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

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Abstract

Tomato fruit paste prepared by hot-or cold-break processing was extracted with water to obtain a water-soluble pectin (HP or CP) and a water-insoluble matter (HR or CR). HP yield was approximately twice that of CP, while those of the water-insoluble matters were similar. Comparing sugar compositions, HP (GalA-rich) was remarkably different from CP (neutral sugars-rich), although their water-insoluble matters were similar. This result indicates that the solubilizations of cell-wall-associated pectins in HB and CB were similar, but the release of GalA by endogenous pectin-degrading enzymes in CB was remarkable.

Fractions separated by a DEAE–cellulose column from HP (HP3, HP4, HP5) were also rich in GalA, while those from CP (CP2, CP3, CP4) were all rich in neutral sugar. However, the ratios of individual sugars of CP fractions were very different. Unlike the neutral sugar-rich fractions, the GalA-rich ones treated with polygalacturonase were mostly degraded. In addition, the chemical structure of HP5 (homogalacturonan) and CP4 (rhamnogalacturonan) were compared after mild acid hydrolysis.

Keywords: Chemical properties of pectins; Water-soluble pectins; Hot-break tomato paste; Cold-break tomato paste

1. Introduction

Pectin is an important component of cell-wall materials of plants, including tomato fruits (Hwang, Pyun, & Kokini, 1993; Ridley, O'Neil, & Mohnen, 2001). The structure of pectin consists of a partially methyl-esterified poly-D-galacturonic acid backbone with interposed L-rhamnose residues. The simple pectin, usually known as homogalacturonan (or smooth region), contains major (1 → 4) linked poly-D-galacturonic acid residues, while the more complex one, known as rhamnogalacturonan (or hairy region), contains repeating unit structures of → 4)-α-D-Gal*p* A-(1 → 2)-α-L-Rha *p*-(1 → with ramified neutral sugars (Renard, Crépeau, & Thibault, 1995; Ridley et al., 2001). This polysaccharide significantly influences the textural and rheological properties of tomato products, because of its great thickening and gel-forming capabilities (MacDougall, Needs, Rigby, & Ring, 1996; Narayanan, Deotare, Bandyopadhyay, & Sood, 2002; Sharma, LeMaguer, Liptay, & Poysa, 1996). Therefore, both the physicochemical properties of pectins (Chou & Kokini, 1987; Luh & Daoud, 1971; Sherkat & Luh, 1976) and the inactivation of endogenous pectin-degrading enzymes have been extensively studied in order to achieve a desired product quality (Anthon, Sekine, Watanabe, & Barrett, 2002; Stoforos, Crelier, Robert, & Taoukis, 2002).

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In general, two types of tomato pastes are prepared and used for maintaining a settled quality of products on manufacture. One is "hot-break" paste (HB), processed at a high temperature, and the other is "cold-break" (CB), processed at a lower temperature. The viscosity of the water-soluble pectin from CB has been reported to be markedly lower than that from HB (Chou & Kokini, 1987; Luh & Daoud, 1971). Usually, both pectin-breakdown and pectin-solubilization during process have been simply attributed to the pectin-degrading enzymes (Anthon et al., 2002; Chou & Kokini, 1987; Luh & Daoud, 1971). Recently, Cámara, Greeve, and Labavitch (1999) have found that the processed tomato-fruit juice, for a prolonged heating, leads to progressive pectin solubilization, even without the involvement of the pectin-degrading enzymes, which has also been reported by Ciruelos, González, Latorre, Ruiz, and Rodríguez (2001). Later, Hurtado, Greve, and Labavitch (2002) have reported that the production of HB is accompanied by not only the solubilization of cell wall-bound pectin, but also the breakdown of pectin polymer. By now, the physicochemical properties of water-soluble pectins from HB and CB have only been compared with levels of the average molecular mass, and the detailed chemical properties of these pectins are still unknown. In this paper, we have compared the chemical properties of the water-soluble pectins obtained from HB and CB, processed at 90 and 70 °C, respectively.

