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Elucidation of the Emulsification Properties of Sugar Beet Pectin

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A protocol has been developed to fractionate sugar beet pectin using hydrophobic affinity chromatography. Three samples eluted from the column using 4 M NaCl as solvent (fractions 1A, 1B, and 1C), two fractions eluted using 2 M NaCl (fractions 2A and 2B), and one fraction eluted using water (fraction 3). The fractions were shown to be very polydisperse, and differences between the GPC refractive index and UV absorbance (214 nm) elution profiles demonstrated chemical heterogeneity. They were found to contain significantly different proportions of protein (1A, 2.79%; 1B, 0.97%; 1C, 0.77%; 2A, 1.41%; 2B, 5.09%; and 3, 5.89%) and ferulic acid (approximately 1A, 0.5%; 1B, 0.5%; 1C, 0.9%; 2B, 1.5%; and 3, 2%). The weight-average molecular mass, M_w, of the fractions also varied (1A, 153 kDa; 1B, 155 kDa; 1C, 306 kDa; 2A, 562 kDa; 2B, 470 kDa; 3, 282 kDa). Three fractions, that is, 1A, 1B, and 3, produced orange oil emulsions with a relatively small droplet size that were stable over a period of weeks. The other three fractions (1C, 2A, and 2B with higher M_w values) produced emulsions with an initially larger droplet size, and the droplet size increased considerably over time. The increased droplet size may be influenced by the viscosity of the aqueous continuous phase. There was no simple relationship between protein or ferulic acid content and emulsification ability. For example, fraction 1B, which contained the lowest proportion of both protein and ferulic acid, produced stable emulsions of similar droplet size to fraction 3, which contained the largest proportion of protein and ferulic acid. The role of protein in the emulsification process was investigated by measuring the amount of protein in the aqueous phase before and after emulsification. It was clearly demonstrated that proteinaceous material adsorbed at the oil−water interface. It is evident that the emulsification properties of sugar beet pectin are influenced by the accessibility of the protein and ferulic acid groups to the surface of the oil droplets, the proportion of ester groups, and the molecular mass distribution of the fractions.

KEYWORDS: Sugar beet pectin; emulsification; molecular mass characterization; gel permeation chromatography; hydrophobic affinity chromatography; droplet size distribution

INTRODUCTION

Pectins are widely used in the food industry because of their gelling properties and their ability to stabilize milk products under acid conditions (*1*). The main sources of commercial pectin are citrus peel and apple pomace, and the world market is estimated to be worth ~£ 220 million ($US 400 million) per annum (*2*). Pectin is also obtained from sugar beet as a byproduct during the extraction of sugar, and >2 million tons of sugar beet pulp are produced annually in the European Union. The pulp consists of cellulose (23%), arabinan (21%), pectin (19%), other sugars (14%), protein, (6%), lignin (5%), fat (1%), and ash (12%) (*3*). Pectins generally consist of linear chains (usually referred to as "smooth regions") of α-1,4-linked galacturonic acid residues, which may be esterified, with a small percentage of rhamnose units, which give rise to kinks (*1*, *4*). The chains also have branches (referred to as "hairy regions") consisting of neutral sugars, particularly galactose and arabinose. Sugar beet pectin differs from other pectins in that it has a higher proportion of hairy regions, a higher acetic acid content on the C2 and/or C3 positions of the galacturonic acid residues, and also phenolic esters (notably ferulic acid) attached to galactose and arabinose units in side chains (*5*). Pectins also contain a small percentage of protein, which is reported to be significantly higher in the case of sugar beet pectin (10.4%) (*6*) compared to

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apple (1.6%) and citrus (3−3.3%), respectively (*7*). Ridley et al. (*8*) reported no evidence of covalent linkages between pectin and protein moieties; however, Kravtchenko et al. (*9*) found hydroxyproline-rich proteinaceous material present that was not removed on purification.

