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Stabilizing Behavior of Soy Soluble Polysaccharide or High Methoxyl Pectin in Soy Protein Isolate Emulsions at Low pH

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The stability of emulsions prepared with soy protein isolates was investigated as a function of pH in the presence of two negatively charged polysaccharides: high methoxyl pectin (HMP) and soy soluble polysaccharide (SSPS). Both polysaccharides are composed of a backbone which contains galacturonic acid but, when added to soy protein isolate-stabilized emulsions, SSPS showed a different behavior than that of HMP. At neutral pH and above a critical concentration of stabilizer (0.05%), HMP caused flocculation of the emulsion droplets via a depletion mechanism. On the other hand, the emulsions containing a similar amount of SSPS did not show creaming or flocculation. At acidic pH (<4.0) the addition of pectin caused extensive droplet aggregation, while no aggregation was observed with the addition of SSPS. The differences in the stabilization behavior between the two polysaccharides can be attributed to their differences in charge, neutral sugars side chains, and molecular weight.

KEYWORDS: Soy soluble polysaccharide; high methoxyl pectin; interactions; soy protein isolate emulsions

1. INTRODUCTION

The increasing demand for protein-based acidic beverages has resulted in a large number of new products being introduced in the market in recent years. The use of soy proteins as ingredients in food formulations is particularly attractive because of their functionality and nutritional value. However, the utilization of soy protein isolates (SPI) at low pH is hindered by their low solubility at acidic pH (*1*).

SPI may be used to stabilize oil-in-water emulsions because of the surface properties of their constitutive proteins: the storage globulins 7S (β-conglycinin) and 11S (glycinin) (*2*). It has been shown that soy proteins decrease the interfacial tension between the water and oil and help stabilize emulsions by forming a physical barrier at the oil/water interface (*3*). However, environmental factors such as pH, ionic strength, or the presence of other ingredients will affect the stabilizing behavior of soy proteins in food emulsions (*4*, *5*).

It has been previously reported for various emulsion systems that the emulsion stability at low pH can be improved by forming multiple layers around the oil droplets. For example, the addition of negatively charged polysaccharides interacting with positively charged proteins may form a thick layer at the interface which prevents coalescence (*6*, *7*). The behavior of charged polysaccharides added to protein-stabilized oil-in-water emulsions depends on the pH. For example, at a pH below the isoelectric point of the protein the negatively charged pectin interacts via electrostatic interactions with β-lactoglobulin adsorbed at the interface (*6*). In a study of whey protein emulsions with high methoxyl pectin (HMP), it was shown that below a critical concentration of HMP the molecules can bind to more than one oil droplet, causing bridging flocculation (*7*). On the other hand, noninteracting polysaccharide molecules present in the dispersed phase can cause destabilization of the emulsion droplets via a depletion mechanism. At neutral pH and above a critical concentration the presence of HMP in β-lactoglobulin-stabilized emulsions causes flocculation and creaming at neutral pH (*7*).

Pectin is an acidic polysaccharide composed of 80% galacturonic acid obtained by aqueous extraction of plant material (usually apple or citrus) and is one of the most commonly used polysaccharides in acidic environments. Pectin is usually distinguished by the number of methyl groups that esterify the galacturonic acid backbone.

Soy soluble polysaccharide (SSPS), another negatively charged polysaccharide, is often employed in acidic emulsion-based beverages. SSPS is a water soluble polysaccharide, extracted from soybean cotyledons, which contains galacturonic acid (about 18% of total sugar) (*8*). It has been recently demonstrated that SSPS contains a small amount of protein which contributes to the interfacial activity of the polysaccharide (*9*).

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The understanding of protein−polysaccharide interactions is fundamental in predicting the stability and rheology of emulsions. The primary emulsifying agent of a soy-protein-based emulsion is a mixture of proteins, and whether the interactions with the polysaccharide are attractive or repulsive depends on environmental conditions such as pH and ionic strength and on the chemical structures of the proteins adsorbed at the interface.

Insufficient information is currently available on the interactions of negatively charged polysaccharides with soy-protein-stabilized emulsions. The objective of this research was to compare the effect of the addition of HMP or SSPS to soy-protein-stabilized emulsions at different pH values. While the behavior of HMP in protein-stabilized emulsions is somewhat known, at least in other protein-stabilized emulsion systems (*7*, *10*, *11*), the effect of the addition of SSPS to soy-protein-stabilized emulsions has not yet been studied.

2. MATERIALS AND METHODS

2.1. Materials. Soybean soluble polysaccharide (SSPS type Soyafibe-S-DA300) and HMP (Genu type YM-100L) were obtained from Fuji Oil Co., Ltd. (Osaka, Japan), and Cp Kelco (San Diego, CA), respectively. Other reagents were purchased from Sigma Chemical (St Louis, MO). Milli-Q water was used to prepare all solutions.

Soy protein isolate (SPI) was prepared from defatted soy flakes with a PDI of 90 donated by Cargill Inc. (Bloomington, IL). The flakes were milled under controlled conditions to avoid overheating and screened (mesh no. 20, 35, and 60). The flour from mesh 60 was used for protein extraction and stored in the freezer (−20 °C) until needed. Soy flour was extracted with 100 mM Tris-HCl buffer at pH 8.0 in a 1:10 ratio (w/v) and stirred at room temperature for 1.5 h. The insoluble fraction was removed by centrifuging for 30 min at 10 °C and 12 000*g* with a laboratory centrifuge (Model J2-21, Beckman Coulter, Fullerton, CA). The supernatant was brought to pH 4.8 with 2 M HCl, and the dispersion was kept at 4 °C for 2 h before centrifuging as described above. The protein precipitate was washed twice with 10 mM sodium acetate buffer at pH 4.8 (1:8 w/v ratio). Before freeze-drying the suspension was brought to pH 6.8. The freeze-dried SPI was stored in the freezer until use. The amount of protein in the samples was 94.6% (dry basis) as measured using the Dumas method (6.25% N/g − approved Method 46-30, AACC 2000).

