Turnover of White Asparagus Cell Wall Polysaccharides during Postharvest Storage

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The main polysaccharides involved in asparagus cell wall turnover have been indentified. Homogalacturonans are lost from both the apical and basal sections of the spear. Galactans are mobilized from the cellulose residue of the apical section and recovered in the KOH-soluble fractions while they are lost from the cellulose residue of the basal section. Xyloglucans are incorporated in the apical region and degraded from the basal one. Cellulose is incorporated in the basal region and lost from the apical one, and acidic xylans are incorporated in high amounts in the basal section of the spear.

Keywords: *White asparagus; polysaccharides; cell wall; xyloglucan; xylan; galactan; homogalacturonan; cellulose*

INTRODUCTION

Major changes occur in both cell wall structure and composition during postharvest storage of fruits and vegetables (Van Buren, 1979), and these changes have important implications in the overall quality of the food. One distinctive characteristic of both processes is that whereas fruits soften, vegetables become tougher.

The postharvest turnover of cell wall polysaccharides of fruits has been extensively studied. The clearest change that accompanies fruit softening is the decrease in wall-bound polyuronides and the concomitant increase of soluble ones. In most cases fragmentation of hemicelluloses has also been detected together with losses of some neutral sugars such as galactose and arabinose. Many hydrolases including polygalacturonase, pectinmethylesterase, glucanases, and glycosidases have been related to the softening process, although a clear picture of the relative contribution of each of these enzymes to turnover is still unavailable (Bartley and Knee, 1982; Lavabitch 1981; Gross 1989; Heredia et al., 1992; King and O'Donoghue, 1995; Fernández-Bolaños et al., 1995)

Much less work has been done in the postharvest storage of immature vegetables such as asparagus. Losses of cell wall bound galactose and increases of xylans and cellulose have been detected during postharvest storage of selected tissues from green asparagus (Waldron and Selvendran, 1992). The potential contribution of cell wall hydrolases to the postharvest textural and metabolic changes is unexplored (King and O'Donoghue, 1995). Knowledge about cell wall turnover during vegetable storage could show us new methods to improve postharvest quality in a similar way as is being done with fruits (Fischer and Bennett, 1991).

In a previous paper (Rodríguez et al., 1999) we reported on changes in the overall composition of white asparagus cell wall during storage, and it was pointed out that cell wall polysaccharides underwent important turnover. The aim of the present study has been to identify the main kind of polysaccharides that are involved in that turnover.

MATERIALS AND METHODS

Plant Material. Asparagus (*Asparagus officinalis* L.) was obtained from a horticultural unit in Alcalá del Río (Sevilla, Spain). The asparagus were stored at 4 °C and under saturated humidity for 21 days.

Cell Wall Isolation and Fractionation. The cell wall material (CWM) was prepared as described by Jiménez et al. (1994) with some modifications (Figure 1). The fractionations were carried out by successive extractions of CWM as indicated by Sánchez-Romero et al. (1998) with some modifications: (i) 0.5 M imidazole−hydrochloric acid buffer (imidazole-soluble fraction, ISF), (ii) 0.05 M sodium carbonate, 0.01 M sodium borohydride (carbonate-soluble fraction, CSF), (iii) 1 M potassium hydroxide (1 M potassium-soluble fraction, K1SF), and (iv) 4 M potassium hydroxide, 0.01 sodium borohydride (4 M potassium-soluble fraction, K4SF) (Figure 2).

Ion-Exchange Chromatography. The isolated fractions were fractionated on two subfractions of neutral and acid polysaccharides. A Hi-Trap Q-Sepharose column (5 × 2.0 cm) (Pharmacia Fine Chemicals, Uppsala, Sweden) was used. The exchanger was equilibrated in 0.01 M imidazole−hydrochloric acid buffer at pH 7, and the neutral polysaccharides were eluted, followed by 2 M imidazole−hydrochloric acid buffer at pH 7, and then the acidic polysaccharides were eluted. Neutral sugars' reaction with anthrone (Dische, 1962) and uronic acid's reaction with *m*-hydroxybiphenyl (Blumenkrantz and Asboe-Hansen, 1973) were quantified colorimetrically in the different fractions.

Analysis of the Glycosyl Composition. The glycosyl composition of the acidic and neutral subfractions was determined by gas chromatography (GC) (Englyst and Cumming, 1984) after hydrolysis with 2 N trifluoroacetic acid, reduction, and acetylation. The derivatives were identified by GC in a Series II Hewlett-Packard 5890 instrument, capillary column (30 m × 0.25 mm). The oven temperature was programmed beginning at 180 °C and then was raised subsequently at 4 °C/min to 240 °C, at which it was maintained for 20 min. Helium was the carrier gas, at 1 mL/min.