High-ester pectins from citrus and apple pomace form gels at low pH (∼3−4) in the presence of high concentrations of soluble solids (e.g., sugar) (*1*, *4*). Chain association, which results in network formation, is believed to be due to both hydrophobic interaction between ester groups and hydrogen bonding between hydroxyl groups. On the other hand, low-ester pectins form gels at low pH in the presence of calcium ions due to calcium bridging (*1*, *4*). Sugar beet pectin, however, does not form gels either in the presence of high sugar concentrations or with calcium ions (*9*, *10*). This may be due to the presence of a high proportion of acetyl groups (*11*), its apparently lower molecular mass (*12*, *13*), or the larger number of side chains (*14*). An alternative application for sugar beet pectin, which would significantly increase its commercial value, is as a food emulsifier. Recently it has been reported that sugar beet pectin is very effective at stabilizing rape seed oil and orange oil emulsions (*15*). It was found that the molecular mass and protein and acetyl contents significantly influenced the emulsifying ability. Analysis of the aqueous phase before and after emulsification showed that the fraction that adsorbed onto the oil was protein-rich. Deacetylation of the beet pectin did not have any significant influence on the emulsifying ability. Leroux et al. (*15*) and Ahktar et al. (*16*) also found that protein-rich components in citrus pectin were adsorbed at the oil−water interface. They carried out studies on depolymerized citrus pectin and found that the optimum M_w for producing stable emulsions was 70 kDa. Pectins with lower or higher molecular mass, M_w, were not as effective. The poorer emulsification properties of the higher molecular mass fractions may be due to flocculation induced by calcium cross-linking pectin molecules on different oil droplets. Sugar beet pectin was more efficient than citrus pectin. They also found that pH had a significant influence on emulsion stability. A recent study concerning the ability of apple, citrus, and sugar beet pectin to stabilize citrus oil emulsions using dynamic surface tension measurements has shown that although all three pectins reduce surface tension, sugar beet pectin produces the greatest reduction (*17*). The results are consistent with data reported by Leroux et al. (*15*), who performed measurements on paraffin oil/2% pectin solutions using the Du Nouy ring method. These workers also showed that the interfacial tension of 2% sugar beet pectin was similar to that obtained for 15% gum arabic.

This paper reports the fractionation of sugar beet pectin using hydrophobic affinity chromatography and the physicochemical characterization and emulsification properties of the fractions.

MATERIALS

Sugar beet pectin was kindly supplied by Degussa Texturant Systems, Baupte, France. The dry sugar beet pulp was hydrated with warm water and heated under acid conditions with stirring for a period of 3−5 h. Solid material was removed by centrifugation and filtration, and the resulting syrup was concentrated by ultrafiltration. The pectin was precipitated out by addition of isopropyl alcohol (IPA), washed with pure IPA, and dried. The sample had a moisture content of 9.7%, determined by drying in an oven at 105 °C to constant weight. The amount of nitrogen in the sample was determined by Kjeldahl analysis and found to be 0.59%, giving a protein content of 3.7%.

The orange oil was provided by Degussa Texturant Systems. Ester gum 8BG, which is a purified glycerol ester of wood rosin, was used as a weighting agent to increase the density of the oil phase and was obtained from Hercules Inc.

METHODS

Fractionation of Sugar Beet Pectin by Hydrophobic Affinity Chromatography. The procedure used to fractionate the pectin was based on a previous method established for the fractionation of gum arabic (*18*). Pectin (4.5 g/150 mL) was dissolved in 4 M NaCl and passed down a HiLoad 26/10 phenyl−Sepharose HP column (Amersham Biosciences, Little Chalfont, U.K.), using a peristaltic pump at a flow rate of 1 mL/min. The amount of pectin eluting from the column was monitored using UV absorbance at 214 nm. The eluent was collected as three discrete fractions (fractions 1A, 1B, and 1C) until no further pectin eluted. Two molar NaCl was then passed down the column, and some pectin molecules, which had adsorbed onto the column matrix, desorbed and were collected (fractions 2A and 2B). When no further pectin eluted, water was passed down the column, facilitating further desorption, and the eluent was collected (fraction 3). The samples were dialyzed using dialysis tubing with a 10000 M_w cutoff and freeze-dried.