The molecular weight of the polysaccharides used in the study was measured using size exclusion chromatography coupled with a multi angle light scattering (MALS) detector. The HPSEC-MALS system consisted of an HPLC system with degasser, autosampler, and UV detector (Thermofinnigan, Mississagua, Ontario, Canada), an in-line filter (0.1 μm pore size, Millipore, Fisher Scientific), and MALS and refractive index detectors (DAWN EOS and Optilab Rex, respectively, Wyatt Technology, Santa Barbara, CA). To eliminate baseline variations, the mobile phase (50 mM sodium nitrate, pH 5.6) was degassed and filtered through 0.2 and 0.1 μm filters (Millipore, Fisher Scientific). Before analysis, pectin (0.5%) and SSPS (1%) were dissolved in the mobile phase at 50 °C and equilibrated at room temperature. Samples were filtered through disposable 0.45 μm filters (Millipore, Fisher Scientific) before injection, and aliquots (50 or 100 μL) were injected on a Polysep-GFC-P linear column (Phenomenex, Torrance, CA) with an operating separation range between 1000 and 10 000 000 Da. The specific refractive index increment value used for both SSPS and HMP was 0.132 (*12*). The value for SSPS was determined with the refractive index detector injecting eight increasing concentrations (ranging from 0.1 to 1.2 mg/mL) in sodium nitrate and calculating the slope of the increment using ASTRA (version 5.1.2.0, Wyatt Technology, Santa Barbara, CA). Elution data were also processed using ASTRA software, and the molecular weight was calculated as weight average (M_w) of the aggregate peak and the polydispersity as ratio of M_w/M_n, where M_n is the number average. The HMP used in this work had a M_w of 245 (± 8) kDa, and a polydispersity index of 1.35, while SSPS showed two populations during the chromatographic elution (with 1.0 as polydispersity value) with a M_w of 839 (± 25) and 294 (± 22) kDa, respectively (results are the average of four independent injections).

2.2. Emulsion Preparation. Appropriate amounts of SPI were dispersed in 10 mM imidazole/acetate buffer (pH 7.5, containing 0.002% NaN_3 as an antimicrobial) by mixing for 2 h at room temperature to ensure complete hydration. The SPI suspension was then centrifuged (Model J2-21, Beckman Coulter) at 7000*g* for 15 min at 4 °C. Soy oil (10 wt %) was added to the supernatant, and the sample was dispersed with a high-speed blender (PowerGen 125, Fisher Scientific, Nepean, Canada) for 1 min. The mixture was then homogenized (high-pressure valve homogenizer, EmulsiFlex-C5, Avestin, Ottawa, Canada) with five passes at 35 MPa. The emulsions were refrigerated overnight. Preliminary experiments were carried out at varying concentrations of SPI, from 0.25% to 2.0%, and 1.5% SPI was used in the interactions studies, as it showed a monomodal particle size distribution and was stable to creaming at neutral pH for the entire storage period (15 days).

2.3. Addition of Polysaccharide. SSPS and HMP were gradually added to water preheated at 70 °C to prepare a 1% (w/w) bulk solution, and the solution was stirred for at least 2 h to ensure complete hydration. Emulsions were then diluted 1:1 with appropriate amounts of polysaccharide (either SSPS or HMP) and water, under continuous stirring with a magnetic stirrer, to a final concentration of 0.75% SPI, 5% oil, and a range of polysaccharide concentrations from 0.005 to 0.3%. The pH of the samples was adjusted to pH 3.0, 3.5, 3.8, 4.0, and 7.5 while stirring, and the final volume was adjusted with MilliQ water at the target pH. Aliquots (10 mL) of emulsions were transferred into test tubes, which were tightly sealed and stored at refrigeration temperatures (4 °C). Samples were observed every day for creaming or serum separation. For each pH, a control sample with no polysaccharide was also prepared. Results presented are the average of three independent experiments unless otherwise indicated.

2.4. Droplet Size Distribution. The particle size was evaluated using a laser diffraction instrument (Mastersizer X, Malvern Southborough, MA) using a relative refractive index of the particles of 1.06, sample absorption of 0.001, and a refractive index of the solvent of 1.33. This instrument measures the angular dependence of the intensity of laser light scattered by a dilute emulsion, and then it finds the particle size distribution that gives the best agreement between theoretical predictions and experimental measurements. To avoid multiple scattering effects, emulsions were diluted approximately at a ratio of 1:200 using deionized water adjusted to the sample pH. The changes in particle size were evaluated checking either the shape of the particle size distributions or the average droplet size reported as equivalent volume mean particle diameter $D(4,3) = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$, where n_i is the number of droplets of diameter d_i. Droplet size distribution measurements were performed after 24 h storage at refrigeration temperature and were repeated after 14 days storage at 4 °C.

