Postharvest Changes in White Asparagus Cell Wall during Refrigerated Storage

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The postharvest changes of the white asparagus cell wall have been studied in relation to the toughening process along the length of a spear that was divided into three sections: apical, middle, and basal. Polysaccharides underwent significant turnover during storage. Uronic acid concentration decreased in all sections and in almost all polysaccharide fractions, while neutral sugars increased very slightly in the apical section, decreased in the middle, and increased in the basal one. Xylose, glucose, and galactose are the main neutral sugars implicated in the turnover; xylose accumulated in the hemicellulose fractions of the middle and basal sections, glucose decreased in the hemicellulose and cellulose fractions of the middle section and increased in the cellulose fraction of the basal one, and galactose disappeared mostly from the cellulose fractions of the three sections. Lignin increased most in the middle section and least in the basal one. No increase was detected in the apical one. No important increases of wall phenolics were detected in any part of the spear. The hardening process was limited only to the basal section.

Keywords: *Asparagus; postharvest; storage; cell wall; polysaccharides*

INTRODUCTION

Texture is probably the most important quality factor of white asparagus for both the fresh product and the processed one. During postharvest storage, asparagus undergoes a process of hardening, resulting in a loss of quality. Furthermore, if the spear is going to be processed, the amount of product lost during peeling is proportional to the extent of hardening. In some extreme cases, almost 50% of the fresh product needs to be peeled off. Therefore, it is obvious that an understanding of the chemistry and biochemistry of asparagus toughening is of important practical interest.

The basis of hardening is related to biochemical modifications of the cell wall composition (constitued by pectic and hemicellulosic polysaccharides, cellulose, protein, and phenols) and has always been associated with lignification. However, there have been very few works dealing specifically with these cell wall modifications. For this reason, very little is known about this subject. As far as we know, only three papers that studied the asparagus cell wall have been published, and all of them deal with green asparagus (Waldron et al., 1990a,b, 1992).

White spears contain more tough tissue than green ones of the same length and diameter (Lipton, 1990). The morphology of the vascular bundles, as shown by microscopy, seems also to be very different (Chang, 1983). During storage, the toughening process is faster for the white spear as compared with the green; some authors have suggested that these different rates could be related to the differences found in some enzymatic activities such as phenylaminolyase (Lipton, 1990). It is a known fact from the industry, and also from the home, that the cooking time needed for white asparagus is always longer than that for green asparagus (Sánchez-Pineda, 1996). All the above could be related with differences in cell wall composition between green and white asparagus.

The focus of this work has been to study the modifications of the main components of white asparagus (polysaccharides, lignin, protein, and phenolic compounds) during postharvest storage as a means of gaining knowledge about the toughening mechanism.

MATERIALS AND METHODS

Sample. Asparagus (*Asparagus officinalis*), having an average maximum diameter of 2.2 cm, was harvested in the property "Pedro Parias" (Alcalá del Río, Sevilla) and washed with sodium hypochlorite (500 ppm active Cl_2). The average weight of the spears was determined in 10 groups of three asparagus each. Their moisture was determined by drying the sample at 60−70 °C to a constant weight under a partial vacuum of about 25 mm. For storage, the asparagus spears were cut 15 cm from the tips and stored for 21 days at 4 °C in an airtight container with an air current that was saturated with humidity. To control the size of the spears, they were marked at a distance of 14 cm from the tips before storage, and the possible apical elongation was checked periodically.

Isolation of the Asparagus Cell Wall. Cell walls (CW) were isolated from three sections (apical, middle, and basal) of each spear (3 × 5 cm). CW were prepared as described by Selvendran and O'Neill (1987) and by Selvendran and Ryden (1990) with some modifications. Asparagus (1 kg) was blended for 2 min at 4 °C with 1000 mL of 0.2 M phosphate buffer (pH 7.0). The mixture was filtered through a nylon filter, and the residue was extracted once more with phosphate buffer, filtered again, and then washed with 500 mL of distilled water and refiltered. The three filtrates were combined and dialyzed (dialysis tubing MWCO 1000) against water and designated as the phosphate-soluble fraction. The residue obtained by filtration was washed twice with distilled water and then mixed for 18 h at 4 °C with 1000 mL of 1% (w/v) sodium dodecyl sulfate (SDS). The mixture was filtered, and the

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residue was washed with 1000 mL of distilled water and then filtered. The two filtrates were combined and dialyzed against water (MWCO 12000) to give the sodium dodecyl sulfate-soluble fraction. The residue was washed twice with distilled water and then treated with 500 mL of phenol−acetic acid−water (PAW, 2:1:1) for 3 h at 25 °C. The mixture was filtered, and the residue was washed with 500 mL of distilled water and filtered. These filtrates was dialyzed as before and constituted the PAW-soluble fraction. The latter two fractions contained proteins and phenols. The residue obtained was washed with distilled water and dried with acetone.

