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# Ferulic Acid Crosslinks in Asparagus Cell Walls in Relation to Texture

ROCIO C. RODRÍGUEZ-ARCOS,† ANDREW C. SMITH, AND KEITH W. WALDRON*

Institute of Food Research, Norwich Research Park, Colney, Norfolk NR4 7UA, UK

Post-harvest toughening of asparagus spears is associated with a large increase in monomeric and diferulic acids in the cell walls of stem tissues. The purpose of this study has been to investigate the distribution of these phenolic components among cell wall polymers and the role they play in the formation of associated pectic−xylan−phenolic complexes in relation to post-harvest toughening. The phenolic esters are found in all the extractable polysaccharide fractions, particularly the 0.5 M KOH fraction, as well as the insoluble cellulose-rich residue. The storage-related increase occurs in all fractions but is most prominent in the 0.5 M KOH-soluble components. Degradation of 0.5 M KOH subfractions with pure polysaccharide degrading enzymes has confirmed the occurrence of pectic-xylan-phenolic complexes in which ferulic acid and its dehydrodimers are attached to the xylan component but not to the pectic component. Studies on cell separation show that the maturation- and storage-related increase in thermal stability of cell adhesion (and therefore texture) is probably due to an increase in phenolic cross linking of xylans mainly in the parenchyma tissues. This overcomes the thermal lability of the pectic polysaccharides that are responsible for cell adhesion in immature tissues. The storage-induced appearance of some of the diferulic acid moieties in a number of wall polymer fractions supports the hypothesis that the storage affect is a wound-induced response rather than a continuation of maturation-related activity.

**KEYWORDS: Asparagus; cell wall complexes; storage; texture**

## INTRODUCTION

The texture of edible plant organs and the physicochemical properties of dietary fiber are dependent on the composition, structure, and interaction of cell wall (CW) components as modified during growth, development, post-harvest, and processing. There is much information on the composition and structure of cell wall polysaccharides (*1*) and on the occurrence of interpolymeric interactions (*2*, *3*). Cell wall interpolymeric complexes have also been studied in edible plant tissues (*4*, *5*). For example, fractionation of cell walls from several tissues has highlighted the existence of xylan−xyloglucan complexes in the CW of olive pulp (*6*) and cauliflower stems (*7*, *8*). However, relatively less is understood about the precise structure−functional properties of interpolymeric cross-linking. One area in which progress has been made concerns cell adhesion in relation to texture. Hydroxycinnamic acids, and in particular, ferulic acid dehydrodimers, can play a functional role in cell adhesion and thermal stability of texture in several edible organs from food plants (*3*, *9*−*12*), particularly those of monocotyledonous plants. Asparagus is a monocotyledounous plant that is grown for its edible stems (spears). Post-harvest studies on asparagus spears (*5*, *13*, *14*) strongly indicate that storage-related toughening is associated with the formation of a specific class of polymer complexes involving pectic polysaccharides, xylans, and unidentified phenolic components. These changes were thought to be relevant to the initial stages of lignification. Further studies have highlighted a range of changes in post-harvest glycosidic composition (*15*, *16*). Detailed investigations on the phenolic composition of stored asparagus have shown that the cell walls contain considerable quantities of ferulic acid and its dehydrodimers. These increase appreciably during maturation (basipetally) and extensively during post-harvest storage (*17*, *18*). We have suggested that these may play an important role in the post-harvest toughening of asparagus spears by mediating the formation of interpolymeric cross-links that stabilize cell adhesion (*18*).

The purpose of this study has been to investigate the distribution of these phenolic components among the cell wall polymers, the role these phenolics play in the development of post-harvest toughening, and in the formation of associated pectic−xylan−phenolic complexes. For this purpose, cell wall material has been isolated from the bottom section of fresh and stored asparagus, and the component polymers have been sequentially extracted, fractionated and analyzed. Similar extraction conditions have been employed on intact tissues to assess the role the wall components might have in cell adhesion.

* To whom correspondence should be addressed. Fax: +44(0)-1603-507723. E-mail: keith.waldron@bbsrc.ac.uk.

† Current address: Instituto de la Grasa, Avenida Padre García Tejero, 4, 41012 Sevilla (Spain).

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**Table 1.** Sugar Composition of Fresh and Stored Asparagus Cell Walls

| fractions | | recovery (mg/g) | sugars (mol %) | | | | | | | | total (ug/mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | UA | |
| imidazole | fresh | 16.60 | 2.50 | 0.10 | 14.70 | 13.90 | 1.70 | 5.80 | 1.60 | 59.70 | 522.5 |
| | stored | 101.10 | 0.89 | 4.66 | 10.37 | 18.02 | 0.59 | 6.71 | 0.59 | 58.17 | 883.3 |
| CDTA | fresh | 44.40 | 0.50 | 0.00 | 5.90 | 4.40 | 4.70 | 5.10 | 1.10 | 78.40 | 392.7 |
| | stored | 117.00 | 1.87 | 0.00 | 5.30 | 2.09 | 0.00 | 2.17 | 0.44 | 88.13 | 632.9 |
| $Na_2CO_3$(1) | fresh | 66.00 | 5.00 | 0.00 | 14.30 | 6.40 | 0.20 | 9.50 | 2.00 | 62.60 | 576.1 |
| | stored | 47.90 | 5.03 | 0.00 | 10.13 | 3.87 | 0.33 | 10.97 | 0.74 | 70.82 | 733.5 |
| $Na_2CO_3$(2) | fresh | 50.00 | 3.80 | 0.00 | 15.50 | 2.80 | 1.80 | 14.70 | 0.80 | 60.60 | 746.4 |
| | stored | 63.80 | 4.06 | 0.00 | 12.51 | 4.82 | 3.33 | 6.95 | 0.98 | 67.36 | 582.2 |
| 0.5 M KOH | fresh | 45.80 | 5.30 | 0.30 | 20.10 | 26.10 | 1.00 | 7.30 | 12.00 | 28.00 | 673.7 |
| | stored | 63.00 | 1.49 | 0.12 | 9.27 | 51.16 | 0.59 | 1.64 | 12.34 | 23.40 | 663.3 |
| 0.5 M KOH S | fresh | 38.80 | 5.90 | 0.50 | 20.40 | 26.00 | 1.40 | 3.80 | 15.00 | 26.90 | 716.0 |
| | stored | 58.50 | 2.13 | 0.21 | 14.10 | 38.64 | 0.44 | 2.31 | 20.43 | 21.74 | 753.0 |
| 0.5 M KOH P | fresh | 7.00 | 4.30 | 0.00 | 18.80 | 25.10 | 0.40 | 8.10 | 11.00 | 32.40 | 631.3 |
| | stored | 4.50 | 0.75 | 0.00 | 3.58 | 69.37 | 0.80 | 2.67 | 0.86 | 21.97 | 579.9 |
| 1 M KOH | fresh | 46.60 | 1.50 | 0.20 | 10.00 | 51.30 | 1.00 | 6.90 | 10.00 | 19.00 | 670.1 |
| | stored | 58.80 | 1.73 | 0.09 | 10.20 | 65.80 | 1.35 | 5.07 | 9.61 | 14.15 | 816.1 |
| 1 M KOH S | fresh | 33.30 | 1.70 | 0.20 | 9.20 | 48.90 | 1.50 | 3.10 | 12.00 | 19.50 | 606.2 |
| | stored | 53.20 | 3.02 | 0.19 | 17.53 | 48.24 | 1.93 | 2.91 | 9.06 | 14.19 | 783.6 |
| 1 M KOH P | fresh | 13.30 | 1.20 | 0.10 | 9.40 | 47.10 | 0.80 | 3.60 | 17.00 | 20.70 | 733.9 |
| | stored | 5.60 | 0.48 | 0.00 | 2.57 | 76.48 | 1.05 | 2.27 | 0.68 | 16.46 | 848.6 |
| 4 M KOH | fresh | 58.80 | 0.60 | 0.50 | 3.90 | 67.20 | 0.80 | 6.40 | 5.70 | 15.10 | 685.8 |
| | stored | 95.80 | 0.78 | 1.50 | 6.87 | 67.60 | 0.97 | 4.13 | 6.45 | 11.71 | 687.4 |
| 4 M KOH S | fresh | 38.80 | 0.90 | 1.10 | 6.70 | 69.70 | 1.00 | 14.40 | 10.70 | 13.40 | 599.5 |
| | stored | 79.80 | 0.87 | 4.07 | 13.92 | 62.06 | 1.58 | 9.46 | 15.84 | 10.63 | 610.8 |
| 4 M KOH P | fresh | 20.00 | 0.40 | 0.20 | 2.50 | 74.50 | 0.70 | 3.20 | 2.10 | 16.50 | 772.0 |
| | stored | 16.00 | 0.82 | 0.00 | 3.13 | 80.50 | 0.69 | 1.11 | 1.16 | 12.58 | 764.0 |
| residue | fresh | 410.00 | 0.20 | 0.00 | 7.20 | 18.80 | 3.10 | 6.30 | 41.80 | 22.70 | 903.6 |
| | stored | 372.00 | 0.85 | 0.45 | 5.81 | 22.21 | 3.85 | 3.12 | 49.61 | 14.11 | 895.6 |
| residue S | fresh | 11.10 | 0.00 | 0.00 | 7.60 | 6.10 | 0.00 | 10.30 | 0.00 | 75.90 | 254.3 |
| | stored | 5.30 | 3.18 | 0.00 | 13.09 | 20.74 | 10.30 | 7.61 | 8.32 | 46.04 | 408.8 |