2.5. Emulsion Creaming. The emulsions were stored for 15 days at 4 °C, and the visible separation layer was recorded each day. The total height of the emulsions H_E and the height of the serum layer H_S were measured. The extent of creaming was characterized as % serum $= H_S/H_E \times 100$. The % serum provided indirect information about the extent of droplet aggregation in the emulsion: the more aggregation, the larger the particles, the faster the creaming, and the greater the % serum (*11*). All measurements were made on at least three freshly prepared samples unless otherwise indicated.

2.6. Microstructure Characterization. Microstructural analyses were carried out after 24 h of storage at refrigeration temperature. Selected emulsions were analyzed using phase contrast optical microscopy (Olympus BX 60, Markham, Ontario). Emulsions were gently agitated in their test tube before analysis to ensure homogeneous sampling. Aliquots (5 μL) of emulsions at pH 3.0, 3.5, 3.8, 4.0, and 7.5 were diluted with 20 μL of 10 mM imidazole-acetate buffer containing 0.002% sodium azide (to prevent microbial growth) at the same pH of the emulsions. Images were acquired using a digital camera (Sensys, Carsen Group Inc., Markham, Ontario) and digital image acquisition software (Image-ProPlus, Media Cybernetics Inc., MD).

Emulsions were encapsulated in agar sleeves for transmission electron microscopy (TEM) using a method described by Allan-Wojtas and Kalab (*13*) with minor modifications. Capillary tubes (5 μL volume) were substituted for Pasteur pipets to decrease sample size. The emulsions were fixed at room temperature overnight (in the fume hood)

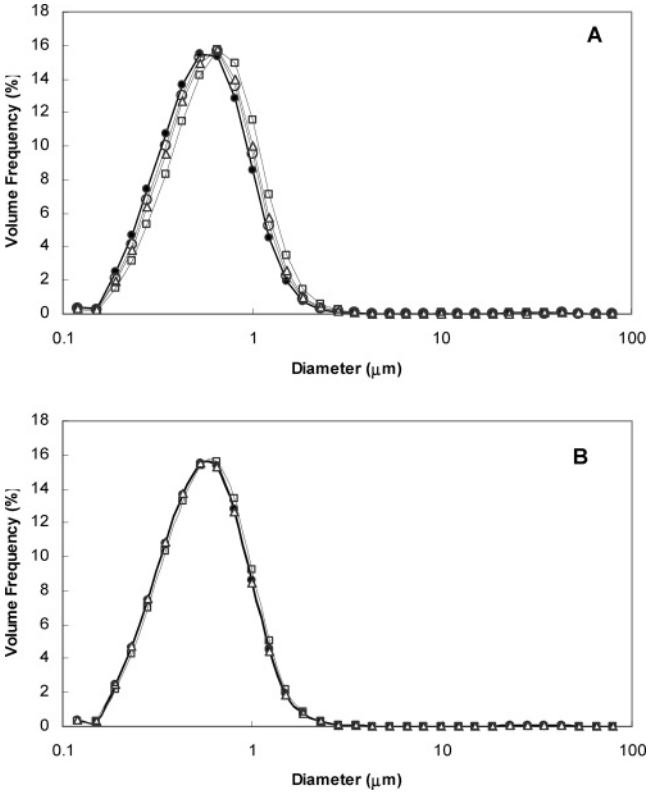

Figure 1. Effect of HMP (**A**) or SSPS (**B**) concentration on the particle size distribution of 5% soy oil emulsions containing 0.75% SPI at pH 7.5. Control (no polysaccharide added) (●); 0.05% (○); 0.10% (△); 0.30% (□). Distributions are the average of four independent experiments.

with a 2% glutaraldehyde solution in 0.07 M Sorensen's phosphate buffer (pH 6.8) by immersion. Samples were washed three times with Sorensen's phosphate buffer. Samples were immersed in 1% osmium tetroxide for 1 h and then washed three times with buffer. Samples were dehydrated into anhydrous ethanol and then washed, two times, in 100% propylene oxide. The propylene oxide was replaced with EPON 812 (Canemco, St. Laurent, QC, Canada) with a series of exchanges, 1:1, 1:2 propylene oxide:EPON 812, and finally incubated overnight with 100% EPON 812. The samples were placed in molds and polymerized in the oven at 60 °C for 24 h. The blocks were sectioned (70−90 nm thick) using a microtome (Reichert Ultracut S, Leica microsystems, Vienna, Austria), and sections were collected on copper grids (300 mesh) where they were stained with saturated uranyl acetate in 50% ethanol for 10 min, followed by Reynold's lead citrate for 10 min. The electron microscope (Hitachi H-7100, Nissei Sangyo, Tokyo, Japan) was used at 75 kV. Images were acquired using AnalySIS (Soft imaging system, CO) digital image capture system.

3. RESULTS

Emulsions were prepared at pH 7.5 with different amounts of SPI to investigate the effect of concentration on the particle size distribution. Laser diffraction analysis (Malvern Mastersizer X) showed that all concentrations resulted in a monomodal size distribution after 24 h of storage; however, emulsions with 1.5% and 2% SPI had a lower average size ($D(4,3)$) (data not shown). The emulsions containing 10% oil and 1.5% SPI were chosen as the model emulsion for the study of the interactions of SSPS and HMP with the SPI-stabilized oil droplets.

