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Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design

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Abstract

An experimental design was used to study the influence of different parameters (pH, temperature, time, and type of acid) on extraction of pectins from fresh sugar beet. The extraction conditions have important effects on the features of extracted pectins. Their composition (neutral and acidic sugars, degrees of esterification, amounts of ferulic and diferulic acids) and some physicochemical properties (molar mass, intrinsic viscosity) were determined. The type of acid used (HCl or HNO_3) had no effect on the characteristics of extracted pectins. Different kinds of pectins can be obtained with good yields at pH 1. Galacturonic acid amounts of the extracted pectins were nearly constant whatever the extraction conditions, whereas the degrees of methylation and acetylation showed large variations. At pH 1, the extracts were particularly rich in rhamnogalacturonan regions, the nature and the quantity of side chains differing according to extraction conditions. The molar masses of extracted pectins were higher than those obtained from sugar beet pulp; beside the sole impact of raw material, possible cross-linking of pectic molecules through diferulic bridges is discussed.

Keywords: Pectin; Sugar beet; Experimental design; Acidic extraction; Ferulic acid

1. Introduction

Pectic substances are important polysaccharides of the cell walls of higher plants. They mainly consist of a backbone of (1 → 4)-linked partly methylesterified α-D-galacturonic acid units. This linear chain may be interrupted by (1 → 2)-linked α-L-rhamnopyranosyl units bearing some side chains mainly composed of galactose and arabinose residues (Voragen, Pilnik, Thibault, Axelos & Renard, 1995). Pectins form gels under certain conditions and this property is used in jams, jellies and marmalade, confectioneries and acid mild products (May, 1990; Pilnik & Voragen, 1992). Highly methoxylated pectins (degree of methylation (DM) DM > 50%) gel with sucrose at low pH, and lowly methoxylated pectins (DM < 50%) in the presence of calcium. Industry traditionally uses citrus peels and apple pomace as raw materials for pectin production. Alternative sources are currently investigated. For example, in Europe, more than two million tons of sugar beet pulp are generated annually by the sugar industry. Its high content in pectins (20–25%), low cost, and availability make sugar beet pulp a potential source of pectins (May, 1990).

Sugar beet pectins have however poor gelling properties under the usual conditions, which have been ascribed to a relatively low molar mass, a high degree of acetylation (DA) or a high amount of side chains (Pippen, McCready & Owens, 1950; Roboz & Van Hook, 1946). The presence of ferulic acid ester-linked to the arabinose and galactose residues of the side chains (Guillon, Thibault, Rombouts, Voragen & Pilnik, 1989; Ralet, Thibault, Faulds & Williamson, 1994) can, however, be used for chemical cross-linking of pectins, leading to gel formation (Guillon & Thibault, 1990; Rombouts & Thibault, 1986).

Sugar beet pectins may be obtained on a laboratory scale by extractions of the cell-wall material by cold and/or hot water or buffer solutions, cold and/or hot solutions of chelating agents, hot diluted acids, and cold diluted sodium hydroxide (Rombouts & Thibault, 1986). Sugar beet contains a low amount of water extractable pectins (Arslan, 1995; Dea & Madden, 1986; Oosterveld, Beldman, Schols & Voragen, 1996; Rombouts & Thibault, 1986). Extraction with chelating agents has the disadvantage that it is difficult to remove residual chelatos. Alkaline extraction could decrease the DM, DA and feruloylation and the length of main chain of galacturonic acid by β-elimination (Rombouts &

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Table 1
Levels (the lower and upper level (−1.1) of the variables correspond, respectively, to 1 and 3 for pH, 75 and 95°C for temperature, 30 and 90 min for time and HCl and HNO_3 for the nature of the acid) of the extraction variables in the factorial design (Yates matrix)

	pH	*T*	*t*	Acid
A	−1	−1	−1	−1
B	1	−1	−1	−1
C	−1	1	−1	−1
D	1	1	−1	−1
E	−1	−1	1	−1
F	1	−1	1	−1
G	−1	1	1	−1
H	1	1	1	−1
I	−1	−1	−1	1
J	1	−1	−1	1
K	−1	1	−1	1
L	1	1	−1	1
M	−1	−1	1	1
N	1	−1	1	1
O	−1	1	1	1
P	1	1	1	1
CP1[a]	0	0	0	−1
CP2	0	0	0	1
SP1[b]	−1	0	0	−1
SP2	1	0	0	−1
SP3	0	−1	0	−1
SP4	0	1	0	−1
SP5	0	0	−1	−1
SP6	0	0	1	−1

[a] Central points are duplicated for each acid.
[b] Star points are performed with HCl.

