Cell Wall Chemistry of Carrots (*Daucus carota* Cv. Amstrong) during Maturation and Storage

Annie Ng, Adrian J. Parr, Lindsay M. Ingham, Neil M. Rigby, and Keith W. Waldron*

Department of Food Biophysics, BBSRC Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom

The aim of this study was to examine the changes of cell wall chemistry of carrots (*Daucus carota* cv. Amstrong) during maturation and storage in relation to textural properties. Alcohol-insoluble residues were prepared and extracted sequentially with water and cyclohexane-*trans*-1,2-diaminetetraacetic acid (CDTA) to leave residues. The extracts and residues were analyzed for their carbohydrate composition and, when appropriate, degree of methylesterification (DM). During maturation, the water-soluble pectic polysaccharides decreased from ≈23% in immature (8-week-old) carrots to 16% in mature carrots. This was accompanied by an increase in the levels of CDTA-soluble and CDTA-insoluble pectic polysaccharides. During storage, the proportions of water-soluble uronide continued to decrease (at all temperatures) to <8% after 3 months; this was accompanied by a decrease in the DM of the soluble uronide to ≈30%. A concomitant increase in the levels of CDTA-soluble pectic polysaccharides occurred.

Keywords: *Carrot; cell walls; pectic polysaccharides; maturation; storage*

INTRODUCTION

Carrots are a biannual dicotyledon plant, the edible portion of which is an overwinter storage organ (root). The state of maturity and the conditions of storage are important factors affecting the quality of vegetables that can be manipulated to meet the continuous market supply (Avon, 1979). Carrots are prone to damage during harvest; they deteriorate in quality and lose firmness during storage (Burton, 1982). Most aspects of plant growth and development involve the modification of cell wall structure, and continued maturation during storage may affect the textural properties (Waldron and Selvendran, 1992; Brett and Waldron, 1996).

Carrots have attracted attention as a valuable source of dietary fiber (Robertson et al., 1979a,b). The effect of long-term storage on the dietary fiber of carrots has been studied to obtain a better understanding of the physiological effect (Margareta et al., 1994). Most maturation and storage studies of carrots that have investigated the changes in carbohydrate composition have concentrated solely on the soluble carbohydrate (Nilsson, 1987a,b). There have been detailed studies on the chemical composition of carrot cell walls (Steven and Selvendran, 1984; Ng and Waldron, 1997), but little, if any, definitive information exists on the changes in the cell walls of carrots during maturation and post-harvest. The purpose of the present investigation is to compare changes in the cell wall carbohydrate and phenolic ester composition that occur during maturation with those that occur during storage under a range of conditions and relate them to heat-induced changes in texture.

MATERIALS AND METHODS

Materials. Carrots (*Daucus carota* cv. Amstrong) were grown at the Institute of Food Research Norwich, Norfolk, U.K., during the 1993 season. They were cultivated on a sandy soil (no pesticides were used) and were harvested regularly for the first 22 weeks, by which time the roots were mature. At that point, whole carrots were stored at 0.5 °C [ice bank, 99% relative humidity (RH)], 1 °C (85% RH) 4 °C (99% RH), 8 °C (90% RH), and 10 °C (95% RH) in darkness and sampled monthly for up to 3 months.

Tissue Types. When appropriate, carrots were transversely cut in two at the midpoint of the entire length of the root, to give top and bottom sections. In addition, sections of outer cortex (3 mm from the skin), intermediate (between the outer and inner rings), and center (inside the ring) of the carrot tops and bottoms were also collected.

Precooking and Cooking. Disks (5 mm diameter) of mature carrots (150 g) were subject to cooking (100 °C, 30 min), precooking (50 °C, 30 min), or precooking (50 °C, 30 min) followed by cooking (100 °C, 30 min) as described by Ng and Waldron (1997). All samples were frozen in liquid nitrogen.

Unless otherwise stated, all chemicals were of AnalaR grade.

Dry Weight. The dry weight of carrots (10 g) was determined in duplicate by freeze-drying; dried material was then dried in a ventilated oven for 24 h at 60 °C in the presence of a desiccant (silica gel) and measured gravimetrically.

Firmness Measurement. The field tenderometer (Bertuzzi Brugherio, Milan, Italy), a type of extrusion cell that determines the tenderness by measuring the force necessary to shear through a standard grid, was used for firmness measurement of fresh and processed carrot disks (10 disks). Reported values of carrot firmness (kilograms) are the average of seven replicates.

Preparation of Alcohol-Insoluble Residue (AIR). Carrots were extracted for AIR as described by Ng and Waldron (1997). Frozen carrots were homogenized in a Waring blender (Fischer Scentific Instrument, U.K.) with hot ethanol (85% v/v final concentration, 85 °C; Fisons), reducing particle size to <5 mm. The homogenate was transferred to a stainless steel beaker and homogenized with an Ystral homogenizer (Scientific Instruments Ltd., Eastleigh, U.K.) before boiling in a water bath for 5 min. The homogenate was filtered through 100 μm nylon mesh (John Stannier and Co., U.K.). The residue was further homogenized twice in 85% (v/v) ethanol

* Author to whom correspondence should be addressed [fax +44(0) 1603 507723; e-mail keith waldron@bbsrc.ac.uk].

S0021-8561(97)00992-8 CCC: $15.00 Published 1998 by the American Chemical Society
Published on Web 07/11/1998

and boiled for 1 min. The AIR was washed with acetone (Fisons) and air-dried in a fume cupboard.