3.1. Interactions between SPI-Stabilized Droplets and Polysaccharides at Neutral pH. Control emulsions (5% soy oil and 0.75% SPI without additional polysaccharide) at pH 7.5 showed a monomodal distribution of sizes with an average diameter of 0.6 μm. After 24 h, the dependence of the emulsion

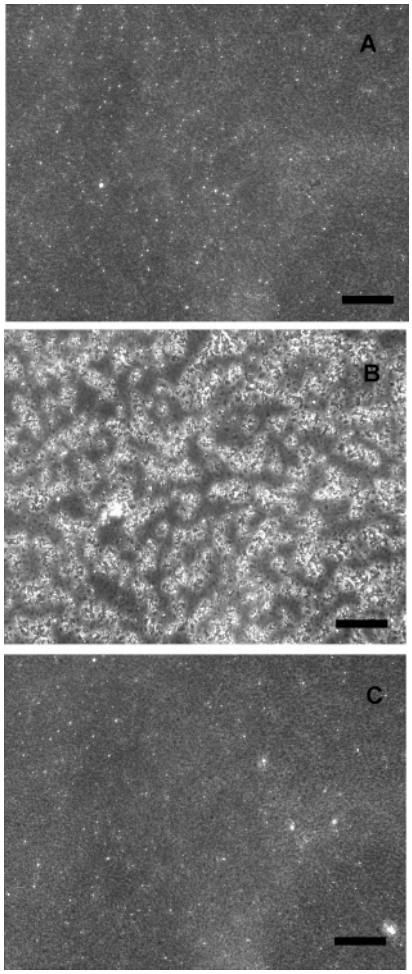

Figure 2. Phase contrast microscopy images of the emulsions containing 0.75% SPI, 5% soy oil at pH 7.5. Control emulsion (**A**); emulsion containing 0.2% pectin (**B**) or 0.2% SSPS (**C**). Bar size is 50 μm.

droplet size on different amounts of SSPS and pectin (up to 0.3%) was measured. **Figure 1** depicts the effect of the addition of polysaccharides to emulsions at pH 7.5. The average size of the emulsion droplets was not affected by the addition of the negatively charged polysaccharides. Although particle size distribution measurements showed the absence of droplet coalescence, the emulsion stability was affected by the addition of HMP. Above a critical concentration (0.05% w/v HMP), emulsions showed separation. Serum separation was caused by a depletion flocculation mechanism, as HMP and the protein-covered oil droplets are both negatively charged. On the other hand, SSPS showed not only a monomodal distribution of particle sizes (**Figure 1**) but also stability to creaming at pH 7.5, at all the concentrations tested. These results indicated that although negatively charged, SSPS did not cause depletion flocculation at concentrations up to 0.3%.

These results were supported by microscopic observations (**Figures 2** and **3**). Optical phase contrast microscopic images indicated that the addition of HMP to SPI emulsions at pH 7.5 caused flocculation of the oil droplets, as shown by the uneven distribution of the oil droplets in **Figure 2B**. On the other hand, emulsions containing SSPS (**Figure 2C**) were not different from control emulsions (**Figure 2A**).

TEM results (**Figure 3**) showed excellent preservation of protein and oil droplets. **Figure 3A** depicts a control SPI solution at pH 7.5, while **Figure 3B** shows a control emulsion stabilized by SPI at the same pH. In control emulsions, SPI was mainly

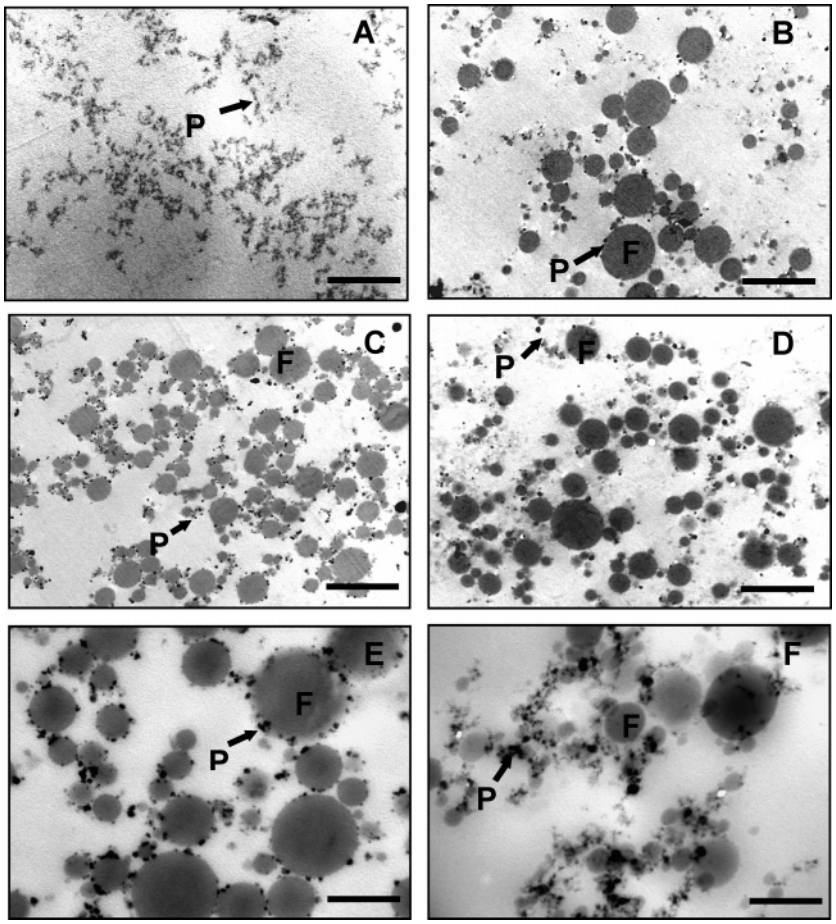

Figure 3. Transmission electron micrographs of emulsions with 0.75% SPI, 5% soy oil at pH 7.5. SPI solution at pH 7.5 (**A**), control emulsion (**B**), 0.2% pectin (**C**, **E**), 0.2% SSPS (**D**, **F**). Bar size is 2000 nm for micrographs **A**–**D** and 500 nm for micrographs **E** and **F**. Protein and oil droplets are indicated by P and F, respectively.

