On the gelling behaviour of 'nopal' (*Opuntia ficus indica*) low methoxyl pectin

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Abstract

Fully de-esterified pectin with excellent gelling properties was isolated in the sodium-salt form from fresh 'nopal' cactus (*Opuntia ficus indica*) pads (0.6% w/w yield of fresh weight) using an alkaline extraction medium in the presence of a sequestering agent. Sugars composition of the cactus pectin alkaline extract (CPAE) was: 85.4% uronic acids, 7.0% galactose, 6.0% arabinose and minor quantities of rhamnose and xylose. Features of the Fourier-transformed infrared spectrum were nearly identical to those of commercial citrus pectin as well as to homogalacturan-rich pectin isolated from prickly pear, lime peel, and sugar beet pulp. The gelling behavior of this material was studied as a function of amount of Ca^{2+} added and temperature by dynamic oscillatory rheology. Addition of Ca^{2+} at 85 °C was adjusted at varying stoichiometric ratios, R (=2 [Ca^{2+}]/[COO^{-}]), namely 0.07, 0.21, 0.35 and 0.42 and fixed pectin concentration (16 g/L), and a temperature dependent behavior of the system on cooling was imposed. Evolution of the viscoelastic storage (G') and loss (G'' moduli on cooling revealed unequivocal set up of a gel network under a highly cooperative sol-gel transition at $R \leqslant 0.21$. At greater R values, the Ca^{2+}-mediated dimeric association of pectin chains led to formation of a gel network even at 85 °C. On heating, pectin gels melted partially at temperatures notably greater than those at which they were formed. Thermal hysteresis observed between the cooling and heating temperature traces is explained in terms of helix–helix aggregation. The gelling behavior of this system is interpreted in terms of the formation of two distinct types of junctions mediated by the stoichiometric amount of calcium (R). Namely, short 'egg-box' type junctions formed directly at high temperature on addition of calcium (limited zones are related to high mobility of the chains) and highly cooperative 2_1 helix junctions followed by aggregation formed at lower temperature under a thermal conformational transition driven by charge neutralization and lower chain mobility (related to stabilization by H-bonds).

Keywords: Pectin; *Opuntia ficus indica*; Gel formation

1. Introduction

Pectin is widely used in the food industry as a hydrocolloid gelling gum. Commercially, it is commonly derived from fruit waste mainly apple and citrus peel. Generically, the term ''pectin'' represents a family of structural polysaccharides which occur as constituents of the primary cell wall of plant cells and intercellular regions of higher plants where they function as hydrating agent and cementing material of the cellulosic network (Lau, McNeil, Darvill, & Albersheim, 1985). The main pectin component is a central, linear backbone chain composed of α-D-galacturonic acid units linked by (1 → 4) glycosidic bonds. This linear (or 'smooth') structure is interrupted by highly branched regions ('hairy' zones) (Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000; Thibault, Renard, Axelos, Roger, & Crepeau, 1993; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

Depending on the botanic origin and the extraction procedure used, the carboxylic groups are partially esterified with methanol, and in certain pectins the hydroxyl groups are partially acetylated. Neutral sugars, such as rhamnose,

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arabinose, galactose, xylose and glucose are also usually present in a 5–10% proportion of the galacturonic acid weight. These neutral residues comprise the highly branched side chains (arabinan and galactan), a small part of the central chain (rhamnose) or occur as contaminating polysaccharides (glucans and xyloglucans) (Rolin, 1993). Depending on the degree of esterification (DE, expressed conventionally as a percentage of the content of esterified units compared with the content of total uronic acids), pectins can form gel networks either in an acid medium and under high sugar concentrations (high DE pectins, more than 50%) or by interaction with divalent cations, particularly Ca^{2+} (low DE pectins, less than 50%) (Durand, Bertrand, Clark, & Lips, 1990; Evageliou, Richardson, & Morris, 2000). In these gels, the macromolecules are cross-linked by divalent calcium ions (Thibault & Rinaudo, 1984a; Thibault & Rinaudo, 1984b; Thibault & Rinaudo, 1985; Thibault & Rinaudo, 1986; Turquois, Rinaudo, Taravel, & Heyraud, 1999). In both cases, gelation and gel properties depend upon many factors, including pH, temperature, DE, sugar content, calcium content and pectin content.

The occurrence of polysaccharides that generically have been called "pectins" in various *Opuntia* species from Mexico has been documented for almost three decades (Villarreal, Rojas, Arellano, & Moreno, 1963), with yield of soluble pectin within a wide range of 0.13–2.64% in wet basis (1.00–23.87% in dry-weight basis). Two types of water-soluble materials can be extracted from *Opuntia* spp. pads and fruits, namely a mucilage material easily recognized as a slimy fluid that appears as soon as cacti is cut and a structural cell-wall component. The sugar residues composition and linkage geometry of the mucilage from the fruits and pads of *Opuntia* spp. cacti have long been studied using different chromatographic techniques (Amin, Awad, & El-Sayed, 1970; Majdoub et al., 2001; McGarvie & Parolis, 1979a; McGarvie & Parolis, 1979b; McGarvie & Parolis, 1981a; McGarvie & Parolis, 1981b; Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000; Trachtenberg & Mayer, 1981). However, up to now, the mucilage polysaccharides in *Opuntia*, do not seem to be chemically associated, either covalently or otherwise, to the structural cell-wall pectins (Goycoolea & Cárdenas, 2003). The physicochemical and rheological properties of mucilage extracted from *Opuntia* spp. have been studied by several Groups (Amin et al., 1970; Forni, Penci, & Polesello, 1994; Majdoub et al., 2001; McGarvie & Parolis, 1979a; McGarvie & Parolis, 1981a; Medina-Torres et al. 2000; Mindt, Saag, Sanderson, Moyna, & Ramos, 1975; Trachtenberg & Mayer, 1981; Trachtenberg & Mayer, 1982). The evidence is consistent in that this material has no gelling capacity. By contrast, the polysaccharide material addressed in this study is the far less studied pectin extracted from the cell-wall with a very low DE and an unequivocal capacity to form a gel network in the presence of calcium ions. It is hypothesized that the underlying mechanism for this phenomenon is the consolidation of a gel network that involves the association of junctions mediated by Ca^{2+} crosslinking of two distinctive types, namely short dimeric "egg-box" type structures (Grant, Morris, Rees, Smith, & Thom, 1973) formed at high temperature followed by a highly cooperative mechanism comprising ordered perhaps 2_1 double helical structure with aggregation at low temperature. This study opens new horizons to the use of cactus pads as a potential source of pectin, with gelling properties of enormous technological importance to the food industry, particularly in arid and semi-arid zones.

