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Modification of cell-wall polymers of onion waste—Part II. Effect of divalent cations

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Abstract

The purpose of this study was to investigate the effect of divalent cations on the chemistry and mechanical properties of onion cell walls as modified by heating. Pre-soaking the outer fleshy scale leaves of onion (*Allium cepa* L. cv. Delta) in solutions of Ca^{2+} or Sr^{2+} ions reduced the rate of thermal softening. This was accompanied by a reduction in wall swelling, and an enhancement of cell adhesion, particularly at the edges of the cell faces. To investigate this effect on wall polymer chemistry, cell-wall material (AIR) was prepared from these tissues, heated in water or solutions of Ca^{2+} and then extracted in cyclohexane-trans-1,2-diaminetetra-acetate (CDTA) to leave a residue. The samples were analysed for their carbohydrate composition and the molecular size of selected soluble polysaccharides. Divalent cations reduced the heat-induced solubilization of wall polymers by reducing the propensity for β-eliminative degradation and depolymerization, and thereby increasing the thermal stability of the cross-linked pectic polysaccharides, including those involved in cell–cell adhesion. Increasing the levels of calcium availability in onion cell-wall material resulted in an increase in the molecular weight of some species of heat-treated, CDTA-soluble, polysaccharides and a reduction in the thermal solubility of CDTA-insoluble polysaccharides.

Keywords: Divalent cations; Cell-wall polymers; Onion waste

1. Introduction

In some processes such as canning, the extent of softening of vegetable tissues can be modulated and reduced by the addition of calcium (Hoogzand and Doesburg, 1961; Van Buren, 1968). This effect is thought to be due to the interaction between calcium ions and cell-wall pectin. In contrast, under certain conditions, various cations and anions have been shown to enhance the β-elimination of pectin (Keijbets and Pilnik, 1974; Van Buren, 1983; Van Buren et al., 1990). The metal ion-enhanced β-eliminative degradation of purified carrot pectin increased in the order $Zn^{+} > Ca^{++} > Cd^{++} \sim Sr^{++} > Mg^{++} \sim Na^{+} \sim K^{+} > NH_4^{+}$ (Sajjaanantakul et al., 1993).

As described in Part I of this study (Lecain et al., 1998), heat-induced softening of onion (*Allium cepa* L.) cell walls results in an increase in the ease of cell separation and is associated with dissolution of cell-wall pectic polymers. This is accompanied by, and probably due to the β-elimination degradation of the pectic polymers (Sajjaanantakul et al., 1989; Sajjaanantakul et al., 1993).

As part of a larger study involving the exploitation of onion waste, the objective of this study has been to investigate the effects of divalent cations on thermally-induced changes in the firmness, structure and cell-wall chemistry of outer fleshy scale leaves of onions.

2. Materials and methods

2.1. Materials

Onions (*Allium cepa* L. cv. Delta) were obtained from local onion producers (Lingarden, Spalding, Lincolnshire, UK) and stored at 4°C. The two outer fleshy scale leaves of the onions (Delta; 100 g; 1 cm × 1 cm × 2.5 cm) were presoaked in $CaCl_2$ (0.1 M; Sigma, Poole, UK) or $SrCl_2$ (0.1 M; Sigma) containing 0.02% sodium azide (Sigma) to prevent bacterial growth at 20°C for 16 h and with or without subsequent pressure-cooking (120°C, 50 min). The thermally-treated onion tissue was cooled briefly in air, frozen in liquid N_2, and stored at −40°C.

Unless otherwise stated, all chemicals are of AnalaR grade.

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2.2. Firmness measurement

Firmness measurement of fresh, presoaked and pressure-cooked onion tissue (20 g per sample, 10 samples per analysis) were determined using an Instron (Model 1122, High Wycombe, UK), with a Kramer shear cell (Kramer and Hawbecker, 1966) as described previously (Ng and Waldron, 1997). The crosshead speed and chart drive speed on the Instron were 50 mm/min and 50 mm/min, respectively. The area under the force–displacement curve (work done) expressed in N.m was used for firmness measurement.

2.3. Light microscopy

Fresh, presoaked, and pressure-cooked onions tissues were fixed in 3% (v/v) glutaraldehyde (Agar Scientific, Stansted, UK) in 0.05 M cacodylate (TAAB Lab., Reading, UK) buffer (pH 7.4) for 3 h. The samples were dehydrated in an ethanol (Fisons, Leicester, UK) series with three changes in 100% ethanol, and then infiltrated with the acrylic resin LR White (London Resin Co, Reading, UK). The samples were transferred to gelatine capsules containing fresh resin, which was polymerized for 24 h at 60°C. Sections, 1–2 μm thick, were cut with glass knives, dried down onto glass slides and stained with 1% (v/v) toluidine blue (Aldrich, Poole, UK) in 1% (v/v) borax (BDH, Poole, UK) (pH 11).

