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Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts

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Abstract

The extraction of pectins from sugar beet pulp was carried out in an aqueous acid medium under different conditions using a full two-state experimental design for three extraction parameters (pH, temperature and time). The yields of the extracted pectins ranged from 4.1% to 16.2%. Their contents in pectin constituents were 35.2–76.3% galacturonic acid, 6.8–32.9% neutral sugars, 2.0–4.2% methoxy groups, 0.8–3.8% acetyl groups, and 0.1–0.7% ferulic acid. Moreover, protein residues were present in all the extracts within the range of 0.9–6.8% and varied with the extraction conditions. On high performance size exclusion chromatography, the elution pattern of the acid-extracted pectins showed a wide molar mass distribution consisted of two relatively broad peaks. Their weight-average molar mass values determined by HPSEC-RALLS ranged widely from 20,200 to 90,100 g/mol. Most of the extracted pectins were surface-active, and some of them were quite able to produce and stabilize with effectiveness oil-in-water emulsions. Thus, it was inferred that yield, physico-chemical characteristics and surface properties of acid extracted pectins from sugar beet pulp were influenced by the extraction conditions.

Keywords: Sugar beet pulp; Pectin; Surface and emulsifying properties

1. Introduction

Pectins are complex polysaccharides from higher plants, consisting mainly of D-galacturonic acid, and neutral sugars, such as L-rhamnose, L-arabinose, and D-galactose. They are organized in a chain-like combination wherein the D-galacturonic acid residues are covalently $\alpha(1 \rightarrow 4)$ linked to form a linear backbone. This main chain is interrupted in places by single residues of 2-O-linked-α-L-rhamnose (Aspinall, Craig, & Whyte, 1968) which can bear neutral sugar side chains, primarily arabinans and/or arabinogalactans (De Vries, Rombouts, Voragen, & Pilnik, 1984; De Vries, Voragen, Rombouts, & Pilnik, 1986). Moreover, the galacturonic acid residues could be partly methyl-esterified at *C*-6 (Pilnik & Voragen, 1970), and the hydroxyl groups at position *O*-2 and/or *O*-3 partly acetyl-esterified (Rombouts & Thibault, 1986). Pectins extracted from several plant by-products are widely used in the food industry as gelling agents (May, 1990; Pilnik & Voragen, 1992). Depending on their degree of methoxylation (DM), pectins are referred to as high methoxy pectins (HMP) (DM $\geqslant$ 50) or low methoxy pectins (LMP) (DM $<$ 50). HMP form gels in an acidic medium (pH 2.0–3.5) if sucrose is present at a concentration $>$ 55% wt. LMP can gel in a larger pH range (2.0–6.0) in the presence of divalent ion, such as calcium (Ca^{++}). In this case, the presence of sucrose is not necessary for gel forming. Pectins are also capable of stabilizing protein in acidic media through conjugation or complexation (Al-Hakkak & Kavale, 2002; Dalev & Simeonova, 1995; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Mishra, Mann, & Joshi,

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2001; Neirynck, Van der Meeren, Gorbe, Dierckx, & Dewettinck, 2004). These applications account for the substantial consumption of pectin worldwide. The main sources for commercial pectin production are apple pomace and citrus peels (May, 1990). Another source also attempted was sugar beet pulp, co-product from the sugar industry, owing to its high pectin content (15–30%) on dry weight basis, and its availability in large quantities. However, because pectin extracted from sugar beet pulp exhibited poor gelling properties (Pilnik & Voragen, 1992), it is not fully utilized. These poor gelling properties have been attributed to the presence in beet pectin of large amount of acetyl groups, to its relatively small size (Arslan, 1995; Pippen, McCready, & Owens, 1950), to its high neutral sugar content (Keenan, Belton, Matthew, & Howson, 1985; Rombouts & Thibault, 1986), and to its relatively low average molecular weight (Michel, Thibault, Mercier, Heitz, & Pouillaude, 1985; Phatak, Chang, & Brown, 1988; Rombouts & Thibault, 1986). In contrast, these features seemed to "confer" to beet pectin, good surface-active and emulsifying properties. Sugar beet pectin has been reported to act as an effective emulsifier in the oil-in-water emulsions (Dea & Madden, 1986; Endreß & Rentschler, 1999; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). However, until now, the origin of the emulsifying ability of beet pectin has remained unknown and conclusions reached by previous workers do not agree with one another. According to Endreß and Rentschler (1999), the emulsifying ability of beet pectin could be attributed to the presence of acetyl groups (4–5%). On the contrary, Leroux et al. (2003) reported that the presence of acetyl groups was not an absolute requirement for (beet) pectin to act as an effective emulsifier and/or emulsion stabilizer, and explained the more favourable emulsifying properties of beet pectin in comparison with citrus pectin by the presence of a higher amount of protein within the former. It is then not yet clearly established what may cause beet pectin to possess efficient surface properties. On the other hand, extraction conditions have been reported to influence the physico-chemical properties of beet pectin (Levigne, Ralet, & Thibault, 2002). Therefore, it could be hypothesized that extraction conditions may also influence beet pectin surface properties (surface-activity, emulsifying and/or emulsion stabilizing abilities). The scope of this work was then to select optimum conditions to isolate from sugar beet pulp, pectin with a good yield, of acceptable purity, and displaying effective surface-active and emulsion stabilizing properties.