Thibault, 1986). The highest amount of pectin (yields > 20%) is generally obtained by hot acid extraction (Arslan, 1995; Michel, Thibault, Mercier, Heitz & Pouillaude, 1985; Rombouts & Thibault, 1986). It is also the most convenient approach for industrial extraction of pectin (May, 1990).

Previous works (Michel et al., 1985; Phatak, Chang & Brown, 1988) reported that temperature, pH, time and the nature of acid could modify the quantity as well as the quality of the extracted pectins from beet pulp. Generally proposed extraction conditions ranged from pH 1 to 3, 75 to 95°C and 30 to 90 min (Michel et al., 1985). Pectins from citrus, apple or sugar beet pulp are mainly extracted with mineral acids (Aravantinos-Zafiris & Oreopoulou, 1992), the most used being hydrochloric and nitric acids.

We have studied the optimisation of the acidic extraction of sugar beet pectins, their composition (neutral and acidic sugars, degrees of esterification), and some of their physicochemical properties (molar mass, viscosity).

The starting material chosen was fresh sugar beet instead of pulp because the different physical treatments applied to the beet roots, especially drying, could have some deleterious effects on the cell walls (i.e. collapse). To study the influence of extraction parameters (pH, temperature, time and type of acid), an experimental central composite design was constructed. This statistical approach allowed the quantification of these parameters and their potential interactions. We report here on the identification of extraction conditions for pectins differing in their chemical and physical characteristics.

2. Experimental

2.1. Material

Fresh sugar beet roots were sampled in the experimental station of INRA in Mons (France) during the 1999 campaign. The roots were first washed with tap water to eliminate the bulk of sand and other inorganic materials. Immediately after manual peeling and rasping in a Braun kitchen grinder, pieces of sugar beet roots (1 kg) were immersed in 96% boiling ethanol (31) for 20 min. The slurry was filtered through G3 sintered glass and the insoluble material was left under magnetic stirring for 30 min with 70% ethanol (21) and filtered again. This step was repeated until the filtrate gave a negative reaction to the phenol sulphuric acid test (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). The residue was dried by solvent exchange (ethanol, aceton) and left overnight at 40°C to give alcohol insoluble residue (AIR). This process excluded low-Mr sugars, amino acids, organic acids and many inorganic salts, and also inactived enzymes, which could degrade polysaccharides.

2.2. Experimental design

A factorial composite design (Table 1) was used to determine the effect of the four extraction variables (pH, temperature, time and nature of the acid) on the characteristics of the extracted pectins. The experimental design was constructed from a full two-level factorial design for four factors. The variables were standardised and coded as levels (−1, +1). The coefficients were tested for significance using Student's *t*-test at a significance level of $P = 0.05$. The estimated regression equation was tested for the adequacy of fit using the Fisher *F*-test at a significance level of $P = 0.05$.

Central points were performed in duplicate for both acids. The quadratic effects were determined by six 'star' points for HCl. The model coefficients reflected the linear, quadratic and interactive effects.

2.3. Pectin isolation

2.3.1. Aqueous extraction

AIR (1 g) was stirred for 30 min in water at 25°C and pH 4.5 (60 ml). The residue was separated by filtration through G3 sintered glass. The extraction was carried out three times. The supernatants were pooled, concentrated under vacuum, and extensively dialysed against distilled water. The extract was freeze-dried, stored in a vacuum oven at 40°C and weighed.

Table 2
Yield (mg/g) and chemical composition (mg/g) of AIR from fresh sugar beet

Yield	42
Galacturonic acid	200
Rhamnose	20
Fucose	2
Arabinose	172
Xylose	11
Mannose	10
Galactose	45
Glucose	188
Ferulic acids[a]	8.2
Feruloyl dimers[b]	1.5
DM[c]	50
DA[d]	58

[a] Ferulic acids *cis* and *trans*.
[b] Feruloyl dimers = 8.5′ (18%), 5-5′ (53%) and 8-*O*-4′ (29% of total dimers).
[c] Degree of methylation.
[d] Degree of acetylation.

2.3.2. Acidic extractions

AIR (1 g) was stirred in an acid solution (30 ml) in a glass reactor bearing a reflux condenser. The temperature was regulated using an oil bath. The extraction was carried out three times. The residue was separated by filtration through G3 sintered glass. The supernatants are pooled, adjusted to pH 4.5 with 2 M NaOH, concentrated under vacuum at 35°C, and extensively dialysed against distilled water. The extract was freeze-dried, stored in a vacuum over at 40°C and weighed. The required pH (1–3) of the acid solution, the temperature (75–95°C) and the time of extraction (30–90 min) were set according to the different values of the composite design.

