Impact of Heating on Carrot Firmness: Changes in Cell Wall Components[†]

L. Carl Greve, Richard N. McArdle,[‡] James R. Gohlke,[‡] and John M. Labavitch[*]

Department of Pomology, University of California, Davis, California 95616

The impact of heating in boiling water on tissue firmness retention of the carrot genotypes Danvers and B9304 was measured. Both genotypes showed a rapid early loss in firmness that was followed by a slower, more prolonged, softening. Softening was greater in Danvers carrots. Cell wall carbohydrate compositions of the two varieties were similar, but wall component dissolution (solubilization and depolymerization) was more extensive in Danvers. The breakdown of soluble and wall-bound pectins involved β-elimination reactions. A survey of several carrot genotypes indicated that those carrots with chelator-soluble pectins of relatively lower molecular weight tended to soften more extensively during heating.

Keywords: *Carrot; pectin; texture; β-elimination*

INTRODUCTION

A considerable amount of research has been directed toward understanding the tissue factors that contribute to the eating texture of fresh and cooked fruits and vegetables. The primary focus has been on changes in the plant cell wall as tissue firmness decreases during ripening, storage and processing. Pectic polysaccharides, acidic polymers that are found throughout the primary wall and appear to be particularly prominent in the middle lamella between cells, have been the focus of much of this research.

Studies of processed carrots have indicated that heat treatments cause changes in pectin solubility, size, and charge density (Lee et al., 1979; Plat et al., 1988, 1991; Ben-Shalom et al., 1992). Work with extracted pectins (Sajjaanantakul et al., 1989) suggests that some of these changes are due to β-elimination reactions. The fact that various treatments that should lower the potential for β-elimination of pectins in intact carrots (e.g., Bourne, 1987; Heil and McCarthy, 1989; Van Buren and Pitifer, 1992) reduce tissue firmness loss supports this idea. In the present work, we have examined several properties of cell wall pectins from different breeding lines and cultivars of carrots and related these properties to the impact of continuous heating on their texture.

MATERIALS AND METHODS

Plant Materials. Carrots of the cultivars Danvers (D) and B9304 (a USDA breeding line; Simon et al., 1990) used for most of this work were grown near Modesto, CA, and harvested at 5.5–7.5 months of age. Carrots were harvested in the early morning and transported rapidly to Davis, where they were trimmed and washed (30 min) in 0.01% sodium hypochlorite, rinsed with distilled water, and blotted dry. They were then held in plastic bags and stored at 0 °C until used. Carrots used in the comparison of texture retention with chelator (*trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid; CDTA)-soluble pectin size distribution were grown near El Centro, CA, and transported to Davis overnight.

Carrot Heating. Radially cut disks of carrot 1 cm in thickness were made from portions of roots ranging from 2.1 to 3.0 cm in diameter. Sets of three disks were placed in 150 mL beakers containing 75 mL of boiling distilled water. Disks were removed with forceps at intervals and placed on crushed ice. Cooled disks were subjected to firmness testing and homogenized for cell wall analysis. Aliquots of the cooking water were also analyzed. Water samples were made 80% in ethanol and held overnight at 4 °C. Insoluble material was collected by centrifugation, redissolved in water and assayed for uronic acid using the colorimetric procedure of Blumenkrantz and Asboe-Hansen (1973).

Firmness measurements were made with an Instron force analyzer in the penetrometer mode. Firmness was recorded as the force required to drive a 2.2 mm probe 3 mm into the carrot disk with a cross-head velocity of 50 mm/min. All measurements were made at room temperature (ca. 22 °C).

The rate at which the interior of the carrot disk reached 100 °C was followed in early experiments. The thermocouple-tipped probe (Type K) of an Omega HH-26K digital thermometer was inserted through the side of a carrot disk so that the tip was at the disk's center. The impaled disk was then suspended in boiling water so that only the insertion point was not fully immersed and the temperature was recorded.

Three-disk samples were sliced into pieces and homogenized (Polytron) in 70 mL of chilled deionized water. The homogenate was centrifuged (25000*g*) for 15 min at 4 °C. Twenty milliliters of the supernatant was frozen; the rest was discarded. The insoluble pellet was suspended in acetone and recentrifuged (two times). Acetone-insoluble material (crude cell wall) was allowed to air dry in a fume hood overnight and then held in a vacuum oven at 50 °C until constant weight was achieved.

Uronic acid in cell wall material was determined colorimetrically after solubilization in sulfuric acid (Ahmed and Labavitch, 1977). Noncellulosic polymers were hydrolyzed in 2 N trifluoroacetic acid (TFA) as described by Albersheim et al. (1967) and monosaccharides produced were converted to alditol acetates (Blakeney et al., 1983) which were analyzed by gas chromatography (isothermal separation at 210 °C using a 30 m × 0.25 mm DB-23 capillary column). Material remaining insoluble after TFA treatment was dissolved in 67% sulfuric acid and assayed for cellulose content using anthrone reagent (Dische, 1962) with a standard of dissolved cellulose powder.

The dried crude cell wall was extracted by stirring at room temperature for 16 h in 0.05 M CDTA in 0.05 M sodium acetate buffer (pH 6.0). Following extraction, the CDTA-soluble pectin (CSP) and one deionized water wash of the pellet were collected by centrifugation. Separate aliquots of the CSP were assayed

* Author to whom correspondence should be addressed (e-mail jmlabavitch@ucdavis.edu).

† This work was supported by a grant from Kraft-General Foods.

‡ Present address: Kraft-General Foods, Tarrytown, NY 10591.