present around the oil droplets and adsorbed in aggregated form. Microscopy observations at pH 7.5 were in agreement with the particle size distributions measurements by light scattering reported in **Figure 1**. The dark character of the oil droplets was caused by fixation with osmium tetroxide. No differences in the microstructure were shown between control emulsions and emulsions containing HMP (**Figure 3C**) and SSPS (**Figure 3D**), all reported at the same magnification. All emulsions also showed some evidence of smaller droplets bridged via protein aggregates. This can be observed more closely in the higher magnification images of SPI emulsion with added HMP (**Figure 3E**) or added SSPS (**Figure 3F**).

While oil droplets in emulsions containing pectin were similar to control samples, emulsions containing SSPS seemed to have a smaller amount of protein adsorbed at the interface (compare parts **E** and **F** of **Figure 3**). SSPS emulsions showed a larger amount of protein bridged between the oil droplets, with fewer protein aggregates at the interface. This could be caused by the interfacial activity of SSPS, which has been previously reported to cause some exchanges with the protein from the oil/water interface (*14*). However, these observations were not tested by quantifying the amount of protein present at the interface after the addition of SSPS; the competitive adsorption behavior of SSPS and SPI is currently under investigation. In addition, the displaced protein may be interacting with SSPS to form a protein−polysaccharide complex which could be present in the dispersed phase. Evidence of this protein association was seen in the transmission electron micrographs (**Figure 3F**).

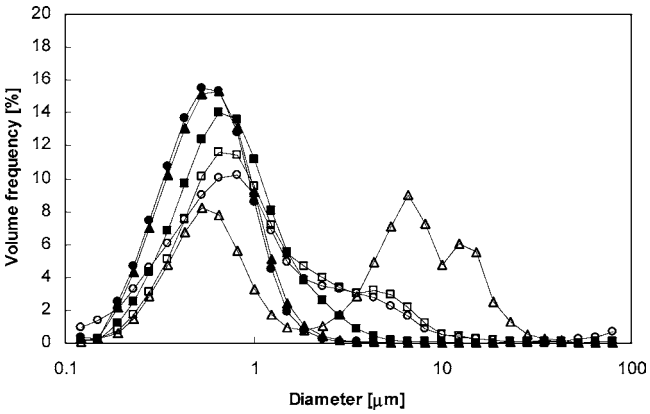

Figure 4. Particle size distribution of 10% soy oil emulsion and 1.5% SPI adjusted at pH 7.5 (●); pH 6.5 (□); pH 4.0 (△); pH 3.8 (○); pH 3.5 (■); pH 3.0 (▲). Results are the average of three independent experiments.

3.2. Interactions between SPI-Stabilized Droplets and Negatively Charged Polysaccharides at Low pH. To investigate the effect of acidification on the stability of SPI emulsions, the primary emulsions made at pH 7.5 were adjusted to various pH values ranging from 6.5 to 3.0. The changes in the average particle size distribution of SPI-stabilized emulsions as a function of pH are summarized in **Figure 4**. Decreasing pH caused droplet aggregation when the pH values were close to the isoelectric point of the proteins because of the decreased charge present on the surface of the oil droplets. SPI emulsions adjusted to pH 3.0 showed a monomodal distribution comparable

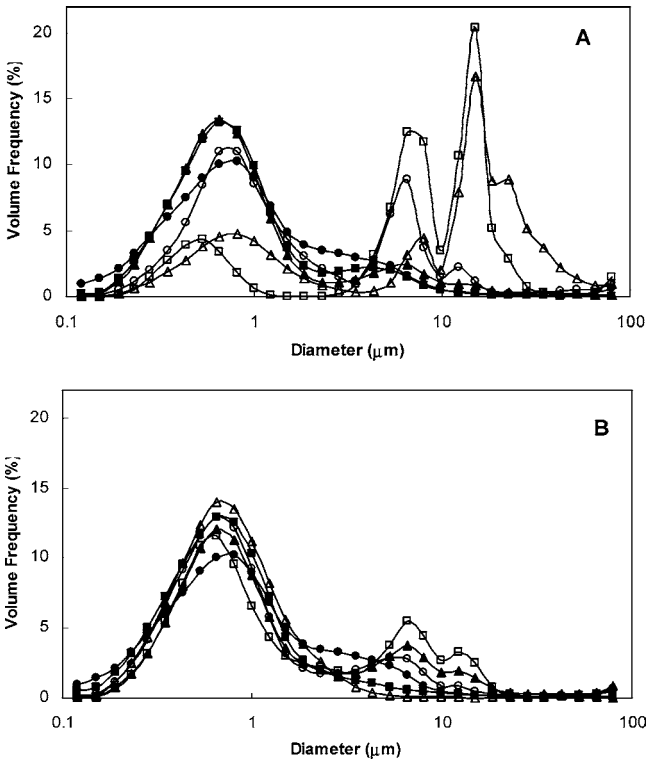

Figure 5. Effect of HMP (**A**) or SSPS (**B**) concentration on the particle size distribution of 5% soy oil emulsions containing 0.75% SPI at pH 3.8. Control emulsions (●); 0.005% (■); 0.01% (▲); 0.025% (○); 0.1% (□); 0.3% (△). Distributions are the average of four independent experiments.