2.4. Analytical

The galacturonic acid content was determined by the automated *m*-hydroxybiphenyl method (Thibault, 1979) and by the method of Ahmed and Labavitch (1977) in the soluble and in the insoluble materials, respectively.

Individual neutral sugars were analysed as their alditol acetate derivatives (Blakeney, Harris, Henry & Stone, 1983) by gas chromatography after hydrolysis by 1 M H_2SO_4 at 100°C for 2 and 6 h. Six hours of hydrolysis were needed for a total rhamnose release (Micard, Renard & Thibault, 1996), but some degradation of arabinose and galactose could occur in this condition. A prehydrolysis step in 72% H_2SO_4 (30 min, 25°C) was used in the case of insoluble materials (Saeman, Moore, Mitchell & Millet, 1954).

Methanol and acetic acid were released by alkaline deesterification (0.5 M NaOH) for 1 h at 4°C. These components were separated and quantified by HPLC on C18 Superspher eluted at room temperature with sulphuric acid solution at pH = 3.5 at a flow rate of 0.7 ml/min. Isopropanol was added as internal standard. DM and DA were calculated as the molar ratio of methanol and acetic acid to galacturonic acid, respectively.

Phenolic compounds were determined by HPLC after saponification and extraction. The pectins were saponified by 2 M NaOH at 35°C under argon during 30 min in the dark. After an internal standard (*o*-coumaric acid) has been added, the solution was neutralised with 2N HCl. Phenolic compounds were extracted with ether. The ether phase was evaporated at 40°C, 1 ml of methanol/H_2O (1:1v/v) was added and samples 20 μl were injected on an HPLC system equipped with a C18 column (Purospher, Merk). Gradient elution was performed using methanol/acetic acid (1:0.01) (A) and H_2O/acetic acid (1:0.01) (B) at 0.7 ml/min at 25°C: (0 min, A = 20%; 20 min, A = 60%; 21 min, A = 80%; 30 min, A = 80%; 31 min, A = 20%). Phenolic compounds were detected at 320 nm. Response factors were determined relatively to *o*-coumaric acid (The response factor for ferulic acid was 0.54 and for the diferulic acid: 5-5′ = 0.57; 8-*O*-4′ = 1.17; 8-5′ = 1.22).

2.5. Molar mass

The pectins were solubilised in 50 mM $NaNO_3$ solution (3 mg/ml) at room temperature for 3 h under magnetic stirring, filtered over a 0.45 μm Minisart RC15 Sartorius membrane and injected at 25°C on a high performance size exclusion chromatography (HPSEC) system constituted of one Shodex SB-G pre-column followed by two Shodex OH-pak SB HQ 804 and 805 columns eluted at 0.7 ml/min with 50 mM $NaNO_3$, containing 0.02% NaN_3 as a preservative. On-line molar mass and intrinsic viscosity determinations were performed at room temperature using a multiangle laser light scattering (MALLS) detector (miniDawn, Wyatt, Santa Barbara, CA, USA) operating at three angles (41, 90 and 138°C), a differential refractometer (ERC 7517A) ($dn/dc = 0.146$ ml/g) and a differential viscometer (T-50A, Viscotek). Molar masses were determined using an universal calibration curve (pullulans 5×10^3–1600×10^3 g/mol).

3. Results

3.1. Composition of the cell wall material

The yield of AIR was 42 mg/g of fresh sugar beet (Table 2). It was mainly composed of polysaccharides (650 mg/g) and its composition was similar to that of AIR from sugar beet pulp (Micard, Renard & Thibault, 1997; Renard & Thibault, 1993). It was rich in galacturonic acid (200 mg/g) and the main neutral sugars were arabinose (172 mg/g) and glucose (188 mg/g). The DM (50) and acetylation (58) and the galacturonic acid content are typical of sugar beet pulp (Micard, Renard & Thibault, 1994). The amounts of ferulic acids and dimers were 8.2 and 1.5 mg/g of the AIR, respectively, values close to those determined in the pulp by

Table 3
Yield of extract (mg/g of AIR) composition (mg/g) and degrees of esterification of the pectins

