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Structural modifications of sugar beet pectin and the relationship of structure to functionality

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ABSTRACT

The emulsifying properties of sugar beet pectin (SBP) were investigated in relation to its molecular structure. SBP has been subjected to an enhancement process, and this material was here compared with conventional non-enhanced SBP. The oil-in-water emulsification properties of both were compared at 1.5% concentration at pH 3.25, using 15% middle-chain triglyceride as the oil phase. Their emulsification behavior after various enzyme treatments decreased in the order: protease > arabinanase/galactanase mixture > polygalacturonase. The enzyme treatment also decreased the molecular weight of SBP. Protease degraded the high molecular weight carbohydrate–protein complex. Arabinanase/galactanase mixture was more effective in decreasing the emulsification performance than polygalacturonase. The results confirm the key role of protein as the anchor for the oil droplets and identify also the contribution of the neutral lateral chains in stabilizing emulsions by forming a hydrated layer. Protein also aggregates, which functions as a linker for the association of the carbohydrate chains consequent to the enhancement process.

1. Introduction

Pectin is widely used in the food industry as a gelling agent, thickener, and stabilizer (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Commercial pectins are extracted from citrus peel and apple pomace in most instances, and pectin is also obtained from sugar beet pulp as a byproduct during the extraction of sugar. A common feature of pectin molecules is that the backbone consists of α-1, 4-linked D-galacturonic acid units interrupted by the insertion of 1, 2-linked L-rhamnose in adjacent or alternative positions, whereas the lateral chains consist mainly of D-galactose and L-arabinose as found in galactan, arabinogalactan, and arabinan with a considerable chain length, linked glycosidically to O4 and O3 positions of the rhamnose residue. Physicochemical differences between sugar beet pectin (SBP) and other conventional pectins include a higher proportion of neutral lateral chains (Williams et al., 2005), a higher content of acetyl group at O2 and O3 positions within the galacturonic backbone (Rombouts & Thibault, 1986), a higher content of phenolic esters in the lateral chains, particularly arabinose and galactose (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994; Guillon, Thibault, Rombouts, Voragen, & Pilnik, 1989; Ralet, Thibault, Faulds, & Williamson, 1994; Rombouts & Thibault, 1986), and a higher content of proteinaceous materials bound to the lateral chains through covalent linkages (Williams et al., 2005). A schematic figure of SBP is illustrated in Fig. 1. SBP does not form gels even in the presence of high concentration of soluble solids (e. g., sugar) at low pH (<3–4), or in the presence of calcium ions. The main application envisaged for SBP in the food industry is as an emulsifier rather than as a gelling or stabilizing agent.

Two essential functions determine the emulsification effectiveness of food hydrocolloids; providing good emulsifying activity and good emulsion stability. The emulsifying activity of SBP is attributed mostly to the proteinaceous moiety since the hydrocolloid itself is predominantly hydrophilic (Funami et al., 2007). Emulsion stability is generally supported by the carbohydrate fraction through viscosity effects, steric hindrance, and electrostatic interactions (Chanamai & McClements, 2001). In particular for SBP, the chain–chain interactions due to the protruding long chains emanating from the oil droplet surface also add to the stability. As an emulsifier, SBP is advantageous over other food hydrocolloids in that it requires smaller amount to activate the oil-in-water (O/W) interface (Nakauma et al., 2008). Its disadvantages lies, on the other hand, in that emulsion stability is lower than the other food hydrocolloids, particularly when the emulsions are sterilized thermally (Funami et al., 2008). Also, SBP usually shows low compatibility with limonene-based oils, and the emulsification

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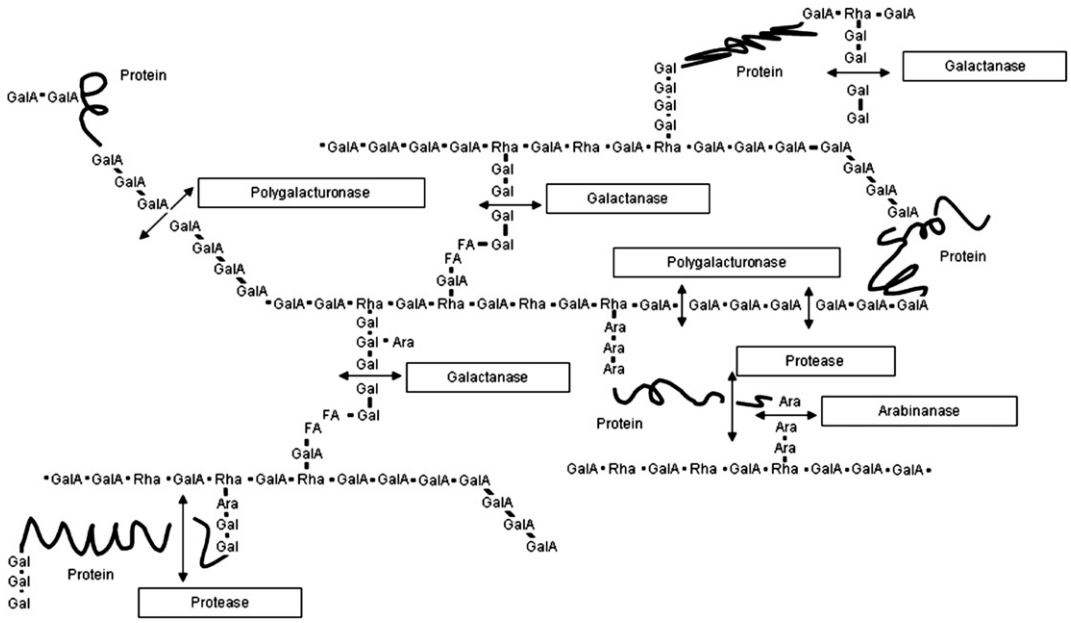

Fig. 1. Schematic figure of sugar beet pectin and enzyme "scissors" used in the experiments. GalA: Galacturonic acid; Rha: Rhamnose; Gal: Galactose; Ara: Arabinose; FA: Ferulic acid.

performance may depend on the production batches due to natural diversity (Funami et al., 2008). These attributes need to be improved for the widespread acceptance of SBP by the food industry. Previously we have described a non-chemical and food acceptable process for treatment of SBP to greatly improve its emulsifying properties (Funami et al., 2008).