2. Materials and methods

2.1. Materials

A batch (~10 kg) of fresh cactus (*Opuntia ficus indica*) pads or cladodes were obtained from a commercial plantation in San Pedro El Saucito, Hermosillo, Sonora. Commercial low-ester citrus pectin was a sample from DANISCO A/S (Copenhagen) (DE < 50%). Chemical reagents were of analytical grade supplied by Sigma Chemicals (Mexico, DF) except sodium hexametaphosphate that was from Monsanto Co. (St. Louis, MO, USA). Bi-distilled water was used throughout.

2.2. Methods

2.2.1. Pectin extraction and purification

Before pectin extraction, the fresh cactus pads were cleaned to remove thorns and cut into small pieces (~1 × 1 cm) with a kitchen knife. Cactus pieces were heated in water at 85 °C for 20 min to inactivate enzymes and left to cool to ambient temperature; neutralized to pH 7.5 from initial pH ~4.0 in order to induce de-esterification of methoxyl groups and filtered through a cloth filter to extract as much mucilage as possible. The solid residue was suspended in a NaOH 50 mM sodium hexametaphosphate 7.5 g/L solution (pH ~ 12.0) stirred continuously for 1 h. Cactus pectin alkaline extract (CPAE) was filtered with cloth and the supernatant recovered. Pectin was precipitated by adjusting the pH to 2 and left to stand overnight at ~5 °C. The precipitate was recovered by centrifugation (Beckman centrifuge, model J2-21) at 8000 rpm for 15 min at 20 °C. The precipitate was re-suspended in water and the pH adjusted to 8 to re-dissolve the pectin. A similar protocol was proposed by Turquois et al. (1999) to extract pectin from sugar beet pulp. The pectin was further purified according to the procedure suggested by Rinaudo (1993): the polymer solution was filtered with 3, 1.2 and 0.8 μm membranes, precipitated by adding absolute ethanol to a final concentration of 50% (v/v) and washed successively with ethanol/water mixtures of volume ratios: 75/25, 80/20, 85/15, 90/10 and absolute ethanol and left to dry at ambient temperature. The sodium pectate final yield was of 6.2 g/kg referred to the weight of fresh cactus tissue (~60 g/kg in dry weight basis).

2.2.2. Monosaccharides composition

Neutral sugars were determined using gas–liquid chromatography after hydrolysis with sulfuric acid and conversion of the sugars to their alditol-acetates according to the protocol described elsewhere (Blakeney, Harris, Henry, & Stone, 1983), using inositol as internal standard. The chromatographic system was a HP 5890 gas chromatograph and a FID detector coupled with a HP 3380-A integrator. The column was a SP2380 macropore (25 m × 0.53 mm) silica column and the oven temperature program was: started at 195 °C and the temperature raised at 2.5 °C/min to 225 °C. The flow rate of the vector gas (N_2) was set at 4 ml min^{-1}. The temperature of the injector was 260 °C.

2.2.3. Equivalent weight (uronic acids content)

The content of carboxylic groups (i.e. uronic acids) was determined using a conductometric technique exposed previously (Thibault & Rinaudo, 1984a; Thibault & Rinaudo, 1984b); a Tacussel CD78 conductivity bridge equipped with a platinum electrode was used. A known weight of pectin is dissolved in presence of a known excess of NaOH; conductimetric titration is performed with HCl solution followed by titration with NaOH; analysis of the curves gives the total –COOH content in the pectin from which the equivalent weight per carboxylic group was determined to be 223.2 g/eq.

2.2.4. Intrinsic viscosity

It was determined in 0.1 M NaCl at 25 °C. Relative viscosity measurements were performed by registering the flow time of the solution in an Ubbelohde capillary viscometer (OB size) at 20 ± 0.1 °C, immersed in a temperature controlled Koehler bath. The intrinsic viscosity value was obtained by joint extrapolation of Kraemer, Huggins and "single point" curves to "zero" concentration using an iterative minimization sub-routine and the Solver tool in Microsoft Excel. Successive dilutions were done directly in the viscometer by volumetric addition of solvent. Relative viscosity of the solutions was within 1.2 and 2.0. The intrinsic viscosity allows to get an approached value for the molecular weight when the Mark–Houwink parameters are known.

2.2.5. Fourier-transformed infrared spectroscopy (FTIR)

The FTIR spectrum of a cactus pectin film was obtained in the transmission mode with a Nicolet Protégé spectrophotometer (460 E.S.P., Madison WI, USA). Base line and the spectrum data were processed with the OMNIC program. A total of 64 interferograms with a 2 cm^{-1} resolution were collected in all cases. The pectin film was prepared as follows: a 20 g/L pectin solution was spread out over a glass surface and cast in a vacuum-oven at 25 °C.

2.2.6. ^{13}C NMR spectroscopy

Spectra were recorded on a Bruker AC-300 instrument at 75.47 MHz at 85 °C with a delay time of 0.5 s and 8 K data points collected. The 50 mg sample was directly dissolved in 1 mL D_2O.

2.2.7. Pectin gel preparation

Pectin gels were prepared by mixing the pectin previously dissolved in water (32 g/L) at pH ~ 6.5 with $CaCl_2$ solutions of varying concentration at 85 °C. Rheological properties were studied at different stoichiometric relations of calcium to pectin, R (=2[Ca^{2+}]/[COO^-]): 0.07, 0.21, 0.35 and 0.42. The final concentration of pectin was 16 g/L throughout.

