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Influence of Cultivar, Cooking, and Storage on Cell-Wall Polysaccharide Composition of Winter Squash (*Cucurbita maxima*)

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Changes in the cell-wall polysaccharides (CWP) of the edible tissues of four winter squash cultivars during storage and after cooking were investigated. A procedure for isolating cell walls of tissues containing high levels of starch was used. The starch-free CWP were sequentially fractionated using CDTA, dilute Na_2CO_3, and 4 M KOH. Cellulose made up 40−42% of the total CWP for three cultivars (Delica, CF 2, and CF 4) at harvest but was 35% in the softer Red Warren. The pectic polysaccharides of Delica, CF 2, and CF 4 cell walls are more branched than those from Red Warren squash. The higher proportion of uronic acid in the pectic polysaccharides of Red Warren squash correlates with its lower firmness. Cooking resulted in an increase in the water-soluble pectins and a decrease in the pectins associated with cellulose. The total CWP content of the squash cultivars remained unchanged for up to 2 months of storage and then markedly decreased between 2 and 3 months of storage. The galactose content of Delica and Red Warren cell walls remained relatively constant from harvest to 2 months of storage and then decreased markedly during 2−3 months of storage.

KEYWORDS: Buttercup squash; winter squash; pumpkin; cell walls; cell-wall polysaccharides; chemical markers of texture

INTRODUCTION

The relative amounts of cell-wall polysaccharides, as well as the type, length, and branching pattern of side chains of cell-wall polysaccharides, have a profound effect on the structure of the cell walls and consequently the texture of fruits and vegetables (*1*−*3*). Their texture is also influenced by other factors such as turgor pressure (*4*). With regard to cell walls, cellulose is thought to provide rigidity, resistance to tearing, and the capability of the plant material to bear substantial stress (*5*). Cellulose microfibrils provide the framework of the cell wall interpenetrated by a cross-linking matrix of hemicelluloses and pectic polysaccharides (*6*). The mechanical strength of primary cell walls can be very largely attributed to the cellulose microfibrils. The middle lamella pectic polysaccharides are thought to be responsible for intercellular adhesion, allowing the cells to move in response to compressive forces (*7*). Moreover, characteristics of the cell walls such as their polysaccharide composition may influence the behavior of the plant tissues during mastication in the mouth, which causes mechanical stress, strain, and failure of the tissues, and the perceived sensory characteristics (*8*).

Changes in the composition of cell-wall polysaccharides associated with softening during storage and ripening have been studied in numerous fruits (*9*−*11*). Such investigations include melons (*12*−*14*), which along with squash are members of the Curcurbitaceae family. Changes in texture and cell-wall composition also occur during cooking (see, for example, refs *1*−*3*, *15*, and *16*).

Aside from our previous study (*17*), no information is available on the changes of cell-wall polysaccharides of winter squash (*Cucurbita maxima*) during storage and after cooking. Such knowledge is needed to develop improved cultivars having desirable texture characteristics and so expand their market share.

In this paper, the monosaccharide compositions of cell walls from raw and cooked edible tissues of two commercial squash cultivars, namely, Delica (a green-skinned buttercup variety) and Red Warren (a red-skinned pumpkin variety), and two buttercup squash breeding lines, CF 2 and CF 4, are discussed in relation to the differences in texture among the cultivars. Changes in the cell-wall polysaccharide composition of raw and cooked Delica and Red Warren squash cultivars stored over various periods are also discussed. Our aim was to identify cultivar-specific differences in cell-wall polysaccharide composition that could be used as markers in a breeding experiment designed to study the heritability of texture characteristics in *C. maxima* (*18*).

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MATERIALS AND METHODS

Plant Material. Winter squash (*C. maxima* D.) cultivars Delica, Red Warren, CF 2, and CF 4 were grown on the farm associated with the New Zealand Institute for Crop and Food Research at Kimberley Road, Levin, New Zealand (geographical location: 40° 39′ S, 175° 16′ E) and harvested in 1998, 50 days after flowering. The harvested squash were washed, dried, and stored at 12 °C and 80−85% relative humidity.

Cell-Wall Isolation from Raw Squash Tissues. Isolation of the cell walls from raw squash tissues was based on the method described by Ratnayake et al. (*17*) with slight modifications. The squash were cut along 10 marked segments. Tissues from alternate segments, excluding the skin, seeds, placental tissues, and tissues damaged by removal of cylinders, were frozen in liquid nitrogen and stored at −80 °C. From each of six squash, one segment was chosen at random. Segments were combined to obtain a representative sample (200 g). The sample was immersed in liquid nitrogen and ground to a powder in a coffee grinder with a rotary blade. All apparatuses in contact with the squash samples was prechilled with liquid nitrogen. The isolation of cell walls was done in duplicate. Frozen powder (50 g) was layered with HEPES buffer (pH 6.8) containing dithiothreitol (DTT) (100 mL). The mixture was allowed to thaw and stirred with a strong glass rod to give a thick slurry. The slurry was homogenized (20500 rpm, 6 × 20 s bursts) in an Ultra-Turrax (T25 Basic, INK Labortechnik Works). The suspension was filtered though a 53 μm nylon mesh and washed with cold HEPES−DTT buffer (pH 6.8) (200 mL). The combined filtrate was designated first HEPES-soluble fraction. The residue was ground (3 × 3 min) in a ring grinder (bench top ring mill, Rocklab, Auckland, New Zealand). After each grinding, a small sample was stained with Ponceau 2R and examined by microscopy for the extent of cell breakage. After grinding, the suspension was immersed in cold HEPES−DTT (100 mL), stirred, and filtered through a 53 μm nylon mesh. The residue was washed with cold HEPES−DTT buffer (200 mL). The two filtrates were combined and designated second HEPES-soluble fraction.

Starch Removal from Cell-Wall Preparation from Raw Tissue. The residue was washed with 90% (v/v) aqueous DMSO (20 mL) and resuspended in a 90% DMSO (200 mL) solution for 24 h with stirring to gelatinize the starch granules. This was followed by filtration and washing as described above. The residue was ground again in a ring grinder (2 × 2 min) to rupture the remaining unbroken cells. The homogenate was filtered through a 53 μm mesh and then washed with HEPES buffer. The two filtrates were combined and designated DMSO-soluble fraction. The residue was resuspended in HEPES buffer (100 mL, pH 6.8) and incubated with porcine pancreatic α-amylase (220 units, type 1-A, Sigma) at 25 °C for 1 h with stirring. The suspension was filtered through glass microfiber filters to obtain the residue (the cell walls). The filtrate was designated the amylase-soluble fraction. Cell walls isolated from squash were sequentially extracted using a modification of the method Selvendran et al. (*19*). Starch was assayed enzymatically (Megazyme, Bray, Ireland) and by I_2/KI staining viewed by light microscopy.

Cell-Wall isolation from Cooked Squash Tissues. The tissues from remaining alternate segments of each squash, excluding the skin, seeds, placental tissues, and damaged tissues, were cooked by steaming until edible (~6−10 min). The cooked squash was frozen immediately in liquid nitrogen and stored at −80 °C. The cooking water, used to generate the steam and which included steam drip, was collected for analysis.

