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SOME PHYSICOCHEMICAL PROPERTIES OF SUGAR-BEET PECTINS MODIFIED BY OXIDATIVE CROSS-LINKING

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ABSTRACT

Sugar-beet pectins were cross-linked through their feruloyl residues by treatment with ammonium persulfate. Gels were obtained for concentrations of pectins higher than 0.8%, and modified pectins having different degrees of cross-linking were obtained.

The swelling (50–180 mL/g) of these cross-linked pectins was favoured by a low degree of cross-linking, low valency of the counterion, complete dissociation of the carboxyl groups, and low concentrations of salt.

INTRODUCTION

Pectins are traditionally used in the food industry as gelling agents. The current sources of commercial pectins are citrus peels and apple pomaces, which both contain highly methylated pectins. These "high methoxyl" pectins form gels in acidic media containing such polyhydric alcohols as sucrose. Pectins having lower contents of methyl ester are prepared by controlled de-esterification and their gelation is induced by the addition of calcium ions[1–3].

Each gelling process requires a close association of the pectic macromolecules which can be sterically hindered if the main chains carry substituents, such as the acetyl groups at positions 2 and/or 3 of the galacturonic acid of sugar-beet pectins[4–6]. This is the main reason why these pectins have poor gelling qualities and cannot be used as substitutes for citrus and apple pectins, although sugar-beet pulps which contain ~25% of galacturonic acids[7] are a potentially rich source of pectins.

The elucidation of the fine structure of the sugar-beet pectins[5,6] revealed that the neutral-sugar side chains, occurring in blocks (so-called hairy fragments), carry some feruloyl groups, as described[8] for spinach pectins. This structural feature offers a third way for a gelling process, since cross-linking reactions through the feruloyl groups can be carried out. Ammonium persulfate[9] and hydrogen peroxide–peroxidase[10] are efficient coupling agents and, for dilute solutions of pectins, only water-soluble products of increased molecular weight can be obtained. Gelation is observed when the pectin concentration is higher than 0.8–1% and we now report

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on some physicochemical properties of the resulting, modified water-insoluble pectins.

MATERIALS AND METHODS

Pectin. — The extraction of pectin from sugar-beet pulp and its characterisation have been described[9,11].

Preparation of the cross-linked pectin. — Solutions (0.8, 1, 1.2, 1.6, 2, and 2.4%) of pectin were made 0.01M in ammonium persulfate and stored at 18–22° for 15 h. Each resulting gel was disrupted, and stirred in an excess of water, and the slurry was centrifuged for 30 min at 20,000*g*. Each supernatant solution was discarded and this procedure was repeated twice unless otherwise stated. The water-insoluble products were washed with aqueous 80% ethanol and centrifuged as noted above, and this procedure was repeated twice. Each modified pectin was dried by solvent exchange (ethanol, acetone, and ether) and ground to a particle size of <0.25 mm.

Ionic properties. — The acid form of each cross-linked pectin was obtained by washing with aqueous 70% ethanol containing 1% of conc. hydrochloric acid, and then repeatedly with aqueous 60% ethanol until the eluate was chloride free. The acid form of the initial pectin was obtained by percolation of a salt-free solution through Amberlite IR-120 (H^+) resin.

The ionic capacities were determined by neutralisation of a known weight of each acid form by different carbonate-free hydroxides and expressed as mequiv./g of dry product (H^+ form). The titrations were followed by pH-metry and conductimetry at 25.00 ±0.01°, using a PHM 84 Radiometer pH meter and a CDM 83 Radiometer conductimeter equipped with a platinised electrode (PP 1042). The values of pH and conductivity were measured after stirring for 3–5 min.

The sodium, potassium, lithium, and calcium forms were obtained by appropriate exact neutralisation of the cross-linked pectins. From the values of the equivalent conductivities of the pectins in lithium, potassium, and calcium forms, the transport parameters for monovalent and divalent ions can be calculated. These parameters are related to the free fraction of the counterions[12].

