Carbohydrate Polymers **8** (1988) 209–223

Characterisation and Oxidative Crosslinking of Sugar-Beet Pectins Extracted from Cossettes and Pulps under Different Conditions

J.-F. Thibault

INRA, Laboratoire de Biochimie et Technologie des Glucides, Rue de la Géraudière, 44072 Nantes Cédex 03, France

(Received 9 March 1987; accepted 11 January 1988)

ABSTRACT

Pectins have been extracted from sugar-beet cossettes by a sequential treatment with water, oxalate, hot dilute acid and cold dilute alkali, and from pulp with different conditions of pH and temperature. All these samples have been chemically characterised and tested for their gelling capacity with persulphate. Pectins from cossettes have characteristics very close to those previously extracted in the same way but are not able to give gels by addition of persulphate. On the other hand, some, but not all, of the pectins from pulp can gel under these conditions. No simple relationship was found between the gelling ability and the chemical characteristics of the pectins.

INTRODUCTION

Apple marks and citrus peels have been until now the only sources of commercial pectins. Substitutes for these pectins have been investigated, mainly from sunflower (Sosulski *et al.*, 1978) and sugar-beet (Kertesz, 1951). Despite the large quantity of beet pulp left after sugar extraction and the high pectin content of sugar-beet pulp, commercial production of beet pectins has failed because they have poor gelling power, which has been ascribed to their acetylesterification (Pippen *et al.*, 1950). Recently, the fine structure of beet pulp pectins has been studied and the presence of feruloyl groups in the part of the rhamnogalacturonan backbone carrying neutral sugar side-chains (the 'hairy regions') has been shown (Rombouts & Thibault, 1986*a*, *b*). This substitution was also reported for spinach pectins (Fry, 1983) but was not found in other

Carbohydrate Polymers 0144-8617/88/$03.50 —

pectins (Rombouts *et al.* 1983). It is possible to take advantage of the presence of feruloyl groups in beet pectins by carrying out 'coupling' reactions, using either hydrogen peroxide-peroxidase (Rombouts *et al.*, 1983) or ammonium persulphate (Thibault & Rombouts, 1986) leading to chemical gelation. These gels can be dried easily and the resulting powders containing crosslinked beet pectins have remarkable water-absorption capacities (Thibault, 1986) in contrast to gels obtained from apple or citrus pectins. This property may lead to new and specific applications for beet pectins.

In the present paper, pectins have been extracted from pulp under different acidic conditions, and from the whole beet by water, oxalate, acid and alkali. The main aims of this work were to examine the effects of the processing of the beet on the chemical characteristics of the pectins, and to study their persulphate-induced gelation. This study should also indicate which conditions are needed for the extraction in order to obtain beet pectins with a good gelling power, and should be considered as a first approach to the role of their chemical structure in their reactivity with persulphate ions.

MATERIALS AND METHODS

Sugar-beet cossettes and pulp

Sugar-beet cossettes were obtained in a dried form from the Sugar Factory in Saint-Martin (France); the pulps and the pellets were purchased from the Sugar Factory in Eppeville (France).

Extraction of pectins from sugar-beet cossettes

The sucrose of the cossettes was extracted by boiling with 80% ethanol under reflux until the filtrate does not give colour in the phenol-sulphuric acid test. From this alcohol-insoluble residue (AIR), pectins were sequentially extracted with water and ammonium oxalate at room temperature, hot (85°C) dilute (0·05N) hydrochloric acid and cold (4°C) dilute (0·05N) sodium hydroxide, giving water-soluble (WSP), oxalate-soluble (OXP), acid-soluble (HP) and alkali-soluble (OHP) pectins, respectively. HP and OHP were purified by precipitation with cupric ions. The extraction conditions and the purification step were exactly those previously used for pulp pectins (Rombouts & Thibault, 1986*a*).