A representative frozen sample (200 g) was immersed in liquid nitrogen and ground into a powder using a coffee grinder. Frozen powder (50 g) was layered with HEPES−DTT buffer (100 mL), and the mixture was allowed to thaw and stirred to produce a thick slurry. The suspension was filtered through nylon mesh (110 μm) and washed with cold HEPES−DTT buffer (150 mL). This step separates single cells from the clumps of cells. The filtrate was filtered again through 53 μm nylon mesh to recover individual unbroken squash cells from the suspension. The filtrate obtained from the second filtration was designated first HEPES-soluble fraction. Both residues, wet with HEPES−DTT (particle size > 110 μm and single cells), were ground separately using a ring grinder (2 × 2 min). The two homogenates were combined and resuspended in HEPES−DTT buffer (100 mL). The suspension was filtered through 53 μm nylon mesh. The residue retained on the 53 μm nylon mesh was ring-ground (2 × 2 min) and filtered as above. The filtrates obtained from the last two steps were combined and designated second HEPES-soluble fraction.

Starch Removal from Cell-Wall Preparation from Cooked Tissue. Residual starch was removed by solubilizing in 90% DMSO as described above. The resulting residue was subjected to a final ring grinding (3 × 2 min) to rupture the remaining unbroken cells. The homogenate was resuspended in HEPES buffer (100 mL) and filtered through a 53 μm nylon mesh. The residue was washed extensively using HEPES buffer (2 × 100 mL aliquot). The combined filtrate was designated third HEPES-soluble fraction. Residual starch in the residue was degraded using α-amylase (420 units) as described above to give the cell walls. A third HEPES-soluble fraction was not required for the cell-wall isolation from cooked tissues of Red Warren.

Fractionation of Squash Cell Walls. Before fractionation, the wet weight of the isolated cell walls was determined, and a weighed portion of cell walls was freeze-dried to obtain the dry weight. The isolation of cell walls of raw and cooked squash was done in duplicate. Fractionation of isolated cell walls was carried out individually. Analyses of cell-wall polysaccharides of individual fractions were done in duplicate. Thus, the cell-wall analysis data in the tables represent the mean of four analyses.

Cell walls isolated from squash were sequentially extracted using a modification of the method of Selvendran et al. (*19*). The never-dried cell walls (equivalent to 1.2−1.4 g of dry weight) were suspended in 0.05 M CDTA containing 0.05 M KOAc buffer (pH 6.8, 100 mL), stirred for 6 h at room temperature, and then centrifuged (1000*g*, 5 min). The pellet was subjected to a second CDTA extraction. The supernatants from the two extractions were pooled to give the CDTA-soluble fraction. The pellet from the CDTA extraction was suspended in 0.05 M Na_2CO_3 (100 mL) containing 0.02 M $NaBH_4$. Both the suspension and headspace were flushed with nitrogen, capped, and stirred for 16 h at 4 °C. This was followed by a second incubation at room temperature for 2 h. The suspension was centrifuged (1000*g*, 5 min), and the supernatants were combined to give the Na_2CO_3-soluble fraction. The pellet from the Na_2CO_3 extraction was suspended in 4 M KOH (100 mL) containing 0.02 M $NaBH_4$ and stirred at room temperature for 4 h under nitrogen. The suspension was centrifuged (1000*g*, 5 min), and the 4 M KOH extraction was repeated. The pellet was washed with deionized water. The supernatants from the two extractions and water wash were pooled to give the 4 M KOH-soluble fraction. The pellet was suspended in deionized water (75 mL) and stirred at room temperature for 1 h, followed by centrifugation (1000*g*, 5 min). The water wash was repeated, and the supernatants were combined to give the residue wash. The remaining pellet was designated the final residue (α-cellulose fraction).

Neutralization and Dialysis of the Fractions. The HEPES-soluble fractions were dialyzed against deionized water. The Na_2CO_3- and KOH-soluble fractions were neutralized to pH 6.8 with acetic acid (1 M) and concentrated HCl, respectively, before dialysis. The residue wash fraction was neutralized with HCl (1 M) to pH 6.8. All fractions, except the CDTA-soluble fraction, were dialyzed for 3 days against deionized water, with three changes of water per day. The CDTA-soluble fraction was dialyzed against ammonium acetate (0.1 M, pH 5.2) for 2 days, with three changes of buffer per day, followed by dialysis in deionized water for 3 days, with three changes of water per day. All dialyses were carried out at 4 °C. Volumes of the dialyzed fractions were recorded and freeze-dried.

Colorimetric Analysis of Uronic Acid. The samples were hydrolyzed as previously described (*20*), and uronic acid content of the cell-wall fractions was determined colorimetrically as anhydrogalacturonic acid (*21*).

Gas—Liquid Chromatography Determination of Monosaccharide Composition of Cell-Wall Fractions. Fractions were hydrolyzed in duplicate using TFA (*22*). Cellulose was determined in the final residue by hydrolyzing in duplicate using a two-stage H_2SO_4 hydrolysis (*23*). Alditol acetates were prepared and quantified by gas chromatography (*22*).

Table 1. Yields (by Dry Weight) of Cell Walls (without HEPES-, DMSO-, and Amylase-Soluble fractions) Isolated from Raw and Cooked Squash Tissues of Cultivars Delica, Red Warren, CF 2, and CF 4 during Storage[a]

		yield (mg/g)	
cultivar	storage	raw	cooked
Delica	harvest	16.88 ± 1.02	17.34 ± 0.92
	1 month	17.22 ± 1.10	17.02 ± 1.11
	2 months	16.91 ± 0.98	17.23 ± 0.99
	3 months	15.11 ± 1.03	15.92 ± 0.87
Red Warren	harvest	13.72 ± 1.10	14.16 ± 0.86
	1 month	14.03 ± 0.95	13.82 ± 0.96
	2 months	13.92 ± 0.79	14.12 ± 1.00
	3 months	12.61 ± 1.01	12.87 ± 0.84
CF 2	harvest	17.92 ± 1.09	18.42 ± 1.21
	1 month	17.72 ± 0.98	18.02 ± 1.02
	2 months	18.19 ± 1.36	18.52 ± 1.24
	3 months	16.50 ± 1.08	17.02 ± 0.98
CF 4	harvest	16.57 ± 0.81	17.06 ± 1.12
	1 month	16.42 ± 1.12	16.92 ± 1.35
	2 months	16.34 ± 0.97	16.86 ± 1.06
	3 months	16.53 ± 1.06	15.43 ± 0.89
P value[b]		<0.001	<0.001
LSD (5%)[c]		0.96	1.09

[a] Values are expressed as mg/g of fresh tissue. Data represent the mean ± standard deviation of two replicates of cell-wall isolation. [b] *P* value for each comparison between the samples within and between cultivars in the respective column. [c] LSD (5%) value for each comparison between the samples within and between cultivars in the respective column.

Statistical Analysis. The yields of cell walls isolated from raw and cooked squash tissues were separately compared using one-way analysis of variance (ANOVA). The difference in yield of cell walls between raw and cooked squash tissues was individually compared statistically using a one-way ANOVA. When ANOVA results were significant ($P < 0.05$), least significant differences (LSD) were used to compare the values among cultivars and storage conditions. The statistical analysis was performed using Genstat 5 software (Genstat 5 committee, 1989; Clarendon Press, Oxford, U.K.).

RESULTS AND DISCUSSION

Yields of Cell Walls from Raw and Cooked Squash Cultivars. Isolation of cell walls using HEPES buffer, DMSO, and α-amylase resulted in starch-free cell walls, as confirmed by the starch assay, and negative staining with I_2/KI, even though squash contain high levels of starch (~15% by fresh weight in Delica) (*24*, *25*).

