pharmaceuticals (*1, 7, 8*).

In principle, food manufacturers could benefit from the already existing natural protection of the soybean oil bodies to obtain a product that has improved stability during food processing, storage, transport, and utilization (*1*). Soybean oil bodies could be utilized in food products as ingredients in place of emulsified soybean oil, for example, in dressings, sauces, dips, beverages, and desserts. Additional advantages of using natural soybean oil bodies in foods, rather than emulsified bulk soybean oil, are that neither emulsifiers nor homogenization procedures are required. The successful utilization of soybean oil bodies in food products requires a thorough understanding of their functional performance under different environmental conditions. In a previous study, we investigated the influence of pH, ionic strength, and thermal processing on the properties and stability of oil bodies extracted from soybeans (*9*). This study showed that oil bodies were stable to aggregation over only a narrow range of pH values (pH <3 and >7) and NaCl concentrations (<50 mM). The relatively poor physical stability of oil bodies to aggregation would severely limit the range of products in which they could be utilized in the food, health care, and pharmaceutical industries. For this reason, we examined the possibility of improving the stability of soybean oil bodies by utilizing an electrostatic deposition method that has proved to be highly successful at improving the aggregation stability of phospholipid- and protein-coated emulsion droplets to environmental stresses (*10–13*). This interfacial engineering method involves coating charged lipid droplets with a layer of oppositely charged polysaccharides.

In this study, we prepared oil body suspensions by aqueous extraction of soybeans and then coated them with a pectin layer. Pectin was selected because it is an anionic polysaccharide ($pK_a \approx 3.5$) that has already been shown to improve the stability of protein-coated lipid droplets (*14, 15*). The major objective of this work was to test the impact of coating oil bodies with pectin on their physical stability over a range of pH values, on increasing the ionic strength, and after freeze–thaw cycling.

MATERIALS AND METHODS

Materials. High-fat soybeans (code 5601T) were obtained from the crops laboratory at the University of Tennessee (Knoxville, TN). Pectin extracted from citrus fruit was purchased from Sigma Chemical Co.

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(lot 91K1420, St. Louis, MO). The degree of esterification of the pectin was reported to be 59% by the supplier. Analytical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were also purchased from Sigma Chemical Co. All other chemicals were obtained from Fisher Scientific. Distilled and deionized water from a water purification system (Nanopure Infinity, Barnstead International, Dubuque, IA) was used for the preparation of all solutions.

For the isolation and characterization of soybean oil bodies, further studies on the impact of the molecular characteristics of pectin would be useful, for example, charge density, hydrophobicity, and molecular weight.

Oil bodies were physically isolated from a total homogenate of mature soybean seeds using an aqueous-based flotation−centrifugation method described previously (*9*). The composition of the cream layer extracted from the soybeans was determined in a previous study by proximate analysis (*9*): moisture = 46.7 ± 0.3%; ash = 0.328 ± 0.002%; fat = 40.1 ± 1.4%; protein = 8.8 ± 0.6%; other = 4.1 ± 1.6%.

Oil Body Suspension Preparation. An oil body suspension was prepared by mixing 8 g (wet weight) of cream layer with 24 g of buffer solution (10 mM sodium phosphate, pH 7) using the Wheaton Potter Elvehjem tissue grinder. Then 30 g of oil body suspension was diluted with 120 g of buffer solution (10 mM sodium phosphate, pH 7). The oil body suspension (5% oil body, 2% oil) was then transferred into a 250 mL conical flask, stored in a water bath at a fixed temperature (90 °C for 30 min), and then cooled to room temperature using ice. The oil body (5 wt % oil body, 2% oil) was adjusted back to pH 7 using 0.1 M HCl. Uncoated (1.25 wt % oil body, 10 mM sodium phosphate buffer) and coated (1.25 wt % oil body, 0.08 wt% pectin, 10 mM sodium phosphate buffer) oil body suspensions were prepared by dilution.

