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CHANGES IN CELL WALL NEUTRAL SUGAR COMPOSITION DURING FRUIT RIPENING: A SPECIES SURVEY*

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Key Word Index—Galactose, arabinose, xylose, fruit softening, senescence, cell wall turnover

Abstract—Non-cellulosic neutral sugar composition of cell walls from seventeen fruit types were analysed during ripening. Galactose was the major non-cellulosic neutral sugar in cell walls of cucurbit and solanaceous fruit, xylose was the predominant non-cellulosic neutral component of berries, and arabinose was the major non-cellulosic component of pome fruits. The major non-cellulosic neutral sugar residue in cell walls of stone fruits varied In nectarine and peach, plum, and apricot, the major sugar was arabinose, galactose, and xylose, respectively In 15 of the 17 types of fruit, a net loss of non-cellulosic neutral sugar residues occurred during ripening No net loss occurred in plums and cucumbers A net loss of cell wall galactose and/or arabinose occurred in 14 of the types of fruit Xylose was the major neutral sugar residue lost from walls of apricot during ripening. In general, berry cell walls were comparatively low in galactose and arabinose content

INTRODUCTION

A substantial loss of firmness occurs in many fruits during ripening. The softening that occurs is primarily due to a change in cell wall carbohydrate metabolism, resulting in a net decrease in certain structural components [1, 2] In many fruit, the most apparent and intensively studied change in cell wall composition is the loss of uronic acid polymers which is accompanied by an increase in soluble polyuronide [2, 3] Polygalacturonase (EC 3 2 1 15), a hydrolytic enzyme that cleaves 1,4-galacturonosyl linkages, has been strongly implicated in the softening process [4–8]. In addition to the solubilization of pectin, a net loss of non-cellulosic neutral sugar residues also occurs during the ripening of pears [9, 10], apples [11], strawberries [12] and tomatoes [13, 14] Recent studies of relatively few species, suggest that the net loss of neutral sugar residues from cell walls during ripening is due to altered turnover rates and seems to be restricted to galactose and/or arabinose containing polymers However, unlike changes in cell wall polyuronide, the neutral sugar composition of fruits has only been studied in a limited number of species The objective of this study

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Table 1 The type of fruit and tissue used for cell wall non-cellulosic neutral sugar analysis

Genus/species	Common name	Cultivar/clone	Tissue type
Lycopersicon esculentum Mill	Tomato	'Rutgers'	Mesocarp
Capsicum annuum L	Bell Pepper	'Midway'	Mesocarp
Capsicum annuum L	Hot Pepper	Cayenne	Mesocarp
Prunus persica (L) Batsch	Nectarine	NY-2603	Mesocarp
Prunus persica (L) Batsch	Peach	'Loring'	Mesocarp
Prunus domestica L	Plum	'Demontford'	Mesocarp
Prunus armeniaca L	Apricot	'Goldcot'	Mesocarp
Malus domestica Borkh	Apple	'Golden Delicious'	Cortex
Pyrus pyrifolia (Burm f) Nakai	Pear	'El Dorado'	Cortex
Cucumis sativus L	Cucumber	'National Pickling'	Mesocarp
Cucumis melo L	Muskmelon	'Honeyrock'	Mesocarp
Cucumis melo L	Melon	'Golden Crispy'	Mesocarp
Cucurbita maxima Duch	Squash	'Early Prolific'	Mesocarp
Vaccinium corymbosum L	Blueberry	G-132	Pericarp
Rubus idaeus L	Red Raspberry	'Chilcotin'	Mesocarp
Rubus spp	Blackberry	'Dirkson Thornless'	Mesocarp
Fragaria × *ananassa* Duch	Strawberry	'Earliglow'	Receptacle