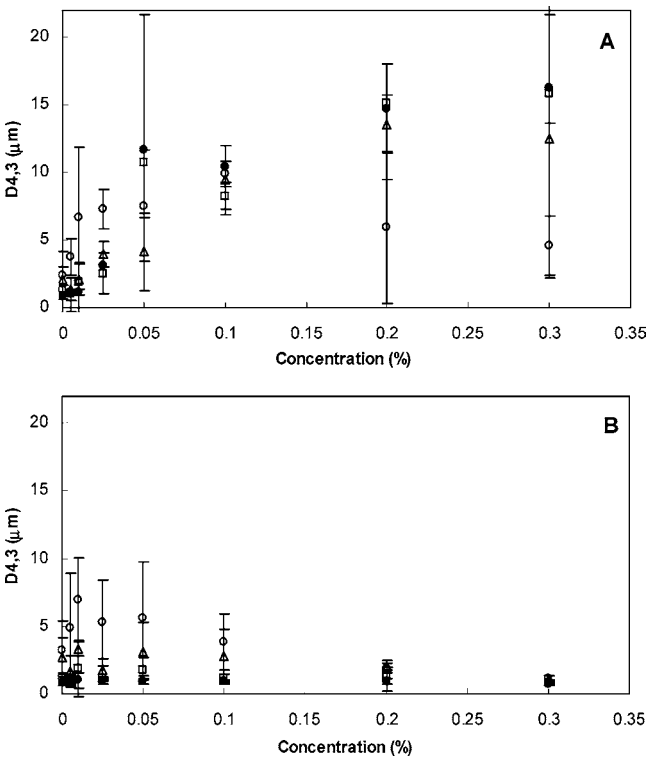

Figure 6. Effect of pectin (**A**) and SSPS (**B**) on the average particle size of emulsions as a function of pH and polysaccharide concentration. Emulsions were adjusted to pH 4.0 (○); pH 3.8 (△); pH 3.5 (□); pH 3.0 (●). Results are the average of four independent experiments; bars represent standard deviations.

to the emulsion prepared at pH 7.5. At pH 3.0 the protein adsorbed at the interface is positively charged, and the droplets are stable because of electrostatic repulsion. These results are in agreement with literature data on the decrease in functionality of SPI with acidification (*5*). However, while in previous reports the emulsions prepared at pH 3.0 showed extensive droplet flocculation, in the present work the emulsions prepared at neutral pH and then adjusted to pH 3.0 showed good stability and particle size distribution.

The effect of polysaccharide concentration on the particle size distribution of SPI emulsions at pH 3.8 is illustrated in **Figure 5**. While above neutral pH the particle size distribution was not affected by the type of polysaccharide (**Figure 1**), when HMP was added to the emulsions at pH 3.8 a second population of droplets with a diameter ranging between 5 and 30 μm was measured with integrated light scattering. The oil droplet aggregation was caused by bridging of the negatively charged pectin molecules between positively charged oil droplets. The behavior of SSPS was different from that of pectin: under the same conditions emulsions containing SSPS showed a better distribution of droplet sizes than that of emulsions containing HMP, at intermediate SSPS concentrations (between 0.01 and 0.1%) a small population of large droplets of about 10 μm of diameter was measured, while above a critical concentration (0.3%) of SSPS the emulsions showed a monomodal size distribution at pH 3.8 and an improved particle size distribution compared to control emulsions at the same pH with no stabilizer added.

Figure 6 summarizes the effect of polysaccharide concentration at various pH values on the average droplet diameter (D(4,3)). Results clearly indicate that HMP caused the aggregation of the emulsion droplets at all pH values (in the range between 3.0 and 4.0). The negatively charged pectin interacted with the positively charged oil droplets stabilized by SPI causing bridging flocculation (**Figure 6A**). In contrast with the results of the emulsions containing HMP, when SSPS was present in the emulsions, the oil droplet average diameter D(4,3) remained small at all pH and concentrations tested. When a sufficient amount of SSPS was added (0.2 and 0.3%), the emulsions maintained a small average size and a monomodal size distribution at all pH levels (in the range between 3.0 and 4.0). However, there seemed to be differences in the stabilizing behavior of SSPS with pH. At all concentrations tested (up to 0.3%) and at pH < 3.8, the addition of SSPS did not show creaming and serum separation.

Figure 7 summarizes the effect of a limited amount of polysaccharide (0.1% HMP or SSPS) on the particle size distribution of SPI emulsions as a function of pH. All samples containing HMP showed extensive droplet aggregation caused by bridging flocculation. Emulsions containing SSPS showed some aggregation at pH 4.0 and 3.8, while at lower pH values (3.5 and 3.0) the emulsions had a size distribution comparable to that of the control emulsions at pH 7.5. SSPS could be interacting with SPI at the interface and stabilizing the emulsion droplets by charge repulsion. It could also be hypothesized that SPI may be displaced from the interface by the surface active SSPS, changing the composition of the oil/water interface and increasing the amount of protein present in the dispersed phase. SSPS seemed to be less effective in stabilizing the emulsion droplets at pH > 3.8. At pH 3.8 and 4.0, a higher amount of SSPS was needed to obtain a small droplet size distribution.