Pectins from sugar-beet pulp

Five other pectin samples were used in this study. Sample 1 is the pectin previously investigated (Thibault & Rombouts, 1986). Samples 2, 3 and 4 were extracted as described by Michel *et al.* (1985). Sample 2 was extracted from an AIR of pulp by hydrochloric acid at pH 1·5 and at 85°C for 4 h. Sample 3 was obtained from pulp by hydrochloric acid extraction at pH 1 and at 95°C for 2 h, and Sample 4 was a pectin extracted from pellets with nitric acid at pH 1·5 and at 85°C for 1 h. Sample 5 was a gift of Copenhagen Pectin Factory (Lot No. 515690).

All the pectin samples were solubilised in water and precipitated by four volumes of 95% ethanol. The precipitates were extensively washed with 60% ethanol before drying at 35°C under reduced pressure.

Chromatography on DEAE-Sephacel

Ion-exchange chromatography was performed on columns (9·5 × 1·6 cm) of DEAE-Sephacel equilibrated with 0·05M sodium acetate buffer pH 4·8. After loading of the sample (3 mg), the column was washed with 50 ml of equilibrating buffer and then eluted by a linear gradient (90 ml) of 0·05–1M sodium acetate buffer pH 4·8. Fractions (4 ml) were analysed for their galacturonic acid content by the automated *m*-hydroxybiphenyl method (Thibault, 1979), for their total neutral sugar content (expressed in arabinose equivalents) by the automated orcinol method (Tollier & Robin, 1979) after correction for interferences of uronic acids, and for their feruloyl group content by spectrophotometry as described below. Chromatograms were recalculated for 1 mg of galacturonic acid residues recovered.

Analytical methods

The contents in methanol and in acetic acid were obtained, after alkaline deesterification in alcoholic suspension, by HPLC on an HPX-87H column as described by Voragen *et al.* (1986). From these contents and the content in galacturonic acid residues as determined above, the degree of methylation (DM) and the degree of acetylation (DA) were calculated and expressed as moles of ester per 100 moles of galacturonic acid residues.

Individual sugars were determined by GLC after hydrolysis of the pectins in 1M H_2SO_4 at 100°C for 1·5 h followed by reduction and acetylation (Albersheim *et al.*, 1967). Proteins were estimated as N (Kjeldahl method) × 6·25.

The amounts of feruloyl groups were measured spectrophotometrically from the absorbance at 375 nm of solutions of pectins in 0·067 M glycine–sodium hydroxide buffer, assuming a molar extinction coefficient of 31 600 (Fry, 1982). Ferulic acid, and other phenolic acids, were also analysed by HPLC on a Rsil C18-5 μm column (25 × 0·46 cm) after alkaline hydrolysis (Sharma *et al.*, 1986). About 25 mg of pectins were deesterified in 25 ml of 2 N NaOH at 35°C for 2 h under argon. The solution was acidified to pH ~ 2 by hydrochloric acid and the phenolic acids were extracted by three volumes of diethylether. The extracts were dried on anhydrous sodium sulphate, evaporated to dryness, and dissolved in 1 ml of methanol. 20 μl of this solution were injected on the column at room temperature. The solvent was methanol/water/acetic acid (26/73/1, v/v) at a flow rate of 0·9 ml min^{-1}, and the eluate was monitored by detection at 280 nm. Standards of coumaric, ferulic, chlorogenic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, vanillic and syringic acids were used.

All the values are on a moisture-free basis.

Intrinsic viscosity measurements

Intrinsic viscosities $[\eta]$ were obtained at 25°C by measuring the flow times of solutions of pectins in an automatic Ubbelholde viscometer (Fica, France). The solvent was 0·155 M NaCl and had a flow time of 171 s. Viscosity-average molecular weights were calculated according to Owens *et al.* (1946).

Kinetics of action of ammonium persulphate

The crosslinking reaction was followed by spectrophotometry and by viscometry with solutions of pectin under different conditions. The acid form of the pectins was obtained by percolating salt-free solutions through a strong H^+ resin (Amberlite IR 120) and the sodium form was obtained by exact neutralisation, monitored by conductimetry, with sodium hydroxide.