Fractionation of the Asparagus Cell Wall. To obtain further information on the nature of the carbohydrate polymers constituting the cell wall complex, chemical fractionation has been used. CW (3 g) was treated with 150 mL of 0.5 M imidazole/HCl buffer (pH 7.0) containing 10 mM sodium metabisulfite for 3 h with constant mixing at 4 °C (×2). The mixture was then centrifuged, and the supernatant was collected and dialyzed to constituted the imidazole-soluble fraction. The residue was treated twice with 150 mL of 0.05 M sodium carbonate containing 10 mM $NaBH_4$; the first treatment for 16 h with constant mixing at 4 °C and the second for 3 h at room temperature. After each treatment, the mixture was centrifuged, and the supernatant was neutralized with acetic acid, dialyzed, and saved as the carbonate-soluble fraction. These fractions contained pectic polysaccharides. The residue was treated with 25 mL of 1 M KOH containing $NaBH_4$ for 16 h at room temperature and then centrifuged. The residue was treated with 25 mL of 4 M KOH containing 10 mM $NaBH_4$ for 16 h at room temperature and then centrifuged. KOH-soluble fractions contained hemicellulose polysaccharides. The residue was washed with distilled water and then dried with acetone. This residue constituted the cellulosic fraction.

Carbohydrate Composition. Neutral sugars (NS) and uronic acids (UA) were determined in each of the fractions of the wall material. The noncellulosic fractions were hydrolyzed with 2 M trifluoroacetic acid (TFA) (Ruiter and Burns, 1987), and the neutral sugars have been quantified by gas chromatography of their alditol acetates (Englyst and Cummings, 1984). The chromatography was carried out on a series II Hewlett-Packard 5890 GC instrument, fitted with a SP2330 capillary column (30 m × 0.25 mm). The oven temperature was programmed beginning at 180 °C, then was raised subsequently at 4 °C/min to 240 °C, where it was maintained for 20 min. Hellium was the carrier gas at 1 mL/min. The cellulose was hydrolyzed with sulfuric acid (Saeman et al., 1954). Uronic acids were measured colorimetrically by the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

Proteins. The protein content was calculated from the total nitrogen analysis according to Kjeldahl's method (AOAC, 1990). The nitrogen was measured using a Kjeltec Auto 1030 analyzer (Tecator, Sweden). Kjeldahl nitrogen was converted into protein using the 6.25 factor.

Lignin. The lignin content was determinated by different methods: (a) spectrophotometrically by the modified acetyl bromide procedure (Iiyama and Wallis, 1990); (b) determining the acid detergent lignin of the acid detergent fiber using Van Soest's method (1963); (c) determining permanganate lignin (Goering and Van Soest, 1973); and (d) determining Klason lignin by acid hydrolysis of the asparagus cell wall using Saeman's method (Saeman et al., 1954).

Phenolic Compounds. The CW (100 mg) was treated with 4 mL of 1 M NaOH for 18 h at room temperature on the dark, afterward 0.4 mL of 11.3 M HCl was added. The mixture was centrifuged at 3000 rpm for 5 min, and the phenolics from the supernatant were analyzed by HPLC. A Waters chromatograph (Milford, MA) was used, comprising a model 600E pump, a model 717 injector, a model 996 UV−Vis array-diode detector, and a Millenium 2010 computer chromatographic data station to control the system. The chromatographic separation was carried out using a Spherisorb ODS-2 column (5 μ, 250 × 4.6 mm, Technokroma, Barcelona, Spain).

Table 1. General Characteristics of the Fresh and Stored Asparagus[a]

section	apical	middle	basal
	Fresh		
fresh weight (g/spear)	16.5 ± 0.2	15.9 ± 0.3	16.1 ± 0.4
water (%)	96.0 ± 0.1	95.7 ± 0.1	94.6 ± 0.1
cell wall (% FW)	1.6 ± 0.1	1.3 ± 0.1	1.7 ± 0.1
	Stored		
fresh weight (g/spear)	13.7 ± 0.5	17.2 ± 0.2	15.5 ± 0.9
water (%)	95.5 ± 0.1	95.3 ± 0.1	94.2 ± 0.1
cell wall (% FW)	1.6 ± 0.3	1.2 ± 0.1	1.6 ± 0.2

[a] Values are the mean of three replicates ± SD.

