Effect of Oxidative Coupling on the Thermal Stability of Texture and Cell Wall Chemistry of Beet Root (*Beta vulgaris*)

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The purpose of this study was to investigate whether peroxidase-mediated oxidative coupling of ferulic acid in beet root cell walls could reduce thermally induced softening by enhancing the cross-linking of polymers involved in cell adhesion. Beet root (*Beta vulgaris*, var. Detroit 2 crimson) tissue was incubated in the presence or absence of hydrogen peroxide (H_2O_2) for 18 h before cooking. Incubation in H_2O_2 increased the time required for tissue to soften at 100 °C from an average of 130 min to 650 min and resulted in a significantly higher tensile strength in heat-treated tissues ($p < 0.05$). This was accompanied by a large decrease in esterified *cis*- and *trans*-ferulic acid and a 2-fold increase (approximately) in 5,5′-, 8-*O*-4′-, and 5,8′-(benzofuran form)-diferulic acid moieties. In addition, the yield of hot-water-soluble wall polymers was much lower, consistent with increased cross-linking of the pectic polysaccharides. However, the carbohydrate composition and degree of uronide methylesterification of cell walls from H_2O_2-treated tissues was similar to that of fresh tissue. Incubation of control samples in the absence of H_2O_2 had no effect on thermal softening or phenolic chemistry of the walls. These results indicate that the H_2O_2-mediated changes in mechanical properties of the walls and the rate of thermal softening result from enhanced phenolic cross-linking of pectic polymers involved in cell−cell adhesion, due to oxidative coupling of ferulic acid moieties.

Keywords: *Beet root; ferulic acid; dehydrodimers; oxidative coupling; hydrogen peroxide; cell walls*

INTRODUCTION

The plant cell wall plays an important role in determining the texture of fruit and vegetable tissue; cell adhesion is central to the maintenance of firmness in plant-based foods (Van-Buren, 1979; Brett and Waldron, 1996; Waldron et al., 1997a). During high-temperature treatments, most edible vegetables soften. This involves an increase in the ease of cell separation, which is accompanied by and probably due to the dissolution of those polymers involved in cell−cell adhesion (Brett and Waldron, 1996).

In certain vegetables such as Chinese water chestnut (CWC), cooking fails to induce cell separation, and the thin-walled parenchyma cells remain firmly attached to each other (Loh and Breene, 1981). The thermal stability of this cell adhesion is thought to result from ferulic acid cross-links between cell wall polymers (Parker and Waldron, 1995; Parr et al., 1996). This cross-linking is likely to result from the action of cell-wall-bound peroxidase in the presence of peroxide (Biggs and Fry, 1987; Wallace and Fry, 1995). It has been proposed that control of phenolic cross-linking may provide a novel route to modulating texture in other tissues (Waldron et al., 1997a).

High levels of ferulic acid dehydrodimers have also been reported in the cell walls of two forms of *Beta vulgaris*, beet root and sugar beet (Waldron et al., 1997b). The results indicated that, as in CWC, the dimers may be involved in increasing the time taken for the tissues to soften when cooked by enhancing the thermal stability of cell adhesion. To shed further light on the functionality of these dimers, we report here how the biochemical enhancement of ferulic acid cross-links in beet root cell walls affects the thermal stability of cell adhesion and therefore tissue texture.

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MATERIALS AND METHODS

Materials. Beet root plants (*Beta vulgaris* var. Detroit 2 crimson) were grown in sandy soil in pots under glass-house conditions at the Institute of Food Research and were harvested at between 25 and 30 weeks. By this time, the beet root weighed between 160 and 180 g and were approximately 18 cm long and 7 cm wide. They were then sliced transversely with a Baker and Nixon Excel slicer (Norwich, U.K.) until two slices of tissue, 2 or 4 mm thick, were obtained at the widest point. From the noncentral areas of these slices, rectangular strips (30 × 3 × 2 and 10 × 10 × 4 mm) were cut.

Mechanical Properties−Tensile Test. A universal mechanical test machine (Stable MicroSystems model TA-XT2, Godalming, U.K., with Texture Expert software), equipped with a 5 kg load cell, was used to perform the various tests for which force data were recorded as a function of distance. Rectangular strips (30 × 3 × 2 mm) with a 1.5 mm notch halfway along the 30 mm length were glued to two metal plates clamped to a rigid former at a fixed separation. After the glue had dried (1 min), the plates were gripped in tensile jaws of the universal mechanical test machine, and the rigid former was removed. Upward movement of the crosshead (0.05 mm/s) caused the tissue to fail. Experiments were carried out on 10 tissue samples in a random order.