2.2.8. Rheological determinations

Small deformation oscillatory rheological measurements of the storage modulus (G'), the loss modulus (G''), complex viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$, where ω is the oscillation frequency) and the tangent of the phase angle ($\tan\delta = G''/G'$) were made with a Rheometrics (Fluids Spectrometer RFS II, Piscattaway, NJ) using a truncated cone-plate geometry (0.04 rad cone angle; $\varphi = 5.0$ cm). Temperature was controlled with a circulation water bath connected to the stationary element. The samples were placed in the rheometer at ~85 °C, their periphery covered with silicon oil to minimize evaporation and then immediately cooled from ~85 to ~5 °C. For these measurements a frequency–temperature ramp program was utilized, whereby at each temperature interval, the viscoelastic moduli were registered at five frequency values varying in the range from $\omega = 1$ to 21.5 rad/s. The sample of $R = 0.35$ was also heated from 5 to 60 °C. The heating and cooling traces were done at 1 °C/min. All determinations were performed at the same strain ($\gamma = 5\%$) within the linear viscoelastic region.

3. Results and discussion

3.1. Chemical composition

The cactus pectin extract afforded by the alkaline process, referred here to as cactus pectin alkaline extract (CPAE), represents 0.6% of the fresh tissue weight. Even when some soluble pectin may have been lost during the first aqueous extraction treatment intended to remove mucilage, the yield obtained for the extraction process was considerably greater than that documented for pectin extracted from prickly pear peel, 0.12% (Forni et al., 1994). CPAE obtained consists of 85.4% uronic acids and 14.6% of neutral sugars (Table 1). Galactose and arabinose predominate as the main constituent neutral sugars with a minor presence of rhamnose and xylose, a profile that accounts for the presence of diverse species of arabinogalactans in the branched regions that are inserted along the homogalacturonan chain. Table 1 compares the general characteristics and composition of the sugar residues of 'nopal' cactus pectin extracted by the alkali procedure (CPAE) with pectins obtained from prickly pear peel extracted by an acid process (Habibi, Heyraud, Mahrouz,

Table 1
Composition of *Opuntia ficus indica* pectin extract obtained by alkaline process with sodium hexametaphosphate as sequestering agent and with acid and other pectins

Sample	Extraction yield (w/w)[a]	Protein[b]	Ash[b]	Fat[b]	Intrinsic viscosity (ml/g)[c]	Uronic acids[b]	DE[d]	Neutral sugars				
								Rha[b]	Ara[b]	Gal[b]	Glu[b]	Xyl[b]
Cactus pectin alkaline extract (this paper)	0.6	N.D.	N.D.	N.D.	234	85.4	0	0.6	6	7	0	1
Prickly pear peel pectin (acid process)[e]	12.4	N.D.	N.D.	N.D.	N.D.	64.5	N.D.	3.7	17.2	4	0	0.9
Potato pulp (alkaline process)[f]	20	6	10.2	0.8	N.D.	46.5	8.9	0.7	9.6	3.1	1.3	N.D.
Commercial LM citrus pectin[f]	N.D.	1.2	6.6	0.2	N.D.	41.2	18	0.7	0.3	1.4	1.9	N.D.

[a] As % of dried raw biomass.
[b] As % of dry weight of extract.
[c] Determined in 0.1 M NaCl at 20 °C.
[d] Degree of esterification calculated on the basis of galacturonic acid content.
[e] Habibi et al. (1994).
[f] Potato pulp pectin extracted with alkali with sodium hexametaphosphate and commercial citrus pectin (Turquois et al., 1999). N.D., not determined.

& Vignon, 2004) as well as with potato pulp pectin obtained by the same extraction protocol using alkali and hexametaphosphate as sequestering agent and with those of a commercial LM citrus pectin. In general, the neutral sugars content of the studied pectin was lower than that reported for pectins sourced from prickly pear fruit peel and potato pulp. This, together with the low rhamnose content, may suggest the existence of regions of long chains of polygalacturonate (homogalacturonan), which are essential for the gelling process, as it is further discussed.

While the content of uronic acids in cactus pectin was greater than that of prickly pear peel pectin obtained by an acidic process, it was roughly twice as high as that of potato pulp and commercial LM citrus pectin. In general, the overall composition profile of the cactus pectin composition coincides well with that of pectin obtained from lemon peel pectin (Ros, Schols, & Voragen, 1996) and sugar beet (Oosterveld et al., 2000; Turquois et al., 1999).

The degree of esterification of the CPAE was investigated by FTIR spectroscopy. Fig. 1 shows the FTIR spectrum of a CPAE film. Inspection of the spectral region between 1350 and 1750 cm^{-1} reveals the total absence of the 1749 cm^{-1} band and the clear presence of two bands centered at 1625 and 1415 cm^{-1}. This result concords perfectly with the complete saponification of the methyl groups and the presence of the Na^+ form of the carboxylate groups pectin at the moment of film formation at a pH above the pK_o of the carboxylic groups ~3.3 (Milas & Rinaudo, 1997). The 1749 cm^{-1} band, assigned to C=O stretching vibrations of the non-ionized carboxylic groups, has been proposed as a probe to quantify the DE in pectin (Chatjigakis et al., 1998). The ionization (via salt formation) leads to its disappearance and to the appearance of two new bands associated to symmetric and asymmetric COO^- stretching vibrations centered approximately at 1600–1650 and 1400–1450 cm^{-1}, respectively (Kamnev, Colina, Rodríguez, Ptitchkina, & Ignatov, 1998). The high content of free carboxylic groups is directly related with the alkaline conditions for pectin extraction. The purity of our CPAE material was not quantified, yet the protocol used to extract and purify the pectin polysaccharide was considered exhaustive enough so as to assure removal of most of mineral, protein and lipid impurities. Fresh cactus pads are known to contain 0.5–1% protein and 1–2% and ash in fresh weigh basis. The FTIR spectrum of CPAE does reveal the presence of some residual amount of protein as can be appreciated from the small shoulder at ~1550 cm^{-1} that can be assigned to amide II.