2. Materials and methods

2.1. Materials

Two processed tomato pastes (HB and CB), supplied by Kagome Co. Ltd., (Japan) were prepared from the same variety (*Lycopersicon esculentum* M.). HB and CB contained 70.1% and 68.3% of moisture, respectively, and were stored at −20 °C prior to use. A series of dextrans (T500, 487 kDa; T110, 105 kDa; T40, 43.5 kDa; and T10, 10.5 kDa) was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

2.2. Extraction of water-soluble pectins

Each thawed paste (25 g) was directly dehydrated by methanol and then defatted sequentially by chloroform-methanol (1:1, v/v) and diethyl ether until the red colour removal was complete. After air-drying, the residue was extracted several times with 2.5 l of distilled water at 4 °C for 12 h, with stirring, followed by centrifugation. The supernatant was concentrated to almost 1/10 of the initial volume, and was then treated slowly with three volumes of ethanol, and kept overnight at 4 °C with stirring. The resulting precipitate was collected by centrifugation, followed by dehydration via solvent exchange, to obtain water-soluble pectin (abbreviated as HP from HB and CP from CB, respectively).

2.3. General methods

Total sugar was determined by the phenol–H_2 SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), galacturonic acid by the *m*-hydroxyldiphenol method (Ahmed & Labavitch, 1977), and degree of methyl-esterification (DE) by the chromotropic acid method (Wood & Siddiqui, 1971). The neutral sugar content was calculated after subtracting the contribution of the galacturonic acid in colorimetry. Glycosyl residues (neutral sugars) of the polysaccharides were analyzed by gas–liquid chromatography (GLC) as alditol acetates after acid hydrolysis (Blakeney, Harris, Henry, & Stone, 1983). In the acid hydrolysis, step for water-insoluble matter, the sample was pretreated with 75% H_2SO_4 at room temperature for 30 min, then diluted (to 1 M H_2SO_4) and heated at 100 °C for 3 h. For a water-soluble pectin, the sample was directly treated with 2 M trifluoroacetic acid (TFA) at 120 °C for 3 h. GLC was carried out on a Shimadzu GC-18A apparatus equipped with a flame ionization detector. A capillary column of CBP-10-M25-025 (0.22 mm × 25 m) was used and operated at 220 °C with a gas flow rate of 0.8 ml/min of nitrogen. Peak areas were calculated with a Chromatocorder-21 (System Instruments Co., Tokyo, Japan). The ratio of neutral sugars was calculated from the GLC peak areas.

2.4. DEAE-cellulose column chromatography

Anion-exchange chromatography was performed on a column (3 × 20 cm) of DEAE–cellulose in HCO_3^- form. After applying the sample (HP or CP, 150 mg) onto the column bed, the column was first washed with distilled water, then eluted stepwise with each 160 ml of 0.1, 0.2, 0.3, 0.4, and 0.5 M NH_4 HCO_3.

2.5. Gel filtration chromatography

The average molecular weight of HP and CP fractions was determined using a Toyopearl HW-60 column (1.5 × 92 cm). Each sample (5 mg) was loaded onto the column and eluted with 0.2 M ammonium oxalate solution at a flow rate of 25 ml/h. Dextrans, T110, T40, and T10 were used as standards.

The distribution of molecular weight of HP and CP fractions after polygalacturonase treatment was determined using a Sephadex G-75 column (2.5 × 90 cm) equilibrated with 0.1 M acetate buffer (pH 4.0) at a flow rate of 25 ml/h. The distribution of molecular weight of HP5 and CP4, before and after mild acid hydrolysis, was determined using the same gel in a column (1.5 × 95 cm) equilibrated with 0.1 M NaCl.

2.6. Polygalacturonase (PG) treatment

Polygalacturonase from *Aspergillus niger* (purchased from Sigama) was purified according to the procedure of Thibault and Mercier (1977). Polygalacturonase treatment of pectin was based on the method of Rombouts and Thibault (1986) with 0.05 M acetate buffer (pH 4.0). The final concentration of substrate was made up to 2 mg/ml in the presence of 1.6 nkat/ml of polygalacturonase, and the reaction mixture was incubated at 30 °C for 24 h before being inactivated at 100 °C for 5 min. One nkat was expressed as liberating 1 nmol of galacturonic acid from polygalacturonic acid per second.