Mattson Test (Cooking Time). Samples (19, each 10 × 10 × 4 mm) were cooked in the Mattson cooker as described by Downie et al. (1997). The 50% cookability (CT50) was determined by the time taken for the 10th plunger to drop. Cooking to completion involved heating until all tissue sections had been penetrated by the metal rods.

Preparation of Cell Wall Material (CWM). Cell wall material (CWM) was isolated from beet root essentially as

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described by Waldron et al. (1997b). Frozen tissue (20 g) was blended in 15 g L^{-1} aqueous sodium dodecyl sulfate (SDS) containing 5 mM $Na_2S_2O_5$ with a homogenizer (Ystral GmbH, Dottingen, Germany) for 5 min. A few drops of octanol were added to reduce foaming. The homogenate was filtered through a 100 μm nylon mesh (John Stannier and Co., Manchester, U.K.), and the residue was ball-milled (Pascall, 0.5 L pot) at 0 °C in 5 g L^{-1} SDS containing 3 mM $Na_2S_2O_5$ for 2 h at 60 rpm to ensure cell rupture and to remove the bulk of remaining intracellular contents. After filtering the homogenate through 75 μm nylon mesh, the residue was suspended in cold water containing 3 mM $Na_2S_2O_5$, homogenized for 5 min, and refiltered. This procedure was repeated three times until the cell wall residue was free of cell contents, as assessed by light microscopy and staining with iodine/potassium iodide. The CWM was stored as a frozen suspension at −20 °C. Prior to extraction of cell wall phenolics, the CWM was further extracted with hot ethanol to remove any alcohol-soluble phenolics, washed three times with acetone, and then air-dried.

Analysis of Carbohydrate Composition. Sugars were released from CWM by dispersing in 72% H_2SO_4 followed by dilution to 1 M and hydrolyzing for 2.5 h at 100 °C. All samples were analyzed in duplicate. Neutral sugars were reduced with $NaBH_4$ and acetylated by the method of Blakeney et al. (1983) using 2-deoxyglucose as an internal standard. Alditol acetates were quantified by gas chromatography as described previously (Coimbra et al., 1995). Total uronic acid content was determined colorimetrically by the method of Blumenkrantz and Asboe-Hansen (1973) after dispersal in 72% H_2SO_4, dilution to 1 M, and hydrolysis for 1 h at 100 °C.

Methanol Analysis. Degree of methylesterification was determined as follows: A sample of CWM (5 mg) was suspended/dissolved in water (2 mL) and sonicated for 10 min. Propanol (Sigma, Poole, U.K.; 0.4 mL of a 0.2% v/v solution) was added as an internal standard. The sample was de-esterified by the 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 (Altech) with argon as the carrier gas flowing at 40 mL/min. Standards of methanol and propanol gave a linear calibration.

Extraction of Phenolic Acids. Wall-bound phenolics were released from isolated CWM by sequential alkaline hydrolysis under progressively more vigorous conditions as described by Waldron et al. (1996). CWM (200 mg) was extracted with 0.1 M NaOH (4 mL) for 1 h at 25 °C under N_2. The suspension was filtered through glass fiber paper, and the residue was retained for further extraction with, sequentially, 0.1 M NaOH (4 mL) for 24 h and then 1 M NaOH (4 mL) for 24 h at 25 °C. *trans*-Cinnamic acid was added to all filtrates as an internal standard, and the solutions were then acidified with concentrated HCl and extracted three times with ethyl acetate (3 vol). The phenolic extracts were evaporated to dryness under a stream of N_2, and the samples were redissolved in 200 mL of 50% (v/v) aqueous methanol prior to analysis by HPLC. The alkali procedures were performed in darkness.