Influence of Pectin Concentration on Oil Body Properties. The influence of pectin concentration on the properties of the soybean oil body suspension was examined. A pectin solution (1%, pH 7) was prepared by dispersing weighed amounts of the powdered material into buffer (10 mM sodium phosphate, pH 7). The oil body suspension was diluted with different ratios of buffer and pectin solutions to make samples with the same oil body concentration (1.25 wt% oil) but different pectin concentrations (0−0.5 wt %). The pH of the oil body suspensions was then adjusted from 7 to 4 by the addition of HCl to promote pectin adsorption. The oil body suspensions were then stored at room temperature for 24 h prior to ζ-potential and light scattering analysis and for 7 days prior to creaming stability analysis.

Influence of Environmental Stresses on Oil Body Properties. The influence of pH and ionic strength variations on the properties of uncoated and pectin-coated oil body suspensions was examined. Final oil body suspensions were prepared by diluting the initial oil body suspension with different ratios of buffer solution, pectin solution, and salt solution at pH 7. The pH was then adjusted by the addition of HCl to give a series of uncoated (0% pectin) and coated (0.08 wt % pectin) samples with similar compositions: 1.25 wt % oil body, 10 mM sodium phosphate, pH 2–8, 0−500 mM NaCl. The oil body suspensions were then stored at room temperature for 24 h prior to ζ-potential and light scattering analysis and for 7 days prior to creaming stability analysis.

Influence of Freeze–Thaw Cycling Stability. The influence of repeated freeze–thaw cycles on the properties and stability of uncoated and coated oil bodies at pH 3 was examined. Oil body samples (10 g) were transferred into plastic sealable cryogenic test tubes (internal diameter = 15 mm, height = 75 mm) and were incubated in a −20 °C freezer for 22 h. After incubation, the emulsion samples were thawed by incubating them in a water bath at 40 °C for 2 h. This freeze–thaw cycle was repeated up to three times, and its influence on the oil body suspension properties was measured after each cycle.

Oil Body Characterization. The ζ-potential (particle electrophoresis), particle size (light scattering), microstructure (optical microscopy), and creaming stability (visual observation) of the oil bodies were determined using experimental methods described in a previous paper (*9*).

Statistical Analysis. Experiments were performed at least twice using freshly prepared samples. Average and standard deviations were calculated from these duplicate measurements.

RESULTS AND DISCUSSION

Influence of Pectin Concentration on Oil Body Properties. The electrical charge, mean particle diameter, and creaming stability of oil bodies containing different pectin concentrations (0–0.5 wt %) were measured (**Figure 1**) to determine the optimum amount of pectin required to produce stable pectin-coated oil body suspensions.

The ζ-potential of the uncoated oil bodies was around +5.7 mV at pH 4, indicating that the oil bodies were below their isoelectric point (**Figure 1a**). The electrical charge became increasingly less positive and eventually changed from positive to negative as the pectin concentration in the oil body suspension was increased from 0 to 0.5 wt %. The negative charge on the oil body reached a relatively constant value of −18 mV when the pectin concentration exceeded about 0.04 wt %. These measurements indicate that anionic pectin molecules adsorbed to the surfaces of the cationic oil bodies and that the oil body surfaces eventually became saturated with pectin.

Measurements of the mean particle diameter and creaming stability of the oil body suspensions indicated that they were highly unstable to aggregation in the absence of pectin (**Figure 1b**,**c**). This aggregation can be attributed to the fact that the ζ-potential of the oil bodies was relatively small, and hence the electrostatic repulsion between them was insufficient to overcome the various attractive interactions (e.g., van der Waals and hydrophobic). In addition, it suggests that the steric repulsion between uncoated oil bodies was insufficiently strong to prevent their aggregation (*16*). Thus, we can conclude that the individual oil bodies were highly flocculated in the absence of pectin. Extensive droplet aggregation and creaming were also observed in oil body suspensions when they contained <0.02 wt % pectin. This type of aggregation can be attributed to the relatively low ζ-potential of the oil bodies (weak electrostatic repulsion), as well as the linking together of the cationic oil bodies by anionic pectin molecules (bridging flocculation) that occurs when the pectin concentration is below saturation coverage (*17*). At pectin concentrations from 0.04 to 0.1 wt %, the mean particle diameter of the oil bodies was relatively small (**Figure 1b**) and the oil body suspensions were relatively stable to creaming (**Figure 1c**). The good stability of the oil body suspensions at intermediate pectin concentrations can be attributed to the ability of the pectin molecules to completely cover the oil body surfaces and form a relatively thick electrically charged coating (**Figure 1d**). This polysaccharide coating increases the electrostatic and steric repulsion between the oil bodies, as well as decreases the van der Waals attraction, thereby increasing their stability to aggregation (*16*). The size of the pectin-stabilized oil bodies determined in this study was similar to that reported for native oil bodies determined by light and electron microscopy in earlier studies (*5–7, 18*). At pectin concentrations exceeding 0.2 wt %, the mean particle diameter gradually increased with increasing pectin concentration (**Figure 1b**), and there was some evidence of cream layer formation at the top of the tubes (**Figure 1c**). The decrease in stability of the oil bodies at high pectin concentrations can be attributed to depletion flocculation induced by the high levels of nonadsorbed pectin in the continuous phase (*17, 19*). These results indicate that stable coated oil bodies can be formed over a range of pectin concentrations (0.04−0.1

