Effect of Steaming on Cell Wall Chemistry of Potatoes (*Solanum tuberosum* Cv. Bintje) in Relation to Firmness

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The aim of this work was to investigate the heat-induced changes in cell wall polysaccharides of potato in relation to texture. Potatoes (*Solanum tuberosum* cv. Bintje) were subjected to a time course of steaming (95 °C) with and without a preliminary low-temperature heat treatment (50 °C, 3 h). Cell wall material was prepared and extracted sequentially with water, NaCl, *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, sodium salt (CDTA), and Na_2CO_3 to leave a residue. These were analyzed for their carbohydrate compositions, their degree of methyl esterification, and the molecular size of selected soluble polysaccharides. Steaming caused the tissues to soften. This was accompanied by an increase in DMSO- and water-soluble pectic polysaccharides and a concomitant decrease in the CDTA, Na_2CO_3-soluble, and residual pectic polysaccharides. In contrast to many vegetables, low-temperature preheating failed to reduce steaming-induced softening, but resulted in a general reduction in the degree of methyl esterification of cell wall pectic polymers and a decrease in the cooking-induced modification to all pectic fractions. Preheating was effective, however, if potato tissues were presoaked in dilute $CaCl_2$. The role of Ca^{2+}-cross-linked polymers in tissue firmness is discussed.

Keywords: *Cell walls; texture; potatoes and processing*

INTRODUCTION

The cell wall is largely responsible for the textural properties of fruit and vegetable tissues (Klockeman *et al*., 1991). Hence, there is much interest in understanding the effects of processing on the chemistry of cell wall polymers in relation to the texture of the final product (Brett and Waldron, 1996). Heat-induced softening of potato tissue is due, mainly, to an increase in the ease of cell separation (Van-Buren, 1979, 1991; Jarvis and Duncan, 1992; Parker and Waldron, 1995). This is accompanied by an increase in the solubility of pectic polysaccharides, probably as a result of β-eliminative degradation (Bartolome and Hoff, 1972; Keijbets, 1974) and changes in the distribution of ions (Van Marle *et al*., 1994). Heat-induced changes in many vegetables may be influenced by pretreatments at moderate temperatures. For example, in carrots and many varieties of potatoes, precooking at 50 °C reduces the propensity of cell separation during subsequent cooking (Schoch and Sloan, 1972; Ng and Waldron, 1997). Precooking may also be important in the manufacture of potato granules by reducing cell-wall rupture and release of intracellular starch (E. Field, personal communication).

The purification and fractionation of fresh potato cell walls has been studied in detail (Ryden and Selvendran, 1990). However, there is little definitive information concerning the origin of heat-induced changes within the cell walls of potatoes or on the effects of pretreatments on cell wall chemistry. In contrast to carrots, preheating the Bintje variety of potatoes does little to enhance firmness of the steamed product. This paper reports changes in the chemistry of cell wall polymers of potatoes (*Solanum tuberosum* cv. Bintje) during heat-induced softening, with and without low-temperature pretreatments. The results are discussed in relation to changes that occur in carrot cell walls when heat treated (Ng and Waldron, 1997) and the possible role of calcium ions in preheating-induced firming.

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MATERIALS AND METHODS

Materials. Potatoes (*S. tuberosum* cv. Bintje) were obtained from a local supplier. Potatoes (250 g, 1 cm cubes) were steamed for 0, 2, 5, 10, 15, and 20 min (S) at 95 °C or were subjected to low-temperature heating (LTH) (50 °C, 3 h) with or without subsequent steaming (95 °C, 20 min). Fresh and processed potatoes were cooled and frozen in liquid nitrogen before being freeze-milled (20 s, speed 5, SPEX Industries, Edison, NJ).

Unless otherwise stated, all chemicals were of AnalaR quality.

Textural Measurement. The firmness of fresh and processed potatoes (25 g) was measured using an Instron universal tester (Model 1122) using a Kramer shear cell (Kramer and Hawbecker, 1966) as described by Ng and Waldron (1997).

Preparation of Cell Wall Material (CWM). The CWM of fresh potatoes and that of processed potatoes were prepared according to the method developed by Ring and Selvendran (1978) with a modification in which the freeze-milled tissues were extracted with phenol−acetic acid−water (PAW) (2:2:1) and 90% (v/v) DMSO (Sigma, Poole, U.K.). A complete removal of starch was achieved by three 90% DMSO extractions (1 × 15 h and 2 × 5 h) with intermittent sonication at temperature below 20 °C. To reduce the solubilization of water-soluble cell wall polymers, DMSO was removed by extraction with 85% ethanol; the final residue was then washed in acetone and air-dried.

Removal of Starch from DMSO-Soluble Cell Wall Polysaccharides. DMSO extracts were dialyzed exhaustively against deionized water to remove the DMSO and then equilibrated against 0.1 M sodium acetate buffer (Sigma) containing 1 M $CaCl_2$ (Sigma), a few drops of deoxycholic acid−sodium salt (Sigma), and 0.02% (w/w) azide (Sigma), pH 5.2, and brought to 100 mL. α-Amylase (3000 units of porcine pancreatic α-amylase; Sigma A-6255) was added, and the mixture was dialyzed for 48 h at 37 °C against the acetate

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buffer, which was changed at 12 h intervals. The remaining dialysate was adjusted to pH 4.5 with HCl (Fisons, Loughborough, U.K.) after which amyloglucosidase (50 units, Sigma A-3514) was added, and the mixture was dialyzed for 18 h at 55 °C against the same buffer. The dialysate was then dialyzed against deionized water for 4 h to remove salts, and the remaining starch-free components were freeze-dried.