Measurement of the swelling of the cross-linked pectins. — The degree of swelling of the cross-linked pectins in water was determined under different conditions, using the bed-volume technique, and expressed as mL of bed volume per g of dry product (H^+ form). Thus, ~50 mg of product was mixed with an excess of water, and the volume of the gel after swelling for 24 h in a volumetric cylinder was read[13]. The values were reproducible to ±10%.

Analytical methods. — The galacturonic acid content was measured colorimetrically by the *m*-hydroxybiphenyl method[14].

The content of feruloyl groups was obtained at pH 10 (67mM glycine–sodium hydroxide buffer) by spectrophotometry at 375 nm. The cross-linked pectins were previously solubilised by *β*-eliminative degradation in 0.05M sodium hydroxide at room temperature for 3 h. The value of the molar extinction coefficient for the feruloyl residues was taken[15] as 31,600.

RESULTS AND DISCUSSION

General characterisation of samples of cross-linked pectins. — In order to obtain gels with different degrees of cross-linking, the pectin concentration was varied in the range 0.8–2.4%, the ammonium persulfate concentration being 0.01M. In more dilute solutions, only solutions of increased viscosity were observed[9]. The gel obtained from a 0.8% solution of pectin was very loose, and only one extraction with water was carried out. Therefore, the relatively high yield in cross-linked pectins from this preparation (Table I) may be ascribed to the presence of water-soluble products. With increasing concentration of pectin, firmer gels were obtained and the yield of cross-linked pectins was roughly constant (63–64%). All of the isolated modified pectins were powders similar to the initial pectin.

From a knowledge of the initial concentration of feruloyl groups and the measurement of its residual concentration in the cross-linked pectins, the percentage of feruloyl residues modified by the persulfate action could be calculated; the water-soluble products did not contain feruloyl residues[9]. The values obtained (Table I) decreased from 74.6 to 66.7% on increasing the concentration of pectin from 0.8 to 2.4%.

The ionic capacities, indicated in Table I, ranged between 1.88 and 1.99 mequiv./g (*cf.* 1.97 mequiv./g for the initial pectin). Some typical examples of titration curves from pH and conductivity measurements are shown in Fig. 1 and Fig. 2; the nature of the counterion (K^+, Li^+, Ca^{2+}) had little effect, indicating that all of the carboxyl groups were accessible to mono- and di-valent ions. These curves show also that the ionic selectivity $K^+ < Li^+ < Ca^{2+}$ observed for the initial pectin was maintained in the cross-linked pectins, but that the mobility of the counterions was significantly decreased. The values of the transport parameter for monovalent ions were 0.68 and 0.44 ±0.07 for the initial pectin and the cross-linked samples, respectively. For divalent ions, these values were 0.34 and 0.22 ±0.05. The differences between soluble and insoluble samples were due to a high local ionic concentration and to a limitation in the diffusion process in the cross-linked samples. This selectivity has also been reported for pectins extracted from other raw materials, for example, apple pomace[12].

The galacturonic acid content of the samples was in the range 56.2–58.3%, values similar to that of the initial pectin.

Determination of the p*K value.* — Potentiometric titration curves (*cf.* Fig. 1) allow for the determination of the number of carboxyl groups. They relate the pH of the external solution to the amount of alkali added to the gel and can be used to gain more information about the dissociation constant of these functional groups.

The curves shown in Fig. 3 relate the apparent dissociation constant (pK_a) to the degree of dissociation (α) as

TABLE I

GENERAL CHARACTERISTICS OF THE SAMPLES OF CROSS-LINKED SUGAR-BEET PECTINS

	Samples					
	1	*2*	*3*	*4*	*5*	*6*
Initial pectin concentration (g/100 mL)	0.8	1	1.2	1.6	2	2.4
Water-insoluble products (%)	74.2	63.9	63.7	62.5	63.7	63.9
Feruloyl modified residues (%)	74.6	74.1	70.0	69.4	67.1	66.7
Ionic capacity (mequiv./g)	1.92 ±0.04	1.99 ±0.02	1.97 ±0.04	1.97 ±0.04	1.88 ±0.03	1.89 ±0.01

