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Interactions of High Methoxyl Pectin with Whey Proteins at Oil/Water Interfaces at Acid pH

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The interactions between whey protein isolate (WPI) and high methoxyl pectin (HMP) at pH 3.5 were investigated in situ using ultrasound (US) and diffusing wave spectroscopy (DWS). HMP was added to 10% oil-in-water emulsions containing 1% WPI. At neutral pH, no protein−pectin interactions were observed as both molecules are negatively charged, while at pH 3.5 bridging flocculation occurred via electrostatic interactions. Four different stages were distinguished during the addition of HMP in WPI-stabilized emulsions at pH 3.5. At a concentration below a critical value, no interactions were observed. At concentrations >0.02% HMP, a change in the l^* factor indicated a change in the ordering of the emulsion droplets, influenced by long-range interactions. At higher concentrations (in the range between 0.04 and 0.06% HMP), attenuation showed significant changes in the surface of the oil droplets, changes which affected the droplet−droplet interactions. At pectin concentrations >0.05%, attenuation of sound and $1/l^*$ decreased, while velocity of sound and particle size increased, as a result of bridging flocculation. These results demonstrated for the first time that methods such as US and DWS combined permit the observation of the early stages of the interactions between two biopolymers at the interface. This is significant in light of increasing efforts in engineering complex interfacial layers.

KEYWORDS: Whey protein; pectin; ultrasound; diffusing wave spectroscopy; emulsion flocculation

INTRODUCTION

To improve the quality, appearance, and shelf life of emulsion-based products, the mechanisms that regulate stability need to be better defined. In general, the design and formulation of new food products are still mainly based on trial and error, and there is a general lack of understanding of the principles that regulate the interactions between ingredients during processing and storage.

Stable emulsions can be prepared by engineering their interfacial membranes, designing a fine balance between the electrostatic interactions and steric interactions occurring on the surface of oil droplets. Within an oil-in-water emulsion, the primary stabilizing agent against droplet coalescence is typically a mixture of adsorbed proteins (*1*). Globular proteins such as whey proteins (more specifically, α-lactalbumin and β-lactoglobulin) are often used because of their emulsifying properties. These proteins are adsorbed at the interface and cover the oil droplets stabilizing them from coalescence, provided that sufficient amount of protein is available to ensure complete coverage (for example, 2 $mg \cdot m^{-2}$ is the minimum surface load for whey protein stabilized emulsions).

While proteins are recognized for adsorbing at the oil−water interface and forming interfacial films, polysaccharides usually impact the stability of the emulsions by modifying the physical properties of the continuous phase. In general, by adding polysaccharides to the emulsion, the viscosity of the continuous phase increases, the shelf life of the emulsion is extended, and flocculation, coalescence, and creaming are prevented (*2*). Most polysaccharides are added to emulsions to modify the properties of the continuous phase although a few have the ability to adsorb at interfaces, as for example gum arabic or soy soluble polysaccharide (*3*, *4*). In general, the adsorption of the polysaccharides occurs via a protein fraction linked to the carbohydrate moieties of the polysaccharide.

Charged polysaccharides interact, under specific conditions, via electrostatic forces with proteins both in solution and at interfaces. These interactions are usually affected by the charge and size of the molecules, their relative concentrations, and the solution conditions (pH and ionic strength) (*1*, *5*−*7*). For example, studies have demonstrated that dextran sulfate, a highly anionic polysaccharide, forms interfacial electrostatic complexes with bovine serum albumin but not with β-lactoglobulin-stabilized oil-in-water emulsions at pH 7.0 (*7*).

Pectins, anionic plant polysaccharides often derived from citrus and apple, are used as gelling agents or stabilizers in low pH foods such as acidified milk drinks and yogurt. Pectins are acidic polysaccharides containing homogalacturonans, rhamnogalacturonans, arabinans, and galactans. Pectins are still negatively charged at acidic pH (around 4.0) because of the presence of a large number of carboxylic groups along the backbone. Pectins can be divided in two main groups, high methoxyl

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pectins (HMP), containing a large number (>50%) of esterified carboxylic acids, and low methoxyl pectins, with a higher number of acid groups (<50% esterified). The degree of esterification of the pectins strongly affects the interactions with proteins at high and low pH (*8*). In addition to their overall charge, the functional characteristics of the pectins are highly influenced by their molecular size and charge distribution (*8*, *9*).