2. Materials and methods

2.1. Sugar beet pulp

Dried and roughly ground sugar beet pulp was a gift from the Warcoing Sugar Industry (Warcoing, Belgium). Moisture content of the pulp was 8%. It was stored at room temperature (23 °C) until used.

2.2. Experimental design

An earlier study using an experimental design based on a two-state Hadamard's factorial matrix for four extraction parameters (pH, temperature, solid–liquid ratio and time) showed that pH, temperature, and time were the main parameters which influenced significantly the experimental responses (unpublished results). However, the Hadamard's matrix used for screening purposes did not permit us to examine the presence of potential interactive effects between the parameters. Therefore, in this work, we built an experimental design based on a full two-state factorial matrix for the three important parameters, namely pH, temperature, and time of extraction (Table 1) in order to study their main and potential interactive effects on the experimental responses. Eight factorial experimental points were then considered and extractions were carried out in double replications for each experimental point (16 experimental units). The three variables were standardized and arbitrarily coded as (−1, +1) for the lower and upper states, respectively. The effects of the parameters were estimated by the "least square" method as fully described by Dagnelie (2003) and tested for significance using the Student's t-test at a significance level of $p = 0.05$. Additionally, data were tested for adequacy of fit to a first-order multiple linear regression equation using, whenever applicable, the Fisher F-test at a significance level of $p = 0.05$.

2.3. Pectin extraction

The extraction of pectins from the dried sugar beet pulp was carried out according to the experimental design shown in Table 1. Dried pulps (solid–liquid ratio; 1:29, w/v) were gently stirred at 250 rpm in a mild acid aqueous solution adjusted to convenient pH (1.5 or 2) with 6 M H_2SO_4 in a stainless steel reactor flask with a magnetic thermostatic stirrer (ETS-D4 Fuzzy, IKA-Werke, GmbH & Co. KG, Janke & Kunkel-Straße, Staufen, Germany) fitted with temperature probe for temperature setting and regulation at 80 or 90 °C. The extractions were carried out for 1 or 4 h. The resulting slurries were allowed to cool to room temperature (23 °C), and centrifuged at 17,675g

Table 1
A full two-sate experimental design used for pectin extraction from sugar beet pulp (based on Hunter's factorial matrix)

	t (X_1)	T (X_2)	pH (X_3)
E1	−1	−1	−1
E2	+1	−1	−1
E3	−1	+1	−1
E4	+1	+1	−1
E5	−1	−1	+1
E6	+1	−1	+1
E7	−1	+1	+1
E8	+1	+1	+1

The lower and upper states (−1, +1) correspond to 1 and 4 h for time (t), 80 and 90 °C for temperature (T) and 1.5 and 2 for (pH), respectively.

for 20 min in a Beckman J2-M1 centrifuge (Beckman Instruments, Inc., Fullerton, CA, USA). The supernatants were filtered through two stacked-up layers of nylon cloth (20 μm) and (11 μm) downwards, respectively. The initial pH of each clarified crude extract was measured before adjusting to pH 3.5 with a known volume of 0.2 M K_2HPO_4. After measuring the whole volume, aliquots (2×30 ml) were sampled and dispersed into 4 volumes of 96% ethanol (final ethanol concentration > 70%) for 1 h, at room temperature. Pectin gels were centrifuged at 17,675*g* for 20 min, washed with 70% ethanol (2×50 ml), centrifuged as before, recovered in water, freeze-dried, vacuum-dried at 40 °C, overnight, and weighed for yield assessment. The remaining material was dispersed into 4 volumes of 96% ethanol for 1 h, at room temperature as above, and pectin gel was washed twice with 70% ethanol (gel–solvent ratio; 1:1, w/w), hand-squeezed in nylon cloth (11 μm) to eliminate ethanol remnant, dried at room temperature, for 48 h, vacuum-dried at 40 °C, overnight and finely ground in an IKA-A10 mill (IKA. GmbH & Co. KG, Janke & Kunkel, Labortechnik, Staufen, Germany) to pass through 60-mesh screen sieve. Homogenous pectin powders were stored at room temperature until used. Extractions were performed in duplicate for each experimental point.

2.4. Pectin purification and characterization

2.4.1. Purification

Before characterization, the extracted pectins were further purified by centrifugation process. Dried pectins were dissolved in fresh Milli-Q water (solid–liquid ratio; 1:200, w/w), prepared from a Milli-Q water purification system (Millipore Co., Milford, MA, USA), for 15 h and centrifuged at 30,074*g* for 20 min, at room temperature in a Beckman J2-M1 centrifuge (Beckman Instruments, Inc., Fullerton, CA, USA) to remove the water insoluble fraction (WIF). The supernatants were filtered through 11 and 3 μm Millipore membranes (Millipore Co., Milford, MA, USA), respectively. The filtrates were freeze-dried to recover the "purified" pectins which were vacuum-dried at 40 °C, for 5 h and weighed. Pectins so obtained were used for the various investigations.