HPLC Phenolic Analysis. Phenolics were detected and quantified by HPLC using an Inertpak ODS2 5 mm reverse-phase column (25 cm × 5 mm; Capital HPLC Ltd., Broxburn, West Lothian, U.K.) with gradient elution employing progressively increasing methanol−acetonitrile levels in 1 mM trifluoroacetic acid (TFA). The most suitable gradient profile for separation of wall-bound phenolic dimers and monomers was as follows: time = 0: 90% solvent A, 5% solvent B, 5% solvent C; time = 25 min: 25% solvent A, 37% solvent B, 37% solvent C (linear gradient); time = 30 min: 0% solvent A, 50% solvent B, 50% solvent C (exponential gradient); time = 45 min: 90% solvent A, 5% solvent B, 5% solvent C (exponential gradient); time = 55 min: 90% solvent A, 5% solvent B, 5% solvent C. Solvent A is 10% (v/v) aqueous methanol plus TFA to 1 mM, solvent B is 80% (v/v) methanol plus TFA to 1 mM, and solvent C is 80% (v/v) acetonitrile plus TFA to 1 mM. Flow = 1 mL/min. All three solvents were sparged with helium. Detection was with a Perkin-Elmer diode array detector 235 °C. Quantitation was by integration of peak areas at 280 nm, with reference to known amounts of pure compounds (Waldron et al., 1996).

Chemicals. Unless otherwise stated, all chemicals were of HPLC-grade purity.

Incubation of Tissues in Hydrogen Peroxide. This approach was based on the method of Schopfer (1996). Beet root tissue strips (all sizes, 60 g) were incubated in 1 L of phosphate buffer (0.1 M, pH 6.0) with or without the continuous addition of 3% H_2O_2 at 1 mL/h for 16 h at 25 °C.

Microscopy of Beet Root Tissue. Autofluorescence of hand-cut, transverse sections from fresh and H_2O_2-treated beet root strips (above) was examined using a Leitz Ortholux II microscope with a HBO 50 W mercury arc lamp and an exciter and barrier filter combination with transmission of 340−380 nm and >430 nm, respectively. The tissues were mounted either in 0.1 M sodium acetate, pH 4.5, or in 20 mM NH_4OH (pH 10) to enhance autofluorescence (Parker and Waldron, 1995). Similar sections mounted in water were also examined by darkfield microscopy.

Hot Water Extraction. CWMs of fresh and treated beet roots were extracted with hot water (1 g of CWM/100 mL) for 30 min at 100 °C. After extraction, the hot-water-soluble polymers and the water-insoluble CWM were freeze-dried.

Statistics. Analysis of variance and means of various samples were calculated. One-way analysis of variance was performed with Minitab software to determine significant differences ($p < 0.05$).

RESULTS

Visual Appearance of Fresh Tissues. The swollen hypocotyl of beet root consisted of concentric layers of vascular tissue derived from secondary cambia, separated by wide layers of loosely packed large-celled storage parenchyma. The parenchyma cells were full of pigment, giving the tissue a red color. Only the relatively sparse (secondary) xylem cells were lignified; when irradiated with UV light at pH 4.5, they emitted a strong blue autofluorescence, while the rest of the tissues gave only a very slight blue autofluorescence. At pH 10, the walls of the parenchyma cells showed a strong greenish-yellow autofluorescence that is due to the presence of large quantities of ferulic acid and its derivatives (Harris and Hartley, 1976; Waldron et al., 1997b).

Yield and Composition of Cell Walls from Fresh Tissues. The yield of CWM from fresh beet root (F; Table 1) was 2.21%, similar to previous findings (Waldron et al., 1997b). The CWM was analyzed for its carbohydrate composition after hydrolysis in 72% sulfuric acid (Selvendran and O'Neill, 1987) and was found to be rich in pectic polysaccharides as indicated by the high levels of uronic acid and arabinose (Table 1). The remaining carbohydrate consisted predominantly of glucose, which is mainly cellulosic in origin (Waldron et al., 1997b). The uronic acid component exhibited a DM of 69%. High levels of simple phenolic acids were released from the isolated CWM by saponification with increasing strengths of alkali (0.1 M NaOH, 1 h, 0.1 M NaOH, 24 h; 1 M NaOH, 24 h; Table 2; Figure 1a). The fresh beet root CWM contained small quantities of p-OH-benzoic acid, vanillic acid, p-OH-benzaldehyde, vanillin, and coumaric acid. However, the bulk of the phenolic complement comprised ferulic acid and its derivatives (Table 2). Approximately 14% of the ferulic acid was present in the form of six identified dehydrodiferulic acid dimers, with the 8-*O*-4′- and 5,8′-