## MATERIALS AND METHODS

**Plant Material.** Fresh asparagus (*Asparagus officinalis*, var 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 top to a distance of 15 cm. For storage, 12 groups, each consisting of 500 ± 30 g of marked asparagus spears were packaged in trays over-wrapped with perforated film. Packs were held at 21 °C for 3 days.

**Preparation of Asparagus Cell Wall Material (CWM).** Cell wall material was isolated from the basal section of both fresh and stored asparagus. Cell walls were prepared as described by Parker and Waldron (*9*), with some modifications. A 1000-g sample of frozen asparagus basal section (cut in small pieces) 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-$\mu$m nylon mesh (John Stannier and Co, Manchester, UK), 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 filtering the homogenate through 75-$\mu$m nylon mesh, the residue was resuspended in cold water containing 3 mM $Na_2S_2O_5$, homogenized for 5 min, and refiltered. This procedure was repeated (five times) until the cell wall material was free of starch granules and other intracellular contents as checked by light microscopy and staining with iodine/potassium iodide. The CWM was stored as a frozen suspension at −20 °C. Prior to analysis of sugar and phenolic composition, the cell wall material was further extracted with hot ethanol, washed with acetone, and air-dried. Then, the cell wall material was ground for 5 min in a Junke and Kunkel mill (Analyzemmuhle, Typ A 10, IKA Labortechnik), to obtain powdered samples that allow a complete extraction of cell wall components.

**Chemical Fractionation of Cell Wall Material.** Bottom sections of asparagus spears were extracted by the method developed for CWM from onion bulbs (*19*) with some modifications. CWM (9 g) was sequentially extracted with 500 mL of (i) 2 M Imidazole, pH 7.0 at 20 °C for 6 h; (ii) 50 mM *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetate (CDTA, Na salt), pH 6.5 at 20 °C for 6 h; (iii) 50 mM $Na_2CO_3$ + 20 mM $NaBH_4$ at 1 °C for 16 h, (iv) 50 mM $Na_2CO_3$ + 20 mM $NaBH_4$ at 20 °C for 2 h; (v) 0.05, 1, and 4 M KOH + 20 mM $NaBH_4$ at 20 °C for 2 h. The alkali extractions were carried out with $O_2$-free solutions under nitrogen. The alkali extracts were filtered (GF/C) then acidified to pH 5 with acetic acid. Any material that precipitated at this stage was recovered by centrifugation and designated as a precipitate by the letter "P" in the extract description. Soluble material was designated by the letter "S" (e.g., in **Table 1**). The cellulose rich residue left at the end was also acidified to pH 5. Soluble components released from the cellulose-rich residue during acidification (residue S) were also recovered. The filtered extracts and cellulose were then dialyzed exhaustively and after separating supernatant and residue, by centrifugation, the polymers obtained were freeze-dried and analyzed for phenolics, proteins and sugars. The polysaccharides extracted with 0.5 M KOH were subjected to graded precipitation with 20, 40, 60, and 80% ethanol, each for 24 h at 0 °C.

**Analysis of Carbohydrate Composition.** Neutral sugars and uronic acid composition of all fractions were determined as described in Parker and Waldron (*9*). Analyses were carried out in triplicate, and standard deviations were less than 4%.

**Methylation Analysis.** The polysaccharides were methylated following the method described by Femenia et al. (*7*).

**Analysis of Phenolic Acids.** Total saponified phenolics were released by alkaline hydrolysis of different fractions. Samples (2 mg) were extracted with 4 m NaOH (4 mL) for 24 h at 25 °C under nitrogen. The suspension was filtered through glass fiber paper, and *trans*-cinnamic acid (1.0 $\mu$g) was added as IS. The filtrate was then acidified to pH < 2 with concentrated HCl and extracted 8 times with ethyl acetate (5 mL). The extracts were evaporated to dryness under a stream of nitrogen, and the samples were dissolved in 100 $\mu$l of 50% (v/v) aqueous methanol. The samples were analyzed by HPLC, using the method of Waldron et al., (*20*).

**Gel Permeation Chromatography.** Selected fractions obtained from the 0.5 M KOH extracts were subjected to gel permeation chromatography. The fractions were dissolved in 3 mL of 50 mM potassium-phosphate buffer and applied to a column (100- × 1.6-cm) of Sephacryl S-400-HR. The elution of the samples was performed at a flow rate of

12 mL/h, and fractions of 2 mL were collected. Carbohydrate and phenolic contents were determined in every fraction. The column calibration was made by using standard dextrans having molecular weights of 2000, 487, and 72 KDa.

**Xylanase Digestion.** 60% EtOH fractions (10 mg) were digested with purified xylanase (EC 3.2.1.8. from *A. niger;* Megazyme), using 50 units of enzyme in 1 mL 100 mM sodium acetate buffer, pH 5.0, overnight at 36 °C. The solutions were dialyzed, centrifuged (12 000 rpm, 10 min), and the supernatants were applied on the Sephacryl S-400-HR column and eluted as described above.

**Polygalacturonase Digestion.** 60% EtOH fractions (10 mg) were digested with purified endo-polygalacturonase (EC 3.2.1.15. from *A. niger*; Megazyme), using 10 units of enzyme in 1 mL of 100mM sodium acetate buffer, pH 4.0, as described for the xylanase.