0021-8561/94/1442-2900$04.50/0 © 1994 American Chemical Society

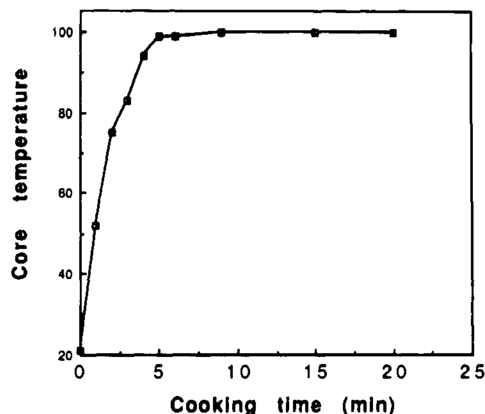

Figure 1. Temperature profile at the center of a carrot disk following its immersion in boiling water. Experiment was performed with B9304 (three times) and Danvers (one time) carrots with similar results.

for uronic acid and subjected to gel filtration chromatography on Sepharose CL-4B in either 0.05 M sodium acetate (pH 6.0) containing 0.2 M NaCl or 1 M imidazole–HCl (pH 7.0), as described in figure legends.

The analysis of heating-related changes in carrot uronic acid solubility followed a different protocol. After homogenization, the crude cell wall was collected by filtration through a 0.05 mm nylon mesh. The soluble filtrate was collected and made 80% in acetone. This was held at 4 °C overnight and then centrifuged to produce water- and acetone-soluble (low molecular weight) and water-soluble, acetone-insoluble (high molecular weight) fractions. The crude cell wall on the mesh was washed several times with cold, deionized water and then acetone. These washings were discarded. The residue was transferred from the mesh to a glass beaker and air-dried in the fume hood overnight. It was then held in a vacuum oven (50 °C) until analysis.

RESULTS AND DISCUSSION

Our primary approach to understanding cell wall change influences on heat-induced texture change was to compare cell walls of carrot genotypes which performed differently in texture-retention tests. The breeding line B9304 (a breeder's selection, not in commercial use) was reported to retain its texture much better than the commonly used Danvers cultivar. This was confirmed in preliminary tests. Carrot disks were plunged into boiling water and reached 100 °C in 3–4 min (Figure 1). Penetrometer tissue firmness measurements made on uncooked samples and on samples collected at intervals during heating (Figure 2) showed that both interior tissue (xylem; X) and external tissue (phloem; P) of B9304 were firmer than their D counterparts initially (differences in X being the clearest) and that B9304 remained firmer throughout heating, with the X remaining most clearly different between the two genotypes. Tissue firmness loss in the first 5–8 min was much more rapid in each variety than in the last 20 min of cooking. This was in agreement with the two-reaction relationship discussed by Bourne (1989).

Varietal differences in overall cell wall carbohydrate composition do not seem to provide an explanation for differences in texture retention. The uronic acid content of D is slightly higher than that of B9304 and its cellulose content is slightly lower (Table 1). Noncellulosic (i.e., 2 N TFA-hydrolyzable) neutral sugar contents of the two varieties are similar (Table 1), as are the overall distributions of the different sugars in hydrolyzed cell wall samples (Table 2). These results are consistent with reports for other carrot lines (Stevens and Selvendran, 1984; Penner and Kim, 1991). Analysis of cell wall components from X and P tissues collected separately or from older and younger portions of the roots revealed differences (not shown). In fact, differ-

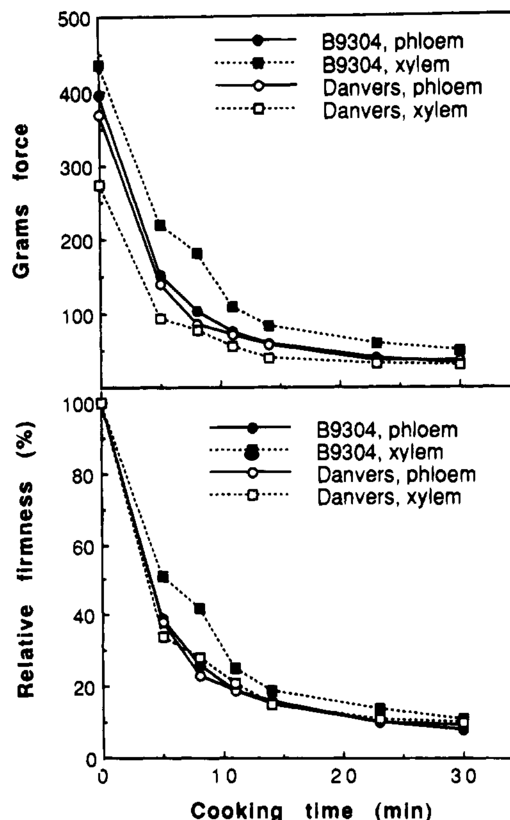

Figure 2. Loss in tissue firmness (three measurements each for xylem and phloem at each time point) during cooking of B9304 and Danvers carrot disks in boiling water. The panel showing relative firmness expresses all values as a percentage of the zero-time value. Data are for a single experiment. (The experiment has been performed several times with similar results, although as storage time increases, the initial firmness increases and the difference between varieties decreases.)

Table 1. Distribution of Carbohydrate Constituents in Cell Walls of B9304 and Danvers Carrots[a]

	carrot variety	
component	Danvers	B9304
total neutral sugar	24.1	23.4
total uronic acid	38.2	34.8
cellulose	28.8	31.0

[a] Noncellulosic neutral sugars were measured by GLC and are listed individually in Table 2. Uronic acid was determined colorimetrically [Blumenkrantz and Asboe Hansen, 1973; as modified by Ahmed and Labavitch (1977)]. Cellulose was measured by anthrone assay (Dische, 1962) of the cell wall material which remained insoluble after 2 N TFA hydrolysis (Albersheim et al., 1967). All data are expressed as a percent dry weight and are means of duplicate analyses. No duplicates varied by more than 5%.