The yield of cell walls from raw tissues of CF 2 squash was significantly higher ($P < 0.001$) than from other cultivars, whereas the CWP yield from raw tissues of Red Warren was significantly lower ($P < 0.001$) value than from other cultivars (**Table 1**). The larger size of the Red Warren parenchyma cells and larger intercellular spaces together with their thinner cell walls (*18*) could account for their lower yield of CWP. Yields of CWP of cooked squash tissues were not significantly different from the CWP yields of the corresponding raw squash. This shows that steaming did not alter the extraction of total CWP in squash cultivars. All cultivars showed no change in CWP yields for up to 2 months of storage, but then all yields decreased significantly ($P < 0.001$).

Cell-Wall Fractions from Raw Squash Cultivars. The first HEPES- and second HEPES-soluble fractions contained small quantities of starch for the buttercup squash cultivars Delica, CF 2, and CF 4, and virtually no starch was found in these fractions for Red Warren (**Table 2**). The DMSO dissolved mainly starch with only small amounts of CWP (<1% total CWP) found in this fraction. The proportions of CWP recovered from the CDTA-soluble fractions were rather low (57−61%) for all cultivars. The recoveries of the remaining fractions were 75−98% of the fraction for all cultivars.

The amounts of CWP (by analysis for neutral sugars, uronic acid, and cellulose) extracted by the combined first HEPES- and second HEPES-soluble fractions of Delica, Red Warren, CF 2, and CF 4 squash were 9, 12, 8, and 9% of the total CWP, respectively (**Table 2**). Galactose was the major neutral monosaccharide in these fractions for all cultivars at harvest (**Table 2**). This was followed by smaller proportions of arabinose and glucose. The CWP extracted in these early fractions was expected to be mainly water-soluble pectic polysaccharides (*17*). This was confirmed by a high content of uronic acid, which accounted for 25% in Delica and 22% of total uronic acid, each for Red Warren, CF 2, and CF 4 squash extracted by combined first HEPES- and second HEPES-soluble fractions (**Table 2**). The results indicate that the water-soluble pectic polysaccharide released from the cell walls during isolation and purification of CWP was substantial and that it should be collected and analyzed; otherwise, the amount of CWP would be underestimated. However, the smaller amounts of CWP extracted from combined DMSO- and amylase-soluble fractions (≤2% of total CWP) could be ignored.

Combined CDTA- and Na_2CO_3-soluble fractions extracted 14% (by analysis) of the total CWP for Delica, Red Warren, and CF 4 squash, whereas the combined fractions were only 11% of the total CWP for CF 2 squash (**Table 2**). These fractions accounted for 34 and 39% of total uronic acid present in raw squash of Delica and CF 4, respectively. Equivalent combined fractions for Red Warren and CF 2 squash represented 31 and 32% of total uronic acid, respectively, at harvest. In all cultivars, the CDTA- and Na_2CO_3-soluble fractions consisted of >70% uronic acid. Galactose and arabinose were the major neutral monosaccharides, and rhamnose was also present in all squash cultivars at harvest. These results suggest that CDTA- and Na_2CO_3-soluble fractions extracted mainly galacturonan with minimal side chains consisting of possibly galactans, arabinans, and arabinogalactans. The results are similar to those previously obtained for Delica squash (*17*), raw onions (*27*), and raw potatoes (*28*).

Unlike the other cultivars, the CDTA-soluble fraction of CF 2 has a higher UA:(Ara + Gal) ratio than its $NaCO_3$-soluble fraction, indicating that the Na_2CO_3-soluble fraction contained pectic polysaccharides with a higher proportion of arabinan and galactan side chains. Moveover, the previous fractions (all water-soluble fractions) extracted less CWP for CF 2 than for other cultivars (**Table 2**). Together these results indicate that the pectic polysaccharides in the CF 2 squash tissues are more tightly associated with other cell-wall polysaccharides such as hemicelluloses and cellulose than in other cultivars. This may be another reason (other than CF 2 squash tissues containing more CWP) that the CF 2 tissues are firmer than those of other cultivars (*18*).

The ratios of UA:(Ara + Gal) of CDTA-soluble fractions and Na_2CO_3-soluble fractions for Red Warren were 5.5 and 6.7, respectively (**Table 2**). These were much higher values than the similar ratio in equivalent fractions of other cultivars, indicating that CDTA- and Na_2CO_3-soluble fractions of Red Warren contained fewer branched pectic polysaccharides than that in equivalent fractions of other cultivars. This is consistent

Table 2. Neutral Sugar Composition and Starch, Cellulose, and Uronic Acid Contents of the Different Fractions of Raw Squash (*C. maxima*) Cultivars at Harvest[a]