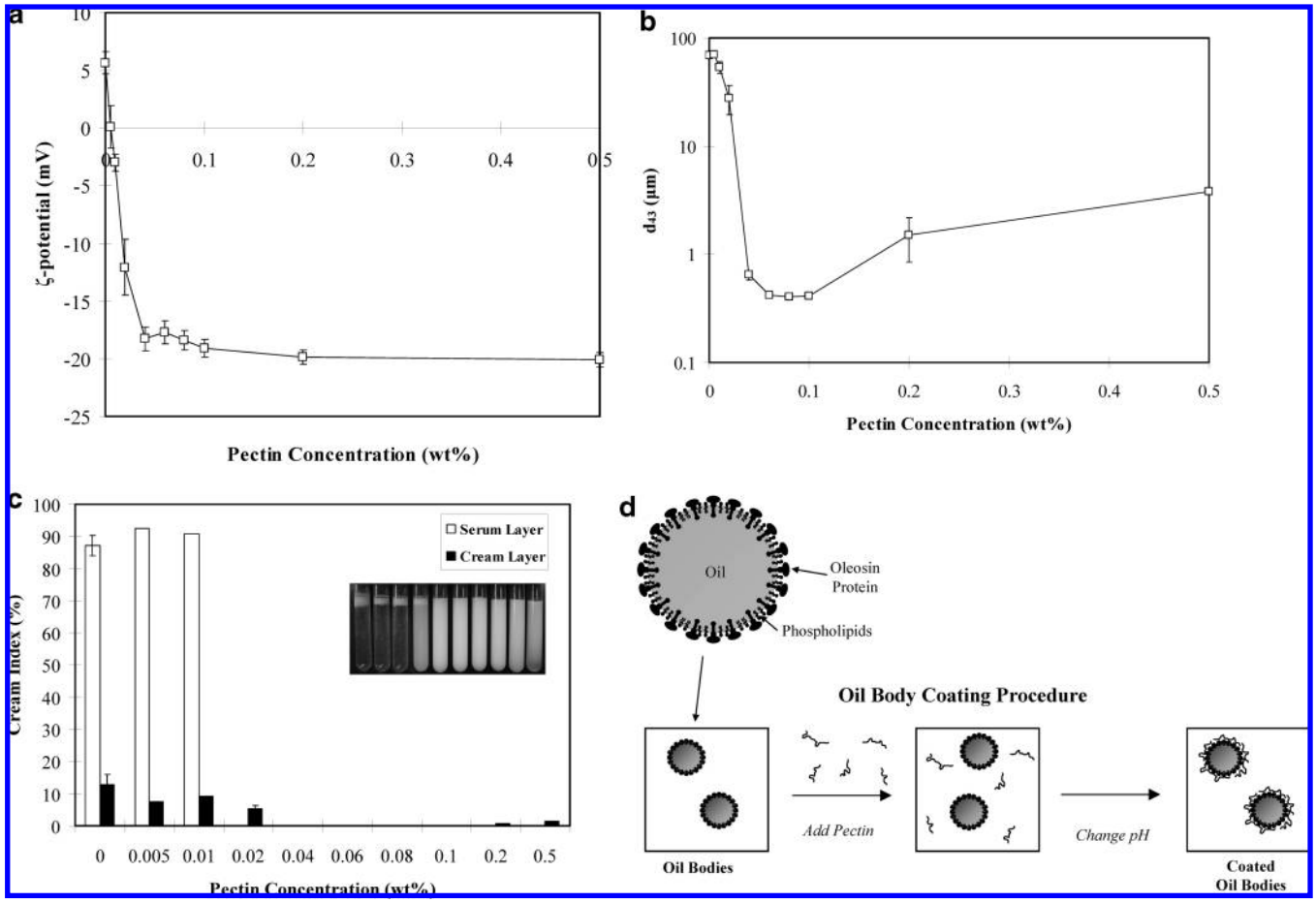

Figure 1. Dependence of the electrical charge (ζ-potential) (**a**), mean particle diameter (d_{43}) (**b**), and creaming stability (**c**) of soybean oil body suspensions on pectin concentration (1.25% oil bodies, 10 mM sodium phosphate, pH 4.0). The relative heights shown are of the serum and cream layers observed in the oil body suspensions after 1 week of storage at room temperature. The pectin concentrations in the photograph increase from left to right as marked on the *x*-axis of the figure. (**d**) Schematic diagram of coating of oil bodies by polysaccharide.

wt %). Consequently, we used a pectin concentration of 0.08 wt % to prepare pectin-coated oil bodies for the subsequent stability studies.

Influence of pH on Oil Body Properties. The purpose of this series of experiments was to determine the influence of pH on the properties of uncoated and coated oil bodies. The electrical charge, mean particle diameter, and creaming stability of oil body suspensions were measured (**Figure 2**). An understanding of the pH dependence of these properties provides valuable insights into the range of solution conditions under which coated oil bodies can be formed, as well as providing guidelines to the type of food matrices (e.g., acid, neutral) with which coated oil bodies can be successfully utilized. The ζ-potential of the uncoated oil body suspension changed from around −18 mV at pH 8 to around +25 mV at pH 2, with the point of zero charge being around pH 4.5 (**Figure 2a**). This kind of behavior is consistent with protein-stabilized lipid droplets (*14, 20*) and suggests that the oleosin and maybe other proteins remained around the oil bodies after the aqueous extraction procedure. Previous studies have found that the isoelectric point of oil bodies extracted from various plant sources was around pH 6 (*5, 6, 8, 21*), which is about 1.5 pH units higher than the value determined in our study. This difference has previously been attributed to the presence of enzymes in the oil body extracts that catalyze biochemical changes that increase the negative charge on the oil bodies, for example, lipases or phospholipases (*9*).

