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Effect of Cooking and Pre-Cooking on Cell-Wall Chemistry in Relation to Firmness of Carrot Tissues

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Abstract: The aim of this work was to investigate heat-induced changes in cell wall polysaccharides of carrot in relation to texture. Discs of fresh carrot (*Daucus carota* cv Amstrong) tissue were subjected to cooking (100°C, 20 min), with or without a pre-cooking treatment (50°C, 30 min). Alcohol-insoluble residues were prepared from the tissues and were extracted sequentially with water, NaCl, CDTA, Na_2CO_3 and 0·5 M KOH to leave a residue. These were analysed for their carbohydrate compositions, their degree of methyl esterification and the molecular size of selected soluble polysaccharides. Cooking caused tissues to soften. This involved cell separation, an increase in water- and salt-soluble, high-molecular-weight pectic polysaccharides and a concomitant decrease in the pectic polymers in all wall extracts and the residue. Pre-cooking prior to cooking enhanced cell–cell adhesion and reduced the extent of softening. This was accompanied by a general reduction in the degree of methylesterification of cell-wall pectic polymers, and a decrease in the cooking-induced modification to all pectic fractions. The firming effect of pre-cooking could be reversed by extracting the precooked + cooked tissue with CDTA, a chelating agent. The role of Ca^{2+} cross-linked polymers and pre-cooking in the enhancement of firmness are discussed.

Key words: cell walls, texture, pre-cooking, carrot, calcium

INTRODUCTION

The structure and textural properties of fruit and vegetable tissues are dependent, largely, on the cell wall (Klockeman *et al* 1991). Hence, there is much interest in understanding the effect of processing on the chemistry of cell wall polymers, in relation to texture of the final product (Brett and Waldron 1996). Cooking-induced softening of carrots is due to an initial loss of turgor (Greve *et al* 1994a) followed by an increase in the ease of cell separation (Van-Buren 1979). As for many vegetables, this can be reduced by pre-treatments at moderate temperatures (Van-Buren 1979; Chang *et al* 1993). Investigations into processing of carrots have shown that heat-induced softening is accompanied by changes in the solubility, size and charge density of pectic polysaccharides (Lee *et al* 1979; Plat *et al* 1988, 1991; Ben-Shalom *et al* 1992), probably as a result of β-eliminative degradation (Sajjaanantakul *et al* 1992; Greve *et al* 1994a, b). However, there is relatively little information concerning the origin of heat-induced changes within the cell walls of carrots, or on the effects of texture-modifying pre-treatments on cell-wall chemistry.

This paper reports changes in the chemistry of cell wall polymers of carrots during heat-induced softening, with and without firmness-enhancing pre-treatments.

MATERIALS AND METHODS

Materials

Carrots (*Daucus carota* cv Amstrong) were obtained from a local supplier. Discs (5 mm) were excised from

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the top (top quarter) and bottom (bottom quarter) regions of the organs. Fresh (F) tissue was frozen immediately in liquid N_2 and stored at $-40°C$ until required. Carrots (150 g) were subjected to cooking (100°C, 30 min), or precooking (50°C, 30 min) followed by cooking (100°C, 30 min) in 1 litre of distilled water. Cooking liquors were collected and dialysed. Processed carrots were cooled in air, frozen in liquid N_2 and stored at $-40°C$.

Unless otherwise stated, all chemicals were of AnalaR quality.

Textural measurement

Instron (Model 1122), using a Kramer shear cell (Kramer and Hawbecker 1966), was used for firmness measurement of fresh and processed carrots (20 g). The crosshead speed and chart drive speed on the Instron were 50 mm min^{-1} and 50 mm min^{-1}, respectively. The area of force (work done) expressed in N m was used for firmness measurement.

Scanning electron microscopy (SEM)

Fresh and processed carrot discs (5 mm) were bent gently so that they fractured across the centre of the slice revealing the outer cortex and vascular tissue in longitudinal view. The fracture face was cut away from the rest of the slice and fixed in 30 g glutaraldehyde $litre^{-1}$ in 0·05 M cacodylate buffer (pH 7·3) for 2 h. The fixed tissue was dehydrated in an ethanol series, then transferred to acetone before being critical point dried using liquid CO_2. The dry tissues were then mounted, fracture surface upwards, onto aluminium stubs using silver conducting paint. All samples were sputter coated with a layer of gold, approx 25 nm thick, and photographed in a Leica Cambridge Stereoscan 360 scanning electron microscope.

Preparation of alcohol-insoluble residue (AIR)

Carrot samples were extracted for AIR as described by Martin-Cabrejas *et al* (1994).

Sequential extraction of AIR

AIR (0·5 g, milled to pass through a 500 μm mesh) was suspended in water (50 ml, pH 4·8) and stirred for 2 h at 20°C. The water-insoluble residue was further extracted in NaCl (50 ml, 0·136 M, pH 6·5) for 2 h at 20°C. The residue was then extracted in CDTA, Na_2CO_3 and 0·5 M KOH as described by Waldron and Selvendran (1992). The supernatants were filtered, neutralised where required, and dialysed exhaustively prior to concentration and freeze-drying.

Sugar analysis

Cell-wall neutral sugars and uronic acids were analysed as described previously (Coimbra *et al* 1995).