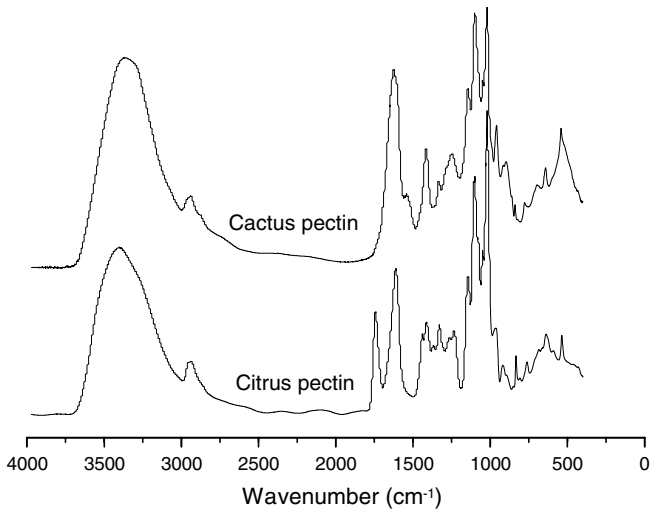

Fig. 1. Absorbance spectra of FTIR spectroscopy for cactus pectin and commercial citrus pectin films obtained in transmission mode.

^{13}C NMR spectroscopy confirmed the presence of two distinct types of materials in this cactus pectin. A major carbohydrate constituent that gives rise to six main C signals including the –C=O signal at 176.1 ppm characteristic of the uronic acid (Fig. 2), and a minor component to which also six carbon signals not well defined can be assigned. The integrals of the small signals relative to the large signals one represent about 11.0% (w/w). It was gratifying to confirm that this value is in close agreement with that obtained for the proportion of neutral sugars obtained after complete hydrolysis. Hence, the large signals can be ascribed to poly-D-galacturonic acid, while small signals

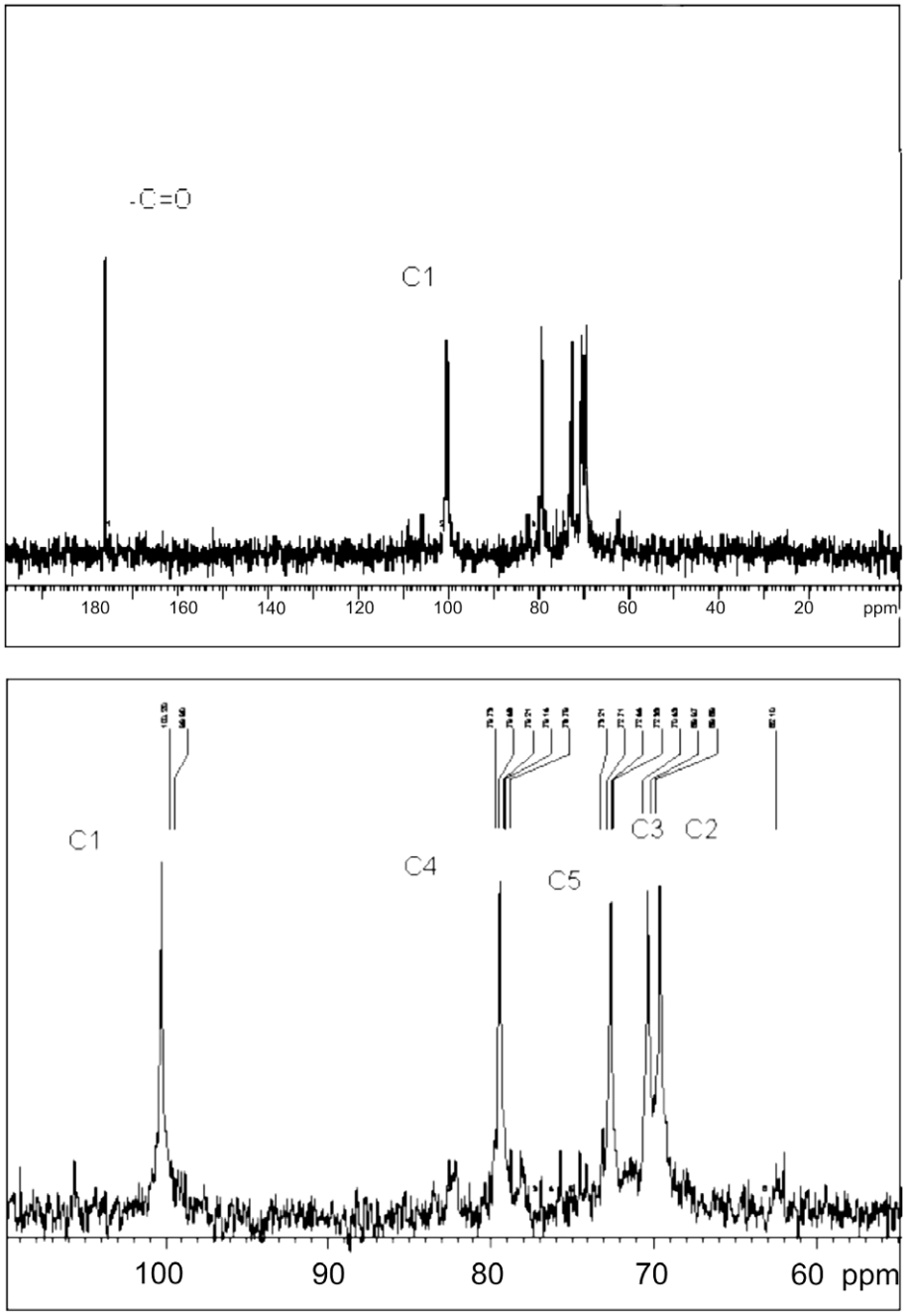

Fig. 2. ^{13}C NMR (75 MHz) spectrum recorded in D_2O at 85 °C of cactus pectin. The lower spectrum corresponds to an amplification of the region 55–109 ppm of the upper one.

can be assigned to the various neutral sugars present (Table 1) (Davis, Derouet, & Herve du Penhoat, 1990; Habibi et al., 2004). It is interesting to point out that no evidence of the presence of rhamnose was detected (i.e. absence of characteristic signals in the anomeric region and for the C6 methyl group at 99 and at 17.4 ppm, respectively), which is in agreement with the low contents found for this monosaccharide (Table 1). In addition, nonappearance of a signal at 20.2–20.7 ppm for $-CH_3$ also reveals that neither methyl ester nor *O*-acetylated residues are present in CPAE.