In the present study, the emulsifying properties of SBP were investigated in relation to its molecular structure. Conventional non-enhanced SBP and SBP subjected to our process to give what we refer to here as "enhanced SBP" were structurally modified enzymatically using protease to degrade the proteinaceous moiety, polygalacturonase to cleave the carbohydrate backbone, and the mixture of arabinanase/galactanase to cleave the lateral chains. In this way, the contribution of each of these structural units to emulsification could be assessed for the discussion of the structure–function correlation of SBP as an emulsifier. Nuclear magnetic resonance (NMR) was also used to identify the molecular association which is introduced during the enhancement process.

2. Materials and methods

2.1. Materials

2.1.1. SBP

A commercial product (Vistop® D-2250, San-Ei Gen F.F.I., Inc., Osaka, Japan) was used as non-enhanced SBP after the purification by alcohol precipitation as described (Funami et al., 2007). Non-enhanced SBP was processed to obtain enhanced SBP according to the details given in patents (Funami, Kataoka, & Hiroe, 2006; Hayashi, 2002).

2.1.2. Enzymes

For the enzymatic treatments of SBP, pepsin A (E.C.3.4.23.1, Sigma–Aldrich Inc., St. Louis, MO) and a food grade of acid protease (Amano Enzyme Inc., Aichi, Japan) were used to decompose the proteinaceous materials. Endo-polygalacturonase (E.C.3.2.1.15, Megazyme Ltd., Wicklow, Ireland) was used to cleave the polygalacturonan backbone, and endo-arabinanase (E.C.3.2.1.99, Megazyme Ltd.) and endo-1, 4-β-galactanase (E.C.3.2.1.89, Megazyme Ltd.) were used to cleave the lateral chains of arabinan and galactan (Fig. 1). For each enzyme, the optimal pH was 3–4, and the optimal temperature was 40 °C.

For the analysis of constitutional sugars and the estimation of the thickness of hydrated layer, Macerase (Calbiochem Co., Ltd., Darmstadt, Germany), a mixture of hemicellulase (E.C.3.2.1.), cellulase (E.C.3.2.1.4), and pectinase (E.C.3.2.1.15), was used.

2.1.3. Others

For chemical analysis of SBP, the reagent grades of ferulic acid, neutral sugars, including rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), and xylose (Xyl), and galacturonic acid (GalA) were used without further purifications (Wako Pure Chemical Ind., Ltd., Osaka, Japan). For preparing the emulsions, medium-chain triglyceride (MCT) (Nisshin Oillio Group, Ltd., Tokyo, Japan) synthesized from triglyceride and a mixture of C8 fatty acid and C10 fatty acid at a mixing ratio of 75:25 was used as the oil phase. The density of MCT was 0.95 g/mL.

2.2. Enzymatic treatments of SBP

2.2.1. Protein decomposition

SBP (with or without enhancement) was dispersed at 1.0 w/v% in 100 mM citrate buffer (pH 3.0) with mechanical shearing at 2000 rpm for 15 min. Into these dispersions, pepsin A (700 unit per 1.0 g SBP) and the food grade of acid protease (70 unit per 1.0 g SBP) were added and incubated at 40 °C for 14 h with gentle stirring.

2.2.2. Backbone cleavage

SBP (with or without enhancement) was dispersed at 1.0 w/v% in 100 mM citrate buffer (pH 3.5) with mechanical shearing at 2000 rpm for 15 min. Into these dispersions, endo-polygalacturonase (10 unit per 1.0 g SBP) was added and incubated at 40 °C for 20 h with gentle stirring.

2.2.3. Lateral chains cleavage

SBP (with or without enhancement) was dispersed at 1.0 w/v% in 100 mM citrate buffer (pH 3.5) with mechanical shearing at 2000 rpm for 15 min. Into these dispersions, endo-arabinanase

(7 unit per 1.0 g SBP) and endo-1, 4-β-galactanase (5 unit per 1.0 g SBP) were added and incubated at 40 °C for 24 h with gentle stirring.

2.2.4. Dialysis

The hydrolysate mixture was heated at 90 °C for 5 min to stop the enzymatic reaction and dialyzed against distilled de-ionized water at 20 °C through a dialysis membrane with a 10 kg/mol molecular weight cut off, followed by lyophilization. The lyophilized samples were stored in a desiccator at 20 °C until use.

In the present study, "control" means a sample without any enzymatic treatment.

2.3. Characterization of SBP

2.3.1. Constitutional sugars

SBP (with or without enhancement) was dispersed at 0.5 w/v% in distilled de-ionized water (10 mL) with mechanical shearing at 2000 rpm for 15 min. Into these dispersions, Macerase (1200 unit per 1.0 g SBP) was added and incubated at 40 °C for 24 h with gentle stirring. One mL of 3 M H_2SO_4 was then added to the mixture and heated at 98 °C for 2 h. The mixture was neutralized with 1 mL of 6 M NaOH and diluted to a definite concentration (100 ppm) with distilled de-ionized water. After filtering through PTFE membrane filters of 0.2 μm pore size, sample solutions (25 μL) were injected to the high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) system as described (Funami et al., 2007).

2.3.2. Protein (Bradford method)

Bradford protein assay kit Pierce (Thermo Fisher Scientific Inc., Rockford, IL, USA) was used to determine the total protein content in SBP (with or without enhancement) using absorbance at 595 nm, which is the color formed as a result of the reaction between protein and Coomassie Brilliant Blue G-250. Bovine serum albumin was used as a standard for calibration within a working range between 0 and 750 μg/mL. This assay was to confirm the action of the enzymes because the assay is generally insensitive to low molecular weight proteins and polypeptides and also can be influenced by bound materials with protein.

2.3.3. Ferulic acid

Ten mg of SBP (with or without enhancement) was decomposed in 0.5 M NaOH (5 mL) under N_2 gas at 20 °C for 24 h and acidified with 6 M HCl (0.75 mL). Ferulic acid was recovered from the mixture in four steps using ethyl acetate as a solvent (4 mL for each step), and the residue was reconstituted in distilled de-ionized water (10 mL) after the evaporation of the solvent. Ferulic acid content was determined from the absorbance of test solutions measured at 310 nm at 20 °C using a V-560 spectrophotometer (JASCO International Co., Ltd., Tokyo, Japan) after suitable dilution.