	Yield	GalA	Rha	Ara	Gal	DM	DA
A	280	295	28	294	68	34	37
B	25	445	11	114	33	94	39
C	350	455	41	31	85	65	28
D	56	445	14	159	27	83	36
E	332	402	35	127	80	68	28
F	37	400	15	132	26	61	43
G	354	460	43	6	65	55	15
H	101	440	15	229	29	54	25
I	329	380	30	273	68	54	28
J	23	440	11	101	35	84	39
K	319	460	45	13	78	71	23
L	67	380	12	175	27	77	42
M	309	460	30	32	81	63	19
N	41	410	13	113	35	76	39
O	331	465	50	3	60	42	6
P	138	390	18	320	40	74	37
CP1	358	355	30	406	61	59	31
CP1	362	358	25	477	46	47	25
CP2	274	388	26	464	53	51	21
CP2	296	364	26	401	51	58	27
SP1	336	347	54	13	95	65	10
SP2	67	528	15	202	41	50	34
SP3	100	407	16	265	32	51	43
SP4	219	392	21	365	44	51	32
SP5	87	304	14	268	32	77	43
SP6	186	377	19	357	40	46	28

Micard et al. (1997). The proportions of dimers observed here (5-5′ diFA: 6%, 8-*O*-4′ diFA: 16%, 8-5′ diFA: 78% of total dimers) differed however from data relating to the pulp (Micard et al., 1997); and from data relating to the cell walls (Waldron, Ng, Parker & Parr, 1997). Moreover, we have not detected the 8-8′ and 4-*O*-5′ dimers. Those discrepancies could be explained by the use of different separative methods (GC and HPLC) or different response factors or by the nature of the starting material. 8-5′ was however the most abundant ferulic dimer detected in the AIR as reported in the pulp (Micard et al., 1997).

4. Extracted pectins

The water extract represented 50 mg/g of the dry AIR, in agreement with published data (Dea & Madden, 1986; Oosterveld et al., 1996; Rombouts & Thibault, 1986). Only low proportions (4.2, 2.2, 0.7, and 1.2%) of the galacturonic acid, rhamnose, arabinose and galactose, respectively, initially present in the AIR were extracted. The degrees of methylation (54%) and acetylation (35%) observed for the water soluble pectins were slightly higher than those previously reported (Guillon & Thibault, 1990). An intrinsic viscosity of 399 ml/g and a molar mass of 120×10^3 g/mol were observed, these values being at least twice as high as those obtained for water soluble pectins from pulp (Rombouts & Thibault, 1986). Since, these water soluble pectins represented only a minor amount of AIR, this step was omitted and acidic extractions were directly carried out on AIR.

A first full experimental design was developed without star points. It was shown that the type of acid used (HCl or HNO_3) had no effect on the characteristics of extracted pectins, since *P*-value observed for each characteristic was much greater than 0.05. Furthermore, the predictive ability of this first order model using linear effects was of low significance. A second order model including star points was therefore constructed. As pointed out above, the type of acid had no influence on the chemical and physicochemical characteristics of the extracted pectins thus star points experiments were only performed on HCl.

4.1. Yield

Yield expressed as dry weight of the extract varied from 23 to 354 mg/g of the AIR dry matter (Table 3). The highest yields were obtained at pH = 1. Yields observed are much higher than those obtained from the pulp at pH 1 by Michel et al. (1985) (172–207 mg/g) indicating the importance of the starting material. The variance analysis showed that pH was the main influent parameter influencing yield. Yield increased with decreasing pH. Data were fitted to a second order empirical model with a *R*-squared 82% and it was shown that the quadratic effects of temperature and time of extraction were quite important. The regression equation derived for pectin yield was

$$\text{yield} = 25.72 - 12.66X_1 + 3.06X_2 + 2.12X_3 + 4.31X_1^2 + 4.88X_2^2 - 7.18X_3^2$$

where X_1, X_2 and X_3 are the coded values of pH, temperature and time of extraction, respectively.

4.2. Composition

The galacturonic acid content varied from 295 to 528 mg/g of extract (Table 3). These values are slightly lower than those obtained for pectins extracted from the pulp, (around 650 mg/g, Arslan, 1995; Michel et al., 1985). These discrepancies can be attributed to the different recovery means (dialysis against water in this study and ethanol precipitation in the other studies), which for example will determine the extent of arabinan removal. There is only a moderate influence of pH, corresponding quadratic effect, temperature and interaction pH-temperature on galacturonic acid contents with slightly higher contents at pH 3, 95°C, 60 min. Galacturonic acid content of the extract was not related to the extraction yields. The amounts represented generally the same proportion of the extract but the extractions were more efficient at low pH 41–81%, 13–67% and 5–27% of the galacturonic acid present in the AIR were extracted at pH 1, 2 and 3, respectively.