Phase contrast microscopy images confirmed the results obtained with integrated light scattering and creaming stability experiments (**Figure 8**). While control emulsions and emulsions containing HMP showed extensive flocculation at pH 3.8 (**Figure 8A**,**B**), emulsions containing a sufficient amount of SSPS (0.2%) were stable to flocculation (**Figure 8C**). Transmis-

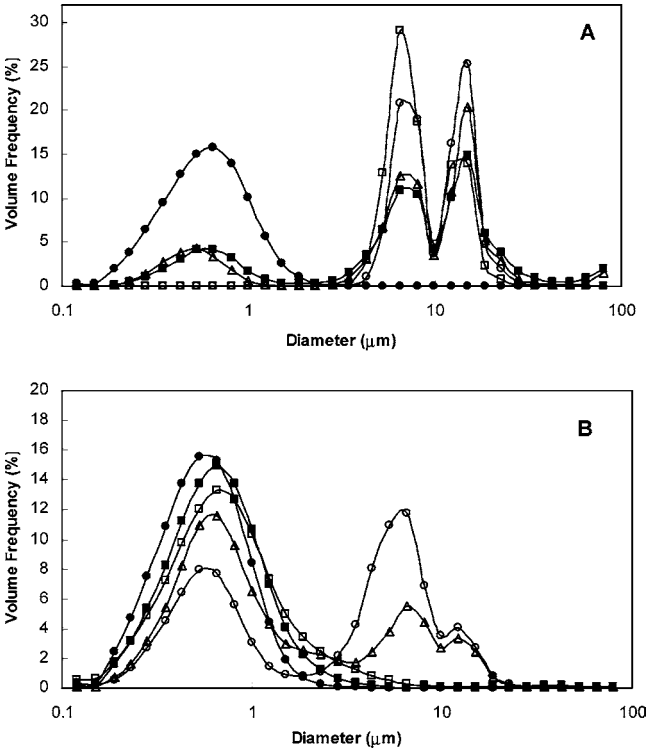

Figure 7. Effect of 0.1% HMP (**A**) or SSPS (**B**) on the particle size distribution of 5% soy oil emulsions containing 0.75% SPI as a function of pH: pH 7.5 (●); pH 4.0 (○); pH 3.8 (△); pH 3.5 (□); pH 3.0 (■). Results are the average of four experiments.

sion electron micrographs of the emulsions at pH 3.8 showed the presence of individual droplets surrounded by smaller droplets and covered by large protein aggregates (**Figure 9**). Emulsions containing pectin showed large clusters of oil droplets, confirming that, at low pH, pectin caused bridging flocculation (**Figure 9B**). Emulsions containing SSPS showed a microstructure similar to that of control emulsions at the same pH (**Figure 9A**,**C**); however, in emulsions with SSPS the protein seemed to be present in smaller aggregates than in control emulsions.

4. DISCUSSION

Emulsions stabilized by SPI prepared from mildly heat-treated, defatted soy flakes showed better stability to coalescence than emulsions prepared under similar conditions with commercial SPI (*15*, *16*). The SPI used in this study showed an emulsifying behavior similar to that previously reported by other authors (*5*). SPI-stabilized emulsion droplets were resistant to coalescence, mostly because of their globular structure and their adsorption as aggregates at the interface. Transmission electron microscopy observations clearly showed the presence of SPI at the interface. Large protein aggregates were present both at the interface and in the dispersed phase, and some small oil droplets were bridged via SPI to large oil droplets.

While there was no evidence of droplet aggregation at pH 7.5 in the absence of polysaccharide, lowering the pH affected the stability of the emulsions. Only when the pH of the emulsion was sufficiently away from the isoelectric point of the protein (for example, when the emulsion pH was adjusted to pH 3.0) were the emulsions containing SPI stable.

At neutral pH, the addition of a negatively charged polysaccharide to the negatively charged SPI-covered oil droplet may cause depletion flocculation (*7*, *10*). It has been previously

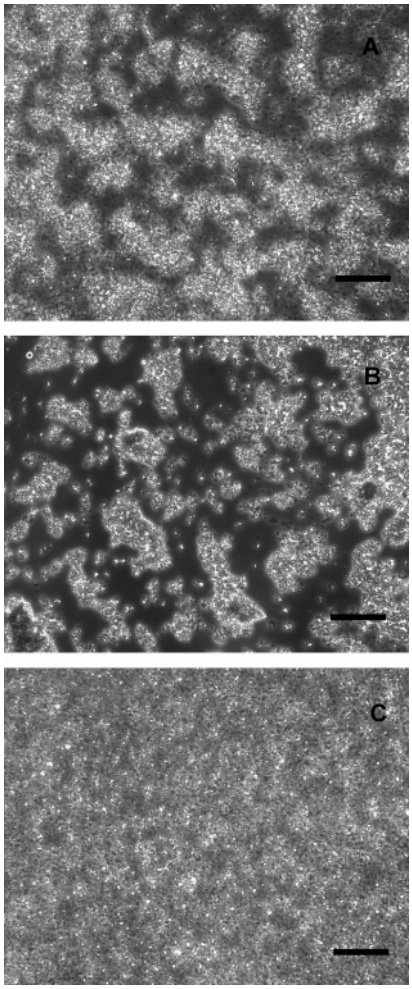

Figure 8. Phase contrast microscopy images of the emulsion containing 0.75% SPI, 5% soy oil at pH 3.8. Control emulsion (**A**); emulsion containing 0.2% pectin (**B**) or 0.2% SSPS (**C**). Bar size is 50 μm.