Preparation of Cell Wall Material (CWM) for Phenolic Analysis. Immature (8-week-old), mature (22-week-old), and stored (10 °C for 3 months) carrot CWM were prepared as described by Parker and Waldron (1995) with modification in which frozen tissues (100 g) were blended in aqueous 1.5% sodium dodecyl sulfate (SDS; Sigma) containing 0.005 M $Na_2S_2O_5$ (Sigma) in an Ystral homogenizer for 5 min. A few drops of octan-1-ol (Sigma) were added to prevent foaming. The homogenate was filtered through 100 μm nylon mesh, and then the residue was ball-milled (500 mL pot, The Pascall Engineering Co. Ltd, Crawley, U.K.) in cold 0.5% SDS (400 mL) containing 0.003 M $Na_2S_2O_5$ for 2 h at 60 rpm at 1 °C to remove the bulk of the intracellular compounds and starch. The homogenate was filtered through 75 μm nylon mesh, and the residue was further ball-milled in 0.5% SDS containing 0.003 M $Na_2S_2O_5$ for 14 h at 60 rpm at 1 °C. CWM was recovered by filtering through 15 μm nylon mesh and washed three times in 1 L of cold distilled water containing 0.003 M $Na_2S_2O_5$. The CWM was further extracted with hot ethanol for 1 min to remove any alcohol-soluble phenolics, washed three times with acetone, and then air-dried.

Sequential Extraction of AIR. AIR (0.5 g) was suspended in water (50 mL, pH 4.8) and stirred for 2 h at 18 °C. The water-insoluble residue was recovered by centrifugation at 12000*g* for 30 min. It was then further extracted in cyclohexane-*trans*-1,2-diaminetetraacetic acid (CDTA, sodium salt; Sigma; 0.05 M, 50 mL, pH 6.5) first for 6 h at 18 °C and then for 2 h at 18 °C as described by Ng and Waldron (1997). In all cases, the supernatants were filtered and dialyzed exhaustively prior to concentration and freeze-drying.

Sugar Analysis. Cell wall neutral sugars were analyzed as alditol acetates by GC, and uronic acids were analyzed colorimetrically as described previously by Ng and Waldron (1997). Sugars were hydrolyzed from CWM by dispersing in 72% (w/w) H_2SO_4 (Fisons) for 3 h at room temperature, followed by diluting to 1 M and hydrolyzing for 2.5 h at 100 °C. 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 GC as described by Ng and Waldron (1997). Total uronic acid contents of CWM were determined colorimetrically according to 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. All samples were analyzed in duplicate.

Methanol Analysis. Degree of methylesterification (DM) was determined essentially as described by Ng and Waldron (1997). A sample (≈5 mg) was dispersed in distilled water (2 mL) and sonicated for 10 min. Propanol (0.4 mL, 0.2%) was added as an internal standard. The sample was de-esterified by addition of NaOH (0.8 mL, 2 M) for 1 h at 20 °C before being neutralized by the addition of HCl (0.8 mL, 2 M). It was then allowed to equilibrate for 15 min at 25 °C. Methanol was quantified by gas−liquid chromatography (Ng and Waldron, 1997).

Phenolic Acid Analysis. Phenolic acids were extracted and determined as described by Waldron et al. (1996). CWM (1 g) was sequentially extracted with 0.1 M NaOH (1 h; HPLC grade; BDH), 0.1 M NaOH (24 h), 1 M NaOH (24 h), and 2 M NaOH (24 h) under N_2 (O_2-free) at room temperature. The supernatant was filtered (GF-A, Whatman, Maidstone, U.K.) and neutralized with HCl (Fisons). *trans*-Cinnamic acid (Sigma; 100 μL, 1 mg/mL 50% methanol) was added as an internal standard, and the filtrate was extracted three times with ethyl acetate (Fison; HPLC grade). The ethyl acetate extracts were combined and dried under a stream of N_2. The sample was dissolved in 50% methanol (200 μL; HPLC grade; BDH) and analyzed by HPLC as described in Waldron et al. (1996).

Klason Lignin. The sample (50 mg) was dispersed in 72% H_2SO_4 for 3 h at room temperature followed by dilution to 1 M and hydrolysis for 2.5 h at 100 °C. The residue was recovered by centrifugation (12000*g* for 10 min), washed three times in distilled water, and lyophilized. The Klason lignin was then quantified gravimetrically.

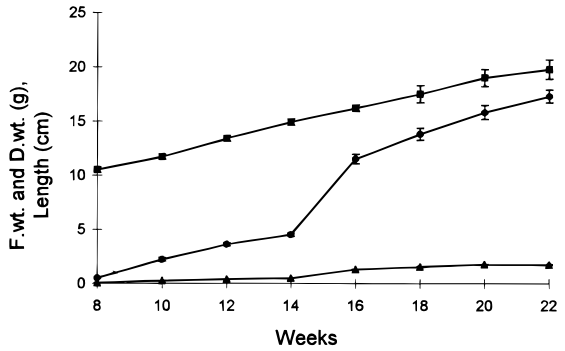

Figure 1. Changes in fresh weight (Fwt), dry weight (Dwt), and length of carrots during maturation: (●) Fwt; (▲) Dwt; (■) length.

