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COMPOSITION OF ETHANOL-INSOLUBLE POLYSACCHARIDES IN WATER EXTRACTS OF RIPENING TOMATOES

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; fruit softening; cell wall; carbohydrates; galactose; β-galactosidase.

Abstract—The carbohydrate composition of the 80% ethanol-insoluble polysaccharides (EIP) from water extracts of 'Rutgers,' *rin* (ripening inhibitor) and *nor* (non-ripening) tomatoes has been determined. The amount of EIP extracted from 'Rutgers' fruit increased from 0.34 to 0.61 mg/g fr. wt during ripening; little change occurred in *rin* or *nor* fruit. The carbohydrate composition (μg/g fr. wt) of EIP from mature green fruit was: galacturonic acid (48); rhamnose (3); arabinose (20); xylose (48); mannose (31); glucose (139); galactose (51). The most obvious changes that accompanied ripening were a 7.4-fold and 4-fold increase in galacturonic acid and rhamnose content, respectively. These changes were attenuated in the ripening mutants. EIP was fractionated into three major peaks by using DEAE-cellulose ion exchange chromatography. The first peak, which was not retained by the column, contained predominantly glucose and mannose, with lower amounts of galacturonic acid and galactose. The two retained peaks which eluted at 0.1 and 0.2 M sodium chloride contained primarily galacturonic acid, xylose, galactose and arabinose. The galacturonic acid content of these two fractions increased substantially during ripening, whereas the other components decreased. No changes were evident in the ripening mutants. No increase in water-soluble polysaccharides high in galactose content was observed during ripening.

INTRODUCTION

An increase in polygalacturonase (EC 3.2.1.15) [1] and soluble polyuronide [2, 3], as well as a decrease in cell wall galacturonosyl residues [4, 5] occur during ripening of tomato fruit. Polygalacturonase has been implicated in tissue softening through its ability to hydrolyse α-1,4-galacturonosyl linkages found in the wall of tomato fruit [4, 6–9].

In addition to the solubilization of wall uronides, a net loss of galactosyl and arabinosyl residues from cell walls occurs during the ripening of tomatoes [4, 5, 10] as well as many other fruit types [11, 12]. However, the significance of the loss of wall galactose and arabinose is unclear.

Decreases in cell wall galactosyl residues may result from reduced synthesis and/or structural modification during *de novo* galactan synthesis [9, 12–14]. An isozyme of β-galactosidase (EC 3.2.1.23) has been found in tomatoes that is capable of hydrolysing terminal β-1,4-galactosyl linkages from tomato cell wall β-1,4-galactan [15, 16]. The increase in free, monomeric galactose that occurs in tomato fruit during ripening [17, 18] may be due to the action of this enzyme. However, it is not clear if wall galactosyl residues are solubilized by other mechanisms as well.

The aim of this study was to characterize the water-soluble polysaccharides (ethanol-insoluble; *ca* DP > 10) released from the walls of tomato fruit during ripening in an effort to clarify the mechanism(s) involved in the loss of cell wall galactosyl residues.

RESULTS AND DISCUSSION

The amount of material in water-extracts of 'Rutgers,' *rin* and *nor* tomato pericarp tissue that was insoluble in 80% ethanol, ranged from 0.36 to 0.80 mg/g fr. wt (Table 1). From 76 to 82% of this material was carbohydrate and was designated ethanol-insoluble polysaccharides (EIP). The EIP represents water-soluble polysaccharides with a degree of polymerization of *ca* 10 or greater. The amount of EIP increased 79% in 'Rutgers' tomato fruit from the mature green to the red ripe stage, whereas little increase occurred in *rin* and *nor* fruit at a similar chronological age (Table 1), suggesting that the changes were ripening-related.

Carbohydrate composition studies showed that the amount of uronic acid in EIP increased 7.4-fold during ripening of 'Rutgers' tomato fruit (Table 2). The increase in uronic acid content was accompanied by a 4-fold increase in the content of rhamnose, an integral component of pectic polysaccharides in cell walls that links galactose- and arabinose-containing side chains to the main galacturonic acid backbone in many dicots [19]. The galactose and arabinose content of EIP increased 20 and 85%, respectively, during ripening. These are relatively insignificant increases compared to those of galacturonic acid and rhamnose, supporting the suggestion that the loss of galactosyl residues from the wall during ripening [5] is not directly related to polygalacturonase activity [4, 10], as is the case with arabinose in 'Bartlett' pears where the loss of pear wall arabinosyl residues is due to the solubilization of a pectic arabinan by polygalacturonase [20]. The increase in galacturonic acid content of EIP occurred, but to a lesser extent in *rin* and *nor* fruit at similar chronological ages (Table 2). The uronic acid content of EIP in *rin* and *nor* fruit increased 1.6- and 1.8-fold, respectively. No significant increase in other components was evident.

