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Role of Protein and Ferulic Acid in the Emulsification Properties of Sugar Beet Pectin

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The ability of sugar beet pectin to stabilize 20% w/w limonene oil-in-water emulsions has been investigated. The size of the oil droplets as determined by laser diffraction measurements decreased from about 15 μm to about 6 μm when the pectin concentration increased from 0.05% to 2% w/w but leveled off thereafter, suggesting complete coverage of the oil droplets by the polymer at this optimum concentration. Isotherms for the adsorption of pectin, protein, and ferulic acid were constructed. The adsorption capacities at the oil–water interface of ~1.4 and ~0.2 mg/m^2 for protein and ferulic acid, respectively, compared to ~9.5 mg/m^2 for pectin revealed that the adsorbed fractions of the pectin sample were rich in protein (14.7%) and ferulic acid (2.1%) given that there were only 2.7% protein and 1.06% ferulic acid present in the whole pectin sample. Direct measurements on the adsorbed fraction recovered from the oil droplets via desorption with SDS confirmed that it contained 11.1% protein and 2.16% ferulic acid. The results suggest that one or both of these two functional groups adsorb onto the surface of the oil droplets and stabilize the emulsions. High molecular mass fractions adsorbed preferentially onto oil droplets during emulsification. As compared to those made with gum arabic, the emulsion samples made with sugar beet pectin samples exhibited similar (or even slightly higher) stability.

KEYWORDS: Sugar beet pectin; emulsification; protein; ferulic acid; adsorption

1.0. INTRODUCTION

Pectin is a heteropolysaccharide with a chain structure of (1→4)-linked α-D-galacturonic acid units interrupted by the insertion of (1→2)-linked L-rhamnopyranosyl residues (*1*, *2*). Some of the acid groups of the (1→4)-linked α-D-galacturonic acid units in the linear chain structure ("smooth" region) are present as methoxyl (methyl) ester (*3*) and the term degree of esterification (DE) is used to describe the percentage of acid groups present in the ester form. The pectin molecule can contain 200–1000 linked galacturonic acid units (*4*). Sugar constituents such as D-galactose, L-arabinose, D-xylose, D-glucose, D-mannose, L-fucose, and D-glucuronic acid are attached in side chains ("hairy" region). Side chains are glycosidically linked to O-4 and/or O-3 of L-rhamnopyranose or O-2 or O-3 of some of the galacturonosyl residues (*3*). These side chains (neutral sugars) are not evenly distributed along the backbone.

Pectin has traditionally been used in food applications. Historically, pectin has been used as a gelling agent for jam or similar fruit-containing, or fruit-flavored, sugar-rich systems. Citrus peels and apple pomace are the two main industrial sources of pectin used for gelled systems, with the former being the preferred one due to its higher yield compared to the latter. Two different models have generally been used to describe the gelling mechanism of pectin. Walkinshaw and Arnott (*5, 6*) modeled the high-ester pectin (when DE > 50%) gelation as the alignment of molecular helices that are held together by hydrogen bonding and hydrophobic interactions. High-ester pectin forms gels at low pH, typically below 3.5, and in the presence of high solid contents, typically sucrose at a concentration of 55% or more, by weight. At high pH, the carboxylic groups on the pectin chains are dissociated and this leads to electrostatic repulsion between the chains, which is unfavorable for gel formation. It was initially suggested that the cosolute acts as a bridging agent by forming hydrogen bonding between two different polymer chains (*7*) but Rees (*8*) later suggested that the cosolute creates low water activity conditions that promote pectin chain−chain interaction.

Unlike high-ester pectin, low-ester pectin (when DE < 50%) does not need a high solids content or low pH to form gels. Instead it requires the presence of divalent cations such as calcium ions. Low-ester pectin gelation results from the cross-linking of carboxylate groups from different pectin molecules by calcium ions. The mechanism of gelation is described by the "egg-box" model (*9*) involving two (dimer) or more (multimer) polymer chains with the calcium ions binding preferentially and stronger to polyguluronate and polygalacturonate than to polymannuronate due to structural/conformational differences. This mechanism involves the formation of junction

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Table 1. Characteristics of the Sugar Beet Pectin and Gum Arabic Samples

sample	galacturonic acid, %	degree of esterification (de), %	acetic acid, %	Ca content, ppm
SBP1	n/d[a]	n/d	n/d	n/d
SBP2	74.1	59.5	3.19	500
SBP3	71.2	57.5	5.0	n/d
GA	n/a[b]	n/a	n/a	6500

[a] n/d: not determined. [b] n/a: not applicable.

zones between unbranched nonesterified galacturonan blocks bound together by calcium ions (*6*). The findings by Morris et al. (*10*) and Rees (*11*), however, show that the structure is of a dimeric rather than a multimeric association. Molecular modeling studies (*12, 13*) examining the interaction of calcium ions and pectin (polygalacturonate) and alginate (polyguluronate and polymannuronate) and NMR spectroscopy investigation of the binding of Mn^{2+} ions with pure mannuronate and guluronate blocks (*14*) were performed and the findings compared to the consistency of the "egg-box" model. More recently, Siew et al. (*15*) proposed new insights into the gelation mechanism of low-ester pectin (and alginate) involving charge annihilation and reversal.