Sequential Extraction of CWM. The amounts of material extracted are based on one sequential extraction of each CWM. CWM (1 g) was suspended in water (50 mL, pH 4.8) and stirred for 2 h at 20 °C. The water-insoluble residue was further extracted sequentially with (i) NaCl (100 mL, 136 mM, pH 6.5; Sigma) for 2 h at 20 °C; (ii) *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid sodium salt (CDTA; Sigma), pH 6.5, at 20 °C for 6 h (CSP-1); (iii) CDTA, pH 6.5, at 20 °C for 2 h (CSP-2); (iv) Na_2CO_3 (100 mL, 50 mM; Sigma) at 0 °C for 16 h (NSP-1); and (v) Na_2CO_3 (100 mL, 50 mM) at 20 °C for 2 h (NSP-2). The supernatants were filtered, neutralized when required, and dialyzed exhaustively prior to concentration and freeze-drying. Measured aliquots of the CDTA-insoluble residue (CIR) and NSP-2-insoluble residue (RES) were also neutralized, dialyzed, and freeze-dried.

Sugar Analysis. Sugars were released from cell wall material by dispersion in 72% (w/w) H_2SO_4 (Fisons) for 3 h at 20 °C, followed by dilution to 1 M, and hydrolysis for 2.5 h at 100 °C. All samples were analyzed in duplicate. Neutral sugars were reduced with $NaBH_4$ (Sigma) and acetylated according to the method of Blakeney *et al.* (1983) using 2-deoxyglucose (Sigma) as an internal standard. Alditol acetates were quantified by gas chromatography as described previously (Coimbra *et al.*, 1995).

Methanol Analysis. Degree of methyl esterification 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 (Sigma; 0.4 mL of a 0.2% v/v solution) 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 neutralized 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 4 m × 4 mm column packed with HayeSep P 80−100 mesh (Alltech) with argon as the carrier gas flowing at 40 mL/min. Standards of methanol and propanol gave a linear calibration.

Gel Filtration Chromatography. DSP, water, and NSP-1 fractions of F, S, LTH, and LTH+S potatoes were investigated for molecular weight (MW) profiles by chromatography on Sepharose CL-4B (Sigma). The bed volume (V_t) of the column was 280 mL, and the void volume (V_0) was 27 mL. The column elution profile was calibrated with dextran standards of between 352.3 and 2 000 000 Da at a flow rate of 10 mL h^{-1}. The elution buffer was 1 M imidazole buffer (pH 7.0, containing 0.2% sodium azide; Aldrich, Gillingham, U.K.; Mort *et al.*, 1991). Fractions (2.5 mL) were collected by LKD Bromma 2111 multirack fraction collector (15 min per fraction) and assayed for total carbohydrate using the phenol−sulfuric acid method of Dubois *et al.* (1956). Samples (approximately 2 mg) were dissolved in 1 mL of buffer and dialyzed against buffer prior to application to the column.

Vortex-Induced Cell Separation (VICS). Fresh potato sections (10 × 10 × 1 mm approximately) were presoaked with or without $CaCl_2$ containing 0.02% sodium azide at 20 °C for 16 h or extracted with 50 mM CDTA (Na salt, pH 6.5, containing 0.02% sodium azide) at 20 °C for 16 h. Fresh and processed tissues were tested for VICS as described by Parker and Waldron (1995). After each extraction/treatment, the tendency for cell separation was determined by placing two tissue sections into each of two screw-capped test tubes with 3 mL of water, vortexing the tissue sections for 1 min, and shaking the tubes vigorously 10 times. The following scores (number of "+") were assigned according to the degree of disruption: (0) each tissue section intact; (1) each tissue section broken into 3−5 clumps; (2) each tissue section broken into 6−7 clumps; (3) tissue sections broken into many clumps, some separate cells; (4) tissue sections disrupted into clumps of approximately 20−30 cells or fewer, many separated cells; (5) tissue completely disrupted, many clumps of fewer than 5-10 cells, mostly single separated cells (total VICS). Intermediate values were apportioned if necessary.

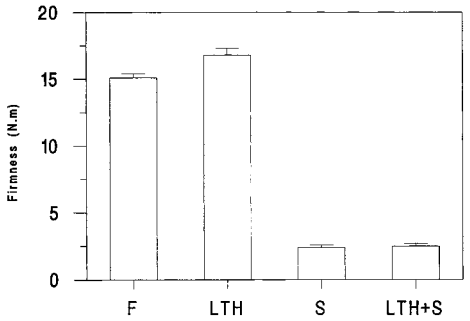

Figure 1. Firmness of potato tissues: fresh (F); tissue after low-temperature heating at 50 °C for 3 h (LTH); tissue after low-temperature heating at 50 °C for 3 h followed by steaming at 95 °C for 20 min (LTH+S); tissue after steaming at 95 °C for 20 min (S).