The rate of disappearance of feruloyl groups in the presence of ammonium persulphate (0·01–0·02 M) at 25°C was followed at 375 nm in alkaline conditions as previously described (Thibault *et al.*, 1987). The apparent rate constant (k_{app}) was determined by

$$\log \frac{A_t - A_\infty}{A_0 - A_\infty} = k_{app} t$$

where A_0, A_t and A_∞ were absorbances initially, at time t, and at infinity, respectively. A_∞ was found to be equal to 18–20% of A_0. The rate constant (k_2) was therefore obtained from

$$k_2 = k_{app}/[\text{ammonium persulphate}]_0$$

The increase in viscosity was followed at 25°C as a function of reaction time, in an automatic Ostwald viscometer (Fica, France, ϕ 0·58 mm), as previously described (Thibault & Rombouts, 1986). The mixtures contained 0·48% of pectin, 0·01 M ammonium persulphate and the initial pH value was adjusted to 4·4–4·5.

Preparation and swelling of crosslinked pectins

Solutions of pectins were made 2·4% in 0·01 M ammonium persulphate and left at room temperature. Resulting gels were disrupted and washed with water in order to separate water-soluble and water-insoluble materials (Thibault, 1986). The crosslinked water-insoluble pectins were converted to the acid form by washing with aqueous 70% ethanol containing 1% concentrated hydrochloric acid, and the modified pectinic acids were converted to the sodium form by exact neutralisation, followed by conductimetry, with sodium hydroxide.

The degree of swelling was determined by the bed volume technique and expressed as ml of bed volume per g of dry product in the H^+ form (Thibault, 1986).

RESULTS

Characterisation of pectins extracted from sugar-beet cossettes

The yield in AIR from the whole sugar-beet was 17·7% and from this AIR, ~ 30% of pectic material can be extracted sequentially with water, oxalate, acid and alkali (Table 1) with high amounts of HP and OHP and low amounts of WSP and OXP. The crude extracts were also characterised by their galacturonic acid, neutral sugars and feruloyl contents. WSP and OXP were richer in galacturonic acid and poorer in neutral sugars and feruloyl groups than HP and OHP (Table 1).

The extracts were chromatographed on DEAE-Sephacel columns (Fig. 1). The unbound material amounted to < 1%, < 0·5%, 9% and 2% of the injected pectins for WSP, OXP, HP and OHP, respectively. The bound pectins were eluted with a linear gradient. WSP and HP were eluted as a sharp and homogeneous peak whereas OXP was eluted as a

TABLE 1
Yields of Pectins Extracted from AIR of Sugar-Beet Cossettes and Their Contents of Galacturonic Acid, Neutral Sugars and Feruloyl Groups

	% AIR	*% galacturonic acid*	*% neutral sugars*	*% feruloyl groups*
Water-soluble pectins (WSP)	2·1	45·7	8·7	0·29
Oxalate-soluble pectins (OXP)	1·2	51·6	6·7	0·22
Acid soluble pectins (HP)	17·4	38·0	23·5	0·70
Alkali-soluble pectins (OHP)	8·4	37·6	15·7	0·59