For AIR material, samples were embedded in 1% agarose (Sigma) after fixation in glutaraldehyde in 0.05 M cacodylate buffer overnight. The fixed samples were then dehydrated and embedded as above.

2.4. Preparation of alcohol-insoluble residue (AIR)

Frozen outer scale leaves were homogenized in a Waring blender (Fischer Scientific Instrument, Loughborough, UK) with cold ethanol (85% v/v final concentration), reducing the particle size to less than 5 mm. The cold homogenate was transferred to a stainless-steel beaker and homogenized with an Ystral homogenizer (Scientific Instruments, Manchester, UK) for 1 min. The homogenate was filtered through 100 μm nylon mesh (John Stannier and Co., Manchester, UK). The residue was further homogenized twice in 85% (v/v) ethanol before being extracted with phenol (BDH) and buffered with Tris (250 ml, pH 7; Sigma) for 45 min (Huber, 1991). The buffered phenol was prepared by addition of 100 ml 500 mM Tris (pH 7.5), to 200 g phenol. The suspension was stirred and allowed to stand for 8 h and the lower layer was used. The residue was recovered by centrifugation and washed three times with ethanol (85% v/v), acetone (Fisons), and dried overnight in a fume cupboard.

The ethanol extracts were reduced by rotary-evaporation, and then lyophilised. The buffered phenolic extracts were dialysed against acetic acid: water (1:1) and then water (three changes) prior to lyophilisation.

2.5. Sequential extraction of AIR

AIR (1 g) was suspended in water (100 ml, pH 5.1) and stirred for 2 h at 20°C. The water-insoluble residue was further extracted in CDTA (Na salt, 0.05 M, 100 ml, pH 6.5; Aldrich), as described by Waldron and Selvendran (1992). The supernatants were filtered and dialysed exhaustively with water prior to concentration and freeze drying.

2.6. Sugar analysis

Cell-wall neutral sugars and uronic acids were analysed as described previously (Parr et al., 1997). Sugars were released from cell-wall material by dispersing in 72% (v/v) H_2SO_4 (Fisons) for 3 h followed by dilution to 1 M and hydrolysing as described by Blakeney et al. (1983) using 2-deoxyglucose (Sigma) as an internal standard. Alditol acetates were quantified by gas chromatography as described in Parr et al. (1997). All samples were analysed in duplicate.

Uronic acid samples were determined colorimetrically by a modification of the method of Blumenkrantz and Asboe-Hansen (1973) in which each sample was dispersed in 72% (v/v) H_2SO_4 for 3 h at room temperature, diluted to 1 M H_2SO_4, and hydrolysed for 1 h at 100°C.

2.7. Gel filtration chromatography

Molecular weights were determined using a Sepharose CL-4B column (Sigma) as described previously (Ng and Waldron, 1997). Samples (approximately 2 mg) were eluted with imidazole buffer (1 M, pH 7, containing 0.02% sodium azide) at a flow rate of 10 ml/h and collected by an LKD Bromma 2111 multirac fraction collector (15 min per fraction; St. Albans, UK). The collected fractions (2.5 ml) were assayed for total carbohydrate using the phenol/sulphuric acid method of Dubois et al. (1956). Samples were dissolved in 1 ml of buffer and dialysed against buffer before being applied to the column. Dextran (2 mg, Sigma) of size approximately 72 000 and 2 000 000, and sucrose (2 mg, Sigma) of size 342.3 were used for calibration. The bed volume (V_t) of the column was 280 ml, and the void volume (V_0) was 27 ml.

2.8. Effect of calcium and strontium ions on AIRs

AIRs (100 mg; Delta variety of onion) were extracted in 0.1 M $CaCl_2$ or $SrCl_2$ (10 ml) either with or without subsequent heating (100°C, 30 min). The supernatants were filtered and dialysed with water prior to concentration and freeze drying.

2.9. Quantification of reducing groups

The numbers of reducing groups were measured as described by Nelson (1944) with modification by Somogyi (1952). AIR (100 mg; Delta; with or without presoaking

with $CaCl_2$ or $SrCl_2$) were suspended in Tris buffer (10 ml, 0.3 M, pH 6; Sigma) before being subjected to heat treatment (100°C for 30 min) and were subsequently extracted with CDTA (1 M, pH 6.5; 20°C, 6 h). Soluble samples were filtered and dialysed exhaustively with water prior to appropriate dilution before analysis. The soluble polysaccharides (1 ml, containing approximately 40 μg reducing sugar) were mixed with copper reagent (1 ml; Nelson, 1944), and heated at 100°C for 20 min. After rapid cooling to room temperature, arsenomolybdate reagent (1 ml) was added and the mixtures were shaken until carbon dioxide was no longer evolved. The solutions were allowed to stand for 10 min before addition of water (2 ml) and the absorbance was determined at 500 nm. Galacturonic acid was used as a standard. The degree of polymerization of soluble polymers was calculated from the amount of reducing end groups, based on the assumption that the reducing groups were derived from polygalacturonic acid.