2.3.4. Molecular weight distribution

SBP (with or without enhancement) was dispersed at 0.05 w/v% in 50 mM $NaNO_3$ and 0.01 w/v% NaN_3 and passed through a high-pressure homogenizer Nanomizer (Yoshida Kikai Kogyo, Aichi, Japan) at 50 MPa. SBP solutions (100 μL) were injected to the size-exclusion chromatography coupled with multiangle laser light-scattering (SEC–MALS) system after filtering through PTFE membrane filters of 0.45 μm pore size as described (Funami et al., 2007). The MALS measurements were carried out at 25 °C using a DAWN-EOS (Wyatt Technology Co., CA, USA) with linearly polarized light of $\lambda = 690$ nm (semiconductor laser) to determine the weight-average molecular weight M_w, the z-average root-mean-square radius of gyration R_g, and the polydispersity index. The increase in refractive index with concentration (dn/dc in mL/g) was 0.135 mL/g (Williams et al., 2005).

2.4. Emulsion tests

2.4.1. Preparation of O/W emulsions

One point 5 g of SBP (with or without enhancement) was dispersed in distilled de-ionized water (80 g) using a Polytron type homogenizer at 24 000 rpm for 1 min, into which 10% benzoic acid (1 mL) and the same amount of 10% citric acid were added, and mixed using the homogenizer at 8000–10000 rpm for 1 min. MCT (15 g) and distilled de-ionized water were added to give 100 g, then mixed again with the homogenizer at 24 000 rpm for 1 min to prepare crude emulsions. The crude emulsions were applied with two passes through the high-pressure homogenizer at 50 MPa. The final pH of the emulsions was 3.25.

2.4.2. Oil droplet size and interfacial parameters

The droplet size distribution of the O/W emulsions was determined by a computer-controlled laser diffraction apparatus SALD-2100J (Shimadzu Co., Kyoto, Japan) and was analyzed using the software Wing SALD-2100 ver. 1.10. After suitable dilution using distilled de-ionized water, the surface-volume mean diameter $d_{3,2}$ of the oil droplets was determined immediately after preparation and after storage at 60 °C for 3 days. The ratio of the diameter over 3 μm was also determined as an indicator for the distribution of oil droplet size. The specific surface area S_v of the whole emulsion was calculated (Puppo et al., 2005):

$$S_v = 6\phi/d_{3,2}\,(\mathrm{m^2/mL\ emulsion})$$

Table 1
Physicochemical properties of sugar beet pectin (SBP).

	Constitutional sugars (%)						Protein (%)	Ferulic acid (%)
	Rha	Ara	Gal	Glu	Xyl	GalA		
Non-enhanced SBP								
Control (W/O any enzymatic treatment)	6.02	7.36	11.13	0.48	1.11	45.27	5.38	1.12
+Protease	9.13	10.50	13.24	2.36	1.19	47.43	0.56	1.07
+Polygalacturonase	17.92	20.98	26.04	3.66	3.23	20.18	3.24	0.88
+Arabinanase/Galactanase	14.58	2.40	1.86	3.13	1.18	73.46	2.56	0.40
Enhanced SBP								
Control (W/O any enzymatic treatment)	7.42	7.96	12.08	1.86	1.25	46.58	5.63	1.19
+Protease	9.75	10.34	13.16	2.58	1.29	49.43	0.42	1.13
+Polygalacturonase	15.72	19.05	19.06	1.89	2.74	23.68	3.86	0.92
+Arabinanase/Galactanase	13.70	4.39	5.72	1.37	1.08	71.05	2.08	0.49

Each datum is on a dry base.

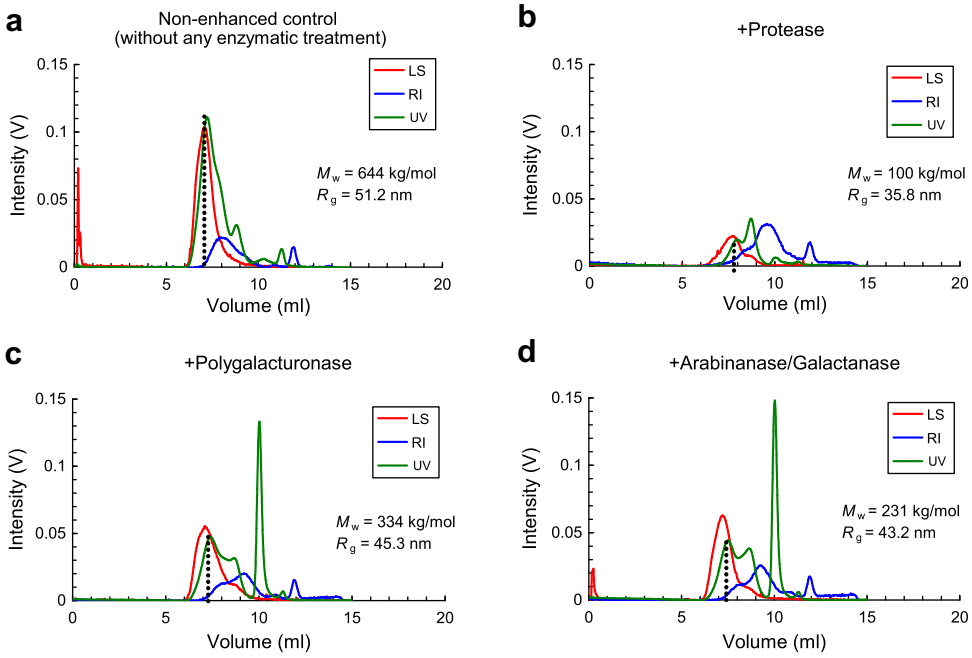

Fig. 2. SEC–MALS profiles of non-enhanced sugar beet pectin.

where ϕ represents the volume fraction of the dispersed phase determined by density measurement using a DMA 48 density meter (Anton Paar, Tokyo, Japan):

$$\phi = (\rho_{aq} - \rho_{em})/(\rho_{aq} - \rho_{oil})$$

where ρ_{aq}, ρ_{em}, and ρ_{oil} represent the density of aqueous phase, emulsion, and oil, respectively (Buffo, Reineccius, & Oehlert, 2001).