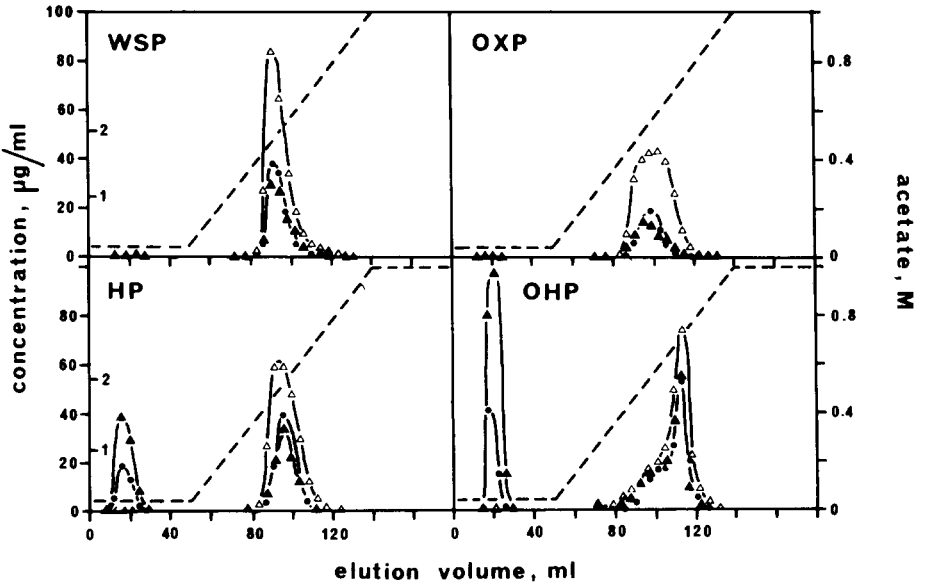

Fig. 1. Ion-exchange chromatography of pectins from sugar-beet cossettes on DEAE-Sephacel; △, galacturonic acid residues; ▲, neutral sugars; ●, feruloyl groups.

more tailing peak, and OHP was partly irreversibly bound to the gel as indicated by the yields of 7% and 28% in galacturonic acid and neutral sugars, respectively. Feruloyl groups were found in the unbound (up to 0·4%) as well as in the bound material, where they coeluted with galacturonic acid and neutral sugars.

Due to the different amounts of contaminating neutral polysaccharides, WSP and OXP were not purified, whereas HP and OHP were purified by the copper precipitation method. This procedure led to the elimination of materials containing mainly arabinose, galactose and glucose in a molar ratio of 75/16/6 and 66/15/11 for HP and OHP, respectively, with only traces of galacturonic acids.

The composition of the purified pectins is shown in Table 2. The galacturonic acid contents were in the range 45·7–56·8% while the neutral sugar contents varied between 12·5–25%, with the predominant presence of arabinose (7·3–13%) and galactose (2·7–11%), and minor amounts of rhamnose, fucose, xylose, mannose and glucose. The molar ratio galactose/arabinose varied between 1 (OHP) and 0·3 (WSP, OXP).

Acceptable agreement was found in the feruloyl contents (0·2–0·7%) determined by HPLC and spectrophotometry. The HPLC showed minor amounts ($< 0{\cdot}01\%$) of other phenolic acids such as vanillic, syringic and *p*-coumaric acids.

TABLE 2
Characteristics of Purified Pectins Extracted from Sugar-Beet Cossettes

	Water-soluble (WSP)	*Oxalate-soluble (OXP)*	*Acid-soluble (HP)*	*Alkali-soluble (OHP)*
Galacturonic acid[a]	45·7	51·6	56·8	56·1
Total neutral sugars[a]	13·5	12·5	25·0	25·0
Rhamnose + fucose	1·0	1·3	3·3	4·2
Arabinose	7·6	7·3	13·0	8·7
Xylose	0·5	0·3	0·3	0·3
Mannose	0·4	0·2	0·1	0·2
Galactose	2·7	2·9	7·9	11·0
Glucose	1·3	0·5	0·4	0·6
Feruloyl groups[b]	0·29	0·22	0·71	0·60
Feruloyl groups[c]	nd	nd	0·71	0·51
Proteins	nd	nd	10·4	19·6
Degree of methylation	72·7	64·1	50·4	< 1
Degree of acetylation	27·4	20·6	31·6	1·6
Intrinsic viscosity (ml g^{-1})	280	386	278	207
Huggins constant	0·48	0·65	0·53	0·44
Viscosity-average molecular weight	54 400	64 000	50 100	40 200

[a]Sugars are expressed in anhydrous form (% w/w).
[b]Measured by spectrophotometry.
[c]Measured by HPLC.
nd = not determined.