2.10. Measurement of β-elimination

The concentrations of 4,5-unsaturated uronides were measured in diluted samples by UV absorbance at 235 nm (Model UVIKON 860, Kontron Instruments, Zurich, Switzerland). An average molar extinction coefficient (ε) of 5412 M/cm was used to calculate the amount of unsaturated uronides from the UV absorbance (Nagel and Wilson, 1969; Voragen, 1972). The amounts of calculated unsaturated uronides were expressed as μmoles per μmole of galacturonic acid.

2.11. Statistical analysis

Analysis of variance and means among samples prepared by various methods were calculated. Duncan's multiple range test was used to determine significant differences ($P < 0.05$).

3. Results and discussion

3.1. Effect of divalent cations on thermal softening and wall morphology

Previously (Part I of this study; Lecain et al., 1998), we demonstrated that pressure-cooking onion tissue (Delta variety) at 120°C for 50 min reduced the tissue firmness from 7.6 to 0.42 N.m. Here, the effect of divalent cations on tissue firmness was investigated by presoaking the fresh onion tissue with $CaCl_2$ or $SrCl_2$ and measuring firmness before and after pressure-cooking. Presoaking in $CaCl_2$ and $SrCl_2$ increased the tissue firmness to 8.7 and 9.3 N.m, respectively, and significantly reduced the thermal softening ($P < 0.05$; Fig. 1). The treatment also reduced heat-induced colouration (slight browning) which may have implications for processing applications. The appearance of cell walls in the presoaked tissues was similar to that of the control tissues in that the walls were approximately 2.5 μm thick and were tightly adhered to each other (Fig. 2a and Fig. 2b).

Heating at 120°C for 50 min, however, resulted in much less wall swelling in the cation-treated tissues (Fig. 2c and Fig. 2d) compared with the untreated tissues (Part I of this study; Lecain et al., 1998). The thermal swelling of the cell walls was reduced by approximately 40% and cell adhesion was clearly maintained at the edges of the cell faces. The reduction of swelling is probably due to the influence of ion-exchange between matrix polysaccharides (Gieringer et al., 1995).

So that the effect of divalent cations on thermal changes in cell-wall chemistry could be investigated in detail, alcohol-insoluble residues (AIRs) were heated at 100°C either in water, or in 0.1 M $CaCl_2$ (see Materials and Methods). The effect of the cations on thermally-induced wall swelling in the AIRs was also investigated by microscopy. As for intact tissues, heating resulted in thermal swelling in the AIR cell walls (Fig. 3a and

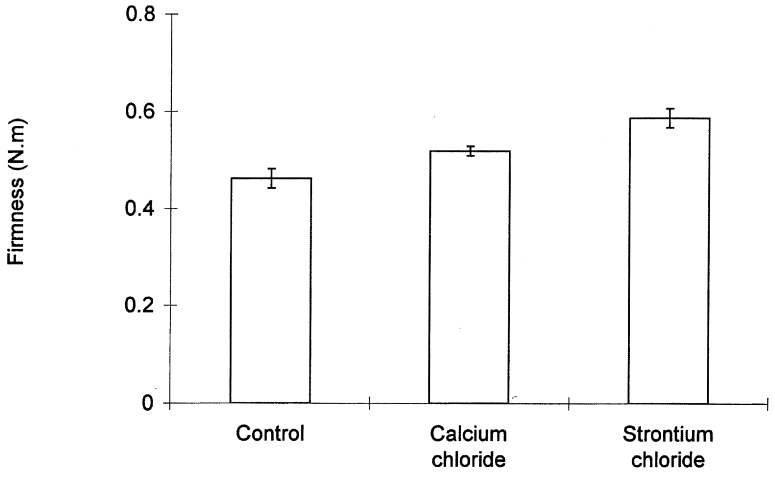

Fig. 1. Effect of metal ions on firmness of pressure-cooked onion (Delta, 50 min).