Table 2 Parameters used for determining stage of ripeness

Fruit	Stage I	Stage II	Stage III
Tomato Bell pepper Hot pepper	Mature/green	Turning	Red/ripe
Apple Pear	Pre-climateric	Climateric	Post-climateric
Cucumber Melon Muskmelon	Immature/green, seeds undeveloped	Mature/turning, seeds developed	Mature/ripe, yellow
Yellow squash	Immature/light yellow	Mature/Yellow	Overripe
Nectarine Peach Plum Apricot	Immature/green, pit hardening nearly complete	Mature/turning, pit hardening complete	Ripe/soft, flesh yellow
Blueberry	Green	Green/blue, turning	Dark blue/ripe
Blackberry	Green/firm	Red	Black/soft
Strawberry Red raspberry	White/firm	Pink	Red/ripe, soft

was to characterize and compare changes in cell wall neutral sugar composition of fruit from various species during ripening

RESULTS AND DISCUSSION

Tomato, bell pepper and hot pepper fruit lost 39%, 42% and 56%, respectively, of their total cell wall neutral sugar during ripening (Table 3) Galactose was the major neutral monosaccharide present in unripe fruit accounting for 14%, 11% and 14% of the walls of tomato, bell pepper and hot pepper, respectively The net loss of neutral sugar residues during ripening involved primarily galactose and arabinose containing polymers Although the content of non-cellulosic glucose declined during ripening, this may be due to the presence of small, undetectable amounts of starch in wall preparations of unripe fruit rather than representing a true decrease in wall glucose residues Previous studies with tomato did not indicate a loss of glucose residues from cell walls during ripening [14]

Cell wall composition of stone fruits varied substantially (Table 4) Arabinose was the major neutral sugar in walls of nectarines and peaches, although these fruits also contained high levels of galactose In comparison, xylose and galactose were the predominant neutral monosaccharides in walls of apricots and plums, respectively A substantial net loss of wall galactose and arabinose residues occurred in nectarine and peach fruit during ripening, while the cell wall neutral sugar composition of plums remained relatively constant There was a 30% decrease in the content of xylose and glucose in walls of apricots during ripening.

Similar to previous studies [11, 15], apple and pear cell walls contained relatively high levels of arabinose and xylose, galactose was also a major wall component of apples but not pears (Table 5) The net loss of non-cellulosic neutral sugar residues from cell walls during

Table 3 Changes in non-cellulosic neutral sugar composition of cell walls from various solanaceous fruits

		Non-cellulosic neutral sugar (mg/100 mg wall)†							
Fruit	Stage*	Total	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
Tomato	I	28 3	1 2	ND‡	4 0	3 2	1 7	4 5	13 9
	II	24 9	1 3	ND	4 0	3 2	1 7	4 6	10 3
	III	17 2	1 2	ND	3 0	4 3	2 4	2 2	4 0
Bell pepper	I	24 6	0 7	Tr‡	3 8	4 3	1 2	3 7	10 9
	II	18 5	0 9	Tr	3 5	4 1	1 2	3 2	5 6
	III	14 3	1 0	Tr	2 9	4 2	1 1	1 6	3 5
Hot pepper	I	29 7	1 1	ND	4 8	3 4	1 1	5 6	13 8
	II	20 6	1 0	ND	3 5	4 5	1 3	5 5	4 7
	III	13 0	0 6	ND	1 9	4 7	1 1	1 8	2 9

*Stages are described in Table 2

†Data represent the mean of three analyses, triplicate injections were made of each sample

‡ND, none detected, Tr, trace (less than 0 1 mg/100 mg wall)

Table 4 Changes in non-cellulosic neutral sugar composition of cell walls from various stone fruits