2.4.2. Characterization

Analyses of "purified" pectins were performed in duplicate unless otherwise specified.

Moisture content of pectins was determined by drying pectin samples in an air-circulated oven at 106 °C, for 24 h. All values were calculated on a dry-weight basis.

Galacturonic acid (GalA) content of pectins was determined by high performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) according to Garleb, Bourquin, and Fahey (1991) method with a slight modification, after a complete hydrolysis of pectin constituents to monomers as fully described in Garna, Mabon, Wathelet, and Paquot (2004). Hydrolysates (25 μl) were injected into the Dionex system (DX-500 Bio-LC, Dionex Corp., Sunnyvale, CA, USA) equipped with CarboPac PA-100 column (250×4 mm) in combination with a guard CarboPac PA-100 column (50×4 mm), Dionex Corp., Sunnyvale, CA, USA. Elution was carried out with 0.1 M NaOH and 0.17 M NaOAc at a constant temperature of 30 °C, and at a flow rate of 1 ml/min. The column was washed post-injection with 0.5 M NaOH over 17 min followed by 20 min re-equilibration before the next injection. Pure galacturonic acid monohydrate (purity > 99%, Fluka Chemie AG., Buchs, Switzerland) was used as an external standard.

Individual neutral sugars were released from pectin molecules by acid hydrolysis with 1 M H_2SO_4 at 100 °C for 3 h and converted to alditol acetate according to Blakeney, Harris, Henry, and Stone (1983) method. Alditol acetate derivatives were separated and quantified by gas chromatography using a high performance capillary column, BP1-methylsiloxane (30 m L × 0.32 mm ID, 0.25 μm film thickness, Scientific Glass Engineering, S.G.E. Pty. Ltd., Melbourne, Australia) mounted on a Hewlett–Packard HP-6890 series GC system (Hewlett–Packard Co., Palo Alto, CA, USA). 2-Desoxy-D-glucose (purity > 99.5%, Sigma Chemical Co., St. Louis, MO, USA) was used as an internal standard. Total neutral sugars (NS) were calculated as the sum of the individual neutral sugars.

Phenolic compounds (expressed as ferulic acid) were analysed by high performance liquid chromatography (HPLC) after saponification and extraction. Pectin samples (50 mg) were saponified in 3 ml of 2 M NaOH under nitrogen in the dark at room temperature, overnight, after which they were neutralised to pH 2 with 2 M HCl, then phenolic compounds were extracted into ethyl acetate (3×3 ml). The ethyl acetate phase was evaporated under nitrogen at room temperature and residue dissolved into 1 ml of methanol/H_2O (1:1, v/v). Twenty five microliters of this material was injected into a 2690 HPLC system (Waters Inc., Milford, MA, USA) equipped with a C_{18}-reversed phase column (250 mm L × 4.6 mm ID, 5 μm particle size, 300 Å pore size, Vydac, The Separations Group, Hesperia, CA, USA) and coupled on line with a Waters 996 photodiode array detector (DAD). Elution was carried out with 75% solvent A (Milli-Q water + 0.05% TFA) and 25% solvent B (acetonitrile + 0.05% TFA) at constant temperature of 35 °C and at a flow rate of 1 ml/min. Phenolic compounds were detected at 320 nm. Ferulic acid (purity > 99%, Sigma–Aldrich Chemie GmbH., Steinheim, Germany) was used as an external standard.

Methoxy and acetyl groups were released from pectins by saponification with 0.2 M NaOH, at 4 °C, for 2 h, separated and quantified by HPLC on an Aminex HPX-87 H ion exchange column (300 mm L × 7.8 mm ID, BioRad, Hercules, CA, USA) according to Voragen, Schols, and Pilnik (1986) method. Elution was carried out with 5 mM H_2SO_4 solution at a constant temperature of 25 °C, and at a flow rate of 0.6 ml/min. Degrees of methoxylation (DM) and acetylation (DA) were expressed as the percent

molar ratio of methanol (MeOH) or acetic acid (AcOH) to GalA, respectively, using GalA quantified by the HPAEC-PAD method.

Protein content ($N \times 6.25$) was determined by the Kjeldahl procedure (AOAC, 1984). Sugar beet pulp protein content was determined after it was finely ground and sieved to pass through 60-mesh screen.