RESULTS AND DISCUSSION

Firmness of Potato Tissues. Fresh (F) potato tissues exhibited firmness values of 15 N·m. Steaming for 20 min (S) reduced the tissue firmness to approximately 2 N·m and involved the loss of turgor and/or complex chemical changes in the cell wall matrix polysaccharides (Van-Buren, 1979; Greve *et al.*, 1994). LTH did not result in a loss of firmness (Figure 1), even after 180 min. LTH followed by steaming (LTH+S) resulted in softening similar to that caused by steaming alone (Figure 1). This contrasts with precooking-enhanced firmness of cooked carrot tissues (Ng and Waldron, 1997).

To report the effects of S, LTH, and LTH+S on the cell walls of potatoes, the carbohydrate chemistry of the cell walls of F tissues is described, followed by the changes induced by heat treatments.

Fresh Potatoes: PAW- and DMSO-Soluble Components. The yield of PAW-extracted material (PSP) was 0.1% [fresh weight (FW)]. Of this, 10% consisted mainly of pectic polymers as inferred from the UA, Gal, Ara, and Rha components (Table 1). These may have been solubilized due to the chelating effect of the PAW (Huber, 1991). The Glc and Man are likely to have been derived from intracellular sucrose or starch. The remaining 90% is likely to have comprised intracellular protein.

Preliminary investigations into the use of α-amylase and amyloglucosidase showed that the use of both of these enzymes was essential for almost complete removal of starch from the dialyzed DMSO extracts (results not shown). The yield of DMSO-extracted material (DSP) was 0.14% (FW). Of this, 50% consisted mainly of pectic polymers (Table 1; Figure 2); the low level of glucose may be due to a small quantity insoluble, residual starch. The degrees of methyl esterification (DM) of PSP and DSP were 18% and 12%, respectively.

CWM. The absence of starch in the CWM was confirmed by negative staining with I/I_2 and by the release of <10% of the glucose after hydrolysis with 1 M sulfuric acid (Table 1; Selvendran and O'Neill, 1987), producing a yield of 8.4 g/kg of FW. The CWM was mainly carbohydrate (97%, 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,

Table 1. CHO Composition of PAW Extracts, DMSO Extracts, and CWM of Potatoes during Processing

treatment	total CHO (mg/g of FW)	carbohydrate (mol %)								total (μg/mg of sample)	DM %	ratio NS:UA
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA			
PSP												
0 min	0.1	2	2	12	3	8	24	28	20	101	18	2
2 min	0.1	2	2	11	3	6	24	31	20	96	19	2
5 min	0.2	2	2	11	2	6	23	34	19	106	21	2
10 min	0.3	1	1	10	2	6	23	36	20	168	21	2
15 min	0.4	2	1	10	2	7	21	34	22	187	29	2
20 min	0.5	2	2	11	1	7	21	34	21	296	34	2
LTH	0.3	3	2	12	2	11	25	24	20	62	22	2
LTH+S	0.3	2	2	13	3	10	25	25	19	91	24	2
DSP												
0 min	1.4	1	t	3	3	2	51	15	24	577	12	2
2 min	1.5	1	t	4	3	2	36	19	34	491	15	1
5 min	1.6	1	t	4	3	1	31	25	34	468	28	1
10 min	1.7	1	t	4	3	1	30	23	35	451	32	1
15 min	1.7	1	t	4	3	1	30	22	38	449	35	1
20 min	1.8	1	t	3	3	2	28	23	39	471	36	1
LTH	1.5	1	t	3	4	2	33	32	27	491	56	1
LTH+S	1.6	1	t	5	4	2	32	27	28	530	59	1
CWM												
0 min	7.3	1	t	6	3	1	35	28 (2)[a]	26	870	53	2
2 min	7.0	1	t	6	3	1	37	29 (2)	22	880	53	2
5 min	6.9	1	t	6	3	1	40	30 (3)	18	802	51	2
10 min	6.8	1	t	6	3	1	40	31 (4)	17	792	51	3
15 min	6.7	1	t	6	3	1	40	32 (4)	16	762	46	3
20 min	6.5	1	t	6	4	1	37	35 (9)	15	774	44	3
LTH	7.0	1	t	5	2	2	36	28 (2)	25	867	32	2
LTH+S	6.9	1	t	5	2	1	40	26 (3)	24	867	30	2

[a] 1 M sulfuric acid hydrolysis.

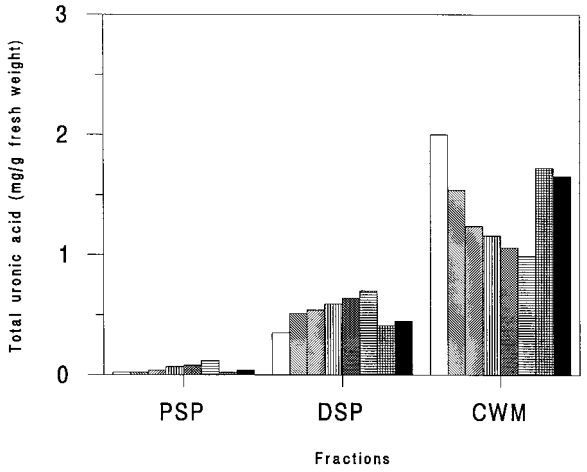

Figure 2. Yield of uronic acid (mg/g of FW) from buffered phenol and DMSO extracts and cell wall material of potatoes during steaming and processing: (open bar) 0 min S; (slashed bar) 2 min S; (backslashed bar) 5 min S; (vertically striped bar) 10 min S; (cross-hatched bar) 15 min S; (horizontally striped bar) 20 min S; (screened bar) LTH; (black bar) LTH+S.