Table 2. Sugars in the Noncellulosic Fraction of Carrot Cell Walls[a]

	carrot variety	
sugar	Danvers	B9304
rhamnose	8.0	8.7
fucose	0.7	0.9
arabinose	34.1	35.5
xylose	3.2	3.3
mannose	1.9	2.4
galactose	46.8	46.5
glucose	5.2	3.0

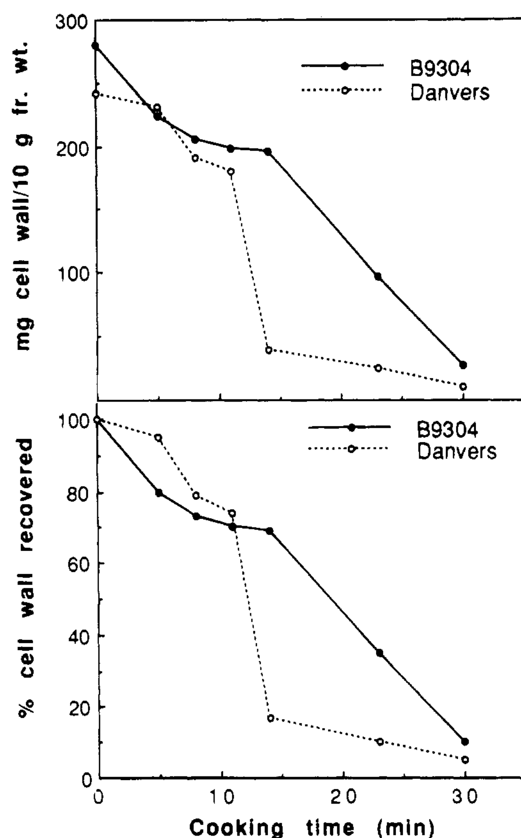

Figure 3. Loss of cell wall during heating of carrot disks. All wall values are the dry weight of that fraction of carrot disk homogenates that does not pass a 0.05 mm mesh nylon screen. Each point represents a single measurement. Relative cell wall recovery is the percentage of recovery from uncooked samples. The experiment was performed two times for each carrot variety.

ences in cell wall composition due to tissue location or age were at least as great as any differences between genotypes. Because these data seem to indicate that gross compositional differences are not responsible for the varietal differences in texture retention, more subtle physical differences must exist in the cell walls of B9304 and D carrots.

It is clear that cell wall components are altered during heating in boiling water. The yield of cell wall from homogenates of heated carrots decreases steadily as treatment time increases (Figure 3). This loss is slow in the first 10 min and more rapid thereafter, especially for D carrots. A portion of the material that is lost from the insoluble cell wall (i.e., that which is trapped on a nylon mesh) is recovered in soluble form (Table 3). Uronic acid (a rough quantitative indicator of pectin backbones) was measured separately in the cooking water (as 80% ethanol-insoluble material) and in the supernatants collected following homogenization of carrot disks in water [in 80% acetone-soluble (low molecular weight) and acetone-precipitable (high molecular weight) form]. Prior to heating, the amount of uronic acid in insoluble form (i.e., crude cell wall pellet-associated) is much more (8 and 5 times, respectively, for B9304 and D) than the amounts found in the soluble fractions of tissue homogenates. After 30 min of boiling, the amount of uronic acid remaining in association with the crude cell wall (normalized to fresh weight of unheated samples) is greatly reduced (12% and 7% of the initial uronic acid contents for B9304 and D, respectively) and the amounts of uronic acid recovered in the soluble fractions are now greater than the amounts remaining cell wall bound (2.2 times for D and 4.5 times for B9304). From the total recoveries indicated in Table 3 it is clear that uronic acid solubilization is extensive and, also, that uronic acid destruction (conversion to a form not detected by our assay) is occurring. The patterns of uronide recovery in various soluble fractions are also instructive. Uronic acid-containing polymers accumulate in the boiling water. [Because of the very high concentration of neutral sugars (monomers and small oligomers) in the 80% ethanol-soluble fraction of the cooking water samples, we could not measure their uronic acid content.] Presumably, this 80% ethanol-insoluble material represents pectins solubilized from cell walls and leached from the tissue. Some tissue-associated uronic acid is freely soluble prior to heating (proportionally more in D than B9304) and this fraction (Table 3, columns B and C) increases during the early part of heating of both carrot varieties. As heating continues the amounts of soluble uronide begin to fall. Some of the decrease must be due, at least in part, to continued leaching into the boiling water (Table 3, column D), but for D carrots the