Characterization of the Fractions. *UV Absorbance.* The UV absorption spectrum of 0.01% solutions of the fractions was determined from 190 to 600 nm using a Uvikon 860 UV spectrophotometer with 1 cm quartz cells.

Amino Acid Analysis. The amino acid analysis was undertaken by Alto Bioscience (University of Birmingham, U.K.) and involved HPLC−ion exchange of the acid hydrolyzed fractions using ninhydrin detection.

Molecular Mass Distribution. A linear Hema-Bio Column (Polymer Standards Service GmbH, Mainz, Germany) protected by a guard column was used. The eluent from the column was attached to a Dawn multiangle light scattering apparatus in conjunction with a Wyatt Technology Optilab DSP interferometric refractometer (Optochem Instruments Ltd., Nercwys, U.K.) and a Pharmacia UV-500 UV detector at wavelengths of 214 and 254 nm. A 100 μL injection loop was used in all experiments. Runs were carried out at room temperature with a flow rate of 0.5 mL/min. Solutions (0.1%) of the samples were filtered through 0.45 μm filters before injection onto the column. The mobile phase was 0.1 M NaCl and was filtered through a 0.22 μm filter and degassed before use. The data were analyzed using the Debye method, and a value of 0.131 was used for the refractive index increment (*19*). Analysis was undertaken using the refractive index profiles because the UV profiles do not accurately reflect the pectin concentration due to the presence of a range UV-absorbing species.

Emulsification Properties of Sugar Beet Pectin and Its Fractions. Pectin solutions were prepared for emulsification by dissolving sugar beet pectin or its fractions in water by gently heating at 50 °C with stirring to ensure complete dissolution. Ester gum was mixed with orange oil at a 1:1 ratio with gentle heating at 50 °C to ensure a homogeneous blend. Emulsions were prepared by mixing with an Ultra-Turrax mixer for 4 min. It had been previously established that this was the optimum time to produce the minimum droplet size. Droplet size was measured using the Malvern Mastersizer 2000 (Malvern, U.K.). A few drops of the emulsion were added to water in the dispersion unit on the instrument until the obscuration was ∼15%.

Measurements were carried out to determine the effect of pectin concentration on droplet size distribution and stability. Emulsions were prepared using 0.5−5% w/w whole sugar beet pectin and 20% orange oil without ester gum and also with 10% orange oil and 10% ester gum. Twenty gram solutions were mixed for 4 min. using the Ultra-Turrax mixer. Droplet size distribution was determined after storage for 24 h.

For the fractions, to conserve the sample, 10% emulsions were prepared by adding 0.5 g of orange oil/ester gum to 5 g of 1% pectin solution and mixing for 4 min. The emulsions were stored in a refrigerator, and the droplet size was measured over a period of weeks.

Preferential Adsorption of Specific Pectin Fractions during Emulsification. A 2% w/w pectin solution was prepared by gentle heating in water with stirring. A proportion of the sample was used to prepare a 20% orange oil emulsion by mixing using the Ultra-Turrax

Table 1. Percent Recovery of the Fractions

fraction	concn (M) of NaCl for elution	wt (g) recovered (72% total recovery)
1A	4	0.79 (26.0%)
1B	4	0.43 (14.3%)
1C	4	0.58 (19.3%)
2A	2	0.06 (2.0%)
2B	2	0.21 (7.0%)
3	water	0.10 (3.3%)

mixer for 4 min. The emulsion was then centrifuged at 2500 rpm for ~10 h until it had separated into two layers. The top oil layer was removed and discarded. The remaining aqueous layer was retained and a proportion of it used to produce a second emulsion. The procedure was repeated a third time. The droplet sizes for each of the emulsions were determined, and the experiment was performed in duplicate.

The amount of protein present in solution before and after each emulsification stage was determined using Coomassie blue solution (Bradford method). The reagent was made by dissolving 100 mg of Coomassie blue in 50 mL of 95% ethanol. The solution was then mixed with 100 mL of 85% phosphoric acid and made up to 1 L with distilled water.