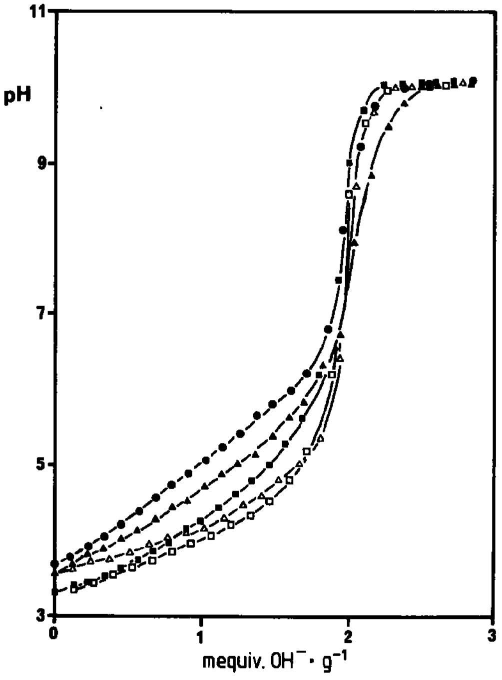

Fig. 1. Neutralisation curves obtained at 25° by pH-metry for the initial pectin (□, ■, 2 mequiv./L) and for cross-linked pectins (△, ▲, sample No. 5, 1.9 mequiv./L; ●, sample No. 2, 1.7 mequiv./L). Open symbols and full symbols refer to neutralisation by calcium hydroxide and potassium hydroxide, respectively.

$$pK_a = pH_{ext} + \log(1 - \alpha)/\alpha, \qquad (1)$$

where pH_{ext} is the pH of the external solution.

The intrinsic pK value (pK_0), characteristic of the carboxyl dissociation, can be obtained by extrapolation to zero charge[16]. The results were compared to those obtained with the initial pectin. The sugar-beet pectins behaved towards monovalent ions as polyelectrolytes with a low charge density, since the value of the charge parameter, as defined by Lifson and Katchalsky[16], was 0.76.

Plots obtained for the initial pectins when they were neutralised by potassium hydroxide can be regarded as straight lines and the pK_0 value could be determined as 3.1–3.2, in agreement with results reported for pectins with similar degrees of esterification[17,18]. Plots observed for cross-linked pectins were also straight lines, but they were ~0.5 pK unit higher, and the apparent pK value decreased with the degree of cross-linking for a given degree of dissociation. These shifts reflect directly the Donnan exclusion phenomenon[19,20]. In the presence of external salts, all the apparent pK values were lowered and the titration curves approached the ideal behaviour of a monofunctional acid[19] and tended to become superimposed in the presence of a high concentration of salt. This result is explained by the fact that

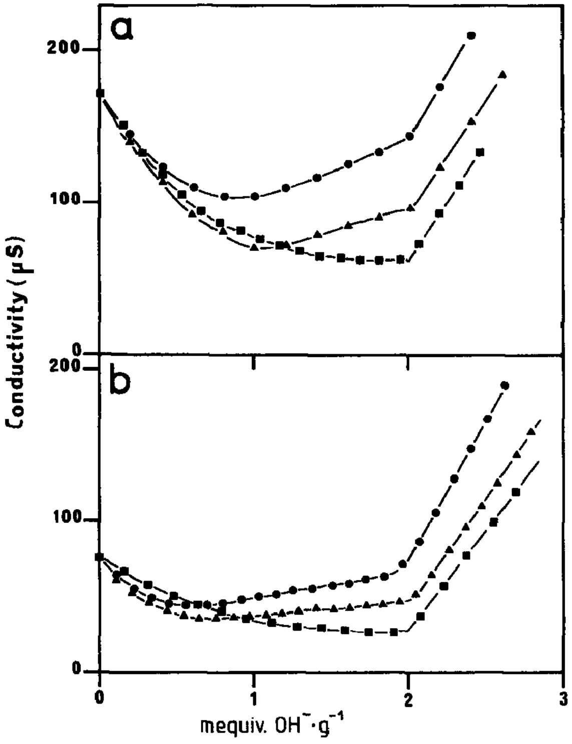

Fig. 2. Neutralisation curves obtained at 25° by conductimetry for the initial pectin (a) and for sample No. 4 (b); the neutralisation was effected with potassium hydroxide (●), lithium hydroxide (▲), and calcium hydroxide (■).

the Donnan effect as well as the polyelectrolyte effect are suppressed at high ionic strengths[20].