xylose, and mannose. Since only a small proportion of the glucose could be released by hydrolysis in 1 M H_2SO_4 only (Table 1, values in parentheses), the majority of the glucose may be inferred to be cellulosic in origin (Waldron and Selvendran, 1992). The composition was comparable to that reported previously for potato tissues by Ryden and Selvendran (1990). The DM of CWM from F potatoes was 53%. The relative yields of the non-starch carbohydrate in the PSP, DSP, and CWM are shown in Table 1.

Sequential Extractions of CWM. This approach was based on the method of Redgwell and Selvendran (1986) with a modification in which water-insoluble residue was extracted with 136 mM NaCl to quantify separately the salt and CDTA-soluble polysaccharides (SSP and CSP). The procedure was designed to minimize β-eliminative degradation of pectic polymers during the initial stages of extraction and to solubilize the cell wall polymers in as close to their native form as possible. Ryden and Selvendran (1990) found that strong alkali extracted only small amounts of xyloglucan-rich polysaccharides, so these were not investigated in this study.

The polysaccharides released by the sequential extractions were predominantly pectic in nature (Table 2). 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; Ng and Waldron, 1997). A relatively small amount of glucose was detected in many of the extracts, similar to that observed by Ryden and Selvendran (1990). Trace amounts of Xyl were observed in most extracts; this is consistent with the findings of Ryden and Selvendran (1990), who detected (1−4)-linked Xyl in CDTA-soluble pectic polysaccharides.

The relative yields of extracted carbohydrate, on a fresh-weight basis, are shown in Table 2. Most of the extractable pectic polymers were solubilized by the water, CDTA-1, Na_2CO_3-1, and Na_2CO_3-2 extractions, while smaller quantities were released by NaCl and CDTA-2 (Table 2; Figure 3). The NS:UA (neutral sugar: uronic acid) ratio was highest in Na_2CO_3-2-soluble polysaccharides (NSP-2) as found by Ryden and Selvendran (1990), who characterized much longer arabinan and galactan side chains in these pectic polysaccharides. The DM values of WSP and CSP-1 polymers were approximately 41% and 56%, respectively. The DM of the CIR was 57% (Table 2).

MW Profiles. The gel filtration profile of DSP from fresh potatoes consisted of one broad peak with a peak MW of approximately 130 000 (Figure 4). The water-soluble polysaccharides (WSP) contained two peaks with MW of approximately 110 000 and 60 000 (Figure 5). In contrast, the CSP-1 fraction resolved only one sharp peak with MW of approximately 110 000 (Figure 6). Gel

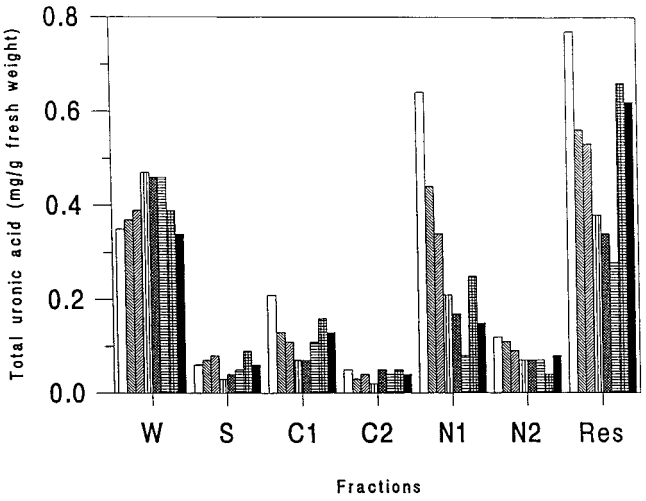

Figure 3. Yield of uronic acid (mg/g of FW) from extracts from CWM of potatoes during steaming and processing: symbols as for Figure 2.

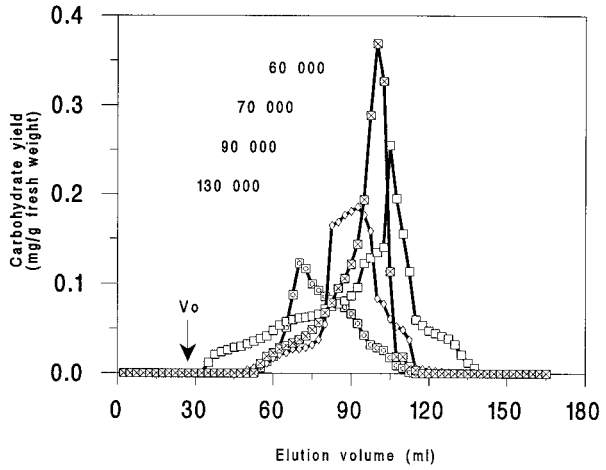

Figure 4. MW profiles of DMSO-soluble polysaccharides from potatoes during steaming and processing: (circle in square) 0 min S; (square) 20 min S; (diamond) LTH; (crossed square) LTH+S.