At a pH below their isoelectric point, milk proteins are positively charged and form complexes with pectins. These complexes are driven by electrostatic forces. For example, it has been demonstrated that during acidification, HMP adsorbs onto casein micelles at a pH as low as 5.3 and with decreasing pH the casein micelles are fully covered, with minimum bridging between them (*10*). At low pH (3.8−3.5), HMPs form soluble and insoluble complexes with β-lactoglobulin depending on the molecular size and charge of the polysaccharide (*8*, *9*). These electrostatic interactions can be exploited to modify emulsion stability and texture by forming multilayered membranes composed of protein and negatively charged polysaccharide (*1*, *11*−*13*).

In β-lactoglobulin-stabilized emulsions at low pH, pectin molecules adsorb onto oil droplets via electrostatic interactions with the protein. The charge interactions could be modulated to obtain mixed interfacial layers onto oil droplets. In β-lactoglobulin-stabilized emulsions at pH 3, there is a decrease in droplet surface charge from +33 mV when only β-lactoglobulin is present to about −19 mV with added pectin (*11*). The decrease in charge is caused by the gradual adsorption of pectin onto the oil droplet surface. At a high enough concentration, the pectin molecules are likely adsorbed to the surface of more than one emulsion droplet causing bridging flocculation (*11*). On the other hand, at high pH, most of the pectin does not approach the surface of the oil droplet as a result of the charge repulsion between the protein and the polysaccharide molecules, and the actual concentration of pectin surrounding the oil droplets is smaller than the concentration in the bulk. This creates an osmotic pressure difference and at high enough concentrations of pectin, the oil droplets aggregate via depletion flocculation. Usually, depletion flocculation occurs at much higher pectin concentration than bridging flocculation.

The ability to modulate the interactions between proteins and polysaccharides would permit the design of novel structures and textures; however, using conventional light scattering, the early stages of flocculation and creaming cannot be easily identified, mainly because the oil droplet size is measured after extensive dilution (*14*).

This research focused on the study of the interactions of HMP with whey protein isolate (WPI) in oil-in-water emulsions in situ using two spectroscopic techniques, diffusing wave spectroscopy (DWS) and ultrasonic spectroscopy (US). The main advantages of these techniques are that they are noninvasive and nondestructive; this allowed for the first time the observation of the effect of the addition of pectin on WPI-stabilized emulsions under more realistic conditions.

MATERIALS AND METHODS

Sample Preparation. WPI (Alacen 895, donated by New Zealand Milk Proteins, Mississagua, Ontario) solutions were prepared by dispersing 1.1% (w/w) WPI in 20 mM imidazole buffer, pH 7.0 with continuous stirring. The protein solution was filtered through a 0.8-μm filter (Millex-HV Millipore Co., Billerica, MA). A stock solution of 1% (w/w) pectin (72 DE, AMD 783, donated by DANISCO, Scarborough, ON) was prepared by adding the pectin to MilliQ water at 60 °C and continuously stirring at room temperature until complete solubilization. After 24-h storage at 4 °C, the pH of the pectin solution was adjusted using HCl or NaOH (Sigma, St. Louis, MO).

Emulsion Preparation. Ten percent oil-in-water emulsions were prepared by adding 10 mL of soybean oil to 90 mL of WPI solution. The samples were premixed for 1 min using a high-speed blender (PowerGen 125, Fisher Scientific, Co., Nepean, ON). Emulsions were then prepared at room temperature using a laboratory scale high-pressure homogenizer (Emulsiflex C5, Avestin, Ottawa, ON) with two passes at 40 MPa. After storage at 4 °C for 24 h, the emulsion pH was adjusted using HCl. Experiments were carried out in triplicate.

Particle Size Measurement. Different concentrations of pectin were added to the emulsions and kept at room temperature for 1 h with moderate mixing. The maximum volume of pectin stock solution added to the emulsion resulted in a 10% dilution, bringing the protein concentration down to 0.91%. After overnight storage at 4 °C, the particle size distribution of the emulsions was measured using laser light scattering (Mastersizer X, Malvern Inst., Brookhaven, MA) with a value of 1.06 for relative refractive index, 0.001 absorption, and a refractive index of the solvent of 1.33. The angular dependence of the intensity of the scattered light was measured by diluting the emulsion with MilliQ water previously adjusted to pH 3.5, unless otherwise indicated. Measurements are reported either as full distributions or as surface volume mean diameter $D_{3,2} = n_i D_i^3/\sum n_i D_i^2$ where n_i is the number of droplets of diameter D_i.