Viscometric parameters and viscosity-average molecular weights are also given in Table 2. The data show that the molecular weights are in the range 40 000–64 000.

Characterisation of pectins extracted from sugar-beet pulp

Five other pectin samples (1–5) were used in this study. They were extracted from pulp or pellets under acidic conditions. These samples were not purified because their contents of contaminating neutral polysaccharides were low (< 2%) as judged by ion-exchange chromatography, and because they were considered in this study as representative of potentially commercial samples. Their characteristics are shown in Table 3. The galacturonic acid content varied between 64·0–73·7% and the neutral sugar content between 10·3–20·6%. The molar ratio galactose/arabinose had values between ≈ 5·7 (Sample 1) and ≈ 1·5 (Samples 4 and 5). Spectrophotometry and HPLC gave similar values of content of feruloyl groups (range 0·28–0·83%) and HPLC revealed the presence of traces of *p*-coumaric, vanillic, hydroxybenzoic, and syringic acids. The viscosity-average molecular weights were in the range 13 300–48 300.

Kinetics of the action of ammonium persulphate

Previous work (Thibault *et al.*, 1987) showed that the reaction of persulphate on sugar-beet pectins followed a pseudo-first order law with respect to pectin and a pseudo-first order law with respect to persulphate. It was also demonstrated that the pH value has a profound influence on the reaction rate, in contrast with the conformational state of the pectins, and that the presence of buffer ions may inhibit the reaction (Thibault *et al.*, 1987). For these reasons, the second-order rate constant k_2 was determined on all the pectin samples in acidic and in sodium forms. The kinetics of the disappearance of feruloyl groups followed for all the pectic samples a pseudo-first order law with respect to pectin concentrations; the k_2 values are listed in Table 3.

The ionic form of the pectin had a great influence on the values of the overall rate constant except for HP and OHP, which are characterised by constant and low values. Marked differences were shown between Sample 1 which had a high rate constant, and the other samples which reacted very slowly with persulphate ions, and especially pectins extracted from the cossettes.

The course of the reaction was also followed by viscometry for all the samples at the same concentration and at approximately the same initial pH value. Sample 3 did not show any increase in viscosity up to 40 h of

TABLE 3
Characteristics of Pectins Extracted from Sugar-Beet Pulp

	Samples				
	1	*2*	*3*	*4*	*5*
Galacturonic acid[a]	66·0[d]	67·4	73·7	64·0	66·7
Total neutral sugars	17·1[d]	15·5	10·3	20·6	17·0
Rhamnose + fucose	2·8	4·2	3·1	4·7	4·6
Arabinose	1·7	1·7	1·2	5·2	3·2
Xylose	0·2	0·3	1·4	0·6	0·3
Mannose	0·2	0·1	1·2	0·1	0·1
Galactose	11·9	8·8	3·4	9·5	8·5
Glucose	0·3	0·4	2·0	0·5	0·3
Feruloyl groups[b]	0·83	0·61	0·28	0·66	0·57
Feruloyl groups[c]	0·79	0·59	nd	0·49	0·57
Proteins	4·1[e]	9·5[e]	nd	1·9	8·2
Degree of methylation	55·7	49·3	nd	35·5	61·4
Degree of acetylation	19·7	15·3	nd	12·8	19·2
Intrinsic viscosity (ml g^{-1})	138	207	47	182	265
Huggins constant	0·47	0·61	0·11	0·52	0·45
Viscosity average molecular weight	29 700	40 200	13 300	36 500	48 300

[a]Sugars are expressed in anhydrous form.
[b]Measured by spectrophotometry.
[c]Measured by HPLC.
[d]Values from Thibault & Rombouts (1986)
[e]Values from Michel *et al.* (1985).
nd = Not determined.

