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Degradation of Pectic Substances in Carrots by Heat Treatment

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Changes in the pectic substances of carrots were studied after heat treatment. Differences in soluble pectin and calcium pectate were observed after ion-exchange chromatographic separation on DEAE (diethylaminoethyl) cellulose. Different "fingerprints" were found in both pectic fractions after heat treatment. The ratio of neutral sugars to uronic acids was almost unchanged in the soluble pectin fraction, but the relative amounts of glucose and rhamnose increased after heat treatment by about 10- and 3-fold, respectively. The ratio of neutral sugars to uronic acids in the calcium pectate increased after heat treatment from 0.11 to 0.27. On the average, all the neutral sugars increased about 3-fold while rhamnose increased about 8-fold. The increase in the relative amount of the rhamnose compared with other sugars in the heated tissue indicates possible degradation of pectins in the "hairy region".

Pectin is a polysaccharide responsible for the texture of fruits and vegetables (Jarvis, 1984). The cohesion of the pectin gel is probably the critical factor in determining fruit texture (Williams and Knee, 1980; Jarvis, 1984). The structure of the cell wall polymers of the carrot root was studied intensively by Stevens and Selvendran (1984). Results of their investigation showed that the preponderant polymers in the cell wall were pectic polysaccharides with associated arabinans and galactans. Changes in the noncellulosic cell wall polysaccharides of the carrot during growth in suspension culture were studied by Asamizu et al. (1983). The polyuronoid polymers, unlike other carbohydrates, are very susceptible to degradation by β elimination upon heating at neutral or weakly acidic pH (Albersheim et al., 1960; Doesberg, 1965). This reaction is catalyzed by several cations and anions (Ben-Shalom et al., 1982). Unsaturated compounds, formed by the trans-elimination reaction, result from the removal of the hydrogen atom at C-5 and of the glycosidic residue at C-4 of the galacturonic acid molecule (Albersheim et al., 1960). Heat-induced degradation by β elimination was found after isolation of cell components in potato (Keijbets et al., 1976) and in cherry (Thibault, 1983). In this study we characterized the changes found in the pectic substances after heating of the carrot tissue (blanching).

MATERIALS AND METHODS

A 10-kg batch (for each treatment) of baby carrot (var. Amsterdam Forcing) that was obtained from the Sunfrost freezing plant in Israel was hand-peeled and divided into two samples, one of which remained untreated while the other was steam heated (blanching) for 4 min, the time found necessary to inactivate the pectin esterase (PE). Alcohol-insoluble solids (AIS) were prepared from the untreated and the blanched tissue by repeated extractions with 70% and 96% alcohol. Soluble pectin was prepared by sequential extraction of the AIS with water at room temperature until no galcturonic acid appeared in the extract. Calcium pectate was extracted from the washed pellet of the soluble pectin with 0.2% EDTA and Tris-HCl (0.02 M, pH 6.2), dialyzed against water, and freeze-dried.

The soluble pectin and calcium pectate (20 mg of galacturonic acid) were solubilized, dialyzed with sodium phosphate buffer (1 mM, pH 6.2), and applied to a column of DEAE-cellulose (Whatman) (1.6 × 20 cm), which had been equilibrated previously with the same buffer. Elution was done initially with 1 mM sodium phosphate (150 mL) and then with the same buffer, in a linear gradient of 0–0.8 M (300 mL). Fractions (3–4 mL) were collected and monitored for galacturonic acid by the *m*-hydroxyphenol method (Blumenkrantz and Asboe-Hansen, 1973) and for total carbohydrate by reaction with phenol–sulfuric acid (Dubois et al., 1956). Total neutral sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose standards. Appropriate fractions eluted from the column were combined, dialyzed, and freeze-dried. The composition and the amount of individual neutral sugars were obtained by hydrolysis in trifluoroacetic acid. The respective alditol acetates were analyzed by gas chromatography as described by Albersheim et al. (1967). Methanol derived after demethylation was converted to methyl nitrite and determined by gas chromatography according to the method of Litchman and Upton (1972), as modified by Versteeg (1979). Molecular weight of the pectic fractions was determined by viscometric measurements according to Christensen (1954).