Statistical Analysis. Analysis of variance and means among samples prepared according to various methods were calculated. Duncan's multiple-range test was used to determine significant differences ($P < 0.05$). The data for firmness measurement of fresh and processed carrots were used to interpret the significant differences during storage by using a two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Root Development and Changes during Storage. Fresh weight (Fwt), dry weight (Dwt), and total root length increased during maturation (Figure 1). Similar results were obtained by Nilsson (1987a). Mature (22-week-old) carrots were crisp and gave a firmness reading of 11.8 kg. During storage, fresh weights decreased. At 0 °C (ice bank), this change was small, being <15% over the 3 months. However, at the higher temperatures moisture loss was considerable, resulting in a weight loss of up to 80% at 10 °C; this was accompanied by a darkening of color. Storage resulted in a large increase in firmness (up to 25 kg). This is probably due to the loss of turgor, as shown by Jackman et al. (1992), who demonstrated that reduced failure force was required to induce tissue failure with increasing turgor of tomato tissue.

Cell Wall Chemistry of Immature Carrots. To describe the changes of cell wall chemistry of whole carrot tissues during maturation and storage, the carbohydrate composition of the cell wall of immature carrots will be examined, followed by studies of the effects of maturation and storage.

Carbohydrate Composition and DM. As carrot AIRs were free of starch, as shown by negative staining with I_2/KI and light microscopy, they were used as the source of the CWM. AIR from immature carrots was prepared and analyzed for its carbohydrate composition. The yield of AIR from immature carrot was 3.7% (Fwt). The bulk of the AIR (≈63%) comprised carbohydrate, mainly pectic polysaccharides as inferred from the levels of uronic acid, galactose, arabinose, and rhamnose, and also cellulosic glucose with minor quantities of xylose and mannose (Table 1). The remainder of the AIR consisted of cell wall or coprecipitated intracellular protein (Martin-Cabrejas et al., 1994). The composition of carrot cell walls was found to be comparable to that previously reported (Steven and Selvendran, 1984; Ng and Waldron, 1997). The absence of starch from AIR was confirmed by comparing glucose released by hydrolysis in 1 M sulfuric acid (Selvendran and O'Neil,

Table 1. Yields, Carbohydrate Composition, and DM of Carrot AIRs during Maturation[a]

weeks	yield (% Fwt)	carbohydrate (mol %) Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	total μg/mg	DM %	ratio UA:Ara+Gal
8	3.7	1	tr[b]	12	3	2	12	37	32	631	18	1.3
14	3.5	1	tr	12	3	2	12	37	32	656	30	1.3
18	3.5	1	tr	11	2	2	12	37	34	647	34	1.5
22	3.3	1	tr	10	2	2	10	33	41	622	40	2.1

[a] The values are the mean of duplicate determinations, and the variations within duplicates were <5%. [b] Trace.

Table 2. Yields, Carbohydrate Composition, and DM of AIR Fractions during Maturation[a]

	weeks	yield (% AIR)	carbohydrates (mol %) Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	total μg/mg	DM %	ratio UA:Ara+Gal
WSP	8	11	1	tr[b]	11	1	1	11	11	63	883	65	2.9
	14	13	1	tr	11	1	2	11	12	60	741	63	2.6
	18	13	1	tr	13	1	1	10	12	61	632	64	2.4
	22	12	1	tr	14	1	1	10	13	59	615	47	2.5
CSP-1	8	15	1	tr	10	1	1	10	15	61	374	31	3.1
	14	16	1	tr	11	1	1	9	15	62	434	37	3.1
	18	17	1	tr	11	1	1	10	15	60	447	38	2.8
	22	18	1	tr	10	1	1	9	15	64	455	39	3.4
CSP-2	8	14	1	tr	10	1	1	10	15	61	184	ND[c]	3.1
	14	15	1	tr	11	1	1	9	15	62	212	ND	3.1
	18	15	1	tr	11	1	1	10	15	60	276	ND	2.8
	22	15	1	tr	10	1	1	8	15	63	256	ND	3.5
CIR	8	60	1	tr	13	4	2	12	46	20	794	3	0.8
	14	56	1	tr	13	4	2	13	44	19	787	7	0.7
	18	55	1	tr	13	4	2	13	45	22	793	10	0.8
	22	55	1	tr	12	3	2	11	43	26	751	37	1.1

[a] The values are the mean of duplicate determinations, and the variations within duplicates were <5%. [b] Trace. [c] Not determined.

1987) with that released after treatment in 72% (w/w) sulfuric acid followed by dilution to 1 M and hydrolysis at 100 °C; the glucose quantified after hydrolysis in 1 M H_2SO_4 was ≈10% of the amount quantified from 72% and 1 M sulfuric acid hydrolysis (Selvendran and O'Neil, 1987). The DM of the uronide was ≈18%.

Sequential Extractions of AIR. The immature carrot AIR was extracted sequentially with water and CDTA. The pectic polysaccharides that were not cross-linked into the cell wall were solubilized by water. Those held in the wall predominantly by Ca^{2+} only were subsequently extracted by CDTA. It is probable that the bulk of the water- and CDTA-soluble pectic polysaccharides are of middle lamella origin (Selvendran and O'Neil, 1987). The procedure was designed to minimize β-eliminative degradation of pectic polymers (Waldron and Selvendran, 1992). The amounts of material extracted are based on one sequential extraction of AIR. Previous studies in carrots showed that most of the extractable pectic polymers were solubilized by water and CDTA and relatively less with Na_2CO_3 and KOH (Ng and Waldron, 1997). The importance of calcium-cross-linked polymers in the texture of carrots had been demonstrated by Greve et al. (1994) and Ng and Waldron (1997); hence, for the purpose of this study, only water- and CDTA-extracted polymers were investigated.