2.7. Mild acid hydrolysis

The pectin solutions of HP5 and CP4, stocked at 4 °C, were used for experiment. The final concentration of the total sugar was made up to 1 mg/ml in the presence of 0.23 M HCl. After incubation at 80 °C for 10 h, the reaction mixture was precipitated slowly (drop-wise) with three volumes of ethanol, and kept overnight at 4 °C with stirring, then separated by centrifugation at 20,000*g* for 30 min.

3. Results and discussion

The yields and sugar compositions of water-soluble pectins (HP and CP) and water-insoluble residues (HR and CR), obtained from hot- and cold-break pastes, respectively are listed in Table 1. The data show that both residues were similar in yield, and had similarly low galacturonic acid and rhamnose contects. This result indicates that the extent of increased water-soluble pectins, that had originally belonged to the cell wall-associated pectins in the tomato fruits for both pastes, were equivalent. Nevertheless, CP showed a rather low yield, in other studies (Chou & Kokini, 1987; Luh & Daoud, 1971), and was significantly lower in galacturonic acid and higher in neutral sugar than was HP. This may be explained by slower inactivation of pectic enzymes such as polygalacturonase (PG) during the cold-break paste process than in the hot-break process (Anthon et al., 2002; Luh & Daoud, 1971; Stoforos et al., 2002). Comparing the ratio of the individual neutral sugars to rhamnose (Ara/Rha, Xyl/Rha, and Gal/Rha) between HP and CP, a reduction in the three neutral sugars (arabinose, xylose, and galactose) in CP was observed, while rhamnose remained high in CP. This result indicates that the enzymatic pectin-degradation occurred mainly in the homogalacturonan region, where some neutral sugars (arabinose, xylose and galactose) were also distributed in side chains (Hwang et al., 1993; Ridley et al., 2001). Table 1 also shows that both pectins were extremely low in degree of methylesterification (DE), which was confirmed by ^{1}H NMR (data not shown). This result was significantly different from the one reported by Chou and Kokini (1987) when the DE of HP reached 62%. However the result seems to be reasonable, since the activation of pectin methylesterase (PME) was considered to be extremely different between in vivo and in vitro (Anthon et al., 2002; Koch & Nevins, 1989; Seymour, Lasslett, & Tucker, 1987). A further investigation of PME during the process would be necessary in order to provide a detailed explanation.

Fig. 1 shows the elution profile of HP and CP conducted on a DEAE–cellulose column. Three major fractions (HP3, HP4, and HP5, separated at 0.3, 0.4, and 0.5 M NH_4HCO_3, respectively) were obtained from HP, and three major fractions, CP2, CP3, and CP4 were obtained from CP. Some properties of these fractions are summarized in Table 2. The ratio of uronic acid to neutral sugar, in both fractionations, increased with the ionic strength. In general, all of the fractions from HP were rich in galacturonic acid, with a high ratio of GalA/Rha, but were different in average molecular size. Hurtado et al. (2002) pointed out that the water-soluble pectin of HB was broken down in molecular mass, although the inactivation of PG had been accomplished at the juice process step. Therefore, it might be possible that a part of the original pectin was impacted by heating beside the action of pectin-associated enzymes, and cleaved into lower molecular homogalacturonan, such as HP3 and HP4, during the

Table 1
Yield and sugar composition of water-soluble pectins and water-insoluble residues from HB and CB

Fraction	Yield[a] (%)	DE[b] (mol%)	Sugar composition (%)							
			GalA	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
HP	6.9	13.3	57.6	4.0 (1.0)[c]	0.2	4.0 (1.0)	2.0 (0.5)	0.9	6.3 (1.6)	2.4
HR	12.1		9.0	0.4	ND[d]	1.4	5.0	ND	4.7	49.3
CP	3.8	11.9	33.1	12.5 (1.0)	0.3	4.5 (0.4)	2.1 (0.2)	0.7	12.8 (1.0)	1.5
CR	11.4		6.4	0.2	ND	1.5	5.6	ND	5.3	53.7

[a] Expressed as the yield of dry-solid.
[b] DE, degree of methylesterification.
[c] The data in parentheses are expressed as the ratio of respective neutral sugar to Rha.
[d] ND, not detected.

