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Physical properties of water-soluble pectins in hot- and cold-break tomato pastes

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Abstract

Physical properties of water-soluble pectins (HP and CP) obtained from hot- and cold-break tomato pastes, respectively, and purified by anion-exchange chromatography were compared on the basis of their viscosities. The differences in viscosities between HP and CP solutions depended, not only on the average molecular mass, but also on the chemical structure. Neutral sugar-rich pectin, CP, was more flexible than galacturonic acid-rich pectin, HP. The three-dimensional structure of homogalacturonan was a hollow helix with ionic strength lower than 5 mM NaCl, which was built through hydrogen bonds between intramolecular free carboxyl groups. Such conformation was destroyed by ionic force (>5 mM NaCl). The effects of kinking structures on the physical properties of the rhamnogalacturonan were remarkable, as a consequence of $(1 \rightarrow 2)$ linkages of the rhamnosyl residues in the pectic backbone, and induced the molecular conformation of the pectin to sphere-like shape, which was unfavourable for Ca^{2+}-gelling.
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1. Introduction

Pectin is an important component of cell wall materials of tomato fruits, as well as various plants (Hwang, Pyun, & Kokini, 1993; Ridley, O'Neil, & Mohnen, 2001). This polysaccharide markedly influences the textural and rheological properties of plant-derived foods, such as tomato products, because of its great thickening and gel-forming capabilities. Therefore, the physical properties of pectins from tomato products have been extensively studied (Foda & Mccollum, 1970; Sharma, LeMaguer, Liptay, & Poysa, 1996; Sherkat & Luh, 1976). The physical properties of water-soluble pectins from hot- and cold-break tomato pastes have also been studied to some extent. Compared with hot-break paste, remarkably lower viscosity of the water-soluble pectin of

cold-break paste has been reported and was considered to be a result of the greater degree breakdown of pectin into low molecular mass by pectin-degrading enzymes, such as polygalacturonase, during processing (Anthon, Sekine, Watanabe, & Barrett, 2002; Luh & Daoud, 1971). Chou and Kokini (1987) have suggested that the hot-break tomato pectin has a random coil conformation while the cold-break pectin has a more rigid conformation. However, most of the published studies have only compared the physical properties of the water-soluble pectins with levels of the average molecular mass (Chou & Kokini, 1987; Luh & Daoud, 1971).

The focus of our work is to compare the physicochemical properties of the purified water-soluble pectins, which were obtained from hot- and cold-break tomato pastes, respectively. Previously, we successfully compared the chemical properties of the water-soluble pectins between both types of pastes processed at 90 and 70 °C, respectively (Lin et al., 2004). In this paper, we

have made an effort to clarify the physicochemical properties of the purified pectins obtained previously from the hot- and cold-break pastes.

2. Materials and methods

2.1. Materials

Water-soluble pectins (HP and CP) were extracted from hot- and cold-break tomato pastes and fractionated by DEAE-cellulose column chromatography (from HP, HP3, HP4 and HP5, and from CP, CP2, CP3 and CP4 were obtained) (Lin et al., 2004). Those fractions, almost free of methylesterification, were used as experimental materials. Some of their properties are listed in Table 1. Fractions from HP are rich in galacturonic acid, while those from CP are rich in neutral sugars.

2.2. Viscosity measurement

The Ostwald viscometer was selected to measure the viscosity of the solution, since the viscosity of pectin in dilute solution is low and, for a dilute solution, the molecular behaviour of pectin is good for observation and comparison. Ten ml of solution were pipetted into the Ostwald viscometer and the flow time (in seconds) was determined. All the measurements were made at 25 °C.