Weight-average molar mass (MW) of the extracted pectins was determined by high performance size exclusion chromatography (HPSEC) method on a Waters 2690-HPLC system (Waters Inc., Milford, MA, USA) equipped with a TSKgel G4000PWXL column (300 mm L × 7.8 mm ID, exclusion limit = 1–700 kDa for dextrans; TosoHaas Co. Ltd., Tokyo, Japan) and coupled on-line with three detectors, a Waters 2410 differential refractometer (RI), a right angle laser light scattering (RALLS) detector, and a differential viscometer (Model T-50A, Viscotek, Houston, TX, USA). Pectin solutions (0.3%, w/w) were centrifuged and filtered through 3 and 0.45 μm membrane filters (Millipore Co., Milford, MA, USA), respectively, for clarification. A constant volume of pectin solutions was sampled and dried to a constant weight in an air-circulated oven at 106 °C to calculate the exact pectin concentration of solutions. Samples were filtered once again through 0.45 μm membrane filters (GHP Acrodisc, Pall Gelman Corp., East Hills, NY, USA), and 50 μl were loaded onto the column. Elution was carried out with 50 mM sodium nitrate ($NaNO_3$) solution containing 0.02% sodium azide (NaN_3) as a bacteriocide at 25 °C, and at a flow rate of 0.7 ml/min. RI detector was used to determine the specific refractive index increment (dn/dc) value for beet pectin. The dn/dc value was subsequently used to determine the absolute MW of pectins using the RALLS detector or the differential viscometer and a universal calibration curve constructed with dextran standards ($MW = 5 \times 10^3 - 670 \times 10^3$ Da). Data from the RI and RALLS detectors were acquired and processed by Millennium V 2.1 software (Waters, Inc., Milford, MA, USA), and those from the viscometer by TriSEC software (Version 2.7, Viscotek, Houston, TX, USA).

2.5. Surface properties

The surface and interfacial tension measurements were performed with a Tensimat Prolabo No. 3 (Prolabo, Paris, France) according to the Wilhelmy Plate Technique using a roughened mica plate. For surface tension measurements, pectin solutions (0.1%, w/v) containing 0.02% sodium azide as a bacteriocide were prepared by dissolving dried pectins in fresh Milli-Q water at pH 3.5, at room temperature without any pH adjustment. A constant volume of pectin solutions was gently poured into a thermostated glass dish at 23 °C. Surface tension was measured from the pull on mica plate over an equilibrium period of 1 h. A surface tension value of 72.5 dyn cm^{-1}, determined with fresh Milli-Q water, was used as reference. Pectin solutions were prepared in triplicate and measurements were performed three times for each solution. Mean values were considered. Interfacial tension measurements were carried out at the oil/water interface using 0.5% (w/w) pectin solution prepared at room temperature by dissolving dried pectin in fresh Milli-Q water containing 0.02% sodium azide as a bacteriocide. A constant volume of 0.5% (w/w) pectin solution was gently poured into a thermostated glass dish at 23 °C, as above. Immediately afterwards, *n*-dodecane (purity > 99%, Sigma Chemical Co., St. Louis, MO, USA) was gently layered on top of the pectin solution. The interfacial tension was measured after 1 h of equilibrium from the pull on the mica plate. Pectin solutions were prepared in triplicate and measurements were performed three times for each solution.

Emulsifying activity and emulsion stability were assessed using Dalev and Simeonova (1995) procedure with a slight modification. Oil-in-water (O/W) emulsions were prepared by adding 3 ml of *n*-dodecane (43% wt final oil level) to 3 ml pectin solutions (0.5%, w/w) containing 0.02% sodium azide as a bacteriocide in 15 ml graduated polypropylene transparent centrifuge tubes (Treff Lab, Treff SA/AG, CH-9113; Degersheim, Switzerland), and treating the whole system in an ultrasonic instrument (Model B-2200 E4, Branson Co., Shelton, CT) for 1 min, at room temperature. The pre-emulsions were spun vigorously in a Labinco L46 vortex mixer (Labinco, Breda, The Netherlands) fitted with a tube holding device and set to a continuous mode at maximum speed, for 1 min, at room temperature to achieve a good emulsion level. The emulsions were finally centrifuged in a Mistral centrifuge (MSE Mistral 2000R, Sanyo Gallenkamp PLC, Leicester, UK) at 527*g*, for 5 min, at 23 °C and the whole volume (Wv) of the system and the emulsified layer volume (ELV) were measured. Emulsifying activity (EA) was calculated as

$$EA\ (\%) = (ELV/Wv) \times 100$$

To study the emulsion stability (ES), another set of emulsions were prepared fourfold for each sample in transparent graduated tubes as above. Two tubes out of the four were cooled to 4 °C, and centrifuged at 527*g*, for 5 min, at 4 °C after which the initial emulsified layer volumes (ELV_i) were measured, and stored at 4 °C. The other 2 tubes were treated the same way but at room temperature and stored at that temperature. After 1 and 30 day(s) of storage, the remaining emulsified layer volumes (ELV_r) were measured after centrifugation and emulsion stability was calculated as

$$ES\ (\%) = (ELV_r/ELV_i) \times 100$$

3. Results and discussion

3.1. Extraction yield

The acid-extracted pectin yield ranged from 4.1% to 16.2% of the dry weight of the pulp (Table 2). The highest

Table 2
Yield (% w/w), chemical composition (% w/w), degree of methoxylation (% mol), degree of acetylation (% mol) and weight-average molar mass (kg/mol) of pectins extracted from sugar beet pulp