Diffusing Wave Spectroscopy. Diffusing wave spectroscopy (DWS) was used to measure the particle size and interactions while titrating the emulsion with increasing amounts of pectin. Pectin was added to the emulsion using a Hamilton syringe and mixed in the cuvette at each subsequent addition. Measurements were taken after the system stabilized (when the average intensity was constant over the run time, about 25 min). Light from a monochromatic solid-state laser with a wavelength of 488 nm and 50 mW of power (Model 532-100MBS, Omnichrome, Chino, CA) was passed through the sample, contained in a rectangular flat-faced glass cuvette, with a 4-mm path length. The cuvette was immersed in a water tank (1 L) maintained at 20 °C using a temperature controller. The water tank was fitted with two glass windows to allow passage of the laser beam and the scattered light. The scattered light was collected with a single-mode optical fiber (Oz Optics Ltd., Carp, ON, Canada) placed directly behind the exit window of the water tank. The fiber optic was bifurcated at its outlet end, and the 50/50 split light signal was fed into two matched photomultipliers (HC120-03, Hamamatsu, Loveland, OH). The signals from the photomultipliers were amplified and fed to a correlator (FLEX2K-12 × 2, Bridgewater, NJ), which performed a cross-correlation analysis. The instrument output was a time-correlation function, which can be interpreted in terms of the dynamics of the particles in the sample (*15*).

Two parameters can be derived from the DWS data, radius and l^*. The radius was derived from the diffusion coefficient, which depends on the decay constant of measured correlation function of the sample. The diffusion coefficient was calculated using a value of 1.33 for the refractive index of water, and the apparent particle radii were calculated using the Stokes−Einstein relationship, with the viscosity of water taken to be 1.0 cp (*16*). l^* is the photon transport mean free path, related to the mean free path of photons between scattering events (l) by

$$l^* = \frac{l}{\langle 1 - \cos\theta \rangle} \tag{1}$$

where θ is the scattering angle and $\langle\ \rangle$ indicates the ensemble average over many scattering events. l^* is the length scale over which the direction of the scattered light is completely randomized. For a turbid solution, where all photons are multiply scattered, l^* can be calculated from the transmission of light:

$$T_i = \frac{I}{I_0} = \frac{5l^*/3L}{1 + 4l^*/3L} \tag{2}$$

where I and I_0 are the initial and transmitted intensities of the laser light (*17*).

For completely noninteracting scatterers (completely uncorrelated spatially), the l^* value will depend on particle size, particle concentra-

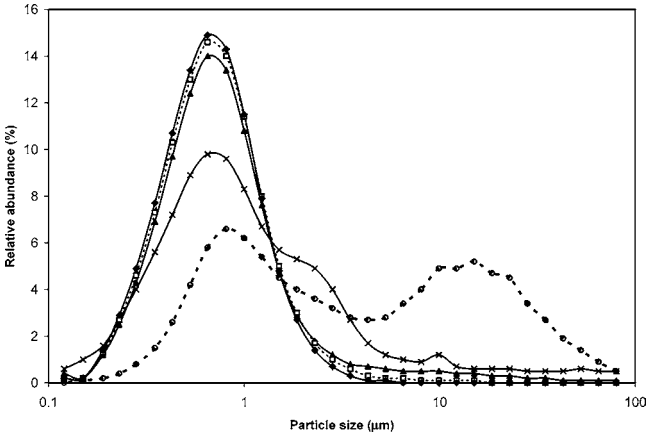

Figure 1. Effect of high methoxyl pectin on the droplet size distribution of emulsions (10% oil-in-water emulsions containing 1% WPI) at pH 3.5 as measured using integrated light scattering. (◆) control emulsion, no pectin; (□) 0.03%; (▲) 0.05%; (X) 0.07%; (○) 0.1% pectin.