**Vortex-Induced Cell Separation (VICS).** This was based on the method developed by Parker and Waldron (*9*). Stem sections, 2 mm in length, were sliced from the midpoint of the top, middle, and bottom sections of the spear. These "rings" contained all the main stem tissue types. They were divided into four quadrants. These were placed in screw-capped soviril tubes (two per tube) and extracted in a sequence of solvents (3 mL) based on those used for extracting cell wall material. After each extraction, the tubes were vortexed for 1 min. Scores were attributed as follows: No "+", no disruption; "+", tissues separated into large pieces, several mm across; "++", tissue disrupted into small pieces, generally less than 1 mm across; "+++", tissue disrupted into very small pieces, all less than 1 mm across, with many small clumps of cells; "++++", tissue completely disrupted into separated cells.

## RESULTS AND DISCUSSION

**Isolation and Fractionation of Asparagus Cell Wall Material.** The cell wall material was isolated by homogenizing and ball-milling in SDS and then filtering through nylon mesh (100 $\mu$m). This method, developed from that of Selvendran and Ryden (*21*) and modified by Parker and Waldron (*9*) gives a cell wall preparation that is essentially free of cytoplasmatic contamination, with minimum degradation of cell wall components. To study changes in sugar and phenolic composition during storage of green asparagus, the cell walls of the bottom section of fresh and stored spears were isolated and sequentially extracted with Imidazole, CDTA, sodium carbonate, and KOH, leaving a cellulosic residue.

Total recovery of cell wall material after sequential extraction was higher for stored (92%) than for fresh samples (76%). Total carbohydrate recovery from the extracts are presented on a cell wall basis in **Figure 1**(**a**), and show that most of the wall carbohydrate was present in the cellulose-rich fractions. The detailed carbohydrate composition of the fractions is presented in **Table 1**. The extracts obtained during the first stages of CWM fractionation contained pectic polysaccharides that were solubilized by Imidazole, CDTA, or $Na_2CO_3$, depending on the nature of their interpolymeric cross-linking. Hemicellulosic polysaccharides rich in xylose were released by treatment with 0.5 M KOH, 1 M KOH, and 4 M KOH. Significant amounts of pectic polysaccharides were also solubilized by 0.5 M KOH (**Table 1**). On neutralizing, a supernatant and precipitate were separated from each of the KOH extracts by centrifugation. Sugar composition of both soluble and insoluble subfractions was very similar in fresh asparagus: the 0.5 M KOH S and P subfractions exhibited similar amounts of Ara, Xyl, Gal, Glc, and UA, consistent with the presence of pectic−xylan−xyloglucan−phenolic mixtures and complexes (*5*). The same sugars were found in 1 M KOH and 4 M KOH fractions, but the amount of xylose was greater, whereas UA content was lower than in the 0.5 M KOH extract (**Table 1**).

In keeping with previous studies (*5*), the key storage-related changes in cell-wall chemistry were found in the 0.5 M KOH-solubilized polymers. Storage resulted in a doubling of the xylose component in this fraction, the majority of which occurred in the insoluble component (0.5 M KOH P). The increase in xylose was accompanied by concomitant decreases in other sugars and larger decreases in galactose and arabinose, reflecting their turnover (*13*). Xylose also increased in the 1 M KOH extract to a lesser extent.

The phenolic composition of each fraction obtained from the CWM was also investigated. The phenolics detected will be those that remain esterified to wall polymers after the sequential extraction process and which could then be released by saponification in concentrated alkali overnight (see materials and methods). Hence, it is very likely that some phenolic esters from the hemicelluloses and cellulose-rich residue will have been lost during the alkaline steps of sequential extraction and subsequent dialysis. Nevertheless, a considerable proportion of phenolics remained to be characterized. The results are shown in **Figure 1b** and **Table 2**.

In fresh asparagus cell walls, the bulk of the phenolic esters on extracted polymers could be detected in the polysaccharides of the 0.5 M KOH-extract and cellulose-rich residue (**Figure 1b**). However, phenolics were detected in significant quantities in all fractions except the CDTA extract. The main phenolic compounds were ferulic acid and its dehydrodimers; these were particularly concentrated in 0.5 M KOH. Significant amounts of other phenolics, such as *p*-hydroxy benzoic acid, vanillic acid, *p*-hydroxy benzaldehyde, vanillin, and *p*-coumaric acid were also detected in polymers extracted from asparagus cell walls. They were mainly concentrated in the KOH soluble polysaccharides. However, it should be noted that they were also found in the imidazole and carbonate soluble fractions. After storage, a large increase of the total saponified phenolics was observed in every faction (**Figure 1b**), in keeping with the increase observed for total wall phenolics (*18*). This increase was more pronounced in the KOH soluble fractions than in the pectic fractions extracted with imidazole and cold carbonate solutions (**Figure 1b**). Indeed, more than the 50% of the total amount of phenolics attached to solubilized polysaccharides were found in the 0.5 M KOH extract. The largest proportion of the storage-related increase in phenolics was due to an increase in dehydrodimers in most phenolic-containing fractions (**Table 2**), particularly the 8−0−4′-DiFA and 8−5′-DiFA (BF). In some fractions, including the $Na_2CO_3$-2, 0.5 M KOH−P, and several higher alkali extracts, there were no diferulates detected in the fresh material, even though considerable quantities were found in the equivalent extracts from stored stems. This is consistent with our previous suggestion that the storage-related increase is not a mere enhancement of maturation-related cross-linking (which undoubtedly occurs, as indicated by the basipetal increase in whole wall diferulates; (*18*), but is due to a wound-induced response by the whole stem.

**Characterization of 0.5 M KOH Extract.** The 0.5 M KOH extract contained the bulk of the phenolics that could be released by sequential extraction (**Table 2**), and more than 85% remained soluble after neutralization. Both the precipitated and soluble components (0.5 M KOH P and 0.5 M KOH S, respectively) demonstrated the same storage-related changes in carbohydrate composition as the parent extract. In the precipitated material, storage resulted in xylose becoming the dominant sugar component, and glucose all but disappeared, consistent with an increase in xylan hemicelluloses. This was confirmed by methylation analysis (results not shown). In the souble material, both xylose and glucose increased, indicating an increase in both xylan and xyloglucan moieties. Storage-related changes in phenolics also showed similar trends to the parent extract, with