The value of the intrinsic viscosity also shown in Table 1, which is an indirect measure of molecular weight, measured in 0.1 M NaCl (234 mL/g), was within the reported range for pectin from diverse sources (110–250 mL/g) (Arslan, 1995; Fishman, Gillepsie, Sondey, & El-Atawy, 1991). By using the Mark–Houwink parameters ($K = 2.43 \times 10^{-2}$ and $a = 0.82$ obtained in 0.1 M sodium phosphate buffer) proposed previously by Kar and Arslan (1999), a viscometric-average molecular weight of 73,400 was estimated. This value can only be regarded as a rough estimate of the molecular weight, as the solvent conditions

between our determinations (0.1 M NaCl) and those in which the Mark–Houwink constants were reported (0.1 M sodium phosphate buffer) were different. However, it is interesting to note that the intrinsic viscosity value obtained in CPAE is substantially inferior to that documented previously for cactus mucilage (840 mL/g, Majdoub et al., 2001). In this respect, it is worth mentioning that the formation of aggregates of large molecular weight has been reported in cactus mucilage (Cárdenas, Goycoolea, & Higuera-Ciapara, 1997). The presence of such aggregates in CPAE however, is discarded due to the micro filtration procedure applied to the extract solution before precipitation with ethanol; in addition, alkaline conditions may also have a slight degradative effect on the molecular weight.

3.2. Gelling properties

The rheological studies were conducted on CPAE gels of fixed polysaccharide concentration of 16 g/L, pH ~ 6.5 and varying amounts of added $CaCl_2$ so as to give stoichiometric ratios, R, of 0.07, 0.21, 0.35 and 0.42.

Fig. 3 shows the dependence of the elastic (storage), G', and viscous (loss), G'', moduli, on frequency, ω, of two representative CPAE gels with R values of 0.21 (Fig. 3a) and 0.42 (Fig. 3b), registered immediately after addition of $CaCl_2$ at 85 °C and before initiation of the cooling program. No time sweeps were recorded at such high temperature before the onset of cooling, however, it can be assumed that the rheological properties of the system remained essentially unchanged with time at least during the time span of the measurements, as it can be appreciated from the virtually negligible dependence of G' and G'' on frequency in the mechanical spectra of the two samples (Figs. 3a and b).

Clearly, both mechanical spectra reveal the predominance of G' over G'' and only little dependence of both moduli on oscillation frequency. Also, notice that doubling in R value is accompanied by a concomitant increase by two orders of magnitude on the values of G' and by a greater difference among the relative values of both moduli (i.e. lower $\tan\delta$ values ($=G''/G'$)). Altogether, these features are diagnostic of the formation of a loose gel network at high temperature, in which the degree of crosslinking is directly governed by the amount of added $CaCl_2$ into the system. In turn, Fig. 4 shows mechanical spectra registered at 5 °C after the completion of the cooling cycle, for the gels of $R = 0.07$, 0.21, 0.42. In line with what was observed at 85 °C, increasing the calcium crosslinking stoichiometry from $R = 0.07$ to 0.21, leads to an increase in G' modulus by more than an order of magnitude (Figs. 4a and b) and to a larger predominance of G' over G'' modulus (i.e. hence lower $\tan\delta$ values). Inspection of the mechanical spectrum of the gel at $R = 0.21$ (Fig. 4b) shows that cooling to 5 °C leads to an increase in the mechanical moduli G' by more than three orders of magnitude with respect to gels at 85 °C (Fig. 3a).

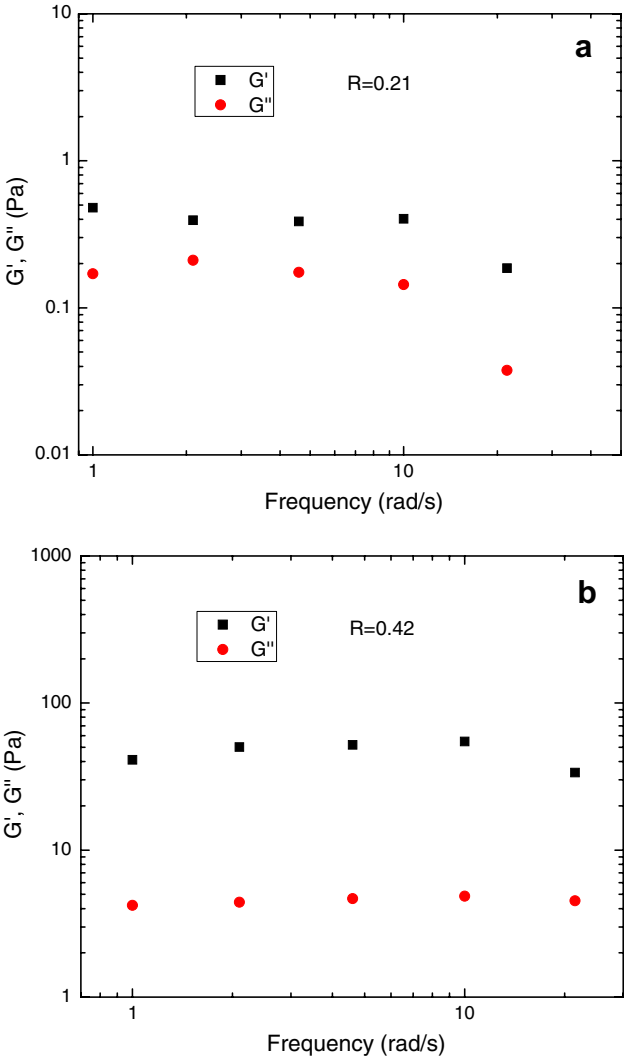

Fig. 3. Variation of the elastic, G' ■, and loss, G'' ●, moduli, in response to the oscillation frequency of pectin gels (at 85 °C, $\gamma = 0.05$) added with $CaCl_2$ at two levels of stoichiometric equivalence R ($=2[Ca^{2+}]/[COO^-]$): (a) 0.21 and (b) 0.42 (polymer concentration 16 g/L).