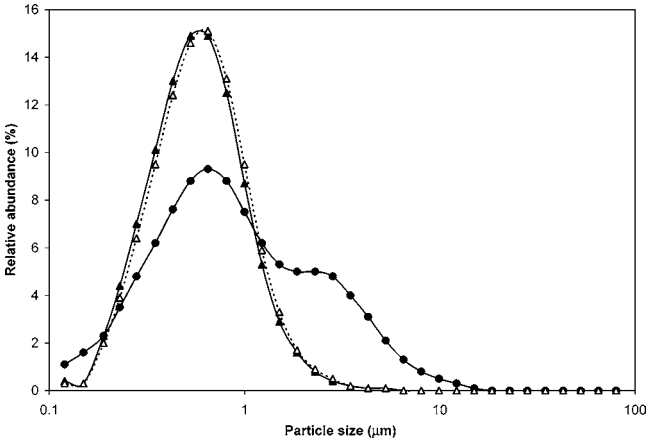

Figure 2. Effect of pH on the droplet size distribution of emulsions containing 0.1% high methoxyl pectin as measured using integrated light scattering. (▲) pH 7.0; (●) pH 3.5; (△) pH 3.5 then adjusted to pH 7.0.

tion, and index of refraction. However, in general, spatial positions and the correlation of the particles will play a role, especially in the optically dense suspensions where DWS is most useful.

Ultrasound. High-resolution ultrasonic spectroscopy HR-US102 (Ultrasonic Scientific, Dublin, Ireland) was also employed to measure the changes of sound velocity and attenuation while adding pectin to the emulsion. Before measurement, the samples were degassed for 20 min in a water bath at 20 °C. Emulsions (1 mL) were loaded in the two cells, kept at 20 °C by a programmable Haake F8 water bath (Thermo-Haake, Georgetown, ON). The equipment passes transverse sound waves through the sample and reference cells and measures both the velocity and the attenuation of the transmitted sound. The equipment configuration has been described in detail by others (*18*). The instrument was tuned to measure six frequencies in the range between 5 and 12 MHz.

Similarly to the measurements with DWS, pectin was added to the emulsion in incremental amounts using a Hamilton syringe, in this case, every 30−60 min, stirring for 5 min at 475 rpm to ensure homogeneous mixing, and then resting the sample for measurements for a minimum of 25 min. Measurements were carried out simultaneously in two cells, both containing the emulsion. In one cell pectin was added, while in the second cell water previously adjusted to pH 3.5 was added as reference. When analyzing the data for attenuation or velocity as a function of concentration, a value was selected for each concentration after 30 min of equilibration after the addition of the pectin. When calculating the attenuation differences with pectin concentration (see **Figure 4**), the values reported are the difference in attenuation between two consecutive additions of either pectin or water, the latter for the reference sample.

RESULTS

To compare the present results with previous reported data on similar systems (for example, pure β-lactoglobulin emulsions and pectin (*11*)), the average size distribution of the emulsion droplets was measured after 24 h using integrated light scattering. **Figure 1** illustrates the effect of different amounts of pectin on the droplet size distribution of WPI-stabilized emulsions at pH 3.5. While without pectin the emulsions were stable and showed a monomodal size distribution with an average size of the droplet of 0.52 μm, addition of pectin affected the droplet size distribution. At concentrations up to 0.05% pectin, no changes were noted on the particle size distribution of the emulsions. When 0.07% of pectin was present in the emulsions, the distribution was no longer monomodal, but bimodal (**Figure 1**), with a second population of droplets with a significantly larger size at about 20 μm. The presence of this second population of larger size droplets indicated aggregation, most likely caused by bridging of pectins between oil droplets.

Although the particle size distribution of emulsions at pH 3.5 was affected by the presence of pectin, at neutral pH, the addition of pectin at concentrations up to 0.1% did not cause changes in the particle size distributions when measured by integrated light scattering. **Figure 2** illustrates the effect of 0.1% pectin on the particle size distributions of emulsions at pH 7.0 and 3.5 and for emulsions acidified at 3.5 and brought back to neutral pH. While at pH 7.0 the negatively charged pectin molecules do not adsorb onto the negatively charged protein-stabilized oil droplets, at acidic pH there is enough pectin (0.1%) to cause bridging flocculation between the droplets. The interactions between pectin and proteins at the interface are reversible, as neutralization of the emulsion system disrupted the flocs and a monomodal size distribution of droplets is measured by integrated light scattering.