cultivar	fraction	FDW (mg)	composition (mg/g)										UA:(Ara + Gal)
			Rha	Fuc	Ara	Xyl	Man	Gal	Glu	starch	cellulose	UA	
Delica	1st HEPES	70.5	0.2	tr	1.7	tr	1.2	3.8	1.0	1.2	ND	49.1	8.8
	2nd HEPES	28.4	0.2	tr	0.7	0.1	0.5	3.0	1.2	2.0	ND	17.4	4.6
	DMSO	19.6	tr	tr	0.1	tr	0.2	0.4	1.3	13.7	ND	1.9	3.7
	amylase	13.9	0.1	tr	0.2	0.1	0.5	0.6	0.4	tr	ND	9.2	11.2
	CDTA	44.9	0.4	UD	1.5	tr	1.2	4.4	tr	ND	ND	19.7	3.3
	Na_2CO_3	114.6	2.2	tr	3.9	tr	1.9	17.0	tr	ND	ND	70.7	3.4
	4 M KOH	79.8	0.3	0.7	3.4	15.8	0.8	20.9	17.2	ND	ND	10.4	0.4
	residue wash	84.4	1.2	tr	4.1	1.0	2.0	28.8	0.7	ND	ND	25.9	0.8
	final residue	542.7	2.6	tr	8.4	4.5	6.3	59.4	23.9	ND	343.0	59.1	0.9
	total[b]	998.8	7.1	0.7	24.0	21.5	14.5	138.5	45.8		343.0	263.3	
Red Warren	1st HEPES	74.9	0.2	tr	5.2	tr	0.3	11.5	3.4	tr	ND	44.6	2.6
	2nd HEPES	43.2	0.1	tr	1.2	0.1	tr	5.4	2.8	tr	ND	25.2	3.8
	DMSO	21.0	tr	tr	tr	tr	tr	0.6	2.0	13.8	ND	2.2	3.3
	amylase	14.1	tr	tr	0.2	0.1	0.2	0.6	1.6	0.0	ND	7.1	9.2
	CDTA	48.4	0.1	UD	0.5	tr	0.6	3.4	0.6	ND	ND	21.8	5.5
	Na_2CO_3	113.5	1.2	tr	2.1	tr	1.2	9.4	tr	ND	ND	77.3	6.7
	4 M KOH	32.0	tr	0.4	1.2	9.1	1.1	3.5	6.3	ND	ND	4.6	0.9
	residue wash	90.0	1.0	tr	3.7	1.3	2.0	9.9	0.7	ND	ND	50.5	3.7
	final residue	546.7	6.2	tr	10.7	5.2	3.2	66.3	29.9	ND	285.0	85.7	1.1
	total[b]	983.7	8.9	0.4	24.8	15.8	8.7	110.7	47.3		285.0	319.1	
CF 2	1st HEPES	42.1	0.5	tr	1.9	tr	0.4	9.4	1.0	1.1	ND	25.9	2.3
	2nd HEPES	33.0	0.5	tr	0.2	0.1	0.6	5.6	2.0	1.2	ND	20.9	3.6
	DMSO	25.7	tr	tr	0.3	0.2	0.3	2.3	1.7	15.5	ND	2.1	0.8
	amylase	3.9	tr	tr	0.1	0.1	0.2	0.8	0.5	0.0	ND	1.4	1.7
	CDTA	41.3	0.3	tr	0.7	tr	tr	3.4	0.9	ND	ND	18.4	4.5
	Na_2CO_3	86.5	1.3	UD	3.1	0.2	tr	16.0	0.5	ND	ND	52.7	2.8
	4 M KOH	57.7	1.4	0.6	3.5	10.7	1.0	15.7	14.0	ND	ND	5.5	0.3
	residue wash	98.0	3.4	0.2	4.4	0.6	tr	29.8	13.9	ND	ND	30.3	0.9
	final residue	569.6	4.4	tr	8.3	4.1	3.6	55.1	31.0	ND	361.6	64.2	1.0
	total[b]	957.7	11.8	0.9	22.5	15.9	6.1	138.1	65.4		361.6	219.3	
CF 4	1st HEPES	56.9	0.3	tr	1.5	tr	tr	6.3	1.9	1.2	ND	33.1	4.2
	2nd HEPES	41.0	0.7	tr	2.1	tr	tr	9.1	1.8	1.3	ND	24.9	2.2
	DMSO	36.2	0.1	tr	1.2	tr	0.2	4.0	1.4	18.7	ND	1.5	0.3
	amylase	4.9	tr	tr	0.1	tr	tr	0.2	1.0	0.0	ND	2.9	11.7
	CDTA	40.9	0.4	tr	1.5	tr	tr	3.2	0.3	ND	ND	17.9	3.8
	Na_2CO_3	111.0	1.4	tr	2.4	tr	tr	15.3	tr	ND	ND	84.5	4.8
	4 M KOH	59.2	0.6	0.6	1.9	12.5	1.9	15.5	15.0	ND	ND	10.0	0.6
	residue wash	88.4	1.2	tr	3.4	1.7	0.0	32.3	5.3	ND	ND	35.3	1.0
	final residue	555.5	3.2	tr	4.9	3.6	3.2	60.7	25.8	ND	345.0	51.8	0.8
	total[b]	994.1	8.0	0.6	18.9	17.8	5.4	146.7	52.5		345.0	261.7	

[a] Expressed as mg/g of dry cell-wall polysaccharide. Data represent the mean of four analyses. tr, trace (<0.1 mg); UD, undetected; ND, not determined; FDW, freeze-dry weight; SD, standard deviation; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose (excluding starch and cellulose); UA, uronic acid. [b] Σ means. SD < 10% of the mean of four analyses.

with a lower firmness associated with Red Warren compared to other cultivars (*18*).

Eight percent (by analysis) of the total CWP was extracted by the 4 M KOH-soluble fraction of Delica squash. Corresponding figures for the other cultivars were 3, 6, and 7% each of total CWP for Red Warren, CF 2, and CF 4, respectively (**Table 2**). The uronic acid content of this fraction was very low for all cultivars, suggesting that the CWP extracted from 4 M KOH-soluble fractions consisted mainly of hemicelluloses.

Xylose made up 20−23% of the 4 M KOH-soluble fractions for Delica, CF 2, and CF 4, squash, whereas in Red Warren, xylose accounted for 34% of the fraction (**Table 2**). Galactose and glucose and lesser amounts of arabinose were also found in this fraction for all cultivars. This fraction of raw Delica at harvest had a glucose:xylose ratio of 1.1, which is lower than the ratio (1.3) typical of xyloglucan from dicotyledon cell walls (*29*). This suggests that the 4 M KOH-soluble fraction contains both xyloglucan and heteroxylans, such as arabinoxylan, whereas the ratio of glucose:xylose ratio of CF 2 squash (1.3) suggests that this fraction of CF 2 contains mostly xyloglucan. The glucose:xylose ratio (1.2) in the 4 M KOH-soluble fraction of CF 4 squash is close to that of Delica, also suggesting the presence of mainly xyloglucan, whereas the very low ratio (0.7) for Red Warren suggests the presence of lower amounts of xyloglucan and more heteroxylans. Furthermore, the yield of this fraction from Red Warren was lower than from the other three cultivars (**Table 2**). The ratio of glucose:xylose of these cultivars correlates with their firmness (*18*). The highest ratio corresponded to the greater firmness of CF 2 squash, whereas the lowest ratio corresponded to the least firm Red Warren, suggesting that tissues containing more xyloglucan and less heteroxylan are firmer.

The presence of mannose in 4 M KOH-soluble fractions together with galactose and glucose may indicate the presence of galactoglucomanans and glucomanans in the squash cell walls. The presence of low proportions of uronic acid, moderate amounts of galactose and arabinose, and traces of rhamnose in 4 M KOH-soluble fraction of all cultivars suggests that this fraction contained some highly branched pectic polysaccharides. The very low ratios of UA:(Ara + Gal) in this fraction (0.1−0.6) (**Table 2**) confirm this. Similar highly branched pectic polysaccharides have been reported in 4 M KOH-soluble

Table 3. Neutral Sugar Composition and Starch, Cellulose, and Uronic Acid Contents of the Different Fractions of Cooked Squash at Harvest[a]