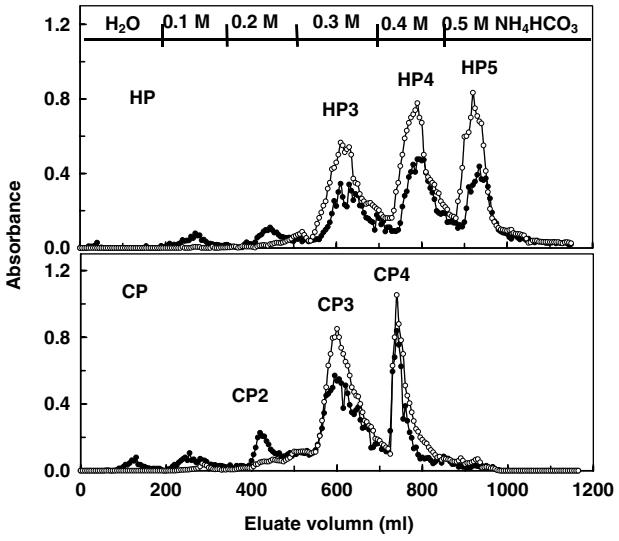

Fig. 1. Elution profile of HP and CP on a DEAE-cellulose column (HCO_3^- form). Closed circles, total sugar; open circles, galacturonic acid. Experimental conditions are described in Section 2.

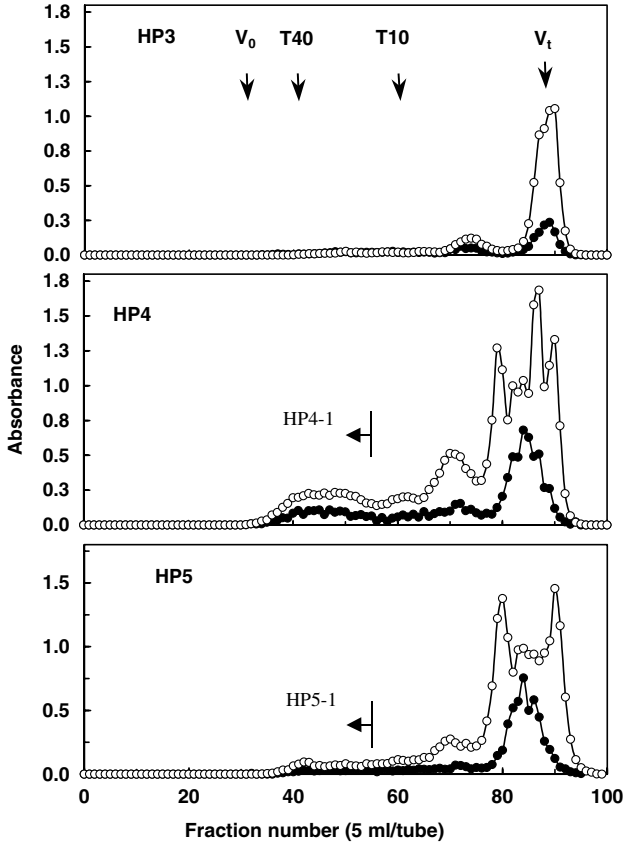

Fig. 2. Chromatography of HP3, HP4 and HP5, after pectinase treatment, on a Sephadex G-75 (2.5 × 90 cm) column eluted with 0.1 M acetate buffer, pH 4.0. Closed circles, total sugar; open circles, galacturonic acid. T500 and D-galacturonic acid were used to estimate the void and the total volume (V_0 and V_t) of the column, respectively. Experimental conditions are described in Section 2.