WPI-stabilized emulsions with HMP represent a good model system for our spectroscopy study, as the light scattering measurements did not estimate any large coalescence of the oil droplets, and no apparent change in particle diameter, only flocculation. However, measurements of particle size distribution in diluted systems do not permit a fine determination of the dynamics of the interactions between the negatively charged carbohydrate polymers and the protein-stabilized oil droplets. To better observe the changes occurring in the emulsions during small, consecutive additions of HMP, ultrasound spectroscopy and diffusing wave spectrometry measurements were carried out.

Figure 3 illustrates the changes occurring in attenuation and velocity of sound during consecutive additions of pectin, at three selected frequencies. At concentrations <0.04%, no changes were shown in velocity and attenuation for emulsions with added pectin when compared to control emulsions (emulsions with water added). The values of attenuation and velocity showed a significant change at a pectin concentration of about 0.05%. Values of attenuation decreased with increasing concentration of pectin, with a significant effect at 0.05% pectin (**Figure 3A**), while adding only water had no significant effect on attenuation. At high pectin concentration (>0.07%), the values of attenuation showed a continuous increase at each measurement interval. The same behavior was shown at all frequencies tested.

Values of ultrasound velocity increased with pectin concentration, while dilution with water did not significantly contribute to a change in sound velocity. **Figure 3B** illustrates the changes

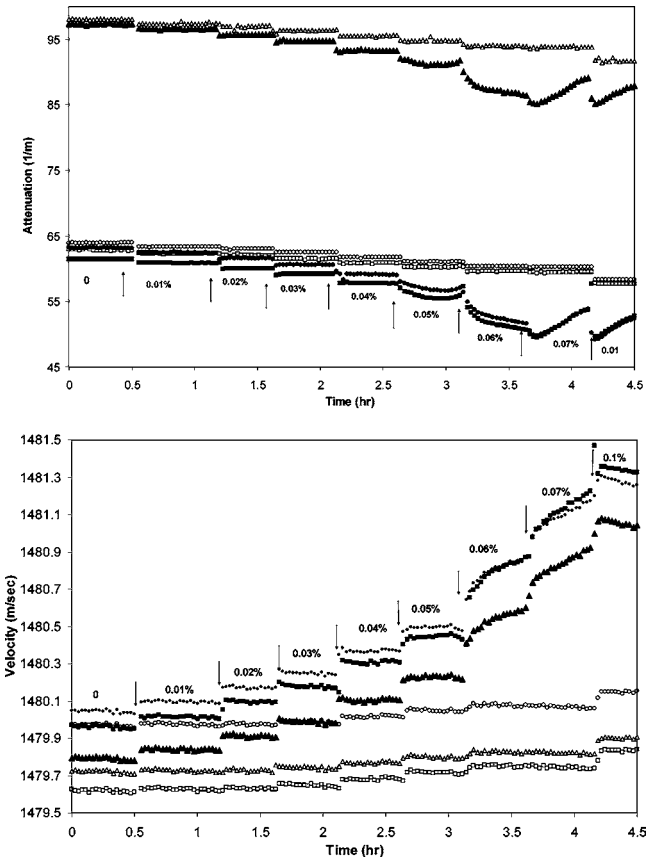

Figure 3. Attenuation (**A**, top) and velocity (**B**, bottom) changes with time while adding HMP (filled symbols) or water at pH 3.5 (empty symbols) to WPI-stabilized emulsions at pH 3.5. Data are shown for three different frequencies (■, □) 7.8 MHz; (●, ○) 8.6 MHz; (▲, △) 11.4 MHz.