A calibration curve was prepared by pipetting various volumes of 2% pectin into a test tube and making up to a total volume of 100 μL with distilled water. Distilled water (100 μL) was pipetted into an additional tube to provide the reagent blank. Sample (40 μL) to be tested was taken and made up to 100 μL total volume in a test tube with distilled water. Five milliliters of reagent was added to each tube and mixed well by inversion. The absorbance at 595 nm was measured against a reagent blank after 1 h.

The molecular mass distributions of the pectin solutions were also determined before and after emulsification by gel permeation chromatography (GPC). Samples were diluted using 0.1 M NaCl (1.5 g/6.5 g of NaCl) prior to measurement. The stock 2% pectin solution was subjected to 4 min of mixing using the Ultra-Turrax to ensure the samples had similar shear histories.

RESULTS AND DISCUSSION

Fractionation of Sugar Beet Pectin. The percent yield of the fractions obtained by hydrophobic affinity chromatography was determined, and the results are given in **Table 1**. The total recovery was ~72%, and it is noted that most of the material recovered (>80%) eluted from the column using 4 M NaCl.

Characterization of Sugar Beet Pectin Fractions. The UV absorbance spectra of the fractions are given in **Figure 1a** and show significant peaks at wavelengths of ~200, 250, 286, and 310 nm. The spectra for fractions 1A and 1B were superimposable, and hence only the 1B spectrum is shown. High-ester pectin derived from citrus peel was found to have only one peak at ~200 nm (data not shown). The peak at ~250 nm will be influenced by the presence of proteinaceous material; however, ferulic acid also has an absorbance peak at this wavelength and additional peaks at ~286 and 310 nm (**Figure 1b**). The intensity of the peaks at 286 and 310 nm for the fractions increases in the order 1A = 1B < 1C < 2B < 3, indicating that the ferulic acid content increases in this order. This is consistent with the order of elution from the column and, hence, increasing hydrophobic character. Assuming that the intensity of the absorbance peak at 310 nm can be used as a direct measure of ferulic acid content, it is calculated that the ferulic acid contents of the fractions are approximately as follows: [1A], 0.5%; [1B], 0.5%; [1C], 0.9%; [2B], 1.5%; and [3], 2%. These values are of the order of those reported in the literature for the whole sugar beet pectin (*7*).

The samples were analyzed for amino acid content, and the results are presented in **Table 2**. The protein contents increase in the order 1C < 1B < 2A < 1A < 2B < 3. The fact that this is not the order of elution from the column indicates that the

