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Effect of Storage on Wall-Bound Phenolics in Green Asparagus

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The cell walls of green asparagus spears have been analyzed for their phenolic and carbohydrate composition as modified by postharvest storage. Esterified phenolic components were released by sequential alkaline hydrolysis and identified and quantified by diode array HPLC. Significant quantities of ferulic acid (FA) and its derivatives were found to increase at least 3-fold during storage, particularly in walls from the lower parts of the stem, where accompanying changes in sugar composition were also observed. In fresh asparagus, >60% of the total FA was in the form of diferulic acid, and this increased to ∼70% after 3 days of storage. The main FA dehydrodimers were 8-8-, 8-*O*-4-, and 8-5-diferulates. These have been detected in other monocotyledonous and dicotyledonous plants, but as a smaller proportion of the total FA. The possible roles phenolic esters might have in relation to the mechanical, textural, and wound-response properties of asparagus spears are discussed.

KEYWORDS: **Cell wall (CW); phenolics; asparagus; storage; ferulic acid; diferulic acid**

INTRODUCTION

Asparagus spears are rapidly growing, edible, immature plant stems that senesce rapidly after harvest (*1*). The physiological changes that occur affect cell wall composition, structure, tissue mechanical properties, and texture (*2*−*5*). Previous investigations in asparagus have demonstrated a high degree of postharvest metabolic activity in the cell walls. For example, harvested spears rapidly lose wall-bound galactose and show an increase in xylans and cellulose (*4*, *6*, *7*). Of particular interest are the major changes that occur in cell walls in the lower parts of the stem. Asparagus contains a range of tissues, some of which are lignified and contribute differently to mechanical properties of the whole spear (*4*, *5*). Lipton (*8*) has suggested that the pericyclic (sclerencyma) fibers play a dominant role in determining the textural characteristics of asparagus. These fibers are thin walled toward the tip of the spears but thick walled and lignified near the base, and they accompany the increase in sensory toughness of this region. Indeed, the basipetal toughening of asparagus has often been attributed to lignification. However, a number of studies have demonstrated that phenolic components other than those involved in lignfication can have a significant impact on the texture of plant tissues (*9*, *10*), especially after thermal processing.

It is well established that the cell walls of a number of nonlignified, edible plant tissues contain phenolic esters. Most notable are the trans-isomers of *p*-coumaric (PC) and ferulic acid (FA) (*11*). FA has been found to be esterified to several polysaccharide families, including arabinoxylans (*12*) and pectic polysaccharides (*13*). FA can be cross-linked as a result of peroxidative activity (*14*), and a number of diferulic acid (DiFA) species have been identified (*13*, *15*). Their role in cross-linking polysaccharides has been implicated in conferring thermal stability of mechanical properties of edible plant tissues including Chinese water chestnut (CWC) (*16*), chufa (*17*), and beetroot (*18*) by reducing the propensity for cell separation during thermal processing. The formation of these dehydrodimers is also likely to have an impact on the other cell wall mechanical properties, including extensibility (*19*, *20*). In grasses (*15*, *21*) they have also been proposed as nucleation sites for lignification, and they are likely to have a role in disease resistance (*10*).

There is little information on the occurrence of such phenolic esters in the cell walls of asparagus. Nevertheless, non-lignin phenolics have been detected, and it is likely that postharvest changes in texture of asparagus tissues are related to the formation of pectic−xylan−phenolic complexes in the cell walls, which may affect cell adhesion (*3*, *4*, *10*). The phenolic components were not identified or characterized. The present study seeks to address this in part by investigating the nature and extent of alkali-soluble phenolics in the cell walls of green asparagus spears as modified by postharvest storage.

MATERIALS AND METHODS

Plant Material. *Asparagus officinalis* L. cv. Franklin was obtained from a local commercial asparagus producer. Spears, ca. 18 cm in length and 1.0−1.5 cm in diameter, were marked at 5 cm intervals from the tip to a distance of 15 cm (upper, middle, and lower sections; tissues below the lower section are referred to as basal tissues). Twelve groups consisting of 500 ± 30 g of asparagus were packaged in trays overwrapped with perforated film (*3*, *4*). Lengths and weights of all spears and their sections were measured at $t = 0$ and after storage at 21 °C for 3 days.

Two different tissue types were dissected from the middle and lower sections: the inner (white) tissue contained mainly parenchyma cells and vascular bundles; the outer (green) tissue consisted of sclerenchyma

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and epidermal cells. Separation of tissues was not practical from the upper stem section.

The separation of stem sections and component tissues was performed at 1 °C, and all tissues were frozen immediately in liquid nitrogen and then stored at −20 °C until required.