Separation of phenolic compounds was achieved by gradient elution using an initial mixture of 95% water, with the pH adjusted to 2.5 with phosphoric acid, and 5% acetonitrile. The flow rate was 1 mL/min. The concentration of acetonitrile was increased to 25% during 30 min, maintained for 10 min, and increased to 50% in the subsequent 5 min. Phenolic compounds were identified by their retention times and absorption spectra in the 200−380 nm range. Phenolic standards were purchased from Sigma (St. Louis, MO).

Texture Determination. The texture of asparagus was measured using an Instron texturometer model 1011 fitted with a computerized system for the analysis of the force-displacement curves (Lipton, 1990). Values were expressed as maximum force (N), determined in a Warner−Blatzer cell, with a blade (0.3 cm) that cut the spear at 200 mm/min (average of 10 replicates).

Statistical Analysis. The data were statistically analyzed by an analysis of variance. The means were compared with Duncan's multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

A very well-known characteristic of the hardening process is that it does not take place homogeneously along the length of the spear (Lipton, 1990). For this reason, three different sections were studied, dividing the originally 15-cm-long spear into three 5-cm parts that were called the apical, middle, and basal sections.

Initially, the average weight of each section was approximately 16 g (Table 1). With storage, there was a 3-g weight loss in the apical section and a weight increase in the middle one of about 1 g, while the basal section did not change ($p < 0.05$). These differences most likely reflect the different respiration rates of the three sections, the apical having a much higher one than the others. The water content was between 95% and 96% of the fresh weight for fresh and stored asparagus. No apical growth was detected in the conditions used in this study.

The cell wall was isolated by treating the fresh tissue with SDS and PAW. The method was developed by Selvendran and Ryden (1990) and gives a cell wall preparation that is essentially free of cytoplasmic contamination with minimum degradation of cell wall components. An initial extraction with phosphate buffer has been included, which allows the study of some soluble polysaccharides that otherwise would be very difficult to recover from the SDS extracts. Cell wall represents between 1.2% and 1.7% (Table 1) of the fresh weight of the spear, depending on the section. In general, the yield is higher in the apical and basal sections than in the middle one for both the fresh and the stored product.

Figure 1 represents the texture of the three different asparagus parts, measured as maximum shear force. In the fresh spear, the texture in the apical section is much lower than that of the middle and basal ones.

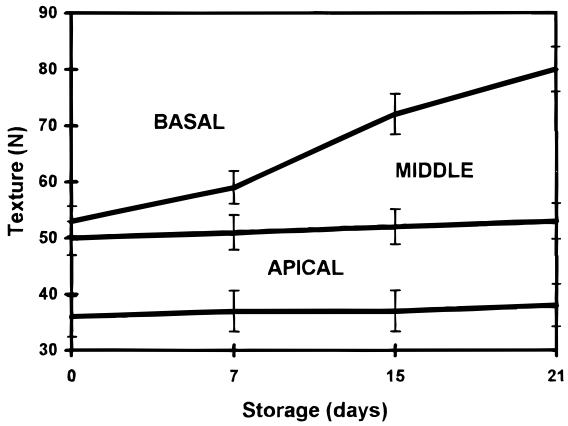

Figure 1. Texture as maximum shear force (N) of the three sections of the spear during storage.

Table 2. Neutral Sugars and Uronic Acids of the Different Cell Wall Fractions of Fresh and Stored Asparagus (μg/Spear)[a]

	apical		middle		basal	
	NS	UA	NS	UA	NS	UA
			Fresh			
PSF	2464	420	2095	260	2010	150
ISF	1125	4180	1175	2750	590	2100
CSF	2640	8030	2649	5750	2311	9300
K1SF	5889	8250	13146	6790	13025	7090
K4SF	12856	2880	24033	2440	21040	3060
CEL	62750	4750	56000	3400	76973	5180
total	87724	28510	99098	21390	115949	26880
			Stored			
PSF	3945	450	5449	240	4480	560
ISF	940	1470	475	1090	765	1100
CSF	3498	6290	2010	3450	1070	3300
K1SF	12678	2460	10202	3250	19443	4260
K4SF	15129	1700	21403	1320	29843	2380
CEL	55355	7450	49140	5540	80190	5610
total	91545	19820	88679	14890	135791	17210

[a] Values are the mean of four replicates (variation <10%).