	E1	E2	E3	E4	E5	E6	E7	E8
Yield	8.1	13.2	9.0	16.2	4.1	8.2	5.1	11.2
Rha	4.1	3.9	5.2	0.7	3.2	3.0	2.9	2.3
Ara	7.9	7.1	9.9	3.5	12.3	11.8	9.8	9.5
Xyl	0.5	0.4	0.5	–	0.6	0.9	1.2	0.8
Man	0.4	0.3	0.7	–	0.7	0.8	1.0	–
Glu	0.7	0.5	0.6	0.1	0.9	1.2	1.3	1.1
Gal	10.1	11.3	8.2	2.5	15.2	12.8	13.9	14.0
NS	23.7	23.5	25.1	6.8	32.9	30.5	30.1	27.7
GalA	54.7	56.4	55.2	76.3	35.2	41.2	40.2	49.2
FA	0.2	0.2	0.1	0.2	0.4	0.3	0.7	0.5
MeOH	3.1	2.5	2.3	2.0	4.2	3.6	3.2	2.9
DM	31.2	24.4	22.9	14.4	65.6	48.1	42.4	32.4
AcOH	3.1	2.2	1.6	0.8	3.5	3.8	2.9	3.3
DA	16.6	11.4	8.5	3.1	29.2	27.1	21.2	19.7
Protein	3.7	2.6	1.9	0.9	8.6	6.1	7.5	5.9
Moisture	7.5	8.6	8.9	9.2	9.3	9.8	9.5	6.8
Mw	85.3	42.7	40.1	20.2	90.1	62.3	66.4	50.1

FA, ferulic acid; MeOH, AcOH; DM, DA; methoxy and acetyl groups; degree of methoxylation and degree of acetylation, respectively; Mw, Weight-average molar mass.

yield was obtained when the dried beet pulp was treated at pH 1.5, for 4 h, at 90 °C. The variance analysis showed no significant interactive effect on the pectin yield. The individual effects of pH and time were the most influential on the pectin yield. Indeed, at constant pH and temperature, the yields of pectin obtained for 1 h of extraction were lower than those for 4 h. On the other hand, the pectin yields from various extractions at pH 1.5 were higher than those at pH 2. Hence, the yield increased significantly ($p < 0.05$) with decreasing pH (or increasing acid strength) of the extraction as previously reported (Levigne et al., 2002). Likewise, at constant pH and temperature, increasing the extraction time significantly increased the yield. In contrast, increasing temperature when pH and time remained constant only slightly increased the pectin yield. The effect of temperature did not actually impact on the pectin yield. This indicated that yield was higher when pectin was extracted from beet pulp at pH 1.5 and 4 h irrespective of the temperature. Compared to the literature data, the pectin yields were close to those of Micard and Thibault (1999) (14%) and Mesbahi, Jamalian, and Farahnaky (2005) (17.9%) under similar conditions. Yield data fitted an acceptable first-order multiple regression equation as a function of pH, temperature, and time of extraction with an adjusted R-squared 95% as follows:

$$\text{Yield } (\%) = 9.39 + 2.81X_1 + 0.99X_2 - 2.24X_3$$

where X_1, X_2, X_3 are the coded variables (−1 and +1) for time, temperature and pH of extraction, respectively.

3.2. Chemical composition of pectins

The chemical composition of the extracted pectins is shown also in Table 2. Their galacturonic acid content ranged from 35.2% to 76.3% (on dry-weight basis). The galacturonic acid amounts were close to data already published under similar conditions (Levigne et al., 2002 (29.5–52.8%); Micard and Thibault, 1999 (63.5–71.9%)). The highest galacturonic acid content was obtained when pectin was isolated at pH 1.5, for 4 h, at 90 °C. However, the extraction yield of pectin was not related to the content in galacturonic acid. The variance analysis revealed that pH was the main parameter influencing the galacturonic acid content. The pectins extracted at pH 1.5 contained more galacturonic acid than those at pH 2, suggesting that the pectin content in galacturonic acid increased with decreasing pH (or increasing acid strength). These results also indicated that the pectins extracted at pH 1.5 were more pure than those at pH 2. The galacturonic acid content of the extracts was only moderately influenced by time, temperature, and time–temperature interaction.

The main neutral sugars present within the pectins were rhamnose (0.7–5.2%), arabinose (3.5–12.3%), and galactose (2.5–15.2%) suggesting that the structure of most of the extracted pectins could consist of a rhamnogalacturonan backbone and arabinan and/or arabinogalactan-rich side chains, consistent with previous reports (Oosterveld, Beldman, Schols, & Voragen, 2000; Sakamoto & Sakai, 1995). Other neutral sugars, such as xylose, mannose and glucose were present, but in low amounts and were therefore assumed to be "contaminants" from hemicellulosic and sugar materials. The total neutral sugar content calculated as the sum of the individual neutral sugars ranged from 6.8% to 32.9% (Table 2). All the extracts, except that obtained at pH 1.5, for 4 h, at 90 °C, were very rich in neutral sugars, a typical feature of beet pectin reported in various studies (Buchholt, Christensen, Fallesen, Ralet, & Thibault, 2004 (28.8%); Guillon and Thibault, 1988 (19.5–20.2%); Thibault, Renard, Axelos, Roger, and Crépeau, 1993 (18.6%)). The highest neutral sugar content