In order to allow for a more specific characterization,

Table 1. Amount of ethanol-insoluble polysaccharide extracted from 'Rutgers,' *rin* and *nor* tomato fruit at two stages of ripeness

Fruit	Stage*	H_2O-soluble, EtOH-insoluble material (mg/g fr. wt)	Total carbohydrate content (%)	Ethanol-insoluble polysaccharide (mg/g fr. wt)
'Rutgers'	MG	0.45	76	0.34
	RR	0.80	76	0.61
Rin	I	0.50	77	0.39
	II	0.60	80	0.48
Nor	I	0.36	81	0.29
	II	0.40	82	0.33

*Stages of ripeness are described in the Experimental.

Table 2. Galacturonic acid and neutral sugar composition of ethanol-insoluble polysaccharides (EIP) from 'Rutgers,' *rin* and *nor* tomato fruit at two stages of ripeness

		Amount [μg/g fr. wt (% EIP)]						
Fruit	Stage*	Galur	Rha	Ara	Xyl	Man	Glc	Gal
'Rutgers'	MG	48	3	20	48	31	139	51
		(14)	(1)	(6)	(14)	(9)	(41)	(15)
	RR	354	12	37	37	12	98	61
		(58)	(2)	(6)	(6)	(2)	(16)	(10)
Rin	I	98	8	20	51	39	109	66
		(25)	(2)	(5)	(13)	(10)	(28)	(17)
	II	158	5	19	53	48	130	67
		(33)	(1)	(4)	(11)	(10)	(27)	(14)
Nor	I	67	6	20	55	17	75	49
		(23)	(2)	(7)	(19)	(6)	(26)	(17)
	II	122	3	23	43	26	59	53
		(37)	(1)	(7)	(13)	(8)	(18)	(16)

*Stages of ripeness are described in the Experimental.

EIP was fractionated by DEAE-cellulose ion exchange chromatography, and the fractions assayed colorimetrically for uronic acid and hexose. Elution profiles of EIP from mature green and red ripe 'Rutgers' fruit are depicted in Fig. 1. A major peak that contained primarily hexose, with smaller amounts of uronic acid, was not retained by the column and eluted during loading and washing (peak A). No apparent changes in the amount or hexose:uronic acid ratio of this carbohydrate peak were evident during ripening. However, striking changes in this ratio occurred with two peaks that eluted at *ca* 0.1 (peak B) and 0.2 M sodium chloride (peak C). The galacturonic acid content increased dramatically during ripening (Fig. 1). This increase did not occur in *rin* (Fig. 2) or *nor* fruit (data not shown), the chromatography profiles of which were similar.

For each of the three peaks(A–C), fractions containing the majority of carbohydrate were pooled and lyophilized (Figs 1 and 2). The carbohydate composition of the three fractions (A–C) from 'Rutgers,' *rin*, and *nor* fruit EIP was then determined (Table 3).

Fraction A contained primarily glucose and mannose, and to a lesser extent, galactose and galacturonic acid. The relative changes in composition that accompanied ripening of 'Rutgers' fruit were an increase in galacturonic acid and glucose content and a decrease in galactose and mannose. No substantial changes in composition occurred in EIP from *rin* and *nor* fruit except for slight increases in glucose content.

The amount of galacturonic acid in fraction B increased 3-fold during 'Rutgers' fruit ripening (Table 3). The relative amounts of arabinose, xylose and galactose decreased, reflecting the large relative increase in galacturonic acid while the total carbohydrate amount changed little. No changes in carbohydrate composition of fraction B occurred in *rin* and *nor* fruit EIP.

Fraction C contained primarily galacturonic acid with lower amounts of rhamnose, arabinose, xylose, glucose and galactose (Table 3). The relative amount of galacturonic acid increased from 50 to 81% during 'Rutgers' fruit ripening, whereas the content of all other components decreased. An 18% increase in the relative content of galacturonic acid occurred in *rin* fruit, but no change occurred in *nor* fruit EIP. No other changes in the composition of fraction C were noted for *rin* or *nor* fruit.