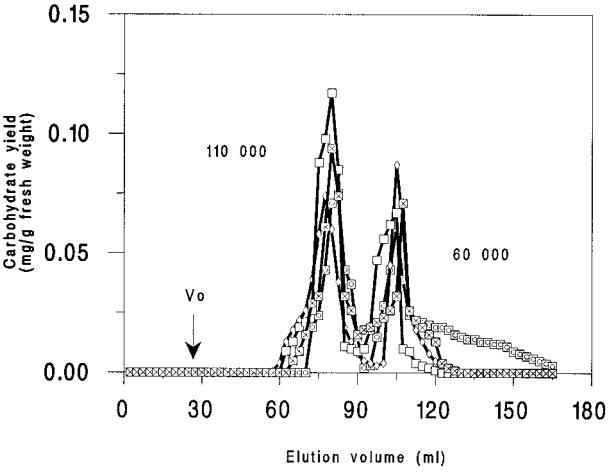

Figure 5. MW profiles of water-soluble polysaccharides from CWM of potatoes during steaming and processing: symbols as for Figure 4.

filtration of Na_2CO_3-soluble polysaccharides 1 (NSP-1) yielded two populations of different MW (approximately 110 000 and 60 000; Figure 7). The carbohydrate compositions of the high MW (HMW) and low MW (LMW) WSP and NSP-1 polymers were broadly similar to those of their parent extracts (Tables 2 and 3). The

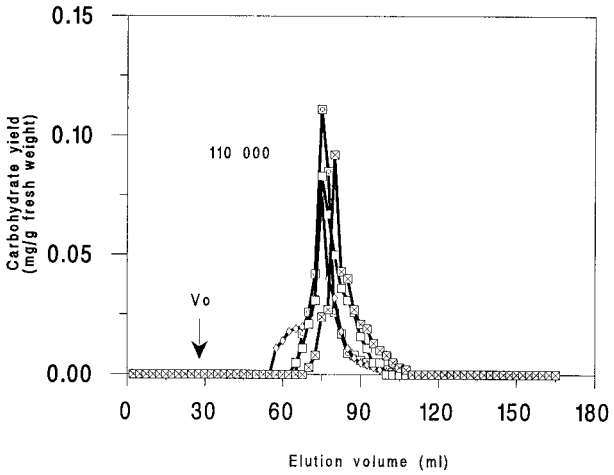

Figure 6. MW profiles of CDTA-soluble polysaccharides from CWM of potatoes during steaming and processing: symbols as for Figure 4.

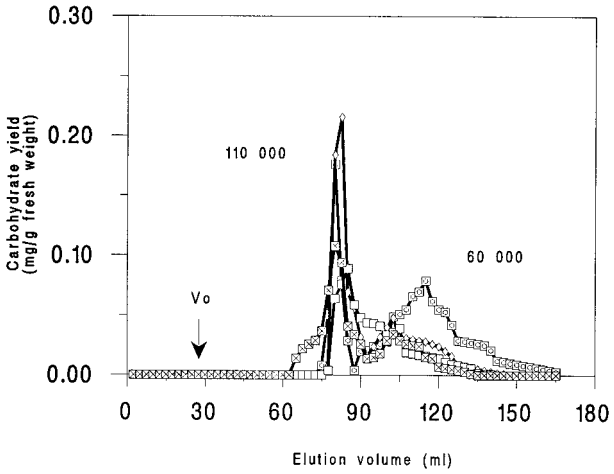

Figure 7. MW profiles of sodium carbonate 1-soluble polysaccharides from CWM of potatoes during steaming and processing: symbols as for Figure 4.

yields of PAW-, NaCl-, and CDTA-2-extracted polymers were small and precluded further analysis by gel filtration.

Effect of Steaming. Steaming resulted in a decrease in the yield of total carbohydrate in the CWM (Table 1). This was due mainly to the solubilization of uronic acid-rich pectic polysaccharides, for example, into the DMSO and buffered phenol fractions (Figure 2). Within the CWM, steaming was also accompanied by an increase in total carbohydrate in the WSP and a decrease in the total carbohydrate in the CSP, NSP, and particularly the residues (CIR and RES; Table 2). Interestingly, although the changes in total carbohydrate in the WSP, CSP, and NSP fractions were relatively small, the changes in the distribution of UA were considerable (Figure 3). Indeed, steaming resulted in a significant increase in WSP uronide and large decreases in NSP and RES uronide. The steaming-induced decrease in uronide levels of the NSP-1 extract is much larger than the decrease in total carbohydrate because of the steaming-induced redistribution of neutral carbohydrate, particularly Gal and small quantities of Ara, from the RES into the NSP-extractable material. Hence, the NS:UA ratio in NSP-1 more than doubles after steaming for 20 min (Table 2). Presumably, these neutral sugar-rich pectic fragments are retained in the CIR by ester linkages. Kiejbets (1974) demonstrated that cooking resulted in an increase in solubilization and