Table 3. Cooking Effects on Cell Wall Uronic Acid Solubility[a]

variety	cooking time (min)	cell wall[b]	acetone insoluble[c]	acetone soluble[c]	ethanol insoluble[d]	total soluble	total (all fractions)[e]
B9304	0	67.3	7.92	0.52	0 (0)	12.5	75.74 (100)
	5	56.2	5.65	0.81	2.98 (4.5)	14.0	65.64 (86.7)
	8	37.9	11.75	1.02	4.77 (6.3)	26.1	55.44 (73.2)
	11	34.2	12.36	1.57	7.23 (9.5)	31.4	55.36 (73.1)
	14	28.0	8.51	0.54	7.22 (9.5)	24.2	44.27 (58.4)
	23	10.1	8.21	0.28	8.68 (11.5)	25.5	27.27 (36.0)
	30	7.8	6.5	0.19	10.59 (14.0)	27.7	25.08 (33.1)
Danvers	0	58.8	11.97	0.42	0 (0)	21.1	71.19 (100)
	5	44.0	12.93	1.02	1.3 (1.8)	25.9	59.25 (83.2)
	8	30.4	13.0	1.41	2.9 (4.1)	29.4	47.71 (67.0)
	11	26.9	13.2	0.92	4.6 (6.5)	31.8	45.62 (64.1)
	14	10.2	12.8	0.47	10.9 (15.3)	41.1	34.37 (48.3)
	23	8.1	9.72	0.35	9.6 (13.5)	33.5	27.77 (39.0)
	30	4.0	9.15	0.27	8.8 (12.4)	31.0	22.22 (31.2)

[a] Uronic acid contents of cell wall and soluble fractions of homogenates of carrots heated for various periods of time in boiling water were determined. Values are given in mg (galacturonic acid equivalents) and are normalized to a zero-time (i.e., unheated) tissue fresh weight of 10 g. Recoveries of soluble uronic acids are expressed as a percentage of the zero-time "cell wall" fraction uronic acid content. "Total uronic acid" values are the sums of all assays of uronic acid (wall-bound and soluble) normalized to the 10 g fresh weight. [b] Uronic acid in insoluble fraction after homogenization in water. [c] Uronic acid in fractions of supernatants from homogenized tissue that have been made 80% in acetone. [d] Material insoluble when heating water is made 80% in ethanol. Values in parentheses are the percentages of the zero time total uronic acid for a given variety. [e] Values in parentheses are the percentages of the zero time total uronic acid for a given variety.

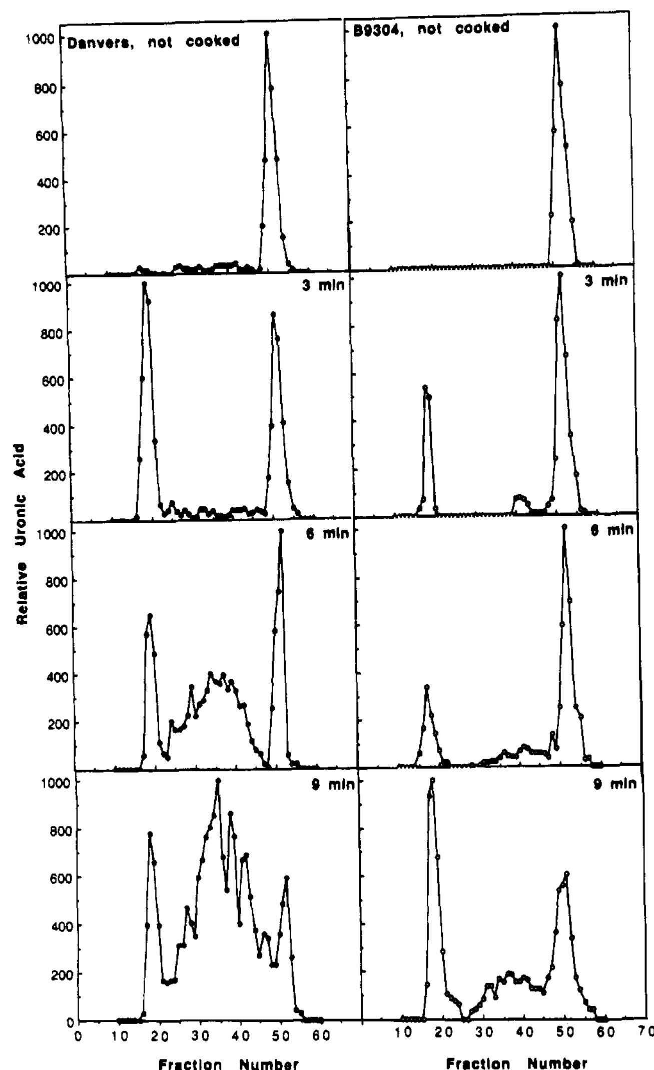

Figure 4. Gel filtration of uronide in the water-soluble fraction (supernatant) of the aqueous homogenates of Danvers and B9304 carrot disks cooked for the indicated times. The 54 × 1.8 cm column of Sepharose CL-4B was equilibrated and eluted with 0.05 M sodium acetate (pH 6.0) containing 0.2 M NaCl. Fraction size was 1.3 mL, and the column's fractionation range was fractions 17−55 (elution of standards). Relative uronic acid values are for the colorimetric assay of Blumenkrantz and Asboe Hansen (1973) and are reported in relationship to the highest reading for the series (=1000). Sampling time is indicated in the upper right corner of each panel.

amounts of uronide in this fraction also fall. Perhaps the difficult-to-assay 80% ethanol-soluble portions of the heating water samples contain the missing uronic acid, but this would represent a substantial amount of material. When β-elimination of pectins occurs, there is conversion of uronosyl residues to the corresponding 4,5-unsaturated residue (Albersheim et al., 1960b). Perhaps such a conversion is occurring as heating proceeds.