Methanol analysis

Degree of methylesterification (DM) was determined essentially as described by Martin-Cabrejas *et al* (1994). A sample of AIR (5 mg) was suspended/dissolved in water (2 ml) and sonicated for 10 min. Propanol (0·4 ml, 1·6 mg ml^{-1}) was added as an internal standard. The sample was de-esterified by addition of NaOH (0·8 ml, 2 M) and incubated for 1 h at 20°C with occasional shaking. Subsequently, the sample was neutralised by the addition of HCl (0·8 ml, 2 M) and allowed to equilibrate at 25°C in a water bath for 15 min. Methanol was quantified by isothermal GLC at 150°C on a 1·3 m × 4 mm column packed with HayeSep 'P' 80–100 mesh (Altech) with argon as the carrier gas flowing at 40 ml min^{-1}. Standards of methanol and propanol gave a linear calibration.

Gel filtration chromatography

Gel filtration chromatography (column 100 cm × 2·0 cm) was carried out using Sepharose CL-4B (Sigma; Barrett and Northcote 1965), eluted with 1 M imidazole buffer (pH 7, containing 0·2 g $litre^{-1}$ sodium azide, Mort *et al* 1991) at a flow rate of 10 ml h^{-1} and collected by LKD Bromma 2111 multirac fraction collector (15 min per fraction). The collected fractions (2·5 ml) were assayed for total carbohydrate using the phenol/sulphuric acid method of Dubois *et al* (1956). The pectic fractions extracted from carrots before and after processing (2 mg) were dissolved in 1 ml of buffer and dialysed against buffer before being applied to the column. Dextrans (2 mg) of size 352·3, 72 200 and 2 000 000 were used for calibration.

Effect of CDTA on Vortex-induced cell separation (VICS)

Fresh and processed carrots sections (10 × 10 × 1 mm approximately) were extracted with 50 mM CDTA (Na salt, pH 6·5 containing 0·2 g $litre^{-1}$ sodium azide) at 20°C for 16 h. Tissues were tested for VICS as described by Parker and Waldron (1995).

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 ($P \leqslant 0{\cdot}05$).

RESULTS AND DISCUSSION

Textural measurement

Fresh (F) carrot tissues from top (FT) and bottom (FB) regions gave similar firmness values (19·4 and 18·7 Nm, respectively; Fig 1). Fracture of F tissues involved cell-wall rupture (Fig 2a). Cooking (C) for 30 min reduced the tissue firmness to approximately 4 Nm ($P \leqslant 0{\cdot}05$; Fig 1). Here, tissue fracture involved cell separation only (Fig 2b) consistent with a heat-related weakening of cell–cell adhesion (Brett and Waldron 1996). However, pre-cooking prior to cooking (PC) resulted in a significantly higher firmness than C alone ($P \leqslant 0{\cdot}05$; Fig 1) and tissue fracture involved a combination of cell rupture and cell separation (Fig 2c). This indicates that enhanced firmness after PC is due to an increase in the thermal stability of cell wall polymers involved in cell–cell adhesion.

In order to report the effects of C and PC on the cell walls of carrot, the carbohydrate chemistry of the cell walls of F tissues are described, followed by the changes induced by the heat treatments.

Fresh carrots

Carbohydrate composition and DM

Carrot tissues were prepared as AIRs and analysed for their carbohydrate composition after hydrolysis in 720 g kg^{-1} sulphuric acid (Saeman hydrolysis; Saeman *et al* 1954). The absence of starch from the AIR was indicated by negative staining with I/I_2 and by the release of only 10% of the glucose after hydrolysis with 1 M sulphuric acid (Selvendran and O'Neill 1987).

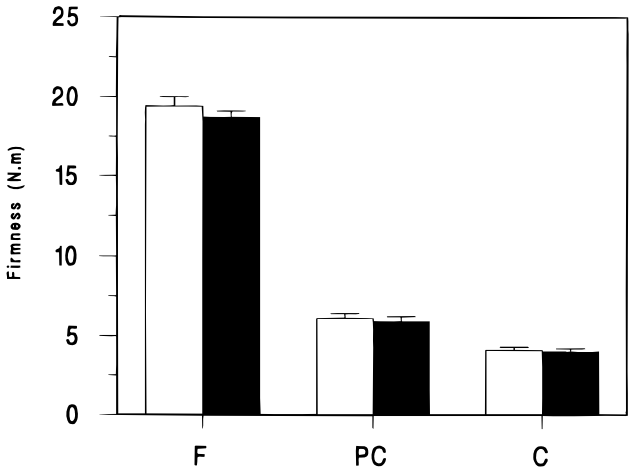

Fig 1. Change of texture of carrots (□) top, and (■) bottom during processing.