The results of this study confirm previous reports [2, 3] that soluble polyuronide content increases in ripening tomatoes and further characterizes the carbohydrate

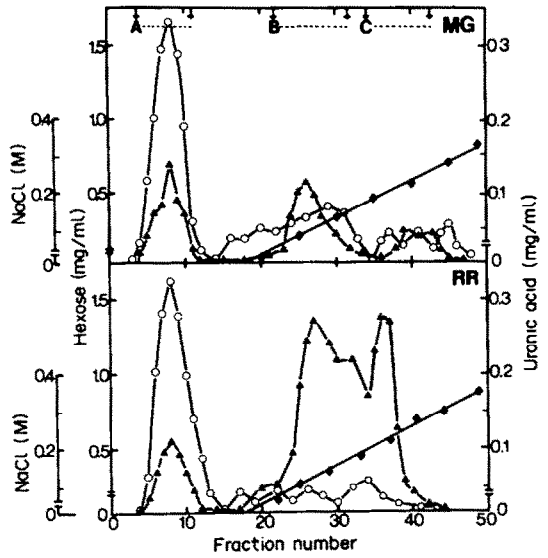

Fig. 1. DEAE-cellulose column chromatography of ethanol-insoluble polysaccharides from water-extracts of mature green (MG) and red ripe (RR) 'Rutgers' tomato fruit. Arrows and dotted lines at the top of the graph represent those fractions which were pooled for subsequent carbohydrate composition analysis. ○——○, Hexose; ▲——▲, uronic acid; ◆——◆, NaCl.

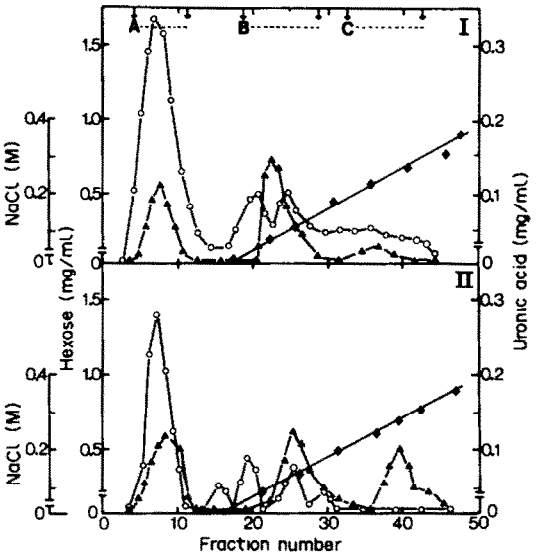

Fig. 2. DEAE-cellulose column chromatography of ethanol-insoluble polysaccharides from water-extracts of Stage I (I) and Stage II (II) *rin* tomato fruit. Stages I and II are analagous to mature green and red ripe 'Rutgers' fruit, respectively, in terms of chronological age. Arrows and dotted lines at the top of the graph represent those fractions which were pooled for subsequent carbohydrate composition analysis. ○——○, Hexose; ▲——▲, uronic acid; ◆——◆, NaCl.

composition of the polyuronide, which is presumably solubilized from the wall by polygalacturonase action. It has been suggested that polygalacturonase or a type of galactanase (*endo*-type action) may solubilize polysaccharides high in galactose content from the cell wall of tomato fruit during ripening and that these solubilized polysaccharides are released as very large polymers [15, 21]. However, the results of this study do not support this contention since no galactose-containing, soluble polysaccharide fractions were found which increased in amount during ripening. Thus, there is no evidence to suggest that polygalacturonase, a galactanase, or mechanism other than β-galactosidase (*exo*-type action) is directly involved in the removal of galactosyl moieties from

Table 3. Carbohydrate composition of the pooled fractions A, B and C from DEAE-cellulose chromatography of EIP

Column fraction	Fruit stage*	Total carbohydrate (mg)	% Total carbohydrate						
			Uronic acid	Rha	Ara	Xyl	Man	Glc	Gal
A	MG	3.88	7	ND†	2	4	23	54	10
	RR	2.51	11	ND	ND	ND	9	76	4
	Rin I	4.62	12	ND	3	8	20	46	11
	Rin II	4.24	8	ND	ND	5	21	56	9
	Nor I	3.50	5	ND	3	7	27	46	12
	Nor II	4.38	8	ND	ND	5	26	51	10
B	MG	3.28	22	ND	12	35	ND	ND	31
	RR	4.29	69	ND	8	9	ND	ND	14
	Rin I	4.35	15	ND	14	40	ND	ND	31
	Rin II	3.03	17	ND	15	39	ND	ND	29
	Nor I	4.21	18	ND	14	36	ND	ND	32
	Nor II	3.23	18	ND	15	36	ND	ND	31
C	MG	1.09	50	7	12	13	ND	10	8
	RR	3.10	81	5	4	4	ND	2	4
	Rin I	0.53	56	5	7	16	ND	7	9
	Rin II	1.23	66	4	6	9	ND	6	9
	Nor I	0.97	67	4	5	10	ND	8	6
	Nor II	0.82	67	4	6	9	ND	8	6