Preparation of Asparagus Cell Wall Material (CWM). CWM was isolated from the different sections and tissue types of both fresh and stored asparagus. Cell walls were prepared as described by Parker and Waldron (*16*) with some modifications. One hundred grams of frozen asparagus sections or tissues was suspended in 15 g/L aqueous sodium lauryl sulfate (SDS) containing 5 mM $Na_2S_2O_5$ and blended with an Ystral homogenizer (Ystral GmbH, Dottingen, Germany) for 5 min. A few drops of octanol were added to reduce foaming. The mixture was filtered through a 100 μm nylon mesh (John Stannier and Co., Manchester, U.K.), and the residue was ball-milled (Pascall 0.51 pot) at 1 °C in 5 g/L SDS containing 3 mM $Na_2S_2O_5$ for 16 h at 60 rpm. After the homogenate was filtered through a 75 μm nylon mesh, the residue was resuspended in cold water containing 3 mM $Na_2S_2O_5$, homogenized for 5 min, and refiltered. This last procedure was repeated (five times) until the CWM was free of cell contents as checked by light microscopy and staining with iodine/potassium iodide. CWM was stored as a frozen suspension at −20 °C. Prior to analysis of sugar and phenolic ester composition, the CWM was further extracted with hot ethanol (100 °C, 5 min), washed with acetone, and air-dried. Particle size was reduced by grinding for 5 min, in a water-cooled Janke and Kunkel mill (Analysemmuhle, type A 10, IKA Labortechnik), in order to obtain powdered samples that would facilitate quantitative extraction of cell wall components.

Analysis of Carbohydrate Composition. Cell wall neutral sugars and uronic acids were analyzed after acidic hydrolysis of the CWM, as described in Parker and Waldron (*16*).

Analysis of Phenolics by HPLC. Wall-bound phenolics were released by sequential alkaline hydrolysis of isolated CWM as described by Hartley and Morrison (*22*). CWM (200 mg) was extracted with 0.1 M NaOH (1 h, 25 °C) under N_2 in darkness. The suspension was filtered through glass fiber paper (GF-A, Whatman, Maidstone, U.K.) and the residue retained for further extraction with, sequentially, 0.1 M NaOH for 24 h, 1 M NaOH for 24 h, and 2 M NaOH for 24 h, each at 25 °C. *trans*-Cinnamic acid (CA) (6.4 μg) was added to all filtrates as an internal standard. The solutions were then acidified to pH <2 with concentrated HCl and extracted three times with ethyl acetate (3 volumes). The ethyl acetate extracts were combined and evaporated to dryness under a stream of nitrogen. The samples were dissolved in 200 μL of 50% (v/v) aqueous methanol and analyzed by HPLC. The residue remaining after saponification of CWM was washed with water until the cell wall residue was free of sodium hydroxide and retained for lignin analysis.

Phenolics were detected and quantified by HPLC using an Interpak ODS2-IK5 reversed-phase column (25 cm × 4.6 mm i.d., 5 μm; Capital HPLC Ltd., Broxburn, West Lothian, U.K.) with gradient elution employing progressively increasing methanol/acetonitrile levels in 1 mM trifluoroacetic acid (TFA) (*23*). The gradient profile used for separation of wall-bound phenolics was formed using solvent A (10% (v/v) aqueous acetonitrile plus TFA to 1 mM) and solvent B (40% methanol, 40% acetonitrile, and 20% water plus TFA to 1 mM) in the following program: initially A 90%/B 10%; linear gradient over to 25 min to A 25%/B 75%; exponential gradient over 5 min to A 0%/B 100%; exponential gradient over 10 min to A 90%/B 10%; held at A 90%/B 10% for a further 2 min. Flow rate was at 1 mL min^{-1}. The solvents were sparged with helium prior to use. Detection was with a Perkin-Elmer diode array detector 235 C DAD. Quantitation was by integration of peak areas at 280 nm, with reference to known amounts of pure compounds.

Klason Lignin of CWM. The lignin contents of asparagus sections and different tissue types were calculated as described by Theander et al. (*24*). Samples of CWM were dispersed in 72% (v/v) H_2SO_4 for 3 h at room temperature, followed by dilution to 1 M and hydrolysis for 2.5 h at 100 °C. While the resulting hydrolysis mixture was still warm, the insoluble material was quantitatively filtered off (Pyrex No. 2 glass filter) and thoroughly washed with hot water (3 × 10 mL). The glass filter was dried until constant weight (80 °C, 18 h) and the filtered material, Klason lignin, quantified gravimetrically.