cultivar	fraction	FDW (mg)	composition (mg/g)										UA:(Ara + Gal)
			Rha	Fuc	Ara	Xyl	Man	Gal	Glu	starch	cellulose	UA	
Delica	cooking water	1.7	tr	UD	tr	UD	tr	0.1	0.2	1.0	ND	0.3	3.8
	1st HEPES	69.9	0.3	tr	2.0	tr	2.0	3.4	0.9	1.0	ND	48.4	9.1
	2nd HEPES	18.0	0.1	tr	0.9	tr	tr	4.8	0.4	1.8	ND	6.9	1.2
	DMSO	41.4	0.3	tr	0.9	tr	0.6	5.7	3.3	14.6	ND	8.2	1.2
	3rd HEPES	38.6	0.6	tr	0.4	tr	0.9	5.6	1.5	1.7	ND	27.2	4.5
	amylase	21.2	0.6	tr	1.3	tr	0.3	4.6	1.0	tr	ND	8.7	1.5
	CDTA	105.2	1.7	UD	2.8	tr	2.3	11.1	2.8	ND	ND	52.3	3.8
	Na_2CO_3	92.6	1.2	tr	2.5	tr	1.3	19.1	tr	ND	ND	61.9	2.9
	4 M KOH	99.7	1.2	0.6	3.4	13.5	2.8	34.3	18.8	ND	ND	5.8	0.2
	residue wash	58.7	2.0	tr	1.9	1.1	0.8	24.0	1.9	ND	ND	22.8	0.9
	final residue	451.9	2.3	tr	5.0	2.9	6.0	34.0	31.4	ND	346.0	19.3	0.5
	total[b]	999.1	10.3	0.6	21.1	17.5	16.9	146.5	62.2		346.0	261.8	
Red Warren	cooking water	5.9	tr	tr	0.1	tr	tr	0.1	0.8	1.8	ND	1.1	4.5
	1st HEPES	98.3	0.3	tr	5.1	tr	3.0	5.9	2.2	1.3	ND	72.4	6.0
	2nd HEPES	59.4	0.1	tr	1.2	tr	tr	5.8	2.6	1.5	ND	33.1	4.7
	DMSO	24.9	tr	tr	0.2	tr	0.4	1.0	0.5	19.0	ND	3.3	2.9
	amylase	17.1	0.6	tr	1.9	tr	0.5	1.7	0.6	1.3	ND	6.8	1.9
	CDTA	60.1	0.1	tr	0.3	tr	0.2	4.7	0.1	ND	ND	35.3	7.1
	Na_2CO_3	92.1	0.8	tr	1.7	tr	0.9	8.6	tr	ND	ND	69.1	6.7
	4 M KOH	50.1	0.3	0.4	1.5	10.9	1.1	5.2	8.0	ND	ND	13.0	1.9
	residue wash	56.6	0.9	tr	2.3	0.9	1.0	8.5	0.5	ND	ND	29.8	2.8
	final residue	527.1	5.5	tr	8.5	6.2	4.1	50.1	32.0	ND	294.0	65.0	1.1
	total[b]	991.5	8.7	0.4	22.8	17.9	11.3	91.5	47.3		294.0	329.0	
CF 2	cooking water	2.1	tr	tr	tr	tr	tr	0.1	0.6	tr	ND	0.7	6.3
	1st HEPES	46.7	0.3	tr	1.6	tr	0.7	6.5	1.7	2.1	ND	25.2	3.1
	2nd HEPES	32.7	0.3	tr	1.5	tr	0.4	5.8	0.9	1.6	ND	14.0	1.9
	DMSO	36.9	0.2	tr	0.6	tr	0.3	4.5	1.3	22.1	ND	1.1	0.2
	3rd HEPES	25.1	0.3	tr	1.0	0.2	0.3	10.2	1.5	1.6	ND	6.2	0.6
	amylase	8.3	tr	tr	0.1	tr	0.1	2.8	1.5	1.0	ND	2.4	0.8
	CDTA	78.4	0.3	tr	1.7	tr	tr	7.7	1.7	ND	ND	29.3	3.1
	Na_2CO_3	98.3	3.1	tr	3.5	tr	tr	15.3	0.4	ND	ND	69.1	3.7
	4 M KOH	66.8	1.4	0.5	3.3	9.9	0.6	17.1	15.0	ND	ND	8.4	0.4
	residue wash	75.1	2.8	tr	5.7	1.3	tr	34.0	3.8	ND	ND	23.7	0.6
	final residue	527.7	4.9	0.4	10.8	4.7	3.7	53.8	23.7	ND	365.0	49.6	0.8
	total[b]	998.1	13.6	0.9	30.0	16.1	6.1	157.8	51.2		365.0	228.8	
CF 4	cooking water	4.3	tr	tr	tr	tr	tr	1.1	0.8	tr	ND	0.9	7.1
	1st HEPES	67.6	0.4	tr	1.8	tr	0.9	8.1	2.1	1.0	ND	47.6	4.8
	2nd HEPES	36.2	0.3	tr	1.9	tr	tr	7.6	1.5	1.3	ND	16.7	1.7
	DMSO	31.6	0.1	tr	1.1	tr	0.5	2.4	2.1	23.9	ND	1.2	0.4
	3rd HEPES	31.3	0.3	tr	0.6	0.3	tr	7.1	1.5	0.9	ND	13.3	1.7
	amylase	11.8	0.1	tr	0.4	0.1	tr	4.4	1.2	tr	ND	2.5	0.5
	CDTA	74.8	0.5	tr	1.1	tr	tr	4.8	0.3	ND	ND	33.1	5.6
	Na_2CO_3	92.4	1.8	tr	3.0	tr	tr	15.3	1.1	ND	ND	63.6	3.5
	4 M KOH	61.1	0.8	0.6	2.0	10.6	1.3	18.2	12.0	ND	ND	7.4	0.4
	residue wash	87.2	0.8	0.1	3.7	2.0	tr	26.7	4.6	ND	ND	29.8	1.0
	final residue	499.8	1.7	0.3	4.9	2.2	2.5	49.8	24.6	ND	349.0	28.5	0.5
	total[b]	998.1	7.1	1.0	20.6	15.2	5.1	145.5	51.8		349.0	243.5	

[a] Expressed as mg/g of dry cell-wall polysaccharide. Data represent the mean of four analyses. tr, trace (<0.1 mg); UD, undetected; ND, not determined; FDW, freeze-dry weight; SD, standard deviation; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose (excluding starch and cellulose); UA, uronic acid. [b] Σ means. SD < 10% of the mean of four analyses.

fractions from many plant cell walls including onions (*27*), potatoes (*28*), and taro (*16*).

The residue wash-soluble fraction extracted 8−10% (by analysis) of the total CWP for all cultivars, whereas the final residue contained 58−63% of total CWP. Cellulose made up 40% of total CWP each for Delica and CF 4 squash at harvest for 35 and 42% of total CWP for Red Warren and CF 2, respectively (**Table 2**). Simandjuntak et al. (*13*) reported that the CWP from two muskmelon cultivars, members of the Cucurbitaceae family, had similar cellulose contents (35−39%).

The ratio of UA:(Ara + Gal) of the residue wash fraction for Red Warren was 3.7. However, the ratios of equivalent residue wash fractions of other cultivars were much lower, indicating that in these three cultivars the residue wash contained highly branched pectic polysaccharides, whereas in Red Warren the pectic polysaccharides in this fraction were less branched. Again, the presence of pectic polysaccharides with few branches in Red Warren correlates with that cultivar's lower firmness (*18*).

Glucose was the predominant neutral sugar in the final residue, accounting for 68−69% for Delica, CF 2, and CF 4 and 58% for Red Warren (**Table 3**). This glucose was almost all derived from cellulose. However, substantial amounts of noncellulosic neutral sugars also remained in the α-cellulose fraction (**Table 2**), and the monosaccharide compositions indicated that the noncellulosic polysaccharides were mostly pectic polysaccharides with side chains probably of galactan, arabinogalactan, and arabinan. The low ratios of UA:(Ara + Gal) of the final residue of all cultivars confirm that the pectic polysaccharides in the final residues were highly branched. Branched pectic polysaccharides have also been found in the

α-cellulose fraction of cell walls of many fruits and vegetables including onions (*27*), potatoes (*3*, *28*), and carrots (*15*).

Overall results of CWP fraction yields for the buttercup squash Delica in the present study are very similar to those of a previous study (*17*). The total uronic acid extracted from Delica and CF 4 squash was 30% each of their total CWP, whereas that extracted from Red Warren and CF 2 squash was 38 and 26% of their total CWP, respectively (**Table 2**). Because pectins and hemicelluloses contribute to plasticity and flexibility, whereas cellulose contributes to rigidity and resistance (*26*), higher cellulose and lower pectin yields for CF 2 squash were most likely major contributors to its greater firmness (*18*). By contrast, the lower cellulose yield and higher pectins for Red Warren squash correlated with its lower firmness (*18*).