2.4.3. Creaming stability

Creaming stability of the O/W emulsions was measured by the backscattering of light from the emulsions as a function of height using a Turbiscan LA*b* Expert (Eko Instruments Co., Ltd., Tokyo, Japan). Backscattered light fluxes were detected at 880 nm during incubation at 60 °C for up to 120 min, and changes in the scattering intensity were determined:

$$\Delta\text{Backscattering}(\%) = \text{Data at the initial}(\%) - \text{Data after each incubation time}(\%)$$

2.4.4. Adsorption of SBP onto the surface of the oil droplets

The O/W emulsions (10 g) were mixed with 15 g of solution A (35% sugar and 5% NaCl in distilled de-ionized water) using a Bortex type mixer for 1 min. The mixture was centrifuged at 24 000 *g* for

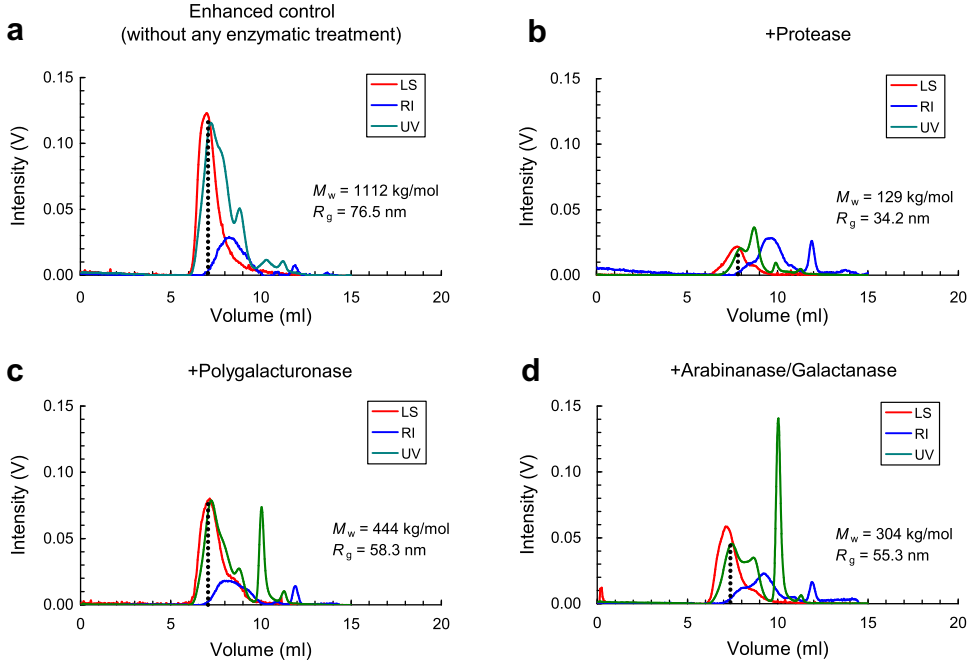

Fig. 3. SEC–MALS profiles of enhanced sugar beet pectin.

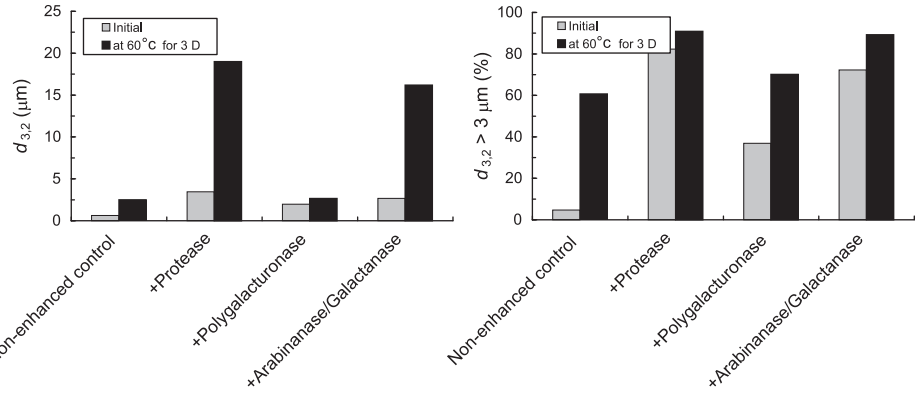

Fig. 4. The volume-surface mean diameter $d_{3,2}$ of oil droplets in oil-in-water emulsions stabilized by non-enhanced sugar beet pectin.

30 min at 10 °C to recover the lower aqueous phase. The residue was centrifuged again at the same condition after mixing with the solution A (total weight 25 g) to recover the lower aqueous phase, which was repeated 3 times. The residue was mixed with solution B (0.1% SDS and 5% NaCl in distilled de-ionized water) (total weight 25 g) using the Polytron type homogenizer at 15 000 rpm for 1 min. Here, the surfactant SDS functions to displace the proteinaceous materials from the O/W interface, transferring to the aqueous phase. The mixtures were centrifuged at 24 000 *g* for 30 min at 10 °C to recover the lower aqueous phase. The residue was centrifuged again at the same condition after mixing with the solution B (total weight 25 g) to recover the lower aqueous phase, which was repeated 3 times. The recovered sample gives the fraction adsorbed on to the oil droplet surface, and the concentration was determined by the RI signal on the SEC profile as shown in 2.3.4. Interfacial concentration Γ of sugar beet pectin at the surface of oil droplets was calculated:

Γ (mg/m^2) = adsorbed pectin concentration C (mg/mL emulsion)/S_v (m^2/mL emulsion) (Puppo et al., 2005).