Pectin from sugar beet is not used in gelled systems due to its poor gelling ability. The presence of a high amount of acetyl groups at the C2 and C3 positions of the galacturonic acid residues, which leads to incompatibility of chain−chain association, is one of the reasons for the poor gelling properties of sugar beet pectin. In recent years, sugar beet pectin has been found to have good emulsification properties (*16–19*) and the potential for sugar beet pectin to be used as a food emulsifier is seen. The emulsification properties exhibited by sugar beet pectin have been linked to the protein moiety (*16–19*) and also the acetyl groups (*17*). Sugar beet pectin appears to have higher protein and acetic acid contents compared to nonbeet pectin. Thibault (*20*) reported 10.4% protein and Axelos and Thibault (*21*) and Levigne et al. (*22*) respectively found 16% and 35% degrees of acetylation in sugar beet pectin. This compares to 1.6% protein and 5% degree of acetylation for apple pectin and 3–3.3% protein and 1.4–1.6% degree of acetylation for citrus pectin reported by Kravtchenko et al. (*23*).

This paper investigates the role of protein and ferulic acid in the emulsification properties of sugar beet pectin.

2.0. MATERIALS AND METHODS

2.1. Materials. Three commercial sugar beet pectin samples (SBP1 and SBP2, from Degussa Texturant, and SBP3, from Herbstreith & Fox) and a commercial gum arabic sample (*Acacia senegal*) (GA from Agrisales Ltd.) have been used and their characteristics provided by the suppliers are given in **Table 1**. The D-limonene oil, sodium dodecyl sulfate (SDS), and propan-2-ol (IPA) used in the studies were obtained from Aldrich, Fisons Laboratory Reagent, and Fisher Scientific, respectively.

2.2. Methods. *2.2.1. Preparation of Pectin and Gum Arabic Solutions.* Stock solutions of 4% w/w were prepared by dispersing the polymers in distilled water in wide screw neck glass powder bottles and leaving overnight on a roller mixer at room temperature. A series of pectin and gum arabic solutions of different lower concentrations were then prepared from these stock solutions via dilution.

2.2.2. Determination of Amino Acid Content. The amino acid content of the samples was determined by Alto Biosystems Ltd., Birmingham, UK, and the total protein content calculated.

2.2.3. Determination of Ferulic Acid Content. The ferulic acid content in the samples was determined by measuring the absorbance at a wavelength of 310 nm (Shidmazu UV–visible recording spectrophotometer UV240, Graphicord). A calibration curve was constructed using ferulic acid [*trans*-4-hydroxy-3-methoxycinnamic acid, $HOC_6H_3(OCH_3)CH{=}CHCO_2H$] obtained from Aldrich.

2.2.4. Determination of Molecular Mass Distribution. The molecular mass distribution of the pectin samples was determined using gel permeation chromatography (GPC), coupled to multiangle laser light scattering (MALLS) (DAWN DSP laser photometer, Wyatt Technology Corp.), reflective index (RI) (Optilab DSP interferometric refractometer, Wyatt Technology Corp.), and ultraviolet (UV) (Agilent 1100 series) detectors. A 100 μL portion of 0.1% polymer solution was passed through a Superose 6 column at a flow rate of 0.5 mL/min with 0.1 M NaCl as eluent. Prior to injection to the GPC system, all samples were filtered through a 0.45 μm nylon filter. Eluent was passed through a degasser (Gastorr 153) before being pumped using a Knauer HPLC pump K-501 into the GPC system. A d*n*/d*c* value of 0.131 mL/g for pectin and 0.141 mL/g for gum arabic was used, and the data were analyzed using the Debye fitting method.

2.2.5. Measurements of Surface Tension. The static surface tensions of 2% w/w polymer solutions were measured using a Kruss tensiometer (model K8) equipped with a Du Nuoy ring.

2.2.6. Preparation of Emulsions. Sixteen grams of pectin solution at various concentrations (0.05–4%) were added to 4 g of limonene oil in 28-mL glass vials with polyethylene caps. The mixtures were subjected to shaking for 30 s immediately prior to homogenization with an Ultra Turrax T25 Basic S2 at a speed of 24 000 rpm for 4 min. Emulsions were stored at room temperature. For emulsion stability studies (over time), 0.1% w/w sodium benzoate was added as an antimicrobial agent to the polymer solutions prior to emulsification.

2.2.7. Measurements of Droplet Size. The droplet size of the emulsions was analyzed using a Malvern Mastersizer 2000. Prior to measurements, all glass vials containing the emulsions were gently inverted repeatedly until homogeneously dispersed. The emulsions were added to the dispersion unit drop by drop until the laser obscuration was 13 ± 0.5%. The sample was dispersed (stirred) at a speed of 2000 rpm. The refractive indexes for the limonene oil and water were 1.472 and 1.330, respectively. All reported values are the average of three measurements with a 30-s delay between measurements.

2.2.8. Determination of Adsorption Isotherms. In order to determine adsorption isotherms, the emulsions were centrifuged using a Mistral 3000i centrifuge at 25 °C at 2500 rpm for 10 h or longer until complete separation of the oil and the aqueous layers. The aqueous layer at the bottom of the centrifuge tube was carefully separated from the oil layer at the top using a needle connected to a syringe and used for measurements. The amount of pectin adsorbed onto the oil droplets was determined by GPC from the change in the intensity of the refractive index elution profile before and after emulsification.

The quantification of protein adsorption onto oil droplets during emulsification was investigated using the Bradford method (*24*). The method is based on the binding of Coomassie Blue dye to protein, which forms a complex that absorbs ultraviolet light at 595 nm. The Coomassie Blue reagent was made by dissolving 100 mg of Brilliant Blue G in 50 mL of 95% ethanol. One hundred milliliters of 85% phosphoric acid was added and the solution made up to 1 L with distilled water. One milliliter of supernatant solution was added to 5 mL of the Coomassie Blue reagent and the solution was gently mixed by inversion. The solution was left at room temperature between 30 min and 1 h before being analyzed with an ultraviolet spectrophotometer at 595 nm. The protein contents were determined on the basis of the individual calibration curves constructed from the initial protein contents of the pectin samples as shown in **Table 2**.