The ζ-potentials of the oil body suspensions in the absence and presence of pectin were similar at pH 7 and 8 (**Figure 2a**), which suggested that the anionic pectin molecules did not adsorb to the surfaces of the anionic oil bodies because of the relatively strong electrostatic repulsion between them (*17*). When the pH was decreased below 6, the ζ-potentials of the oil bodies in the suspension containing 0.08 wt % pectin were appreciably more negative than those of the oil bodies in the suspension containing no pectin (**Figure 2a**). This difference can be attributed to the adsorption of anionic pectin molecules to cationic patches on the proteins adsorbed to the oil body surfaces (*12*). The difference in ζ-potential between uncoated and coated oil bodies was about −25 to −30 mV between pH 2 and 4.

The mean particle diameters (**Figure 2b**) and creaming indices (**Figure 2c**) were relatively low for the uncoated oil body suspensions at low (pH 2) and high (pH 7 and 8) pH values (i.e., $d_{43} < 0.5$ μm, $CI_{SL} = 0\%$, $CI_{CL} = 0\%$), which can be attributed to the relatively strong electrostatic repulsion between the oil bodies preventing their aggregation (*19*). On the other hand, there was a large increase in mean particle diameter and rapid creaming in the oil body suspensions at intermediate pH values (pH 4 and 5), suggesting that extensive oil body aggregation occurred due to the relatively weak electrostatic repulsion between the oil bodies (*12, 20, 22*). The oil body suspensions at pH 3 and 6 were only marginally stable, with a relatively low mean particle diameter but some evidence of serum or cream layer formation after 1 week of storage (**Figure**