Rhamnose content varied from 11 to 54 mg/g (Table 3). The *R*-squared value (92%) indicated that the model

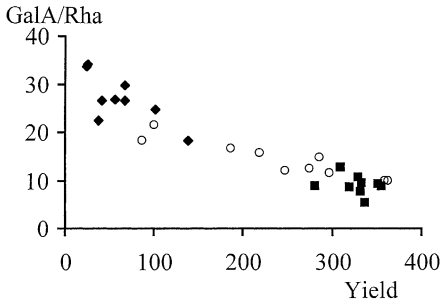

Fig. 1. Molar ratio GalA/Rha according to the yield of the extract (mg/g of AIR) and pH of extraction: pH 1; ■ pH2; ○ and pH 3 ◆.

correlates particularly well with the experimental results. Linear and quadratic pH terms had an important significant effect on the rhamnose contents while the quadratic time term was also significant but to a lesser extent. Higher rhamnose contents were obtained at pH 1, 60 min. Values observed for this pH were higher than those obtained by Rombouts and Thibault (1986) for pectins from AIR of the pulp. The regression equation derived for rhamnose was

$$\text{Rha} = 2.57 - 1.31X_1 + 1.10X_1^2 - 0.7X_3^2$$

Fig. 1 shows that the molar ratio GalA/Rha decreased with an increasing yield. Different kinds of pectins could be extracted according to the conditions: the extracts seemed to be richer in rhamnogalacturonans at pH 1, and richer in homogalacturonans at pH 3.

Galactose content varied from 27 to 95 mg/g (Table 3), this range being in agreement with previously published data (Guillon & Thibault, 1988; Rombouts & Thibault, 1986; Thibault 1988). The model showed a good correlation with the observed results as it explained 91% of the variability in galactose contents. pH and quadratic effects of all factors showed an important effect on galactose contents. The equation of the fitted model was

$$\text{Gal} = 5.11 - 2.37X_1 + 2.05X_1^2 - 0.95X_2^2 - 1.15X_3^2$$

Arabinose contents exhibited a large variation from 3 to 477 mg/g (Table 3). The values were generally higher than

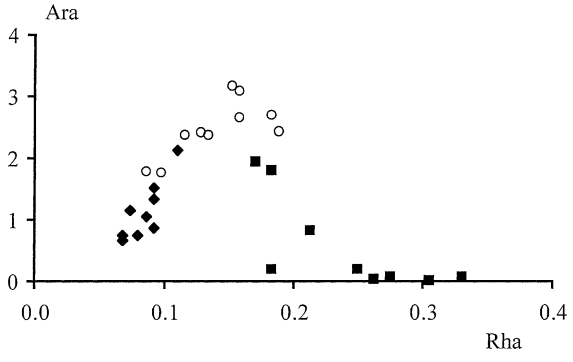

Fig. 2. Arabinose amount (mmol/g of extract) according to rhamnose amount (mmol/g of extract) and pH of extraction: pH 1; ■ pH2; ○ and pH 3 ◆.

those obtained by Rombouts and Thibault (1986). The quadratic pH term was from far the most important for the arabinose content. The simplified regression equation was

$$\text{Ara} = 36.37 - 21.6X_1^2 + 6.6X_1X_2$$

Arabinose content was highest at pH = 2 ($X_1 = 0$).

Fig. 2 showed that different pectin groups could be obtained according to pH. At pH 1, the extracts were rich in rhamnose and poor in arabinose. Degradation of the arabinan chains probably occurred at pH 1, the arabinofuranosyl linkages being particularly acid labile (BeMiller, 1967). At pH 2, the extracts were richer in arabinose and poorer in rhamnose. At pH 3, since conditions extraction of both arabinose and rhamnose was low. The ratio Gal/Rha did not vary much whatever the extraction yield. Except at pH 1, where arabinose side chains were hydrolysed, the Ara/Rha molar ratio was always higher than the Gal/Rha one, confirming the predominance of arabinan side chains in sugar beet pectins. Some conditions can lead to complete removal of the arabinose while the galactose residues were retained within rhamnogalacturonans.