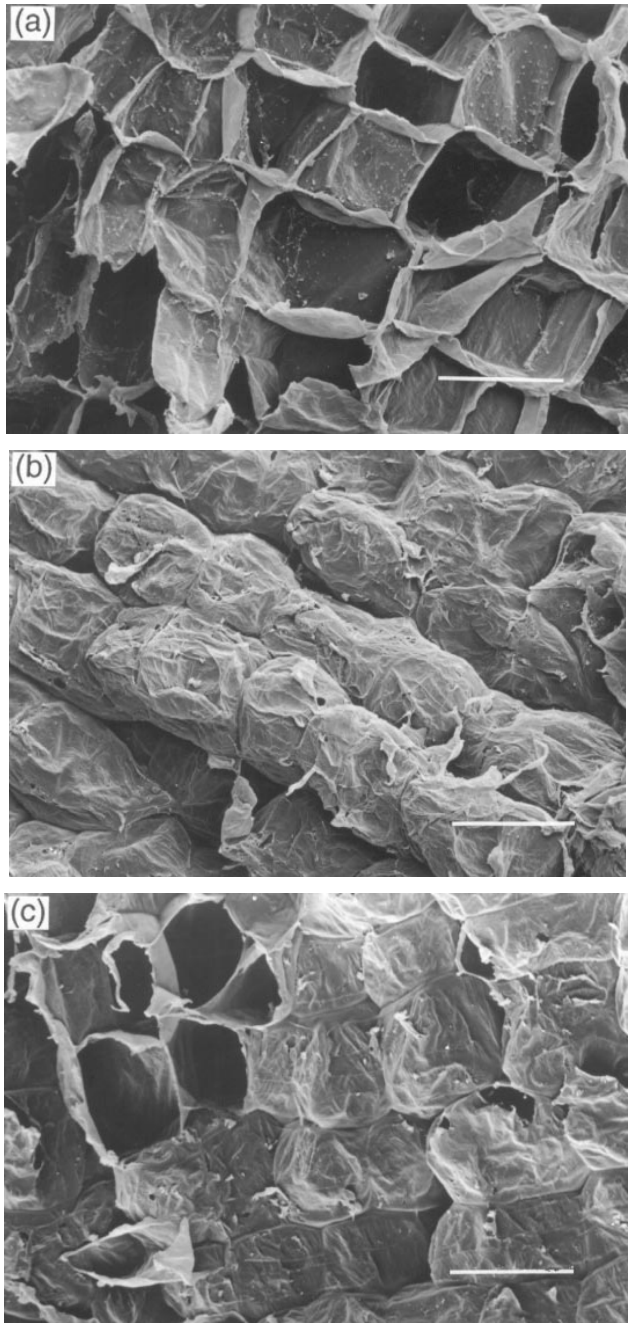

Fig 2. SEM of fracture surface of (a) fresh, (b) cooked and (c) precooked and cooked carrot top tissues. Bar—50 μm.

The yields of AIR from FT and FB carrots were similar at 3% on a fresh weight basis. Approximately 60% of the AIR comprised carbohydrate (Tables 1a and b); the remaining 40% will have contained intracellular protein which would have co-precipitated with the CWM during extraction (Martin-Cabrejas *et al* 1994). The carbohydrate compositions of the FT and FB AIR were similar; in addition to cellulosic glucose, they were rich in pectic polysaccharides as indicated by the levels of rhamnose, arabinose, galactose and uronic acid; they also contained relatively small amounts of xylose and

TABLE 1a
Carbohydrate compositions of alcohol-insoluble residue (AIR) of fresh and processed carrots (top) and fractions obtained by sequential extraction[a]

		Yield (%*AIR*)	*Carbohydrate* (*mol*%)								*Total* ($\mu g\ mg^{-1}$)	*DM* (%)	*Ratio* *UA : NS*
			Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*UA*			
AIR	FT	100	2	t	7	2	3	9	39	36	625	63	2
	PCT	100	2	t	7	2	3	9	41	35	673	41	2
	CT	100	2	t	8	2	3	9	42	32	637	48	2
WSP	FT	13	2	t	9	1	3	16	14	54	562	39	2
	PCT	15	2	t	11	t	2	16	14	54	572	13	2
	CT	20	2	t	11	t	1	17	10	56	581	18	2
SSP	FT	2	2	t	14	t	6	14	14	49	734	ND	2
	PCT	3	2	t	15	t	4	13	15	50	633	ND	2
	CT	3	2	t	14	t	5	13	12	53	776	ND	2
CSP-1	FT	19	1	t	6	t	t	5	15	72	410	38	6
	PCT	20	2	t	6	t	t	6	17	67	414	36	5
	CT	21	3	t	8	t	t	6	17	61	348	35	4
CSP-2	FT	19	1	t	5	t	1	4	12	76	119	ND	7
	PCT	19	1	t	6	2	1	7	13	69	69	ND	5
	CT	16	2	t	14	1	2	13	21	46	74	ND	2
CIR	FT	47	2	t	7	2	2	8	42	36	763	44	2
	PCT	43	1	t	7	2	2	7	48	29	820	35	2
	CT	39	1	t	7	3	3	7	54	24	760	36	2
NSP-1	FT	6	2	t	6	t	t	7	19	67	852	ND	5
	PCT	6	3	t	10	t	t	10	22	52	805	ND	3
	CT	6	3	t	13	t	t	14	21	47	722	ND	2
NSP-2	FT	6	4	t	13	t	t	16	7	59	514	ND	2
	PCT	4	4	t	17	1	t	19	2	55	540	ND	2
	CT	3	5	t	19	1	t	22	3	48	588	ND	1
KSP	FT	4	7	t	20	2	3	18	7	43	562	ND	1
	PCT	4	7	t	22	2	3	17	7	41	607	ND	1
	CT	4	8	t	24	2	3	20	8	33	466	ND	1
RES	FT	31	4	t	10	4	4	8	63	7	998	ND	0·4
	PCT	29	3	t	8	4	4	6	70	5	999	ND	0·4
	CT	27	2	t	6	4	4	5	75	3	912	ND	0·3