Methylation Analysis. Methylation analysis was carried out on the neutral and acidic fractions of PSF, ISF, CSF, K1SF,

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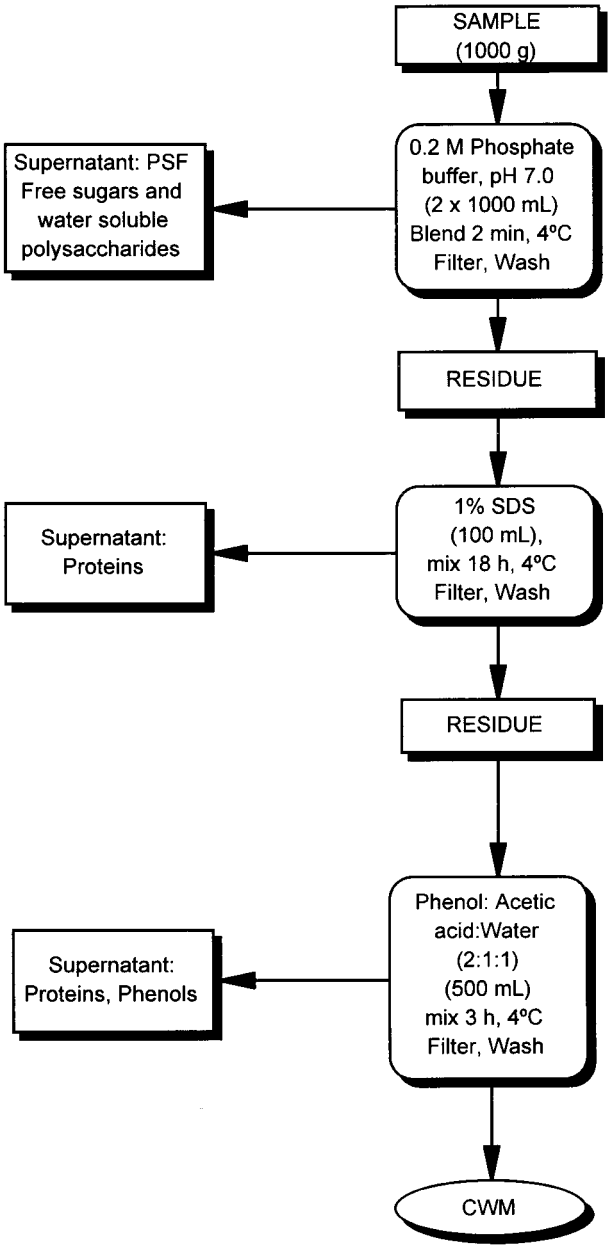

Figure 1. Scheme followed for the preparation of cell walls from the apical and basal sections of white asparagus.