2.2.9. Preparation and Recovery of Nonadsorbed and Adsorbed Fractions. An emulsion was prepared with 320 g of 2% SBP1 and 80 g of limonene oil. The pectin solution and the limonene oil were mixed in a 500-mL bottle and subjected to shaking for 30 s immediately prior to homogenization in a 600-mL beaker with a Silverson L4R homogenizer with a medium-perforation emulsor screen at maximum speed for 5 min. The emulsion was left at room temperature for 24 h before being centrifuged at 2500 rpm at 25 °C for 10 h or until complete separation of the aqueous phase and the oil phase. The aqueous phase, which contained the nonadsorbed fraction of the sugar beet pectin, at the bottom of the centrifuge tube was carefully separated with a needle connected to a syringe. This nonadsorbed fraction was freeze-dried and

Table 2. Amino Acid Profiles of the Sugar Beet Pectin Samples

amino acid	% of total protein		
	SBP1	SBP2	SBP3
hydroxyproline	0.47	0.52	0.76
aspartic acid	0.15	0.17	0.17
threonine	0.17	0.20	0.24
serine	0.17	0.19	0.23
glutamic acid	0.25	0.27	0.29
proline	0.16	0.18	0.21
glycine	0.10	0.11	0.14
alanine	0.12	0.13	0.14
cystine	0.01	0.02	0.00
valine	0.19	0.22	0.26
methionine	0.00	0.01	0.01
isoleucine	0.06	0.07	0.08
leucine	0.10	0.12	0.13
tyrosine	0.16	0.17	0.23
phenylalanine	0.07	0.09	0.08
histidine	0.15	0.17	0.24
tryptophan[a]	n/d	n/d	n/d
lysine	0.21	0.24	0.36
arginine	0.11	0.13	0.12
total protein, %	2.67	3.01	3.69

[a] Tryptophan usually suffers complete loss during acid hydrolysis and was therefore not quantified in the analysis. n/d: not determined.

analyzed. The oil phase, which contained the adsorbed fraction of the sugar beet pectin, was collected into a 500-mL bottle and sodium dodecyl sulfate (SDS) solution was added such that the amount of oil phase was 20% of the total weight. SDS of about twice the amount of the sugar beet pectin adsorbed (based on the results obtained from the adsorption isotherms) was used in the desorption process. This excessive amount of SDS was used to ensure that all adsorbed sugar beet pectin was desorbed from the oil phase. This mixture was homogenized using Silverson L4R for 1 min with 30 s of shaking immediately prior to homogenization. The dispersion was centrifuged at 2500 rpm at 25 °C until complete separation of the oil and the aqueous phases after being left at room temperature for 24–48 h. The aqueous phase at the bottom of the centrifuge tube, which contained the adsorbed fraction of the sugar beet pectin, was carefully separated from the oil phase using a syringe connected to a needle. The adsorbed fraction of the sugar beet pectin was precipitated with propan-2-ol (IPA) and collected after brief centrifugation. This recovered adsorbed fraction of sugar beet pectin was then repeatedly (five or six times) subjected to dissolution in water (~3−4% w/w) followed by precipitation with IPA (of a final concentration ~80% w/w) to remove SDS. Foaming is an indication of the presence of SDS. The nonadsorbed and the adsorbed fractions of the sugar beet pectin recovered from the above procedure were analyzed for ferulic acid and protein contents.

3.0. RESULTS AND DISCUSSION

3.1. Characterization of the Pectin Samples. The amino acid compositions of the pectin samples given in **Table 2** show that hydroxyproline is the major amino acid present, contributing 17–21% of the total protein. Similar results were reported by Williams et al. (*18*), where all six different adsorbed sugar beet pectin fractions obtained via hydrophobic affinity chromatography fractionation had hydroxyproline as the major amino acid ranging from 9 to 20%. The GPC elution profile of the SBP1 sample in **Figure 1** shows the signals of light scattering at 90° (LS#11), RI (AUX1), and UV absorbance at 310 nm (AUX2). Whereas the intensity of the RI signal is proportional to the concentration of eluting species, the UV absorbance at 310 nm is proportional to the concentration of ferulic acid eluting. The strong UV absorbance signal in **Figure 1** is evidence of the presence of ferulic acid in the SBP1 sample. SBP2 and SBP3 samples had GPC elution and signal profiles (data not shown) similar to those of SBP1, also with a strong UV signal confirming the presence of ferulic acid.

The weight-average molecular masses (M_w) and ferulic acid and protein contents of the sugar beet pectin and gum arabic samples are given in **Table 3**. The molecular masses of the SBP1 pectin and GA gum arabic samples were 7.53×10^5 g/mol and 5.27×10^5 g/mol, respectively. The ferulic acid content of the sugar beet pectin samples was found to be ~1%, which is similar to the values reported by a number of other workers (*19, 22, 25*). However, Yapo et al. (*26*) reported lower values of 0.1–0.7%, depending on the extraction conditions (pH, temperature, and time) of the pectin from the sugar beet pulp. It has been established that ferulic acid is mainly attached to the neutral sugar units of arabinose (~50%) and galactose (~50%) (*27*) found in the "hairy region" of the pectin molecule. Ferulic acid can either exist as a monomer attaching to one arabinose residue or as dimer cross-linking two arabinose residues (*27–30*).