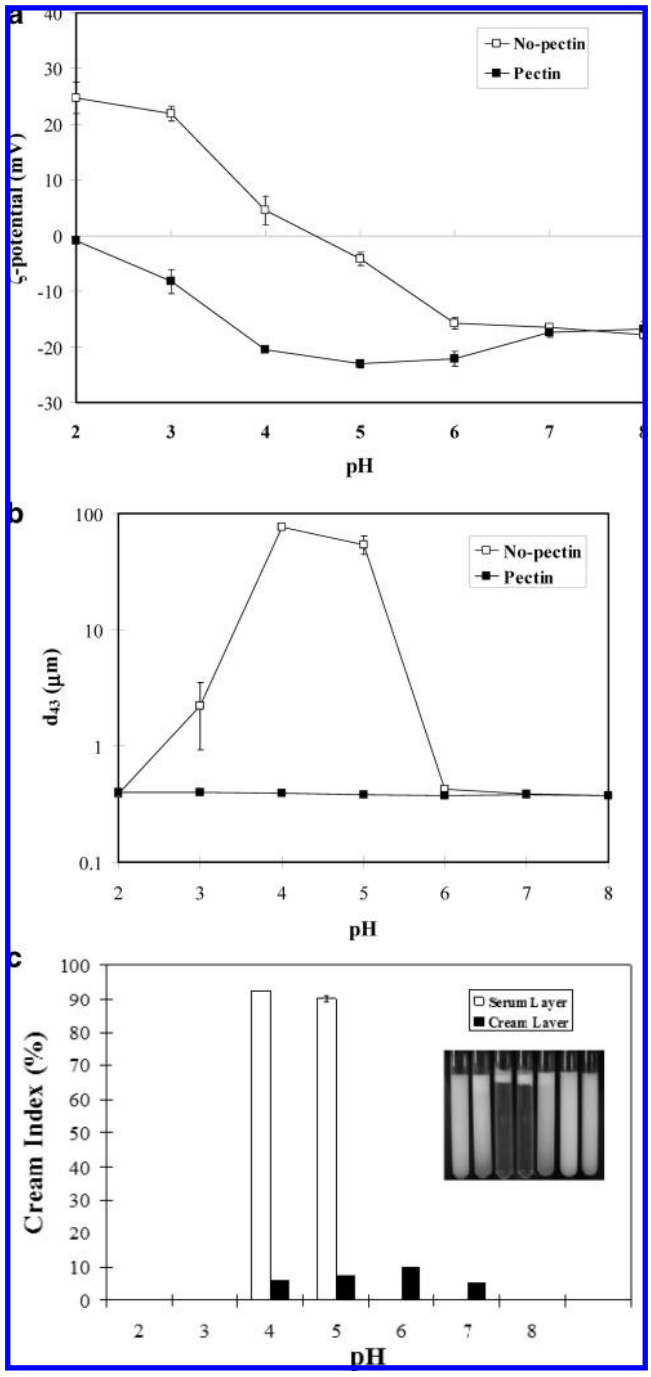

Figure 2. pH dependence of the electrical charge (ζ-potential) (**a**), mean particle diameter (d_{43}) (**b**), and creaming stability (**c**) of uncoated (0 wt % pectin) and coated (0.08 wt % pectin) oil body suspensions (1.25% oil bodies, 10 mM sodium phosphate). The relative heights shown are of the serum and cream layers observed in the oil body suspensions after 1 week of storage at room temperature. The photograph shows the uncoated oil body suspension with the pH values increasing from left to right as marked on the *x*-axis of the figure.

2c). This was probably because the energy barrier in the interaction potential versus droplet separation profile was not sufficiently high (>20 kT) to completely prevent droplet aggregation due to the relatively low ζ-potential in these systems (*19*). The mean particle diameter of the coated oil body suspension was relatively low at all pH values studied (i.e., $d_{43} < 0.4$ μm), and there was no evidence of creaming instability ($CI_{SL} = 0\%$, $CI_{CL} = 0\%$). As mentioned above, this increased stability to aggregation can be attributed to the relatively thick charged polysaccharide layer that surrounds the coated oil bodies because this increases the repulsion and decreases the attraction between them (*16*). Nevertheless, it should be noted that the coated droplets were more stable than the uncoated droplets even at pH values at which the magnitude of the ζ-potential on the uncoated droplets ($|\zeta| = 22$ mV at pH 3) was higher than that on the coated droplets ($|\zeta| = 8$ mM at pH 3). This suggests that the increased stability of the coated droplets was primarily due to strengthening of steric repulsion or weakening of van der Waals attraction, rather than increasing electrostatic repulsion (*16*). Overall, these results clearly show that coating the soybean oil bodies with a pectin layer can greatly extend the range of pH values at which they remain stable to aggregation.