was obtained at pH 2, for 1 h, at 80 °C which corresponded to the mildest extraction conditions. The neutral sugar content of the pectins extracted at pH 2 was higher than those at pH 1.5 indicating that at pH 1.5, some degradation of pectin side chains could occur. pH had the most influential effect on the neutral sugar content. It seemed that the neutral sugar content increased with increasing pH. On the other hand, the galactose content of most of the extracts was higher than that of arabinose suggesting either the predominance of arabinogalactan side chains and/or a higher degradation of arabinan side chains, probably due to the fact that arabinofuranosyl linkages are the most acid-labile (BeMiller, 1967). It could also be seen that the neutral sugar content of the pectin isolated at pH 1.5, for 4 h, at 90 °C was particularly low. Moreover, its rhamnose content (0.7%) was the lowest of all the extracts. Hence, under much harsher extraction conditions, the degradation of pectin also affected the rhamnogalacturonan backbone. It is possible that at pH 1.5 and 90 °C, a time of extraction longer than 4 h could lead to the obtaining of a polygalacturonic acid. Indeed, based on different sensitivities to acid hydrolysis of the glycosidic linkages in pectins (GalA–GalA > Gal–Rha > Rha–GalA > neutral sugar-neutral sugar), Thibault et al. (1993) reported that at pH values < 2, a fast hydrolysis of pectin side chains to oligomers and monomers occurs, after which the backbone, more resistant, could be cleaved preferentially between galacturonic acid and rhamnose, thus giving rise to an insoluble material which almost entirely consists of galacturonic acid residues (homogalacturonic acid) after 72 h of hydrolysis.

Low amounts of ferulic acid were present in all extracted pectins (Table 2). Indeed, it has been reported that beet pectin chains could be linked through diferulic bridges with arabinose residues (Levigne et al., 2002; Oosterveld et al., 2000). Ferulic acid content of the extracted pectins was not especially influenced by the extraction conditions. However, the pectins isolated at pH 2 were slightly richer in ferulic acid than those at pH 1.5. But apparently, this had nothing to do with the arabinose content contrary to the report of Levigne et al. (2002).

The methoxy content of pectins ranged from 2.0% to 4.2%, and corresponding degree of methoxylation from 14.4 to 65.6 indicating the extraction of high and low methoxy pectins from the sugar beet pulp. The acetyl content ranged from 0.8% to 3.8%, and corresponding degree of acetylation from 3.1 to 29.2. In comparison with literature data, the degree of acetylation was consistent with those reported in previous works (Buchholt et al., 2004; Levigne et al., 2002). The lowest degrees of methoxylation and acetylation were obtained when pectin was extracted at pH 1.5, for 4 h, at 90 °C, probably because harsher conditions of temperature and pH increased the deesterification of polygalacturonic chain (Mort, Qui, & Maness, 1993). pH had a more marked effect than temperature on the methoxy and acetyl contents of pectins. Furthermore, the extracts and sugar beet pulp were analysed for their content in proteinaceous compounds. The quantification of protein residues in the initial pulp used for pectin extraction allowed us to assess the different amounts of protein residues "co-extracted" following the extraction conditions. About 7.5% of the dry weight of the sugar beet pulp consisted of proteinaceous material. This amount was in good agreement with values (6–10.3%) found in the literature (Broughton, Dalton, Jones, & Williams, 1995; Sun & Hughes, 1999). The protein content of the pectins varied with the extraction conditions (Table 2). These values were slightly higher than those of Leroux et al. (2003) (2–2.3%) under similar conditions, probably due to differing techniques of pectin recovering and purification (ethyl alcohol precipitation and washing in our study against ultrafiltration prior to isopropyl alcohol precipitation in their study). The pectins extracted at pH 1.5 contained more protein residues than those at pH 2 confirming the higher purity levels of pectins extracted at pH 1.5. It seemed that the extraction of beet pectin at pH 2 and its recovering and purification by ethanol precipitation and washing was less selective. The higher protein content of the pectins isolated at pH 2 could probably be due to more interactions between protein and pectin at pH 2 than at pH 1.5, thereby co-precipitating in higher amounts at pH 2 and/or to the fact that larger quantities of proteins were "co-extracted" at pH 2 (4.6–8.8%) than at pH 1.5 (1.9–4.0%), and recovered together with pectin by ethyl alcohol precipitation. Pectins from various sources have been reported to contain small proteinaceous material. By means of preparative chromatographic methods, this material has been shown to be a "true" protein, either in the free form or linked to pectin (Kravtchenko, Berth, Voragen, & Pilnik, 1992; Kravtchenko, Voragen, & Pilnik, 1992; Oosterveld, Voragen, & Schols, 2002). It could also be the same way in beet pectin.