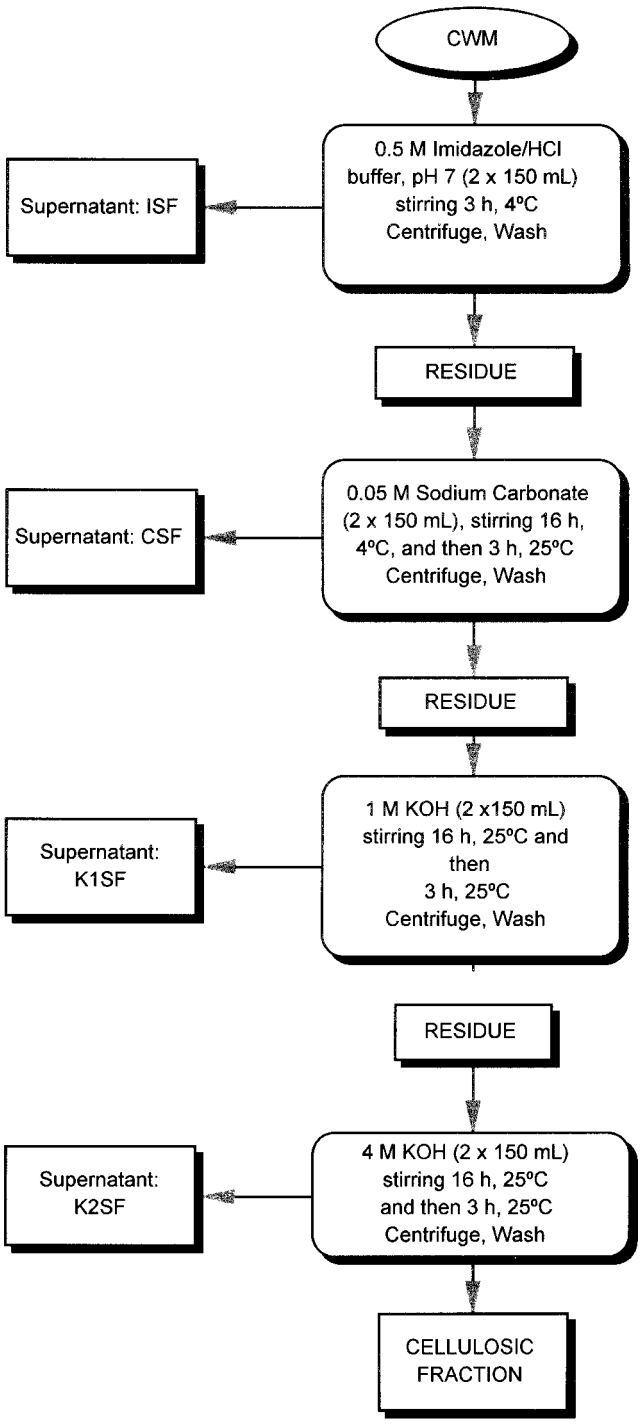

Figure 2. Scheme followed for the preparation of polysaccharide fractions from the apical and basal sections of white asparagus.

and K4SF. Methylation analysis was carried out without reduction of uronic acids. Each fraction was methylated according to a modification of the method of Hakomori (1964): 0.1 mL of butyl-lithium (2.5 M in hexane, Aldrich) was added to samples dissolved in 0.4 mL of dimethyl sulfoxide (Sigma). The mixture was then shaken for 1 h (Kvernheim, 1987). After the samples had been frozen, 0.2 mL of ethyl iodide (Fluka) was added, and the samples were allowed to thaw at room temperature and later shaken for 15 h. The permethylated polysaccharides were separated and purified by reverse phase chromatography using a C_{18} Sep-Pak (Varian). The polysaccharides were hydrolyzed, reduced, and acetylated (York et al., 1986). The partially methylated alditol acetates were separated by GC/mass spectrometry (GC/MS) in a Series II Hewlett-Packard 5890 instrument, which was coupled to a Hewlett-Packard 5972 selective mass detector with a fused silica capillary column (30 m × 0.25 m, SPTM 2330, Supelco) in splitless mode. The oven temperature program was the same as the one used by York et al. (1986). For quantification, molar effective response factors were used (Carpita and Shea, 1989).

RESULTS AND DISCCUSSION

To study polysaccharide turnover during storage of white asparagus, the cell walls of apical and basal sections from both fresh and stored spears were isolated and sequentially extracted with phosphate buffer, imidazole−HCl, sodium carbonate, 1 M KOH, and 4 M KOH, leaving a residue of insoluble material called cellulose. The glycosyl composition of all the supernatants and the cellulose residue has been determined, and the changes during storage have been quantified.

Figure 3 summarizes the main changes on cell wall sugars during storage. In the apical section of the spear,

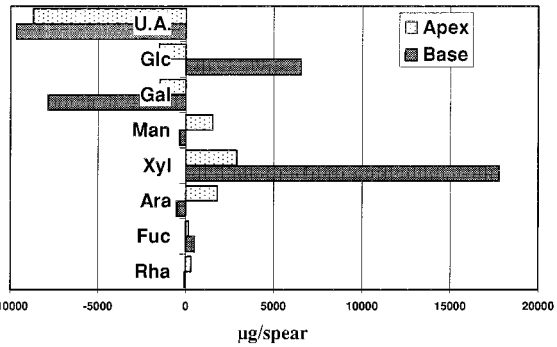

Figure 3. Overall changes on cell wall bound sugars during storage of the apical and basal sections of white asparagus.