2.4.5. Dynamic light scattering for the thickness of the hydrated layer

The O/W emulsions immediately after preparation were diluted 4000 times with distilled de-ionized water and filtered through the PTFE filters of 0.2 μm pore size. Enzyme mixture of Macerase (100 μL) was added to the diluted emulsions (2 mL) at 0.2%. Hydrodynamic radius R_h of the oil droplets was determined using a QELS (Wyatt Technology Co.) every 5 min after the sample apply. From the change in the R_h, thickness of the carbohydrate layer d_H around the surface of the oil droplets was estimated (Funami et al., 2008):

$$d_H = \{R_{h(0)} - R_{h(min)}\}/2$$

where $R_{h(0)}$ and $R_{h(min)}$ represent R_h prior to the enzymatic treatment and at the minimum, respectively.

2.4.6. Nuclear magnetic resonance (NMR)

^{1}H and ^{13}C NMR spectra were measured at various temperatures in D_2O on a JMN-ECA 600 spectrometer (JEOL Ltd., Tokyo, Japan) operating at 600.17 MHz and 150.91 MHz, respectively. No-enhanced SBP and enhanced SBP with or without the protease treatment were subjected to the tests after de-esterification in an alkaline condition to exclude the influence of acetyl and methyl groups bound to the galacturonate backbone. For de-esterification, each SBP sample (200 mg) was dispersed in distilled de-ionized water (20 mL), to which 6 M NaOH (4 mL) was added. The mixture was incubated at 20 °C for 2 h with gentle stirring and neutralized with 6 M HCl (4 mL). It was dialyzed against distilled de-ionized water at 20 °C through the dialysis membrane with a 10 kg/mol molecular weight cut off, followed by lyophilization. Lyophilized sample was treated repeatedly with D_2O to exchange the labile protons with deuterons prior to the spectrometry. Chemical shifts were recorded in ppm (δ) and reference to sodium trimethylsilyl-2, 2, 3, 3,-d_4-propionate (TSP).

3. Results

3.1. Characterizations of SBP

The content of galacturonic acid was less than 65% (which is the limiting of the JECFA specification) even for non-enhanced control SBP (Table 1) although the ash content was not taken into account.

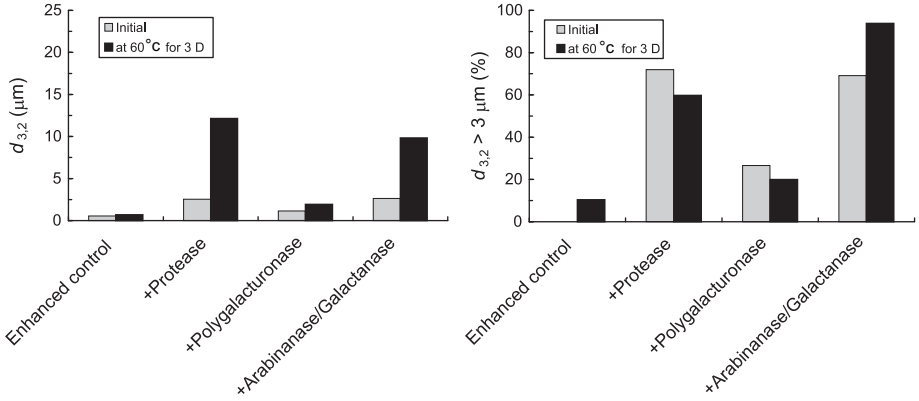

Fig. 5. The volume-surface mean diameter $d_{3,2}$ of oil droplets in oil-in-water emulsions stabilized by enhanced sugar beet pectin.

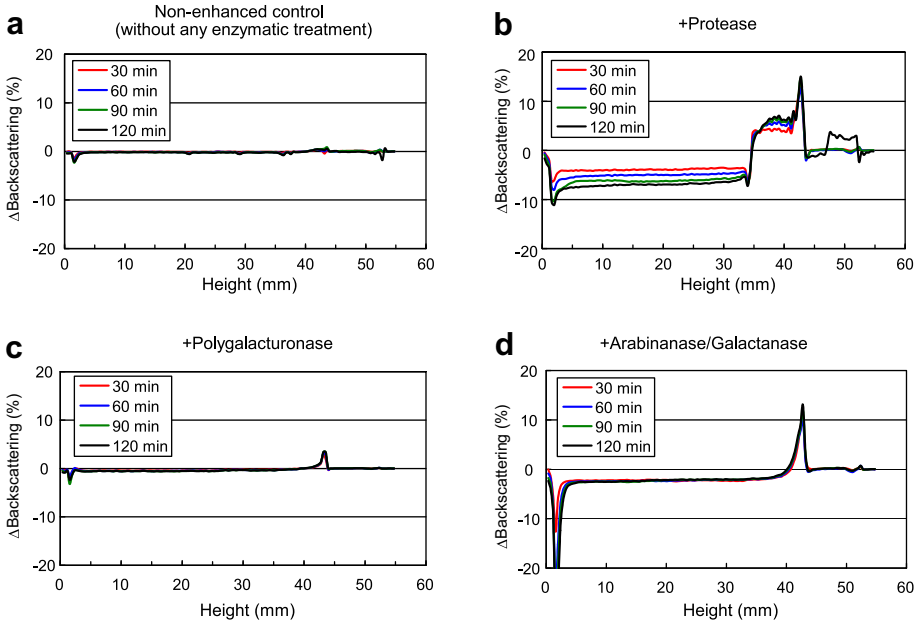

Fig. 6. Creaming stability of oil-in-water emulsions stabilized by non-enhanced sugar beet pectin.

Each enzymatic treatment decomposed effectively the target component. Protease decreased the protein content to ca. 10% or less of the control value for each type of SBP. Polygalacturonase decreased the galacturonic acid content to ca. 45–50% of the control value for each type of SBP. This enzymatic treatment also decreased the protein and the ferulic acid contents to ca. 60% and 80% of the control value, respectively for non-enhanced SBP, whereas to ca. 70% and 80% of the control value, respectively for enhanced SBP. Arabinanase/galactanase mixture decreased the arabinose and the galactose contents to ca. 30% and 15% of the control value, respectively for non-enhanced SBP, whereas to ca. 55% and 45% of the control value, respectively for enhanced SBP. This enzymatic treatment also decreased the protein and the ferulic acid contents to ca. 50% and 35% of the control value, respectively for non-enhanced SBP, whereas ca. 35% and 40% of the control value, respectively for enhanced SBP. The decreases in the protein and the ferulic acid contents were greater for arabinanase/galactanase than that for polygalacturonase for each type of SBP. Also, each enzymatic treatment decreased the M_w in comparison with the control for each type of SBP (Figs. 2 and 3). The decrease in the M_w was greater in the order: protease > arabinanase/galactanase > polygalacturonase, and the effect of each enzyme was more apparent for enhanced SBP due to increased control value. The decrease in the intensity of the protein signal detected in the high molecular weight fraction (see broken lines in Figs. 2 and 3) was greater also in the same order for each type of SBP.