Table 1. Weights (Grams) of Green Asparagus during Shelf Life

	days of shelf life			
	0		3	
section	mean	SD	mean[a]	SD
upper	2.40	0.14	2.30**	0.18
middle	3.21	0.21	3.11*	0.24
lower	4.14	0.26	3.82***	0.31
basal	4.40	0.38	3.75***	0.42
whole spear	14.08	0.72	12.94***	0.68

[a] ***, **, and * denote significant differences compared with day 0 value at $P < 0.001$, < 0.0025, and < 0.25, respectively.

Statistical Analysis. All data were analyzed using ANOVA General Linear Model from EXCEL, with the following factors: position, upper, middle, and lower; tissue type, internal and external; process, fresh and stored.

RESULTS

Freshly harvested asparagus spears were stored as described under Materials and Methods. Length and weight were compared before and after 3 days of storage at 21 °C. Spears exhibited an 8.1% decrease in weight, and this occurred mainly in the lower and basal stem sections (**Table 1**), which lost 7.7 and 14.8% of their weights, respectively. The top and middle sections exhibited a smaller degree of weight loss (4.2 and 3.1%, respectively).

Storage-related increases in length were observed mainly in the upper stem section, which, after 3 days of storage, had increased from 5.0 cm to an average of 5.5 cm. In the other sections, extension growth was minimal.

Cell walls were prepared from upper, middle, and lower sections from both fresh and stored samples and from outer (sclerenchyma-rich) and inner (parenchyma- and vascular tissue-rich) tissues. These were investigated for their phenolic and sugar composition.

The yield of CWM was between 1.76 and 2.12% on a fresh-weight (FWt) basis and increased basipetally (**Table 2**). The yield of CWM was found to be greater in the outer (sclerenchya-rich) layers than in the inner (parenchma and vascular) tissues (**Table 2**). As in the case of whole sections, storage resulted in an increase in the yield of CWM. The carbohydrate composition of the cell walls was ∼80% (**Table 2**). During storage, carbohydrate content increased by ∼10%.

As reported previously (*4*), storage resulted in changes in cell wall carbohydrate composition. In particular, storage was accompanied by a considerable decrease in the levels of galactose (Gal) and arabinose (Ara), and this was accompanied by a relative increase in glucose (Glc), uronic acid (UA), and xylose (Xyl). Similar trends were found in the different sections of the spear. Interestingly, the storage-related increases in UA and Xyl occurred predominantly in the outer tissues (**Table 2**) and, consistent with the earlier results, were greatest in the lower section. However, the increase of Xyl was not as large as in white asparagus, where the most prominent change of the wall carbohydrate is seen in the lower parts of the spear (*7*).

Noncellulosic sugar composition was also investigated by hydrolyzing CWM with 1 M H_2SO_4 only and calculating the crystalline cellulose content by difference. These analyses revealed that in excess of 85% total wall glucose was in the form of cellulose. Furthermore, the storage-related increase in cellulosic glucose occurred predominantly in the inner tissues.

Table 2. Yield and Sugar Composition of Cell Walls from Green Asparagus Tissues[a]

		yield (%)	mg/gCWM									
		(FWt)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Glc (1 M)	UA	total
						Whole Sections						
top	fresh	1.76	21.95	6.66	112.59	51.09	28.94	89.25	269.98	(24.80)	148.00	728.46
	stored	1.83	27.95	7.99	83.01	69.60	27.32	46.76	338.31	(29.77)	230.60	831.52
middle	fresh	1.76	13.89	4.87	69.75	40.44	18.49	98.11	233.64	(34.01)	218.60	697.79
	stored	1.81	18.30	5.70	44.90	55.50	27.40	34.20	295.5	(83.63)	261.60	743.10
lower	fresh	1.95	9.93	5.54	65.67	74.88	30.96	140.72	295.53	(29.45)	228.19	851.42
	stored	2.12	20.48	6.97	54.17	96.35	34.93	58.63	422.29	(73.43)	269.11	962.94
						Excised Tissues						
middle	fresh	1.82	18.6	5.8	55.3	78.8	27.3	149.0	314.0	(33.13)	214.0	862.8
(inner)	stored	1.91	21.6	8.2	47.9	91.0	37.9	45.0	392.9	(41.96)	206.5	850.9
middle	fresh	1.89	19.9	6.6	73.5	58.7	35.5	138.0	406.6	(39.78)	145.6	884.3
(outer)	stored	2.00	20.5	7.9	52.2	92.5	34.3	48.3	435.5	(58.91)	259.9	951.1
basal	fresh	1.86	21.6	7.5	56.4	67.0	33.4	180.2	400.9	(63.73)	192.7	959.6
(inner)	stored	1.97	23.8	7.9	40.7	76.2	34.9	51.6	450.4	(67.04)	191.3	876.9
basal	fresh	1.94	17.1	5.7	78.6	75.7	28.6	123.4	352.3	(41.63)	135.3	816.7
(outer)	stored	1.98	26.6	11.1	61.6	144.5	63.2	44.3	378.5	(76.00)	214.5	944.3

[a] Values are the means of four replicates. SD < 5%. Data in parentheses correspond to noncellulosic glucose.