Table 1. Changes (Micrograms per Spear) of Cell Wall Sugars during Storage in the Different Polysaccharide Fractions Isolated from the Apical and Basal Sections of White Asparagus[a]

	Ara	Xyl	Man	Gal	Glc	UA
			Apex			
phosphate	***	***	***	***	842	***
imidazole	***	***	***	***	***	−2710
carbonate	681	***	***	***	***	−1740
1 M KOH	1673	1943	***	2067	1010	−5790
4 M KOH	***	816	***	***	***	−1180
cellulose	−858	***	1666	−3995	−4210	2700
			Base			
phosphate	***	**	***	***	862	***
imidazole	***	***	***	***	***	−1000
carbonate	***	***	***	−751	***	−6000
1 M KOH	***	8311	***	−1935	***	−2830
4 M KOH	***	9247	***	***	−1148	−680
cellulose	−2332	***	***	−5443	6982	***

[a] A positive number denotes an increase during storage, whereas a negative one indicates a decrease. Asterisks denote that the changes (positive or negative) are <200 μg/spear.

turnover is characterized by an important decrease of uronic acids (UA; 9000 μg/spear), slight decreases of glucose and galactose (1500 μg/spear each), and slight increases of mannose, xylose, and arabinose (1500, 2900, and 1800 μg/spear respectively). Uronic acids (Table 1) increased in the cellulose residue (~3000 μg/spear) and decreased in almost all of the other polysacchride fractions, althought the most important decreases took place in the 1 M KOH-soluble fraction (~6000 μg/spear). Arabinose, galactose, and glucose decreased in the cellulose residue and increased in the 1 M KOH-soluble fraction, whereas xylose increased in the two KOH-soluble fractions and mannose slightly increased in the cellulose residue.

In the basal section (Figure 3) cell wall changes involved decreases of uronic acids (10000 μg/spear) and galactose (8000 μg/spear) and important increases of glucose (6000 μg/spear) and xylose (18000 μg/spear). Uronic acids (Table 1) decreased the most in the carbonate-soluble fraction (6000 μg/spear) and in the 1 M KOH-soluble one (~3000 μg/spear), whereas galactose decreased in the cellulose residue (~5000 μg/spear) and in the 1 M KOH-soluble fraction (2000 μg/spear). Glucose increased in the cellulose residue (7000 μg/spear) and decreased in the 4 M KOH-soluble fraction. Xylose increased in the two KOH-soluble fractions.

To determine which wall polymers are involved in turnover, the different polysaccharide fractions isolated from the apical and basal sections of the spear have been further resolved by ion-exchange chromatography on a Q-Sepharose column as described under Materials and Methods. In the conditions used in this study (on−off chromatography) each polysaccharide fraction is divided into a neutral fraction (the one that does not bind to the column at low ionic strength) and an acidic one that eluted with buffer of high ionic strength (2 M imidazole−HCl buffer).

Phosphate-Soluble Polysaccharides. Table 2 shows the glycosyl composition of the different fractions isolated from the phosphate-soluble polysaccharides. Good recovery (>80%) for all sugars except glucose in the stored spear was found. Glucose in the apical section of the stored spear was recovered only at 11% and in the basal one at 39%. Most of the glucose was lost as an insoluble residue during the step of preparation for anion-exchange chromatography (dialysis with 10 mM imidazole buffer); this is not surprising because glucose in these fractions is in the form of β-glucans (see below) and these polysaccharides tend to precipitate once extracted from the cell wall due to intermolecular interactions.

In all cases the majority of the polysaccharides were recovered in the acidic fractions, although the relative importance of the neutral ones increased with storage. Except for the small amount of polysaccharides isolated from the apex of the fresh spear, in which mannose, glucose, and rhamnose are the main sugars, the major sugars in all of the other fractions are galactose and arabinose together with smaller quantities of mannose in the neutral fractions.

Methylation analysis of the acidic fractions (Table 3) suggests the presence of arabinogalactans of the type II, which are highly branched polysaccharides constituted by a backbone of β-1,3-linked galactose units (3-Gal), which in some cases are substituted at position 6 (3,6-Gal) with (1→6)-linked β-D-galactopyranose side chains (6-Gal), which are in turn branched with ara-

Table 2. Glycosyl Composition of the Two Fractions Isolated from the Phosphate-Soluble Polysaccharides of the Apex and Base of Fresh and Stored White Asparagus[a]

	fraction	total sugars (μg/spear)	recovery (%)	glycosyl composition (%)							
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
				Fresh							
apex	neutral	101	3.5	16	21	0	0	45	6	12	0
	acidic	2782	96.5	4	0	26	3	2	47	3	15
base	neutral	224	10.4	0	0	42	3	4	46	4	0
	acidic	1932	89.4	6	0	33	0	0	51	3	8
				Stored							
apex	neutral	891	20.0	5	0	28	8	17	31	11	0
	acidic	2932	66.7	9	0	38	0	0	38	0	15
base	neutral	1255	24.9	8	0	24	7	11	36	14	0
	acidic	3768	74.8	11	1	27	3	3	36	4	15

[a] Recovery is the percentage that each fraction represents in relation with the total sugars loaded in the ion-exchange column.