These results can be interpreted as the set up of a denser and more elastic gel network, the consequence of a larger number of long calcium-mediated junctions in the gel structure. However, this process seems to proceed up to a maximum point, beyond which the degree of connectivity in the gel no longer increases. Indeed, further increase in R up to 0.42 (Fig. 4c) led to only a moderate increase in G' modulus when compared with $R = 0.21$, as G'' maintained the same dependency on ω.

The minimum interpretation to account for the observed variation of G' in the gels initially formed at 85 °C and reinforced at 5 °C as a function of the degree of crosslinking with Ca^{2+}, R, is in reference to the "egg box" model previously proposed to explain the interaction of sequences of poly-α-D-galacturonic and poly-α-L-guluronic acids in Na^+ form in pectin and alginate, respectively (almost mirror-image structures, differing only in their orientation of O(3)) with Ca^{2+}. Both materials exhibit a parallel behavior

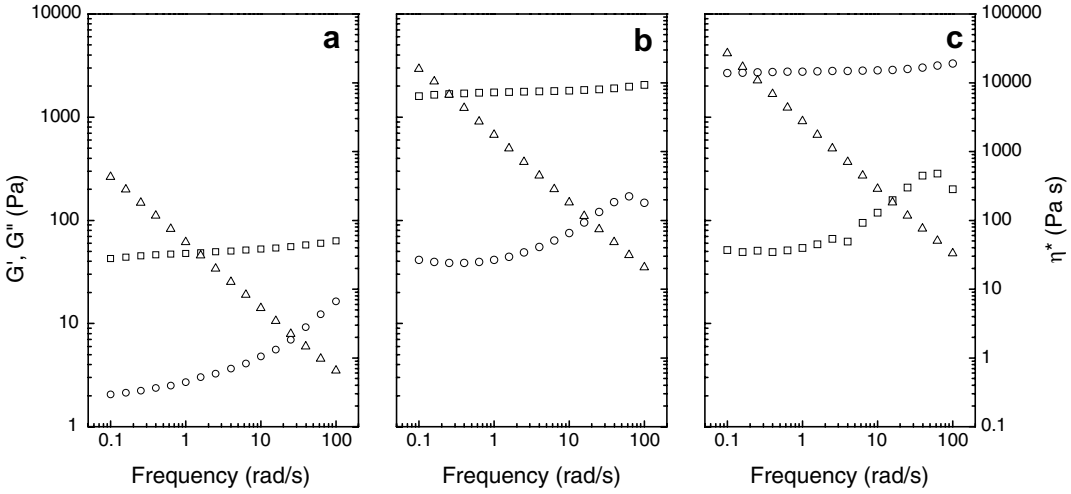

Fig. 4. Variation of the elastic, G' □, and loss, G'' ○, moduli (all in same scale as left axis of (a)) and of the complex viscosity, η^* △ (own right axis), in response to the oscillation frequency of pectin gels (at 5 °C, $\gamma = 0.05$) added with $CaCl_2$ at three levels of stoichiometric equivalence R (=2[Ca^{2+}]/[COO^-]): (a) 0.07, (b) 0.21 and (c) 0.42 (polymer concentration 16 g/L).

in their interaction with calcium, particularly in terms of the quantity resistant to being displaced by monovalent ions, which corresponds exactly to half the total stoichiometry (Morris, Powell, Gidley, & Rees, 1982; Morris, Rees, Thom, & Boyd, 1978).

This evidence, together with previous conformational studies using circular dichroism on calcium pectate films, was consistent with the formation of a stable dimeric structure in which the participating sequences adopt a twofold (2_1) zig-zag type conformation and the ion chelation takes place along the inner faces of both chains to form highly cooperative molecular assemblies (Grant et al., 1973). The establishment of this type of "egg-box" dimeric junction zones is responsible to a great extent for the connectivity and density of the gel network extended throughout the system (Durand et al., 1990; Garnier, Axelos, & Thibault, 1991) and it is expected to exhibit high thermal stability.

In previous studies addressing the rheological properties of pectin gels of low degree of esterification (Durand et al., 1990), it has been proposed that bound calcium is distributed between cooperative-type links of at least seven consecutive polygalacturonate residues and non-cooperative-type ones (with shorter sequences), of which only the former contribute predominately to the connectivity of the network and hence to the storage modulus.

It is possible that the dependence of G'' on ω, observed at the three levels of calcium crosslinking may reflect the existence of more than one mode of relaxation process in the gel network, due to the presence of such two distinctive types of locally ordered structures.

It is proposed that at 85 °C, shorter locally ordered junction zones are formed and partially stabilized due to lower chain mobility; on cooling, a conformational transition takes place and the new formed structure becomes now stabilized by H bonding (i.e. in a similar way as observed for hyaluronic acid at a temperature ~40 °C by Haxaire, Buhler, Perez, and Rinaudo (2002)) involving cooperative Ca^{2+} immobilization. This process is accompanied by a sharp increase in G' on cooling.

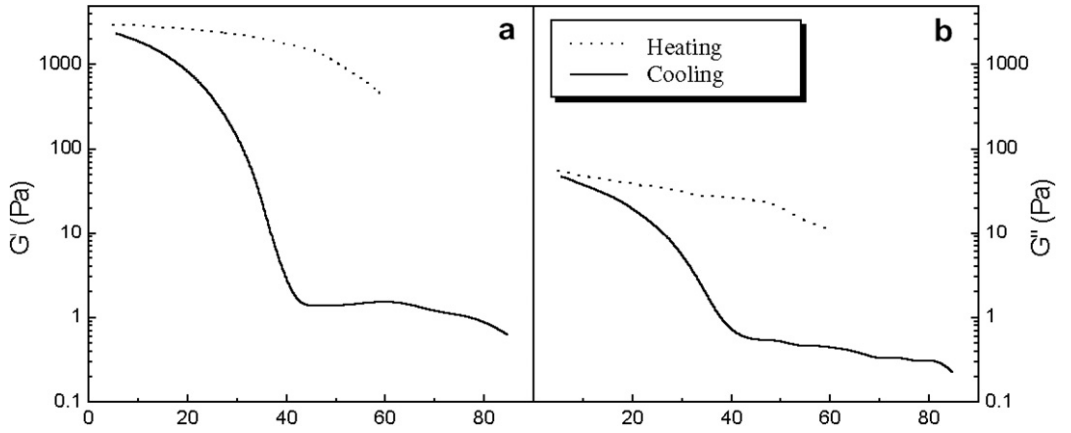

Fig. 5. Evolution of the (a) storage (G') and (b) loss (G'') mechanical moduli ($\omega = 1$ rad/s, $\gamma = 0.05$) during cooling (continuous line) and heating (dotted line) for cactus pectin gels at $R = 0.35$ calcium levels (conc. 16 g/L).