With storage, no significant changes were observed neither in the middle section nor in the apical one, while important increases were found in the basal section, indicating that the hardening process was limited to this section.

Polysaccharides. Cell wall polysaccharides were sequentially extracted with an imidazole/HCl buffer, sodium carbonate and potassium hydroxide of 1.0 and 4.0 M. The final residue contains mostly cellulose, although some other residual polysaccharides were found (see below). The first two extractions released most of the pectic polysaccharides from the cell wall; however, as will be discussed later, a substantial amount of pectic polysaccharides is extracted together with the hemicellulosic material that is released by potassium hydroxide, and an additional polysaccharide remains in the final residue with the cellulose.

In the fresh spear, the total amount of sugars (Table 2) increased from the tip to the base, uronic acids being higher in the apical and basal sections and slightly lower in the middle one. During storage, the total amount of uronic acids decreased in all the sections by about the same magnitude (30%); the net losses were 8.7, 6.5, and 9.7 mg/spear for the apical, middle, and basal sections, respectively. The neutral sugars, on the other hand, increased slightly in the apical section (3.8 mg/spear), decreased in the middle one (10.4 mg/spear), and increased in the basal one (19.8 mg/spear).

Table 3. Neutral Sugars Composition of the Phosphate-Soluble Fraction for the Fresh and Stored Asparagus (μg/mg)[a]

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
			Fresh				
apical	123	0	943	93	250	1003	76
middle	93	0	763	81	100	921	142
basal	124	8	769	56	97	872	65
			Stored				
apical	185	0	1145	189	222	1290	918
middle	208	0	772	336	540	1018	2631
basal	464	0	1178	266	279	1442	856

[a] Values are the mean of four replicates (variation <10%).

Table 4. Neutral Sugars Composition of the Imidazole- and Carbonate-Soluble Polysaccharides for the Fresh and Stored Asparagus (μg/Spear)[a]

Imidazole-Soluble Fraction

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
			Fresh				
apical	72	0	580	62	61	345	57
middle	101	67	412	159	0	391	67
basal	30	0	179	63	13	249	59
			Stored				
apical	38	0	484	61	47	253	62
middle	47	0	190	56	17	132	35
basal	84	0	328	130	0	223	0

Carbonate-Soluble Fraction

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
			Fresh				
apical	352	0	947	191	86	916	146
middle	351	41	660	259	56	1129	146
basal	265	0	613	209	0	1095	159
			Stored				
apical	533	0	1628	228	0	971	133
middle	349	27	723	174	51	605	82
basal	135	14	330	149	36	344	65

[a] Values are the mean of four replicates (variation <10%).

Phosphate buffer extracted only a small proportion of cell wall polysaccharides from the fresh asparagus and about the same amount from every section (around 3% of the total neutral sugars and less than 1% of total uronic acids) (Table 2). The ratio UA/NS in this fraction is very low since neutral sugars represent more than 80% of the total. During storage, the amounts of neutral sugars increased in all sections and especially in the middle and basal ones where the increases exceeded 100%. The neutral sugars composition (Table 3) reveals that the polysaccharides in the fresh product are rich in arabinose and galactose, most likely in the form of arabinogalactans. During storage, the neutral sugar that increased the most was glucose, which suggests the incorporation of glucans and possibly of callose in this fraction.

The imidazole/HCl buffer extracted less than 1% of total neutral sugars and between 8 and 15% of the total uronic acids from the fresh spear (Table 2). They are pectic polysaccharides with a high ratio of uronic acids to neutral sugars (from 2.3 in the middle section to 3.5 and 3.7 in the basal and apical ones, respectively) and rich in arabinose and galactose (Table 4). The total amount of these polysaccharides decreased from the tip to the base mainly due to the decrease of uronic acids and also decreased at the base due to the decrease of arabinose. During storage, the changes in amounts and composition of these polysaccharides were different in the apical region as compared to the other regions. A

Table 5. Neutral Sugars Compostion of 1 and 4 M KOH-Soluble Polysaccharides for the Fresh and Stored Asparagus (μg/Spear)[a]