HB manufacture (Table 2). CP2 also showed a high GalA/Rha ratio (12.2), but the galactose, as the most dominant neutral sugar, was quantitatively equivalent to galacturonic acid. Galactan has been reported as a major side chain of pectin in tomato fruits (MacDougall et al., 1996). The galactan, as present in this fraction, seems to be attached to the homogalacturonan region. On the other hand, CP3 and CP4 were both rich in rhamnose, but different in the ratio of the individual neutral sugars and average molecular weight (Table 2). This result means that the types of rhamnogalacturonans (hairy regions) were various in the original pectins.

During polygalacturonase treatment, all of the fractions (HP3, HP4, HP5, CP2, CP3, and CP4) obtained by performance liquid chromatography showed that the release of galacturonic acid increased with incubation time until 20 h, although releases from CP fractions (neutral sugar-rich) were weaker (data not shown). Figs. 2 and 3 show the elution patterns of the resulting products after incubation for 24 h on a Sephadex G-75 column. Corresponding to their GalA/Rha ratio, the degradation of HP fractions was rather marked in contrast to those of CP fractions. As shown in Figs. 2 and 3, there were still somehigh molecular fractions (notably as HP4-1, HP5-1, CP2-1, CP3-1, and Cp4-1) apart from HP3. Furthermore, on an anion-exchange chromatography column,

Table 2
Some properties of fractions obtained from HP and CP on the DEAE–cellulose column

Fraction	DE[a] (mol%)	Mw[b] (×10⁴)	Sugar composition (%)							
			GalA	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
HP3	4.8	0.91	53.0	3.7	0.2	4.0	1.6	0.2	5.8	0.7
HP4	ND[c]	2.75	61.3	4.9	ND	1.8	1.2	0.1	2.3	1.0
HP5	ND	11.70	68.9	2.3	0.1	1.5	1.6	0.2	2.5	1.5
CP2	17.5	1.32	24.3	2.0	0.1	3.2	1.5	0.1	22.5	0.9
CP3	7.0	1.70	39.4	11.9	0.7	4.8	2.3	ND	7.5	0.6
CP4	ND	16.20	43.4	18.6	0.2	1.2	1.9	ND	2.3	0.2

[a] DE, degree of methylesterification.
[b] Mw, average molecular weight.
[c] ND, not detected.

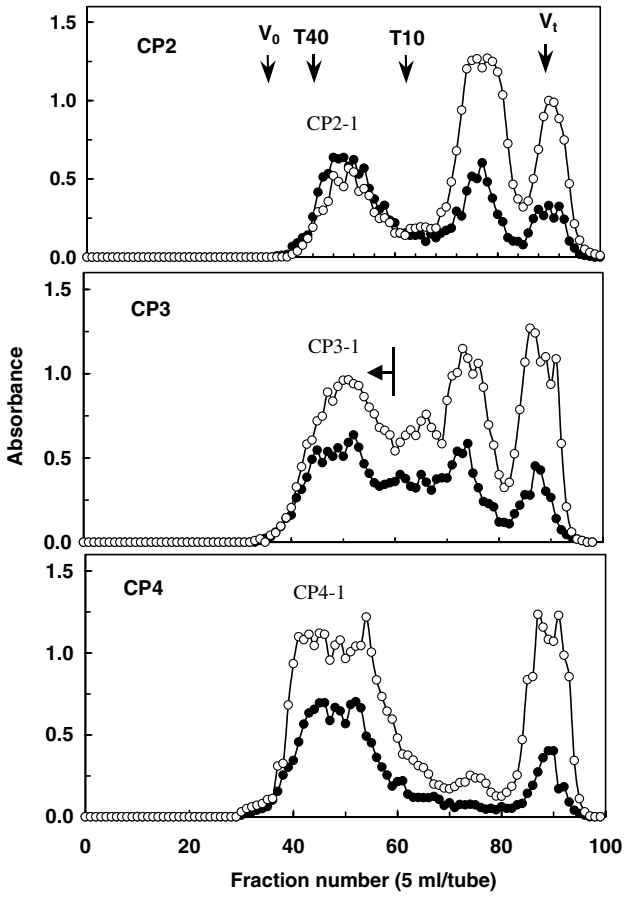

Fig. 3. Chromatography of CP2, CP3 and CP4, after pectinase treatment, on a Sephadex G-75 (2.5 × 90 cm) column eluted with 0.1 M acetate buffer, pH 4.0. Closed circles, total sugar; open circles, galacturonic acid. T500 and D-galacturonic acid were used to estimate the void and the total volume (V_0 and V_t) of the column, respectively. Experimental conditions are described in Section 2.