Pectins are often regarded as the primary components of the middle lamella, the intercellular cement that holds cells together (Albersheim et al., 1960a; Knox et al., 1990), so it is reasonable to expect that changes in pectin characteristics could affect tissue texture. Thus, the data presented above may provide insight into the differences in B9304 and D texture retention. The proportion of tissue uronide that is freely soluble is greater in D than B9304 and solubilization during heating appears to be more extensive. We explored this

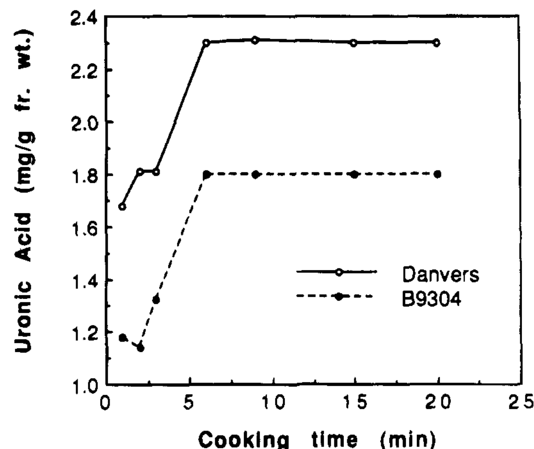

Figure 5. Total CDTA-soluble uronic acids in cell walls prepared from samples of B9304 and Danvers carrots heated for various periods of time. While CSP increases on a fresh weight basis, the yield of cell wall decreases with extended boiling.

possibility by analyzing soluble and wall-bound pectins further.

Preliminary observation indicated that the aqueous supernatants obtained by homogenizing boiled carrots and removing cell wall residues by centrifugation were viscous if treatment time had been short but that supernatant viscosity decreased with prolonged treatment. Viscosity in preparations from D carrots seemed to decrease after shorter heating times than were required for viscosity reduction in B9304 supernatants. Samples of water soluble uronide (combinations of materials described by columns B and C, Table 3) were subjected to gel filtration chromatography on Sepharose CL-4B, a medium that has a nominal fractionation range for dextrans of 5×10^6−3×10^4 Da.

All samples (Figure 4) show a strong peak of uronic acid assay reactive material at the column's fully included volume (fractions 50−55). This should be ignored. The peak is a false positive caused by the high amount of low molecular weight (primarily neutral) carbohydrate that is water soluble in the tissue. The intensity of this peak decreases with treatment time, presumably because lower molecular weight material is rapidly leached into the boiling water. Furthermore, the peak disappears when samples are extensively dialyzed against water prior to chromatography (not shown). There is little water-soluble polymeric uronide present in the zero-time homogenates of the two carrot varieties. As heating proceeds, however, differences in the size of pectins in this fraction become evident. For B9304 (Figure 4) at 3 min, most of the uronic acid voids the column. With increased boiling times the relative size of the void peak decreases while increasing amounts of uronic acid are found in the mid-size fractions collected. For D (Figure 4), the pattern is more pronounced. As treatment continues a substantial amount of uronide is found in the mid-range. The accumulation of mid-sized pectins as treatment lengthens could indicate depolymerizaton of pectins or increased solubilization of smaller, wall-bound pectins. In any case, this change is more extensive in D carrots. Dissolution of pectins is often associated with cooking-associated texture loss (Plat et al., 1988, 1991; Ben-Shalom et al., 1992) and several authors have proposed that β-eliminative pectin cleavage is responsible for the solubilization (Bourne, 1989; Sajjaanantakul et al., 1989). β-Elimination is often inferred spectrophotometrically by

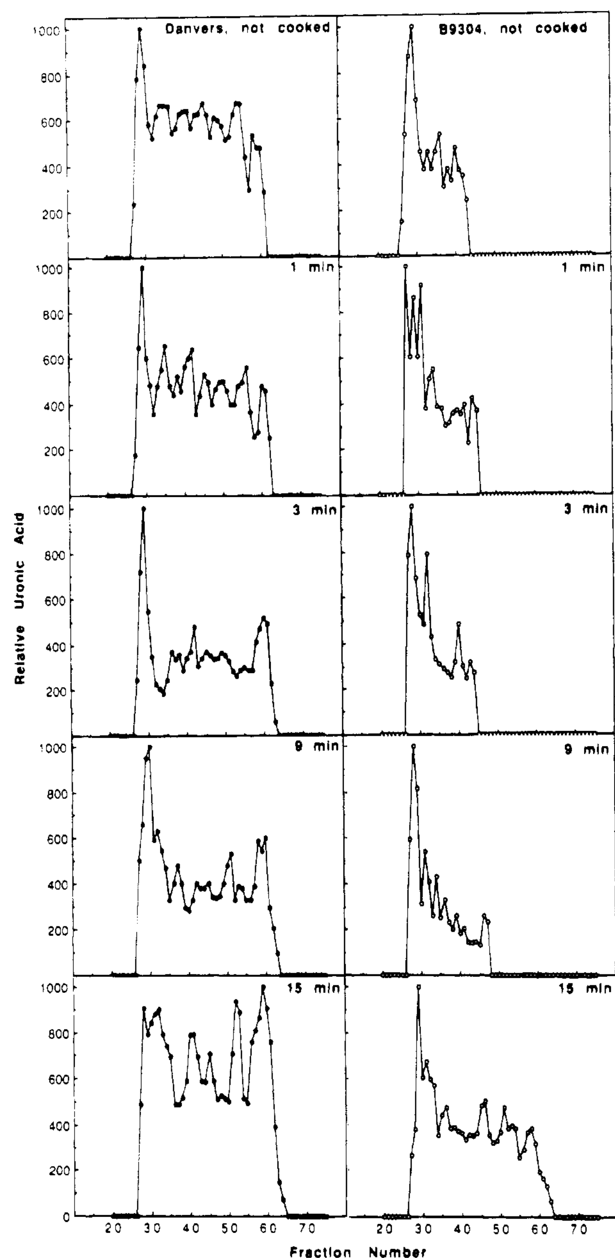

Figure 6. Gel filtration chromatography of CSP preparations from cell walls of unheated and boiled B9304 and Danvers carrots. Chromatographic conditions and data presentation as described in the legend for Figure 4. Sampling time is indicated in the upper right corner of each panel.