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
1 M KOH-Soluble Fraction							
Fresh							
apical	359	82	1431	1789	124	1373	731
middle	710	126	1897	6149	0	3516	726
basal	656	146	1866	5922	277	3283	956
Stored							
apical	460	149	3104	3732	0	3440	1741
middle	301	0	1467	6304	125	1308	697
basal	558	187	2126	14233	0	1303	1037
4 M KOH-Soluble Fraction							
Fresh							
apical	0	442	1471	4654	812	1457	4020
middle	229	818	2368	8212	1679	2531	8180
basal	233	593	2093	8621	1045	2300	6143
Stored							
apical	0	528	1663	5470	921	1648	4866
middle	121	454	3554	12799	367	2279	1829
basal	0	742	3374	17868	764	2055	4995

[a] Values are the mean of four replicates (variation <10%).

decrease of uronic acids was observed in the apical region (from 4 to less than 1.5 mg/spear) together with increases of arabinose, galactose, and glucose. In the middle and basal sections, however, there was a lower decrease of uronic acids (Table 2), and the other sugars undergo slight variations.

The polysaccharides extracted by sodium carbonate have a very similar composition to those soluble in imidazole, even though they represent a higher percentage of the cell wall. In the fresh asparagus, the amounts of neutral sugars are about the same in the three sections (Table 2), and their glycosyl compositions are also very similar (Table 4). The amounts of uronic acids, however, are lower in the middle section when compared to the other two sections. Storage induced a general decrease in uronic acids in all of the sections, this decrease being more pronounced in the basal section where about 6 mg/spear was lost.

It is remarkable that the amounts of uronic acids recovered in these two fractions are less than 50% of the total cell wall uronic acids, suggesting that pectic polysaccharides in asparagus are very strongly bound to the cellulosic and hemicellulosic matrix and need harsh conditions for release. The same applies for the stored spear.

The 1 M KOH extracted a far higher proportion of the neutral sugar residues from the middle and basal sections of the fresh spear than from the apical one, while the amounts of uronic acids are not statistically significant (Table 2). The neutral sugars composition (Table 5) shows that the amounts of xylose recovered are higher in the middle and basal sections of the spear as compared to the apical section. The same is true for galactose, although to a lesser extent. Storage induced different changes in each section. In the apical one there was a general increase in glucose, xylose, galactose, and arabinose together with a significant uronic acid decrease. In the middle section, the only noticeable changes were a decrease in uronic acids that was lower than that on the apical section and also a decrease in the amounts of galactose. Finally, in the basal section, together with the uronic acid decrease (which was of the same magnitude than that of the middle section), a very important increase on the amounts of xylose was detected from 5.9 to 14.2 mg/spear (more than a 100% increase). The other sugars did not change appreciably except for galactose, which decreased slightly.

Table 6. Neutral Sugars Composition of the Cellulose Fraction for the Fresh and Stored Asparagus (μg/Spear)[a]

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Fresh							
apical	0	0	3138	0	0	6275	53338
middle	0	0	3360	0	0	5040	47600
basal	0	0	2332	0	0	5443	69198
Stored							
apical	0	0	2280	0	1666	2280	49128
middle	0	0	1966	0	1474	1474	44226
basal	0	0	0	0	0	0	76180

[a] Values are the mean of four replicates (variation <10%).

Compared to the 1 M KOH fraction, the ratio of neutral sugars to uronic acids in the 4 M KOH-soluble fraction is much higher (Table 2). In the fresh asparagus, the amounts of uronic acids are very similar in all sections (between 2.5 and 3 mg/spear), while the amounts of neutral sugars are much higher in the basal and middle sections than in the apical section. Uronic acids decreased in all sections after storage, and neutral sugars increased in both the apical and basal sections but decreased in the middle section. The composition of neutral sugars (Table 5) of the middle and basal sections in the fresh spear are very similar while in the apical section the levels of almost all the sugars are lower. Storage induced a very important increase of xylose in the middle and basal sections that is parallel to a decrease of glucose. Quantitatively the decrease of xylose in the basal section is higher than that of the middle one while the opposite is true in the case of glucose. In the apical section, there was a small but statistically significant increase of xylose as well as an increase of glucose, suggesting that, while in the middle and basal sections deposition of xylans is taking place, in the apical sections xyloglucans are being incorporated. Galactose remains constant in all sections while arabinose increased in both the middle and the basal sections and mannose decreased.

The main polysaccharide fraction present in the asparagus cell wall is cellulose, representing between 50 and 60% of the total polysaccharides recovered from the cell wall (Table 2). The maximum amount of cellulose was found in the basal section (around 77 mg/spear), and the lowest was found in the middle section (56 mg/spear). In all sections, uronic acids were found together with arabinose and galactose (Table 6), which suggests the presence of pectic polysaccharides. Storage induced a general decrease of galactose and to a lesser extent of arabinose, while the amounts of uronic acids increased especially in the apical section. Glucose decreased slightly in the apical and middle sections and increased in the basal section.