Table 2. CHO Composition of Potato CWM Extracts and Insoluble Residues during Processing

treatment	total CHO (mg/g of fresh wt)	yield (% CWM)	carbohydrate (mol %)								total (μg/mg of sample)	DM %	ratio NS:UA
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA			
WSP													
0 min	0.7	11	2	t	5	1	1	34	5	51	630	41	1
2 min	0.8	11	2	t	6	1	1	36	5	48	698	41	1
5 min	0.8	12	2	t	5	1	1	38	5	47	697	41	1
10 min	0.8	12	2	t	5	t	t	34	4	53	733	40	1
15 min	0.9	12	2	t	6	t	t	36	4	51	755	40	1
20 min	0.8	10	2	t	5	t	t	36	5	50	769	39	1
LTH	0.8	12	2	t	4	1	1	31	9	51	653	19	1
LTH+S	0.6	9	1	t	5	1	1	35	9	53	716	11	1
SSP													
0 min	0.2	10	t	t	6	t	t	41	22	30	191	ND	2
2 min	0.3	10	t	t	7	t	t	44	23	25	293	ND	2
5 min	0.3	9	t	t	7	t	t	40	27	25	357	ND	2
10 min	0.2	10	t	t	7	t	t	42	27	23	152	ND	2
15 min	0.2	10	t	t	7	t	t	48	20	24	178	ND	2
20 min	0.2	11	t	t	7	t	t	48	20	24	185	ND	2
LTH	0.2	10	2	t	6	t	1	49	24	17	294	ND	3
LTH+S	0.2	9	1	t	3	1	1	46	27	20	271	ND	2
CSP-1													
0 min	0.5	10	1	t	5	1	1	34	12	45	457	56	1
2 min	0.3	9	2	t	5	1	1	32	13	45	310	57	1
5 min	0.2	10	2	t	5	1	1	34	14	42	249	58	1
10 min	0.2	9	1	t	4	1	1	34	16	42	181	58	1
15 min	0.2	10	2	t	6	1	1	34	16	39	204	58	1
20 min	0.3	11	2	t	6	1	1	34	15	40	250	58	1
LTH	0.4	6	1	t	5	2	1	36	11	43	647	29	1
LTH+S	0.3	8	1	t	5	4	2	37	12	38	429	17	1
CSP-2													
0 min	0.1	6	6	t	4	t	t	28	23	38	212	ND	1
2 min	0.1	6	2	t	5	3	t	28	30	31	187	ND	1
5 min	0.1	7	2	t	3	2	3	30	27	32	205	ND	1
10 min	0.1	5	1	t	3	t	2	30	30	33	137	ND	1
15 min	0.1	6	1	t	5	1	1	26	27	38	217	ND	1
20 min	0.1	5	1	t	6	1	1	27	34	29	256	ND	1
LTH	0.1	8	4	t	7	3	1	23	27	34	179	ND	1
LTH+S	0.1	8	4	t	2	2	1	24	24	41	167	ND	1
CIR													
0 min	6.3	63	1	t	6	2	1	35	27	27	993	57	1
2 min	6.3	64	1	t	5	2	1	37	30	23	992	54	2
5 min	5.9	62	1	t	6	2	1	43	33	13	955	58	3
10 min	6.0	63	1	t	6	2	1	42	35	12	964	57	4
15 min	5.7	62	1	t	6	2	1	42	37	10	918	50	4
20 min	5.4	63	1	t	6	2	1	39	43	7	861	49	6
LTH	6.1	64	1	t	7	1	t	38	30	22	951	36	2
LTH+S	5.8	66	2	t	7	2	1	35	31	21	888	32	2
NSP-1													
0 min	1.3	13	1	t	5	t	1	29	13	50	985	ND	1
2 min	1.0	13	2	t	5	t	1	31	14	45	752	ND	1
5 min	1.0	12	2	t	7	t	1	41	14	34	838	ND	1
10 min	1.0	13	2	t	8	t	t	55	14	20	805	ND	3
15 min	1.1	12	2	t	8	t	t	60	12	17	838	ND	4
20 min	1.1	13	2	t	10	1	t	67	11	8	809	ND	9
LTH	0.7	7	1	t	9	1	t	36	14	38	934	ND	1
LTH+S	0.7	9	2	t	8	1	1	51	13	23	725	ND	2
NSP-2													
0 min	0.9	10	2	t	6	1	t	65	12	13	893	ND	5
2 min	0.7	10	2	t	8	1	t	60	14	14	789	ND	5
5 min	0.8	9	2	t	8	t	1	62	13	13	837	ND	5
10 min	0.9	10	2	t	9	t	t	67	13	8	870	ND	10
15 min	0.9	10	2	t	9	t	t	69	11	8	893	ND	10
20 min	1.0	11	2	t	9	t	t	70	11	7	873	ND	11
LTH	0.5	6	2	t	9	t	t	70	9	9	797	ND	9
LTH+S	0.6	7	2	1	6	t	t	67	11	13	909	ND	7
RES													
0 min	3.7	40	1	t	6	2	1	39	29	21	915	ND	2
2 min	3.5	41	1	t	6	3	1	40	32	16	861	ND	2
5 min	3.3	41	1	t	6	2	1	40	33	16	816	ND	3
10 min	3.3	40	1	t	6	3	1	38	38	12	802	ND	3
15 min	2.8	40	1	t	6	3	1	37	39	12	707	ND	3
20 min	3.1	39	1	t	5	3	1	39	41	9	796	ND	5
LTH	3.9	51	1	t	6	3	1	35	36	17	762	ND	3
LTH+S	4.1	50	1	t	5	2	1	34	40	15	825	ND	3

the dissolution of pectic polysaccharides. However, little information was provided about the nature of the cell-wall polymers (only UA was measured). Steaming resulted in a decrease in the DM of the CWM (Table 1).