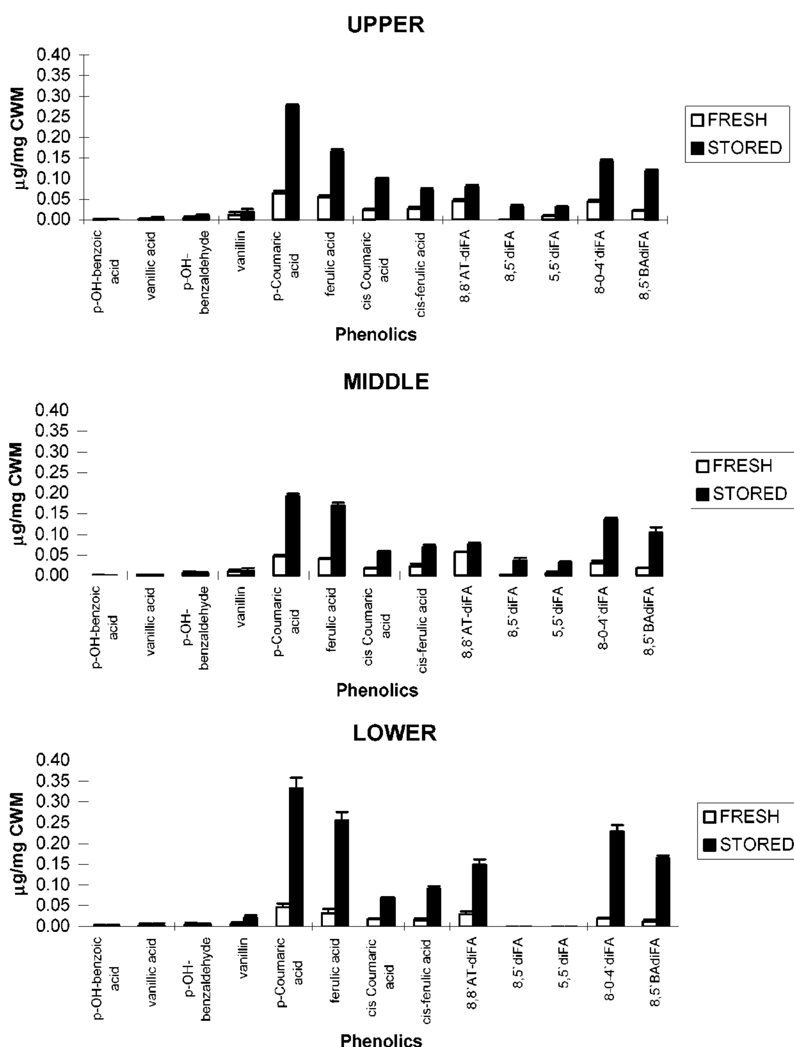

Figure 1. Total esterified phenolics (micrograms per milligram of CWM) from fresh and stored asparagus (upper, middle, and lower sections).

The storage-related decreases in Gal and Ara occurred in both inner and outer tissues.

Phenolic Analysis. *Total Phenolics.* Phenolic monomers and dimers were released by sequential alkaline extraction from the CWMs that had been prepared from different sections of the stem (**Figure 1**). They comprised mainly *cis*- and *trans*-CA, *cis*- and *trans*-FA, and its diferulate derivatives.

In fresh asparagus, the total amounts of saponified phenolics quantified were <0.02% of the CWM (**Figure 1**). They were higher in the upper (316 μg/g of CWM) than in the middle (231