reported that HMP added in sufficient amount to milk-protein-stabilized emulsions at neutral pH causes destabilization by this mechanism (*7*, *10*, *11*). In emulsions containing SPI, HMP showed a similar behavior, and concentrations greater than 0.05% caused depletion flocculation. On the other hand, SSPS did not show the same behavior, and at all the concentrations tested the emulsions containing SSPS were stable to creaming. Although HPSEC-MALS determinations showed that SSPS had a higher average molecular weight than pectin, it has been previously reported that SSPS in solution exhibits low viscosity (*17*), and this could be the cause for the difference in behavior between the two polysaccharides at high pH. In addition, previous work has reported that the structure of SSPS is more branched than that of HMP (*8*), with SSPS having large branched neutral chains, mainly composed of galactan and arabinan. The large molecular weight portion of SSPS contains a peptide fraction which has been shown to play an important role in the surface behavior of SSPS (*9*). It could not be excluded that depletion flocculation of emulsions by SSPS did not occur as the polysaccharide interacted with SPI at the interface (or competed with SPI for the interface) also at neutral pH.

The neutral side chains and the lower content of galacturonic acid of SSPS molecules (*8*) compared to HMP are the cause of the different behavior between the two polysaccharides not only at neutral pH but also at acidic pH. At low concentration, HMP caused bridging flocculation in all the emulsions tested (in the pH range from 3.0 to 4.0). This behavior has been previously

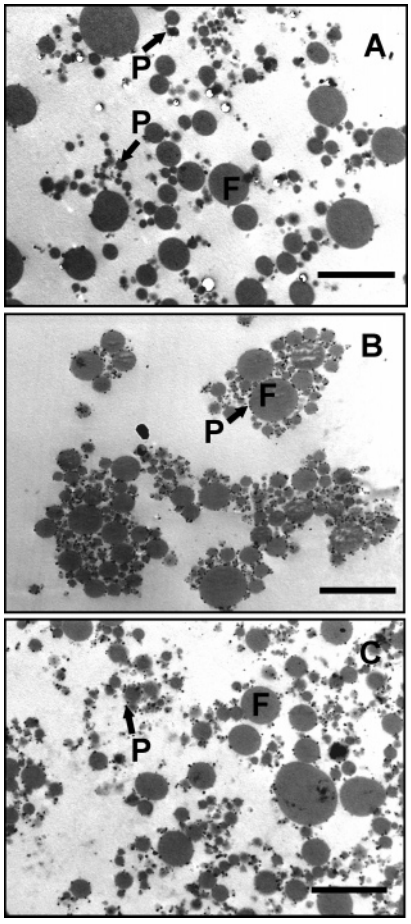

Figure 9. Transmission electron micrographs of emulsions with 0.75% SPI, 5% soy oil at pH 3.8. Control emulsion (**A**); emulsion containing 0.2% pectin (**B**) or 0.2% SSPS (**C**). Bar size is 2000 nm. Protein and oil droplets are indicated by P and F, respectively.

reported for other emulsions stabilized with whey proteins (*7*, *11*), and it is caused by electrostatic forces which drive pectin molecules to adsorb as a secondary layer on the SPI-covered oil droplet. Although charge interactions are the main cause of binding between SPI and HMP, it remains unclear why there were no differences in the interactions with pH, unless one hypothesizes that there was a shift in the balance of charges between pH 4 and pH 3: at pH 4 HMP was still negatively charged, although SPI was quite close to the isoelectric point of some of the protein subunits, while at pH 3 HMP was less charged and SPI more positively charged.

The behavior of SSPS was distinct from that of HMP in acidified SPI emulsions. At pH < 4.0, the droplet size distribution of the emulsions remained small and stable with the addition of SSPS. At low SSPS concentrations and pH 3.8 or 4.0, a population of aggregates was present. However this result was not different from the particle size distribution of control emulsions at the same pH. At these pH values, more SSPS was needed to obtain a stable emulsion. It was hypothesized that also in the case of SSPS the presence of a galacturonan chain was the cause of the pH dependence of the interaction. At lower pH values, all the emulsions were stable, and no bridging flocculation could be observed with integrated light scattering measurements. The mechanism responsible for the stabilization of SPI emulsions by SSPS at acidic pH is still not fully understood, but it could be caused by a balance between electrostatic interactions of SSPS with SPI and interfacial adsorption of SSPS. It has been previously shown that SSPS stabilizes oil-in-water emulsions at low pH values, and the average droplet size for these emulsions is not affected by pH (*18*). The branched portions of the SSPS molecules may stabilize the oil droplets more effectively by steric stabilization.

In conclusion, the results reported herein demonstrate for the first time the difference in the behavior between SSPS and HMP when added to SPI emulsions. At high pH, pectin caused depletion flocculation, while SSPS at the same concentrations did not. Both polysaccharides contain galacturonic acid and are negatively charged at acidic pH. At pH < 4.0, pectin and SSPS molecules interact with positively charged SPI at the interface. However, while pectin at low concentrations caused bridging flocculation, SSPS did not cause aggregation of the oil droplets.

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