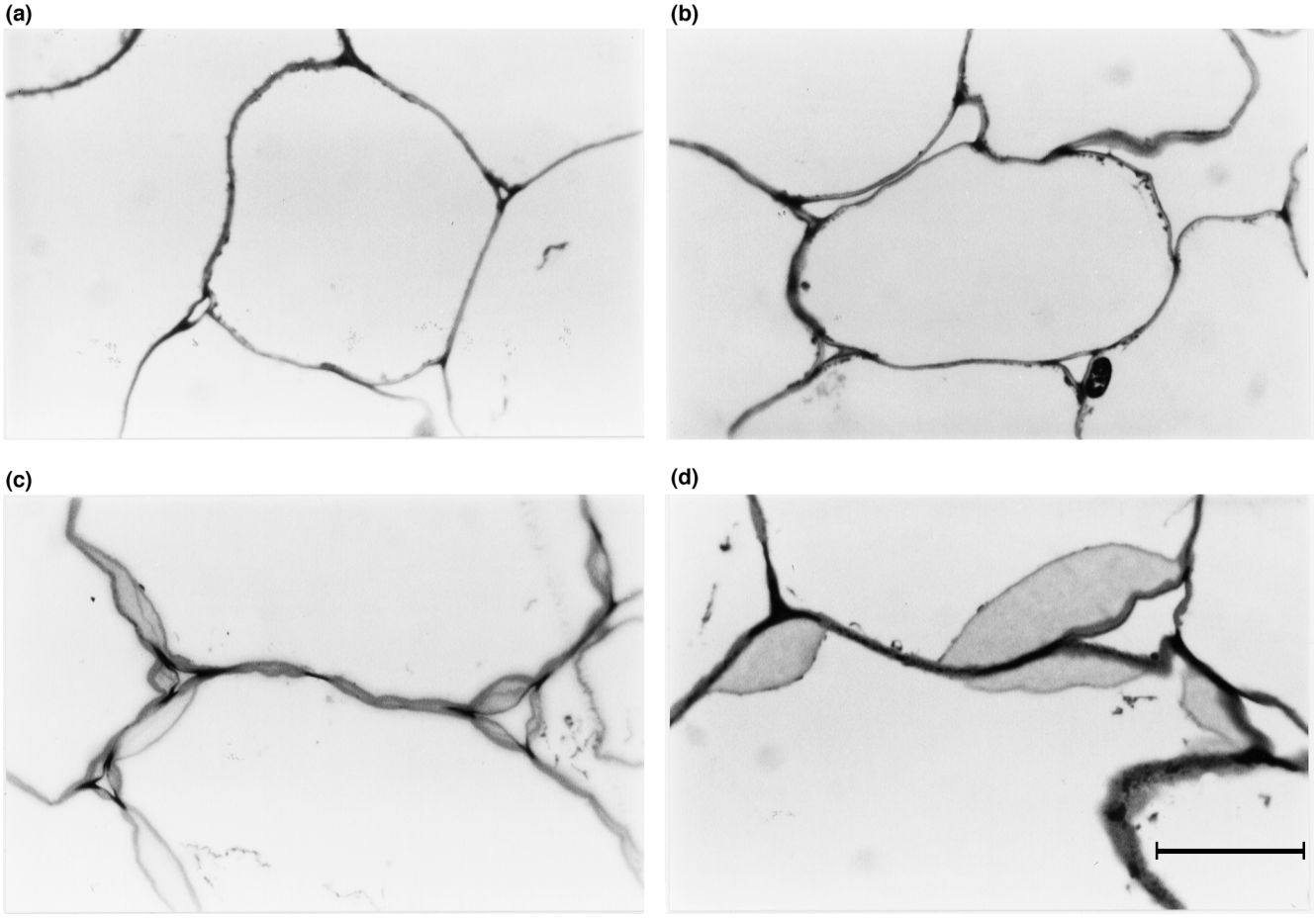

Fig. 2. Light micrographs of transverse sections through onion (Delta) outer scale leaves: (a) presoaked with $CaCl_2$; (b) presoaked with $SrCl_2$; (c) presoaked with $CaCl_2$ and pressure-cooked for 50 min; (d) presoaked with $SrCl_2$ and pressure-cooked for 50 min. Bar = 50 μm.

Table 1
Effect of metal ions on the carbohydrate composition of fresh onion AIR and fractions obtained by sequential extraction

		Yields	Carbohydrate (mol%)								Total	Ratio
		(% AIR)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	μg/mg	UA:NS
AIR	—	100	1	1	2	3	1	17	26	47	754	3
WSP	—	5	t	t	2	1	3	10	9	74	782	6
	$+CaCl_2$	5	1	1	20	2	1	21	25	29	534	1
	$+SrCl_2$	5	1	1	17	3	2	21	23	32	524	1
CSP-1	—	21	t	t	2	t	1	2	1	93	774	23
	$+CaCl_2$	21	t	t	3	t	t	2	1	93	852	19
	$+SrCl_2$	20	t	t	2	t	t	2	1	94	897	23
CIR	—	74	2	1	3	3	2	23	34	32	752	1
	$+CaCl_2$	74	1	1	3	3	2	23	34	33	749	1
	$+SrCl_2$	75	1	2	2	5	2	22	34	32	737	1
HWSP	—	15	t	t	2	2	6	1	19	56	618	3
	$+CaCl_2$	5	1	1	14	4	7	18	15	40	504	1
	$+SrCl_2$	5	1	1	10	3	7	12	15	51	566	2
HCSP-1	—	21	t	t	t	t	t	4	2	93	604	23
	$+CaCl_2$	20	t	t	t	t	t	3	1	95	659	23
	$+SrCl_2$	20	t	t	t	t	t	4	3	92	672	23
HCIR	—	64	2	1	3	3	2	20	34	35	851	2
	$+CaCl_2$	75	1	1	2	3	1	21	31	41	775	2
	$+SrCl_2$	75	1	2	3	5	1	20	32	36	778	2

t, trace.