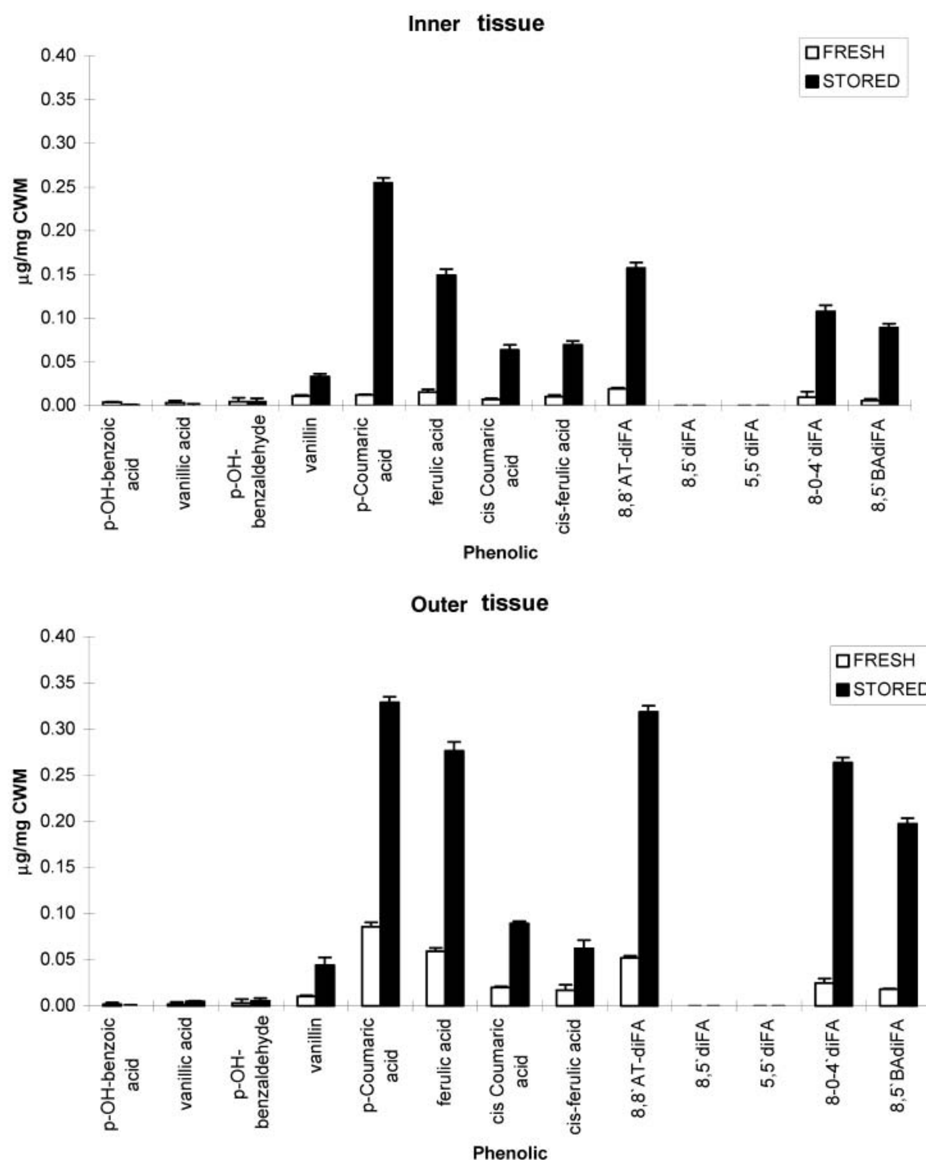

Figure 2. Total esterified phenolics (micrograms per milligram of CWM) from fresh and stored asparagus tissues separated from the lower section of the stem.

μg/g of CWM) and lower sections (187 μg/g of CWM). The phenolic esters in these sections comprised approximately 60% FA and its derivatives and 30% coumaric acid. Interestingly, the dehydrodimers of FA comprised >60% of the total ferulate complement. They consisted of 5,5′-diferulic acid (5,5′diFA), 8-*O*-4′-diferulic acid (8-O-4′diFA), 8,5′-diferulic acid open form (8,5′diFA), 8,5′-diferulic acid benzofuran form (8,5′BAdiFA), 8,8′-diferulic acid open form (8,8′diFA), and 8,8′-diferulic acid aryltetralyn form (8,8′ATdiFA) as described by Ralph et al. (*15*). The most prominent of these were 8,8′ATdiFA, 8-*O*-4′diFA, and 8,5′BAdiFA; only small quantities of 5,5′diFA were found in the different sections of the spear.

Storage of asparagus spears resulted in a considerable increase of phenolic compounds in each section of stored material, particularly in the lower section (1000 μg/g of CWM in the upper section, 874 μg/g of CWM in middle section, and 1317 μg/g of CWM in the lower sections). This was accompanied by an increase in the proportion of diferulic acid moieties in the middle and lower sections.

Analysis of the different tissue types showed that in fresh asparagus, wall phenolic esters were located mainly in the outer tissues. However, storage resulted in a large increase in phenolics in both tissue types. Results are presented for the basal stem section in **Figure 2**.