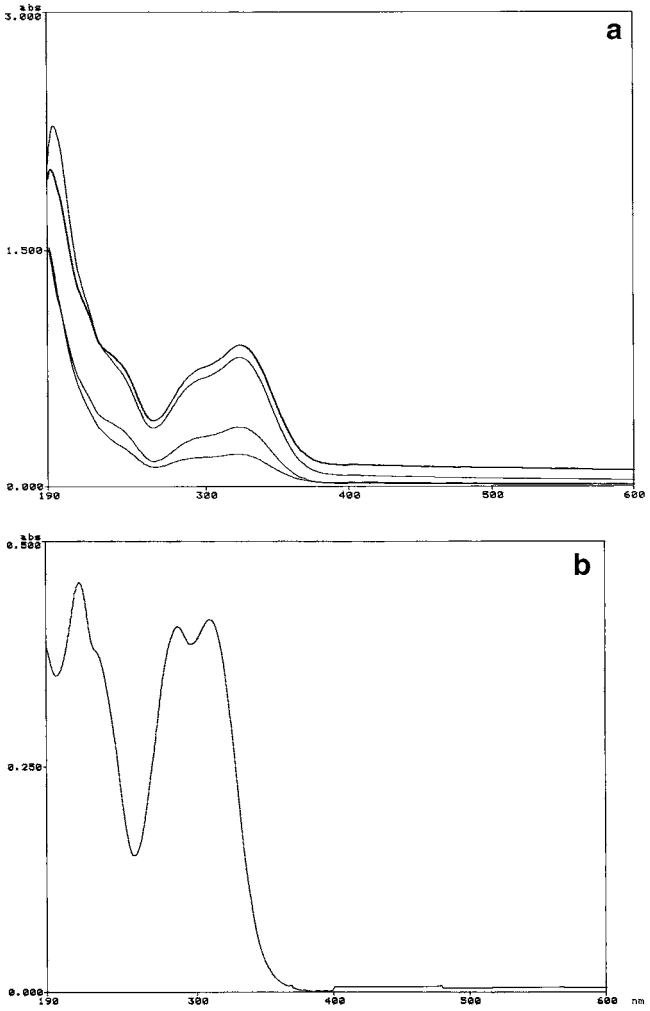

Figure 1. (**a**) UV absorption spectra for sugar beet pectin fractions (0.05%) (from bottom to top, 1B, 1C, 2B, 3); (**b**) UV absorption spectra for ferulic acid (0.005%).

Table 2. Amino Acid Analysis of Pectin Fractions (Milligrams per 100 g)

	fraction					
	1A	1B	1C	2A	2B	3
cysteic acid	0	0	0	0	0	0
hydroxyproline	440	140	70	170	680	1200
aspartic acid	200	70	60	110	260	320
threonine	180	80	50	90	390	420
serine	170	70	80	110	330	420
glutamic acid	270	100	110	160	370	380
proline	150	50	30	70	310	330
glycine	90	40	50	70	210	180
alanine	130	60	50	70	190	240
cystine	0	10	10	10	190	50
valine	190	60	40	80	290	380
methionine	40	20	10	10	90	60
isoleucine	70	20	20	30	70	100
leucine	140	40	30	40	120	230
tyrosine	180	40	20	80	330	480
phenylalanine	70	20	10	30	130	90
histidine	180	60	50	110	350	400
tryptophan	0	0	0	0	0	0
lysine	210	60	50	120	370	460
arginine	80	30	30	50	410	150
total	2790	970	770	1410	5090	5890

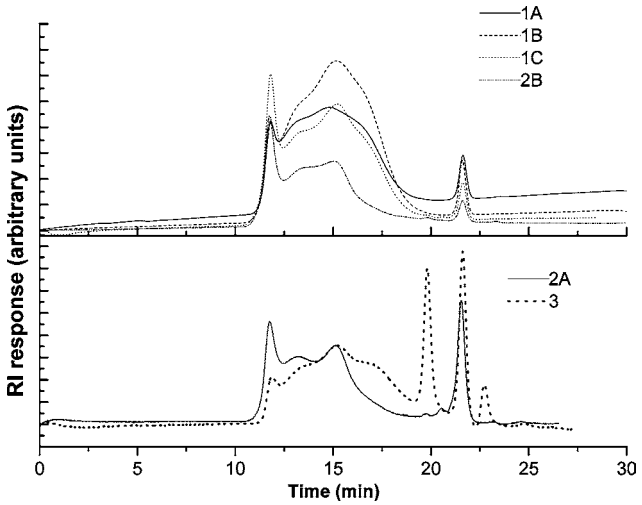

Figure 2. GPC elution profiles for sugar beet pectin fractions using RI detection.

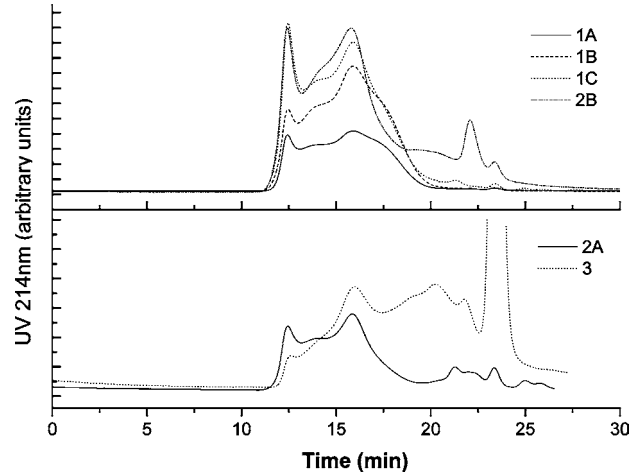

Figure 3. GPC elution profiles for sugar beet pectin fractions using UV absorbance (214 nm) detection.

presence of ferulic acid plays a major role in terms of the overall hydrophobic character of the fractions. The main amino acid in the fractions was hydroxyproline.