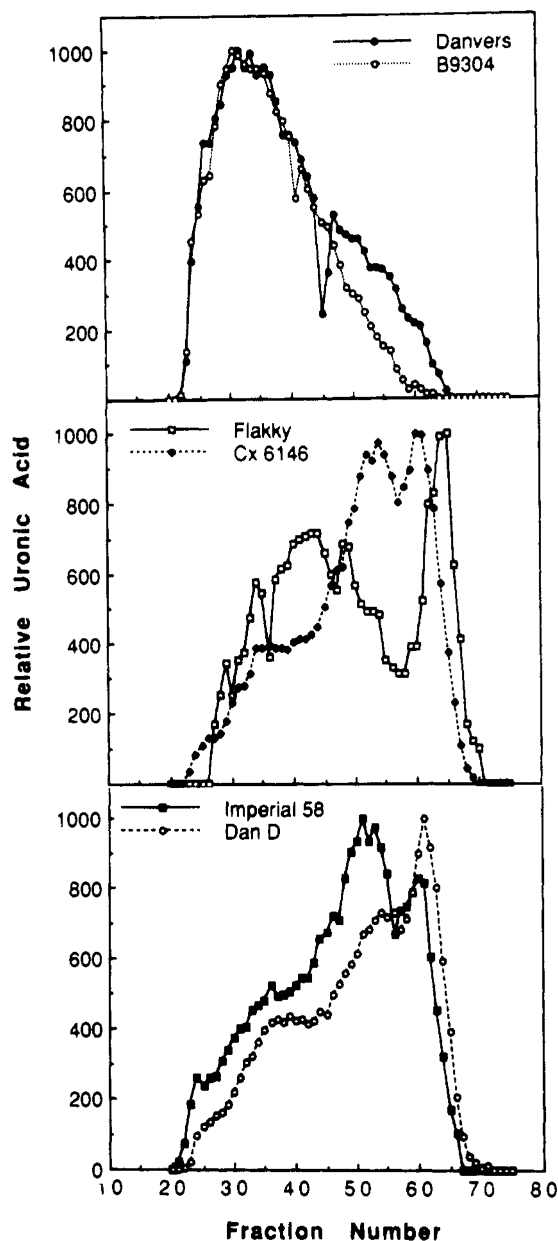

Figure 7. Gel filtration chromatography of CSP preparations from cell walls of unheated samples of several carrot varieties. The 54 × 1.8 cm column of Sepharose CL-4B was equilibrated with 1 M imidazole−HCl (pH 7.0). Fraction size was approximately 1.1 mL, and the column's fractionation range was fractions 21−68. Relative uronic acid values are based on colorimetric assay with the maxium value in a series set to 1000.

measurement of the absorption at 235 nm due to the accumulation of 4,5-unsaturated products (Albersheim et al., 1960b). We therefore tested whether the accumulation of water-soluble pectins in carrot homogenates was the consequence of β-elimination by measuring A_{235} in our collected fractions. Absorbance closely followed (not shown) the patterns of uronic acid distribution, suggesting that β-elimination reactions were responsible for the pectin solubilization observed.

Sajjaanantakul et al. (1989) have shown that pectins extracted from carrot cell walls using the chelator EDTA were subject to heat-catalyzed β-elimination. This observation suggested to us that the water-soluble pectins (described above) might be derived from a CSP population that remained with the cell wall material following homogenization. We therefore subjected CDTA-soluble materials from raw and heated D and B9304 carrots to a series of tests. The concentrations (fresh weight basis) of CSP in the cell wall preparation from unheated D carrots was about 40% greater than that of B9304 cell walls (Figure 5) and the concentrations of CSP in both varieties increased quickly during heating. This could only be explained by a heating-related solubilization of more tightly bound wall pectins and subsequent (during heating or tissue homogenization) association of these polymers into chelator-soluble (presumably Ca^{2+}-mediated) complexes. The CSP from raw D carrots contained smaller polymers than did the preparation from untreated B9304 (Figure 6). As for the water-soluble pectins, when CSP samples from carrots boiled for increasing periods of time were analyzed, the proportions of smaller uronides increased (Figure 6). These samples, too, contained material detectable by measuring A_{235}. A_{235} absorbance appeared earlier for samples from D carrots than for those from