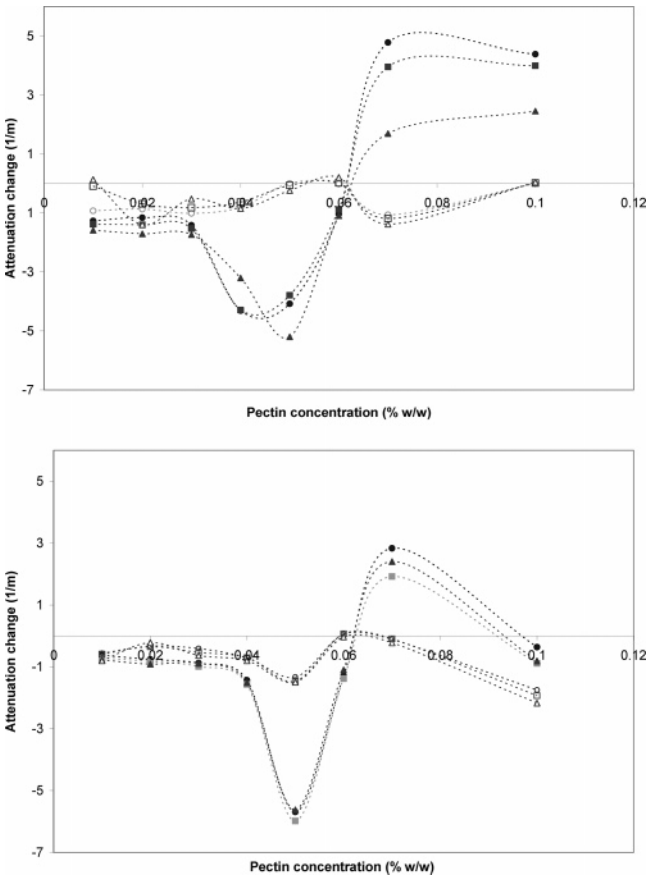

Figure 4. Attenuation differences (calculated by subtracting values obtained from subsequent additions) in emulsions with addition of pectin (filled symbols) or with water (reference, empty symbols). Results are shown for two separate replicate experiments (top, bottom), measured at (●, ○) 7.8 MHz; (■, □) 8.6 MHz; (▲, △) 11.4 MHz. Lines are drawn to show trends.

in velocity at three selected frequencies. Although velocity and attenuation can be considered independent parameters, as in attenuation, there was no effect on ultrasonic velocity up to 0.04% pectin. Adding pectin above 0.05% caused a much higher change in velocity than that attributable to a step increase in pectin concentration.

Figure 4 illustrates the difference in the values of attenuation between two consecutive additions of pectin for three frequencies. The changes are compared to those of control emulsions, to distinguish the change in attenuation caused by pectin addition to those of simple dilution. Two separate replicates are shown in **Figure 4**. These results clearly indicated that while adding water had no significant effect on the attenuation change at each step, up to the eighth dilution step, there was a marked change of attenuation at a pectin concentration >0.04%. At very low pectin concentrations <0.04%, the change in attenuation was not significantly different from that of the dilution step; however, at a concentration of 0.05 and 0.06% each additional amount of pectin caused a change in attenuation of about 5 m^{-1}. At 0.05 and 0.06% pectin, a substantial decrease in attenuation was shown. The negative difference in attenuation at each step of pectin addition turned into a positive difference at higher pectin concentration (>0.06%).

The frequency dependence of velocity and attenuation values of the emulsions for three pectin concentrations compared to those of control samples are shown in **Figure 5**. As expected, the ultrasound attenuation increased linearly with increasing frequency (**Figure 5A**), in this small range of frequency tested. Velocity of sound did not show frequency dependence at the high concentration of pectin (**Figure 5B**); however, this seemed to be dependent on pectin concentration: in reference emulsions or in samples containing low pectin concentration (up to 0.05%), there seemed to be some frequency dependence. These results agreed with previously reported data on emulsions (*19*, *20*).

Figure 6 summarizes the results obtained during measurements with diffusing wave spectrometry. Changes in droplet radius and 1/*l** are indicated for emulsion droplets as a function of pectin concentration. No change in droplet radius was shown up to 0.06% pectin. The behavior of 1/*l** was quite different. At low concentration of pectin, up to 0.04%, 1/*l** showed a small decrease. At 0.04% pectin, the change in 1/*l** was more pronounced, up to 0.06%. This behavior could indicate that the pectin present in solution is changing the surface charge of the system. Positively charged protein-covered emulsion droplets are now being screened by the presence of the negatively charged pectin. At these concentrations, the interactions are not strong enough to induce flocculation, but enough to induce ordering in the relative position of the particles. This is shown clearly by a change in the 1/*l** behavior which is linked to particle correlation. At concentrations of pectin >0.06%, the droplet size increased dramatically, which indicated droplet aggregation. When the concentration of pectin was sufficient to cause a dramatic increase in droplet radius, the values of 1/*l** reached a steady state: 1/*l** leveled off to a plateau which remained fairly constant to the last pectin addition. This may indicate that the emulsion droplets are trapped in place and adding more pectin increases the number of bound molecules