The polysaccharides released by water and CDTA extractions were rich in pectic polysaccharides as shown by the high levels of uronic acid, galactose, and arabinose (Table 2). There was a relatively small amount of glucose in the water- and CDTA-soluble polysaccharides (WSP and CSP), similar to previous results in carrots (Ng and Waldron, 1997). It is possible that this is due to the presence of small quantities of nonstarch glucans (Ng and Waldron, 1997) and has not been further investigated. To provide information on the ease of extraction of the cell wall pectic polysaccharides, the relative yields of extracted pectic polysaccharides, as a function of the total AIR pectic polysaccharides, are shown in Figure 2. This showed that relatively more pectic polysaccharides were solubilized by water than CDTA. The ratio of uronic acid to arabinose + galactose (UA:Ara+Gal) was higher in CSP, indicating that these were less branched than the polysaccharides extracted by water (Ng and Waldron, 1997). The DM values of WSP and CSP-1 were 65 and 31%, respectively (Table 2).

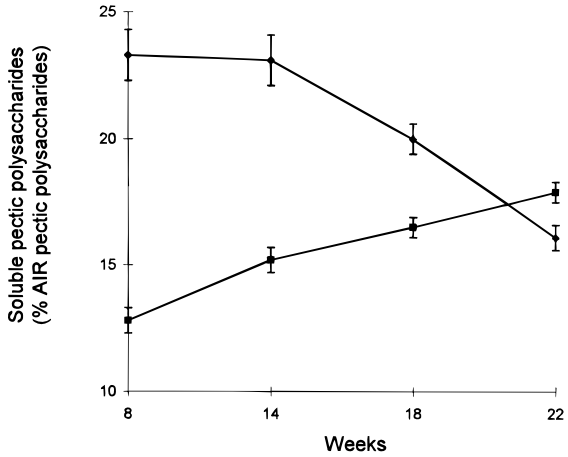

Figure 2. Solubility of pectic polysaccharides (% AIR pectic polysaccharides) during maturation of carrots: (◆) WSP; (■) CSP.

The yield of CDTA-insoluble residue of immature carrots (CIR) was ≈60% (Table 2). They were rich in glucose (≈46%), the remaining carbohydrate consisting mainly of pectic components. The ratio UA:Ara+Gal was lower than the AIR and probably reflects the insolubility of more highly branched pectic polysaccharides in the residue. Similar results were obtained by Ng and Waldron (1997).

Wall-Bound Esterified Phenolic Acids and Lignin. Recent studies on the ester-linked phenolic components

Table 3. Total Esterified Cell Wall Phenolic Acids of Immature, Mature, and Stored Carrots by Sequential Hydrolysis up to 1 and 2 M NaOH[a]

	μg/g of wall carbohydrate					
	1 M NaOH			2 M NaOH		
	immature	mature	stored	immature	mature	stored
4-hydroxybenzoic acid	161.3	180.6	411.5	44.3	33.6	124.6
vanillic acid	17.3	16.9	20.7	0.8	1.2	3.0
4-hydroxybenzaldehyde	4.9	4.0	4.2	2.4	0.6	0.9
vanillin	16.3	13.5	18.4	6.0	4.1	5.6
8,8′-diferulic acid (aryltetralin form)	0	0	0	0	2.8	2.0
p-coumaric acid	0	1.1	1.8	0	0	0.4
trans-ferulic acid	45.4	21.4	21.3	21.5	3.8	5.7
5,8′-diferulic acid	0	tr[b]	tr	0.8	0.6	0.5
cis-ferulic acid	21.7	12.0	11.0	7.0	2.9	4.0
8-*O*-4′-diferulic acid	11.9	16.3	13.7	9.8	4.2	5.5
5,8′-diferulic acid (benzofuran form)	0	2.1	1.9	4.0	0.6	1.9
unknown[c]	2.5	1.9	1.7	10.9	0.6	0.8
total	281.3	269.8	506.2	107.5	55.0	154.9

[a] The values are the mean of duplicate determinations, and the variations within duplicates were <5%. [b] Trace. [c] Assuming response factor equal to that of ferulic acid.

Table 4. Yields, Carbohydrate Composition, and DM of AIRs of Different Tissue Types of Mature Carrots[a]

		carbohydrates (mol %)										
section	yield (% Fwt)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	total μg/mg	DM %	ratio UA:Ara+Gal
top	4	2	tr[b]	9	2	2	9	32	44	649	39	2.4
bottom	4	2	tr	8	2	2	9	38	39	639	39	2.3
outer top	7	2	tr	8	2	3	8	31	47	593	33	2.9
outer bottom	7	2	tr	8	2	3	8	35	41	597	33	2.6
intermediate top	4	2	tr	9	2	3	8	36	39	611	37	2.3
intermediate bottom	3	2	tr	7	1	3	9	39	38	666	43	2.4
center top	4	2	tr	9	4	2	10	33	39	604	40	2.1
center bottom	2	2	tr	7	6	2	10	32	39	611	47	2.3

[a] The values are the mean of duplicate determinations, and the variations within duplicates were <5%. [b] Trace.