3.3. Weight-average molar mass of pectins

When analysed by HPSEC, the elution pattern of the acid-extracted pectins exhibited a wide molar mass distribution. An example of molar mass distribution for two differing extracts is given in Fig. 1. Similar elution pattern has been previously reported for acid-extracted beet pectins (Hatziantoniou & Howell, 2002). Generally, two fraction peaks of high and relatively low size materials were eluted approximately at 10.5 and 12.5 ml, respectively. The weight-average molar mass values of pectins ranged widely from 20,200 to 90,100 g/mol (Table 2). These values are lower than those reported by Levigne et al. (2002) (70,000–355,000 Da), probably due to differences in starting materials (fresh sugar beet roots in their study). The weight-average molar mass values of pectins from various extractions at pH 2 were higher than those at pH 1.5, possibly due to the higher degree of esterification (Fishman, Pfeffer, Barford, & Doner, 1984; Levigne et al., 2002; Morris, Foster, & Harding, 2000) of pectins extracted at pH 2. The effects of pH and time were stronger than those of tem-

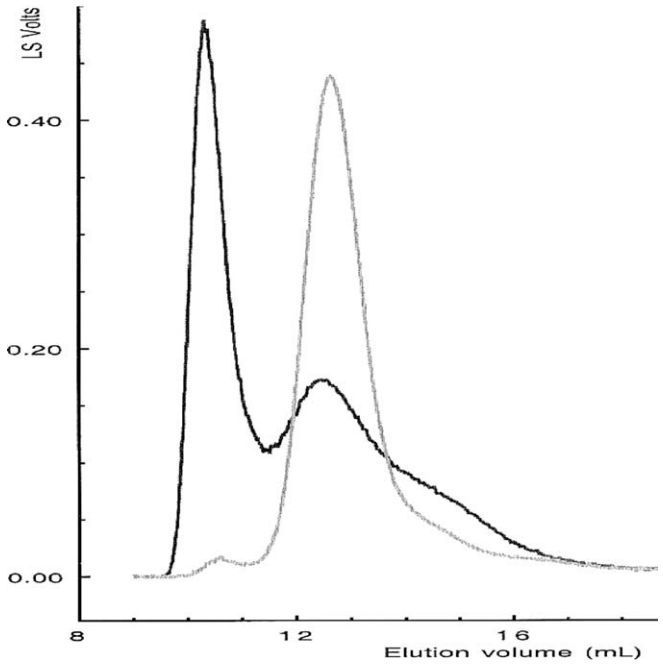

Fig. 1. HPSEC elution profile of the pectins extracted at pH 1.5, for 1 h, at 90 °C (—) and, at pH 1.5, for 4 h, at 90 °C (—).

perature and time–temperature interaction on weight-average molar mass. Furthermore, it was found that high size material fraction present within the whole pectin extracted at pH 1.5, for 4 h, at 90 °C was very low, which could partly explain the fact that this extract had the lowest weight-average molar mass (20,200 g/mol). This weight-average molar mass value was rather close to the value of 19,000 g/mol which has been published for an homogalacturonic acid isolated from a whole beet pectin (Thibault et al., 1993), suggesting therefore the potential isolation of pectin resembling a polygalacturonic acid directly from the beet pulp. It sounded as if at pH 1.5 and 90 °C, a small fraction of high size material was isolated along with a larger fraction of small size material. It could also be that the higher size material was initially preponderant within the extract, but owing to the harsh conditions of pH and temperature, it was degraded almost entirely into small size material, thus making eventually larger that latter fraction after 4 h of extraction.

3.4. Surface properties

3.4.1. Surface and interfacial tensions

The surface tension was determined at the air/water interface at room temperature (23 °C) with 0.1% (w/v) pectin solutions. The surface tension values of the pectin samples ranged from 48.3 to 58.7 dyn cm^{-1} (Table 3) indicating that most of the extracts were surface-active, in agreement with previous studies (Dea & Madden, 1986). The highest surface tension reductions were observed with the pectins extracted at pH 1.5, for 1 h, at 80 °C, and at pH 2, for 1 h, at 80 °C, probably because these extracts displayed in

Table 3
Surface tension of 0.1% (w/v) pectin solutions at pH 3.5 and at 23 °C

	Surface tension (dyn cm^{-1})
Milli-Q water	72.5
E1	50.4
E2	56.1
E3	54.0
E4	58.7
E5	48.3
E6	54.7
E7	53.9
E8	55.8

solution a higher surface coverage owing to their much higher weight-average molar mass. The presence of acetyl groups and/or protein residues could also be implicated in the ability of the extracted pectins to be surface-active. However, the surface tension reductions of pectin samples were not related to their content in acetyl groups or protein residues. Time and temperature exerted the most influential effects on the surface-activity. It seemed that pectin was more surface-active when extracted at 80 °C and for 1 h regardless of the pH value. On the other hand, the surface tension decreased with increasing pectin concentrations from 0% to 0.1% (w/v) (Fig. 2). After this value, the surface tension remained constant until 2% (w/v) pectin concentration after which it trended to increase (not shown in the figures), probably due to the fact that pectin solutions became more viscous. In addition, measurements were found less accurate with highly viscous solutions. An analogous phenomenon has been previously observed with another hydrocolloid, fenugreek gum (Brummer, Cui, & Wang, 2003) and the tendency of the surface tension to increase after a certain concentration was explained by an excessive viscosity development.