Table 1. Carbohydrate Composition of Fresh and Treated Beet Root[a]

	yield	carbohydrate (mol %)								total	DM	ratio
	% fwt	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	(μg/mg)	(%)	NS:UA
CWM of Fresh Tissue												
F	2.21	1	t	39	1	2	5	20	32	904	69	1
W	2.05	1	t	38	2	2	5	20	32	887	62	1
B	1.84	1	t	41	2	2	5	20	30	881	73	1
H	2.21	1	t	39	2	2	6	22	29	832	71	1
CWM of Cooked Tissue												
FC	0.73	1	1	37	4	3	6	38	11	548	29	4
WC	1.00	1	1	34	4	3	5	40	13	583	32	3
BC	1.10	1	t	38	4	2	5	40	10	696	36	4
HC	1.15	1	1	33	3	2	7	42	11	659	41	4
HSP from CWM of Fresh Tissue												
F-HSP	0.20	2	t	44	1	1	3	1	48	879	66	1
W-HSP	0.10	2	t	40	1	3	3	1	49	875	62	1
B-HSP	0.13	2	t	38	5	1	2	1	50	832	67	1
H-HSP	0.04	2	t	35	3	1	3	1	54	972	74	1
HIR from CWM of Fresh Tissue												
F-HIR	2.01	2	t	41	2	2	3	22	28	904	67	2
W-HIR	1.92	2	t	41	2	2	4	18	30	884	60	2
B-HIR	1.71	2	t	41	2	2	3	19	30	910	75	2
H-HIR	2.17	2	t	42	2	2	4	17	30	879	66	2

[a] F, fresh control tissue; W, water-incubated tissue; B, buffer-incubated tissue; H, hydrogen peroxide-incubated tissue; HSP, hot-water-soluble polymers; HIR, hot-water-insoluble residue; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid; t, trace.

Table 2. Effect of Hydrogen Peroxide on Composition of Esterified Phenolics of Beet Root[a]

	μg/g fresh weight							
	fresh				completely cooked			
	F	W	B	H	FC	WC	BC	HC
p-OH-BA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
VA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
p-OH-B	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
V	0.7	0.5	0.9	0.8	0.2	0.5	0.3	0.6
t-CoA	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1
8,8′-AT-DiFA	3.0	3.2	2.2	2.9	0.4	0.4	0.7	1.6
t-FA	143.5	127.7	109.5	64.9	69.1	43.9	27.1	14.4
8,8′-DiFA	2.7	2.4	2.5	2.0	0.2	0.5	0.4	0.7
c-FA	13.1	12.8	10.3	6.6	10.6	10.3	12.0	6.5
5,5′-DiFA	3.6	4.1	2.3	4.9	0.9	1.2	0.5	1.7
8-*O*-4′-DiFA	8.5	5.1	5.4	14.3	4.1	3.2	2.1	8.4
5,8′-B-DiFA	6.9	7.4	6.3	13.6	1.0	0.8	0.2	1.9

[a] F, fresh control tissue; W, water-incubated tissue; B, buffer-incubated tissue; H, hydrogen peroxide incubated tissue; C, cooked; p-OH-B, *p*-hydroxybenzoic acid; VA, vanillic acid; p-OH-B, *p*-hydroxybenzaldehyde; V, vanillin; *t*-CoA, *trans*-coumaric acid; 8,8′-AT-DiFA, 8,8′-diferulic acid (aryltetralin form); *t*-FA, *trans*-ferulic acid; 8,8′-DiFA, 8,8′-diferulic acid; *c*-FA, *cis*-ferulic acid; 5,5′-DiFA, 5,5′-diferulic acid; 8-*O*-4′-DiFA, 8-*O*-4′-diferulic acid; 5,8′-B-DiFA, 5,8′-diferulic acid (benzofuran form).

(benzofuran form) dimers being most prominent. As found previously (Waldron et al., 1997b), the majority of the ferulic acid dehydrodimers were released by the 0.1 M NaOH extractions (Figure 1a) except for the 8,8′-DiFA and 8,8′-DiFA−aryltetralin forms, which were mainly solubilized by 1 M NaOH for 24 h.