of carrot cell walls highlight their possible role in influencing the textural properties (Waldron et al., 1997; Parr et al., 1997). Such phenolics are probably esterified to cell wall polysaccharides (Waldron et al., 1997). The cell walls of immature carrots were analyzed for their phenolic composition (Table 3). In keeping with the earlier work, the results show that carrot CWMs contained significant amounts of simple phenolics, of which the majority comprised 4-hydroxybenzoic acid. In addition, a small amount of ferulic acid (trans and cis) and 8-*O*-4′-diferulic acid were present, probably esterified to the cell walls (Mueller-Harvey et al., 1986). The compositions were comparable to that reported previously for carrot tissues by Massiot et al. (1988) and Parr et al. (1997). The ferulic acid moieties comprised ≈24% (w/w) of the wall-bound esterified phenolic, and ≈15% of the ferulic acid is in dimer form (Parr et al., 1997). The majority of the ferulic acid was extracted by the 1 M NaOH/24 h step, whereas much of the 8-*O*-4′-diferulic acid was extracted by the 2 M NaOH/24 h step; this may reflect differences in the strength of the ester linkages (Parr et al., 1997; Waldron et al., 1997). The results also confirm the appearance of an unknown ferulic acid-like phenolic seen in previous studies on mature carrot CWM (Parr et al., 1997). In this study, the method of CWM preparation was modified from that of Parker and Waldron (1995) to include further ball-milling for 14 h and resulted in a highly purified water-insoluble CWM; this effectively eliminated the interference caused by residual intracellular phenolic acids and proteins, ensuring a more accurate quantification of phenolic acids, particularly for those present in small amount (Ng, Karaoulanis, Ingham, Parr, and Waldron, unpublished results). The occurrence of 5,8′-diferulic acid was, for the first time, identified in immature carrot CWM after sequential hydrolysis with 2 M NaOH/24 h (Table 3). In addition, there was a small amount of Klason lignin present in immature carrot AIR (≈4% on carbohydrate basis).

Cell Wall Carbohydrate Composition of Different Tissue Types. The structural organization of carrot and its constituent tissues (such as stele and phloem parenchyma) may influence the overall cell wall composition. It is of interest to investigate the carbohydrate composition of the outer cortex, intermediate, and center regions of mature carrot top and bottom. The key differences were that the AIR yield of outer cortex of mature carrots (≈7% Fwt) was higher than in the intermediate and central regions (≈3% Fwt; Table 4). The level of xylose (mole percent) was higher in the central stele than in the outer and intermediate regions. The uronic acid from the center of the root exhibited a higher DM, and this was greater in the bottom regions. A similar trend was obtained with other carrots (English Class 1; data not shown). These results reflect the heterogeneity of the root tissues during carrot development. The center of the root is rich in xylem vessels, which will undergo secondary thickening and some lignification during development; the surrounding tissues consist mainly of epidermal and subepidermal parenchyma cells, which increase during root swelling (Brett and Waldron, 1996).

Effect of Maturation on Carrot Cell Wall Chemistry. The yields of AIR decreased slightly from 3.7 to 3.3% (Fwt) during maturation (Table 1). During maturation, there was also an increase in pectic polysaccha-

Table 5. Yields, Carbohydrate Composition, and DM of AIRs of Representative Samples of Carrot Tissues Stored for 3 Months[a]

	yield	carbohydates (mol %)								total		ratio
	(% Fwt)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	μg/mg	DM %	UA:Ara+Gal
ice bank	3	2	tr[b]	10	2	2	9	33	41	684	43	2.2
4 °C	8	2	tr	11	2	2	10	29	41	655	45	1.9
8 °C	9	2	tr	11	2	2	9	28	45	715	44	2.2
10 °C	37	2	tr	10	2	2	9	32	42	691	45	2.2

[a] The values are the mean of duplicate determinations, and the variations within duplicates were <5%. [b] Trace.

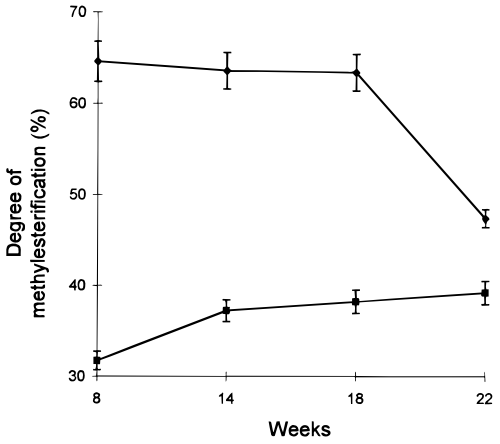

Figure 3. DM during maturation of carrots: (◆) WSP; (■) CSP.

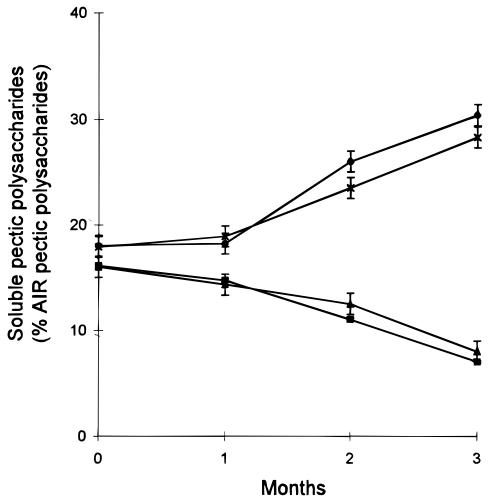

Figure 4. Effect of storage on carrots in the solubility of pectic polysaccharides (% AIR): (■) WSP (ice bank); (▲) WSP (10 °C); (×) CSP (ice bank); (●) CSP (10 °C).

rides, which was accompanied by a concomitant decrease in other polysaccharides, particularly xylose and glucose. These changes can be explained by the lateral growth of the carrot, resulting in a relatively lower contribution of thin highly vascularized initial root. The ratio UA:Ara+Gal increased during maturation, indicating less branched pectic polysaccharides. This may be similar to the breakdown of the galactan and arabinan side chains in asparagus during maturation (Waldron and Selvendran, 1992). The DM of the uronide increased during maturation, indicating that cell extension involved the deposition of methyl-esterified pectic-rich polysaccharides).