4.3. Substitution

DM varied from 34 to 94 (Table 3). Central points and star points values were close to those determined by Michel et al. (1985) for pectins extracted from the pulp and similar conditions. The data fitted a second order empirical model with a *R*-squared 71%; the interaction pH-time was the main significant parameter. The time of extraction, its quadratic effect, and pH also had a slight effect on DM. The regression equation for DM was

$$\text{DM} = 54.7 + 5.5X_1 - 6.9X_3 - 10.75X_1X_3 + 8.99X_3^2$$

The results showed that very highly methylated pectins exist in the cell wall. The values of DM increased with increasing pH, as described by Joye and Luzio (2000). The highest values were obtained for short extraction times.

DA varied from 6 to 43 (Table 3). pH had a more marked effect on DA than temperature and time. The variance analysis showed that DA could be fitted to a first order model, but a second order model explained the variability in DA as the quadratic effects of pH and temperature were relatively important. The regression equation derived for DA was

$$\text{DA} = 29.8 + 5.9X_1 - 5.4X_2 - 4.35X_3 - 8.98X_1^2 + 6.5X_2^2 + 4.3X_3^2$$

The highest values were obtained at pH = 2 at lower temperature. A DA of 58% can be calculated for the AIR, assuming that acetyl groups esterify only GalA residus. All the values of the extracted pectins were lower, indicating that acetic acid was released during extraction and/or that it esterified polymers other than the acid-soluble pectins.

Ferulic acid monomer contents varied from 2.6 to

Table 4
Ferulic components (μg/g) and proportion of dimers (% of total dimers)

	Ferulic acid	Diferulic acids	5-5′	8-*O*-4′	8-5′
A	7990	1060	11	42	47
B	2600	120	12	25	63
C	5970	690	9	51	41
D	3490	310	6	23	70
E	6680	1130	10	40	50
F	3240	110	14	25	61
G	5050	430	10	63	28
H	5020	100	2	31	67
I	9520	1800	12	38	50
J	5320	500	25	37	37
K	90	50	34	22	44
L	4270	190	7	21	71
M	5460	640	12	50	37
N	2820	100	9	41	50
O	2490	590	8	26	66
P	6880	290	9	37	54
CP1	12,000	390	23	64	13
CP1	11,170	520	18	44	37
CP2	13,270	540	24	50	26
CP2	13,820	400	20	57	23
SP1	6510	1060	68	28	4
SP2	4640	160	18	32	51
SP3	9610	130	26	44	30
SP4	9780	230	18	60	21
SP5	7030	120	20	28	52
SP6	13,200	330	52	33	15

16.7 mg/g (Table 4); this range being in agreement with previously published data on sugar beet pulp (Oosterveld, Beldman, Schols & Voragen, 2000). The *R*-squared value (88%) indicated that the model showed a particularly good correlation with observed values. pH and its quadratic effect were the main factors influencing ferulic acid contents. The highest values were obtained at pH = 2, 85°C and for 90 min.

The presence of ferulic dimers in the extracted pectins was shown, suggesting that some extracted pectins may still be cross-linked. The sum of dehydroferulic acids varied from 0.1 to 1.8 mg/g of the extract (Table 4). Highest values were obtained at pH 1 and an extraction time of 60 min. pH and its quadratic effect were the important factors. Dimers resulting from 8-5′, 5-5′ and 8-*O*-4′ radical couplings were detected. The 8-5′ and 8-*O*-4′ radical coupling represented the main dimers. The proportions of these dimers varied widely according the extraction conditions: the weight ratio of dehydrodiferulic acids to ferulic acid varied from 0.01 to 0.60.

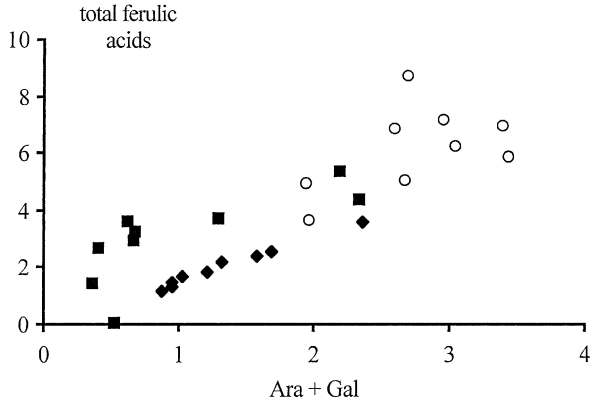

Fig. 3. Total ferulic components (mmol/g of extract) according to arabinose and galactose amounts (mmol/g of extract) and pH of extraction: pH 1; ■ pH2; ○ and pH 3 ◆.