The interfacial tension measured with 0.5% (w/w) pectin solutions prepared with the pectins extracted at pH 1.5, for 1 h, at 80 °C, and at pH 2, for 1 h, at 80 °C is shown in Table 4. The interfacial tension values at the oil/pectin dispersed aqueous interface were significantly lower, as compared with that at the pure oil/water interface

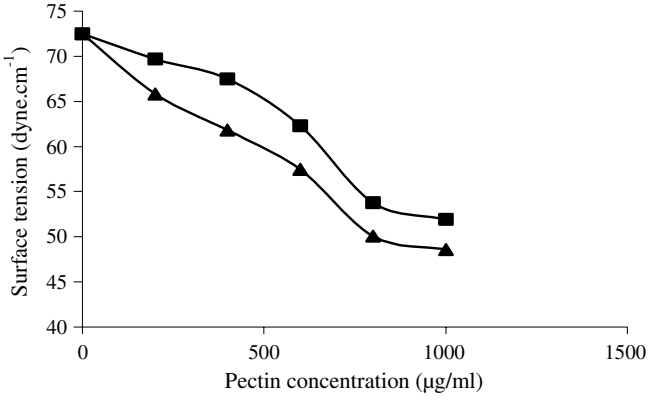

Fig. 2. Evolution of the surface tension of the pectins extracted at pH 1.5, for 1 h, at 80 °C (■) and at pH 2, for 1 h, at 80 °C (▲) with pectin concentration at 23 °C, and at pH 3.5.

Table 4
Interfacial tension, emulsifying activity and emulsion stability of oil/0.5% (w/w) pectin solutions

	Interfacial tension (dyn cm^{-1})	Emulsion activity (%)	Emulsion stability (%)			
Storage time			1 day		30 days	
Temperature	23 °C	23 °C	4 °C	23 °C	4 °C	23 °C
Milli-Q water	50.2	–	–	–	–	–
E1	14.2	43.2	78.1	65.5	78.1	65.3
E5	13.1	47.1	80.3	70.1	80.1	70.1

E1 and E5: pectins extracted at pH 1.5, for 1 h, at 80 °C and at pH 2, for 1 h, at 80 °C, respectively.

(50.2 dyn cm^{-1}) showing the effectiveness of those pectin samples to act as surface-active hydrocolloids. The interfacial tension values were close to the value published by Huang, Kakuda, and Cui (2001) (12.4 dyn cm^{-1}) for a non-depolymerized pectin, but slightly lower than that of Leroux et al. (2003) (19.4 dyn cm^{-1}). Discrepancies with the latter value could probably be due to differing concentrations of pectin solutions used for interfacial tension measurements (0.5% (w/w) in this study against 2% (w/w) in their study). In addition, a longer equilibrium time (1 h in our case versus instantaneous measurement in their study) could partly explain the obtaining of lower values in our study.

3.4.2. Emulsifying properties

The emulsifying activity and emulsion stability of the pectins extracted at pH 1.5, for 1 h, at 80 °C, and at pH 2, for 1 h, at 80 °C were studied with emulsions prepared with 0.5% (w/w) pectin solutions. After centrifuging the emulsions, three phases were observed, a very small oil phase on the top, a pectin dispersed aqueous phase at the bottom, and between the two phases, an emulsified layer phase. The emulsifying activity values of the emulsions prepared with the pectins extracted at pH 1.5, for 1 h, at 80 °C, and at pH 2, for 1 h, at 80 °C were 43.2% and 47.1%, respectively (Table 4). These values were higher than that found (30.3%) in the literature for a non-depolymerised pectin in similar conditions (Huang et al., 2001). Moreover, the emulsions were 78.1–80.3% stable at 4 °C and 65.5–70.1% at 23 °C after 1 day of storage. After 30 days of storage, the emulsion stability ranged from 78.1% to 80.1% at 4 °C and from 65.3% to 70.1% at 23 °C (Table 4) showing the high stability levels of the emulsions under the two different temperatures of storage. However, emulsions were slightly steadier when stored at 4 °C. In comparison with data published, these values were higher than that found (62%) in the literature for a non-depolymerised pectin (Huang et al., 2001). From these results, it could be inferred that under certain extraction conditions, acid-extracted pectins from sugar beet pulp could produce and stabilize efficiently oil-in-water emulsions.

4. Conclusions

The extraction yield, chemical and physico-chemical characteristics, and surface properties of acid-extracted beet pectins were significantly influenced by the extraction conditions. Under very harsh extraction conditions, the pectin yield and galacturonic acid content were high. However, its weight-average molar mass was low. The contrary is true. Acid-extracted beet pectins which had weight-average molar mass in the order of 85,000–90,000 g/mol were more surface-active and possessed effective emulsion stabilizing properties. Therefore, the weight-average molar mass of beet pectin could play a key role in its ability to display good surface properties. By considering the pectin yield, galacturonic acid content (which is indicative of pectin purity), and ability to favour surface-active and emulsifying properties, the acid extraction of beet pectin at pH 1.5, for 1 h, at 80 °C could be suitable.

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