Similar observations were noted when the samples were neutralised by calcium hydroxide (data not shown), although lower apparent p*K* values were obtained. The lower apparent p*K* values reflected the stronger interactions of calcium ions and pectins, which tended to increase the acidity of the carboxyl groups.

From a quantitative point of view, the p*K* values ranged between 3.60 and 3.30 in the presence of 0.1 and 0.5M NaCl, respectively, and were ~3.20 in the presence of 0.1M $CaCl_2$. Increase of the ionic strength of the external solution led to a decrease of the pK_0 value down to that of the initial pectin. The acetyl groups did not appear to play an important role in the dissociation of the carboxyl group, in agreement with the results of Kohn and Malovikova[21] who found only a slight increase or decrease in the pK_0 values of acetyl derivatives of pectic acids, depending on the extent of acetylation.

Swelling of the cross-linked pectins. — The cross-linked pectins did not dissolve in water, but swelled. Swelling represents a balance between the tendency of the polar and ionic groups to be hydrated and the mechanical resistance of the

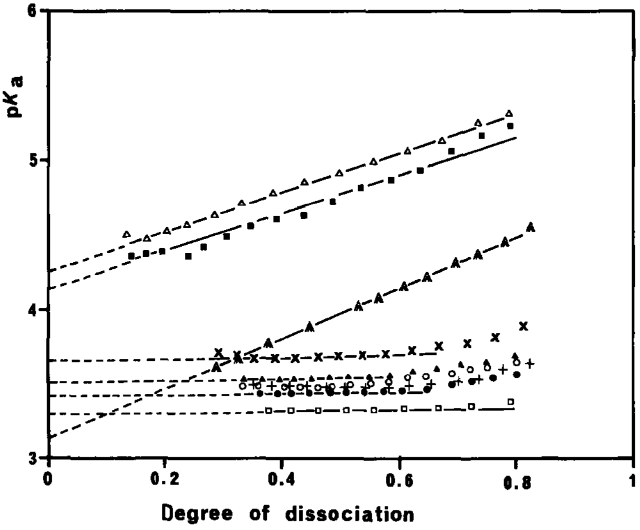

Fig. 3. Dependence of apparent pK on the degree of dissociation, with potassium hydroxide, of the initial pectin and cross-linked pectins in H_2O and KCl solution at 25°. Initial pectin in H_2O (▲, 2 mequiv./L) and in 0.5M KCl (□, 2 mequiv./L); sample No. 5 in H_2O (■, 1.9 mequiv./L), in 0.1M KCl (▲, 1.7 mequiv./L), and in 0.5M KCl (●, 1.7 mequiv./L); sample No. 2 in H_2O (△, 1.7 mequiv./L), in 0.1M KCl (×, 1.7 mequiv./L), and in 0.5M KCl (+, 1.7 mequiv./L).

matrix[22]. The swelling, as measured by the bed-volume technique, was tested on the modified pectins as a function of the degree of neutralisation of the carboxyl groups, the nature of the counterion, and the ionic strength of the surrounding solution. Results are shown in Table II and Fig. 4.

In the acid forms, the cross-linked pectins swelled to a rather limited extent (45–60 mL/g). When they were fully neutralised by sodium hydroxide, the bed volumes were multiplied by factors of 2 and 3 for the samples with the highest and lowest degree of cross-linking, respectively. The maximum value was ~180 mL/g. The nature of the monovalent counterion (K^+, Li^+, Na^+) did not have a profound

TABLE II

EFFECTS OF THE NATURE OF THE COUNTERION AND OF THE IONIC STRENGTH OF THE EXTERNAL SOLUTION ON THE SWELLING (mL/g) OF CROSS-LINKED PECTINS