3. Results and discussion

Fig. 1 shows the concentration-dependence of reduced viscosities (η_{sp}/C) for HP and CP fractions. The data show that the η_{sp}/C of HP was obviously higher than that of CP, which was similar to published studies (Chou et al., 1987; Luh et al., 1971). For a dilute solution, the η_{sp}/C is primarily related to the molecular size. As concentration increases, the polymer coils start to overlap and become entangled, with the viscosity show-
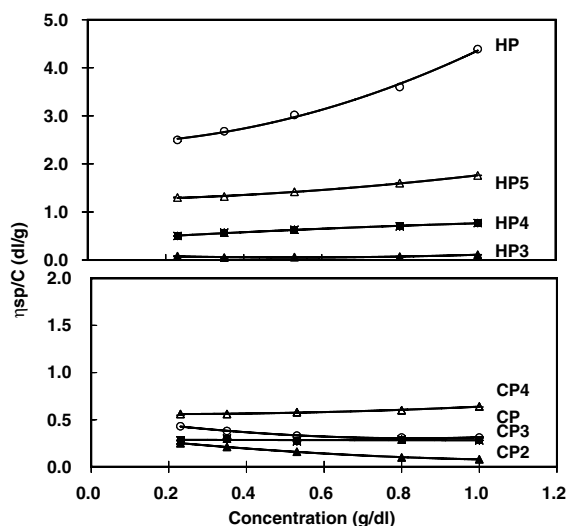

Fig. 1. Concentration-dependence of reduced viscosity of HP and CP fractions in 0.05 M ammonium oxalate. The data are the average of three determinations per point.

ing a more marked dependence on concentration. The η_{sp}/C of HP and HP5 increased with pectin concentration, indicating that the extent of coil entanglement of these pectins increased as concentration increased. Irrespective of the high average molecular mass, CP4 showed rather low η_{sp}/C and less coil entanglement. This result was in agreement with a published study by Chou and Kokini (1987), demonstrating that HP and HP5 had a random coil conformation, while CP4 had a rigid conformation. Furthermore, HP showed higher values of η_{sp}/C than all of its separated fractions, increasing the likelihood that various low molecular weight molecules were efficient cross-linking bridges for high molecular weight molecule crossing.

Fig. 2 gives the plot of log $[\eta]$ vs log Mw for HP and CP fractions. The slope of the available linear plot exhibited the exponent α, which represented the molecular conformation of polymers in the equation of $[\eta] = kM^{\alpha}$. From both dotted lines, A and B, the exponent α for HP fractions was estimated roughly to be in the range of 0.69–1.46, whereas the slope of the solid line drawn by the linear first-order least-squares method through the three HP fractions is 1.02. For homogalacturonan, with a similar molecular mass to HP5, its α value, reported by Garnier, Axelos, and Thibault (1993) was 0.78, which was in the α value range for HP fractions. The exponent α of pectin seems to increase with low molecular mass. Unlike HP fractions, the exponent α of CP fractions (with a value of 0.34) was rather low. The high and low values of the exponent α demonstrate that the molecules of the HP fractions acted as rod-like shapes, in which the molecular conformation of pectin transforms tendentiously toward coil shape with high molecular mass, while that of the CP fractions acted as a sphere-like shape.

Table 1
Some properties of fractions obtained from HP and CP

Fraction	$[\eta]^a$ (ml/g)	Mw^b ($\times 10^4$)	Sugar composition (%)				
			GalA	Rha	Ara	Xyl	Gal
HP	261	–	57.6	4.0	4.0	2.0	6.3
HP3	9	0.9	53.0	3.7	4.0	2.0	5.8
HP4	48	2.8	61.3	4.9	1.8	1.2	2.3
HP5	130	11.7	68.9	2.3	1.5	1.6	2.5
CP	43		33.1	12.5	4.5	2.1	12.8
CP2	25	1.3	24.3	2.0	3.2	1.5	22.5
CP3	26	1.7	39.4	11.9	4.8	2.3	7.5
CP4	57	16.2	43.4	18.6	1.2	1.9	2.3