The content of ferulic acids increased with an increasing amount of arabinose and galactose (Guillon et al., 1989; Ralet et al., 1994). At pH 3, the extraction conditions were not efficient to extract side chains and ferulic components. At pH 2, the conditions led to higher yields and extracts were rich in side chains and ferulic components. At pH 1, the ferulic acid contents were low. At this pH, the degradation of arabinan chains probably led to a loss of ferulic components (Fig. 3).

4.4. Physico-chemical properties

When analysed by HPSEC-MALLS, pectin samples gave a large light-scattering signal at the excluded volume but a negligible refractive index signal. This was attributed to the presence of aggregated materials (Kravtchenko, Berth, Voragen & Pilnik, 1992), which led to an overestimation of molar mass. Molar masses were therefore determined by on-line viscosimetry through universal calibration.

Intrinsic viscosity varied from 172 to 493 ml/g (Table 5). The model explained 84% of the variability, showing a good correlation with the observed results. The analysis of variance showed that pH and its quadratic effect, temperature and interaction pH-temperature had important effects on viscosity. Highest values were obtained at pH 1.5, 85°C for 30 or 60 min. The simplified regression equation was

$$\text{viscosity} = 350.8 - 34.2X_1 - 42.1X_2 - 103.5X_2^2 + 36.1X_1X_2$$

Except for extremely harsh extraction conditions, the viscosity values observed are much higher than those observed for pectins extracted from pulp (Arslan, 1995; Rombouts & Thibault, 1986), showing again the importance of the starting material.

The molar mass varied from 70 000 to 355 000 g/mol. (Table 5) The *R*-squared values indicated that the model explained only 61% of the variability in molar masses. pH, its quadratic effect and the interaction pH-temperature were found to be significant. The simplified regression equation was

$$M_w = 272,000 - 42,000X_1 - 128,800X_1^2 + 37,700X_1X_2$$

The highest molar masses were observed at pH 2 and at 75°C.

Polydispersity index varied from 1.7 to 4.7 but the model did not adequately represent the data; only 55% of the variability was explained by the second order model.

Table 5
Macromolecular characteristics of pectins

	Molar mass (g/mol)	Intrinsic viscosity (ml/g)	Mark–Houwink coefficient	Polydispersity
A	355,000	342	0.3	3.6
B	128,000	454	1.1	1.6
C	202,000	304	0.5	3.4
D	118,000	351	1.0	1.6
E	273,000	337	0.4	1.8
F	177,000	416	1.1	1.6
G	119,000	234	0.9	2.4
H	122,000	265	0.8	2.0
I	311,000	372	0.3	3.5
J	129,000	493	1.1	1.6
K	237,000	240	0.5	4.1
L	130,000	394	0.9	1.7
M	249,000	321	0.5	4.0
N	150,000	428	1.1	1.7
O	700,00	172	1.3	2.0
P	258,000	333	0.4	2.9
CP1a	352,000	324	0.3	4.4
CP1b	312,000	322	0.3	4.5
CP2a	343,000	339	0.3	4.4
CP2b	377,000	293	0.3	4.7
SP1	780,00	210	1.2	1.7
SP2	120,000	319	0.9	1.7
SP3	189,000	397	0.6	2.6
SP4	307,000	275	0.3	3.6
SP5	172,000	374	0.6	1.2
SP6	292,000	295	0.3	3.5

The Mark–Houwink coefficient varied from 0.28 to 1.19. As for M_w and viscosity, the important parameters were pH and its quadratic term. The highest values were obtained at the lowest and highest pH levels and at the central point for temperature and time of the extraction.

5. Discussion

Pectins were recovered from fresh sugar beet by acidic extractions of the alcohol insoluble residue, under different conditions. The amount of acid-extracted sugar beet pectins varied from 23 to 354 mg/g of the initial material. For similar extraction conditions, significantly higher amounts of pectins were extracted from fresh sugar beet AIR than from pulp (Arslan, 1995; Michel et al., 1985; Rombouts & Thibault, 1986). A better extractability of the pectic polymers was evidenced. Renard and Thibault (1991) have suggested that a secondary network of oxidized polyphenols could be formed during the juice extraction and drying of apple. The different treatments applied to the beet during the sucrose extraction may induce some artefacts and modify the solubility of polysaccharides even if a drying process was also applied during the preparation of the AIR.