Waldron and Selvendran (1990b) found a progressive increase in the maturation state of tissues from the tip to the base of green asparagus spears. They concluded that the apical section is a region of cell division and elongation while the base is a region of secondary thickening. The differences in carbohydrate composition reflected these different maturation states. They found an important decrease of arabinose from the tip to the base and an increase of xylose and glucose especially at the base. It was suggested that arabinose-rich pectic

polysaccharides of middle lamella origin from the apical region were being replaced in the middle and basal sections by other pectic polysaccharides richer in galactose and by hemicellulosic and cellulosic polysaccharides as the primary and secondary cell wall developed (Bolwell, 1988). In white asparagus, based on the results presented in this paper, a similar situation seems to happen. However, looking at the overall changes of cell wall carbohydrates, some differences can be inferred. Arabinose did not change very much from the tip to the base, and at the same time the majority of it is not of middle lamella origin since it needs alkaline conditions to be extracted (sodium carbonate and potassium hydroxide). Uronic acids, on the other hand, decreased from the tip to the base, and most of it is of middle lamella origin (soluble in phosphate buffer and imidazole buffer). This suggest that, in white asparagus, the pectic polysaccharides that are replaced are mainly homogalacturonans instead of arabinose-rich polysaccharides as in green asparagus. Xylose and glucose followed the same trend as the green asparagus with increases of about 100 and 30%, respectively.

During storage, the results of this paper suggest that polysaccharides undergo important turnover; uronic acids decreasing in all sections and almost in all polysaccharide fractions, while neutral sugars increased very slightly in the apical section, decreased in the middle, and increased in the basal one. Xylose, glucose, and galactose are the main neutral sugars implicated in the turnover. Xylose accumulated in the hemicellulosic fractions of the middle and basal sections, while glucose is lost from the hemicellulosic and cellulosic fractions of the middle section and accumulated in the cellulosic fraction of the basal one. Finally, galactose is lost from the cellulosic fractions of the three sections. Some differences can be found between these results and those reported for green asparagus during storage (Waldron and Selvendran, 1990b, 1992). No uronic acids decrease has been found in green asparagus stored in aerobic conditions. In fact, an increase was reported of about 34 mg/g cell wall. Arabinose decreased by about 30 mg/g cell wall in green asparagus while no changes were observed for white asparagus. The increase of xylose was more important in the white spear (around 20 mg/g cell wall) than in the green spear (1 mg/g cell wall; Waldron and Selvendran, 1992).

It can be concluded that, except for some minor differences, qualitatively the process that take place in white asparagus during storage is very similar to that of green asparagus; however, quantitatively, the deposition of polysaccharides, specially xylans, seems to be much more intense. These differences, neverthless, could be of consequence as variables such as conditions of storage (3 days at 21 °C for the reported values of green asparagus and 21 days at 4 °C for those reported in this paper) and the variety. A deeper study with similar conditions of storage and the same variety of asparagus would be needed to establish unambiguous comparisons between these two types of asparagus.

Other Cell Wall Components. *Lignin.* Lignin is the cell wall component most frequently associated with hardening. Its measurement in plant material is generally difficult to achieve, and no general method has universal application for a given product. In this study, four different methods were evaluated for estimating the quantity of lignin in asparagus. Three are gravimetric: acid detergent lignin (ADL) (Van Soest, 1963), permanganate lignin (Goering and Van Soest, 1973), and Klason lignin. The last one is spectrometric: acetyl bromide lignin (Iiyama and Wallis, 1990).

Table 7. Comparison of Different Methods for Lignin Quantification (mg/Spear)[a]

	fresh	stored
acid detergent lignin (ADL)	33 ± 5	59 ± 3
permanganate lignin	32 ± 2	60 ± 2
Klason lignin	57 ± 2	80 ± 4
acetyl bromide	105 ± 2	126 ± 2

[a] Values are the mean of three replicates ± SD.

The first two use the acid detergent fiber (ADF) as the starting material (Van Soest, 1963), The ADL method estimates lignin as the residue after treatment with sulfuric acid, while the permanganate method measures weight loss after the oxidation of lignin with potassium permanganate. The main problem when using ADF is that after the plant material is heated with acid detergent for an extended period of time, a variable amount of lignin becomes soluble or dispersible in the extraction media. In some cases, the losses can reach 50% (Lowry et al., 1994).