Influence of NaCl on Oil Bodies. The purpose of this series of experiments was to examine the influence of salt on the stability of the uncoated and coated oil body suspensions because this would provide insights into the type of food matrices with which oil bodies could be successfully utilized in the food industry. After preparation, oil bodies were stored at room temperature in the presence of salt (0−500 mM NaCl, pH 3.0), and then their electrical charge (**Figure 3a**) and mean particle diameter (**Figure 3b**) were measured after 24 h of storage and their creaming stability was measured after 1 week of storage (**Figure 3c**).

The ζ-potential of the uncoated oil bodies was relatively insensitive to salt concentration (**Figure 3a**); that is, it remained virtually unchanged at +22 ± 0.6 mV regardless of added NaCl concentration (0−500 mM). The ζ-potential of colloidal suspensions usually decreases with increasing ionic strength due to electrostatic screening effects (*19*). The relative insensitivity of the ζ-potential of oil bodies to the addition of NaCl has previously been attributed to the presence of endogenous salt in the system or to charge regulation effects (*9*). The ζ-potential of the coated oil bodies was more negative than that of the uncoated oil bodies due to the presence of the anionic pectin layer. As mentioned above, the magnitude of the ζ-potential on the uncoated droplets (22 mV) was higher than that on the coated droplets (8 mV), which suggested that the increased stability of the coated droplets was due to strengthening of steric repulsion and/or weakening of van der Waals attraction, rather than an increase in the electrostatic repulsion.

The mean particle diameters of the uncoated oil bodies were relatively large ($d_{43} > 5$ μm), and the oil body suspensions were unstable to creaming ($CI_{CL} > 6\%$) at all NaCl concentrations studied (**Figure 3b**,**c**), which can be attributed to the relatively low electrostatic repulsion between the oil bodies at this pH (*9*). On the other hand, the mean particle diameter remained relatively small ($d_{43} < 2$ μm) at all salt concentrations for the pectin-coated droplets, and there was no evidence of creaming instability ($CI_{SL} = 0\%$, $CI_{CL} = 0\%$). These results show that the pectin coating was able to greatly improve the salt stability of oil body suspensions, which may have important implications for their utilization in food products.

Influence of Freeze–Thaw Cycling. The purpose of this series of experiments was to examine the influence of freeze–thaw cycling (−20 °C/+40 °C) and sucrose addition (0 or 10% w/w) on the stability of uncoated and coated oil body suspensions (**Figure 4**). This knowledge is of practical importance because many food products undergo some kind of freezing and thawing during their storage and application, for example, frozen desserts, sauces, or beverages.

The number of freeze–thaw cycles or the addition of sucrose caused no change in the ζ-potential of the uncoated oil bodies with ζ = +22 ± 0.8 mV (**Figure 4a**). In the absence of sucrose,