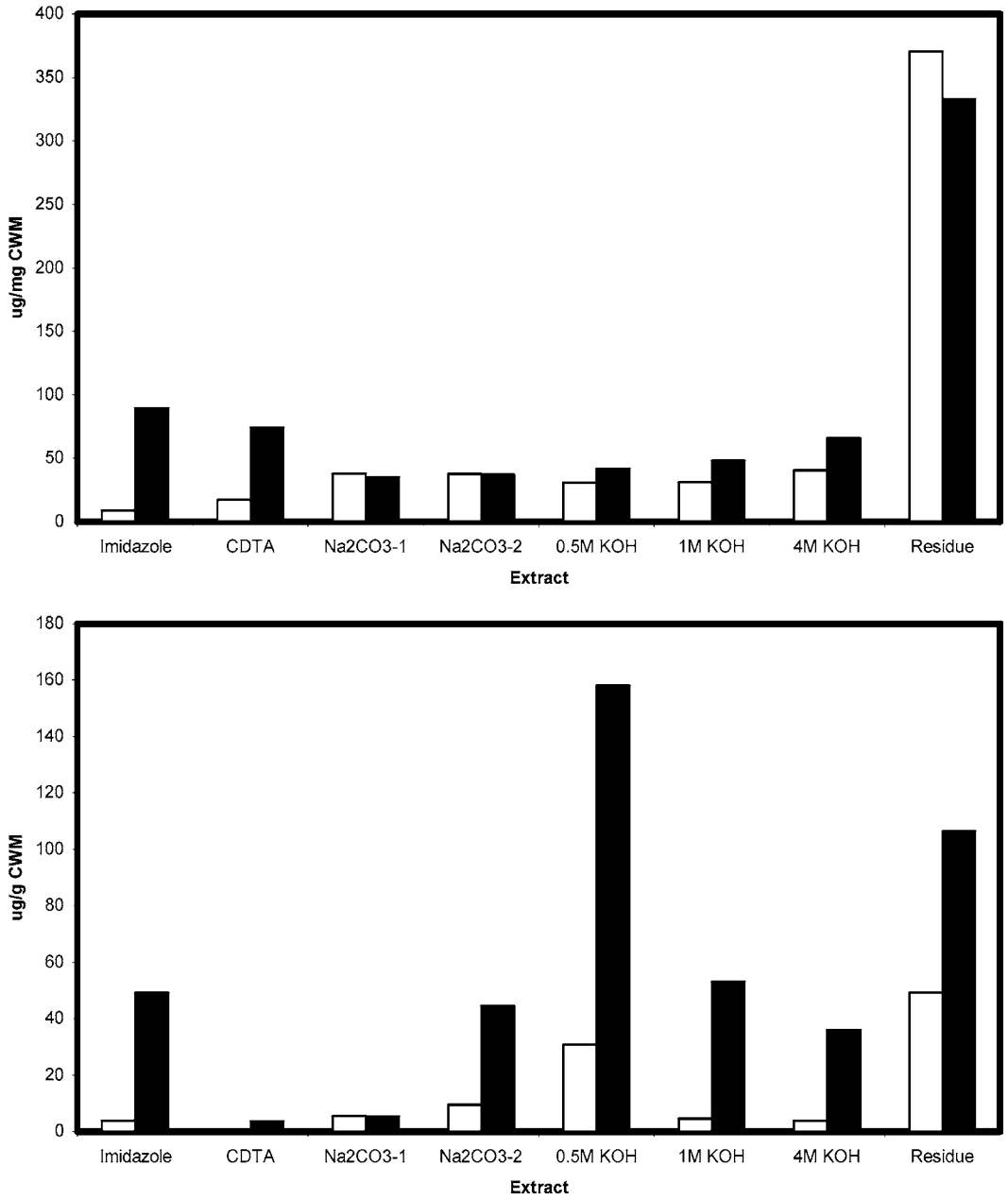


**Figure 1.** Carbohydrate recovery (**a**) and phenolic acid recovery (**b**) on a cell wall basis in sequential extracts and residue. White bars, fresh tissue; black bars, stored tissue.

large increases in the 8−8′-DiFA (AT), 8−0−4′-DiFA, and 8−5′-DiFA (BF), particularly in the soluble material.

The large quantities of associated phenolics (**Table 2**) is consistent with the presence of carbohydrate−phenolic complexes. Complexes of phenolics and carbohydrate have been reported in cauliflower stems (*7*) and in walls of cultured rose cells (*21*, *22*). In asparagus tissues, the most abundant ferulic dimers comprised 8−8′-DiFA (AT), 8−0−4′-DiFA, and 8−5′-DiFA (BF), while other ferulic acid derivaties such as 8−8′-DiFA; 8−5′-DiFA, and 5−5′DiFA were barely detected. The large increase in phenolic moieties on storage is consistent with the storage-related increase in firmness of cooked spears (*18*).

To gain further information on the structure and composition of the possible polysaccharide−phenolic complexes extracted with 0.5 M KOH, selected graded-ethanol-precipitated subfractions were submitted to gel filtration chromatography on Sephacryl S-400-HR and investigated for their glicosidic and phenolic composition as modified by pure wall-degrading enzymes.

**Graded Precipitation with Ethanol.** The components in the 0.5 M KOH (soluble) extracts from fresh and stored asparagus were precipitated in increasing concentrations of alcohol. 40% EtOH failed to precipitate any material. However, 60% EtOH resulted in precipitation of 17% (fresh) and 27% (stored), and 80% EtOH resulted in precipitation of 25% (fresh) and 22% (stored). 80% EtOH-soluble material remaining accounted for 21% (fresh) and 20% (stored). The sugar composition of the 60% EtOH-insoluble (60i), 80% EtOH-insoluble (80i), and soluble (80s) subfractions are shown in **Figure 2**, parts **a**, **b**, and **c**, respectively. The 60i precipitate contained the highest levels of carbohydrate. The sugar compositions indicate a similar range of polymer species (xylan, xyloglucan, pectin) to the parent 0.5 M KOH (soluble) extract, albeit with lower levels of ara. In comparison, the 80i material was rich in pectic moieties, as inferred from the high level of uronic acid, and the final supernatant was rich in xylan−pectic mixtures, which may be highly branched, as indicated by the low uronic acid levels. The

**Table 2.** Phenolic Composition of Cell Walls from Fresh and Stored Asparagus

| | | phenolics ($\mu$g/g CW) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| fraction | | PBZ | VA | PBD | V | PCA | FA | CCA | CFA | 8,8′AT | 8-O-4 | 8,5′BF | total |
| imidazole | fresh | 0 | 12 | 12 | 17 | 57 | 41 | 44 | 28 | 0 | 11 | 5 | 226 |
| | stored | 0 | 14 | 14 | 34 | 71 | 88 | 40 | 40 | 0 | 142 | 44 | 486 |
| CDTA | fresh | 58 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| | stored | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 12 | 0 | 0 | 0 | 31 |
| $Na_2CO_3$ (1) | fresh | 29 | 17 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 82 |
| | stored | 0 | 0 | 0 | 13 | 41 | 23 | 20 | 16 | 0 | 0 | 0 | 112 |
| $Na_2CO_3$ (2) | fresh | 0 | 19 | 64 | 25 | 18 | 16 | 15 | 4 | 0 | 0 | 0 | 189 |
| | stored | 0 | 0 | 0 | 28 | 100 | 93 | 35 | 52 | 85 | 246 | 58 | 698 |
| 0.5 M KOH | fresh | 0 | 25 | 23 | 74 | 43 | 68 | 43 | 63 | 66 | 107 | 59 | 670 |
| | stored | 82 | 18 | 25 | 142 | 191 | 275 | 134 | 180 | 275 | 709 | 477 | 2508 |
| 0.5 M KOH S | fresh | 0 | 33 | 32 | 88 | 63 | 99 | 55 | 86 | 289 | 214 | 117 | 1076 |
| | stored | 164 | 37 | 33 | 188 | 257 | 381 | 168 | 277 | 550 | 969 | 655 | 3678 |
| 0.5 M KOH P | fresh | 0 | 17 | 15 | 60 | 23 | 36 | 30 | 39 | 42 | 0 | 0 | 263 |
| | stored | 0 | 0 | 17 | 96 | 125 | 170 | 100 | 83 | 0 | 449 | 298 | 1339 |
| 1 M KOH | fresh | 18 | 13 | 6 | 25 | 10 | 13 | 7 | 5 | 0 | 0 | 0 | 96 |
| | stored | 13 | 34 | 19 | 112 | 79 | 101 | 62 | 55 | 113 | 232 | 82 | 903 |
| 1 M KOH S | fresh | 36 | 10 | 11 | 25 | 13 | 16 | 9 | 10 | 0 | 0 | 0 | 130 |
| | stored | 0 | 68 | 37 | 160 | 106 | 145 | 79 | 71 | 226 | 321 | 111 | 1324 |
| 1 M KOH P | fresh | 0 | 15 | 0 | 24 | 8 | 9 | 6 | 0 | 0 | 0 | 0 | 62 |
| | stored | 25 | 0 | 0 | 065 | 53 | 58 | 46 | 38 | 0 | 144 | 54 | 483 |
| 4 M KOH | fresh | 0 | 4 | 0 | 21 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 66 |
| | stored | 0 | 24 | 13 | 50 | 37 | 30 | 21 | 16 | 67 | 97 | 21 | 376 |
| 4 M KOH S | fresh | 0 | 7 | 0 | 29 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 110 |
| | stored | 0 | 36 | 16 | 54 | 31 | 25 | 15 | 25 | 26 | 99 | 24 | 353 |
| 4 M KOH P | fresh | 0 | 4 | 0 | 14 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| | stored | 0 | 11 | 10 | 46 | 42 | 35 | 28 | 7 | 107 | 95 | 17 | 399 |
| residue | fresh | 0 | 10 | 8 | 24 | 35 | 26 | 10 | 8 | 0 | 0 | 0 | 120 |
| | stored | 0 | 23 | 20 | 61 | 38 | 66 | 16 | 34 | 28 | 0 | 0 | 286 |