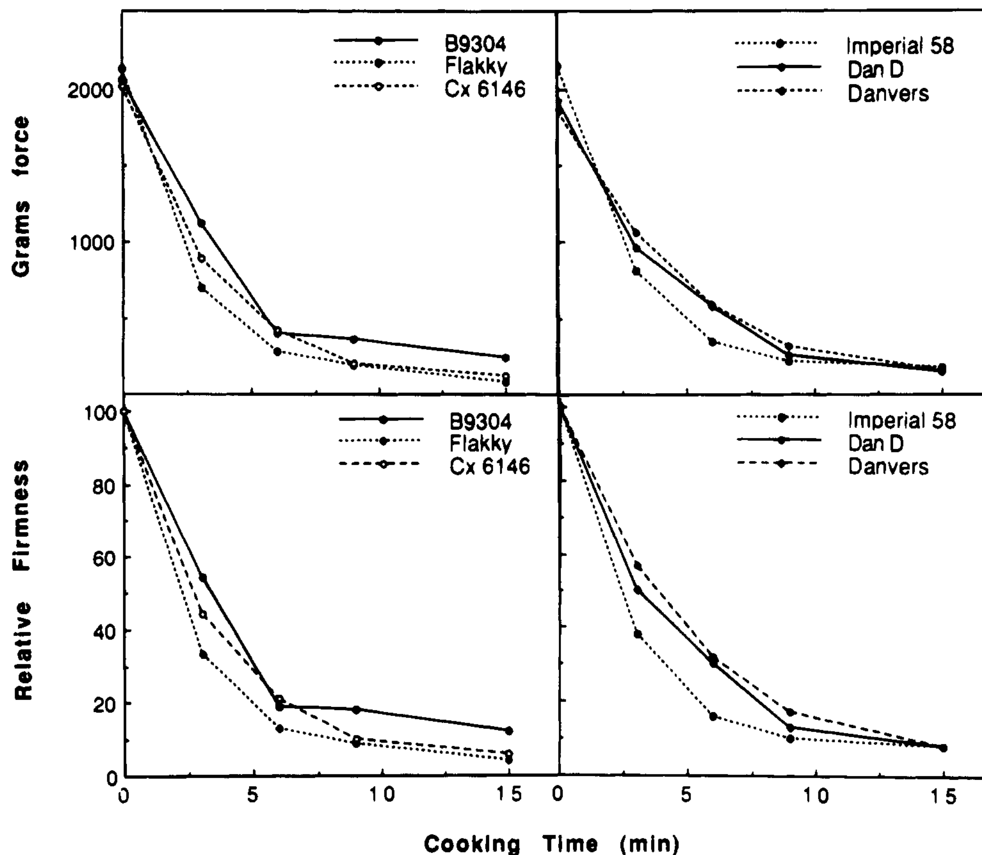

Figure 8. Loss in tissue firmness (three measurements for each time point, only phloem values shown) during treatment of disks cut from several varieties of carrots. Relative firmness shows values after various periods of heating in relationship to the unheated carrot values.

B9304 and, again, coeluted with the smaller uronides in these extracts (not shown).

These results taken together demonstrate that loss of texture in cooked carrots is accompanied by and perhaps caused by a substantial dissolution, depolymerization, and, apparently, destruction of cell wall pectins. One factor in this breakdown is β-eliminative cleavage of polymers that is evident both in pectins that lose their association with the cell wall and those that remain bound to the wall via chelator-sensitive bonds. The breakdown of pectins is more extensive in D than in B9304 carrots and, as described above, the former also lose texture more quickly during heating. Why β-elimination might be more extensive is not clear. A higher pectin ester content would make elimination more likely (Sajjaanantakul et al., 1989), but we have found no varietal differences in overall pectin ester content (data not presented). Our observation is consistent with the results of others who pretreated carrots to inactivate pectin esterase (Heil and McCarthy, 1989) or de-esterify native pectins (Van Buren and Pitifer, 1992) and obtained a cooked product of firmer texture.

An interesting observation we have made is that as storage (at 4 °C and 100% relative humidity) is prolonged, the firmness of both varieties increases and the varietal difference in postcooking firmness retention disappears. This correlates with several changes in cell wall components, including increases in the small amounts of hydroxyproline and ferulic and p-coumaric acids present (data not shown).

The viscosity of a pectin solution increases as the length of the polymers increases. In the cell wall, also, increased pectin size might increase the possibility of an assortment of inter-polymer associations and, thus, wall strength. This, in turn, could have an effect on tissue firmness. Therefore, the presence of CDTA-soluble pectins of relatively smaller size in D cell walls might also be taken to be a predictor of their texture retention vis-a-vis B9304 carrots. This correlation was tested by comparing the patterns of heating-induced texture loss for several carrot varieties and the size of CDTA-soluble pectins prepared from the walls of unheated samples. Gel filtration (Figure 7) demonstrated that these pectins could be quite different in size distribution. The difference between D and B9304 noted in earlier analyses of different carrot samples remained (Figure 7) but was much less distinct (see comments below). The difference in texture retention was also greatly reduced (Figure 8). Several of the varieties tested had pectin sizes much smaller than the B9304 (Figure 7) and, invariably, they retained less of their original firmness (Figure 8). In order to make a rough quantitative comparison of pectin size distributions, the amounts of uronic acid (area under the patterns shown in Figure 7) in large, medium and small-sized polymers for each variety were measured (Table 4). It appears that reduced amounts of large polymers in the CSP fraction correlate with poorer retention of firmness upon heating. As for the B9304 and D samples described above (Figure 6), CSP pectin size decreases upon cooking.

Samples for the comparisons presented in Figure 7 were chromatographed in a different buffer system than that used for the D versus B9304 comparison shown in Figure 6. Because the large number of samples delayed analysis, samples were stored frozen. After several

Table 4. Genotype Effects on Size Distribution of Chelator-Soluble Pectins[a]

variety	A	B	C	A/(B + C)
B9304	67.0	32.6	0.4	2.03
Danvers	59.7	37.2	3.1	1.48
Imperial 58	26.9	60.8	11.6	0.37
Dan D	21.6	56.8	21.5	0.28
CX 6146	19.3	61.6	19.1	0.24
Flakky	27.1	47.7	25.3	0.37

[a] Distributions of CDTA-soluble uronic acids from unheated carrots fractionated into large (A), medium (B), and small (C) pectins by chromatography on Sepharose CL-4B. Refer to Figure 7 for gel filtration profiles. Integration for larger pectins was from fraction 20 to 40, for mid-size pectins was from fraction 40 to 60, and for smaller pectins was from fraction 60 to the end. Values presented are from one analysis per variety and are expressed as a percentage of total recovered uronide. The ratio in the right-hand column is a rough indicator of the distribution of larger pectins in a given sample.

freeze–thaw cycles, gels formed in the samples. These were readily dissolved by diluting gel suspensions into 1 M (final concentration) imidazole–HCl (pH 7.0) buffer. Samples were chromatographed in the same buffer. This had no impact on uronic acid assays but may have contributed to differences seen for the chromatographic comparisons of (different samples of) D and B9304 CSP shown in Figures 6 and 7.