Fruit	Stage*	Non-cellulosic neutral sugar (mg/100 mg wall)†							
		Total	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
Nectarine	I	29 4	0 9	Tr‡	11 9	4 7	0 9	1 2	9 8
	II	14 4	0 3	0 4	3 5	5 5	0 5	0 9	3 3
	III	17 9	0 4	0 6	4 4	5 8	0 9	1 4	4 4
Peach	I	34 3	1 2	Tr	15 4	4 4	0 9	1 1	11 3
	II	25 8	0 7	0 7	9 1	5 6	1 0	1 5	7 2
	III	24 6	0 6	0 9	8 3	6 4	0 9	1 5	6 0
Plum	I	29 3	0 9	Tr	10 5	3 4	0 4	1 0	13 1
	II	32 2	1 0	0 3	10 5	3 5	0 8	0 9	15 2
	III	29 9	0 9	0 3	10 2	3 4	0 9	0 9	13 5
Apricot	I	23 4	0 8	Tr	5 1	9 0	1 4	2 0	5 1
	II	20 7	0 6	Tr	4 0	8 5	1 3	1 7	4 6
	III	20 5	0 8	Tr	6 4	6 4	1 1	1 4	4 4

*Stages are described in Table 2
†Data represent the mean of three analyses, triplicate injections were made of each sample
‡Tr, trace (less than 0 1 mg/100 mg wall)

Table 5 Changes in non-cellulosic neutral sugar composition of cell walls from various pome fruits

Fruit	Stage*	Non-cellulosic neutral sugar (mg/100 mg cell wall)†							
		Total	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
Apple	I	41 1	2 0	ND‡	14 9	7 1	1 4	2 4	13 3
	II	37 0	2 1	ND	15 4	7 5	1 4	2 4	8 2
	III	35 3	2 3	ND	15 1	8 2	1 2	2 0	6 5
Pear	I	38 3	1 4	ND	15 3	15 3	1 3	2 5	2 5
	II	29 6	1 2	ND	10 0	14 1	1 1	1 2	2 0
	III	28 4	0 7	ND	4 4	19 9	0 8	1 0	1 6

*Stages are described in Table 2
†Data represent the mean of three analyses, triplicate injections were made of each sample
‡ND, none detected

ripening was quite specific with losses involving primarily galactose in apple fruit and arabinose in pears

Fruit cell wall compositions of the cucurbits studied were similar in that they contained relatively low levels of arabinose and xylose and high levels of galactose (Table 6) Melon, squash and muskmelon cell walls lost substantial amounts of galactose and smaller amounts of arabinose residues during maturation and ripening, whereas the non-cellulosic neutral sugar composition of cucumber cell walls remained fairly constant

Xylose was the predominant neutral monosaccharide constituent in cell walls of berries (Table 7) Cell wall galactose and arabinose content of these fruits was quite low In general, the net loss of neutral sugar components from walls during ripening was less than most of the other fruits in this study Blueberry, red raspberry, blackberry and strawberry fruit lost 9%, 6%, 17% and 30%, respectively, of their total neutral wall residues during ripening. The losses involved primarily arabinose and/or galactose-containing polymers

These results show that with the exception of the stone fruit, little species variation existed within each botanical group of fruit, in regard to the major cell wall non-cellulosic neutral sugar component However, the neutral sugar composition of cell walls varied between the botanical groups

The primary factor considered when sorting fruit into stages of ripeness was to select three stages which represented the spectrum of ripening for each species Thus, although comparisons of cell wall neutral sugar composition within a species represents relative changes during ripening, comparisons between species at specific stages must be interpreted with caution

In 15 of the 17 types of fruit, a net loss of non-cellulosic neutral sugar residues occurred from cell walls during the developmental period tested The degree of loss ranged from 6% (raspberry) to 56% (hot pepper) No loss of neutral sugar residues occurred during the ripening of plums or cucumbers It is notable that in the present study, no loss occurred in cucumbers because in a recent study, cucumber fruit cell walls lost 51% of their total non-cellulosic sugar residues during 12 days at 12 5° after harvest [16] This sharp contrast apparently reflects differences between cucumbers on and off the vine

Of the 15 fruits in which a net loss of non-cellulosic neutral sugar residues occurred, galactose was the major residue lost in seven of the species and arabinose was the primary residue lost in another seven In apricot, xylose