storage-related increase in xylose occurred only in the 60i subfraction. Phenolic analysis revealed that all three subfractions contained significant quantities of ferulic acid and its dehydrodmers and *p*-coumaric acid (**Figure 3**). However, although the 60i xylan-rich fraction showed a storage-related increase in phenolics, a greater increase was seen in the 80i fraction, consistent with a storage-related increase in xylan−pectic−phenolic complexes. Furthermore, little change in diferulic levels were observed in the 80s fraction.

**Gel-Permeation Chromatography.** The 60i subfractions from fresh and stored asparagus samples were analyzed by gel filtration chromatography on Sephacryl S-400-HR. The elution profiles are shown in **Figures 4** and **5**.

The 60i fraction from fresh asparagus (60if) was resolved into a single peak by chromatography on Sephacryl S-400-HR. The molecular weight of this fraction was equivalent to 70 Kda, as measured with dextran markers, and the elution profile contained a spread of uronic acid and phenolic moieties (**Figure 4a**). Storage of asparagus resulted in a similar profile (**Figure 5a**), although the uronic acid and phenolic profiles showed peaks at slightly lower molecular weights than did the total carbohydrate profile. The 60i (fresh) fraction was digested with a commercially available, highly purified xylanase, followed by dialysis and application to the Sephacryl S-400-HR column. The eluted material was free of phenolic components, and the uronic acid component was almost completely removed (**Figure 4b**), indicating their loss during dialysis. Digestion of the 60i (fresh) fraction with polygalacturonase also reduced the uronic acid component, but had little impact on the phenolic profile (**Figure 4c**). Results were similar for the digested 60i (stored) fractions (**Figure 5**, parts **b** and **c**). These results support previous suggestions of pectic−xylan−phenolic complexes in asparagus cell walls, and indicate that the phenolics are attached only to a xylanase-labile arabinoxylan, to which are attached pectic components.

Limited quantities of the material precluded further analysis in this study. The remaining, relatively unmodified material probably comprises xyloglucan, as indicated by the significant levels of Glc in the 0.5 K subfractions and detailed linkage analysis of 0.5 K subfractions in previous studies (*5*). The results indicate that this material is unlikely to be complexed with nonsugar components because their removal had little effect on the molecular weight profile, and it was separate to the phenolic and uronic-rich material in **Figure 5a**.

**VICS.** VICS studies were carried out to provide information on any relationships between the classes of sequentially extracted phenolic-substituted polysaccharides and the stability of cell adhesion, which underlies thermal softening during processing (*3*, *17*, *18*). Asparagus stem tissue sections were sequentially extracted in a manner similar to the cell wall preparations as described in the Materials and Methods section. The tissues were then subjected to VICS to assess the weakening of intercellular adhesion. The results, in order of extraction, are shown in **Tables 3** and **4**. Additional thermal treatments (separate to the sequential series) are shown at the bottom of the tables. Although the present study has investigated the cell wall composition of tissues from the bottom of the stem, VICS was carried out on tissues from the top, middle, and bottom sections, so as to relate this study to maturation effects highlighted previously (*13*, *14*, *17*, *18*).

**Water and CDTA Extraction.** Treatment with cold or hot water had no impact on VICS of immature tissues (top stem section). Subsequent extraction for 16 h in CDTA also had no effect. However, heating in CDTA caused significant (75%) VICS (**Table 3**). This involved disruption of virtually all the parenchyma tissues into clusters but left the immature sclerenchyma tissues mostly intact. These results indicate that in the immature internal tissues, cell adhesion was dependent on calcium cross-linked, thermally labile pectic polysaccharides. The degree of hot CDTA-dependent VICS was reduced to 50% in the more mature middle and bottom sections in fresh asparagus and was reduced further by storage. This is consistent with the maturation- and storage-induced formation of additional intercellular cross-links within the parenchyma and vascular