Effect of Heating on Cooking Time. The required time at 100 °C to induce softening was investigated with a Mattson-style cooker. This apparatus is used to measure the time taken for tissue to soften sufficiently to allow a weighted rod to pass through it and drop (Downie et al., 1997). Preliminary experiments indicated that suitable dimensions for strips of beet root were 10 × 10 × 4 mm. Strips of 2 mm or thinner allowed the tissue to bend around the rod, resulting in a false reading. A sample of 19 strips (performed twice) revealed a mean cooking time of 177 min and a median cooking time (CT50) of 115 min. The variation in the cooking time is clearly shown in Figure 2; all sections were cooked by 10 h. The variability reflects the heterogeneity within beet root tissue and is likely to result from the different tissue types. During cooking, much of the red pigment leached into the cooking liquor. Subsequent examination of the broken surfaces of Mattson-cooked tissues by light microscopy revealed that thermal softening involved cell separation (results not shown).

Effect of Heating on Cell Wall Chemistry. Mattson-cooking resulted in a considerable decrease in the yield of CWM on a fresh-weight basis (FC; Table 1), probably due to the increase in water content of the tissue sections during heating. This was reflected also in the lower levels of wall phenolics on a fresh-weight basis (FC; Table 2; Figure 1b). The CWM of completely cooked tissue contained much lower proportions of pectic components, particularly uronic acid, in relation to cell wall Glc, indicating their solubilization from the tissues during the cooking process (FC, Table 1). The uronic acid component remaining in the CWM of the cooked beet root had a much lower degree of methylesterified uronic acid (DM value; 32%) than the fresh control. This is likely to be due to chemical de-esterification, as observed during the heating of pectic polysaccharides

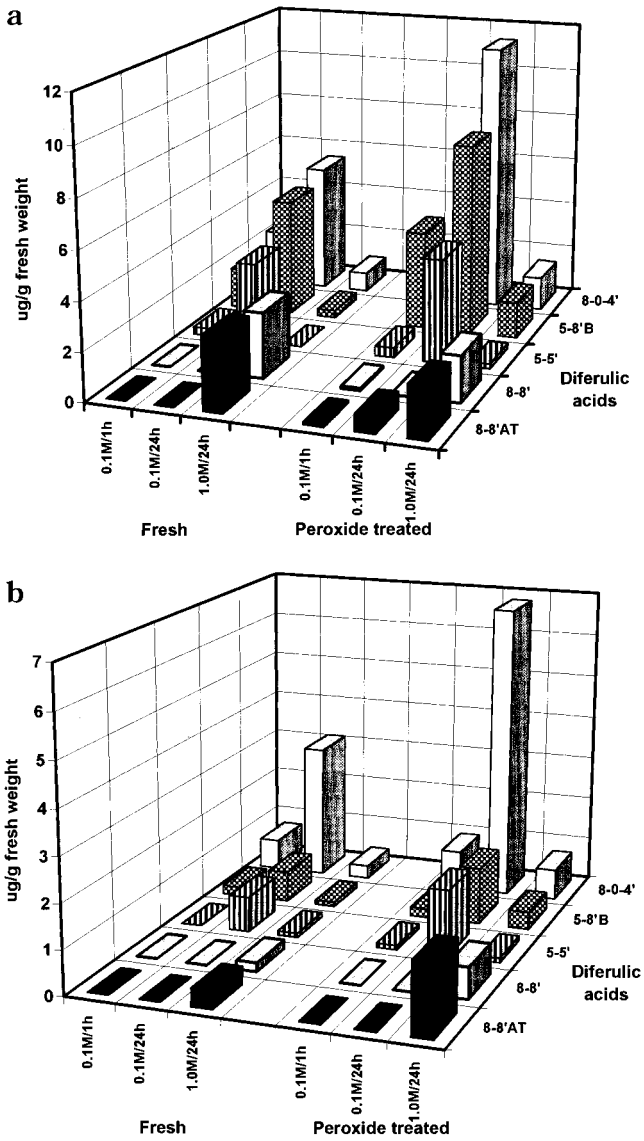

Figure 1. Effect of alkali treatment (0.1 M NaOH for 1 or 24 h and 1.0 M NaOH for 24 h) on solubilization of ferulic acid dimers from fresh- and peroxide-incubated beet root tissue (a) before and (b) after cooking to completion. The dimers are distinguished by their linkages.

(Sajjaanantakul et al., 1989) and cooking of carrots (Ng and Waldron, 1997a).