Our work verifies and extends the work of others who have investigated cooking-related loss of carrot texture. We have shown that pectins are solubilized and depolymerized and that at least some of these changes are due to β-elimination reactions. By comparing the performance of different genotypes, we have found a correlation between the sizes of pectic polymers and texture retention. So far we can only speculate about why pectin depolymerization is greater in some varieties than others. [Companion work (Vreeland, personal communication) using a variety of anatomical tools has indicated differences in cell and tissue architecture and cell–cell associations in B9304 and D, and these may hold a key to understanding differences in texture of these carrots both before and after they are heated.] Our study suggests to us that most of the cell wall changes which occur become apparent only after several minutes heating; that is, after tissue temperature has reached 100 °C and much firmness has been lost. While changes in cell wall pectins that are below our threshold of detection may occur early in heating (and contribute to the early, rapid loss of texture), it has become clear that factors other than the cell wall are also important. The impact of heating on the cell turgor component of carrot texture is discussed in a companion paper (Greve et al., 1994).

ABBREVIATIONS USED

CSP, CDTA-soluble pectin; CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid; D, carrot cultivar Danvers; P, phloem; TFA, trifluoroacetic acid; X, xylem.

ACKNOWLEDGMENT

Special thanks to Jay Schafer (Alf Christianson Seed Co., Mt. Vernon, WA) for providing several of the carrot genotypes used in this work.

LITERATURE CITED

Ahmed, A.; Labavitch, J. M. A simplified method for accurate determination of cell wall uronide content. *J. Food. Biochem.* **1977**, *1*, 361–365.

Albersheim, P.; Mühlethaler, K.; Frey-Wyssling, A. Stained pectin as seen in the electron microscope. *J. Biophys. Biochem. Cytol.* **1960a**, *8*, 501–506.

Albersheim, P.; Neukom, H., Deuel, H. Splitting of pectin chain molecules in neutral solution. *Arch. Biochem. Biophys.* **1960b**, *90*, 46–53.

Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. *Carbohydr. Res.* **1967**, *5*, 340–345.

Ben-Shalom, N.; Plat, D.; Levi, A.; Pinto, R. Changes in molecular weight of water-soluble and EDTA-soluble pectin fractions from carrot after heat treatments. *Food Chem.* **1992**, *45*, 243–245.

Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide 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.

Bourne, M. C. Applications of chemical kinetic theory to the rate of thermal softening of vegetable tissue. In *Quality Factors of Fruits and Vegetables, Chemistry and Technology*; Jen, J. J., Ed.; American Chemical Society: Washington, DC, 1989.

Dische, Z. Color relations of carbohydrates. In *Methods In Carbohydrate Chemistry*; Academic Press: New York, 1962; pp 475–514.

Greve, L. C.; Shackel, K. A.; Ahmadi, H.; McArdle, R. N.; Gohlke, J.; Labavitch, J. M. The impact of heating on carrot firmness: the contribution of cellular turgor. *J. Agric. Food Chem.* **1994**, *42*, 2896–2899.

Heil, J. R.; McCarthy, M. J. Influence of acidification on texture of canned carrots. *J. Food Sci.* **1989**, *54*, 1092–1093.

Knox, J. P.; Linstead, P. J.; King, J.; Cooper, C.; Roberts, K. Pectin esterification is spatially regulated both within cell walls and between developing tissues of root apices. *Planta* **1990**, *181*, 512–521.

Lee, C. Y.; Bourne, M. C.; Van Buren, J. P. Effects of blanching treatments on the firmness of carrots. *J. Food Sci.* **1979**, *44*, 615–619.

Penner, M. H.; Kim, S. Nonstarch polysaccharide fractions of raw, processed and cooked carrots. *J. Food Sci.* **1991**, *56*, 1593–1596, 1599.

Plat, D.; Ben-Shalom, N.; Levi, A.; Reid, D.; Goldschmidt, E. Degradation of pectic substances in carrots by heat treatment. *J. Agric. Food Chem.* **1988**, *36*, 362–365.

Plat, D.; Ben-Shalom, N.; Levi, A. Changes in pectic substances in carrots during dehydration with and without blanching. *Food Chem.* **1991**, *39*, 1–12.

Sajjaanantakul, T.; Van Buren, J. P.; Downing, D. L. Effect of methyl ester content on heat degradation of chelator-soluble carrot pectin. *J. Food Sci.* **1989**, *54*, 1272–1277.

Simon, P. W.; Peterson, C. E.; Gabelman, W. H. B493 and B9304, Carrot inbreds for use in breeding, genetics, and tissue culture. *HortScience* **1990**, *25*, 815.

Stevens, B. J. H.; Selvendran, R. R. Structural features of cell-wall polysaccharides of the carrot *Daucus carota*. *Carbohydr. Res.* **1984**, *128*, 321–326.

Van Buren, J. P.; Pitifer, L. A. Retarding vegetable softening by cold alkaline pectin deesterification before cooking. *J. Food Sci.* **1992**, *57*, 1022–1023.

Received for review April 14, 1994. Accepted October 4, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1994.