The values of protein content for the sugar beet pectin samples used in the current studies (2.67–3.69%) are reasonably similar to those reported by Leroux et al. (*17*) (1.95%), Williams et al. (*18*) (3.7%), and Funami et al. (*19*) (1.56%) but lower than the value of 10.4% reported by Thibault (*20*). Oosterveld et al. (*31*) reported the presence of an arabinogalactan protein (AGP) in hop pectin and suggested that this AGP was linked to the pectin based on the molecular mass distribution profiles of the pectin sample after degradation with endopolygalacturonase plus methyl esterase. More recently, Immerzeel et al. (*32*) suggested that at least part of carrot AGPs from the cell walls may be covalently linked to pectin containing a homogalacturonan structural element. This was based on the findings that pectin methylesterase and pectin galacturonase released high amounts of galacturonic acid from the AGPs that were isolated from carrot cell walls with Yariv phenylglycoside. Kirby et al. (*33*), who used atomic force microscopy (AFM), have suggested that the protein moiety is attached to one end of the pectin chains.

3.2. Static Surface Tension. The static surface tensions of 2% polymer solutions of the pectin and gum arabic samples together with SDS and nonbeet pectin samples are shown in **Table 4**. The sugar beet pectin and gum arabic samples showed a significant reduction in surface tension as compared to apple and citrus pectin samples, which may be due to their higher protein and/or ferulic acid contents. For gum arabic it has been postulated that the more hydrophobic protein component anchors the molecules to the surface of the oil droplets while the more hydrophilic carbohydrate moieties protrude into solution, preventing droplet aggregation by electrostatic and steric repulsion (*34, 35*). Gum arabic and sugar beet pectin, however, are not as effective as sodium dodecyl sulfate at reducing the surface tension.

3.3. Effect of Polymer Concentration on Droplet Size. The droplet sizes of 20% w/w limonene oil in water emulsions prepared using varying concentrations of sugar beet pectin and gum arabic and aged for 24 h are shown in **Figure 2**. It was noted that the droplet size decreased as the polymer concentration increased up to ~2% and then remained fairly constant, indicating full coverage of the oil droplets by the polymer molecules. Williams et al. (*18*) reported similar findings on the effects of polymer concentrations on the droplet sizes of emulsion samples made with 20% orange oil and orange oil/ester gum with various sugar beet pectin concentrations. The droplet size ($D[0.5]$) 72 h after being prepared decreased from 18 and 28 μm for orange oil/ester gum and orange oil, respectively, at 0.5% pectin with increasing pectin concentration

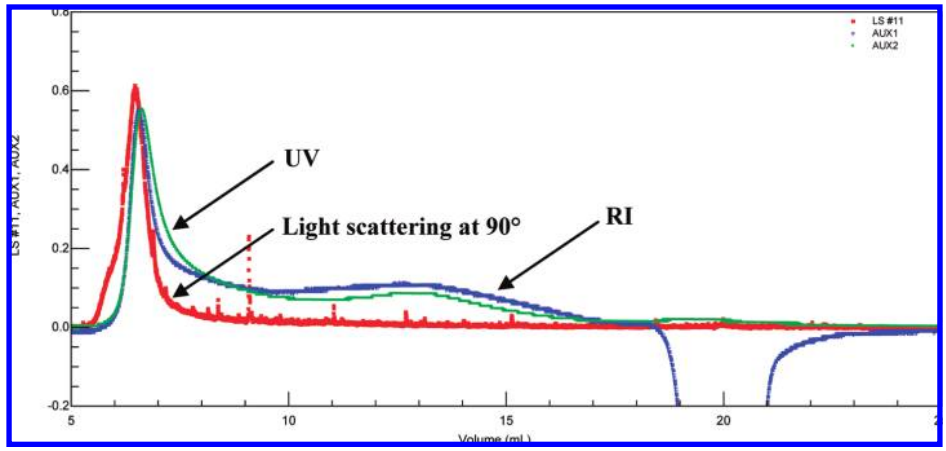

Figure 1. GPC elution profiles of the SBP1 pectin sample.

Table 3. Weight-Average Molecular Masses and Ferulic Acid and Protein Contents of the Sugar Beet Pectin and Gum Arabic Samples

sample	$M_w \times 10^5$ g/mol	ferulic acid, %	protein, %
SBP1	7.53	1.06	2.67
SBP2	n/d[a]	1.01	3.01
SBP3	n/d	0.78	3.69
GA	5.27	n/a[b]	2.15

[a] n/d: not determined. [b] n/a: not applicable.

Table 4. Static Surface Tension Measurements of the Sugar Beet Pectin and Gum Arabic Samples Compared to Some Nonbeet Pectin Samples and SDS

sample	surface tension, mN/cm
water	71.1
SBP1	54.5
SBP2	56.1
SBP3	n/d[a]
GA	57.1
high-methoxyl citrus pectin	62.7
low-methoxyl amidated apple pectin	69.2
high-methoxyl apple pectin	66.9
sodium dodecyl sulfate (SDS)	37.9

[a] n/d: not determined.

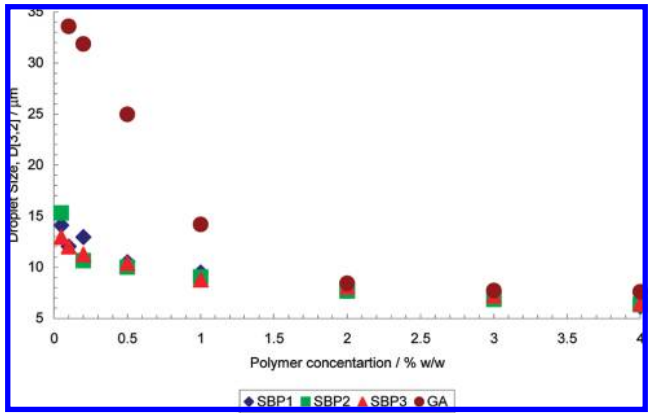

Figure 2. Droplet size (D[3,2]) profiles of the limonene oil emulsions stabilized by sugar beet pectin and gum arabic as a function of polymer concentration.

and eventually leveled off at 3% and 1.5% pectin, respectively, for orange oil/ester gum and orange oil with both having the similar droplet size of 5 μm.