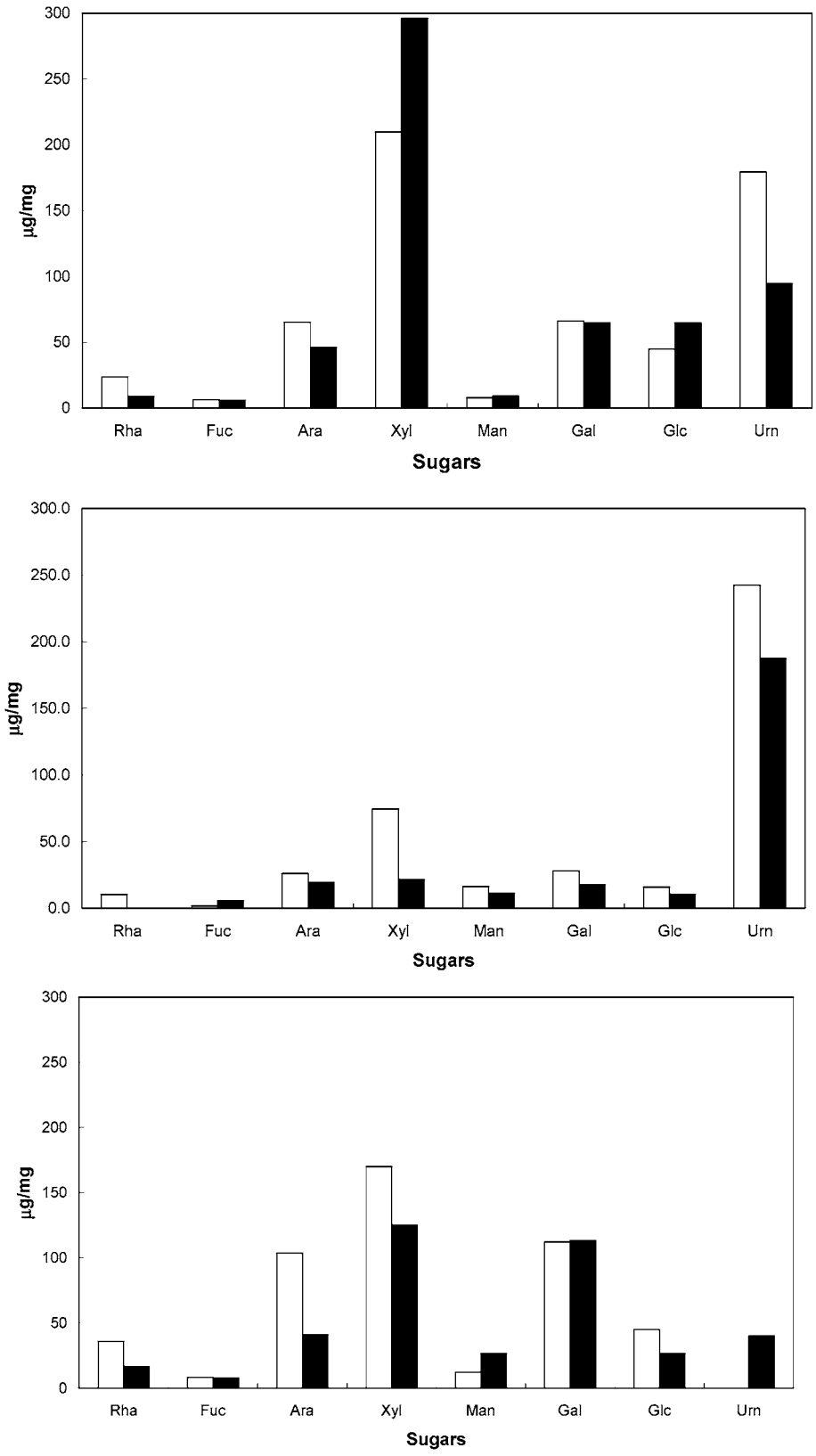


**Figure 2.** Sugar composition of (**a**) 60% Et OH subfraction, (**b**) 80% EtOH subfraction, and (**c**) supernatant obtained from 0.5 M KOH soluble fraction of fresh (white bars) and stored (black bars) asparagus ($\mu$g/mg fraction).

bundle tissues (including diferulic acid) that are resistant to chelating agents and thermal treatments.

**$Na_2CO_3$ Extraction.** Extraction of CDTA-extracted fresh tissue sections in $Na_2CO_3$ at 0 °C resulted in 50% VICS, mainly in parenchyma regions. This indicates that weak-ester linkages may have some involvement in cell adhesion. However, the effect was completely prevented by maturation (middle and bottom sections) and storage, indicating the formation of additional weak-alkali-stable cross-links, consistent with the increase in FA and its dimers. Subsequent thermal treatment

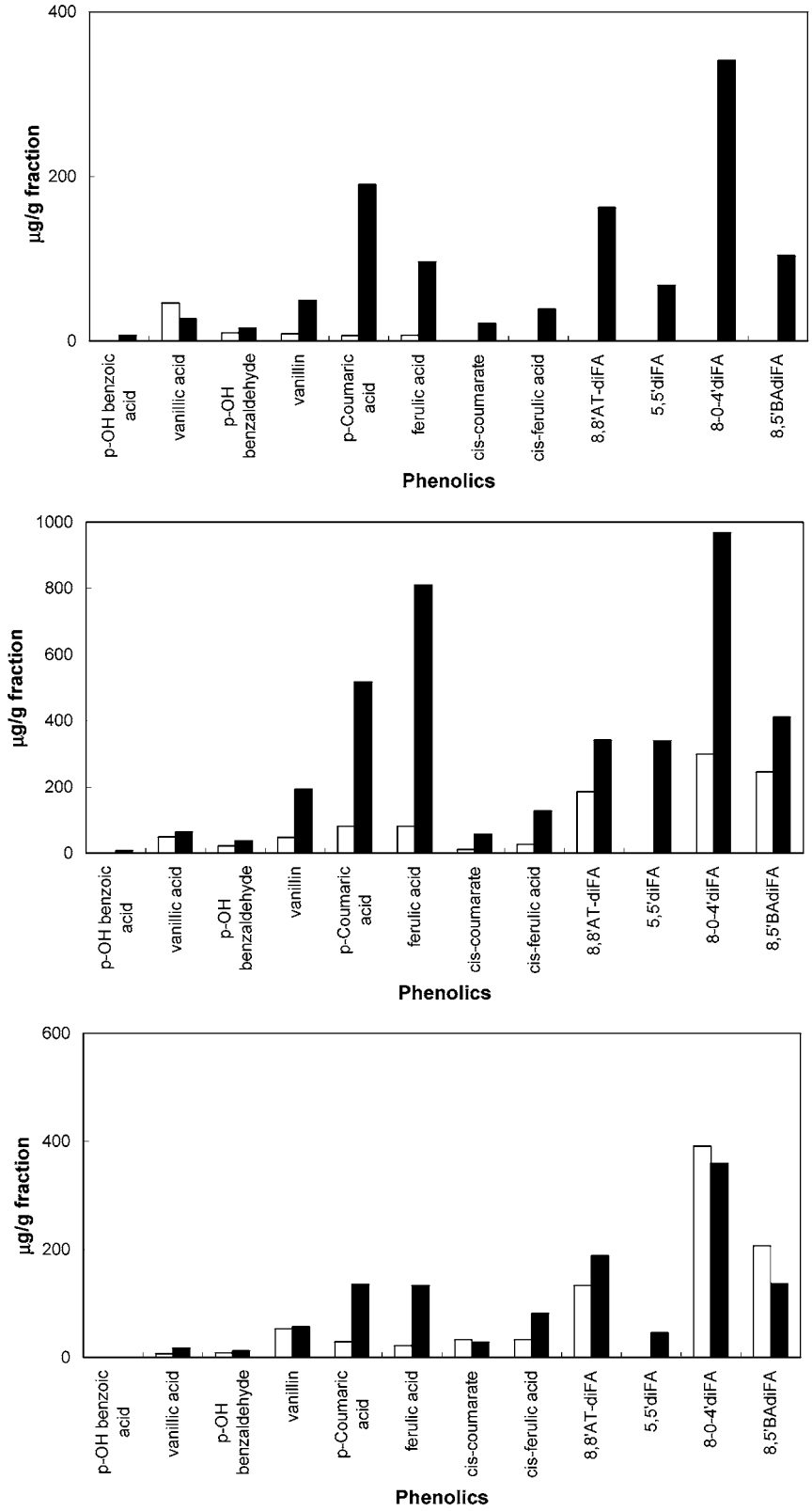


**Figure 3.** Total esterified phenolics of (**a**) 60% EtOH subfraction, (b) 80% EtOH subfraction, and (**c**) supernatant obtained from 0.5 M KOH soluble fraction of fresh (white bars) and stored (black bars) asparagus ($\mu$g/g fraction).

with $Na_2CO_3$ gave similar results to that of thermal treatment with CDTA, leaving only the sclerenchyma zone intact in immature stem material. The same degree of VICS was induced by $Na_2CO_3$ treatment at room temperature. A further extraction in $Na_2CO_3$ for 20 h increased VICS a little, particularly in the more mature tissues.