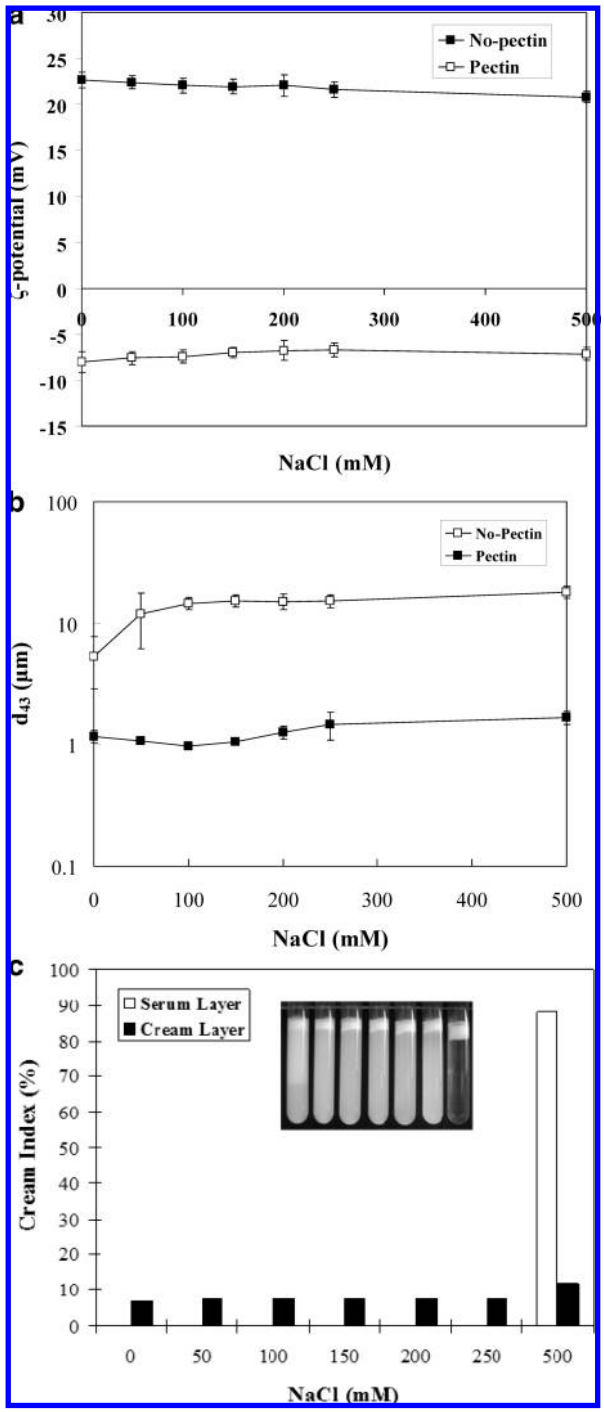

Figure 3. Salt dependence of the electrical charge (ζ-potential) (**a**), mean particle diameter (d_{43}) (**b**), and creaming stability (**c**) of uncoated (0 wt % pectin) and coated (0.08 wt %) oil bodies (1.25% oil bodies, 10 mM sodium phosphate, pH 3.0). The coated oil bodies were stable to creaming at all salt concentrations. The photograph shows the uncoated oil body suspension with the NaCl values increasing from left to right as marked on the *x*-axis of the figure.