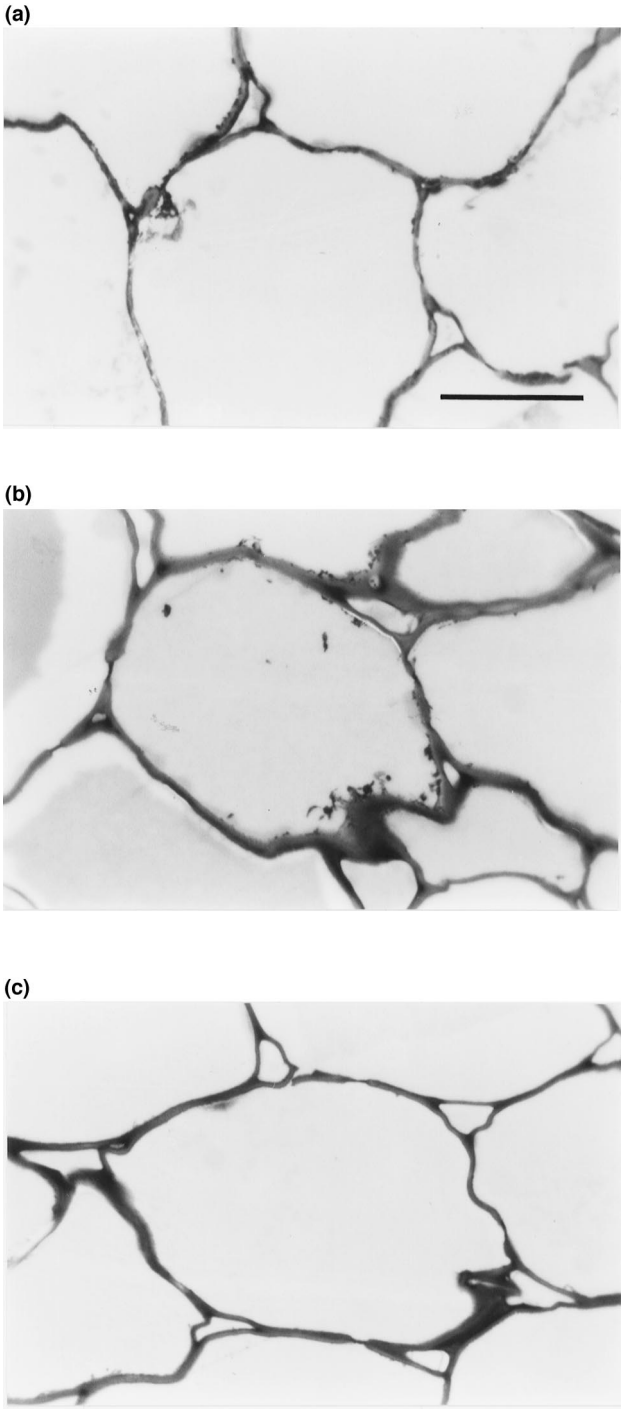

Fig. 3. Light micrographs of transverse sections through onion (Delta) AIR: (a) AIR; (b) AIR heated for 30 min at 100°C; (c) AIR heat-treated in $CaCl_2$ for 30 min at 100°C. Bar = 50 μm.

Fig. 3b). A similar result was obtained from onion during extrusion-cooking in which the cell-wall materials were highly swollen (Ng et al., unpublished). This may be due to heat degradation of matrix polysaccharides opening up the structure so that more hydroxyl groups become accessible to accommodate more fixing reagent (Chatterjee and

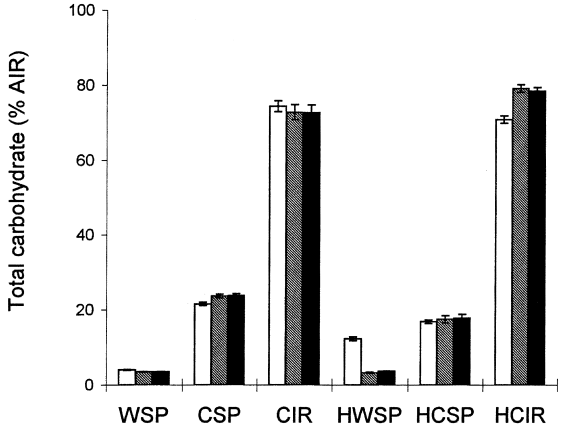

Fig. 4. Effect of metal ions on onion (Delta) AIR during heat treatment. □ control; ▨ + $CaCl_2$; ■ + $SrCl_2$.