**KOH Extraction.** After $Na_2CO_3$ extraction, the remaining intact material from immature fresh asparagus comprised sclerenchyma tissue with some adhering parenchyma and vascular cells. Material from middle and bottom stem sections additionally contained significant amounts of adhered parenchyma and vascular tissues. Extraction of these remaining tissues

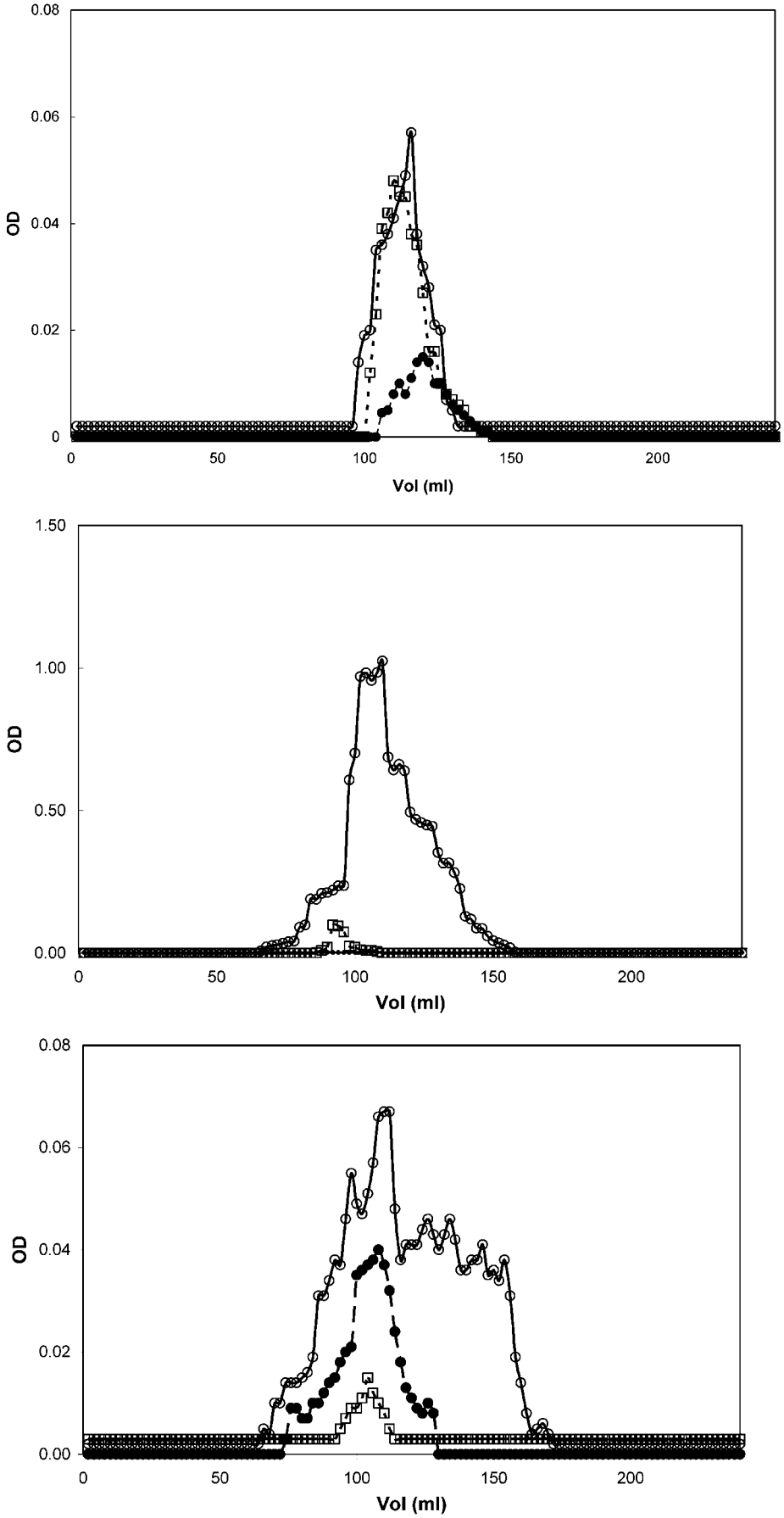


**Figure 4.** Gel permeation chromatography on Sephacryl S-400 HR of (**a**) 60% EtOH subfraction (native), (**b**) 60% EtOH subfraction endo-xylanase digested, and (**c**) 60% EtOH subfraction endo-polygalacturonase digested. Symbols: ○, OD 490 nm (total sugars); ●, OD 280 nm (phenolics); □, OD 520 nm (uronic acids) from cell wall material of fresh asparagus. $V_o$ and $V_t$ fall at 70 and 170 mL respectively.