Cell-Wall Fractions from Cooked Squash Cultivars. The amount of CWP dissolved in the cooking water from steaming the four squashes is negligible (**Table 3**). In contrast, a large amount of pectic polysaccharides was extracted from the cooking water of taro (*16*), which may have been due to the fact that the taro was cooked immersed in water. The cooking water and first, second, and third HEPES-soluble fractions contained small amounts of starch for all cultivars of cooked tissues, whereas the DMSO-soluble fraction contained relatively high quantities of starch for all cultivars (**Table 3**).

The proportions of CWP in the combined water-soluble fractions (cooking water, first HEPES-, second HEPES-, third HEPES-, and amylase-soluble) and DMSO-soluble fraction were 16, 18, 10, and 15% of the total CWP for cooked tissues of Delica, Red Warren, CF 2, and CF 4, respectively (**Table 3**), and were higher than the proportions of CWP in the corresponding fractions of raw squash (9, 12, 8, and 9%). The percentages of uronic acid in the combined water-soluble fractions of cooked tissues for Delica, Red Warren, CF 2, and CF 4 squash tissues were 38, 35, 21, and 33% of the total uronic acid compared with the 22−25% found in water-soluble fractions of raw squash. These results show steaming increased the extractability of water-soluble CWP for Delica, Red Warren, and CF 4 squash but not for CF 2. This may be attributed to the presence of less galacturonan and more neutral sugar-rich pectic polysaccharides in CF 2 squash tissues compared with other cultivars. Higher levels of neutral sugar-rich pectic polysaccharides and less galacturonan for CF 2 squash could contribute to its greater firmness (*18*).

Galactose was the major neutral monosaccharide extracted from the first and second HEPES-soluble fractions for all cultivars. Uronic acid composed 70−85 and 53−77% (w/w) of these fractions, respectively, for all four cultivars, indicating these two fractions contained mostly polygalacturonan. Galactose was also the major neutral monosaccharide extracted from DMSO-soluble fractions for all cultivars. The DMSO-soluble fractions contained large amounts of glucose compared to the other fractions, presumably derived from starch.

The galactose content of the third HEPES-soluble fraction of cooked CF 2 squash was 52% of the fraction, whereas the galactose contents in similar fractions of Delica and CF 4 were 15 and 30%, respectively (**Table 3**). The higher amount of galactose in the third HEPES-soluble fraction for CF 2 squash compared with other cultivars, together with relatively higher arabinose in the same fraction, suggests the pectic polysaccharide in CF 2 consists of side chains composed of galactose and arabinose. These branched pectins could be bound to, or entangled with, other cell-wall polysaccharides, which would also account for the increased the firmness of the CF 2 compared with other squash cultivars (*18*).

Galactose accounted for 27, 13, 47, and 50% (w/w) of the cooked α-amylase fractions of Delica, Red Warren, CF 2, and CF 4, respectively (**Table 5**). These proportions were much higher than those in the corresponding fractions from raw squash. The arabinose contents of amylase-soluble fractions of cooked squashes were also relatively high. These results together with relatively low uronic acid in these fractions indicate that the amylase fractions contained branched pectic polysaccharides. Because branched pectic polysaccharide were found in the CDTA- and Na_2CO_3-soluble fractions of the cell walls of raw squash, the pectic polysaccharides in the amylase fractions of the cooked squash were probably similar to the pectic polysaccharides in the CDTA- and Na_2CO_3-soluble fractions from raw squash, but made more soluble by the cooking process.

The amount of neutral sugars in the combined water-soluble fractions (cooking water-, first HEPES-, second HEPES-, DMSO-, third HEPES-, and amylase-soluble fractions) increased by 8, 4, 5, and 7% for Delica, Red Warren, CF 2, and CF 4, respectively, from raw to cooked stages. As discussed earlier, the uronic acid content of the combined water-soluble fractions increased by 9−10% for Delica, Red Warren, and CF 4 from raw to cooked stages, whereas those extracted in combined water-soluble fractions of CF 2 squash showed no change from raw to cooked. Collectively, these results show that steaming increased the extractability of cell-wall polysaccharides, especially galacturonan and branched pectic polysaccharides for Delica, Red Warren, and CF 4 squash. As CF 2 contained relatively low amounts of uronic acid compared to other cultivars, steaming had less effect on the extractability of branched pectic polysaccharides in CF 2 squash. This may be the reason the tissues of CF 2 squash retained more of their firmness on cooking (*18*).

The combined CDTA- and Na_2CO_3-soluble fractions contained 18% of the total CWP for cooked buttercup squash Delica (**Table 3**), whereas equivalent combined fractions for all other cooked cultivars contained 15% each of the total CWP. The equivalent combined fractions for raw Delica, Red Warren, and CF 4 contained 14% each of the total CWP, whereas the equivalent combined fraction for CF 2 was only 11%. The values for Red Warren and CF 4 were unchanged by cooking. The combined CDTA- and Na_2CO_3-soluble fractions of Delica and CF 2 contained 43% of the total uronic acid (**Table 4**), which was ∼10% higher than those of raw, whereas those for Red Warren and CF 4 remained constant with cooking. Together, these results show that steaming increased the extractability of CDTA- and Na_2CO_3-soluble pectic polysaccharides from Delica and CF 2 but not Red Warren and CF 4.

Total CWP extracted by 4 M KOH from cooked squash was 0.5−2% greater than from raw squash, depending on cultivar. Similarly, the residue wash fractions of cooked squash contained 1−2% less CWP than did raw squash, suggesting that cooking increased the extractability of pectic polysaccharides associated with cellulose in raw squash. This idea is confirmed when one notes that the final residue contained 50, 56, 59, and 55% of total CWP for Delica, Red Warren, CF 2, and CF 4 squash, respectively (**Table 4**), relatively lower values than those of raw squash (59, 60, 63, 58%). However, cellulose contents of cooked squash tissues were almost identical to those for raw tissues of all four cultivars (**Tables 2** and **3**), indicating cooking does not change the cellulose content of squash cell walls.

Xylose made up 17−20% of the 4 M KOH-soluble fraction of cooked Delica, CF 2, and CF 4 but 27% of Red Warren (**Table 3**). However, the glucose:xylose ratios in the 4 M KOH-soluble fractions of all cooked squash cultivars were similar to