Nguyen, 1985). Thermally-induced wall swelling was reduced by the presence of divalent cations (Fig. 3c). In order to describe the effect of divalent cations and heating on cell-wall chemistry, the carbohydrate chemistry of the AIR will be described, followed by the effects of heating and the modulating effects of the divalent cations.

3.2. Carbohydrate composition of fresh onion AIR

As described in Part I of this study (Lecain et al., 1998), the AIR was mainly carbohydrate (Table 1) and contained significant quantities of galactose- and uronic acid-rich pectic polysaccharides. It also contained large quantities of glucose and relatively low amounts of arabinose, xylose and mannose. The polysaccharides released by water and CDTA were predominantly pectic in nature (Table 1). The relative yields of extracted pectic polysaccharides are shown in Fig. 4. A subsequent extraction with CDTA only solubilized a further 3%, and is not considered in this study. The UA:NS ratio was highest in CSP-soluble polysaccharides, indicating that these polymers were less branched than the polysaccharides extracted by water alone (Ng and Waldron, 1997).

3.3. Molecular weight profiles

WSP and CSP of AIRs (Delta) were investigated for molecular weight (MW) profiles by chromatography on Sepharose CL-4B as described in Part I (Lecain et al., 1998). The MW profile of WSP contained one peak with a maximum at MW of approximately 140 000 (Fig. 5a), and the CSP fraction yielded two peaks with maxima at MW of approximately 140 000 and 90 000 (Fig. 5b).

3.4. Effect of heating on sequential extraction of AIR

Extraction of AIR with hot water (100°C, 30 min) resulted in a much higher quantity of water-soluble polysaccharides (HWSP) and this was accompanied by a

Table 2
Effect of metal ions on the carbohydrate composition of high- and low-MW fractions from CDTA-soluble polysaccharides of fresh onion (Delta) AIR

	Yields	Carbohydrate (mol%)								Total	Ratio
	(% AIR)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	μg/mg	UA:NS
High MW											
Control	65	t	t	1	t	1	t	1	96	823	48
+ $CaCl_2$	38	t	t	2	t	t	1	1	95	877	32
+ $SrCl_2$	33	t	t	2	t	t	1	1	95	896	32
Heated	60	t	t	t	t	t	3	1	95	480	24
+ $CaCl_2$	42	t	t	1	t	t	3	1	94	436	24
+ $SrCl_2$	40	t	t	t	t	t	3	1	95	519	24
Low MW											
Control	35	t	t	1	t	t	t	1	97	600	48
+ $CaCl_2$	62	t	t	2	t	1	t	t	96	768	32
+ $SrCl_2$	67	t	t	2	t	t	1	1	95	777	32
Heated	40	t	t	1	t	t	2	1	95	772	32
+ $CaCl_2$	58	t	t	2	t	t	1	1	95	454	32
+ $SrCl_2$	60	t	t	1	t	t	2	1	95	769	32

t, trace.