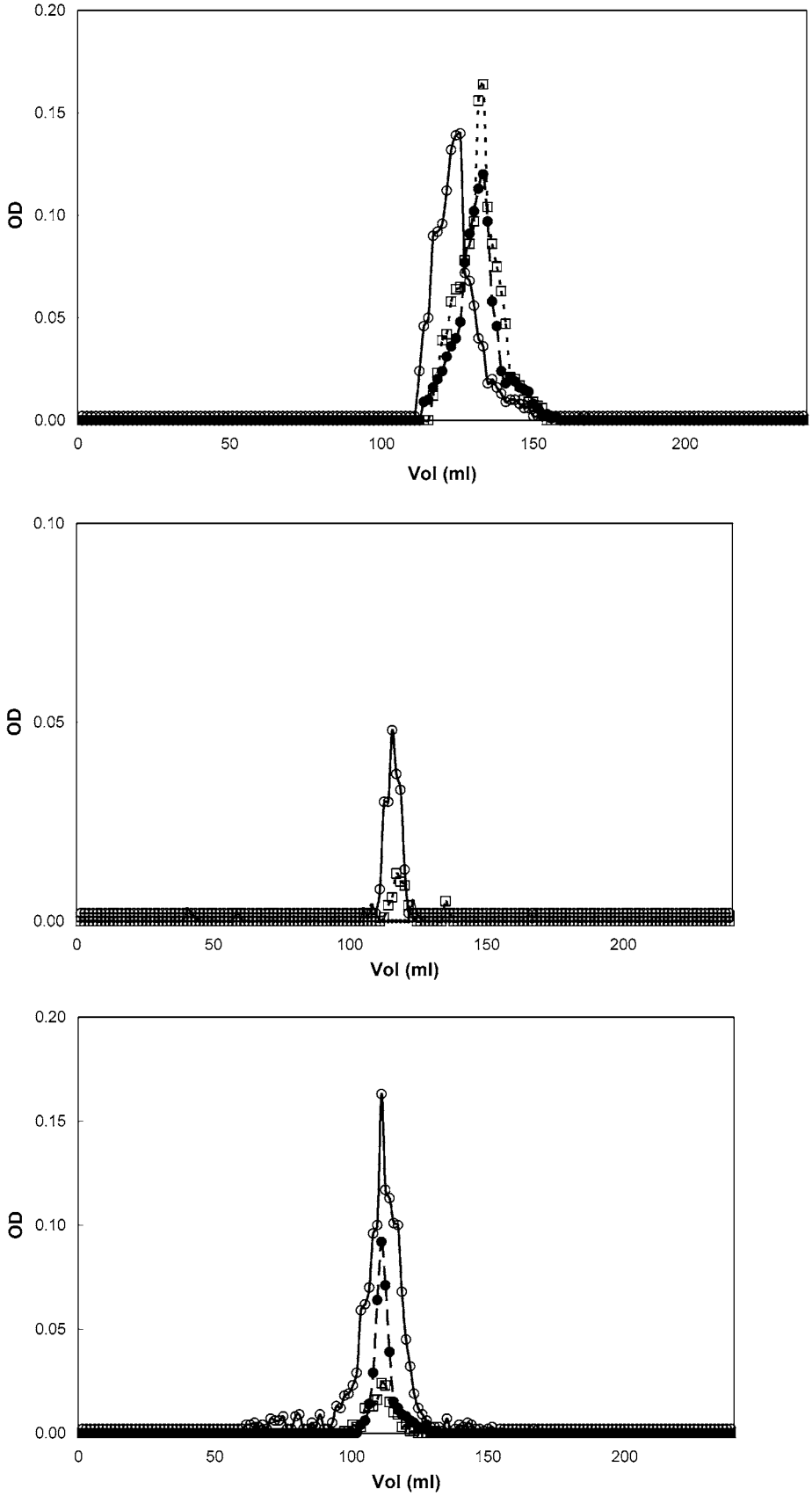


**Figure 5.** Gel permeation chromatography on Sephacryl S-400 HR of (**a**) 60% EtOH subfraction (native), (**b**) 60% EtOH subfraction endo-xylanase digested, and (**c**) 60% EtOH subfraction endo-polygalacturonase digested from cell wall material of stored asparagus. Symbols: same as those for **Figure 4**.

in 0.5 M KOH for 2 h resulted in total VICS in immature fresh tissues (i.e., the sclerenchyma tissue was entirely disrupted). However, maturation inhibited this completely. Maturation also reduced considerably the VICS caused by the 0.5 M KOH,

**Table 3.** Vortex-Induced Cell Separation (VICS) from the Different Sections of Fresh Asparagus

| treatment | top | middle | bottom |
|---|---|---|---|
| water, 16 h | ---- | ---- | ---- |
| CDTA, 20 °C, 16 h | ---- | ---- | ---- |
| $Na_2CO3$, 0 °C, 16 h | --++ | ---- | ---- |
| $Na_2CO3$, 20 °C, 2 h | -+++ | --++ | --++ |
| 0.5 M KOH, 20 °C, 2 h | ++++ | ---- | ---+ |
| 0.5 M KOH, 20 °C, 16 h | ++++ | --++ | --++ |
| 1.0 M KOH, 20 °C, 2 h | ++++ | --++ | --++ |
| 1.0 M KOH, 20 °C, 16 h | ++++ | ++++ | ++++ |
| thermal treatments | | | |
| post-water: 100 °C, 30 min | ---- | ---- | ---- |
| post-CDTA: 100 °C, 30 min | -+++ | --++ | --++ |
| post- $Na_2CO_3$, 0 °C: 100 °C, 30 min | -+++ | --++ | --++ |
| post $Na_2CO_3$, 20 °C: 100 °C, 30 min | -+++ | -+++ | --++ |

**Table 4.** Vortex Induced Cell Separation (VICS) from the Different Sections of Stored Asparagus

| treatment | top | middle | bottom |
|---|---|---|---|
| water, 16 h | ---- | ---- | ---- |
| CDTA, 20 °C, 16 h | ---- | ---- | ---- |
| $Na_2CO_3$, 0 °C, 16 h | ---+ | ---- | ---- |
| $Na_2CO_3$, 20 °C, 2 h | --++ | ---+ | ---+ |
| 0.5 M KOH, 20 °C, 2 h | --++ | ---- | ---- |
| 0.5 M KOH, 20 °C, 16 h | -+++ | --++ | --++ |
| 1.0 M KOH, 20 °C, 2 h | -+++ | --++ | --++ |
| 1.0 M KOH, 20 °C, 16 h | ++++ | ++++ | ++++ |
| thermal treatments | | | |
| post-water: 100 °C, 30 min | ---- | ---- | ---- |
| post-CDTA: 100 °C, 30 min | -+++ | ---+ | ---+ |
| post- $Na_2CO_3$, 0 °C: 100 °C, 30 min | -+++ | ---+ | ---+ |
| post $Na_2CO_3$, 20 °C: 100 °C, 30 min | -+++ | --++ | --++ |

16 h and the 1 M KOH 2 h extraction, consistent with the basipetal increase in phenolics (*18*). Nevertheless, a further 1 M KOH 20 h treatment resulted in total VICS in the remaining (sclerenchyma-rich) tissues consistent with the conditions required to de-esterify much of the ferulic acid moieties in the cell walls.

These results show that in immature tissues storage had an impact on the extent of VICS by all extractions up to the final 1 M KOH (2 h). In the middle and bottom stem sections, storage had an effect only on VICS that could be induced by CDTA (hot and cold) and $Na_2CO_3$ (hot and cold). It had little effect on adhesion stability at the higher alkali concentrations.

## DISCUSSION

Previous studies with Chinese waterchestnut tissues have shown that 0.5 M KOH (16 h) is more than enough to extract ferulic ester cross-links involved in cell adhesion (*12*).

Therefore, it is likely that the storage and maturation-related reduction in $Na_2CO_3$ and 0.5 M KOH-induced VICS of asparagus parenchyma (**Table 3**) is attributable to the large increase in ferulic acid cross-links in the cell walls. The observation that storage and maturation also reduce the thermal enhancement of parenchyma VICS after CDTA and $Na_2CO_3$ extractions is also consistent with the role of these phenolic esters in the post-harvest and basipetal toughening of cooked asparagus tissues. Rodriguez et al. (*18*) demonstrated that a storage-related increase in Klason lignin of about 30% occurred only in the outer sclerenchyma tissues. In conjunction with the VICS data, this indicates that only ferulic acid cross-linking is responsible for the storage-related increase in toughness of the inner parenchyma tissues (*17*).

The study has shown the following: 1. The phenolic esters in asparagus cell walls, particularly ferulic acid and its dehydrodimers, are found in all the extractable polysaccharide fractions, particularly the 0.5M KOH fraction, as well as the insoluble cellulose-rich residue. 2. The storage-related increase in wall phenolic esters occurs in all fractions; however, it is most prominent in the 0.5 M KOH-soluble components that have been previously implicated in texture (Waldron and Selvendran, 1992). 3. The storage-induced appearance of some of the diferulic acid moieties in a number of wall polymer fractions, which in fresh asparagus, contained no diferulic acid, suggests that the storage affect is a wound-induced response rather than a continuation of maturation-related activity. 4. Degradation of 0.5 M KOH subfractions with pure polysaccharide degrading enzymes has confirmed the occurrence of pectic−xylan−phenolic complexes. The phenolic components of these complexes comprise mainly ferulic acid and its dehydrodimers, which are attached to the xylan component but not to the pectic component. 5. Treatment of intact tissues with extractants used in the sequential extraction of CWM has shown that the maturation- and storage-related increase in thermal stability of cell adhesion (and therefore texture) is probably due to an increase in phenolic cross linking of xylans mainly in the parenchyma tissues, overcoming the thermal lability of the pectic polysaccharides that are responsible for cell adhesion in immature tissues.

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