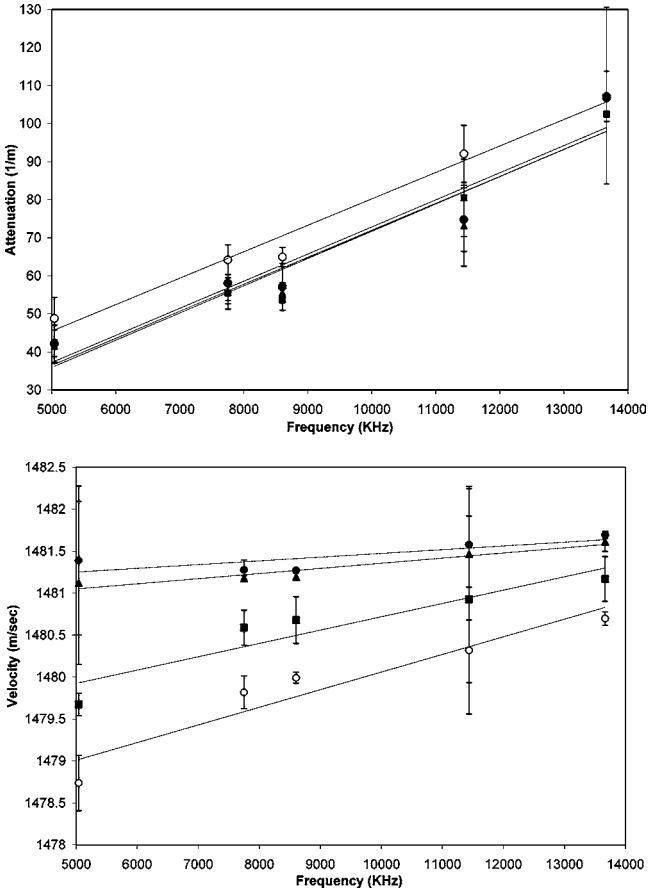

Figure 5. Attenuation (**A**, top) and velocity (**B**, bottom) values as a function of frequency in WPI-stabilized emulsions at pH 3.5 at different HMP concentrations: (○) no pectin; (■) 0.05%; (▲) 0.07%; (●) 0.1%.

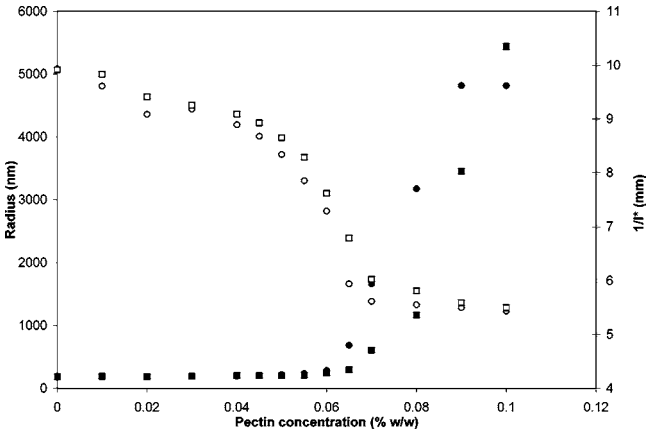

Figure 6. Changes in 1/*l** (□, ○) and radius (■, ●) of the WPI-stabilized emulsion droplets at pH 3.5 as a function of pectin concentration as measured by DWS. Data are shown for two replicate experiments.

with no further changes to the special arrangement. These results suggested that the addition of pectin caused a change in the emulsion droplet dynamics even at low pectin concentration, even before an actual size difference of the oil droplet.

DISCUSSION

As previously reported, the addition of pectin to protein-stabilized emulsions at acidic pH (for example β-lactoglobulin or in this study WPI) caused bridging of the oil droplets (*5*, *11*). Measuring the particle size distribution using integrated light scattering showed bridging at 0.07% pectin to a 1% protein solution, in agreement with previous studies. Moreau et al. (*11*) reported an increase in particle size distribution for 0.04% pectin added to 0.5% protein emulsion. At pH below the isoelectric point of the protein, the protein carries a net positive charge and electrostatic forces drive the interactions with the negatively charged polysaccharide. At a pH above the isoelectric point of the protein, interactions are unfavorable, since both macromolecules are negatively charged (*5*). At pH 7, the same concentration range (0−0.1%) of pectin used for the studies at low pH did not cause changes in droplet size distribution. The reversibility of the electrostatic interactions was demonstrated by adding the polysaccharide at pH 3.5 and then adjusting the pH to neutral pH (**Figure 2**). These emulsions, containing WPI-covered oil droplets and pectin molecules, were chosen as a model for the US and DWS studies.