RESULTS AND DISCUSSION

The chromatogram of soluble pectin and calcium pectate (Figures 1 and 2) on the DEAE column showed three main fractions: nonabsorbed material, which was washed with

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Table I. Content of Total Galacturonic Acids and Neutral Sugars after Separation on a DEAE Column in the Soluble Pectin Fraction of Untreated and Heated Carrot Tissue (Sugar Content Expressed in Micrograms and as a Percent of Total Pectic Substances)

	sugar content											
		B_1		B_2		B_3		B_4		C		total sugars
treatment	A: μg	μg	%	μg	%	μg	%	μg	%	μg	%	μg	%
						Untreated Tissue							
neutral sugars	170	8929	28.7	403	1.3	531	1.7	95	0.3	35	0.1	10173	32.7
galacturonic acids	108	1701	5.5	1008	3.2	8435	27.1	9549	30.7	250	0.8	20943	67.3
pectic substances		10630	34.2	1411	4.5	8966	28.8	9647	31.0	285	0.9	31116	100.0
						Blanched Tissue							
neutral sugars	294	7980	25.3							3250	10.3	11230	35.6
galacturonic acids		18360	58.2							1980	6.3	20340	64.4
pectic substances		26340	83.5							5230	16.6	31570	100.0

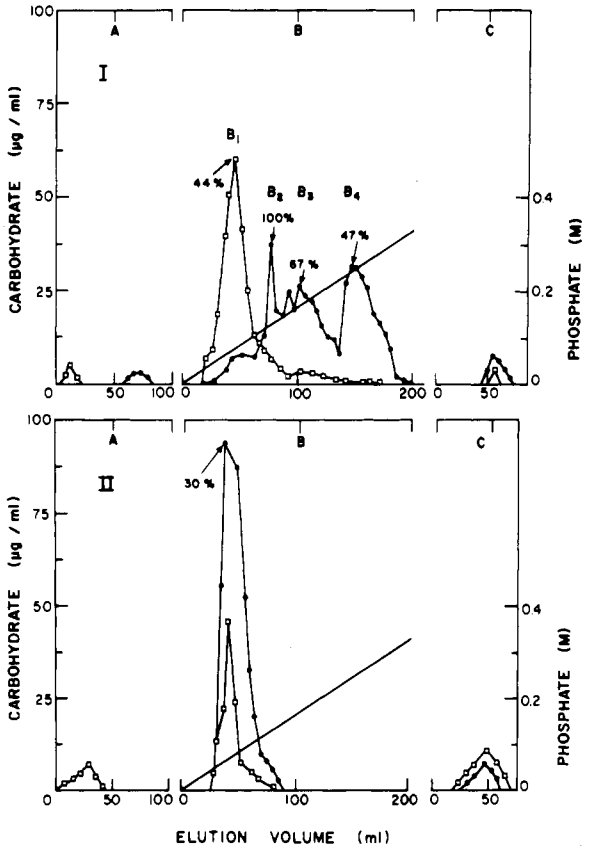

Figure 1. Separation of soluble pectin on DEAE-cellulose. The chromatograms of untreated carrot tissue (I) and of heated tissue (II) were obtained by following the content of uronic acids (●) and of total sugars (○). Three main fractions were shown in each chromatogram: (A) nonabsorbed material, which was washed with 1 mM phosphate buffer, pH 6.2; (B) absorbed material, which was eluted with a linear gradient of sodium phosphate; (C) residual pectin (still bound to the column), which was eluted with 0.05 M NaOH. The percent given in the figure is the degree of esterification.