[a] Intrinsic viscosity, obtained from Fig. 1.
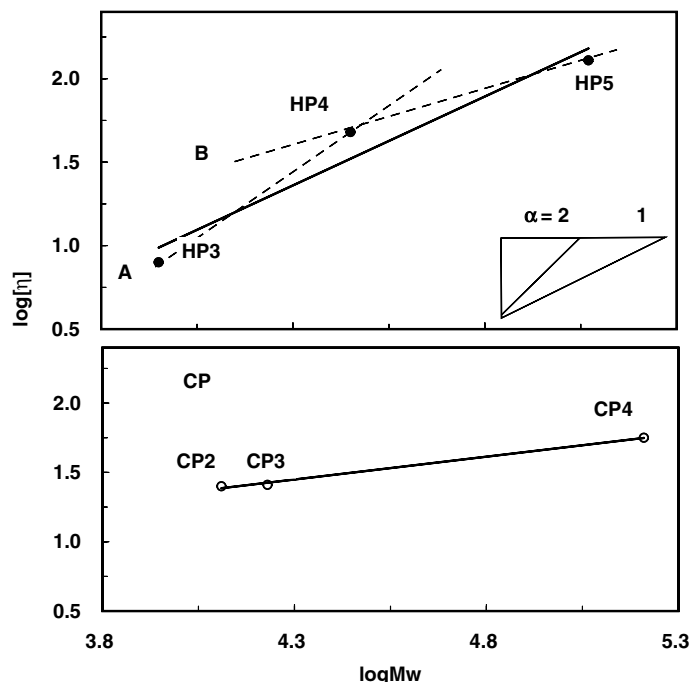[b] Mw, average molecular weight.

Fig. 2. Log [η] vs log Mw of HP and CP fractions. Solid lines were drawn by the linear first-order least-squares method. Dotted lines A and B were drawn for HP3–HP4 points with a slope of 1.46 and for HP4–HP5 points with a slope of 0.69, respectively. The error bar was determined by taking the average of the standard deviation of the data points from the curve of Fig. 1.
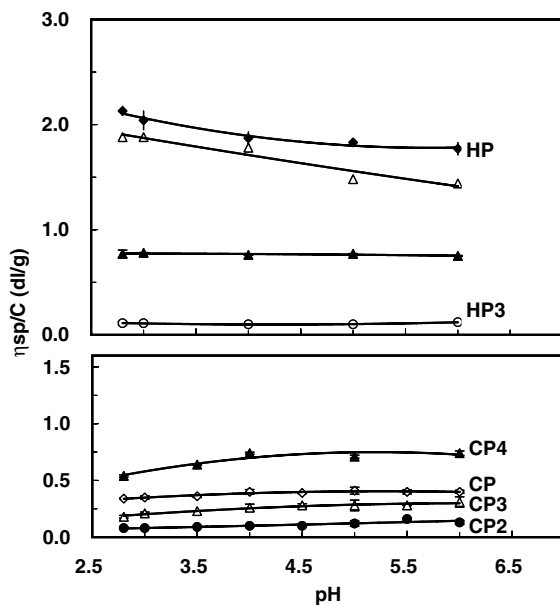

Fig. 3. Effect of pH on reduced viscosity of HP and CP fractions in 0.02 M sodium acetate. Concentration of each sample was made up to 0.4%. The data are the averages of three determinations per point.

Fig. 3 illustrates the effect of pH on η_{sp}/C for HP and CP fractions. The pectin concentration was made up to 0.4% in 0.02 M sodium acetate buffer with a pH range from 2.7 to 6. As shown in Fig. 3, only the η_{sp}/C of HP5 and HP increased as the pH decreased, whereas the phenomenon for HP was considered to result from the presence of HP5. Although HP3 and HP4 were also

rich in galacturonic acid, no change was observed. For the low-methoxyl pectin, a low pH may facilitate the intermolecular interaction through hydrogen bonds, but the molecular size seems to be a decisive factor for efficient cross-linking. The η_{sp}/C of CP4 was not affected by pH, implying that the sphere-shape conformation was unfavourable for intermolecular interaction.