Samples of CWM prepared from fresh beet root were also heated in water at 100 °C for 30 min. The yields of hot-water-soluble (F-HSP) and insoluble (F-HIR) polymers and their compositions are shown in Table 1. In all cases, the hot-water-soluble polymers comprised pectic polysaccharides rich in uronic acid and arabinose.

Effect of Peroxide Treatment on Visual Appearance. Initial experiments involved incubating tissue strips (10 × 10 × 4 mm) for 16 h. To confirm that any changes were due to H_2O_2-dependent peroxidation alone, two additional control incubations were performed, one in water and one in buffer. The strips of beet root tissue incubated in water and buffer retained their bright-red color (Figure 3a). H_2O_2 treatment resulted in a loss of pigmentation from the outermost cells of the strips, probably by bleaching. Seen in cross-section, this colorless layer was about 1 mm deep and comprised about nine cell layers (Figure 3b). At the interface between the colorless layer and the pigmented

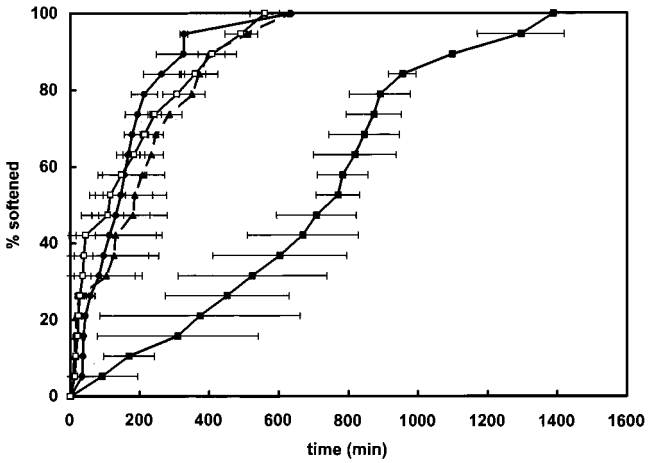

Figure 2. Mattson cooking profiles of fresh (F) beet root tissue (□) and after incubation in water (W, ●), buffer (B, ▲), and H_2O_2 (H, ■).

center, the cells were colored light yellow; this is likely to be due to the ongoing bleaching process. Examination of the autofluorescence of H_2O_2-treated strips showed that the walls of the outer, bleached cells were strongly autofluorescent at pH 10, as were those of the inner, unbleached (red) tissue. However, the walls of the two or three rows of cells with yellow contents were not autofluorescent (Figure 3c).

Effect of Peroxide Treatment on Thermal Softening. Incubation of beet root tissue in H_2O_2 for 16 h led to an increase in cooking time in the Mattson cooker to an average of 750 min and a maximum cooking time of nearly 1500 min (Figure 2). Examination of Mattson-cooked tissues by light microscopy showed that softening involved cell separation (not shown). The H_2O_2-induced increase in cooking time was due, therefore, to an increase in the thermal stability of cell−cell adhesion. In contrast, incubation of tissues in either buffer or water had no significant effect on the cooking profile (Figure 2).

To obtain mechanical data on the effect of peroxide treatment, tissue strips (30 × 3 × 2 mm) were incubated in buffer or buffer and H_2O_2 for 16 h and then subjected to a time course of heating in water at 100 °C. Subsequently, the strips were measured for their tensile strength. Prior to heating, strips that had been incubated in the presence or absence of H_2O_2 exhibited maximum tensile strengths of 1.1 and 0.9 MPa, respectively (Figure 4). The values were not significantly different ($p < 0.05$). Heating in boiling water resulted in a time-dependent decrease in strength for both sets of tissues. However, the H_2O_2-treated strips exhibited significantly higher tensile strengths that were double those of the control strips at all times tested. Microscopic examination of fractured surfaces of 4- and 5-h-heated strips showed that both H_2O_2 and buffer-only incubated strips ruptured by cell separation. This indicates that the thermal stability of cell−cell adhesion was significantly greater in the H_2O_2-incubated tissues. These values may be compared to those of fresh tissue, which vary from 0.4 to 0.6 MPa depending on maturity, and fell to 0.1−0.15 MPa after conventional steaming for 20 min.