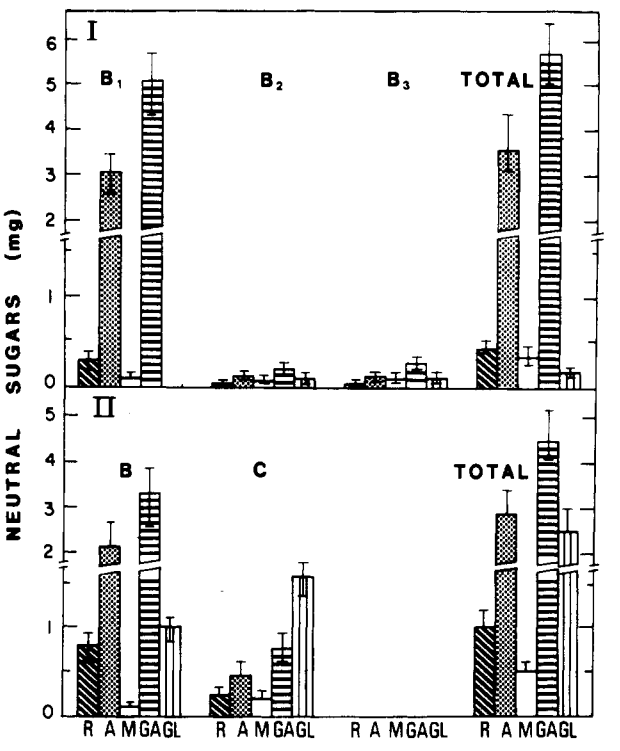

Figure 2. Composition of neutral sugars in the soluble pectin fraction of untreated carrot tissue (I) and of heated tissue (II), after their separation on DEAE-cellulose column. Key: R, rhamnose; A, arabinose; M, mannose; GA, galactose; GL, glucose.

1 mM phosphate buffer, pH 6.2 (A); absorbed material, which was eluted with a linear gradient of sodium phosphate (B); residual pectin (still bound to the column), which was eluted with 0.05 M NaOH (C).

Almost all of the soluble pectin fraction of the nontreated carrot was absorbed on the DEAE (Figure 1I) and eluted with the phosphate buffer with an ionic strength between 0.04 and 0.3 M. Fractions A and C were negligible. Four different peaks (Figure 1, B_{1-4}) were separated by the phosphate gradient. They differed according to the ratio between the uronic acids and the total neutral sugars, their degree of esterification (DE), and their neutral sugars composition. The ratio betwen the galacturonic acid and the neutral sugars (Table I) in B_1 is 0.2; in B_2, 2.5; in B_3, 15.9; and in B_4, 102.3. It can be seen from these ratios that as the ionic strength of the phosphate buffer increased, the eluted pectin contained less and less neutral sugars. The soluble pectin of the heated carrot (Figure 1II) has shown a chromatogram with a completely different pattern as compared with that of the untreated carrot. Peak B_1 of the untreated carrot was eluted at almost the same ionic strength than that of the nonheated carrot. The ratio of total neutral sugars to uronic acids in the soluble pectin was almost unchanged after heating the tissue (Table I), while changes were observed in the individual fraction. The ratio in peak B_1 between the uronic acid and neutral sugars was 0.2 (Table I), compared with 2.3 in the heated one (B). The DE of peak B_1 was 44%, and that of peak B (heated tissue), 30%. None of the other peaks of fraction B (B_{2-4}) of the nontreated tissue were similar to fraction B of the heated tissue in terms of the ratio between uronic acid and neutral sugars and their DE values.

The composition of the neutral sugars in the peaks of fraction B of the soluble pectin (Figure 2I) showed that each peak is different. The most dominant sugars in these peaks were galactose and arabinose; glucose was absent in

Table II. Content of Total Galacturonic Acids and Neutral Sugars after Separation on a DEAE Column in the Calcium Pectate Fraction of Untreated and Heated Carrot Tissue (Sugar Content Expressed in Micrograms and as a Percent of Total Pectic Substances)

	sugar content							
	A		B		C		total sugars	
treatment	μg	%	μg	%	μg	%	μg	%
			Untreated Tissue					
neutral sugars	680	3.1	1260	5.8	180	0.8	2120	9.7
galactouronic acids	761	3.5	18720	85.6	262	1.2	19743	90.3
pectic substances	1441	6.6	19980	91.4	442	2.0	21863	100.0
			Blanched Tissue					
neutral sugars	1796	6.8	3915	14.8	24	0.1	5735	21.7
galacturonic acids	5184	19.6	14616	55.2	954	3.6	20754	78.3
pectic substances	6980	26.4	18531	70.0	978	3.7	26489	100.0