Different kinds of pectins could be extracted according to the conditions of pH, time and temperature. At pH 3, extracted pectins had a composition similar to that of the water-soluble pectins. The yields were low, the extracts were poor in rhamnose and the molar masses were similar (around 130,000 g/mol). Decreasing pH led to the extraction of higher amounts of pectins richer in rhamnogalacturonans. The amount and ratio of arabinan and galactan side chains varied widely with the extraction conditions. Pectins with high DM (extract E, SP5…) and/or low DA (SP1, G…) may also be extracted. Previous studies (Guillon & Thibault, 1988; Rombouts & Thibault, 1986; Thibault, 1988) reported that DM and DA of acid-extracted pectins from sugar beet pulp were generally around 50 -60, and 30, respectively. In this work, DM and DA of the extracted pectins from fresh sugar beet were generally within this range even if low amounts of pectins with high DM values were obtained by extraction at pH 3, in agreement with previously reported data (Joye & Luzio, 2000). High yields of sugar beet pectins of high DM can be obtained by water extraction after thermal or thermomechanical treatments such as autoclaving and extrusion-cooking (Oosterveld et al., 2000; Ralet, Thibault & Della Valle, 1991), indicating that such highly methylated pectins are initially present in the cell walls. Some extraction conditions (G, SP1), led to high yields of pectins with low DA, which might gel under the conditions of high sugar and acid or Ca^{2+}.

Ferulic acids are carried by the neutral sugar side chains of pectins (Rombouts & Thibault, 1986). They esterify about 50–55% of the O-2 position of arabinose moieties and 45–50% of the O-6 position of galactose residues (Colquhoun, Ralet, Thibault, Faulds & Williamson,

1994; Ishii, 1994; Ralet et al., 1994). In this study, the acid-extracted pectins contained some feruloyl groups, the amount of ferulic compounds increasing with the amount of arabinose and galactose residues. The occurrence of ferulic acid in pectins particularly poor in arabinans confirmed that part of the ferulic acid residues esterify galactose moieties in sugar beet pectins. The occurrence of dehydrodiferulic acids was evidenced in most of the pectins studied. The presence of dehydrodiferulic acids has been shown in sugar beet pulp (Micard et al., 1997) suggesting that cross-linking of arabinans and/or (arabino)-galactans side chains may occur in the cell wall. Recently, pectins isolated from sugar beet pulp by autoclaving were shown to contain significant amounts of ferulate dehydrodimers (Oosterveld et al., 2000). The amounts of ferulic acid and diferulic dimers varied from 0.09 to 13.8 mg/g and 0.05–1.8 mg/g of the extract, respectively. Only three dimers (8-5′, 5-5′ and 8-*O*-4′) were detected, 8-5′ and 8-*O*-4′ being the major ones. The predominance of these two compounds is in disagreement with the results obtained on water extracted pectins after sugar beet pulp autoclaving in which 8-*O*-4′ dimer is from far the major dimer (0.53 mg/g of extract), 8-5′ dimer being present in small amounts (0.30 mg/g of extract) (Oosterveld, Grabber, Beldman, Ralph & Voragen, 1997).

The molar masses of the different extracts varied often in a wide range, the highest values being obtained at pH 2. Viscosities and molar masses observed were much higher than those obtained for pectins from the pulp (Rombouts & Thibault, 1986). The starting material may have an influence as mentioned before. Another reason may be found in the high content in ferulic dimers, which can cross-link pectic chains, and which could also explain the high intrinsic viscosities and molar masses observed. Some extracts exhibiting high molar masses were particularly rich in dimers (extracts A, E, I, M). Further work must be carried out to determine the exact location of these dimers. No correlation between the viscosity and the molar mass of the extracts was evidenced. This was mainly due to the large variation of the Mark–Houwink coefficient. For extracts containing high amounts of galactose and arabinose side chains, the Mark–Houwink coefficient was generally below 0.5, revealing a quite compact coil structure. A Mark–Houwink coefficient value of 1–1.3 was observed for pectins particularly rich in homogalacturonans or with low amounts of neutral sugar side chains. This reveals a more extended and rigid conformation. Analyses of these characteristics are complicated by the polyelectrolyte nature of pectins, as the molecules could adopt different conformations according to their DM. Pectic molecules are thought to become less extended and more coiled as the DM increases (Morris, Foster & Harding, 2000; Thibault, Guillon & Rombouts, 1991). The DA and the presence of the dehydroferulic dimers could also have some influence.

6. Conclusion

We have shown that extraction conditions have important effects on the features of extracted pectins. The quantity as well as the quality and the characteristics of the extracted pectins varied often in a large range according to the experimental conditions of extractions. It was possible to obtain pectins with specific characteristics (high M_w, high DM, low DA, …) and thus to increase their potential uses. The impact of ferulic acid dimers on molar mass needs to be investigated further.

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