The molecular mass distributions of the fractions were determined by gel permeation chromatography. The RI and UV (214 nm) elution profiles are presented in **Figures 2** and **3**, respectively. All fractions were found to be polydisperse and had peaks at elution volumes of ~12.5 and 16.0 mL. Fractions 2A, 2B, and 3 also showed a significant proportion of low molecular mass material eluting at elution volumes of >20 mL. The higher relative intensities of these peaks as observed by UV absorbance compared to RI detection indicate that they are rich in ferulic acid and/or protein.

The weight-average molecular masses, M_w, of the fractions are given in **Table 3** and show significant variations.

Emulsification Properties of Sugar Beet Pectin. The droplet size of 20% orange oil and orange oil/ester gum emulsions prepared with various pectin concentration was measured after 72 h. The results are plotted in **Figure 4** and show that the minimum droplet size ($d_{0.5}$) occurs at pectin concentrations of ~2%.

A 20% orange oil emulsion was prepared using 2% pectin and the droplet size measured. The oil from this emulsion was separated off, and further emulsions were produced using the recovered pectin; again, the droplet size was measured. The

Table 3. Molecular Mass of Pectin Fractions

fraction	M_w	M_n	M_w/M_n
1A	153000	45000	3.4
1B	155000	27000	5.6
1C	306000	43800	7.0
2A	562000	135000	4.2
2B	470000	125000	3.8
3	282000	46000	6.1

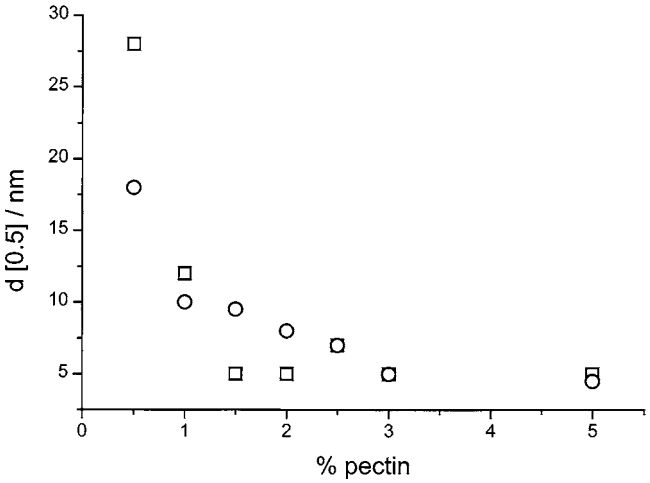

Figure 4. Droplet size ($d_{0.5}$)/nm for 20% orange oil (squares) and 20% orange oil/ester gum (circles) emulsions as a function of sugar beet pectin concentration.

Table 4. Droplet Size Distributions of 20% Emulsions Prepared by Reusing the Pectin

	droplet size (nm) fresh	% protein in solution before preparation of the emulsion
first emulsion		
expt 1	4.5, 4.5, 4.5	0.074
expt 2	5.0, 4.8, 4.8	
second emulsion		
expt 1	4.1, 3.9, 3.7	0.027
expt 2	3.9, 3.8, 5.8	
third emulsion		
expt 1	4.1, 4.0, 4.0	0.020
expt 2	3.6, 3.7, 3.7	
after making third emulsion		0.008

results are reported in **Table 4** and show that, surprisingly perhaps, the droplet size remains constant for all three emulsions.

The amount of protein present in solution before and after emulsification was determined using Coomassie blue solution, and the results are also given in **Table 4**. The results show that the percentage of protein left in solution decreases after each emulsification stage, implying that protein is involved in the emulsification process. The involvement of proteinaceous components has also been reported by Leroux et al. (*15*) for sugar beet pectin and by Ahktar et al. (*16*) for citrus pectin and is analogous to the behavior of the proteinaceous moieties within gum arabic (*18*).

The molecular mass distributions of the samples were determined after each emulsification stage, and the GPC chromatograms are presented in **Figures 5** (RI) and **6** (UV 214 nm).