[a] Abbreviations: DM, degree of methylesterification; UA : NS, uronic acid : neutral sugar (arabinose + galactose); AIR, alcohol-insoluble residue; WSP, water-soluble polysaccharides; SSP, salt-soluble polysaccharides; CSP, CDTA-soluble polysaccharides; CIR, CDTA-insoluble residue; NSP, Na_2CO_3-soluble polysaccharides; KSP, 0·5 M KOH-soluble polysaccharides; RES, residue; FT, fresh top; FB, fresh bottom; PCT, precooked and cooked top; PCB, precooked and cooked bottom; CT, cooked top; CB, cooked bottom. ND, not determined.

mannose (Tables 1a and b). The compositions were comparable to that reported previously for carrot tissues by Massiot *et al* (1988a and b). The DM of the uronic acid from FT and FB AIRs were 63 and 69%, respectively.

Sequential extractions of AIRs

The AIRs were extracted sequentially with water (20°C), NaCl 0·136 M (20°C), 0·05 M CDTA (20°C), 0·05 M Na_2CO_3 (1°C and 20°C) and 0·5 M KOH (20°C). This approach was based on the method of Redgwell and Selvendran (1986) with a modification in which the water-insoluble residue was extracted with NaCl solution in order to quantify separately the salt and CDTA-soluble polysaccharides (SSP and CSP). The procedure was designed to minimise β-eliminative degradation of pectic polymers (Waldron and Selvendran 1992). The amounts of material extracted are based on one sequential extraction of each AIR. Preliminary studies indicated that little, if any, cooking-related changes occurred in 1 and 4 M KOH-extracted polymers which comprised mainly heat-stable xyloglucan hemicelluloses, so these were not investigated in this study.

The polysaccharides released by the sequential extractions were predominantly pectic in nature (Tables 1a and 1b). The carbohydrate recovery from the CDTA-1 and 2 extracts was relatively low and was probably due to the incomplete removal of CDTA during dialysis (Sene *et al* 1994). This was confirmed by IR and Raman spectra (results not shown). The rela-

TABLE 1b
Carbohydrate compositions of alcohol-insoluble residue (AIR) of fresh and processed carrots (bottom) and fractions obtained by sequential extraction[a]

		Yield (%*AIR*)	*Carbohydrate* (*mol*%)								*Total* ($\mu g\ mg^{-1}$)	*DM* (%)	*Ratio UA : NS*
			Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*UA*			
AIR	FB	100	2	t	8	2	3	9	44	31	576	69	2
	PCB	100	2	t	8	2	3	9	44	31	606	52	2
	CB	100	2	t	8	2	3	10	44	30	624	57	2
WSP	FB	16	3	t	10	1	5	22	15	41	492	49	1
	PCB	17	3	t	14	t	2	21	14	45	639	22	1
	CB	22	4	t	14	t	1	21	13	46	646	28	1
SSP	FB	2	3	t	15	t	6	15	20	37	655	ND	1
	PCB	2	2	t	17	t	3	16	10	48	659	ND	1
	CB	3	2	t	17	t	3	13	10	51	674	ND	1
CSP-1	FB	21	3	t	8	t	t	8	17	61	310	54	4
	PCB	19	2	t	8	t	t	7	19	64	387	41	4
	CB	21	2	t	9	t	t	8	21	59	319	41	3
CSP-2	FB	20	3	t	7	1	2	7	18	61	125	ND	4
	PCB	20	2	t	8	1	1	7	16	65	79	ND	4
	CB	14	2	t	10	1	2	9	21	54	60	ND	3
CIR	FB	41	2	t	7	2	2	7	45	34	786	46	2
	PCB	42	2	t	7	2	2	7	50	28	761	32	2
	CB	40	1	t	7	3	3	7	53	26	777	35	2
NSP-1	FB	6	2	t	8	t	t	9	18	61	870	ND	3
	PCB	5	2	t	10	t	t	12	20	53	963	ND	2
	CB	6	3	t	17	t	t	14	23	41	761	ND	1
NSP-2	FB	4	4	t	16	1	t	19	2	58	632	ND	2
	PCB	5	5	t	18	1	t	19	4	52	455	ND	1
	CB	2	5	t	21	1	1	27	4	41	582	ND	1
KSP	FB	4	8	t	20	1	3	18	7	42	498	ND	1
	PCB	5	6	t	23	2	3	19	6	40	433	ND	1
	CB	4	7	t	23	2	2	21	8	37	372	ND	1
RES	FB	27	3	t	8	4	4	6	68	8	716	ND	0·6
	PCB	26	3	t	7	3	4	6	69	7	768	ND	0·5
	CB	28	2	t	7	4	4	6	74	4	666	ND	0·3

[a] For abbreviations, see footnote *a* to Table 1a.

tively small amount of glucose in many of the extracts, similar to that observed by Plat *et al* (1991), may be due to small quantities of non-starch storage polymers; this has not been investigated further. Trace amounts of Xyl were observed in most extracts; this is consistent with the findings of Stevens and Selvendran (1984) who detected (1 → 4)-linked Xyl in hot-water-soluble pectic polysaccharides.