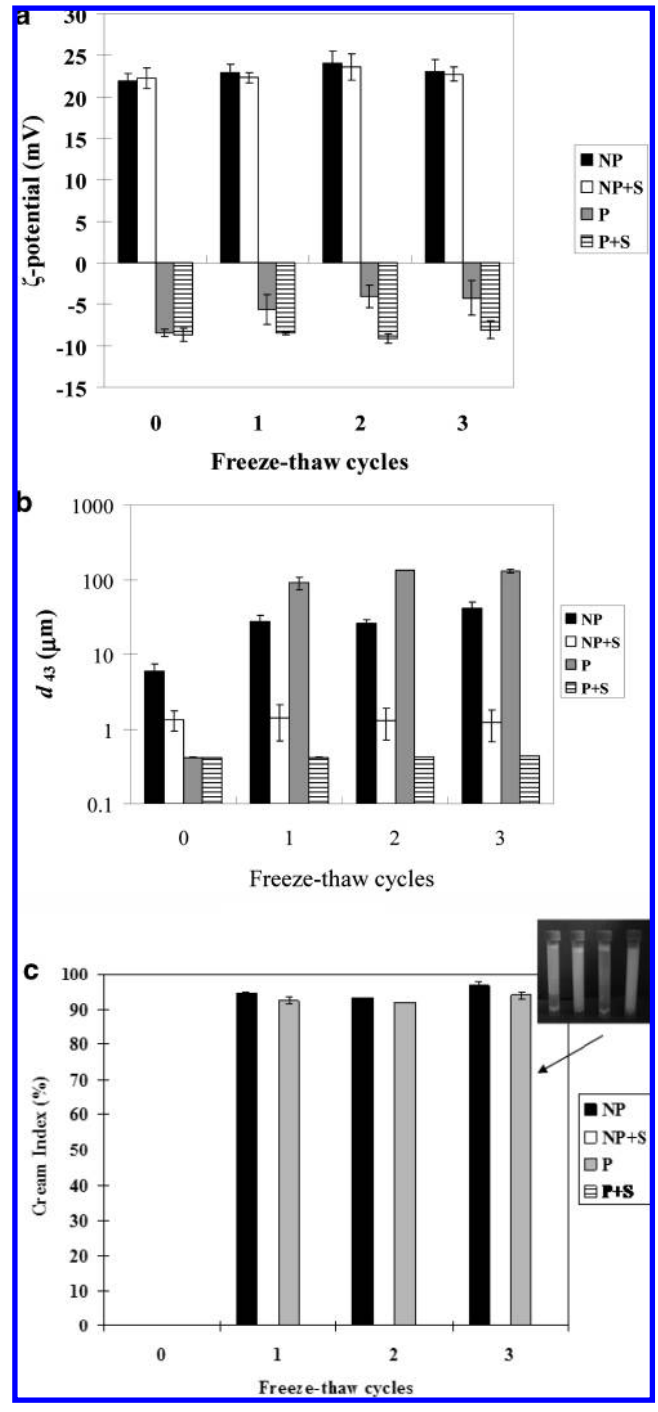

Figure 4. Dependence of the electrical charge (ζ-potential) (**a**), mean particle diameter (d_{43}) (**b**), and creaming stability (**c**) of uncoated (0 wt % pectin) and coated (0.08 wt % pectin) oil bodies (1.25% oil bodies, 10 mM sodium phosphate, pH 3.0) on number of freeze–thaw cycles and sucrose addition. The photograph shows the coated oil body suspension containing no sucrose with the number of cycles increasing from left to right as marked on the *x*-axis of the figure. NP, no pectin; P, pectin; NP+S, no pectin + sucrose; P+S, pectin + sucrose.

the ζ-potential of the coated oil bodies decreased slightly with increasing number of freeze–thaw cycles, for example, from −8.5 ± 0.5 mV at zero cycles to −4.2 ± 2.0 mV after three cycles. In the presence of 10% w/w sucrose, the ζ-potential of the coated oil bodies was independent of the number of freeze–thaw cycles (**Figure 4a**). The change in ζ-potential of the coated oil bodies in the absence of sucrose may have been due to some desorption of pectin molecules from the oil body surfaces during freezing−thawing, or it may have been because of the large change in particle aggregation after freezing.

In the absence of sucrose, there was an appreciable increase in the mean particle size and creaming instability of both the coated and uncoated oil bodies after only one freeze−thaw cycle, which suggested that the pectin coating was unable to protect the oil bodies from freeze–thaw cycling on its own. On the other hand, both the coated and uncoated oil body suspensions were

much more stable in the presence of 10 w/w % sucrose, with there being little evidence of droplet aggregation after three freeze–thaw cycles (**Figure 4b**,**c**). Previous studies have shown that sugars can increase the stability of emulsified lipids through a number of mechanisms, including their ability to modify the freezing behavior of water, modulate colloidal interactions, increase aqueous phase viscosity, and alter emulsifier functionality (*23*–*25*). In particular, sugars increase the fraction of unfrozen water present in the continuous phase of emulsions, thereby decreasing the tendency of lipid droplets to be forced into close proximity. The mean particle diameters and creaming stability of the coated oil bodies indicated less aggregation than the uncoated oil bodies (**Figure 4b**,**c**), which can be attributed to the ability of the pectin layer to improve the aggregation stability of the oil bodies in the absence of freezing. Consequently, it seems that the combination of sucrose and pectin provides a system that is stable to freeze–thaw cycling, with the sucrose increasing the freeze–thaw stability and the pectin increasing the stability of the reconstituted system. Again, these experiments clearly show that the presence of a pectin coating around the oil bodies can greatly improve their stability to environmental stresses.

In this study, we used a particular type of citrus pectin to stabilize the oil bodies. It would be useful in future studies to examine the impact of different molecular characteristics of pectin on its ability to stabilize oil bodies, for example, charge density, hydrophobicity, and molecular weight. In addition, it would be useful to examine the ability of other kinds of charged polysaccharides to stabilize these systems.

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