Figure 2 also shows that at low polymer concentrations, sugar beet pectin samples exhibited higher emulsification properties

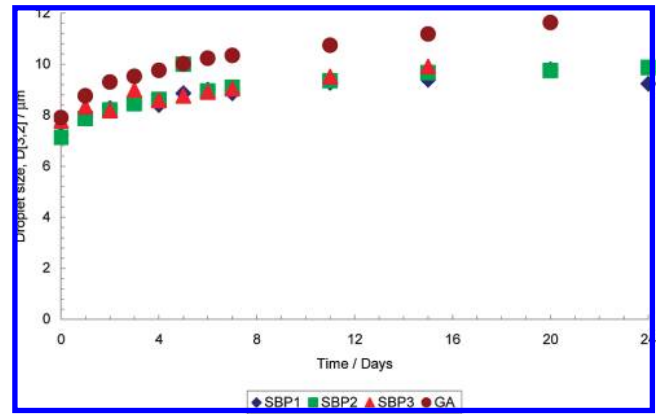

Figure 3. Droplet size (D[3,2]) profiles of the limonene oil emulsions stabilized by sugar beet pectin and gum arabic at optimum polymer concentration (2%) as a function of time.

than gum arabic. The droplet size of the gum arabic-stabilized emulsion sample was much higher than those that were stabilized by sugar beet pectin samples. Similar droplet size was observed for all emulsion samples when the polymer concentration was at and above 2%, suggesting complete coverage of the oil droplet surfaces by both pectin and gum arabic.

3.4. Emulsion Stability at Optimum Polymer Concentration. The droplet size of the emulsions prepared with pectin and gum arabic at the optimum polymer concentration (i.e., 2%) was monitored as a function of time, and the results are shown in **Figure 3**. All emulsion samples showed a slight increase in droplet size (D[3,2]) over the 24-day period. Gum arabic produced droplets with a slightly higher initial droplet size and the emulsions showed a slightly greater increase in droplet size over time compared to the sugar beet pectin-stabilized emulsions.

3.5. Pectin Adsorption during Emulsification. The studies of adsorption of pectin onto oil droplets during emulsification were carried out using SBP1. Briefly, emulsion samples of 20% w/w limonene oil were prepared with pectin of various concentrations, namely, 0.1−2.5%. Upon preparation, the emulsion samples were left at room temperature for at least 10 h and then centrifuged for 10 h or more until a clear aqueous phase was obtained at the bottom of the centrifuging tube. The aqueous layer was carefully taken out with a syringe connected to a needle and analyzed with GPC−MALLS−RI. The RI signal is proportional to the concentration of solute (polymer) present and hence the amount of pectin adsorbed was established from the change in intensity of the RI signals. **Figure 4** shows the RI signal profiles of 0.1% SBP1 sample before and after

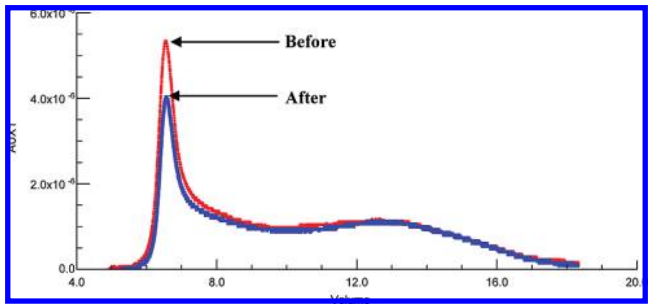

Figure 4. RI elution profiles of the SBP1 pectin sample before and after emulsification with 0.10% w/w initial (before emulsification) polymer concentration.

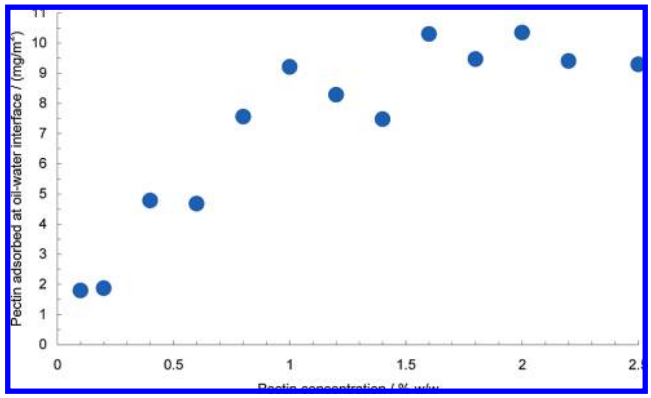

Figure 5. Polymer adsorption isotherm for the SBP1 pectin sample at the oil–water interface during emulsification as a function of added pectin concentration.

emulsification with 20% limonene oil. The signal intensity after emulsification is reduced due to adsorption of some pectin fractions onto oil droplets during emulsification. It is interesting to note that the signal loss was mainly for high molecular mass fractions, indicating preferential adsorption of high molecular mass fractions onto oil droplets during emulsification. This explains the decrease in the molecular mass from 7.53×10^5 g/mol before emulsification to 4.86×10^5 g/mol after emulsification.