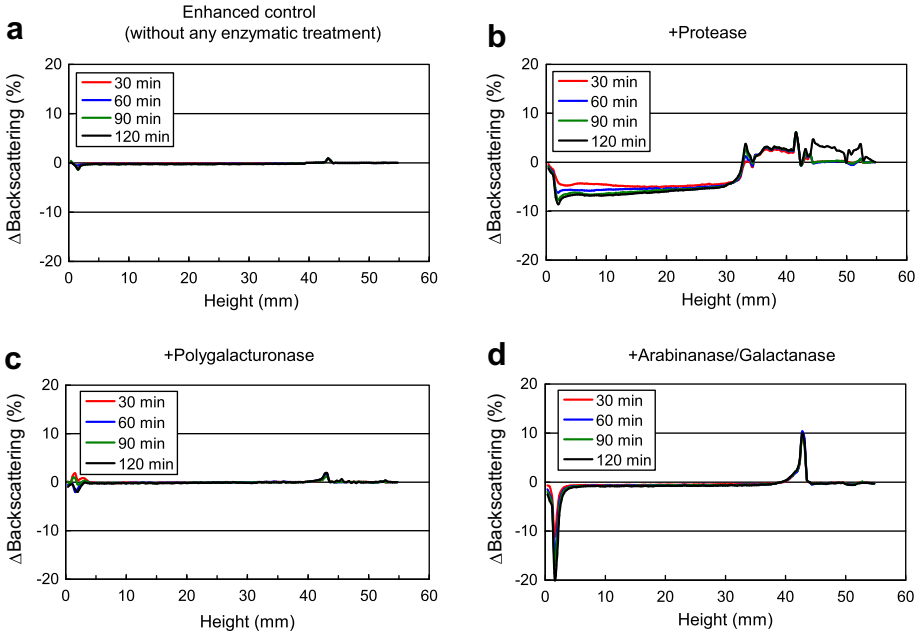

Fig. 7. Creaming stability of oil-in-water emulsions stabilized by enhanced sugar beet pectin.

3.2. Oil droplet size and creaming stability of the O/W emulsions stabilized by SBP

Each enzymatic treatment increased the $d_{3,2}$ either at the initial or after storage at 60 °C for 3 days in comparison with the control for each type of SBP (Figs. 4 and 5). The increase in the $d_{3,2}$ was generally greater in the order: protease > arabinanase/galactanase > polygalacturonase either at the initial or after storage. The increase in the $d_{3,2}$ was more apparent after storage than at the initial, particularly for protease and arabinanase/galactanase. The $d_{3,2}$ for enhanced SBP was smaller than that for non-enhanced SBP either at the initial or after storage, particularly after storage. Also, each enzymatic treatment increased the ratio of $d_{3,2} > 3$ μm either at the initial or after storage at 60 °C for 3 days in comparison with the control for each type of SBP (Figs. 4 and 5). The increase in the ratio was greater in the order: protease > arabinanase/galactanase > polygalacturonase either at the initial or after storage for non-enhanced SBP. For enhanced SBP, the increase in the ratio was greater in the same order at the initial, but arabinanase/galactanase was more effective than protease in increasing the ratio after storage. The ratio for enhanced SBP was smaller than that for non-enhanced SBP either at the initial or after storage, particularly after storage, but this was not necessarily the case for arabinanase/galactanase.

Each enzymatic treatment, except polygalacturonase, decreased notably the creaming stability of the emulsions in comparison with the control for each type of SBP (Figs. 6 and 7). Protease was more effective in decreasing the creaming stability than arabinanase/galactanase. The creaming stability for enhanced SBP was less sensitive to each enzyme than that for non-enhanced SBP.

3.3. Interfacial behavior of SBP

Each enzymatic treatment decreased the interfacial concentration Γ of SBP that had adsorbed on to the oil droplet surface for each type of SBP except non-enhanced SBP treated with polygalacturonase (Table 2). The effect of enzyme on the decrease in the Γ was greater in the order: protease > arabinanase/galactanase > polygalacturonase. The thickness of a hydrated layer d_H that SBP had formed around the oil droplet surface was estimated to be 54.9 nm for non-enhanced control SBP and 74.1 nm for enhanced control SBP (Table 3). Polygalacturonase decreased the d_H to 47.5 nm for non-enhanced SBP and 57.5 nm for enhanced SBP. The d_H for other SBP samples was not determined because the oil droplet diameter was too large to accumulate sufficient data for calculating within a short period due to slow diffusion.

Table 2
Adsorption behavior of sugar beet pectin (SBP) at the oil/water interface.

	Specific surface area S_v (m^2/mL emulsion)	Interfacial concentration Γ (mg/m^2)
Non-enhanced SBP		
Control (W/O any enzymatic treatment)	1.060	1.285
+Protease	0.253	0.610
+Polygalacturonase	0.482	1.378
+Arabinanase/Galactanase	0.328	0.979
Enhanced SBP		
Control (W/O any enzymatic treatment)	1.532	1.571
+Protease	0.307	0.695
+Polygalacturonase	0.736	1.490
+Arabinanase/Galactanase	0.357	1.005

Table 3
Estimated thickness of a hydrated layer for sugar beet pectin (SBP) in relation to the mean diameter of oil droplets.

	Thickness of a hydrated layer d_H (nm)	$1/d_{3,2}$ at the initial (nm^{-1})	$1/d_{3,2}$ after storage at 60 °C for 3 days (nm^{-1})
Non-enhanced SBP			
Control (W/O any enzymatic treatment)	54.9	1.59	0.40
+Polygalacturonase	47.5	1.80	1.47
Enhanced SBP			
Control (W/O any enzymatic treatment)	74.1	0.51	0.38
+Polygalacturonase	57.5	0.87	0.52

$d_{3,2}$: the surface-volume mean diameter of oil droplets.