The relative yields of extracted carbohydrate, as a function of the AIR carbohydrate are shown in Figs 3(a) and (b). This shows that most of the extractable pectic polymers were solubilised by the water, CDTA-1 and Na_2CO_3-1 extractions, whilst relatively little was released by NaCl, CDTA-2, Na_2CO_3-2 or 0·5 M KOH. The UA : NS (uronic acid/neutral sugar) ratio, was highest in CSP and Na_2CO_3-soluble polysaccharides (NSP), indicating that these were less-branched than the polysaccharides extracted by water, salt and 0·5 M KOH (Waldron and Selvendran 1992). Polymers extracted from the AIR of FT tissues generally gave higher UA : NS values than those from FB tissue. This may reflect differences in the state of maturity of the FT and FB tissues.

The DM of the UA from water-soluble polysaccharides (WSP) and CDTA-1-soluble polysaccharides (CSP-1) were approximately 40% for FT and 50% for FB. A similar DM for WSP has been reported by Plat *et al* (1988). The differences between FT and FB may also relate to the degree of maturation. The DM values of CDTA-insoluble residues were approximately 45%; this contrasts with the CDTA-insoluble residues of pear-fruit cell walls in which the uronide DM was 100% (Martin-Cabrejas *et al* 1994).

The yields of KOH-insoluble residue (RES) of FT and FB AIRs were approximately 26% (Tables 1a and b). They were rich in Glc (>60%), the remaining carbohydrate consisting mainly of pectic components.

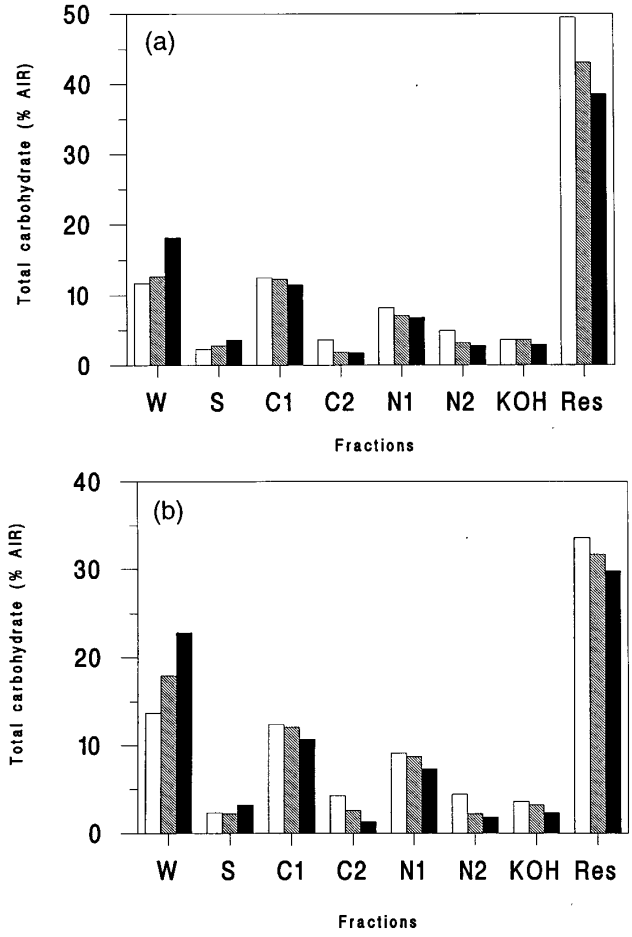

Fig 3. Total carbohydrate (%AIR) from extract and insoluble residues of (a) carrot top, and (b) carrot bottom. Symbols and abbreviations: □, fresh; ▨, precooked and cooked; ■, cooked; W, water; S, salt; C1, CDTA-1; C2, CDTA-2; N1, Na_2CO_3-1; N2, Na_2CO_3-2; Res, residue.

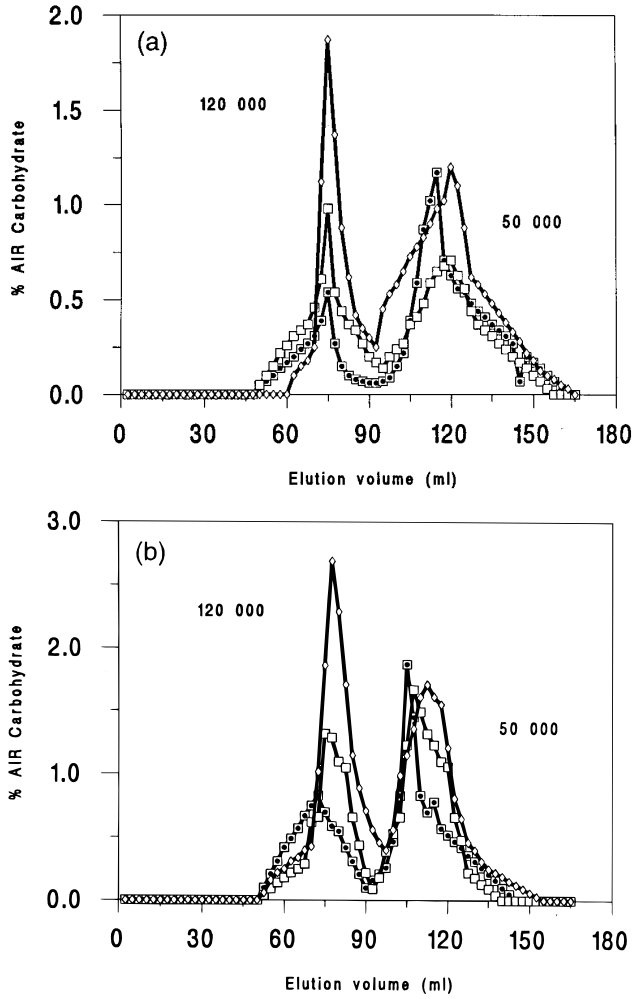

Fig 4. Molecular weight profiles of water-soluble polysaccharides from (a) carrot top, and (b) carrot bottom. Molecular weight values are for fresh carrots. Symbols: —⊡—, fresh; —□—, precooked and cooked; —◇—, cooked.