Extractability of Phenolics. Several differences were found in the extractability of phenolics from fresh and stored asparagus. In the upper (**Table 3**) and middle (**Table 4**) sections of fresh spears, the majority of the phenolics were recovered in the first steps of the sequential extraction (0.1 M NaOH, 2 h; and 0.1 M NaOH, 24 h). However, the majority of phenolics from the lower section (**Table 5**) were released only after prolonged hydrolysis in strong alkali (2 M NaOH, 24 h). Storage of asparagus resulted in an increase in the quantity of phenolics released in every step of the sequential extraction. The majority of phenolics were released with dilute alkali (0.1 M NaOH), and this was the same for all sections (**Tables 3−5**). Analysis of the two different tissue zones separated from the middle and basal portions of the stem revealed that the FA dehydrodimers comprised ~40% of the total FA complement in the internal tissues of fresh asparagus (**Table 6**). This increased to 60% during postharvest storage. The FA dehydrodimers were significantly higher in the outer tissues, comprising 55% of total FA for fresh asparagus and nearly 70% for the stored samples (**Table 7**). Interestingly, after storage, the degree of dimerization

Table 3. Esterified Phenolic Acids (Micrograms per Gram) of CWM from the Upper Section of Fresh and Stored Asparagus[a]

	0.1 M NaOH				1 M NaOH		2 M NaOH	
	1 h		24 h		24 h		24 h	
	fresh	stored	fresh	stored	fresh	stored	fresh	stored
p-hydroxybenzoic acid	0.6	0.3	0.0	0.1	0.0	0.0	0.8	0.0
vanillic acid	0.8	1.4	0.4	0.6	0.3	1.1	0.4	1.6
p-hydroxybenzaldehyde	1.2	3.6	0.1	1.7	1.4	2.6	1.4	1.9
vanillin	3.1	5.1	1.1	3.5	5.9	6.4	3.3	5.9
p-coumaric acid	19.4	119.7	21.1	60.4	16.5	54.9	12.3	37.5
ferulic acid	13.6	50.0	14.4	39.4	12.3	48.1	13.8	28.8
cis-coumaric acid	7.6	33.8	5.9	14.3	5.1	23.7	4.8	19.2
cis-ferulic acid	9.4	23.9	6.4	23.6	6.8	16.1	3.7	12.9
8-8′AT diFA	15.3	50.1	15.0	24.5	7.7	2.	7.2	4.1
8-5′diFA	0.0	10.1	0.0	8.2	0.0	5.9	0.0	8.8
5-5′diFA	4.1	13.0	1.7	5.7	1.7	6.9	2.1	4.1
8-O-4′diFA	14.3	60.7	13.7	33.2	12.2	30.6	6.7	15.1
8-5′BadiFA	6.2	68.6	7.2	27.3	2.4	14.6	5.2	14.1
total	95.6	440.3	87.0	242.5	72.3	212.9	61.7	154.0

[a] Values are the means of four replicates.

Table 4. Esterified Phenolic Acids (Micrograms per Gram) of CWM from the Middle Section of Fresh and Stored Asparagus[a]

	0.1 M NaOH				1 M NaOH		2 M NaOH	
	1 h		24 h		24 h		24 h	
	fresh	stored	fresh	stored	fresh	stored	fresh	stored
p-hydroxybenzoic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
vanillic acid	0.9	0.2	0.3	0.6	0.7	0.6	0.4	1.5
p-hydroxybenzaldehyde	3.6	1.2	1.2	0.5	1.6	1.1	2.0	1.8
vanillin	2.9	1.6	1.9	1.7	1.9	2.5	2.4	5.8
p-coumaric acid	24.6	74.5	11.2	49.2	6.0	22.0	11.7	45.6
ferulic acid	11.9	53.0	9.2	45.1	4.3	25.3	5.4	51.4
cis-coumaric acid	9.4	19.2	3.8	8.9	5.3	10.5	8.6	15.6
cis-ferulic acid	9.2	22.4	4.5	11.5	3.6	10.4	3.6	19.6
8-8′AT diFA	24.2	17.2	10.4	14.5	2.4	8.2	2.9	15.8
8-5′diFA	0.0	6.0	1.9	12.0	1.2	3.5	0.0	15.6
5-5′diFA	3.1	8.7	1.5	16.0	0.6	3.4	1.0	5.5
8-O-4′diFA	17.0	53.4	7.6	35.9	3.1	17.0	4.3	30.4
8-5′BadiFA	17.9	51.4	4.7	31.8	3.7	13.1	4.8	13.2
total	124.7	308.8	58.2	227.7	34.4	117.6	47.1	221.8

[a] Values are the means of four replicates.