The proportion of WSP pectic polysaccharides decreased during maturation (Figure 2). This was accompanied by an increase in their levels in CSP and CIR. This could be due to continued synthesis and insertion of CSP and reduced synthesis and/or turnover of WSP during maturation. The DM of WSP and CSP generally showed little change during growth and development, although the DM of WSP decreased markedly as maturity approached (Figure 3). The DM of CIR increased during maturation (Table 2).

Maturation was accompanied by an increase in the proportion of 8-*O*-4′- and 5,8′ (benzofuran form)-diferulic acid in relation to total ferulic acids (Table 2). The occurrence of 5,8′-diferulic acid after sequential hydrolysis with 1 M NaOH/24 h, and 8,8′ (aryltetralin form)-diferulic acids after sequential hydrolysis with 2 M NaOH/24 h is also in line with a maturation-related increase in phenolic cross-linking (Table 3). The presence of 8,8′ (aryltetralin form)-diferulic acid may have a special role in cell adhesion (Waldron et al., 1997). Maturation of vegetables is often accompanied by an increase in cell wall interpolymeric cross-linking, involving simple phenolics, as demonstrated in asparagus (Waldron and Selvendran, 1992) and cauliflower (Femenia, Waldron, and Selvendran, unpublished results). Maturation had no significant effect on the Klason lignin value, indicating no lignin deposition (Robertson et al., 1979b).

Effect of Storage. During storage, there was no change in the carbohydrate composition of AIR (on a mole percent basis), which was rich in pectic polysaccharides, or in the DM of the uronide (Table 5).

Storage of carrots resulted in a decrease in WSP under all conditions (Figure 4). This was accompanied by an increase in CSP; however, there was no change in CIR. Hence, the overall cell wall components showed no change during storage. Whereas continual synthesis and turnover cannot be ruled out, the results could also be explained by the conversion of WSP to CSP, although the mechanism is not clear. Such a mechanism could involve a number of factors, for example, the availability of calcium required for cross-linking polysaccharides. We have demonstated in onion that increasing the calcium availability does reduce WSP and increase CSP (Ng et al., 1998). Our results could also reflect the ionic movement into cell walls during storage. Storage resulted in a small (albeit significant) decrease of the DM of WSP, and this was accompanied by a slight increase in the DM of CSP ($P < 0.05$; Table 6; Figure 5).

Phenolic acid analysis showed that storage resulted in an increase in the level of 4-hydroxybenzoic acid, possibly due to the presence of pathogen-related elicitors (Schnitzler et al., 1992). Induction of several phenolic compounds of carrots during storage and stress conditions have also been reported (Coxon et al., 1973; Sarker and Phan, 1979; Lafuente et al., 1989). Storage resulted in no change in the level of ferulic acid and its dimers (Table 3). In addition, storage had no significant effect on the Klason lignin value. This contrasts with asparagus, in which storage induced an enzymic-mediated

Table 6. Yields, Carbohydrate Composition, and DM of Extracts and Insoluble Residues during Storage[a]

			yield	carbohydrates (mol %)								total		ratio
	conditions	months	(% AIR)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	μg/mg	DM %	UA:Ara+Gal
WSP	ice bank	1	12	3	tr[b]	11	2	2	13	10	59	615	46	2.4
		2	12	3	tr	13	2	2	16	11	52	460	42	1.8
		3	12	3	tr	15	3	3	18	9	48	308	40	1.5
	10 °C	1	13	3	tr	11	2	2	13	12	55	692	45	2.3
		2	14	3	tr	14	2	2	16	13	48	604	44	1.6
		3	14	3	tr	16	2	2	18	14	42	305	40	1.2
CSP-1	ice bank	1	17	1	tr	9	tr	tr	7	11	71	564	41	4.4
		2	17	1	tr	9	tr	tr	7	10	72	720	44	4.5
		3	17	1	tr	8	tr	tr	7	9	74	767	47	4.6
	10 °C	1	16	1	tr	10	tr	tr	9	11	68	506	41	3.2
		2	16	1	tr	10	tr	tr	9	11	68	723	44	3.6
		3	17	1	tr	10	tr	tr	9	10	69	856	47	3.6
CSP-2	ice bank	1	14	1	tr	10	t	tr	7	11	70	424	ND[c]	4.1
		2	15	1	tr	10	t	tr	7	12	69	416	ND	4.1
		3	15	1	tr	10	t	tr	7	11	70	465	ND	4.1
	10 °C	1	15	1	tr	10	t	tr	7	14	65	428	ND	3.8
		2	15	1	tr	10	t	tr	7	13	65	400	ND	3.8
		3	14	1	tr	10	t	tr	8	15	65	390	ND	3.6
CIR	ice bank	1	57	1	tr	11	3	3	8	41	32	793	40	1.7
		2	56	1	tr	11	3	3	9	39	33	853	42	1.7
		3	56	1	tr	11	3	3	9	38	34	825	43	1.7
	10 °C	1	56	2	tr	11	3	3	9	42	30	827	42	1.5
		2	55	2	tr	11	3	4	10	41	29	825	43	1.4
		3	55	2	tr	11	3	3	9	41	31	848	44	1.6

[a] The values are the mean of duplicate determinations, and the variations within duplicates were <5%. [b] Trace. [c] Not determined.