The pectin adsorption isotherm determined for SBP1 with 20% limonene oil is shown in **Figure 5**. The amount of pectin adsorbed increased with increasing added pectin concentration from ~1.8 mg/m^2 at 0.1% pectin to a plateau ~9.5 mg/m^2 at 2.5% pectin. This value is similar to values reported by Akhtar et al. (*16*), i.e. 10.7, 9.8, and 11.1 mg/m^2 when 2, 3, and 4%, respectively, of depolymerized citrus pectin with 69.7 kg/mol molecular mass was used in the emulsification of 20% rapeseed oil at pH 4.7. The amount of a polymer adsorbed onto a surface depends on a number of factors, including the interaction between the polymer and the surface and also the conformation of the polymer when adsorbed. Typically for many polymer/particle systems the amount adsorbed is on the order of 1 mg/m^2, which is consistent with monolayer coverage. The very high value found by us and others indicates that multilayer adsorption is occurring. We are unable to explain this at the present time, but it may be due to cross-linking of pectin molecules at the surface by calcium ions, ferulic acid, or protein.

3.6. Protein Adsorption during Emulsification. Figure 6 shows the percentages of the protein within the pectin samples adsorbed onto the oil droplets as a function of the pectin concentration. At low added pectin concentrations, nearly all the protein present in the system adsorbed, leaving no or little nonadsorbed protein in solution, indicating high affinity. The

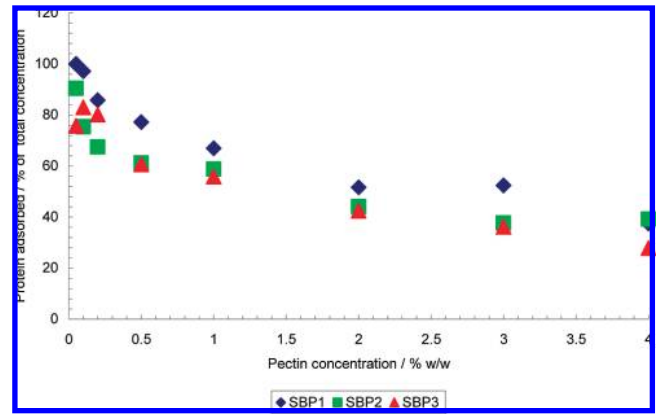

Figure 6. Amount of protein adsorbed (in percentage of total concentration) for the sugar beet pectin samples at the oil–water interface during emulsification as a function of added pectin concentration.

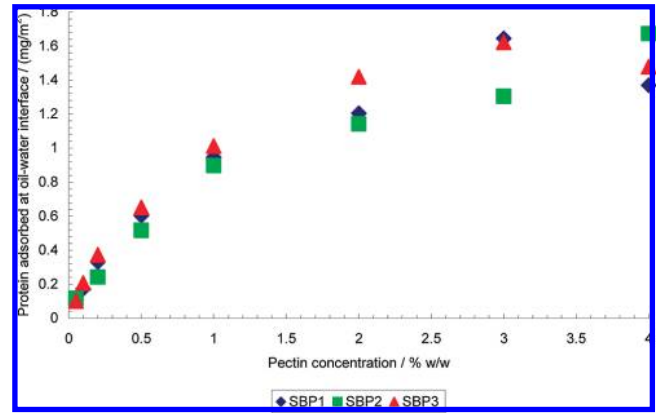

Figure 7. Adsorption isotherms for the protein component of the sugar beet pectin samples at the oil–water interface during emulsification as a function of added pectin concentration.

percentage of the protein adsorbed then decreased gradually with increasing added pectin concentrations, with only about one-third of the protein added adsorbed and the rest remaining free in the system at 4% added pectin concentration.

The isotherms for the adsorption of the protein components onto the oil droplets are given in **Figure 7**. The amount of protein adsorbed increased initially with increasing pectin concentration but leveled off at ~2% addition, which also corresponds to the concentration for minimum droplet size (**Figure 2**). The three sugar beet pectin samples had protein adsorption capacities of ~1.2–1.6 mg/m^2. On the basis of the adsorption capacities of ~9.5 mg/m^2 (**Figure 5**) and ~1.4 mg/m^2 (**Figure 7**) for pectin and protein, respectively, it is calculated that the adsorbed fraction of the SBP1 sample was protein-rich, with a protein content of 14.7%. This compares to a protein content of 2.67% in the whole (original) SBP1 sample. These results are in accordance with the findings that protein plays an important role in the emulsification properties of sugar beet pectin (*16–19*). Akhtar et al. (*16*) studied the emulsification properties of a citrus pectin (at 2, 3, and 3% concentrations) with 20% rapeseed oil at pH 4.7 and found that all the protein (0.60%) present in the samples adsorbed onto the oil droplets during emulsification. Leroux et al. (*17*) studied the emulsification properties of one beet and two depolymerized citrus pectin samples (of different molecular masses and degrees of esterification and protein contents) at 1% pectin concentration with either 20% w/w orange oil or 20% rapeseed oil at pH 3.5. It was found that for all the beet and depolymerized citrus pectin samples, the adsorbed fractions were essentially protein-rich with

21.2% and 7.9% for beet pectin with orange oil and rapeseed oil, respectively, and 7.9% and 7.8% for the first depolymerized citrus pectin with orange oil and the second depolymerized citrus pectin with rapeseed oil, respectively. This compared to the protein contents in the whole samples of 1.95% for beet pectin and 1.32% and 0.77% for the first and second depolymerized citrus pectin samples, respectively. It was also found that a sugar beet pectin with a higher protein (and acetyl) content required a lower polymer concentration compared to a (depolymerized) citrus pectin with a lower protein (and acetyl) content to produce similarly sized oil droplets.