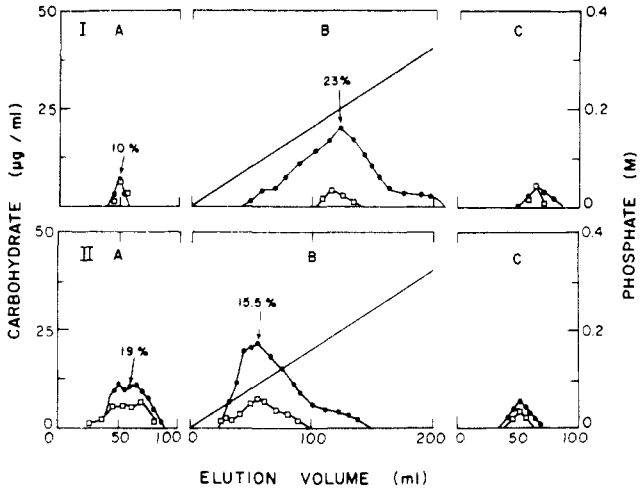

Figure 3. Separation of calcium pectate on DEAE-cellulose. The chromatograms of untreated carrot tissue (I) and of heated tissue (II) were obtained by following the content of uronic acids (●) and of neutral sugars (○). Three main fractions were shown in each chromatogram: (A) nonabsorbed material, which was washed with 1 mM phosphate buffer, pH 6.2; (B) absorbed material, which was eluted with a linear gradient of sodium phosphate; (C) residual pectin (still bound to the column), which was eluted with 0.05 M NaOH. The percent given in the figure is the degree of esterification.

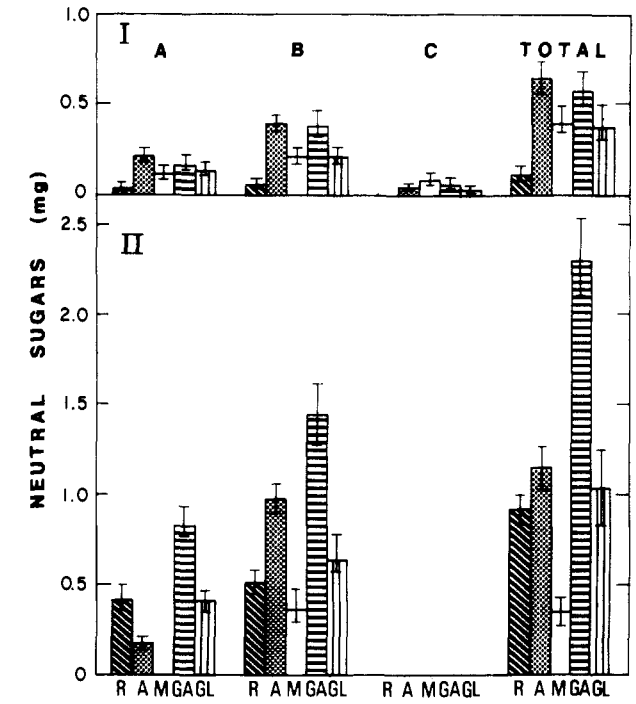

Figure 4. Composition of neutral sugars in the calcium pectate fraction of unheated carrot tissue (I) and of heated tissue (II), after their separation on a DEAE-cellulose column. Key: R, rhamnose; A, arabinose; M, mannose; GA, galactose; GL, glucose.

peak B_1 (Figure 2). The ratio between galactose and arabinose to rhamnose decreased from peak B_1 to peak B_3. These phenomena correlate with the decrease in the ratio between the neutral sugars and the galacturonic acid in these peaks (Table I). This is due to the fact that rhamnose is mainly a part of the backbone of the polygalcturonic acid chain, and not a part of the side chains of neutral sugars.