Table 5. Total Esterified Phenolic Acids (Micrograms per Gram) of CWM from the Lower Section of Fresh and Stored Asparagus[a]

	0.1 M NaOH				1 M NaOH		2 M NaOH	
	1 h		24 h		24 h		24 h	
	fresh	stored	fresh	stored	fresh	stored	fresh	stored
p-hydroxybenzoic acid	0.0	0.0	0.0	0.1	0.0	0.0	0.0	1.8
vanillic acid	0.5	0.1	0.6	0.8	0.8	0.3	0.5	0.0
p-hydroxybenzaldehyde	0.5	1.5	0.6	0.9	1.7	0.6	1.0	1.9
vanillin	1.8	3.0	1.1	3.6	1.2	5.4	2.9	11.7
p-coumaric acid	7.4	139.6	9.9	84.9	7.2	36.3	17.5	68.6
ferulic acid	5.9	102.9	7.4	65.8	7.5	38.8	17.2	53.2
cis-coumaric acid	2.7	27.9	4.1	14.1	3.8	13.0	7.1	16.6
cis-ferulic acid	4.9	37.1	4.7	16.4	2.8	12.3	3.7	28.3
8-8′AT diFA	9.7	50.2	6.5	23.5	4.1	28.3	8.5	38.4
8-5′diFA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5-5′diFA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-O-4′diFA	5.5	102.9	6.1	58.8	2.8	28.7	6.0	29.2
8-5′BAdiFA	2.4	90.5	0.0	43.1	3.8	16.5	5.7	21.0
total	41.3	555.7	41.0	312	35.7	180.2	70.1	21.0

[a] Values are the means of four replicates.

was higher in the fractions released with strong alkali (2 M NaOH). This was clearly shown in the outer tissues from the lower stem section, where the FA dimers comprise ~80% of total FA complement (**Table 7**).

Lignification. Lignin was quantified according to the Klason procedure, which estimates lignin as an acid-insoluble residue. Klason lignin demonstrated a basipetal increase and a storage-related increase (**Figure 3a**). The cell walls of outer tissues

Table 6. Total Esterified Phenolic Acids (Micrograms per Gram) of CWM from the Inner Tissues Separated from the Lower Section of Fresh and Stored Asparagus[a]

	0.1 M NaOH				1 M NaOH		2 M NaOH	
	1 h		24 h		24 h		24 h	
	fresh	stored	fresh	stored	fresh	stored	fresh	stored
p-hydroxybenzoic acid	1.8	0.0	0.7	0.1	0.9	0.2	1.3	0.3
vanillic acid	1.6	0.0	0.6	0.0	1.0	1.1	1.2	0.0
p-hydroxybenzaldehyde	2.3	1.4	1.7	1.2	0.9	1.0	1.1	1.4
vanillin	3.9	7.9	2.2	8.1	2.6	5.4	3.9	12.0
p-coumaric acid	5.9	96.9	2.4	73.3	1.7	33.5	2.4	51.0
ferulic acid	4.5	57.8	3.7	42.5	7.3	35.6	4.4	35.9
cis-coumaric acid	3.0	29.5	0.8	18.2	1.7	14.8	0.0	8.8
cis-ferulic acid	2.6	26.2	2.6	12.4	1.8	0.0	1.9	0.0
8-8′AT diFA	3.4	59.4	2.8	42.4	3.5	11.2	2.4	29.2
8-5′diFA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5-5′diFA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-O-4′diFA	3.7	45.1	2.5	25.8	1.3	12.6	0.9	25.1
8-5′BAdiFA	2.4	67.8	0.9	17.1	0.3	15.6	2.0	4.1
total	35.1	392.0	20.9	241.1	23.0	131.0	21.5	167.8

[a] Values are the means of four replicates.

Table 7. Total Esterified Phenolic Acids (Micrograms per Gram) of CWM from the Outer Tissues Separated from the Lower Section of Fresh and Stored Asparagus[a]

	0.1 M NaOH				1 M NaOH		2 M NaOH	
	1 h		24 h		24 h		24 h	
	fresh	stored	fresh	stored	fresh	stored	fresh	stored
p-hydroxybenzoic acid	0.4	0.1	0.4	0.2	0.1	0.0	0.6	0.0
vanillic acid	1.0	0.0	0.5	1.5	0.3	0.4	0.4	2.2
p-hydroxybenzaldehyde	1.2	3.8	0.7	2.5	0.0	0.9	0.8	1.5
vanillin	2.1	6.5	0.7	13.0	2.2	11.3	2.6	17.6
p-coumaric acid	10.3	67.7	14.3	124.0	10.7	48.0	37.2	66.1
ferulic acid	10.5	56.9	9.8	119.8	13.1	40.8	18.0	63.3
cis-coumaric acid	4.0	19.9	2.7	6.1	2.0	24.8	7.6	39.2
cis-ferulic acid	6.0	39.3	3.9	20.4	2.3	8.4	3.6	0.0
8-8′AT diFA	9.8	57.1	13.9	107.9	10.1	45.7	11.0	120.7
8-5′diFA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5-5′diFA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-O-4′diFA	7.8	61.2	7.3	89.8	2.6	28.7	5.4	76.9
8-5′BAdiFA	3.8	64.1	5.0	74.4	1.5	11.0	3.7	46.9
total	56.9	376.6	59.2	559.6	44.9	220.0	90.9	434.4

[a] Values are the means of four replicates.

contained more Klason lignin than those of the inner tissues and were the source of the storage-related increase (**Figure 3b**).