*Stages of ripeness are described in the Experimental.
†ND, None detected.

tomato cell walls during ripening, as has been suggested [4, 15, 16, 21]. This contention is supported by: (1) the increase in free monomeric galactose in ripening tomatoes [17]; (2) the loss of galactosyl residues from *rin* fruit cell walls in the absence of detectable polygalacturonase or polyuronide solubilization [4, 5, Fig. 2]; (3) the increase in activity of β-galactosidase II during tomato fruit ripening and the enzymes' ability to hydrolyze terminal galactosyl residues from tomato cell wall polysaccharides *in vitro* [16]; and (4) the absence of a ripening-related increase in soluble polysaccharides which have a high galactose content (Table 3). However, the possibility remains that 80% ethanol-soluble galactosyl oligosaccharides could be specifically cleaved from cell wall polymers by the action of a galactanase, but would not be detected in this study because of their ethanol-solubility. Studies to clarify this possibility are in progress.

EXPERIMENTAL

Plant material. Tomato (*Lycopersicon esculentum* Mill.) plants were grown in a greenhouse using standard cultural practices. Flowers were hand-pollinated and tagged at anthesis. Normal-ripening fruit (cv. 'Rutgers') were harvested and sorted into two stages of ripeness based on coloration and days after pollination: mature green, 30 days post-pollination (MG) and red ripe, 55–60 days post-pollination (RR). *Rin* (ripening inhibitor; isogenic to 'Rutgers') and *nor* (non-ripening, isogenic to 'Rutgers') fruit were sorted into stages based on the number of days after pollination as follows: 30–32 days post-pollination (Stage I) and 58–60 days post-pollination (Stage II). To ensure uniformity of development, only two fruit were allowed to develop on each cluster.

Extraction of EIP. Fruit were peeled and the outer pericarp tissue excised. One hundred g fr. wt was homogenized in 100 ml of distilled H_2O at 23° using a Polytron homogenizer and the homogenate filtered through Miracloth. After washing the residue with 50 ml of distilled H_2O, the filtrate and wash were combined and adjusted to 80% EtOH by adding 95% EtOH. The suspension was allowed to stand for 18–24 hr at 4°, and the ppt. was collected by centrifugation at 9000 *g* for 15 min. The pellet (EIP) was dissolved in distilled H_2O, frozen with liquid N_2 and lyophilized. The EIP was stored *in vacuo* over P_2O_5 at 23°.

Ion exchange chromatography. For fractionation of EIP, a DEAE-cellulose column (Whatman DE 52; 10 ml bed vol.) was equilibrated in 5 mM MES–NaOH at pH 6.0. A 25 ml sample of EIP (1 mg/ml) in buffer was loaded at 0.6 ml/min at 23°. After washing with 15 ml of buffer, the retained EIP was eluted with a 60 ml linear gradient of NaCl (0–0.4 M) in 5 mM buffer. Fractions (2.5 ml) were collected beginning at the start of loading and were assayed for uronic acid and hexose using carbazole [22] and anthrone [23], respectively.

Carbohydrate analysis. Neutral sugar composition of EIP was determined using capillary GC/MS. Polysaccharides were hydrolysed with 2 N TFA as described in ref. [24]. The resulting neutral monosaccharides were then made into their aldononitrile acetate derivatives using the procedure of ref. [25] and separated on a cross-linked, 25 m WCOT (0.2 mm i.d.) 5% phenylmethylsilicone column. Helium was used as carrier gas at a linear flow of 1 ml/min. The oven temp. was programmed at 140° for 4 min, then increased to 160° at 2°/min. After 1 min, the temp. was increased at 5°/min to 190° and maintained for 4 min. Other GC conditions were as follows: injection port temp., 225°; sample size, 1 μl; split ratio, 1:50; GC/MS interface temp., 220°. Quantification of sugar derivatives using GC/MS (electron impact) was performed using selected ion monitoring (*m/z* 145) at 70 eV; allose was used as the internal standard.

Uronic acid content of EIP samples was determined by dissolving material in conc H_2SO_4 [26] and assaying aliquots for uronic acids using carbazole [22]; galacturonic acid was the standard.

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