Ionic form	*Samples*					
	1	*2*	*3*	*4*	*5*	*6*
H^+	45	45	60	50	55	55
Ca^{2+}	80	75	100	75	100	125
Na^+	95	110	120	110	140	180
Na^+ in 0.001M NaCl	100	105	120	115	125	160
Na^+ in 0.01M NaCl	70	75	90	80	90	125
Na^+ in 0.1M NaCl	45	40	50	50	60	75

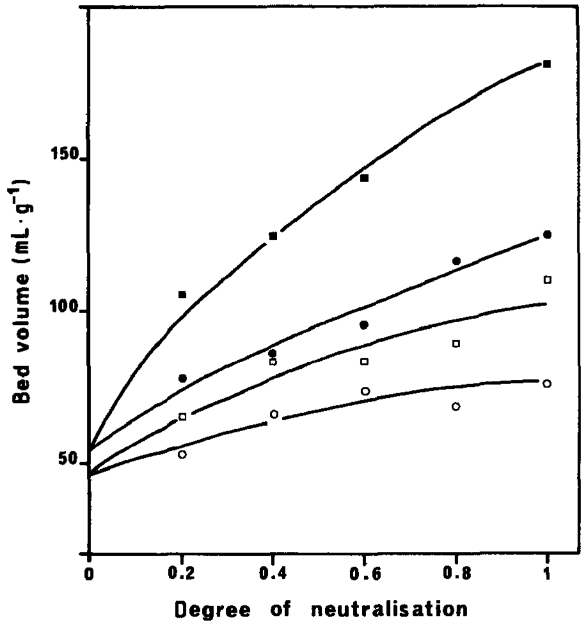

Fig. 4. Dependence of the swelling of cross-linked pectins (■, ●, sample No. 6; □, ○, sample No. 2) on the degree of neutralisation with sodium hydroxide (■, □) and with calcium hydroxide (●, ○).

effect since values obtained under these conditions were in the range of experimental error for the determination of the bed volumes. A more limited swelling was observed when the samples were neutralised with calcium hydroxide, but shrinkages, as reported by Rinaudo[23] for cross-linked alginates, were not observed. This result may be ascribed to the low charge density of the pectic backbone, as compared to that of alginate, and to the acetylation of the galacturonic acid residues[21].

The influence of the degree of neutralisation by sodium and calcium hydroxides on the swelling of two cross-linked pectins is shown in Fig. 4. With increasing ionisation, the negatively charged groups of the matrix set up electrostatic repulsions which progressively expand the network in agreement with results obtained by Rinaudo[23] on cross-linked alginates. In contrast, Katchalsky *et al.*[24] observed a rapid increase of the swelling of cross-linked polymethacrylic acid, even for small degrees of neutralisation.

When the ionic strength (addition of NaCl) of the surrounding solution was increased, the swelling of the modified pectins (Na^+ form) decreased and reached values observed in the acid form when the solution was 0.1M in NaCl (Table II). This fact reflects the role of the screening of the fixed charges, leading to a reduction of the electrostatic repulsion and therefore a reduction of the expansion.

The swelling observed for modified sugar-beet pectins is much higher than that observed for apple or citrus pectins cross-linked by epichlorhydrin (3–15 mL/g)[25–27]. Thus, the cross-linking of sugar-beet pectins through their feruloyl residues yields a weak polyacid network. As the initial pectin contains only ~0.8% of feruloyl groups, the degree of cross-linking is low and the gels obtained from these products are highly swollen. The swelling (range 50–180 mL/g) and the p*K* value (range 3.20–3.60) depend on the degree of cross-linking, the degree of neutralisation of the carboxyl groups, the nature of the counterion, and the ionic strength of the surrounding solutions[22]. All of these variations can be explained by Donnan equilibrium and by polyelectrolyte effects[22]. These effects were theoretically studied on highly swollen gels[19,28,29]. Treatment of the data according to these theories requires a knowledge of the number of monomer residues between two adjacent cross-links, which is not known for our samples.

The swelling properties of the cross-linked pectins could lead to new applications of sugar-beet pectins.

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