The evolution of the mechanical moduli with temperature was recorded at varying frequencies in order to glean further understanding of the molecular mechanisms and processes underlying CPAE gel formation in the presence of Ca^{2+}. Fig. 5 illustrates the formation and melting of a representative gel of R 0.35 on cooling and heating, respectively. The evolution of G' and G'' during cooling program shows a monotonic sigmoidal curve for the increase of both moduli in the range of 10–40 °C. Indeed the shape of the G' and G'' temperature traces resembles that of a sol-gel transition, similar in kind to that which determines the gelling behavior of other polysaccharides, notably agarose (Mohammed, Hember, Richardson, & Morris, 1998), κ-carrageenan (Piculell, Borgström, Chronakis, Quist, & Viebke, 1997), and gellan (Nishinari, 1996). However, this process cannot be regarded strictly as a sol-gel transition, as a pre-existing weak-gel structure is already formed at the onset of the steep increase of the storage and loss moduli. Heating traces of both G' and G'' recorded in the range 10 to ~60 °C are shown in the same plots. Clearly, a large hysteresis is observed between cooling and melting processes and the gel structure was found to persist even at ~60 °C, the highest temperature the system was taken up to. This behavior was found in contrast with previous stud-

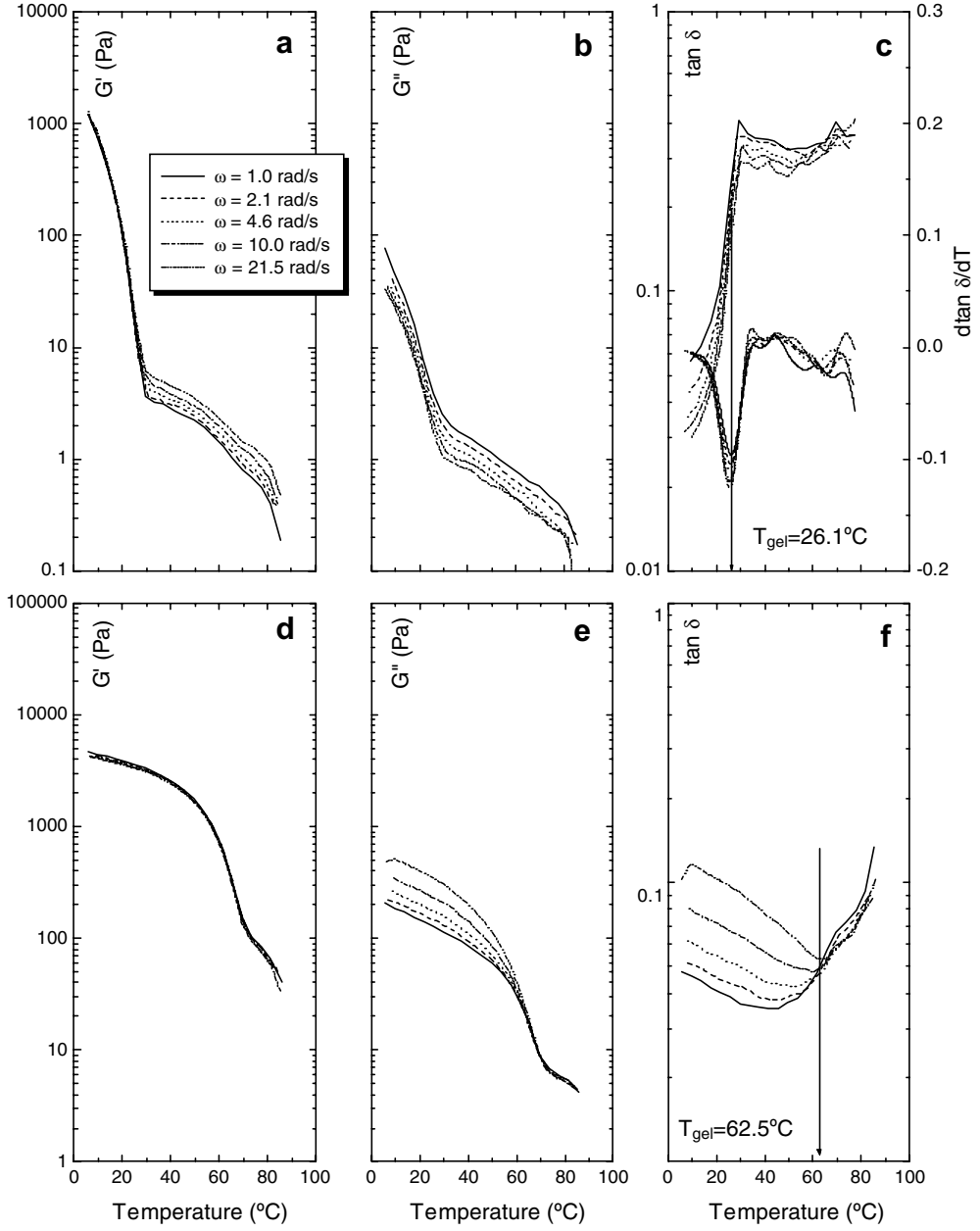

Fig. 6. Variation of the elasticity modulus G' (a and d), the loss modulus (b and e) and the liquid character index $\tan\delta$ (c and f) during cooling (at 1 °C/min strain, $\gamma = 0.05$, at frequencies, ω, indicated in (a)) of pectin gels added with $CaCl_2$ at stoichiometric levels R (=2[Ca^{2+}]/[COO^-]), of 0.21 (a–c) and 0.42 (d–f). (c and f) The critical rheological gelling temperature (T_{gel}).

ies (Gilsenan, Richardson, & Morris, 2000) conducted in pectin of low DE made at very low degrees of calcium conversion ($R \sim 0.01$) at pH in the range 2.5–3.5, where gelling and melting traces, recorded cooling and heating, respectively, were identical independently of the direction of the temperature change. In this case, it has been argued that the gel is stabilized by a thermoreversible H-bonded network involving partially protonated –COOH and –OH groups. However, in the same study, it has also been shown that when the pectin carboxylate groups become fully protonated at pH $\sim$ 2.0 (Gilsenan et al., 2000), melting occurred at temperatures substantially greater than those at which they were formed. As in other gelling polysaccharide systems, such as agarose, κ-carragenan and gellan, the large thermal hysteresis observed can be attributed to the aggregation of ordered structures as they form, favored in neutral polymers (e.g. agarose) or by screening of electrostatic repulsions in polyelectrolyte gels (Morris & Norton, 1983).