Table 6 Changes in non-cellulosic neutral sugar composition of fruit cell walls from various cucurbits

Fruit	Stage*	Non-cellulosic neutral sugar (mg/100 mg wall)†							
		Total	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
Cucumber	I	17 5	0 8	0 2	1 5	2 6	0 8	0 9	10 7
	II	19 7	0 7	0 2	1 2	3 4	1 2	1 2	11 8
	III	19 8	0 6	0 2	1 2	3 4	1 2	1 4	11 8
Muskmelon	I	19 1	0 8	0 3	1 9	3 1	0 9	1 3	10 8
	II	11 2	0 7	0 5	1 1	4 1	0 9	1 4	2 5
	III	11 4	0 5	0 6	0 8	4 7	0 9	1 6	2 3
Melon	I	20 5	0 4	0 2	1 4	3 3	1 3	1 4	12 5
	II	19 8	0 5	0 2	1 8	3 9	1 2	1 7	10 5
	III	15 0	0 5	0 2	1 0	4 1	1 0	1 4	6 8
Squash	I	28 0	1 5	0 4	2 8	3 8	0 6	6 3	12 6
	II	19 1	0 8	0 5	1 0	5 2	0 8	8 6	2 2
	III	21 1	1 3	0 5	1 3	4 8	0 8	6 9	5 5

*Stages are described in Table 2

†Data represent the mean of three analyses, triplicate injections were made of each sample

Table 7 Changes in non-cellulosic neutral sugar composition of cell walls from various berries

Fruit	Stage*	Non-cellulosic neutral sugar (mg/100 mg wall)†							
		Total	Rha	Fuc	Ara	Xyl	Man	Glc	Gal
Blueberry	I	26 7	0 4	ND‡	6 0	14 6	0 7	1 3	3 7
	II	28 3	0 3	ND	1 8	21 0	0 7	1 2	3 3
	III	24 2	0 3	ND	1 1	18 0	0 6	1 1	3 1
Raspberry	I	16 3	0 4	Tr‡	1 5	12 1	0 3	0 7	1 3
	II	15 3	0 3	Tr	1 1	11 8	0 3	0 6	1 2
	III	15 3	0 3	Tr	1 0	11 9	0 3	0 7	1 1
Blackberry	I	17 9	0 6	0 3	3 9	9 4	0 4	1 0	2 3
	II	16 6	0 4	0 2	2 3	11 5	0 3	0 7	1 2
	III	14 8	0 3	Tr	1 4	11 5	0 3	0 6	0 7
Strawberry	I	21 2	1 2	Tr	5 4	6 1	0 9	2 1	5 5
	II	17 2	0 5	Tr	2 8	6 8	1 0	2 6	3 5
	III	14 8	0 4	Tr	1 9	6 4	1 1	2 0	3 0

*Stages are described in Table 2

†Data represent the mean of three analyses, triplicate injections were made of each sample

‡ND, none detected, Tr, trace (less than 0 1 mg/100 mg wall)

was the major monosaccharide lost from walls during ripening Clearly, the loss of arabinose and/or galactose containing polysaccharides is associated with and can be considered a feature of the fruit ripening process in a number of economically important species

Galactose and arabinose are major neutral sugar components of pectic polysaccharides They exist primarily as side chains on the rhamnogalacturonan backbone However, the means by which cell wall neutral sugar-containing polymers are turned over is not as clear as the enzymatic mechanism of polyuronide hydrolysis, i e cleavage of α-1,4-linkages between non-esterified galacturonosyl residues of the pectic portion of fruit cell walls by polygalacturonase Ahmed and Labavitch [10] proposed that the solubilization of pear wall arabinose residues is due to the action of polygalacturonase They demonstrated that *in vitro* hydrolysis of pear cell wall preparations with highly purified polygalacturonase solubilized a pectic arabinan similar to the soluble polymer observed in ripe pear homogenates In tomatoes however, the loss of galactose residues does not seem to be due to polygalacturonase activity because *rin* fruit (ripening-inhibited mutant) cell walls lost a substantial number of galactose residues but contained little or no polygalacturonase activity [14] Tomatoes also contain a β-galactosidase capable of degrading a β-1,4-galactan isolated from tomato cell walls [17] The increase in free, monomeric galactose in tomatoes during ripening [18, 19] may be a product of this enzyme