DISCUSSION

This study has demonstrated that cell walls of asparagus spears contain significant quantities of phenolic esters, particularly FA and its dehydrodimers, which increase considerably during postharvest storage. This occurs in both inner (parenchyma and vascular) tissues and outer (sclerenchyma-enriched) tissues. Lignification (Klason lignin) also increases during maturation and storage but only in the outer tissue zone.

These results are consistent with a role not only of lignin phenolics but also of phenolic esters in the maturation- and storage-related toughening of asparagus. The identification of FA dehydrodimers and their postharvest increase is also consistent with the observed storage-related increase in polysaccharide−phenolic complexes detected previously (*4*).

Cell wall esterified phenolics, particularly FA and FA dehodrodimers, have been implicated in modulating the texture and biodegradability of plant cell walls (*10*). They are also thought to confer thermal stability of cell adhesion, and therefore texture, of other edible plant tissues such as CWC (*16*), chufa (*17*), and *Beta vulgaris* (*13*). In CWC and chufa cell walls, >40% of the FA is in the form of FA dehydrodimers. Beetroot in its immature and edible form exhibited much less thermal stability of engineering toughness (units of J m^{-2}) than CWC (*16*) and chufa (*17*) but also exhibited a lower degree of dimerization (25%). However, increasing dimerization biochemically was accompanied by an increase in thermal stability (*18*). The studies on green asparagus reported here have shown that the tissues of this monocotyledonous plant also contain similar levels of FA dimers (60−70% total FA complement). These compounds were mainly located in the outer tissues separated from the basal section of stored spears and accompanied the deposition of xylans. The proposed role for diferulic acid in enhancing thermal stability of texture may provide an additional mechanism to explain the basipetal toughening of asparagus spears during storage. The interpolymeric cross-linking of wall polymers would complement that resulting from lignification.

The level of wall-bound FA increases markedly in all sections and to a much greater extent than the basipetal increase in fresh

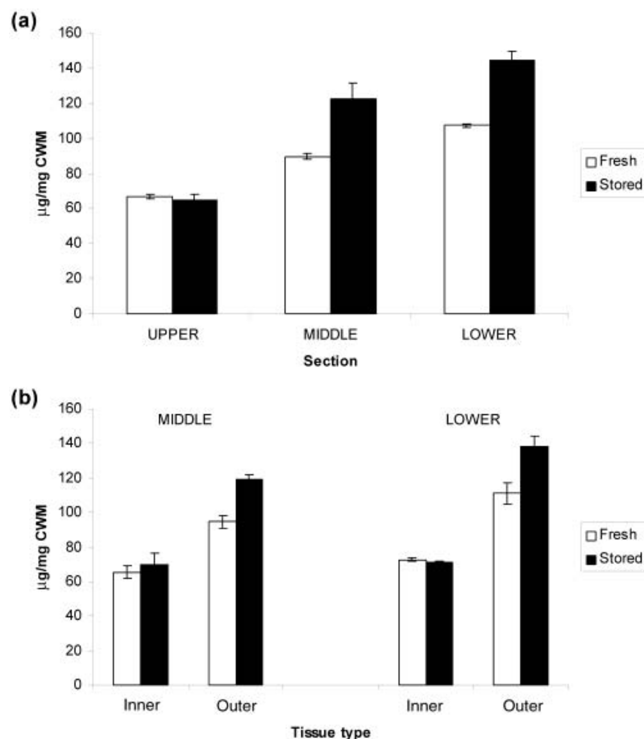

Figure 3. (a) Klason lignin content (micrograms per milligram of CWM) from fresh and stored asparagus sections (upper, middle, and lower); (b) Klason lignin content (micrograms per milligram of CWM) from fresh and stored asparagus tissues separated from the lower section of the stem.

asparagus. This suggests that the relatively rapid increase during postharvest storage is not purely a continuation of developmental changes, but possibly a wound-like response. It is highly likely, therefore, that the nature of the cross-linking that occurs during storage is chemically different from that which occurs during normal extension growth. In addition, there is no indication as to the polysaccharides to which the phenolics are attached or to the impact this has on their extractability. Indeed, the observation that some phenolics are released in 2 M NaOH at the end of the sequential extraction suggests that there may be a small quantity of phenolics remaining esterified to the cell wall. These aspects form part of ongoing studies.

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