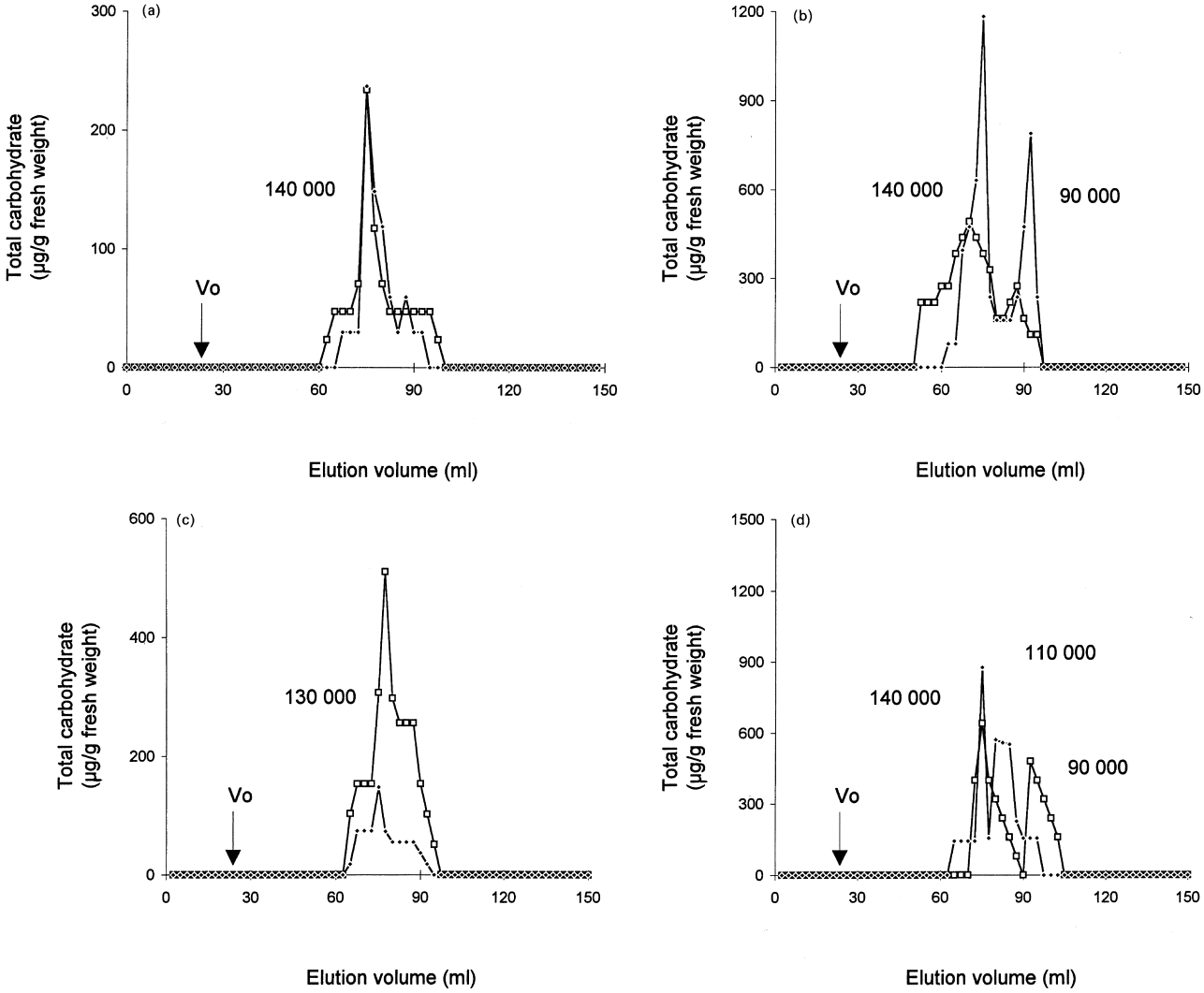

Fig. 5. Effect of metal ions on molecular weight profiles of fresh and heat-treated onion (Delta) AIRs: (a) water-soluble polysaccharides; (b) CDTA-soluble polysaccharides; (c) heat-treated water-soluble polysaccharides; (d) heat-treated CDTA-soluble polysaccharides. – □ – control; – ◆ – + $CaCl_2$.

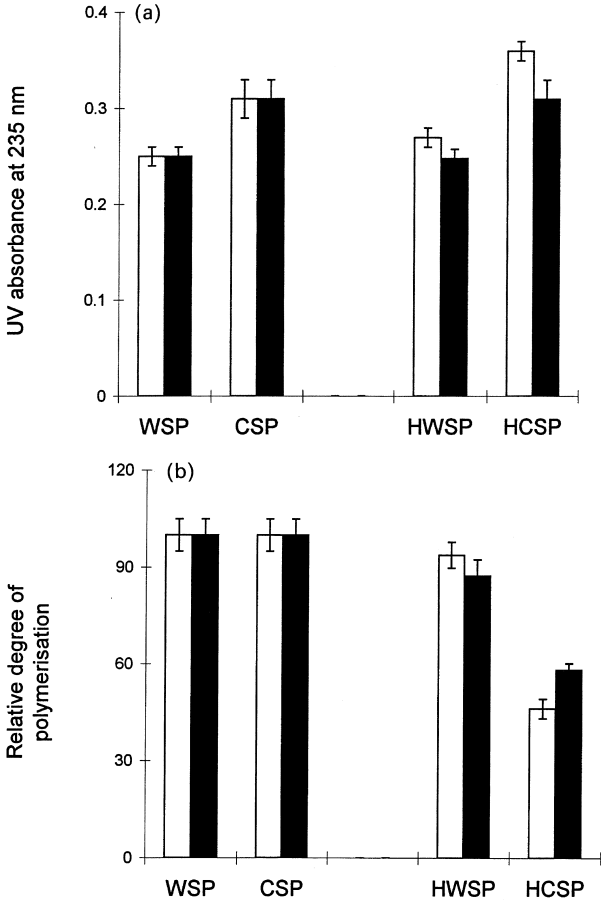

Fig. 6. Effect of metal ions on water- and CDTA-soluble polysaccharides of onion (Delta) AIR: (a) UV absorption at 235 nm; (b) relative degree of polymerisation. □ control; ■ + $CaCl_2$.