The above results illustrate that the molecular conformation of pectin, which directly relates to the viscosity, mainly depends on chemical structure, as well as molecular mass. Particularly, it is obvious for HP5 and CP4, which represent homogalacturonan and rhamnogalacturonan, respectively. As shown in Fig. 4, the effects of polyelectrolyte, for both HP5 and CP4, are clear (Yamanaka, Matsuoka, Kitano, Hasegawa, & Ise, 1990), although the plot of HP5 was less regular.

The solution was made up to 0.5 mg of galacturonic acid (GalA) per ml, which corresponded to 0.083% for HP5 and 0.116% for CP4 (with these concentrations, both showed extensive in H_2O), and the effect of NaCl on η_{sp}/C was examined. As shown in Fig. 5, for HP5 two steps that separated at $\approx$5 mM NaCl appeared. At the separated point, the η_{sp}/C of HP5 dropped vertically from a high level to a rather low level. As mentioned above, for the dilute solutions, the η_{sp}/C is primarily related to the size of the macromolecules. Therefore, the result indicates the molecular size (real occupying volume of molecule, V_r) in <5 mM NaCl, was much higher than that in >5 mM NaCl. The three-dimensional structure of HP5 was obviously
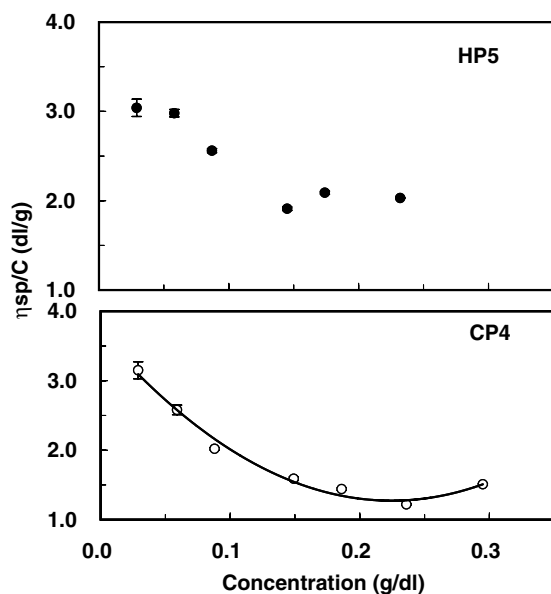
Fig. 4. Concentration dependence of reduced viscosities of HP5 and CP4 in water. The data are the average of three determinations per point.

changed at 5 mM NaCl. Homogalacturonan has been modeled as a left- or right-handed helical (two-, three-, or fourfold) chain with a rather high characteristic ratio (rigid rod-shape), which was accounted for an unperturbed state in 0.1 M NaCl (Pérez, Mazeau, & Penhoat, 2000; Ruggiero, Urbani, & Cesàro, 1995). Since a hollow helical conformation of molecular chain demands a much higher molecular volume than a straight chain,

the three-dimensional structure of HP5 was likely to be a hollow helical shape at <5 mM NaCl, which was built upon the characteristic helical chain. Such a three-dimensional structure may be retained by hydrogen bonds between the intramolecular free carboxyl groups, where the helix of uronic acids along the chain was favourable for forming such a hollow helical structure. With an increase in ionic strength, the hydrogen bonds were broken and the hollow helical structural molecule was transformed to random molecular chain with extremely low viscosity. In contrast, CP4 showed an η_{sp}/C that gradually decreased as ionic strength increased. The tendency of decrease in viscosity with ionic strength seems to be a result of molecular compression, which also appeared in the HP5 solution beside the separated point at approximately 5 mM NaCl. The molecular conformation of CP4 was unchanged by ionic strength.