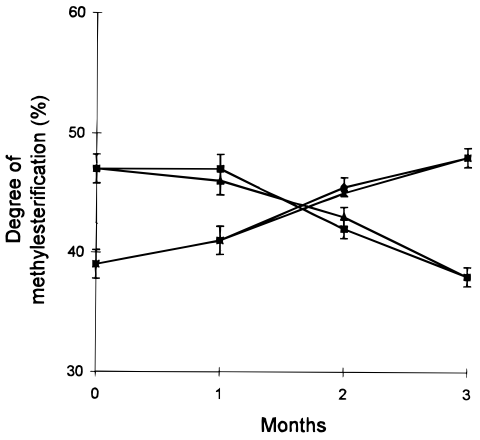

Figure 5. Effect of storage on carrots in the DM: (■) WSP (ice bank); (▲) WSP (10 °C); (×) CSP (ice bank); (●) CSP (10 °C).

lignification (Sharma et al., 1975; Smith and Stanley, 1987; Waldron and Selvendran, 1992).

Effect of Storage on Firming Effect. Long-term storage of many vegetables prior to processing results in a decrease in firmness and color and the development of off-flavors (Okoli et al., 1988; Woolfe, 1991; Collins and Walter, 1992), which makes it difficult for processors to manufacture products of consistent quality throughout the year. Many vegetables also soften dramatically on cooking (Brett and Waldron, 1996). Previous studies had shown that cooking-induced softening of carrot could be reduced by precooking at moderate temperatures (Ng and Waldron, 1997). This firming effect is probably due to an increase in thermal stability of calcium cross-linking of pectic polysaccharides. To assess the effect of storage on precooking-induced firmness, we investigate the change in firmness of precooking and cooking carrot tissues during storage.

Cooking of fresh carrots reduced tissue firmness to ≈1.5 kg. Precooking prior to cooking reduced cooked tissue softness to ≈2.6 kg ($P < 0.05$). This bears similarity to the previous report (Ng and Waldron, 1997). Interestingly, storage of carrots at 10 °C for 3 months resulted in a significant enhancement of the firmness after subsequent cooking to ≈1.8 kg and also resulted in an increase in the firmness of tissue that was precooked prior to cooking to ≈3.6 kg ($P < 0.05$). This effect was not seen in any other of the conditions studied. However, storage for 3 months at all temperature conditions showed an enhancment of carrot firmness after precooking up to 1.4-fold that of fresh carrot ($P < 0.05$). These results may suggest that storage-induced firmness may modulate the firmness during subsequent processing and may relate to the storage-related increase in CDTA-soluble pectic polysaccharides.

CONCLUSIONS

This study has focused attention on the changes in cell wall carbohydrate composition in succulent growing carrot tissues and subsequent storage in relation to the textural quality. Chemical and textural analysis have resulted in the following conclusions:

(1) Maturation of carrots resulted in an increase in less branched pectic polysaccharides and was accompanied by an increase in the degree of total pectin methylesterification, indicating a continual insertion of more highly methyl-esterified pectic polysaccharides into the cell wall during growth and development.

(2) During maturation and storage, the decrease in WSP and increase in CSP were suggestive of an increase in cross-linking of WSP, the mechanism of which is not cleat but may involve calcium.

(3) Maturation was accompanied by an increase in esterified ferulic acid cross-linking, which may have significant structural implications.

(4) Storage-induced firmness may modulate the subsequent processing firmness, such as precooking followed by cooking.

ABBREVIATIONS USED

AIR, alcohol-insoluble residue; CWM, cell wall material; Rha, rhamnose, Fuc, fucose; Ara, arabinose; Xyl,

xylose; Man, mannose; Gal, galactose; Glc, glucose; DM, degree of methylesterification; UA:Ara+Gal, uronic acid: arabinose + galactose; WSP, water-soluble polysaccharides; CSP, CDTA-soluble polysaccharides; CIR, CDTA-insoluble residue; FA, ferulic acid; DiFA, diferulic acid; AT, aryltetralin form; BF, benzofuran form.

LITERATURE CITED

Avon. *Refrigerated Storage of Fruit and Vegetables*; Ministry of Agriculture, Fisheries and Food Reference Book; 1979; p 324.

Blakeney, A. B.; Harris, P. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharides analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.

Brett, C.; Waldron, K. W. *Physiology and Biochemistry of Plant Cell Walls*, 2nd ed.; Chapman and Hall: London, 1996.

Burton, W. G. *Post-Harvest Physiology of Food Crops*; Longman: New York, 1982.

Collins, J. L.; Walter, W. M., Jr. Processing and processed products. In *Fifty Years of Cooperative Sweetpotato Research*; Jones, A., Boukamp, J. C., Eds.; Southern Cooperative Series Bulletin 309; Louisiana State University: Baton Rouge, LA, 1992; pp 71−87.

Coxon, D. T.; Curtis, R. F.; Price, K. R.; Levett, G. Abnormal metabolites produced by *Daucus carota* roots stored under conditions of stress. *Phytochemistry* **1973**, *12*, 1881−1885.

Greve, L. C.; McArdle, R. N.; Gohlke, J. R.; Labavitch, J. M. Impact of heating on carrot firmness. Changes in cell wall components. *J. Agric. Food Chem.* **1994**, *42*, 2900−2906.