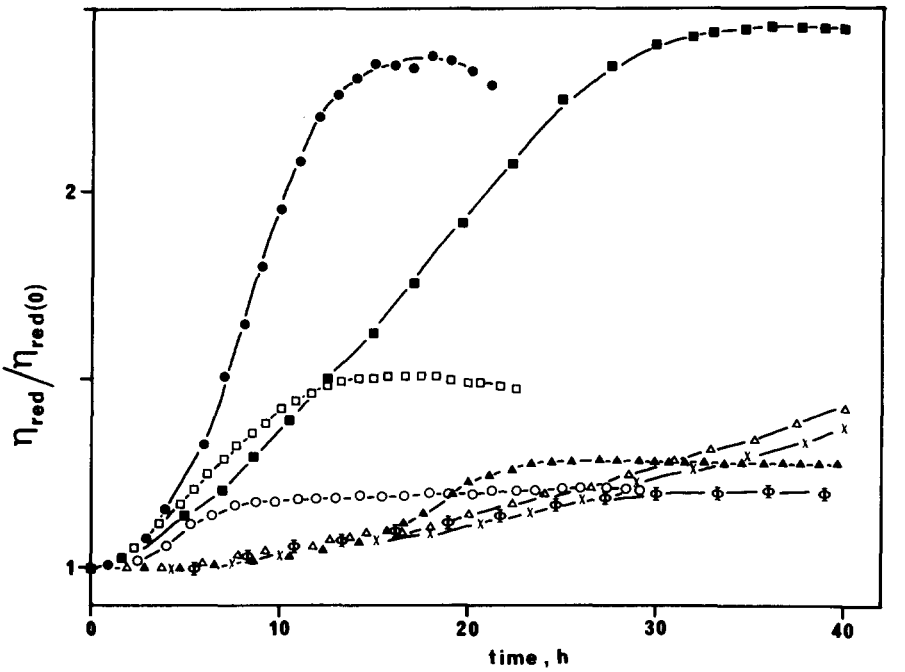

Fig. 2. Changes with time of the ratio of reduced viscosity to initial reduced viscosity of 0·48% solutions of pectins in 0·01 M persulphate at 25°C; ●, Sample 1; □, Sample 2; ■, Sample 4; ▲, Sample 5; ○, WSP; Φ, OXP; X, HP; △ OHP.

reaction time in contrast to the other samples (Fig. 2). Samples 1, 2 and 4 were characterised by rapid increases in viscosity and reached maximum values of reduced viscosity of 1450 ml g^{-1}, 365 ml g^{-1}, 660 ml g^{-1} after 780, 950 and 2250 min, respectively. For Sample 5, WSP, OXP, HP and OHP, a slow increase in viscosity was observed, reaching values of 495, 430, 950, 1450, and 725 ml g^{-1} after 3100, 1490, 2900, 12300, and 5600 min, respectively. No simple relationship exists between the viscometric behaviour and the values of the rate constant; apparently the higher the rate constant, the more rapid the increase in viscosity, but Sample 3 had an intermediate value of the rate constant and was characterised by a constant viscosity.

Gelling of the pectins samples

When solutions were made with a pectin concentration of 2·4% and 0·01 M in persulphate and were left at room temperature, some gelled. Pectins extracted from sugar-beet cossettes and Sample 3 did not gel (up

to seven days). Samples 2 and 5 gelled after a reaction time of ≈ 24 h while Sample 4 gelled after ≈ 40 h and a setting time of ≈ 7 h was previously published for Sample 1 (Thibault & Rombouts, 1986).

Each gel contained water-soluble material (range 22·4–36·1%, Table 4) carrying no feruloyl groups. This fact was also previously reported for Sample 1 (Thibault & Rombouts, 1986; Thibault, 1986).