Table 4. Carbohydrate Compositions and Ratio of UA:(Ara + Gal) of Cell-Wall Fractions of Raw Delica Squash during Storage[a]

storage	fraction	Rha (mg)	Ara (mg)	Xyl (mg)	Man (mg)	Gal (mg)	Glu (mg)	UA (mg)	UA:(Ara + Gal)
harvest	1st HEPES	0.2	1.7	tr	1.2	3.8	1.0	49.1	8.8
	2nd HEPES	0.2	0.7	0.1	0.5	3.0	1.2	17.4	4.6
	DMSO	tr	0.1	tr	0.2	0.4	1.3	1.9	3.7
	amylase	0.1	0.2	0.1	0.5	0.6	0.4	9.2	11.1
	CDTA	0.4	1.5	tr	1.2	4.4	tr	19.7	3.3
	Na_2CO_3	2.2	3.9	tr	1.9	17.0	tr	70.7	3.4
	4 M KOH	0.3	3.4	15.8	0.8	20.9	17.2	10.4	0.4
	residue wash	1.2	4.1	1.0	2.0	28.8	0.7	25.9	0.8
	final residue	2.6	8.4	4.5	6.3	59.4	23.9 (343)	59.1	0.9
	total[b]	7.1	24.0	21.5	14.5	138.5	45.8 (343)	263.3	
1 month	1st HEPES	0.2	1.1	tr	1.7	3.1	1.3	44.3	10.8
	2nd HEPES	0.7	2.2	0.4	1.6	8.2	1.8	18.9	1.8
	DMSO	tr	0.3	tr	0.2	0.7	0.6	2.4	2.3
	amylase	tr	0.2	tr	0.6	0.9	1.0	8.0	7.3
	CDTA	0.4	0.5	0.2	1.2	4.7	tr	19.7	3.8
	Na_2CO_3	2.0	2.3	0.3	2.2	17.8	tr	65.0	3.2
	4 M KOH	0.3	3.5	12.1	1.0	13.4	14.5	7.0	0.4
	residue wash	1.0	8.3	1.2	0.5	36.8	0.4	33.7	0.7
	final residue	3.4	7.6	4.0	8.0	59.2	19.2 (350)	49.6	0.7
	total[b]	8.0	25.9	18.3	17.1	144.9	38.9 (350)	248.6	
2 months	1st HEPES	0.4	2.8	0.2	0.9	4.6	2.0	32.3	4.3
	2nd HEPES	0.3	2.4	0.2	0.3	7.2	0.8	12.4	1.3
	DMSO	tr	0.5	0.3	0.8	0.7	1.1	4.1	3.5
	amylase	tr	0.2	0.1	0.3	0.5	1.0	9.2	14.0
	CDTA	0.2	0.9	tr	1.2	5.9	tr	23.9	3.5
	Na_2CO_3	1.6	3.0	tr	1.3	14.9	1.4	76.5	3.7
	4 M KOH	0.5	3.5	11.0	0.9	14.9	12.8	6.2	0.3
	residue wash	0.7	4.2	1.1	0.7	20.9	1.4	36.9	1.1
	final residue	3.3	8.2	4.7	5.9	62.8	23.5 (346)	56.0	0.8
	total[b]	7.3	25.7	17.5	12.6	137.4	44.2 (346)	258.5	
3 months	1st HEPES	0.5	1.9	tr	0.6	2.7	1.1	40.2	8.6
	2nd HEPES	0.6	2.1	tr	1.4	4.7	1.7	37.2	5.5
	DMSO	tr	0.2	tr	0.3	0.5	2.1	1.9	2.7
	amylase	tr	0.2	tr	0.3	0.4	1.5	6.2	9.8
	CDTA	1.0	2.5	tr	tr	3.8	tr	26.9	4.3
	Na_2CO_3	1.2	2.7	tr	0.8	12.8	tr	69.6	4.5
	4 M KOH	1.1	3.7	10.2	0.9	12.9	8.8	3.5	0.2
	residue wash	0.5	4.2	tr	0.8	20.2	1.1	17.0	0.7
	final residue	2.5	6.1	3.0	4.8	42.2	16.0 (339)	32.1	0.7
	total[b]	7.3	23.6	13.3	9.9	100.2	32.3 (339)	234.5	

[a] Expressed as mg/g of dry cell-wall polysaccharides. Data represent the mean of four analyses. tr, trace (<0.1 mg); Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man. mannose; Gal, galactose; Glu, glucose (values in parentheses are for cellulose); SD, standard deviation. [b] Σ means. SD < 10% of the mean of four analyses.

the ratios in raw squash. These results indicate that most of the xyloglucans and heteroxylans in squash cultivars were heat stable.

As for the raw squash the ratios of UA:(Ara + Gal) in the 4 M KOH- and residue wash-soluble fractions for all cultivars were significantly less than those of the earlier CDTA- and Na_2CO_3-soluble fractions (**Table 3**), indicating that the 4 M KOH- and residue wash-soluble fractions contained more highly branched pectic polysaccharides than in the CDTA- and Na_2CO_3-soluble fractions. Likewise, the ratio of UA:(Ara + Gal) for cooked Red Warren squash was higher than those of the other cultivars, confirming that the Red Warren squash contained less highly branched pectic polysaccharides than other cultivars.

The final residue (α-cellulose) fraction from the cell walls of cooked squash had less uronic acid proportions than those in the equivalent fraction of raw squash. Similarly, it has been reported (*15*) that the total pectic polysaccharide contents of the α-cellulose fraction from carrots decreased on cooking. Glucose accounted for 63−77% of the neutral monosaccharide composition of this fraction, which is significantly higher than in raw squash and consistent with the stabilty of cellulose on cooking.

The ratios of UA:(Ara + Gal) in the α-cellulose fractions from cooked squash were 0.5, 1.1, 0.8, and 0.5 for Delica, Red Warren, CF 2, and CF 4, respectively (**Table 5**) and were lower than those of the α-cellulose fractions in raw squash, except for Red Warren. The latter showed no change on cooking. These results show that the α-cellulose fraction from cooked squash tissues contained pectic polysaccharides with a more highly branched pectic polysaccharide backbone and/or longer neutral side chains than the pectic polysaccharides retained in the α-cellulose fraction from the raw squash, except Red Warren. Likewise, the α-cellulose fraction of cooked carrots contained more highly branched pectic polysaccharides than raw carrot (*15*).

Changes of Carbohydrate Composition of Cell Walls during Storage of Delica and Red Warren. The composition of CWP from raw tissues of all four cultivars was monitored during storage. However, because the pattern of change in CWP of CF 2 and CF 4 squash during storage was very similar to that seen in stored Delica squash, only the results of Delica and Red Warren are reported here (**Tables 4** and **5**). The galactose content of the combined first HEPES- and second HEPES-soluble fractions from both Delica and Red Warren

Table 5. Carbohydrate Compositions and Ratio of UA:(Ara + Gal) of Cell-Wall Fractions of Raw Red Warren Squash during Storage[a]