The composition of the neutral sugars of the soluble pectin in the heated tissue and their ratio are shown in Figure 2 II. They are completely different from the control. The amount of glucose and rhamnose increased about 10 and 3 times, respectively, in the heated carrot. The ratio of rhamnose to other neutral sugars increased significantly in fractions B and C (Figure 2II), as the relative amount of galactose and arabinose decreased. Separating the calcium pectate fraction of the nontreated carrot on DEAE-cellulose (Figure 3I) showed a chromatogram in which about 95% of the calcium pectate is in fraction B. This fraction was eluted in a broad peak with the phosphate gradient buffer of 0.07 and 0.32 M The calcium pectate fraction is characterized by a small amount of neutral sugars (uronic acids to neutral sugar ratio 9.3:1; Table II) and low DE in the fraction (23%; Figure 3). The main difference between fractions A and B is the ratio of uronic acid to neutral sugars, which is 1.1 in A and 14.8 in B (Table II). The relatively high sugar composition of A may be the reason that this fraction was not absorbed onto the column, even though it has a low DE (Figure 3I). From the chromatogram of the calcium pectate fraction on the DEAE and its sugar analysis, it is obvious that it is a separate fraction completely different from the soluble pectin.

Heating the carrot resulted in the formation of a different calcium pectate fraction (Figure 3II). A significant increase in the amount of neutral sugars appeared in the heated fraction. The ratio of uronic acids to neutral sugars changed from 9.3:1 (in the untreated tissue) to 3.6:1 (in the heated tissue) (Table III). The main changes were found in fraction B, in which the neutral sugars increased by about 2.5-fold. In the neutral sugars, the rhamnose ratio increased most markedly (8 times), while the other sugars increased approximately 3-fold (Figures 3II and 4).

The increase in the neutral sugars in fraction A was accompanied by a large increase in the level of uronic acid. Again, the ratio to rhamnose increased most significantly, followed by that of galactose and glucose. The heat treatment did not have a big effect on the DE of the calcium pectate. The DE in fraction A was 19%, and in fraction B, 15.5% (Figure 3II). The low DE in the calcium

Table III. Molecular Weight Determination of the Pectic Substances by Viscometric Measurements before and after Heat Treatment

treatment	soluble pectin	calcium pectate
untreated tissue	50 851	117 021
heat-treated tissue	53 191	106 382

pectate of the heated carrot emphasizes the fact that calcium interacts mainly with low esterified pectic substances. Eluting the calcium pectate with NaOH (fraction C) resulted in a fraction that had a minor amount of neutral sugars and only a little more uronic acid (as compared with the nonheated tissue).

From these data it is obvious that after heat was applied to the carrot tissue, many changes occur in the pectic substances. The soluble pectin and the calcium pectate found in the heated tissue are not similar to the original pectic fraction in the unheated tissue. The question remains as to what mechanism breaks down the pectic substances. Bearing in mind that we are dealing with a tissue with a pH around 6.3, the only mechanism that was proposed in the literature at this pH is the β elimination. In looking for this mechanism, we follow the primary effect of the β elimination hydrolysis: the increase in the unsaturated groups. In spite of all our efforts we could not detect any difference betweeen the untreated and the heated tissue. One of the possible explanations is that most of the breakdown products were extracted during preparation of the AIS. Another possibility to follow the hydrolysis product is to look for the change in the size of the polyuronides. When their molecular weights were determined by viscometric measurements (Table III), there was not much difference before and after the heat treatment, although a significant difference was found between the soluble and the calcium pectate fractions.

One of the main significant observations in this work was the specific increase in the amount of rhamnose and in all the other neutral sugars in general. This phenomenon may be due to an unknown mechanism hydrolizing the glycoside bonds in the neutral sugars. However, if this is the case, the question of why rhamnose increases preferentially arises. Another possibility is that the hydrolysis was in the polyuronide polymers, near a region rich in rhamnose and other neutral sugars. From the new model of the pectic substances (Jarvis, 1984) being accepted, the "hairy region" of the polymer is rich in rhamnose residues inserted in the pectin, side chains of neutral sugars, and uronides with high esterified groups (De Vires et al., 1981).

The absence of unsaturation and lack of change in viscosity after heating is compelling evidence that β elimination is not a significant factor here. β elimination must be accompanied by a decrease in the size of pectin backbone. Since the viscosity of pectin is almost totally dependent on the length of the backbone, it follows that brief heating of carrots must affect primarily the neutral sugar in the "hairy" region.

The changes in the pectic substances with heating seem to be a complicated process. The approach in this paper was to try to follow some of the changes in the pectic substances within the carrot tissue.

Registry No. Pectin, 9000-69-5; Ca pectate, 12672-40-1; glucose, 50-99-7; rhamnose, 3615-41-6.

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