The first point to note is that the intensity of the RI elution peaks (**Figure 5**) does not decrease significantly after emulsification, indicating that only a small percentage of the material

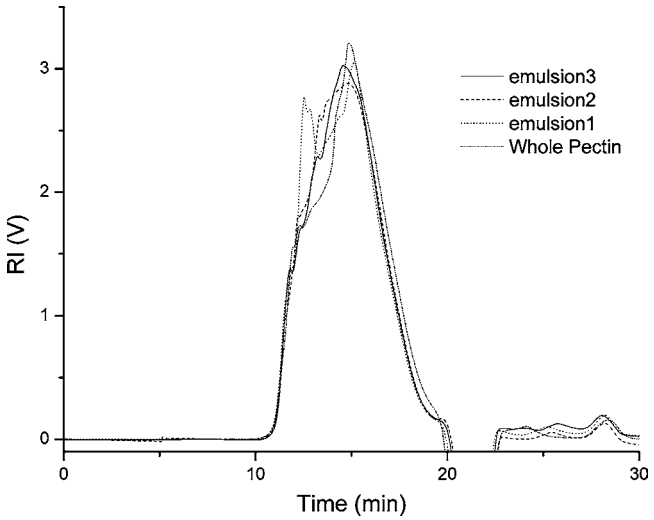

Figure 5. GPC elution profiles for sugar beet pectin before and after emulsification using RI detection.

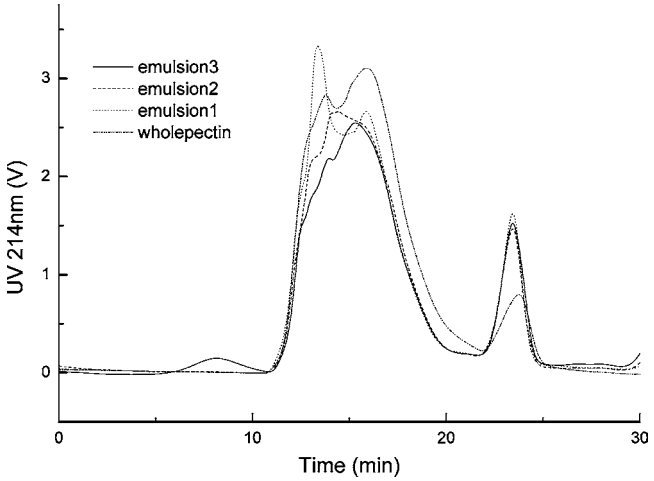

Figure 6. GPC elution profiles for sugar beet pectin before and after emulsification using UV absorbance (214 nm) detection.

actually adsorbs onto the oil droplets. It is interesting that for the first emulsification process the proportion that adsorbs is on the low molecular mass side of the elution peak. Comparison of the RI profiles with the UV 214 nm elution profiles reveals that the proportion that adsorbs must have a strong UV 214 nm absorption peak because the difference between the whole pectin sample and the sample after the first emulsification appears to be greater by UV 214 nm detection than by RI detection. Although it is not possible to conclude with any certainty, it is likely that the fraction which adsorbs initially corresponds to fraction 3. This fraction elutes in this region and has high ferulic acid and protein contents. Also, this fraction is the most hydrophobic because it is most strongly adsorbed onto the hydrophobic column (i.e., last to elute). The elution profiles obtained for the pectin remaining in the aqueous phase after the second and third emulsification processes indicate that high molecular mass pectin material adsorbs onto the oil droplets.

Emulsification Properties of Sugar Beet Pectin Fractions. Ten percent orange oil/ester gum emulsions were prepared using the sugar beet pectin fractions. Droplet size distributions were determined as a function of time, and the results are presented in **Figure 7**. The results show that fractions 1A, 1B, and 3 produced emulsions of similar and relatively small droplet size ($d_{4,3}$) and that the droplet size changed only slightly over a period of up to 4 or 8 weeks. However, fractions 1C, 2A, and