The loss of acid-soluble lignin is much lower in the Klason lignin method since the treatment with sulfuric acid is much shorter than in the ADF. However, incomplete hydrolysis of polysaccharides and the coprecipitation of proteins with lignin can overestimate the quantity of lignin. This problem can be partially overcome by using a pre-extracted material with a reduced protein content (such as the cell wall) as a substrate for analysis.

The extraction of lignin with acetyl bromide and the quantification of its absorbance at 280 nm has proved to be a good estimation of lignin in many plant materials (Iiyama et al., 1990) provided that a suitable starting material is used where interferences by proteins and phenolic compounds are eliminated (Südekum et al., 1997).

Table 7 shows the results of the four different methods applied to whole asparagus both fresh and stored using the cell wall (prepared as described in Materials and Methods) as the starting material for the Klason and acetyl bromide methods. Important differences were found among all methods. ADL and permanganate lignin gave similar results for fresh and stored asparagus, but they are both lower than that of Klason and acetyl bromide lignins, suggesting that a substantial amount of asparagus lignin is readily soluble or dispersible in the acid detergent. The values of lignin for these methods are about 32 and 60 mg/spear for fresh and stored asparagus, respectively. Klason lignin gave an estimate of about 20−25 mg/spear higher than ADL for both fresh and stored asparagus. In this method, the interference of proteins was minimized since, as will be discussed below, the protein content of the cell wall preparation was very low. Finally, the acetyl bromide method gave the highest values for lignin, almost 50 mg/spear higher than that of Klason method. This lack of correlation among the different methods, especially between the acetyl bromide and Klason lignin, is difficult to explain since a relatively pure starting material was used in the determinations. It is possible that the extinction coefficient used in the spectrometric method, which has been calculated for other plant materials (Iiyama and Wallis, 1990), is not applicable to asparagus; this needs to be researched

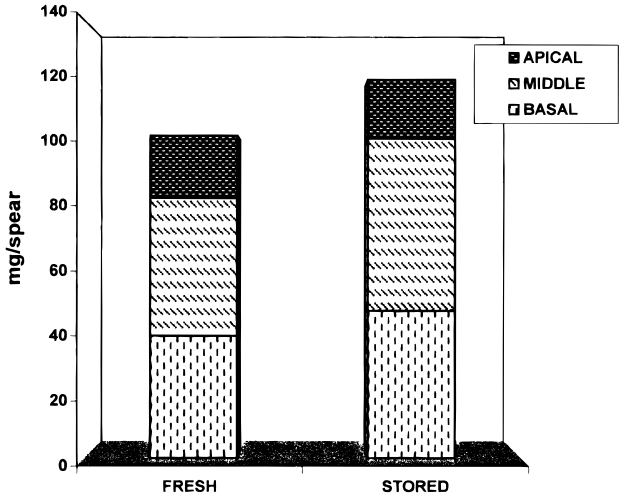

Figure 2. Distribution of lignin (mg/spear) in the three sections of fresh and stored white asparagus.

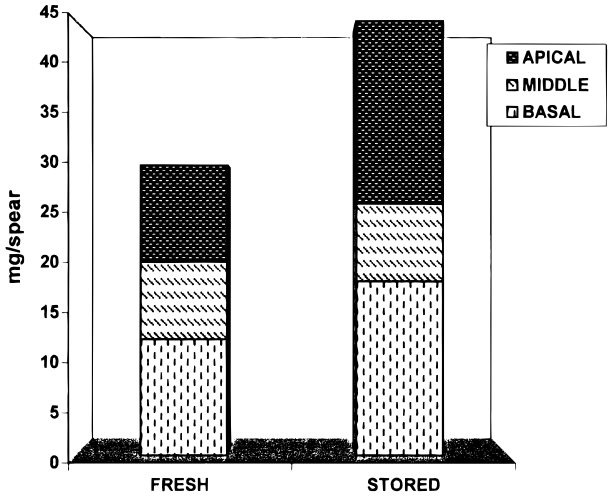

Figure 3. Distribution of cell wall proteins (mg/spear) in the three sections of fresh and stored white asparagus.

more. In any case, no matter what the real value for lignin is, it is remarkable that the increase in lignin content in the asparagus during postharvest storage is about the same, independent of the method used (between 21 and 28 mg/spear).