The solution was also made up to 0.083% for HP5 and 0.116% for CP4 as mentioned above, and the effect of $CaCl_2$ on η_{sp}/C was examined. The Ca^{2+} ions in the initial solutions for HP5 and CP4 were determined to be 1.1 and 1.8 mol% of $Ca^{2+}/GalA$, respectively. The measurement became impossible as sedimentation of pectin occurred at concentrations of more than 31 and 52 mol% of $Ca^{2+}/GalA$ for HP5 and CP4, respectively. As shown in Fig. 6, the η_{sp}/C of HP5 was lower than that of the initial solution at a concentration of less than 21.1 mol% of $Ca^{2+}/GalA$, and then became sharply high with an increase in the $Ca^{2+}/GalA$ ratio. This result indicates that for a low ion strength, the effect of Cl^- anions on the viscosities of HP5 was higher than Ca^{2+}. It seems
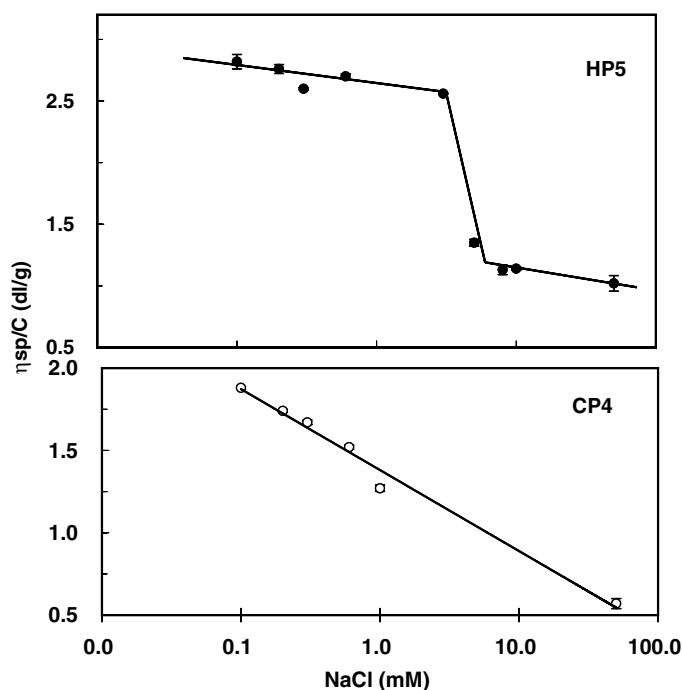

Fig. 5. Effect of NaCl on reduced viscosity of HP5 and CP4 solutions. The concentration was made up to 0.5 mg of GalA per ml, which corresponded to 0.083% and 0.116% for HP5 and CP4, respectively. The data are the averages of three determinations per point.
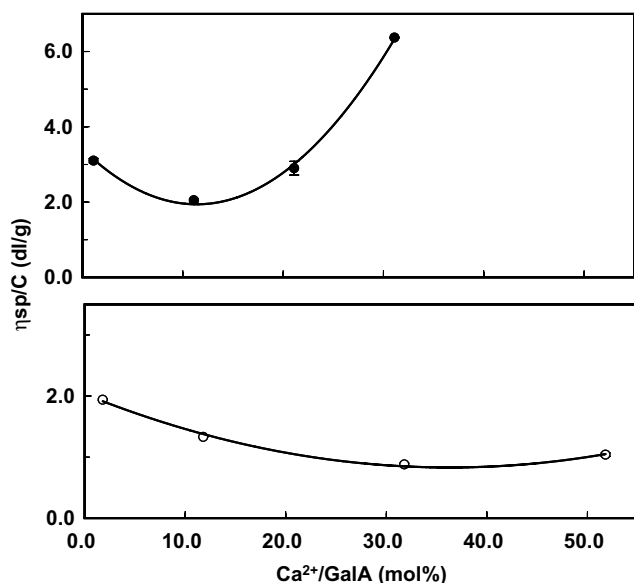
Fig. 6. Effect of CaCl$_2$ on reduced viscosity of HP5 and CP4 solutions. The concentration was made up to 0.5 mg GalA per ml, which corresponded to 0.083% and 0.116% for HP5 and CP4, respectively. The concentrations of CaCl$_2$ were 0.28, 0.57 and 0.85 mM for HP5, and 0.28, 0.85 and 1.42 mM for CP4, which corresponded to 11.1, 21.1, 31.1 and to 11.8, 31.8, 51.8 mol% of Ca^{2+}/GalA, respectively. The data are the averages of three determinations per point.