Effect of Peroxide Treatment on Cell Wall Chemistry and Solubility. There were no significant changes in the yields of CWMs, their carbohydrate compositions, and uronic acid DM following incubation in peroxide or

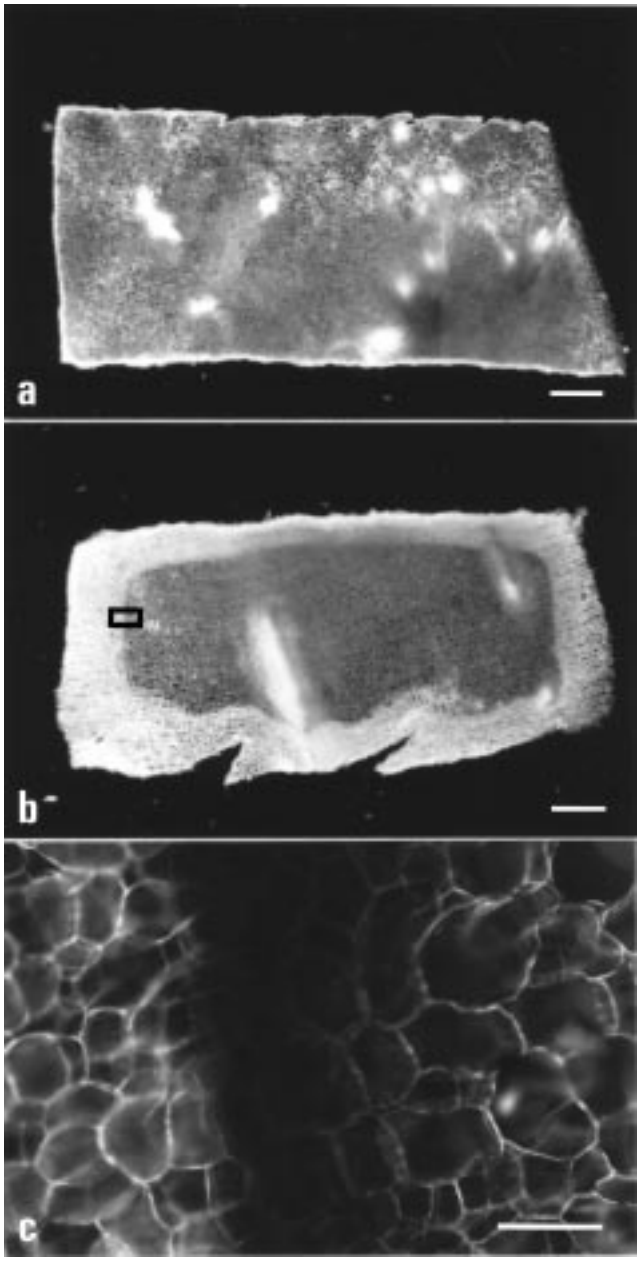

Figure 3. Darkfield and fluorescence micrographs of transverse sections through beet root tissue strips: (a) darkfield micrograph of fresh tissue (bar, 1 mm); (b) darkfield micrograph of H_2O_2-incubated tissues (bar, 1 mm); (c) autofluorescence micrograph of H_2O_2-incubated tissues at pH 10, specifically the region outlined in panel b (bar, 0.2 mm).

control solutions (Table 1). In addition, control incubations (water and buffer) had only a small effect on the levels of phenolic components of the walls (Table 2). However, incubation of tissue in peroxide almost doubled the levels of some of the ferulic acid dehydrodimers, particularly the 8-*O*-4′- and 5,8′-(benzofuran form) dimers. Small quantities of 5,8′-DiFA were also detected but not quantified. As in the CWM from fresh tissues, the majority of these dimers were extracted in 0.1 M NaOH over 24 h (Figure 1a). In addition, the levels of *cis*- and *trans*-ferulic acid moieties decreased by approximately 55% (Table 2). Control incubation of tissues in water and buffer only had no obvious effect on levels of ferulic acid dimers, neither were there any obvious changes in the levels of nonferulic acid phenolic moieties.