decrease in CDTA-soluble polysaccharides (HCSP) compared with CSP and CDTA-insoluble polysaccharides (HCIR; Fig. 4). Heating resulted in a decrease in the UA:NS ratio of WSP, and was accompanied by an increase in the ratio in the residues, indicating that heating solubilized more highly branched pectic polymers. This bears similarity to the changes in pressure-cooking of onion outer tissues in Part I; Lecain et al., 1998. Heating resulted in an increase in the total carbohydrate of the MW profile of WSP (Fig. 5a and Fig. 5c) and a decrease in the total carbohydrate of the high and low MW components of CSP (Fig. 5b and Fig. 5d). Unlike pressure-cooking (Part I; Lecain et al., 1998), heating did not result in a slight decrease in the peak molecular weight of WSP. This may be due to the use of a lower temperature (100°C) in this study; other studies using extrusion have indicated that solubilization of pectic polymers from onion cell walls is greatly dependent on temperature (Ng et al., unpublished). The HCSP polymers exhibited significantly higher absorbances at 235 nm (a reflection of β-eliminative degradation) compared with their cold water counterparts (CSP) and had lower relative degrees of polymerisation ($P < 0.05$; Fig. 6a and Fig. 6b).

3.5. Effect of divalent cations

Addition of $CaCl_2$ to the water used for the control water extraction (WSP) of AIR resulted in a small but significant decrease in WSP (Fig. 4) and this was accompanied by an increase in CSP ($P < 0.05$; Fig. 4, Table 1). The levels of UV absorbance at 235 nm and the relative degrees of polymerization of WSP and CSP components were not affected by $CaCl_2$ in the initial water extraction (Fig. 6a and Fig. 6b). Addition of $CaCl_2$ in the initial water extraction increased the total carbohydrate of the MW profile of CSP, but did not affect the MW profile of WSP (Fig. 5a and Fig. 5b).

Addition of metal ions in the initial water extraction reduced the effect of heating and resulted in much lower levels of HWSP (Fig. 4). This was accompanied by an increase in HCIR. There was no change in HCSP. This suggests that the presence of divalent cations reduces the heat-induced solubilization of wall polymers which are normally CDTA-insoluble. The heat-related increase in the level of UV absorbance at 235 nm of CSP components (HCSP) and the decrease in the relative degrees of polymerization of CSP to HCSP were significantly reduced by metal ions ($P < 0.05$; Fig. 6a and Fig. 6b). Indeed addition of $CaCl_2$ in the initial hot water extraction resulted in an increase in the molecular weight of low MW CSP peak from approximately 90 000 to 110 000 (Fig. 5d) and an increase in the total carbohydrate of the MW WSP (Fig. 5c). Divalent cation-related changes in the ratios of UA:NS of the high MW and low MW CSP fractions reflected the changes in the parent extracts (Tables 1 and 2).

4. General discussion

Our results demonstrate that the calcium- and strontium-enhanced firmness of pressure-cooked onion tissues is accompanied by an enhanced retention of staining density in the middle-lamella region, particularly that which constitutes the edges of cell faces. Increasing the levels of calcium availability in onion AIR had similar morphological effects during heating, and resulted in a decrease in HWSP and an increase in HCIR. This indicates that the conversion of WSP to CIR involves a combination of enhanced cross-linking and a reduction in the β-eliminative degradation of the pectic polymers. It is interesting to note that Knox et al. (1990) demonstrated that the pectic polymers at the edges of cell faces are in carrot tissues, rich in de-esterified polymers which would presumably be more easily cross-linked by divalent cations. The possibility that divalent cations can reduce β-eliminative degradation was highlighted by Keijbets (1974). He demonstrated that calcium cations reduced hot-water solubilization of pectic galacturonan from potato cell walls. However, this effect was dependent on the ratio of divalent cations to carboxyl groups, and at certain ratios, the divalent cations could enhance dissolution by enhancing β-elimination. This has been confirmed by the studies of

Sajjaanantakul et al. (1989, 1993). Clearly, the effect of divalent cations on thermal degradation of pectic polymers requires further work, particularly if the control of processes which cause polymer degradation and solubilization are to be optimized. In relation to firmness of vegetables during heating, the changes in wall chemistry need to be related to polymer localization.

5. Conclusions

The above work has demonstrated that:

1. Thermal softening, polymer dissolution, cell-wall swelling and cell separation are reduced by the divalent cations calcium and strontium.
2. The divalent cations reduce the extent of β-eliminative degradation of pectic polymers, and enhance cell adhesion at the edges of cell faces—the points at which cell separation initiates.

This highlights the importance of the edges of cell faces in the determination of texture.

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