US and DWS measurements can follow the changes occurring to the emulsion droplets during the adsorption of pectin molecules on WPI-emulsions in situ as opposed to methods that require an extensive dilution, such as traditional light scattering. While by measuring the particle size distribution of emulsions at low pH only the late stages of bridging flocculation can be determined, by combining ultrasound and diffusing wave spectrometry four stages can be distinguished when adding pectin. At very low concentrations of pectin, no changes were shown. After addition of 0.02% pectin, 1/*l** started to steadily decrease with concentration of pectin. This indicated the beginning of a change in the interactions of the emulsion droplets, with no aggregation (bridging of the droplets). No large increase in radius was shown at low concentrations, although at >0.05% a steady increase in size of a few nm was observed, representing the binding of pectin. The steady decrease in 1/*l** at the low pectin concentration indicated that the system was gaining order, influenced by long-range interactions, since none of the optical properties of the emulsion changed drastically at such low pectin concentration. For bridging flocculation to occur, a critical amount of pectin is needed in the emulsion. At concentrations <0.05% pectin, these 1/*l** changes were caused by the change in the hydration layer and surface charge of the oil droplet caused by pectin adsorption (*11*).

At these critical low concentrations (0.04−0.05% pectin), changes in emulsions were also shown by US spectroscopy. Velocity and attenuation of sound showed changes compared to the reference samples, where only water was added. Velocity of sound is mainly affected by the compressibility coefficient and the changes in density of the emulsion. Velocity increased at each addition of pectin, simply by the increase in the concentration of the macromolecules and hydration (ordering of the water molecules causes an increase in compressibility). However, at higher concentration of pectin (0.06%), the step increases in velocity of sound were much larger than those caused simply by the increase in concentration of the species. At concentrations >0.05%, bridging flocculation affected the compressibility of the system causing an increase in sound velocity. Ultrasonic attenuation was mainly related to droplet scattering and thermal losses (*19*). At the range of frequencies used in this study, thermal losses affect the attenuation of sound more in smaller particles than in larger particles, while scattering becomes important for larger particles, larger than a few μm (*19*). At low concentration of pectin, the changes in attenuation were mostly caused by thermal losses arising from heat transfer in to and out of the fat globules and indicated binding of pectin molecules to oil droplets, causing a change in the hydration layer of the oil droplet. Droplets getting closer to one another cause their thermal waves to overlap, which results in a decrease

in the attenuation (*20*). Dilution of the emulsion did not affect significantly the velocity and the attenuation of sound, as shown in **Figures 3** and **4**. In the concentration range between 0.04 and 0.06%, the decreasing values of attenuation and the steep decrease in $1/l^*$ with the addition of pectin confirmed our hypothesis that although no aggregation was yet shown (no changes in size), the interactions of pectin with the oil droplets caused the emulsions to gain more order, because of the newly formed interfacial layer: the oil droplet was more hydrated and negatively charged. The change in $1/l^*$ could be in part explained by the change in the oil droplet size; however, the extent of the $1/l^*$ change was higher than that which could be theoretically calculated when correcting for the change in radius. Ultrasound and DWS allowed the determination in situ of the early effects of the interactions between emulsions, before any significant effect in compressibility, bridging flocculation, or particle size distribution.

Higher concentrations of pectin (0.06%) caused bridging flocculation, as shown by the increase in particle size measured by DWS as well as the particle size distribution changes measured by integrated light scattering. At these pectin concentrations, ultrasound velocity and attenuation increased at each additional concentration of pectin. The scattering component of the attenuation becomes important during bridging, when particles are larger than a few μm (*18*). When >0.06% pectin was added, $1/l^*$ did not show any further changes with concentration, indicating that most of the interactions between particles have already occurred.

In conclusion, this research demonstrated how the interactions between pectin and WPI-covered oil droplets and their subsequent bridging flocculation can be observed in situ with nondisruptive techniques which allow the definition of critical concentration ranges for changes in the behavior of the emulsions. Novel methods such as US and DWS can lead us to better understanding of the early stages of the interactions, which are so important in being able to modulate the creation of tailored interfacial systems.

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