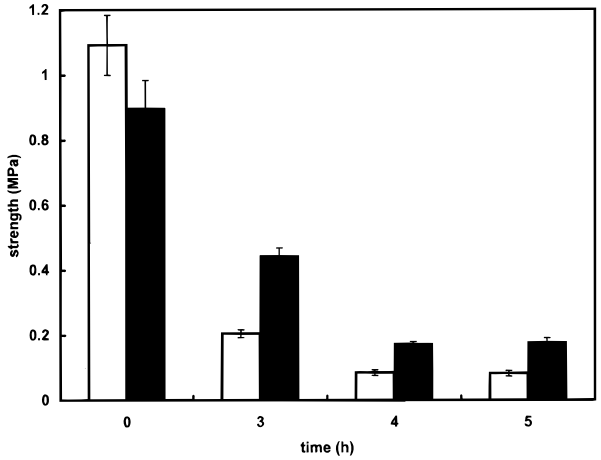

Figure 4. Tensile strength as a function of boiling time for samples incubated in buffer with (black bars) and without (white bars) H_2O_2.

Compared with the fresh tissue and control incubations, the CWM from peroxide-incubated beet root contained much lower levels of hot-water-soluble pectic polysaccharides (Table 1). In addition, the CWM from H_2O_2-incubated, completely cooked beet root contained much higher levels of several diferulic acid species as compared with the corresponding CWM from control, cooked tissues (FC and HC in Table 2; Figure 1b).

DISCUSSION

The results have demonstrated that incubation of beet root tissues in H_2O_2 increases the thermal stability of cell−cell adhesion and consequently increases cooking time. It is likely that this is due to the accompanying formation of diferulic acid moieties that enhance the cross-linking of polysaccharides involved in cell adhesion, since the increase is H_2O_2-dependent. The polysaccharides concerned are likely to be pectic polysaccharides, the neutral side chains of which are substituted with ferulic acid (Ralet et al., 1994). The enhanced cross-linking within the walls is demonstrated by the reduction in hot water solubility of pectic polysaccharides and the increase in the levels of total (pectic) carbohydrate in the water-insoluble residue (Table 1).

Peroxide-mediated cross-linking of soluble, ferulic acid-esterified polymers of the wall has previously been demonstrated in formation of gels from sugar beet pectic polysaccharides (Thibault et al., 1991) and maize arabinoxylans, which are also thermally stable (Ng et al., 1997). However, the possible cross-linking of other wall components including proteins cannot be ruled out; it is conceivable that tyrosine could also be oxidatively cross-linked (Fry, 1986).

The strengthening of cell−cell adhesion is consistent with the formation of the phenolic cross-links in the middle lamella. Previous histochemical studies on cell walls of apple (Ingham et al., 1998) and runner bean (Parker, unpublished) have demonstrated that wall-bound peroxidase enzymes, which are likely to be involved in such cross-linking, are also present in this region, particularly in the plasmodesmata and at the edges of cell faces. The edges of the cell faces are the locations at which thermally induced cell separation initiates (Waldron et al., 1997a). In Chinese water chestnut, which is notable for maintenance of crispness during heating, these wall locations are enriched with thermally stable, ferulic and diferulic acid-rich polymers

(Parker and Waldron, 1995; Parr et al., 1996; Waldron et al., 1997a).

The lack of change in the DM of uronic acid in the walls indicates that the increase in tissue strengthening during incubation in H_2O_2 is not due to the action of pectin-methyl esterase (PME). PME activity may be stimulated during blanching/precooking of certain vegetables such as carrots (Ng and Waldron, 1997a) and potatoes (Ng and Waldron, 1997b). This reduces the DM of pectic polysaccharides involved in cell−cell adhesion, enhancing their capacity for calcium cross-linking and reducing their propensity for β-eliminative degradation during cooking. This can also increase the thermal stability of cell−cell adhesion, although not to the extent shown during H_2O_2 treatment of beet root tissue in this study.

CONCLUSIONS

The results of this study have shown that treatment of beet root tissue with H_2O_2 decreases the rate of thermal softening by enhancing the thermal stability of cell−cell adhesion. The results indicate that this is due to the oxidative cross-linking of ferulic acid-esterified pectic polysaccharides, which become less soluble in hot water as a result. This provides further evidence for the involvement of ferulic acid cross-links in the thermal stability of texture.

ABBREVIATIONS USED

CWC, Chinese water chestnut; CWM, cell wall material; F, fresh tissue; W, tissue incubated in water; B, tissue incubated in buffer; H, tissue incubated in H_2O_2 and buffer; FC, fresh tissue after cooking completely; WC, tissue incubated in water and then cooked completely; BC, tissue incubated in buffer and then cooked completely; HC, tissue incubated in H_2O_2 and buffer and then cooked completely.

LITERATURE CITED

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