The molecular origin of the second gelling process observed on cooling in our system can be traced to a change in the conformation of the polygalacturonate in the extended 2_1 conformation occurring at lower temperature. This conformational transition in low DE pectin has been firmly demonstrated with experimental evidence from X-ray diffraction studies (Walkinshaw & Arnott, 1981), circular dichroism (Ravanat & Rinaudo, 1980), potentiometry, ^{13}C NMR, isothermal calorimetry (Cesàro, Ciana, Delben, Manzini, & Paoletti, 1982) and more recently by dynamic oscillatory rheology (Gilsenan et al., 2000). In an attempt to establish more precisely the relationship between the two processes suggested above, the dependence between the gelling temperatures observed on cooling (T_{gel}) was studied at different R and frequency values. Fig. 6 shows the variation of G' and G'' viscoelastic moduli along with $\tan\delta$ ($=G''/G'$) as a function of temperature during cooling at different ω for gels of $R = 0.21$ (Figs. 6a, b and c, respectively) and $R = 0.42$ (Figs. 6d, e and f, respectively). Notice that the change in the G' and G'' moduli with temperature are much the same as those observed for $R = 0.35$ (Fig. 5). From the $\tan\delta$ traces recorded at varying ω, it was possible to calculate T_{gel}, based on the rheological argument proposed by Winter and Chambon (1986) for the precise establishment of the critical gel temperature, T_{gel}. T_{gel} is defined as the critical point at which G' and G'' have a power-law dependence on ω, and therefore the values of $\tan\delta$ recorded at different ω converge. In the case of some CPAE gels, convergence of $\tan\delta$ (ω) was not observed, as shown for a representative sample of $R = 0.21$ (Fig. 6c). Therefore, an alternative empirical approach to mark the critical gelling temperature, T_{gel}, had to be adopted, by virtue of which the maximum gradient of the temperature trace of $\tan\delta$ in the vicinity of gel formation was computed from the first derivative of the $\tan\delta$ versus temperature trace. In the case of $R = 0.42$, the values of $\tan\delta$ did cross at the same value of T_{gel} (~62.5 °C) (Fig. 6f) which was notably superior to that

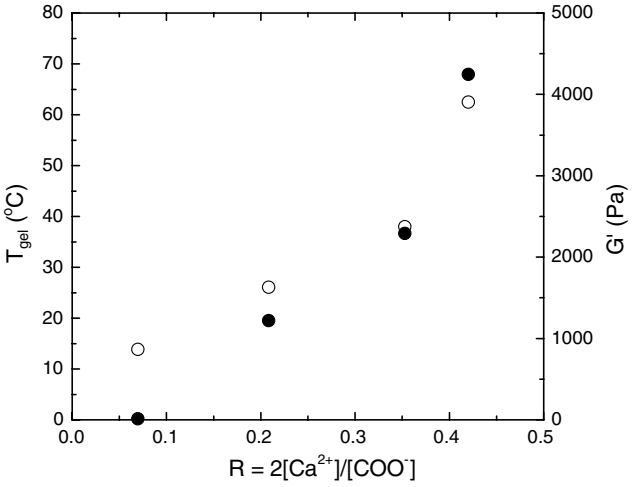

Fig. 7. Dependence of the critical rheological gelling temperature, T_{gel} (○) and the elasticity modulus, G' (●) of cactus pectin gels (5 °C and $\omega = 1$ rad/s) on the stoichiometric relation of the added calcium, R ($\omega = 1$ rad/s, $\gamma = 0.05$).

of $R = 0.21$ (~26.1 °C) and the rest of the gels, indicative of the stabilizing effect of the cross-linking of Ca^{2+} upon the formation of dimeric "egg-box-type" junctions and their successive aggregation. This is more clearly appreciated in Fig. 7, which demonstrates the dependence of the values of T_{gel} and the G' modulus at 5 °C as a function of R.

Clearly, as the contribution of the network formed by Ca^{2+} crosslinking predominates, the entropic cost of the sol-gel transition associated to the lower thermal association is reduced, and therefore the average temperature of said conformational transition, T_m, ($=\Delta H/\Delta S$ when $\Delta G = 0$, with their conventional definitions) increases. In all cases, homogeneous systems are obtained in which it is assumed that the gel networks are formed by the contribution of two mechanisms which coexist throughout the system.

4. Conclusions

Cactus pectin, extracted by means of an alkaline process assisted by a calcium sequestering agent (hexametaphosphate) previously used to extract pectin from other plant matrices, exhibits a low content of neutral sugars (and presumably low rhamnogalacturonan ramification) and inexistent esterification due to the alkaline pH. Addition of calcium at a high temperature to this material, at stoichiometric equivalency levels R ($=2[Ca^{2+}]/[COO-]$), which varied between 0.07 and 0.42 and the subsequent cooling led to the formation of elastic gels under a highly cooperative process whose critical gelling temperature on cooling increases directly with R. The gelling process is partially thermoreversible since the gels melt when heated, with a large thermal hysteresis (approx. 50 °C) at least for the gels with R up to 0.35. Gelling in this system is conceived as the consequence of two co-existing mechanisms at molecular

level: namely, short 'egg-box' type junctions formed directly at high temperature on addition of calcium and highly cooperative 2_1 helix junctions formed at lower temperature associated with a conformational transition driven by temperature, charge neutralization, lower coil mobility and followed by helix aggregation.

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