The distribution of lignin among the three sections studied is shown in Figure 2. For simplication, only the values obtained by the acetylbromide method are presented. In the fresh asparagus, lignin is concentrated in the middle and basal sections representing 44 and 39 mg/spear, respectively; while its content in the apical section is about 50% lower (20 mg/spear). During storage, the lignin content of the apical section remains unchanged, while those of the middle and basal sections increase to 55 and 47 mg/spear, respectively. It is remarkable that the maximum content and also the highest increase is taking place in the middle section of the spear.

These results suggest that the toughening process is not primarily related to the amounts of lignin, as has been previously suggested (Lipton, 1990), since the highest amounts of lignin accumulate in a section of the spear that does not undergo hardening. It is possible that the process of toughening could be related to the macromolecular structure of lignin and to its cross-linking with other cell wall components, either polysaccharides or proteins or both, more than with the total amount present in a given tissue. Similar conclusions have been found in relation to the decreased digestibility of some crops (Südekum et al., 1997).

Proteins. In fresh asparagus, the content of cell wall proteins ranged from 8 to 12 mg/spear, depending in the region considered (4−5% of the cell wall) (Figure 3); its content being higher in the apical and basal sections than in the middle section. During storage, there was a clear increase in the apex and in the base, while no changes were detected in the middle section of the spear.

Wall Bond Phenolics. Monomeric and dimeric phenolic acids have been largely identified as minor components of plant cell walls (Fry, 1988). They are thought to play important roles in cross-linking several wall polymers (Ralph et al., 1994). Cross-link fragments have been isolated and characterized from several plant sources (Ishii, 1997) and have been suggested as key control points of the texture of some fruits and vegetables (Waldron et al., 1997). Since the wall phenolics

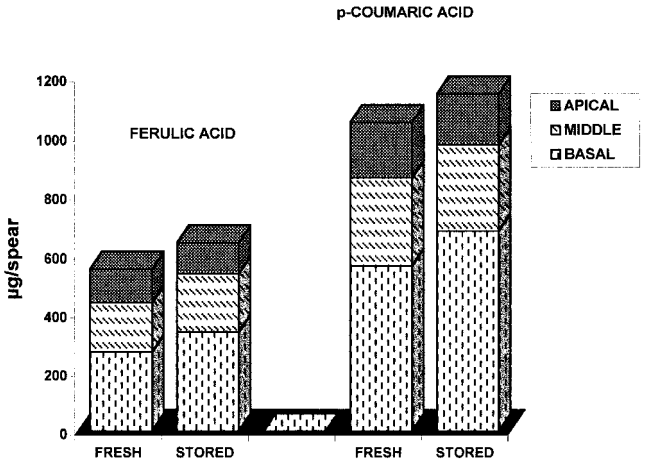

Figure 4. Distribution of cell wall simple phenolics (μg/spear) in the three sections of fresh and stored white asparagus.

are ester bound to the polysaccharides of the cell wall, the easiest way of detecting evidence of phenolics cross-linking in the cell wall is the treatment with 1 M NaOH. If the cross-linkages exist, then, beside ferulic and *p*-coumaric acid, substantial amounts of ferulic acid dimers should be detected. That is the case of Chinese water chestnuts (Parr et al., 1996), where about 20% of the ferulic acid present is in the form of ferulic acid dimers.

In asparagus, using the same conditions as reported by Parr et al. (1996), no evidences of the ferulic acid dimers have been found, and the only wall-bound phenolics detected have been ferulic and *p*-coumaric acids. In fresh asparagus, the highest amounts of ferulic acid were found in the basal region with little change during storage. *p*-Coumaric acids were also concentrated in the basal region of the spear, increasing slightly during storage (Figure 4).

The lack of phenolic dimers in the asparagus cell wall suggests that cross-linking between polysacharides is not very important in the asparagus cell wall. Experiments to confirm this conclusion are presently being carried out.

ABBREVIATIONS USED

CW, cell wall; SDS, sodium dodecyl sulfate; PAW, phenol−acetic acid−water; PSF, phosphate-soluble frac-

tion; ISF, imidazole-soluble fraction; CSF, carbonate-soluble fraction; K1SF, 1 M KOH-soluble fraction; K4SF, 4 M KOH-soluble fraction; CEL, cellulose fraction; UA, uronic acids; NS, neutral sugars; TFA, trifluoroacetic acid; FW, fresh weight; ADF, acid detergent fiber; ADL, acid detergent lignin.

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