The turnover of galactose and arabinose residues in tomatoes does not seem to be directly related to fruit firmness since *rin* fruit soften only slightly although a considerable net loss of galactose-containing polymers occurs [14] Thus, the direct effect of galactan turnover on tomato fruit firmness may be minor Nevertheless, it has been suggested that neutral sugar side chains on the pectic polysaccharide backbone of pear and tomato cell walls may confer protection from polygalacturonase action by

restricting access of the enzyme to the polygalacturonic acid substrate [14, 15] It is notable that cell walls from berries, which are relatively soft fruit, had a low content of galactose

Regardless of the function of and mechanisms involved in cell wall turnover in relation to fruit ripening and softening, this study has shown that the net loss of neutral sugar residues is not restricted to apples [11], strawberries [12], pears [9, 15] and tomatoes [13], but is a relatively general feature of fruit ripening in many, but not necessarily all, species Further, this study provides evidence that substantial variation exists in cell wall composition among tissues and fruits from various botanical groups Care should be taken to avoid generalizations about the 'mechanism' of fruit softening Cell wall composition and metabolism, in relation to softening, may be quite different among various fruits and fruit tissues

EXPERIMENTAL

Plant material With the exception of apples and tomatoes, plants were field-grown using standard cultural practices at the Agricultural Research Center, Beltsville, MD Apples were obtained from a commercial orchard in Pennsylvania and tomatoes were grown in a greenhouse Table 1 lists the species and tissue type used The criteria used for selecting various stages of ripeness differed between species (Table 2) Apples were harvested when mature and stored in air at 0°, ripeness stages were based on the pattern of C_2H_4 and CO_2 evolution which was monitored by using GC Pear ripeness was determined similarly except that fruit were stored at 0° for 6 weeks and then transferred to 20°

Cell wall extraction Fruit were hand-harvested, washed with distilled H_2O, and sorted into three stages of ripeness The skin was removed from all fruit except blueberries, red raspberries, blackberries and strawberries Although the number of fruit used varied between species, five was the minimum number used The appropriate tissue was excised, sliced into small sections, and 10 g was placed into 20 ml 80% EtOH Samples were stored at −70° for up to 1 month prior to cell wall extraction

Samples were homogenized with a Polytron (Brinkmann Instruments), filtered through Miracloth (Calbiochem), and the residue washed with 20 ml 80% EtOH After filtration, the residue was washed with 20 ml 20 mM HEPES–NaOH (pH 6 9) and suspended in 25 ml HEPES–NaOH (pH 6 9) containing 25 mg α-amylase (Sigma Chem Co, type III) and a drop of toluene Samples were incubated at 37° for 24 hr with continuous shaking. After filtration, the residue was washed twice with 25 ml 20 mM HEPES–NaOH (pH 6 9) Cell walls were then extracted with $CHCl_3$–MeOH (1 1) and Me_2CO as previously described [14] Cell walls were dried *in vacuo* at 37° over P_2O_5 for at least 5 days prior to analysis

Cell wall neutral sugar analysis The neutral monosaccharide components of non-cellulosic polysaccharides were determined by using capillary GC Wall material was hydrolysed with 2 N TFA as described by Jones and Albersheim [20] The resulting neutral monosaccharides were then made into their aldononitrile acetate derivatives using the procedure of Lehrfeld [21] and quantified by capillary GC using conditions previously described [19], *myo*-inositol was used as the internal standard

At least three analyses were run for each stage of ripeness of each species Each GC analysis consisted of triplicate injections of each sample, data were averaged over the three injections

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