Table 3. CHO Composition of High and Low MW Fractions from Water- and Na_2CO_3-1-Soluble Polysaccharides of Potatoes during Processing[a]

time (min)	recovery (%)	carbohydrate (mol %)								total (μg/mg of sample)	ratio NS:UA
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA		
high MW WSP											
0	54	1	t	5	t	t	35	3	55	459	1
2	59	1	t	4	t	t	35	3	56	564	1
5	60	1	t	4	t	t	36	2	57	607	1
20	59	1	t	4	t	t	36	2	56	612	1
LTH	52	1	t	5	1	t	31	5	56	480	1
LTH+S	57	1	t	4	1	t	32	4	57	576	1
low MW WSP											
0	46	1	t	6	t	t	36	3	53	387	1
2	41	t	t	5	t	t	33	3	58	402	1
5	40	t	t	5	t	t	32	4	58	409	1
20	41	1	t	5	t	t	29	4	60	463	1
LTH	48	1	t	5	t	t	41	5	47	398	1
LTH+S	43	t	t	4	1	t	35	5	54	427	1
high MW NSP-1											
0	23	1	t	7	t	2	27	5	57	822	1
2	26	1	t	7	t	2	30	5	54	795	1
5	82	1	t	9	1	1	46	3	38	730	1
20	91	1	t	12	2	t	74	2	10	737	8
LTH	56	1	t	4	t	t	57	5	46	788	1
LTH+S	74	t	t	5	1	t	76	4	23	617	4
low MW NSP-1											
0	77	t	t	9	t	5	29	3	53	717	1
2	74	1	t	9	t	4	32	3	50	550	1
5	18	1	t	9	t	4	35	4	46	599	1
20	9	t	t	10	t	4	38	4	43	570	1
LTH	44	t	t	7	t	3	28	10	53	687	1
LTH+S	26	t	t	2	1	1	39	9	47	477	1

The DM of the CSP-insoluble residue (CIR) was also reduced by steaming (Table 2). This decrease probably involved chemical de-esterification of methyl ester groups as reported by Keijbets (1975). However, some de-esterification may have been caused by enhanced pectin methylesterase (PME) activity during the initial stages of heating of the tissues (Tijskens et al., 1997).

While steaming caused an increase in the levels of WSP, their molecular weight profiles showed little change (Figure 5). The molecular weight profiles of CSP were also unaffected (Figure 6). However, for DSP, steaming resulted in a decrease in the peak molecular weight from approximately 130 000 to <60 000 after 20 min (Figure 4). For Na_2CO_3-1-extracted polysaccharides, steaming resulted in a small decrease in both peaks, accompanied by a general redistribution to a molecular weight between the two (Figure 7).

Effect of LTH. Compared to steaming, LTH alone resulted in only a small decrease in the total carbohydrate of the CWM (Table 1). However, the DM of CWM after LTH was less than the levels of DM in CWM from F or S potato tissues (Table 1); this is probably due to LTH stimulation of PME activity. PME activity was found to remain relatively constant during low-temperature heat treatment at 50 °C (Tijskens *et al.*, 1997).

LTH resulted in a significant decrease in the peak MW of DSP (Figure 4), although not to the same extent as that caused by steaming. LTH also caused changes in the MW profiles of NSP-1 (Figure 7), resulting in a decrease in the 60 000 peak. LTH caused little change in the molecular weight profile of carbohydrate from WSP and CSP-1 polysaccharides (Figures 5 and 6). LTH-induced changes in the ratio of NS:UA of the WSP and NSP fractions reflected the changes in the parent extracts (Table 3).

Effect of LTH+S. Compared with F, LTH, and S treatments, the LTH+S treatment resulted in the lowest DM of CWM (Table 1). However, in nearly all other respects, the effect of LTH+S was to reduce the effects of S alone, particularly in relation to uronide and galactose components. LTH+S reduced the steaming-related increase in DSP-extracted UA (Figure 2) and water-soluble UA (Figure 3). LTH+S also reduced the steaming-related decrease in CSP, NSP, and residual UA (Figure 3). The steaming-induced decrease in the RES galactose component, and the corresponding increase in the NSP-1 and NSP-2 galactose components, were substantially inhibited by the LTH+S treatment (Table 2). The reduction in molecular weight of the DSP was less than that caused by S alone (Figure 4), although the level of carbohydrate in the 60 000 Da peak of NSP-1 was still reduced. As in S and LTH treatments, the MS profiles of the water- and CDTA-soluble polymers remained unchanged.

Table 4. Effect of Calcium Ions on Potato Tissues on Ease of Cell Separation

treatment	−0.1 M $CaCl_2$	+0.1 M $CaCl_2$
F	− − − − −	− − − − −
LTH	− − − − −	− − − − −
S	+ + + + +	− − − − −
LTH+S	+ + + + +	− − − − −

VICS of Tissues. Steaming for 20 min resulted in total VICS (Table 4) and was consistent with a heat-related weakening of cell−cell adhesion (Brett and Waldron, 1996). LTH did not reduce VICS during subsequent steaming and is consistent with the absence of a pretreatment induction of firmness (Figure 1). However, presoaking F tissue with calcium ions (0.1 M, 16 h) prior to steaming completely prevented VICS, confirming the work of Keijbets (1975), which indicated that calcium could play a role in tissue firmness in potato.