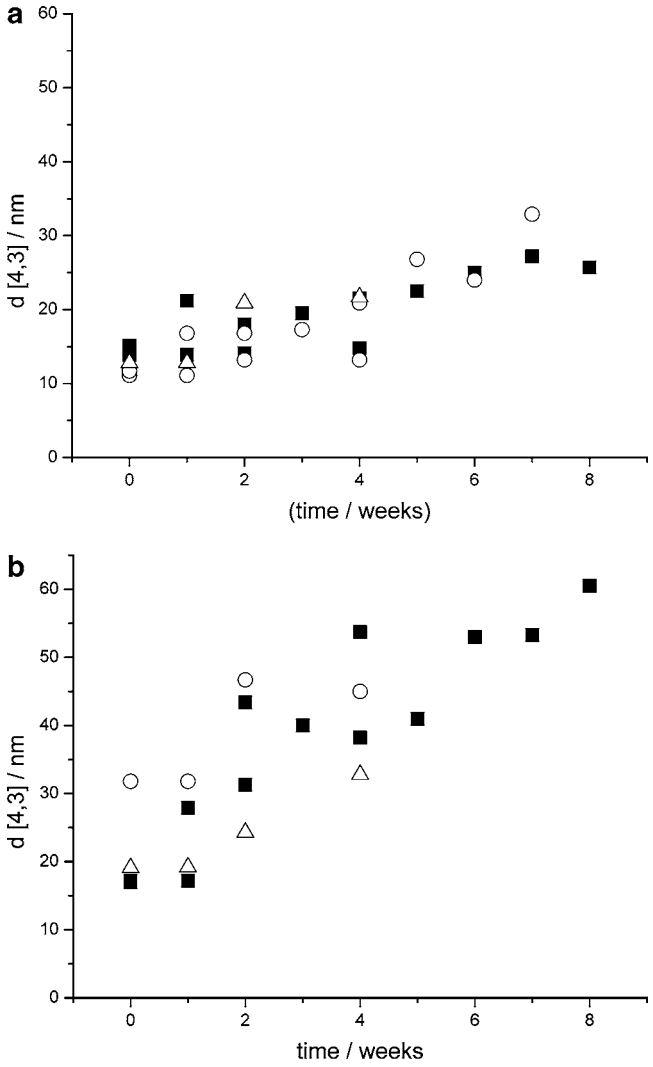

Figure 7. Droplet size ($d_{4,3}$)/nm for 10% orange oil/ester gum emulsions prepared using sugar beet fractions (**a**) 1A (squares), 1B (circles), and 3 (triangles) and (**b**) 1C (squares), 2A (circles), and 2B (triangles).

2B produced droplets with an initially larger droplet size, and the size increased over time. It is interesting to note that the latter three fractions (particularly 2A and 2B) have higher M_w values than the others (see **Table 3**), and hence the higher viscosity of the aqueous phase may be a factor. There does not appear to be any simple relationship between emulsion stability and protein content even though the adsorption experiments have demonstrated that proteinaceous material adsorbs. For example, it is noted that fraction 1C, which has the lowest protein content, produced emulsions with a larger droplet size, which were unstable over time. However, fraction 1B, which also has a comparatively low protein content and low ferulic acid content, produced emulsions with the smallest droplet size, and the size remained constant over time. It is apparent that other factors contribute to the emulsification properties. These might include the distribution of the protein and/or ferulic acid moieties within the fractions and their accessibility for the surface, the presence of other hydrophobic species such as ester groups, or the molecular mass of the surface active species. These conclusions are consistent with those of Leroux et al. (*15*), who also reported that molecular mass and protein and acetyl contents of sugar beet pectin influenced emulsifying ability. The results of Ahktar et al. (*16*) in studies on citrus pectin also support these findings because they found that proteinaceous material adsorbed at the oil−water interface and that pectins with M_w ~70 kDa were

most effective. Because citrus pectin does not contain ferulic acid, it can play at best only a partial role in the emulsification properties of sugar beet pectin.

It is clear that further work is required to elucidate the precise nature of the emulsifying moieties within sugar beet pectin. Of particular importance will be the refinement of the fractionation process to produce an increased number of fractions in greater amounts. The use of both hydrophobic affinity chromatography and gel permeation chromatography to produce more discrete fractions would be advantageous.

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