Williams et al. (*18*) also showed that the protein moiety of sugar beet pectin preferentially adsorbed onto the oil droplets during emulsification of 20% orange oil and 2% pectin. In the studies, the oil phase was separated off and two further emulsion samples were produced using the pectin (aqueous phase) recovered. It was shown that the protein content in the aqueous phase decreased with the number of emulsification processes from 0.074% initial concentration before emulsification to 0.027, 0.020, and 0.008% after the first, second, and third emulsification processes, respectively. It should be noted that the pectin concentration did not decrease significantly after emulsification, indicating that only a small percentage of the material which was protein-rich adsorbed onto the oil droplets. It was however surprising that the droplet sizes remained constant for all three emulsion samples. It could be that the fractions that adsorbed onto the oil droplets during different emulsification processes had different combinations of molecular mass, protein, and other hydrophobic or surface active species such as ferulic acid, acetic acid, and ester groups, thus stabilizing the emulsion at different stages. In the studies, it appeared that, for the first emulsification process, the low molecular mass molecules adsorbed in preference.

Funami et al. (*19*) studied the effects of enzymatic modification using pepsin and protease on the emulsion properties of sugar beet pectin. The emulsion was prepared with 1.5% sugar beet pectin and 15% medium chain triglyceride (MCT) at pH 3.0. It was demonstrated that treatment of a sugar beet pectin sample with a protease enzyme significantly decreased the interfacial pectin concentration from 1.42 mg/m^2 to 0.45 mg/m^2, suggesting that the loss of proteinaceous material lowers the accessibility of the pectin molecules to adsorb onto the emulsion oil droplets. After modification, the amount of the sugar beet pectin adsorbed onto the oil droplets during emulsification decreased significantly from 14.58% for the control sample to 1.22% for the modified sample. The enzyme-modified sugar beet pectin lost most of its emulsifying activity, resulting in a much larger D[3,2] droplet size of 3.00 μm compared to 0.56 μm for the unmodified pectin sample. This was due to the cleavage and release of the protein moiety from the polysaccharide backbone by the protease enzyme resulting in the adsorption of protein only without the polysaccharide moiety onto the oil droplets.

Nakamura et al. (*36*) also found that, for emulsion samples made at pH 4 with 20% soybean oil and 4% trypsin-treated soy-soluble polysaccharide, the droplet sizes increased with increasing hydrolysis of the protein fraction of the polysaccharide, indicating a decrease in the emulsification properties of the polysaccharide. This also reveals, as for sugar beet pectin, that the protein moiety of this structurally pectin-like polysaccharide has a crucial role in the formation and stabilization of emulsion.

It should be noted however that although experiments have demonstrated that the adsorbed pectin fraction is high in protein, there does not appear any simple relationship between emulsion stability and protein content (*18*). Williams et al. (*18*) subjected a sugar beet pectin sample to hydrophobic affinity chromatography and obtained a number of samples with varying molecular mass, protein, and ferulic acid contents. It was found that while one low-protein fraction produced emulsions with larger droplet size that was unstable with time, another low-protein pectin fraction produced an emulsion with small droplet size that remained stable over time. It is therefore important to recognize that, apart from protein content, other factors contribute to the emulsification properties of pectin. This includes the molecular mass; the ferulic acid, acetic acid, and methyl ester contents; and their accessibility for the surface of the oil droplets.

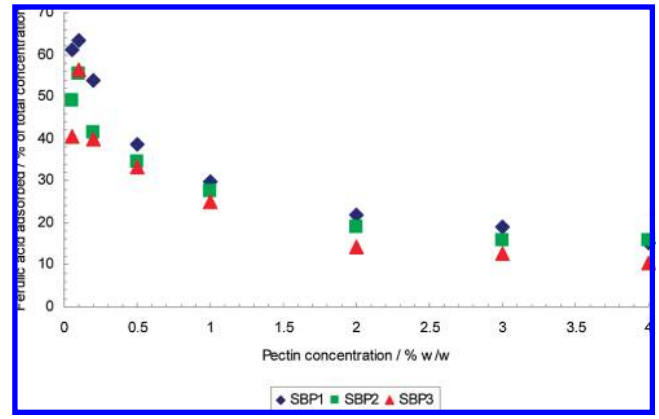

Figure 8. Amount of ferulic acid adsorbed (percentage of total concentration) for the sugar beet pectin samples at the oil–water interface during emulsification as a function of added pectin concentration.

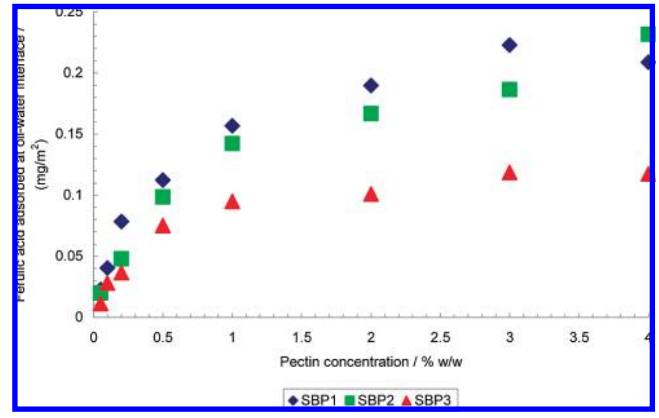

Figure 9. Adsorption isotherms for the ferulic acid component of the sugar beet pectin samples at the oil–water interface during emulsification as a function of added pectin concentration.