The ratio of UA : NS of RES was lower than the AIR and probably reflects the insolubility of more highly branched pectic polysaccharides in the RES.

Molecular weight profiles

WSP, CSP-1 and Na_2CO_3-1-soluble polysaccharides (NSP-1) of F, C and PC (both T and B) were investigated for molecular weight (MW) profiles by chromatography on Sepharose CL-4B. The MW profile of WSP from FT and FB contained two peaks with MW of approximately 120 000 and 50 000 (Figs 4a and b) and carbohydrate compositions similar to the parent WSP (Table 2). In contrast, the CSP-1 fractions only resolved one broad peak with MW of approximately 70 000 (Figs 5a and b). Gel filtration of NSP-1 from FT and FB yielded two populations of polymers; a small well defined peak approximately 110 000 and a broad peak (approximately 75% of the carbohydrate) around 60 000 in each extract (Figs 6a and b). The carbohydrate compositions of the high MW (HMW) and low MW (LMW) NSP-1 polymers were similar within the respective FT and FB extracts, but the FT and FB differences in UA : NS ratios were still evident (Table 2). The yields of NaCl-, CDTA-2-, and 0·5 M KOH-extracted polymers were small and precluded further analysis by gel filtration.

The differences in ease of extraction and MW profiles indicated considerable heterogeneity in the population of pectic polymers, as found in a number of other cell wall studies on asparagus (Waldron and Selvendran 1992); pear (Martin-Cabrejas *et al* 1994) and olive tissues (Coimbra *et al* 1995). Large amounts of pectic polymers remained in the 0·5 M KOH-insoluble residue. These were also insoluble in 1 and 4 M KOH (results not shown), indicating that they were strongly attached to the cellulosic component as found in other walls, eg asparagus (Waldron and Selvendran 1992).

Effect of cooking

Cooking released small quantities of material (1 g kg^{-1} FW) into the cooking liquor (Table 3). The carbo-

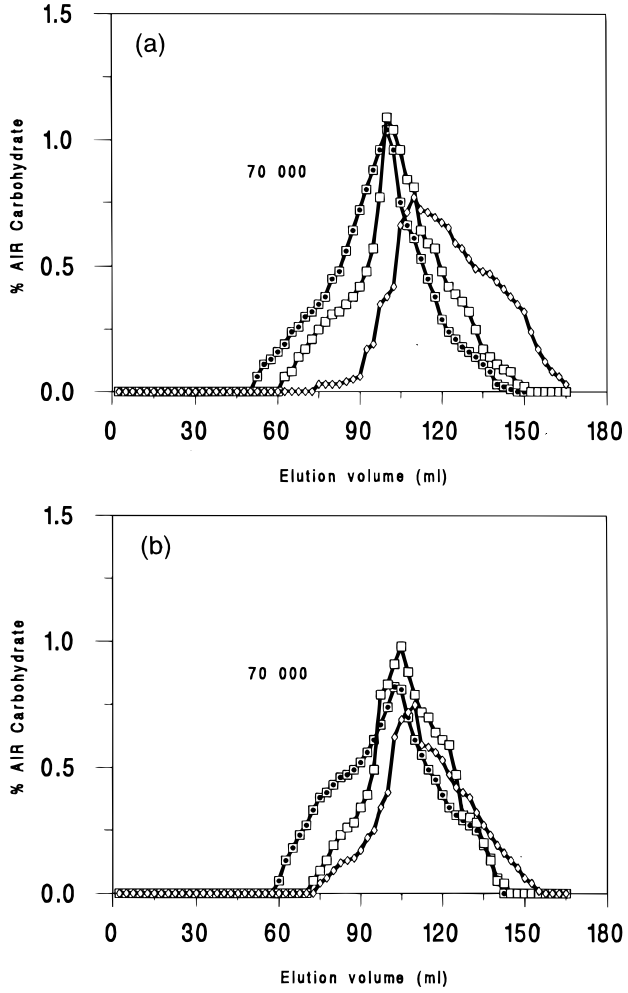

Fig 5. Molecular weight profiles of CDTA-1 soluble polysaccharides from (a) carrot top, and (b) carrot bottom. Symbols—as for Fig 4.