storage	fraction	Rha (mg)	Ara (mg)	Xyl (mg)	Man (mg)	Gal (mg)	Glu (mg)	UA (mg)	UA:(Ara + Gal)
harvest	1st HEPES	0.2	5.2	tr	0.3	11.5	3.4	44.6	2.6
	2nd HEPES	0.1	1.2	0.1	tr	5.4	2.8	25.2	3.8
	DMSO	tr	tr	tr	tr	0.6	2.0	2.2	3.3
	amylase	tr	0.2	0.1	0.2	0.6	1.6	7.1	9.2
	CDTA	0.1	0.5	tr	0.6	3.4	0.6	21.8	5.5
	Na_2CO_3	1.2	2.1	tr	1.2	9.4	tr	77.3	6.7
	4 M KOH	tr	1.2	9.1	1.1	3.5	6.3	4.6	0.9
	residue wash	1.0	3.7	1.3	2.0	9.9	0.7	50.5	3.7
	final residue	6.2	10.7	5.2	3.2	66.3	29.7 (285)	85.7	1.1
	total[b]	8.9	24.8	15.8	8.7	110.7	47.3 (285)	319.1	
1 month	1st HEPES	0.2	9.3	tr	2.0	25.8	3.8	45.1	1.3
	2nd HEPES	0.1	0.7	0.2	0.5	1.9	2.8	34.5	13.4
	DMSO	tr	0.2	0.1	0.2	0.8	2.1	3.9	3.9
	amylase	tr	1.3	tr	0.1	1.7	1.1	4.6	1.5
	CDTA	tr	0.6	tr	tr	2.0	tr	11.7	4.4
	Na_2CO_3	0.9	1.3	tr	tr	8.0	tr	68.4	7.4
	4 M KOH	0.6	2.3	10.4	1.3	7.4	7.8	7.7	0.8
	residue wash	0.5	1.9	1.7	0.7	5.6	1.5	57.0	7.6
	final residue	6.1	9.0	4.9	2.6	57.1	25.5 (283)	90.6	1.4
	total[b]	8.4	26.5	17.2	7.4	110.3	44.5 (283)	323.5	
2 months	1st HEPES	0.3	6.3	0.4	2.7	23.5	3.7	45.4	1.5
	2nd HEPES	0.2	0.9	tr	1.4	3.9	2.2	35.8	7.6
	DMSO	tr	0.5	0.5	0.2	2.3	1.2	6.7	2.4
	amylase	tr	0.4	0.5	0.2	0.7	0.5	9.4	8.8
	CDTA	0.3	0.6	tr	0.2	3.6	0.2	32.2	7.7
	Na_2CO_3	0.9	1.7	tr	0.1	7.1	tr	61.3	7.0
	4 M KOH	tr	1.1	7.0	1.1	2.8	7.2	7.9	1.9
	residue wash	1.5	2.7	1.5	1.7	13.8	1.9	55.5	3.4
	final residue	4.3	5.6	4.7	1.9	52.8	16.6 (294)	68.6	1.2
	total[b]	7.5	19.7	14.6	9.5	110.6	33.4 (294)	322.7	
3 months	1st HEPES	1.0	3.6	tr	1.6	16.8	2.3	54.8	2.7
	2nd HEPES	0.3	1.2	0.4	0.8	3.1	0.9	42.7	9.9
	DMSO	tr	0.3	0.4	0.2	1.0	0.7	5.4	4.4
	amylase	tr	tr	0.1	0.3	0.2	0.6	6.5	36.1
	CDTA	tr	0.6	tr	tr	4.6	tr	36.1	7.0
	Na_2CO_3	1.6	2.2	0.3	tr	9.1	0.5	73.0	6.5
	4 M KOH	0.3	1.0	7.1	0.9	3.1	6.6	4.5	1.1
	residue wash	1.8	2.9	1.3	1.0	4.8	1.4	40.6	5.3
	final residue	2.6	6.1	5.5	2.7	27.3	19.7 (279)	60.5	1.8
	total[b]	7.5	17.7	15.1	7.6	70.0	32.6 (279)	324.3	

[a] Expressed as mg/g of dry cell-wall polysaccharides. Data represent the mean of four analyses. tr, trace (<0.1 mg); Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose (values in parentheses are for cellulose); SD, standard deviation. [b] Σ means. SD < 10% of the mean of four analyses.

increased after 1 month of storage, remained constant between 1 and 2 months, and then decreased after 2 months of storage, indicating that the extractability of water-soluble pectic polysaccharide increased from harvest up to 2 months of storage. Whereas in Delica galactose in the HEPES fractions was distributed about 2:1 between the second and first fractions during storage (**Table 4**), in Red Warren by far the greater proportion of galactose was found consistently in the first fraction during storage (**Table 5**), indicating that the material from Red Warren is more soluble.

Simandjuntak et al. (*13*) reported that the total uronic acids did not change as maturity progressed in muskmelons. Likewise, the amount of total uronic acid remained relatively constant during storage of both squash cultivars (**Tables 4** and **5**). However, its distribution in the various CWP fractions varied with storage time and between cultivars. For example, the uronic acid content of the combined first and second HEPES-soluble fractions of Red Warren increased steadily throughout storage, whereas in Delica it did not increase substantially until between 2 and 3 months of storage. In contrast, the uronic acid content of the combined residue wash and final residue fractions of Red Warren decreased steadily between 1 and 3 months of storage, whereas in Delica the uronic acid content of this combined fraction did not decrease until between 2 and 3 months. Collectively, these results show that the extractability of pectic polysaccharides in Red Warren squash increased sooner with storage (after 1 month) than it did with Delica (after 2 months). These results agree well with the more rapid loss of firmness of Red Warren squash in storage compared with Delica (*18*).

The galactose content in the combined CDTA- and Na_2CO_3-soluble fractions of Delica squash remained unchanged from harvest up to 2 months of storage and then decreased (**Table 4**). Moreover, the ratio of UA:(Ara + Gal) in these fractions showed no change during 1−2 months of storage but then increased during 2−3 months of storage (**Table 4**). It was most likely that the loss of galactose caused this change in the UA:(Ara + Gal) ratio on storage. In contrast, the galactose content in the CDTA- and Na_2CO_3-soluble fractions of Red Warren fluctuated throughout storage, and this was mirrored by the UA:(Ara + Gal) ratios in these fractions (**Table 5**).

The glucose:xylose ratio in the 4 M KOH-soluble fraction of Delica remained constant between harvest and 2 months (1.2) but was then followed by a decrease (0.9) (**Table 4**), indicating degradation and solubilization of hemicellulose in the Delica cultivar after 2 months of storage. The pattern in Red Warren is somewhat different—the greatest increase in the glu-

cose:xylose ratio occurred between 1 (0.75) and 2 months (1.0) of storage (**Table 5**). However, whereas the total xylose content of Delica fell steadily during storage, total xylose in Red Warren showed no clear trend. On the other hand, total arabinose content remained constant in Delica during storage, whereas it fell by 20% in Red Warren between 1 and 2 months of storage. Collectively, these results point to substantial differences in xyloglucan and arabinopectin structures between cultivars.

The amount of cellulose in raw Delica and Red Warren squash did not change during storage (**Tables 4** and **5**). The high contents of uronic acid, galactose, and arabinose in the final residue and residue wash up to 2 months of storage of both cultivars confirmed that significant amounts of branched pectic polysaccharides remain tightly associated with α-cellulose in the cell walls up until this time. However, both cultivars showed substantial losses of galactose from the final residue after 3 months of storage, indicating either that these pectic polysaccharides became less tightly bound to the α-cellulose or that most of the metabolic degradation of galactose occurs in this fraction.

Chemical Markers of Texture. We have achieved our aim of identifying cultivar-specific differences in the cell-wall polysaccharide composition of *C. maxima*. In both raw and cooked squash tissues, the lower firmness, high rigidity, and lower compressibility of Red Warren (*18*) were associated with the least cellulose and neutral sugar contents and highest uronic acid, whereas the greater firmness, lower rigidity, and higher compressibility of CF 2 (*18*) were associated with the most cellulose and neutral sugar contents and the least uronic acid. Delica and CF 4, which have similar textures that are intermediate between Red Warren and CF 2 (*18*), had CWP compositions between the two extremes. Moreover, the lower firmness of Red Warren is associated with the presence of fewer branched pectic polysaccharides. Selected chemical markers of texture will be used in the evaluation of a breeding experiment in which Red Warren has been crossed with CF 2 and CF 4, producing the F1 and F2 and backcross generations of each cross (RW × CF 2 and RW × CF 4) and which was designed to elucidate the heritability of texture attributes in *C. maxima*. Uronic acid and galactose can be used as chemical markers of texture by determining them in the combined HEPES-soluble fractions and in the isolated cell walls from raw squash.

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