these fractions all appeared almost as a sole peak, indicating that they were all essentially pure compounds. Analysis of sugar compositions showed that the ratio of galactose in CP2-1 increased to almost twice that of CP2, while the ratio of GalA/Rha was almost unchanged (data not shown). This result increases the likelihood that the galactan was attached to the homogalacturonan region. In contrast to CP2-1, the rest of the fractions (HP4-1, HP5-1, CP3-1 and Cp4-1) were considered to be ramnogalacturonan, containing mostly both galactuonic acid and rhamnose (Colquhoun, 1990; MacDougall et al., 1996; Renard et al., 1995). As illustrated in Fig. 3, the elution profile of CP3 product covered all of the included volume while, for CP4, only two peaks appeared, at the void and the total volume, which corresponded to their different ratios of the individual sugars. This result implies that the rhamnogalacturonan block in CP4 was simple and concentrated, while CP3 was more complicated.

Apart from the ratio of GalA/Rha (30 for HP5 and 2.3 for CP4), the chemical properties of HP5 and CP4 were similar. Therefore, their chemical structures were compared by mild acid hydrolysis (Thibault, Renard, Axelos, Roger, & Crépeau, 1993). After mild acid hydrolysis, the reaction mixture was separated into two parts, the liberated oligomer and the residual polymer. The contents of both neutral sugar and uronic acid for the two parts were assayed, and the degradation ratio (the liberated part of oligomer) of neutral sugar and uronic acid was calculated. The ratios of the liberated neutral sugar and uronic acid for HP5 were found to be 56.0 and 4.7%, and those for CP4 to be 66.5% and 55.6%. This result indicates that almost all the uronic acid in HP5 remained in the residual polymer, while more than half of the neutral sugar was liberated, confirming that the backbone of HP5 was a homogalacturonan. As shown in Fig. 4, the elution profile of the residual polymer of HP5, obtained on a Sephadex G-75 column, did not transform significantly relative to that of the native one. This result means that the homogalacturonan backbone of HP5 was not broken away during acid hydrolysis. In contrast to HP5, more than half of either the neutral sugar or the uronic acid was liberated. Furthermore, as shown in Fig. 5, the residual polymer of CP4 formed a broad peak shifting to total volume, whereas the total sugar (mainly contributed by neutral sugar) was mainly concentrated in the first half and there was less in the last half of the pattern. These results well support the analysis of enzymatic degradation as

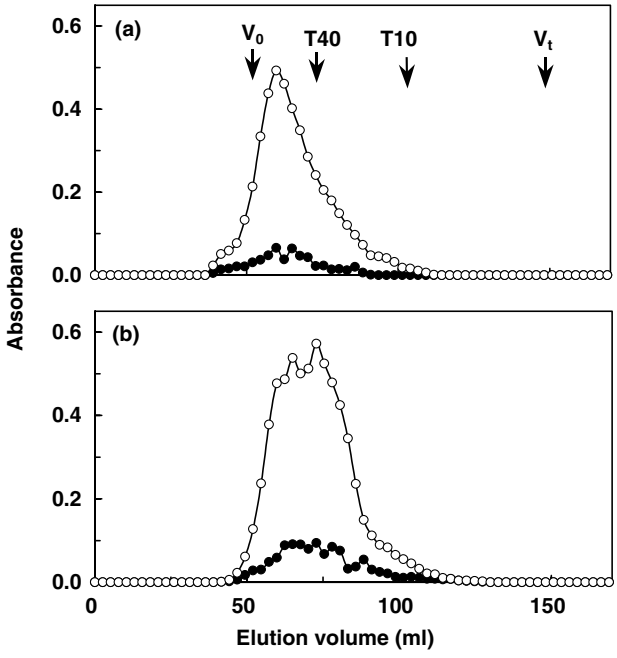

Fig. 4. Chromatography of HP5, before and after acid treatment, on a Sephadex G-75 (1.5 × 95 cm) column eluted with 0.1 M NaCl. a, native; b, after acid treatment. Closed circles, total sugar; open circles, galacturonic acid. T500 and D-galacturonic acid were used to estimate the void and the total volume (V_0 and V_t) of the column, respectively. Experimental conditions are described in Section 2.