3.4. NMR

The ^{1}H NMR spectrum elucidated that all peaks became sharper with elevating temperature up to 80 °C, but significant changes in the chemical shift or broadening were not observed for any of the peaks associated with non-enhanced control SBP or enhanced control SBP (data not shown). The ^{13}C NMR spectrum recorded at 25 °C was essentially the same between non-enhanced control SBP and enhanced control SBP prior to the protease treatment (Fig. 10). The spectrum showed the introduction of two new peaks at 95.1 and 99.1 ppm after the protease treatment for each type of SBP. In this case, the intensity of these new peaks for enhanced SBP was larger than that for non-enhanced SBP.

4. Discussion

4.1. Characterizations of SBP

The content of galacturonic acid observed could be an underestimation, probably due to the instability of this uronic acid during the analysis. The changes in the contents of arabinose and galactose for enhanced SBP are less with arabinanase and galactanase, respectively than for non-enhanced SBP. This indicates the enhancement process provides the neutral lateral chains with greater stability against the enzymatic decomposition. Protein appears to be bound preferentially to the lateral chains rather than to the backbone, and this is also the case of ferulic acid. The decrease in the M_w relates predominantly to the loss of protein, particularly protein bound to the lateral chains. The results suggest that protein linkages or aggregations occur during the enhancement process, making the molecular structure of SBP bulky, particularly through the molecular association among the lateral chains.

4.2. Emulsifying properties of SBP

The initial $d_{3,2}$ and its distribution provide a measure of the emulsifying activity of SBP, whereas the $d_{3,2}$ after storage and its distribution indicate its emulsion stability. Creaming behavior also refers directly to emulsion stability. The results indicate that the most dominant factor controlling the emulsification effectiveness of SBP is the proteinaceous moiety. However, the lateral chains are also important in accounting for the emulsifying properties, particularly emulsion stability. Emulsification is initiated by binding of protein as an anchor on to the oil droplets due to its hydrophobicity relative to the carbohydrate chains, followed by the stabilization through steric effects of the carbohydrate chains, particularly the lateral chains. Arabinanase/galactanase treatment may involve both the decomposition of the lateral chains as a direct

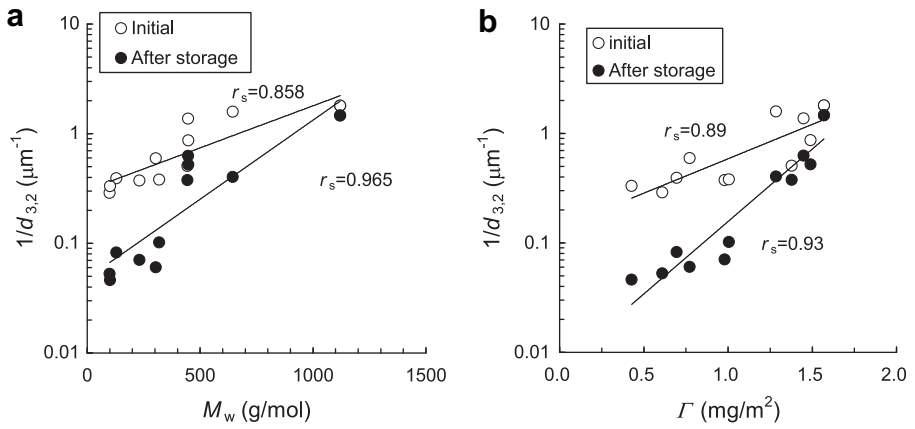

Fig. 8. Particle diameter of oil droplets in relation to the molecular weight (a) and to the interfacial concentration of adsorbed fraction (b) of sugar beet pectin.

effect and the related reduction of protein bound to the lateral chains as an indirect effect as mentioned, and this is why arabinanase/galactanase has a greater impact than polygalacturonase in decreasing the emulsifying performance of SBP. The enhancement process improves the emulsifying performance of SBP, particularly its emulsion stability. This is probably due to enlarged steric effects as a result of the molecular association among the lateral chains, for which protein enables these to become associated. Thus, the decrease in emulsion stability accords well with the decrease in the M_w as already noted. The enhanced SBP is still sensitive to both protease and arabinanase/galactanase degradations, indicating that protein is not embedded completely within the molecules and also that the molecular association do not involve a high degree of covalent linkages.

4.3. Interfacial behavior of SBP

As possible factors influencing the emulsifying properties of SBP, the M_w and the Γ were examined (Fig. 8). The vertical axis represents the reciprocal of the $d_{3,2}$ of oil droplets at the initial and after storage at 60 °C for 3 days. The smaller values are an indication of less effective emulsification. Each parameter show a good relationship with the $d_{3,2}$, particularly after storage, as represented by higher correlation coefficients ($p < 0.05$). These results indicate that either M_w or Γ can be an indicator of the emulsifying effectiveness of SBP, particularly its emulsion stability. Among SBP components, it is the hydrophobic constituents that can work as an effective anchor for the oil droplet surface rather than the hydrophilic carbohydrate moiety. In fact, there is an apparent tendency that the Γ increases with increasing concentration of protein, particularly protein in the high molecular weight fraction (Fig. 9). In contrast, ferulic acid, another hydrophobic constituent, shows a relatively poor correlation with the Γ. These results emphasize the dominant role of protein as a hydrophobic anchor in comparison with ferulic acid.

SBP that adsorbs on to the oil droplet surface can form a hydrated layer with a thickness depending on its molecular size, and so preventing the coalescence among oil droplets. When treated with hemicellulase, the R_h of the oil droplets decreases as a result of the cleavage of carbohydrate chains. When the hydrated layer disappears completely, the R_h inversely grows as a result of the coalescence of the oil droplets. The difference between the initial and the minimum values of the R_h provides an estimation of the thickness of the hydrated layer. The thickness of the hydrated layer increases upon the enhancement process. In relation to the emulsifying properties, the thickness of the hydrated layer can more closely be related to emulsion stability rather than the direct emulsifying activity of SBP although the data is not unequivocal.