Jackman, R. L.; Marangoni, A. G.; Stanley, D. W. The effects of turgor pressure on puncture and viscoelastic properties of tomato tissue. *J. Texture Stud.* **1992**, *23*, 491−505.

Lafuente, M. T.; Cantwell, M.; Yang, S. F.; Rubatzky, V. Isocoumarin content of carrots as influenced by ethylene conentration storage tempeature and stress conditions. *Acta Hortic.* **1989**, *258*, 523−534.

Margareta, E.; Nyman, G. L.; Nilsson, T. Effect of Long-term storage on dietary fibre in different cultivars of carrots. *Acta Agric. Scand. Sect. B. Soil Plant Sci.* **1994**, *44*, 116−122.

Martin-Cabrejas, M. A. M.; Waldron, K. W.; Selvendran, R. R. Cell wall changes in Spanish pear during ripening. *J. Plant Physiol.* **1994**, *144*, 541−548.

Massiot, P.; Rouau, X.; Thibault, J.-F. Isolation and characterisation of the cell-wall fibres of carrot. *Carbohydr. Res.* **1988**, *172*, 217−227.

Mueller-Harvey, I.; Hartley, R. D.; Harris, P. J.; Curzon, E. H. Linkage of p-coumaroyl and feruloyl groups to cell wall polysaccharides of barley straw. *Carbohydr. Res.* **1986**, *148*, 71−85.

Ng, A.; Waldron, K. W. Effect of cooking and pre-cooking on cell-wall chemistry in relation to firmness of carrot tissues. *J. Sci. Food Agric.* **1997**, *73*, 503−512.

Ng, A.; Parker, M. L.; Smith, A. C.; Waldron, K. W. Modification of cell-wall polymers of onion waste. Part II. effect of divalent cations. *Carbohydr. Polym.* **1998**, in press.

Nilsson, T. Growth and chemical composition of carrots as influenced by the time of sowing and harvest. *J. Agric. Sci. Cambridge* **1987a**, *108*, 459−468.

Nilsson, T. Carbohydrate composition during long-term storage of carrots as influenced by the time of harvest. *J. Hortic. Sci.* **1987b**, *62* (2), 191−203.

Okoli, E. C.; Nmorka, O. G.; Unaegbu, M. E. Technical note: Blanching and storage of some Nigerian vegetables. *Int. J. Food Sci. Technol.* **1988**, *23*, 639−641.

Parker, M. L.; Waldron, K. W. Texture of Chinese Water Chestnut: Involvement of cell wall phenolics. *J. Sci. Food Agric.* **1995**, *68*, 337−346.

Parr, A. J.; Ng, A.; Waldron, K. W. The ester-linked phenolic components of carrot cell walls. *J. Agric. Food Chem.* **1997**, *45*, 2468−2471.

Robertson, J. A.; Brydon, W. G.; Tadesse, K, Wenham, P.; Walls, A.; Eastwood, M. A. The effect of raw carrot on serum lipids and colon function. *Am. J. Clin. Nutr.* **1979a**, *32*, 1889−1892.

Robertson, J. A.; Eastwood, M. A.; Yeoman, M. M. An investigation into dietary fibre content of named varieties of carrot at different developmental ages. *J. Sci. Food Agric.* **1979b**, *30*, 388−394.

Sarker, S. K.; Phan, C. T. Naturally occurring and ethylene-induced phenolic compounds in the carrot root. *J. Food Prot.* **1979**, *42*, 526−534.

Schnitzler, J. P., Madlung, J., Rose, A.; Seitz, H. U. Biosynthesis of *p*-hydroybenzoic acid in elicitor-treated carrot cell cultures. *Planta* **1992**, *188*, 594−600.

Selvendran, R. R.; O'Neil, M. A. Isolation and analysis of cell walls from plant material. *Methods Biochem. Anal.* **1987**, *32*, 25−153.

Sharma, S. C.; Wolfe, R. R.; Wang, S. S. Kinetic analysis of post-harvest texture changes in asparagus. *J. Food Sci.* **1975**, *40*, 1147−1150.

Smith, J. L.; Stanley, D. W. Nonenzymatic lignification of asparagus. *J. Texture Stud.* **1987**, *18*, 339−358.

Stevens, B. J. H.; Selvendran, R. R. 1984. Structural features of cell-wall polysaccharides of the carrot (*Daucus carota*). *Carbohydr. Res.* **1984**, *128*, 321−327.

Waldron, K. W.; Selvendran, R. R. Cell wall changes in immature Asparagus stem tissue after excision. *Phytochemistry* **1992**, *31*, 1931−1940.

Waldron, K. W.; Parr, A. J.; Ng, A.; Ralph, J. Cell wall esterified phenolic dimers: Identification and quantification by reverse phase high performance liquid chromatography and diode array detection. *Phytochem. Anal.* **1996**, *7*, 305−312.

Waldron, K. W.; Smith, A. C.; Parr, A. J.; Ng, A.; Parker, M. L. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends Food Sci. Technol.* **1997**, *8*, 213−221.

Woolfe, J. A. *Sweet Potato: An Untapped Food Reserve*; Cambridge University Press: Cambridge, U.K., 1991; pp 251, 317−320.

Received for review November 21, 1997. Revised manuscript received March 26, 1998. Accepted May 20, 1998. This work was funded by the U.K. Biotechnology and Biological Science Research Council and the European Communities (AIR Project NI CT92-0278).

JF9709921