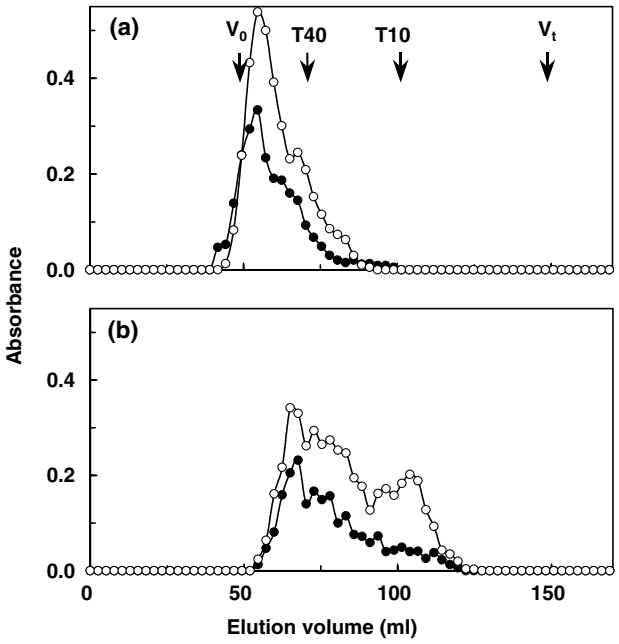

Fig. 5. Chromatography of CP4, before and after acid treatment, on a Sephadex G-75 (1.5 × 95 cm) column eluted with 0.1 M NaCl. a, native; b, after acid treatment. Closed circles, total sugar; open circles, galacturonic acid. T500 and D-galacturonic acid were used to estimate the void and the total volume (V_0 and V_t) of the column, respectively. Experimental conditions are described in Section 2.

mentioned above, indicating that the chemical structure of HP5 was a homogalacturonan (Ridley et al., 2001; Thibault et al., 1993), while CP4 was a rhamnogalacturonan connected with short fragments of homogalacturonans at both ends of the backbone (MacDougall et al., 1996; Ridley et al., 2001), both were also connected with a few neutral sugars as side chains.

From our study, it is clear that the increased water-soluble pectins during the two processings (HB and CB) are very similar. Water-solubilization of pectin in HB was marked although the inactivation of PG was considered to be rapid. Hurtado et al. (2002) reported that there was an absolute loss of pectin during hot-break tomato paste manufacture, but the water-soluble pectin increased markedly during hot-break paste manufacture (the inactivation of PG was said to have been accomplished at the tomato fruits juice step), which was also reported in similar studies (Cámara et al., 1999; Ciruelos et al., 2001). The reason for pectin water-solubilization during tomato paste manufacture appears to be beyond the enzymatic pectin-degradation. Fishman, Chau, Hoagland, and Ayyad (2000) have pointed out that the increase of pectin water-solubilization resulted from compact pectin, aggregated in orange albedo, which was prepared by microwave heating under pressure. The mechanism of pectin water-solubilization during hot-break paste manufacture may be also explained by this concept.

Although enzymatic pectin-degradation was marked during the cold-break paste process, a small quantity of high molecular weight pectin, like CP4, still remained. The distribution of neutral sugars in pectins was disproportionate. Galactan (from CP2), as a side chain, seems to be attached to the homogalacturonan region, in which there were also a few neutral sugars dispersed as side chains. In the rhamnose-rich fragments, CP3 was higher in ramification and smaller in molecular mass than CP4. It is clear that the chemical properties of pectins in the hot-break paste were remarkably different from those in the cold-break paste. These different chemical properties of pectins have led to various physical properties in hot- and cold-break tomato pastes. In a further investigation, the physical properties of those pectins will be studied in detail.

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