4.4. Structural changes during the enhancement process

There are no direct indications on the ^{1}H NMR spectrum that the binding site of the carbohydrate moieties is affected by the enhancement. No deviations in the ^{13}C NMR spectrum indicate essentially the same structure between non-enhanced control SBP and enhanced control SBP prior to the protease treatment. The two new peaks on the ^{13}C NMR spectrum created by the protease treatment can be assigned to the reducing terminals of D-galacturonate moieties for the α anomer (at 95.1 ppm) and for the β anomer (at 99.1 ppm). This indicates that protease cleaves the glycosidic linkages between proteins and galacturonate moieties at the C1 position at the terminal end to set the anomeric carbons free as a result of protein release. The larger intensity of these peaks for the enhanced SBP indicates that the enhancement process promotes the formation of such a protein-associated carbohydrate fraction.

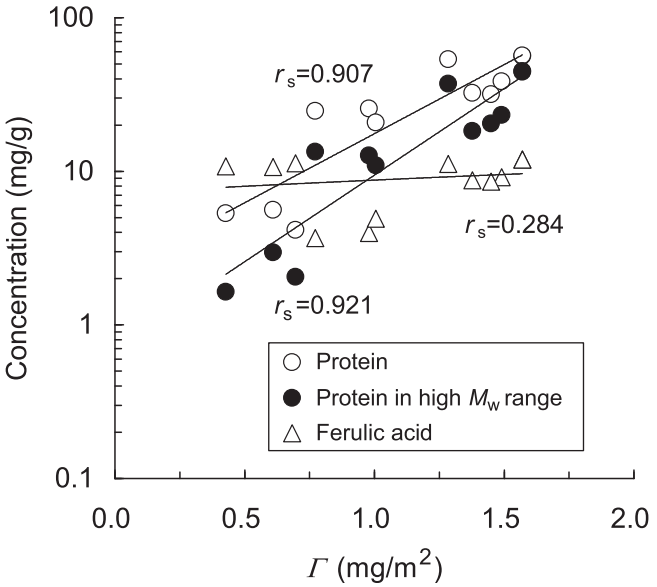

Fig. 9. The amount of adsorbed fraction in relation to some chemical components of sugar beet pectin.

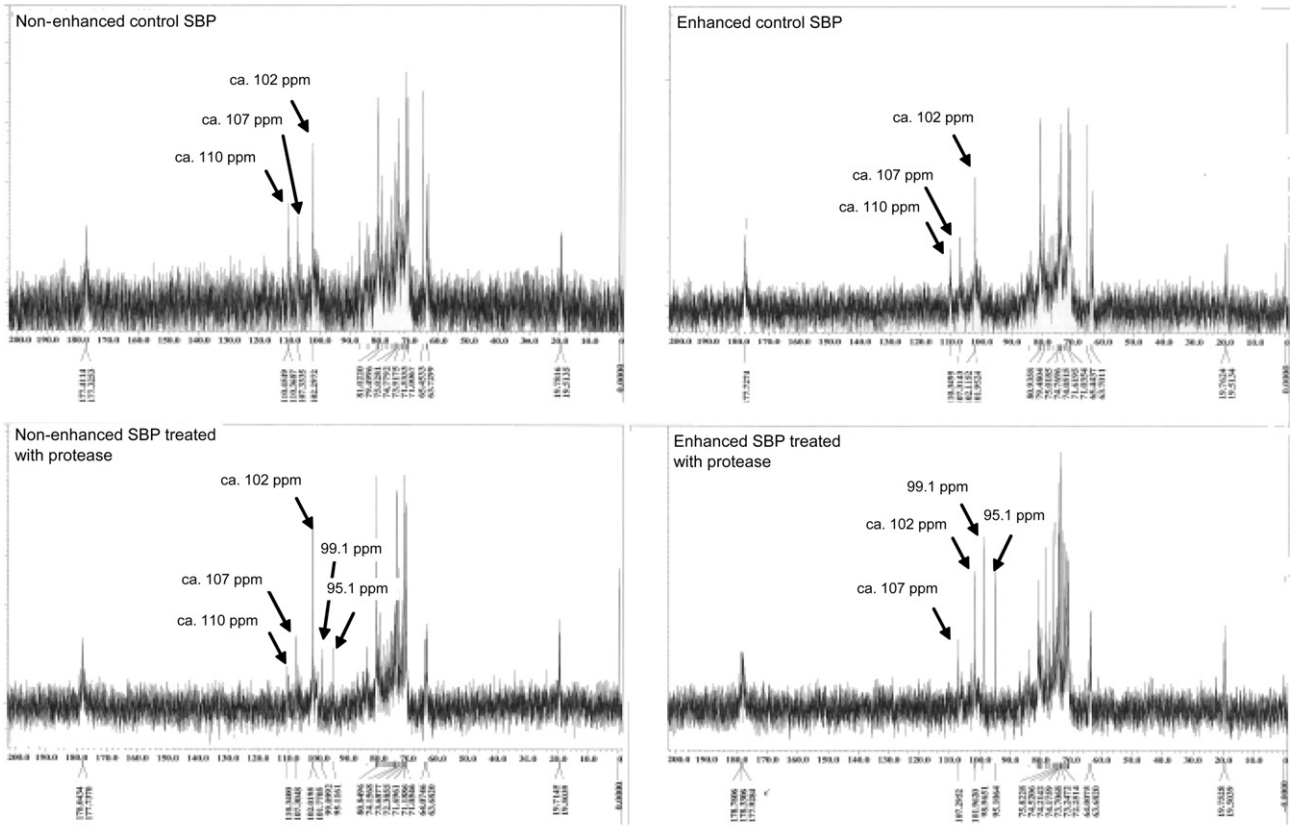

Fig. 10. ^{13}C NMR spectra of sugar beet pectin recorded at 25 °C.

5. Conclusion

Enhancement of SBP using a food-friendly process involves the association of the carbohydrate chains via protein as a linker. This provides food manufactures with a novel SBP emulsifier with improved functionalities, contributing to the progress in the emulsion area.

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