3.7. Ferulic Acid Adsorption during Emulsification. The adsorption of ferulic acid (in percentage of total ferulic acid present) onto oil droplets as a function of pectin concentration for the three sugar beet pectin samples is shown in **Figure 8**. For all three pectin samples, 40–60% of the ferulic acid present adsorbed onto the oil droplets at low pectin concentration. The percentage adsorbed decreased quite drastically with increasing pectin concentration and was ~10–15% at 4% pectin. The amount of ferulic acid adsorbed at optimum adsorption capacity (corresponding to 2% pectin concentration) was 15–20% of the total amount added.

The isotherms for the adsorption of ferulic acid onto limonene oil droplets for the three sugar beet samples are shown in **Figure 9**. The amount of ferulic acid adsorbed increased quite sharply initially with increasing pectin concentration and gradually reached a plateau value at 2% pectin and is in accordance with the pectin plateau adsorption shown in **Figure 5**. The ferulic

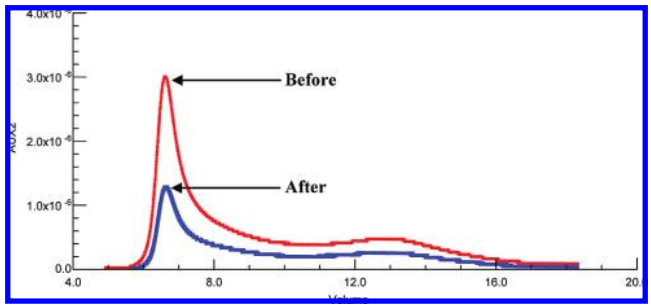

Figure 10. UV elution profiles of the SBP1 sample at 310 nm before and after emulsification with 0.10% w/w initial (before emulsification) polymer concentration.

Table 5. Molecular Mass, Protein, and Ferulic Acid Profiles of the SBP1 Adsorbed Fraction Compared to Nonadsorbed Fraction and Whole Sample

$M_w \times 10^5$ g/mol		protein, %		ferulic acid, %	
whole sample	[a]nonadsorbed fraction	whole sample	adsorbed fraction	whole sample	adsorbed fraction
7.53	4.86	2.67	11.10	1.06	2.16

[a] This was obtained from the supernatant recovered from the emulsion made with 0.1% SBP1 and 20% limonene oil.

acid adsorption capacities at plateau for the three samples were between ~0.1 and 0.2 mg/m^2. Since the plateau pectin adsorption capacity was ~9.5 mg/m^2, it is calculated that the adsorbed fraction had a ferulic acid content of 2.1%. This reveals that the adsorbed fraction was rich in ferulic acid, considering that the ferulic acid content in the whole (original) sample was 1.06%. This was supported by the ultraviolet (UV) chromatograms of the 0.1% w/w SBP1 sample obtained at 310 nm before and after emulsification shown in **Figure 10**. The intensity of the RI signal (**Figure 4**) and the UV signal (**Figure 10**) is proportional to the amount of the pectin and ferulic acid present, respectively. The loss of signal intensity after emulsification was much greater for UV compared to RI, confirming the adsorption of the ferulic acid-rich fractions onto the oil droplets during emulsification. As compared to the RI signal profile shown earlier in **Figure 4**, the loss of UV signal intensity was more evenly distributed across the molecular mass range.

3.8. Molecular Mass, Protein, and Ferulic Acid Profiles of the Adsorbed Pectin Fraction. The nonadsorbed and adsorbed fractions the SBP1 sample onto the oil droplets during emulsification was recovered via centrifugation and desorption with sodium dodecyl sulfate, respectively, and subsequently analyzed for molecular mass, protein. and ferulic acid profiles. **Table 5** shows the results and compares them with those of the whole SBP1 sample (i.e., before emulsification). The weight-average molecular mass of the SBP1 sample was significantly less after emulsification from 7.53 $\times$ 10^5 g/mol for the whole sample to 4.86 $\times$ 10^5 g/mol for the nonadsorbed fraction, confirming our previous findings (**Figure 4**). The reason for the preferential adsorption of high molecular mass material may be linked to its protein and/or ferulic acid contents.

The protein content determined from amino acid analysis (data on amino acid profiles not shown) was four times richer in the adsorbed fraction (11.10%) than was in the whole sample (2.67%). This is consistent with other studies where protein-rich fractions adsorbed onto oil droplets during emulsification (*16–19*). It is noted that the protein content of 14.7% in the adsorbed fraction calculated based on the pectin (**Figure 5**) and protein (**Figure 7**) adsorption isotherms was very close to the 11.10% obtained for the adsorbed fraction of the SBP1 by amino acid analysis (**Table 5**).

The ferulic acid content in the adsorbed fraction was double (2.16%) that of the whole sample (1.06%). This adsorbed fraction was therefore no doubt rich in ferulic acid and this was presumably related to the hydrophobic nature of ferulic acid. It is noted that the ferulic acid content of 2.1% in the adsorbed fraction calculated on the basis of the pectin (**Figure 5**) and ferulic acid (**Figure 8**) adsorption isotherms is the same as the value (2.16%) obtained by direct UV absorbance measurement on the adsorbed fraction shown in **Table 5**.

In summary, sugar beet pectin produces stable limonene oil-in-water emulsions with a smaller droplet size than emulsions prepared with gum arabic at the same concentration. There is preferential adsorption of high molecular mass material that has a high protein and ferulic acid content. The situation is analogous to the model proposed for the adsorption of gum arabic onto oil droplets where the more hydrophobic protein moiety adsorbs at the interface and that the more hydrophilic carbohydrate residues protrude out into solution, providing an electrosteric barrier and preventing droplet aggregation (*34, 35*).

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