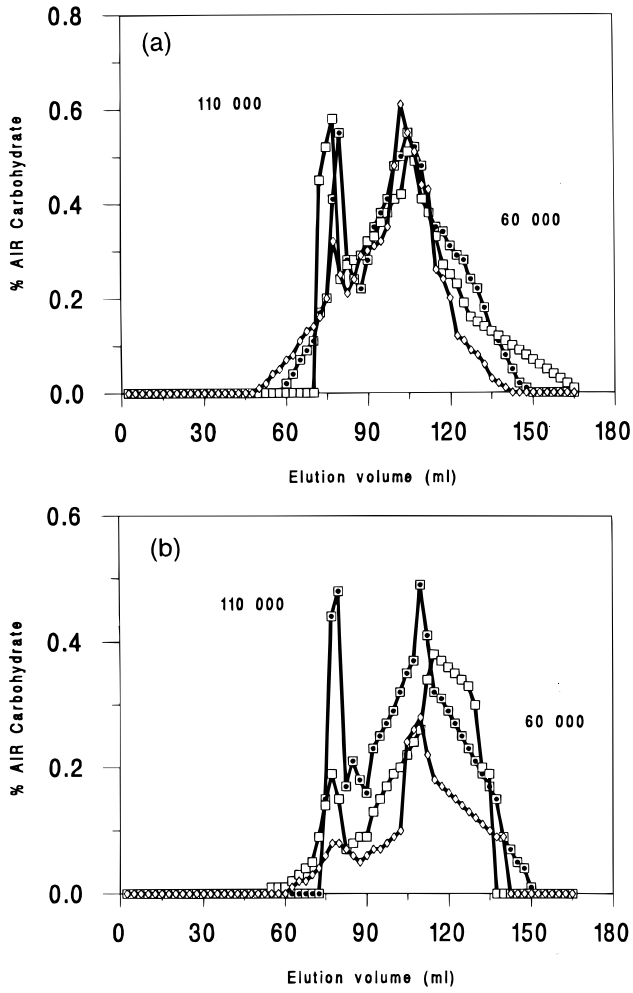

Fig 6. Molecular weight profiles of Na_2CO_3-1 soluble polysaccharides from (a) carrot top, and (b) carrot bottom. Symbols—as for Fig 4.

hydrate component consisted mainly of glucose, uronic acid and mannose. The glucose component is likely to have originated from intracellular storage polymers released from the cut surfaces. The UA was probably pectic in origin, and accounted for approximately 2% of the total carrot uronide. Cooking had little impact on either the yield (2·9% w/w) or overall carbohydrate composition of the AIR from top and bottom regions (CT and CB; Tables 1a and 1b). However, it resulted in a decrease in the DM of the AIR UA component (Table 1a and b). Cooking had a very great effect on the extractability of the cell wall polysaccharides (Fig 3a and b). It resulted in an increase in total carbohydrate of WSP and SSP, and complements the findings of Greve *et al* (1994b). Interestingly, this was accompanied by a decrease in the total (pectic) carbohydrate solubilised by the CDTA, Na_2CO_3, 0·5 M KOH and, in particular, a decrease in the levels of total (pectic) carbohydrate in the residue. This indicates that cooking-induced changes, which are probably due to β-elimination (Sajjaanantakul *et al* 1989; Greve *et al* 1994b) occur in most, if not all, pectic components throughout the cell wall. Cooking also resulted in an increase in the UA : NS ratio of WSP and SSP. This was accompanied by a decrease in the ratio in the subsequent extracts and residues, indicating that the heat treatment was solubilising UA-rich polysaccharide moieties (Tables 1a and 1b); it is likely that the more highly branched pectic polymers are more highly cross-linked, and were more readily retained by the wall residue.

The DM of WSP, CSP and CSP-insoluble residues were reduced by Cooking. This decrease probably involved chemical de-esterification of methyl ester groups as reported by Sajjaanantakul *et al* (1989). However, some de-esterification may have been caused by enhanced pectin methylesterase (PME) activity during the initial stages of heating of the tissues (Ingham and Waldron, unpublished).

Cooking resulted in an increase in the total carbohydrate of the HMW WSP but had little effect on the LMW WSP (Fig 4a and b). This bears similarity to the changes in WSP in carrot, variety B9304, but is

TABLE 2
Carbohydrate composition of high and low MW fractions from water- and Na_2CO_3-soluble polysaccharides of carrots top and bottom during processing[a]

Recovery (%)		*Carbohydrate* (*mol*%)								*Total* ($\mu g\ mg^{-1}$)	*Ratio UA : NS*
		Rha.	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*UA*		
High MW WSP											
FT	30	6	t	7	t	3	14	17	52	203	2
PCT	41	3	t	14	t	2	21	6	53	289	2
CT	48	4	t	13	t	1	20	4	57	170	2
FB	28	4	t	10	t	8	20	14	43	206	2
PCB	42	4	t	15	t	2	24	5	49	193	1
CB	44	6	t	13	t	2	28	6	44	241	1
Low MW WSP											
FT	70	2	t	8	t	2	15	14	58	369	3
PCT	59	4	t	9	t	1	14	4	67	229	3
CT	52	4	t	13	t	1	19	4	58	339	2
FB	72	3	t	10	t	6	19	11	49	279	2
PCB	58	5	t	18	t	3	29	6	38	309	1
CB	56	4	t	13	t	1	19	4	58	347	2
High MW NSP											
FT	33	2	t	7	t	t	8	2	80	164	5
PCT	29	4	t	18	t	t	20	2	55	160	2
CT	11	5	t	24	t	t	27	2	41	157	1
FB	30	3	t	10	t	t	12	1	73	164	3
PCB	28	5	t	21	t	t	25	2	46	235	1
CB	15	5	t	28	t	t	26	2	38	176	1
Low MW NSP											
FT	67	2	t	6	t	t	7	1	83	561	6
PCT	71	3	t	13	t	t	14	1	68	470	3
CT	89	5	t	13	t	t	16	1	64	380	2
FB	70	3	t	10	t	t	10	1	75	554	4
PCB	72	2	t	10	t	t	12	1	74	545	3
CB	85	3	t	18	t	t	16	1	61	413	2

[a] Abbreviations: UA : NS, uronic acid : neutral sugar (arabinose + galactose); WSP, water-soluble polysaccharides; NSP, Na_2CO_3-soluble polysaccharides; FT, fresh top; PCT, precooked and cooked top; CT, cooked top; FB, fresh bottom; PCB, precooked and cooked bottom; CB, cooked bottom.