that the Ca^{2+}-gelling of HP5 occurred in a two-stages. The hollow helical structures of the molecules were first destroyed by Cl$^-$ anions and caused the pectin solution to have a lower viscosity, then, with an increase in the Ca^{2+}/GalA ratio, the random molecular chains liberated by Cl$^-$ anions dimerized through Ca^{2+} ions. Unlike HP5, CP4 showed a η_{sp}/C decrease generally with an increase in Ca^{2+}/GalA ratio. This result reveals that the sphere-like molecular conformation was unfavourable for Ca^{2+}-gelling.

From the above experimental data, the effects of kinking structures on the molecular conformation of the rhamnogalacturonan are significant, as a consequence of $(1 \rightarrow 2)$ linkages of rhamnoyl residues in the backbone, and they induce the sphere-like molecular conformation. This result is in good agreement with some published studies (Hallman & Whittington, 1973; Rees & Wight, 1971; Ruggiero et al., 1995), and seems to conflict with the suggestion that the overall chain conformation of rhamnogalacturonan (the ratio of rhamnose could be up to ~25%) remained relatively unchanged as a consequence of the self-cancellation of the kinking effects of successive paired rhamnose units, which have been reported later by Cros, Garnier, Axelos, Imberty, and Pérez (1996). We suggest that the value of GalA/Rha ratio may be a determinant factor influencing the overall molecular conformation. In a high GalA/Rha ratio range (homogalacturonan), the effects of kinking structures on the whole molecular conformation may be negligible, as suggested by Cros et al.

(1996). With the GalA/Rha ratio decreasing (rhamnogalacturonan), the effects become a decisive factor. Since the molecular polarity of rhamnose is markedly lower than that of galacturonic acid, it may be possible that, in the pectin with low GalA/Rha ratio, intramolecular aggregation occurs among the rhamnose residues in polar solvents and promotes the effects of kinking structures.

HP3 and HP4 are high in GalA/Rha ratio (14.3 and 12.5), and belong to the type of the pectins studied by Cros et al. (1996). CP2 also has a high GalA/Rha ratio (12.2), but the galactose, presented as a side chain (galactan), is quantitatively equivalent to the galacturonic acid. So the neutral sugars may be become a decisive factor influencing the physical properties. CP3 has a low GalA/Rha ratio of 3.3 and its physical properties may be similar to CP4, although the contribution of the neutral sugars (side chains) also seems significant. In general, neutral sugar-rich pectins exhibited a lower α exponent than uronic acid-rich pectins, demonstrating that these pectins are more flexible. Comparing the neutral sugars and GalA, it is not difficult to find that the neutral sugars are free of bulky function groups. However, the C-6 carboxyl groups of galacturonic acids, in their preferred equatorial orientation stabilise the $^4C_1$ conformation of the D-galacturonic acids and lead the homogalacturonan chain into axial–axial $(1 \rightarrow 4)$ α-D-galacturonic acid linkages (Pérez et al., 2000). Such a conformation of the homogalacturonan, therefore, has a high characteristic ratio, reported to be approximately 55 and 150 (degree of polymerization to be ~400) by Cros et al. (1996) and Ruggiero et al. (1995), respectively.

It is clear that the decrease of GalA ratio in pectin promotes flexibility of the pectic molecules and increases the kinking effects of the rhamnose residues in the molecular conformation. This characteristic has been well exhibited in the physical properties of the water-soluble pectin in cold-break paste and causes rather lower viscosities than that of the water-soluble pectin in hot-break paste.

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