In our previous study on carrot tissues, we demonstrated that the precooking-induced firmness of cooked carrot tissues was reversed by chelating agents. This indicated that the precooking effect was due to an enhanced thermal stability of polysaccharides that were

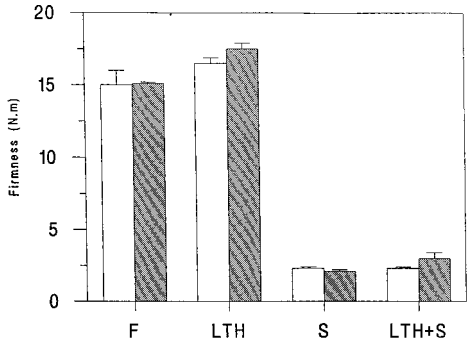

Figure 8. Effect of presoaking potato tissue in 3 mM $CaCl_2$ on firmness of potato tissues, fresh and processed: (open bar) control; (slashed bar) presoaked in $CaCl_2$.

involved in cell adhesion through cross-linking with calcium. In the present study, preheated potato tissues do not exhibit enhanced firmness after subsequent steaming even though, as in carrots, a precooking-induced increase in thermal stability is manifest in nearly all of the pectic polymer fractions of the cell wall, including the CDTA-soluble components which might be expected to bind and cross-link with calcium. This, and the observation that incubation of F potato tissues in 0.1 M $CaCl_2$ prevented VICS after steaming, suggested that the availability of Ca^{2+} required to cross-link pectic polysaccharides might be a limiting factor. Therefore, the effect of soaking cubes of potato tissue in dilute $CaCl_2$ (3 mM) for 24 h prior to LTH, LTH+S, and S treatments was investigated. The results (Figure 8) show clearly that without a $CaCl_2$ presoak, LTH had no effect on steaming-induced softening. However, LTH+S treatment of $CaCl_2$-soaked tissues resulted in a 30% increase in firmness (significant at $P < 0.05$) compared with the S tissues. Furthermore, the enhanced firmness could be reversed by soaking in CDTA (results not shown). Hence, our results indicate that in Bintje potatoes, precooking treatments can enhance the thermal stability of cell wall polymers, but a firming effect fails to occur due to a lack of calcium. This may reflect the relative levels of intracellular calcium in relation to citrate and other organic acids, which can act as natural chelating agents (Keijbets, 1974; Burton, 1989). Certainly, citrate ions can increase the solubility of cell wall pectic polysaccharides from potato cell walls (Keijbets, 1974), and phytate, also present in potato, is a highly efficient chelator of calcium (Holland *et al.*, 1991). Hence, good control of firmness in processed potato (and other vegetables) is only likely if the manipulation of these different aspects of cellular biochemistry is considered. It should also be noted that fresh potato tissues will not undergo VICS in CDTA (although tissues will soften), indicating other aspects of cell wall polymer integrity and cross-linking are also relevant in cell adhesion. These may include cinnamic acid derivatives such as diferulic acid, which plays a key role in the texture of Chinese water chestnut (Parker and Waldron, 1995; Parr *et al.*, 1996).

CONCLUSIONS

We have categorized the polymers of potato 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) Heat-induced softening of potato tissues results in the solubilization of pectic polysaccharides; this is accompanied by the loss of uronic acid from all of the wall fractions studied and is consistent with the general depolymerization of pectic polymers through β-elimination.

(2) LTH+S results in a reduction in the DM of the pectic moieties and reduces steaming-induced modifications to all pectic fractions, similar to carrots.

(3) Unlike carrots, Bintje potatoes do not exhibit a measurable firming effect if they are subjected to LTH+S. This is in spite of the presence of CDTA-soluble polymers, the degradation of which was reduced by the preheating.

(4) However, a firming effect in Bintje potatoes could be induced if the tissues were soaked in dilute $CaCl_2$ prior to the LTH.

This is consistent with the hypothesis that the LTH enhancement of firmness in cooked tissues is due to an increase in the thermal stability of calcium-cross-linked pectic polysaccharides, which are involved in cell−cell adhesion. The results also suggest that optimization of preheat treatments requires calcium.

ABBREVIATIONS USED

CWM, cell wall material; CHO, carbohydrate; DM, degree of methyl esterification; NS:UA, neutral sugar (arabinose + galactose):uronic acid; VICS, vortex-induced cell separation; F, fresh; LTH, low-temperature heating; S, steaming (20 min); LTH+S, low-temperature heating prior to steaming; FW, fresh weight; MW, molecular weight; PME, pectin methylesterase; PSP, PAW-soluble polysaccharides; DSP, DMSO-soluble polysaccharides; WSP, water-soluble polysaccharides; SSP, salt-soluble polysaccharides; CSP, CDTA-soluble polysaccharides; CIR, CDTA-insoluble residue; NSP, Na_2CO_3-soluble polysaccharides; RES, residue; t, trace; ND, not determined.

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