TABLE 3
Carbohydrate composition of processed liquors of carrots top and bottom[a]

	FW ($g\ kg^{-1}$)	*Carbohydrate* (*mol*%)								*Total* ($\mu g\ mg^{-1}$)	*DM* (%)	*Ratio UA : NS*
		Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*UA*			
PTL	0·4	t	t	t	t	9	1	42	30	463	15	30
(P) CTL	1·0	t	t	1	t	8	1	45	44	388	8	22
CTL	1·0	t	t	1	t	8	1	42	47	437	6	23
PBL	0·4	t	t	t	t	9	2	41	29	452	17	29
(P) CBL	1·0	t	t	1	t	9	1	44	44	399	10	22
CBL	1·0	t	t	1	t	9	1	42	46	402	6	23

[a] Abbreviations: DM, degree of methylesterification; UA : NS, uronic acid : neutral sugar (arabinose + galactose); PTL, precooking top liquor; (P) CTL, cooking top liquor after precooking; CTL, cooking top liquor; PBL, precooking bottom liquor; (P) CBL, cooking bottom liquor after precooking; CBL, cooking bottom liquor.

opposite to the changes in WSP from Danvers carrot (Greve *et al* 1994b). In contrast, CSP-1 fractions of CT and CB AIRs showed a lower MW size distribution compared to those from FT and FB (Fig 5a and b). Cooking also reduced the total carbohydrate of high MW NSP-1 fraction of CT and CB AIRs and low MW fraction of CB (Fig 6a and b). Cooking-related changes in the ratios of UA : NS of the HMW and LMW WSP and NSP fractions reflected the changes in the parent extracts (Table 2).

Effect of pre-cooking followed by cooking

Pre-cooking prior to cooking resulted in solubilisation of very small amounts of material into the pre-cooking liquor. These were similar in composition to the material released by cooking alone (Table 2). Further, small amounts of similar material were released during subsequent cooking. PC had no effect on the yield (2·95%) or carbohydrate composition of the AIR (Tables 1a and 1b). However, the DM of the AIR UA in PCT and PCB were less than the levels in CT and CB, respectively.

In nearly all other respects, the effect of PC was to reduce the effects of cooking. In particular, PC reduced the cooking-related increase in WSP and SSP extracted carbohydrate, and the decrease in CSP-, KOH-extractable, and residual carbohydrate (Tables 1a and 1b; Fig 3a and b). Furthermore, the cooking-related changes in the MW profiles of WSP, CSP and NSP were attenuated.

Vortex-induced cell separation (VICS) of tissues

Soaking of PC tissues in 0·05 M CDTA (a chelator of Ca^{2+}) overnight resulted in total VICS. F tissues did not undergo VICS after similar treatment. This indicates that the pre-cooking effect was manifest through enhanced cell–cell adhesion, due to the increase in the thermal stability of Ca^{2+} cross-linked pectic polysaccharides. Our results are consistent with the hypothesis that pre-cooking de-esterifies cell-wall pectic polysaccharides reducing their propencity for β-eliminative degradation, and increasing their potential for Ca^{2+} cross-linking (Van-Buren 1979). This complements and extends considerably the work of Chang *et al* (1993), who attempted to model texture in relation to extractibility of cell-wall polymers during heat treatments in mung bean, radish and snap-bean. However, several of their extraction processes, notably hot water and hot chelating agents, were highly degradative (Stevens and Selvendran 1984), and little information was provided about the chemical nature of the cell-wall polymers. The importance of Ca^{2+} cross-linked polymers in the texture of cooked carrots has also been suggested by Greve *et al* (1994b).

The inability of CDTA to induce VICS in F tissues indicates that in the uncooked carrot, other characteristics of cell-wall polymer integrity and cross-linking, eg simple phenolics (Parker and Waldron 1995; Parr *et al* 1996) are also important in cell–cell adhesion.

CONCLUSIONS

Using methods that minimise degradation, we have categorised the polymers of carrot cell walls by virtue of their extraction properties and MW profiles, and have investigated changes that accompany textural change during heat treatments.

This approach has demonstrated the following:

(1) The similarity between FT and FB tissues in relation to cell wall chemistry and texture during heating.
(2) The heterogenous nature of the pectic polysaccharides.
(3) Cooking-induced softening of carrot tissues results in the solubilisation of HMW pectic polysaccharides; this is accompanied by the loss of pectic polymers from all the wall fractions studied. This is consistent with the general depolymerisation of pectic polymers through β-elimination.
(4) PC of carrots results in firmer tissues as compared to cooked tissues, a reduction in the DM of the pectic moieties, and a decrease in modification to all pectic fractions as compared with the cooked carrot tissue.
(5) Pre-cooking-enhanced firmness of cooked carrot tissues results from an increase in the thermal stability of the Ca^{2+} cross-linked pectic polysaccharides which stabilise cell adhesion.

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