The water-absorption capacities of the modified pectins measured by the bed volume technique are also indicated in Table 4. Values ranged between 55–180 ml g^{-1} in salt-free solutions, depending mainly on the ionisation of the insoluble pectins rather than the nature of the initial pectin. Differences between values recorded for pectins in the acid form and in the sodium form were due to the establishment of electrostatic repulsions between crosslinked pectins when they are ionised. The expansion of the networks can be reduced by addition of salts in the surrounding solution, leading to a screening effect of the fixed charges and, therefore, to a decrease of the swelling. For example, the addition of 0·1 M sodium chloride depressed the swelling of the crosslinked pectins down to values of about 50–75 ml g^{-1}.

DISCUSSION

Comparison of sugar-beet pectins extracted from cossettes and pulps

In order to obtain directly comparable results, the extraction of the pectins from whole sugar-beet was performed exactly in the same way as from sugar-beet pulp (Rombouts & Thibault, 1986*a*). The amount and the distribution of extracted pectins are very similar. The surprisingly low amount of WSP extracted from the cossettes was not reported in other studies (Le Quéré *et al.*, 1981; Dea & Madden, 1986) and may be ascribed to the fact that pectins were extracted in this study from dried cossettes.

The extracts from cossettes and pulp behave very similarly during ion-exchange chromatography and the same conclusions can be made. The amounts of contaminating neutral polysaccharides were not changed, with the exception of HP, for which higher amounts are found in the case of the cossettes. Feruloyl groups were detected in the unbound and in the bound material.

The compositions of each set of pectins are also comparable. Nevertheless, pectins from the cossettes are richer in neutral sugars and especially in arabinose for HP and OXP. They also have higher amounts of proteins and of feruloyl groups. The most distinguishable feature of

TABLE 4
Characteristics of the Reaction of Ammonium Persulphate on the Beet Pectins

	Samples								
	1	*2*	*3*	*4*	*5*	*WSP*	*OXP*	*HP*	*OHP*
$k_2 \times 10^3$ ($M^{-1}.s^{-1}$)									
H^+ form	29·8[a]	2·4	1·1	0·6	0·9	0·5	0·6	0·2	0·2
Na^+ form	1·5[a]	0·4	0·3	0·3	0·3	nd	nd	0·2	0·2
Yield of insoluble product from a 2·4% solution (% w/w)	63·9[b]	77·6	0	76·0	71·7	0	0	0	0
Bed volumes (ml g^{-1})									
H^+ form	55[b]	90		60	100				
Na^+ form	180[b]	190		160	180				
Na^+ form + 10^{-2} M NaCl	125[b]	80		85	105				
Na^+ form + 10^{-1} M NaCl	75[b]	65		50	60				

[a]Values from Thibault *et al.* (1987).
[b]Values from Thibault (1986).
nd = Not determined.

pectins from the cossettes is perhaps their higher molecular weight, particularly for the OXP material.

Gelling ability of sugar-beet pectins

As shown in Table 4, not all the pectins from sugar-beet gel. Only Samples 1, 2, 4, and 5 can form gels, while Sample 3 and pectins extracted from the cossettes are not able to gel in the presence of persulphate. Amongst gelling pectins, there are more or less marked differences in the rate constant for the disappearance of feruloyl groups and in the setting time of the gels.

The rate constant value is not a good criterion for the determination of the gelling ability of the pectins but the ionic state of the macromolecules is an important factor in the value of k_2. Sample 3, WSP and OXP have higher or equal k_2 values than Samples 4 and 5, but they do not gel. This discrepancy may be explained by the fact that k_2 value reflects the rate of disappearance of feruloyl groups but not the rate of crosslinking; other reactions, such as oxidations of the feruloyl residue, may lead to a decrease in the amount of feruloyl groups. Neither is the content of feruloyl groups in the sample an appropriate indicator of gelling ability, since, for example, HP has a higher content in feruloyl groups than Samples 2, 4 and 5, but is not able to gel. The degree of feruloylation calculated from the data in Tables 2 and 3 as the molar ratio of feruloyl groups to neutral sugars of the side-chains does not lead to a separation of the pectins, since similar values are obtained for HP and Sample 4.

All the samples used in this study vary by several parameters and it is difficult to correlate the gelation with some structural features. Nevertheless, it was previously shown (Thibault *et al.*, 1987) that the conformation of the pectins does not have a profound influence on the kinetics, and the results presented here confirm that the values of the degree of methylation and of acetylation do not play an important role on the reaction. Therefore, differences should be found in the structure of the pectins and particularly in the fine structure of the side-chains and the location of feruloyl groups. These groups must be in exposed domains of the side-chains so that they are accessible and crosslinkable by persulphate ions. It is noteworthy that the gelling pectins are generally richer in galactose than in arabinose. One hypothesis could be, therefore, that feruloyl groups are preferentially linked to the galactose residues of the arabinogalactan-like side-chains. An extraction in sufficiently acidic conditions or a technological process such as the leaching of the cossettes may remove some arabinose residues leading to the exposure of feruloyl groups. Therefore, this work should be considered as a first approach to

the study of the effect of molecular structure on the gelation ability of sugar-beet pectins. Complementary work should involve chemical or enzymatic degradations of the side-chains from non-gelling pectins in order to obtain samples varying primarily in the structure of the side-chains.

ACKNOWLEDGEMENTS

The author thanks Mrs M.-J. Crepeau for excellent technical assistance.

REFERENCES

Albersheim, P., Nevins, D. T., English, P. D. & Karr, A. (1967). *Carbohydr. Res.*, **5**, 340.
Dea, I. C. M. & Madden, J. K. (1986). *Food Hydrocolloids,* **1**, 71.
Fry, S. C. (1982). *Biochem. J.,* **203**, 493.
Fry, S. C. (1983). *Planta,* **157**, 111.
Kertesz, Z. I. (1951). In: *The Pectic Substances,* Interscience Publishing, New York, Ch. 21, pp. 463–6.
Le Quéré, J. M., Baron, A., Segard, E. & Drilleau, J. F. (1981). *Sci. Alim.,* **1**, 501.
Michel. F., Thibault, J. F., Mercier, C., Heitz, F. & Pouillaude, F. (1985). *J. Food Sci.,* **50**, 1499.
Owens, H. S., Lotzkar, H., Schultz, T. H. & Maclay, W. D. (1946). *J. Am. Chem. Soc.,* **68**, 1628.
Pippen, E. L., MacCready, R. M. & Owens, H. S. (1950). *J. Am. Chem. Soc.,* **72**, 813.
Rombouts, F. M. & Thibault, J. F. (1986*a*). *Carbohydr. Res.,* **154**, 177.
Rombouts, F. M. & Thibault, J. F. (1986*b*). *Carbohydr. Res.,* **154**, 189.
Rombouts, F. M., Thibault, J. F. & Mercier, C. (1983). French Patent 8,307,208. *Chem. Abstr.,* **102** (1985) 60792u.
Sharma, U., Brillouet, J. M., Scalbert, A. & Monties, B. (1986). *Agronomie,* **6**, 265.
Sosulski, F., Lin, M. J. Y. & Humbert, E. S. (1978). *Can Inst. Food Sci. Technol. J.,* **11**, 113.
Thibault, J. F. (1979). *Lebensm. Wiss. u. Technol.,* **12**, 247.
Thibault, J. F. (1986). *Carbohydr. Res.,* **155**, 183.
Thibault, J. F. & Rombouts, F. M. (1986). *Carbohydr. Res.,* **154**, 205.
Thibault, J. F., Garreau, C. & Durand, D. (1987). *Carbohydr. Res.,* **63**, 15.
Tollier, M. T. & Robin, J. P. (1979). *Ann. Technol. Agric.,* **28**, 1.
Voragen, A. G. J., Schols, H. A. & Pilnik, W. (1986). *Food Hydrocolloids,* **1**, 65.