Table 3. Glycosyl Linkage Composition (Mole Percent) of the Acidic Fractions of Phosphate-Soluble Polysaccharides of the Apex and Base of Fresh and Stored White Asparagus[a]

	apex				base			
	F	aa	S	aa	F	aa	S	aa
T-Rha	2	***5***	3	***8***	3	***7***	3	***13***
T-Araf	20		17		20		14	
T-Arap	0		4		2		5	
3-Ara	4		5		6		7	
5-Ara	6		6		4		3	
3,5-Ara	7	***34***	5	***41***	3	***40***	6	***35***
T-Gal	10		11		9		13	
3-Gal	10		8		9		8	
6-Gal	7		6		8		9	
3,6-Gal	30	***51***	26	***51***	32	***50***	25	***38***
T-Xyl	3	***5***	4	**0**	4	**0**	6	**3**
4-Glc	5	***3***	5	***0***	0	***3***	3	***5***

[a] Column aa represents the mole percent of each sugar calculated by using the method of the alditol acetates. F, fresh; S, stored.

Table 4. Glycosyl Linkage Composition (Mole Percent) of the Neutral Fractions of Phosphate-Soluble Polysaccharides of the Apex and Base of Stored White Asparagus[a]

	apex		base	
	S	aa	S	aa
T-Rha	1	***5***	1	***8***
T-Araf	11		6	
T-Arap	4		7	
2-Ara	3		0	
3-Ara	8		7	
5-Ara	6		3	
3,5-Ara	2	***32***	3	***27***
T-Manp	8		5	
4-Manp	0		0	
4,6-Man	2	***15***	7	***10***
T-Gal	6		9	
3-Gal	3		3	
6-Gal	10		9	
3,6-Gal	11	***29***	9	***33***
T-Xyl	8		7	
4-Xyl	8		12	
2,4-Xyl	2	***8***	0	**8**
3-Glc	1		3	
4-Glc	7	***10***	9	***13***

[a] Column aa represents the mole percent of each sugar calculated by using the method of the alditol acetates. S, stored.

binofuranose and other, less abundant, monosaccharides. In the case of asparagus a large number of the galactose residues in the backbone (>20%) are unsubstituted (3-Gal). The distribution of residues for the arabinose is also compatible with the structure of arabinogalactan II (Fincher and Stone, 1983; O'Neill et at, 1990).

The corresponding methylation analysis of the neutral fractions for the apex and base of the stored spear (Table 4) reveals the presence of mannans and glucans together with polymers of arabinose and galactose very similar to those found in the acidic ones. Glucose is mostly in the form of β-1−4-glucans but is also present in a small amount in the β-1−3 form.

It can be concluded that polysaccharides extracted by phosphate buffer are mainly acidic arabinogalactan II in the fresh asparagus. Storage induced an accumulation of these polysaccharides in the basal region of the spear and also in both the apical and basal regions the deposition of β-glucans, mannans, and arabinogalactans, all of them as neutral polysaccharides.

Table 5. Glycosyl and Glycosyl Linkage Composition (Mole Percent) of Carbonate-Soluble Polysaccharides from the Apex and Base of Fresh and Stored White Asparagus[a]

	apex		base	
	fresh	stored	fresh	stored
total sugars (μg/spear)	10670	9788	11611	4370
glycosyl composition (%)				
Rha	3	5	2	3
Fuc	0	0	0	0
Ara	9	17	5	8
Xyl	2	2	2	3
Man	1	0	0	1
Gal	9	10	9	8
Glc	1	1	1	1
UA	75	64	80	78

linkage	fresh	aa	stored	aa	fresh	aa	stored	aa
T-Ram	3		2		0		4	
2-Ram	4		4		11		0	
3-Ram	3		3		0		0	
2,4-Ram	3	***13***	4	***15***	6	***12***	0	***11***
T-Araf	11		25		16		14	
T-Arap	4		5		7		6	
2-Ara	2		6		0		9	
3-Ara	0		6		4		0	
5-Ara	20		8		10		14	
3,5-Ara	21	***39***	10	***50***	18	***33***	27	***29***
T-Gal	5		6		11		9	
3-Gal	2		2		0		0	
6-Gal	0		1		0		0	
2,4-Gal	3		2		0		0	
3,6-Gal	0		5		4		2	
4,6-Gal	7	***32***	2	***25***	6	***29***	9	***43***
T-Xyl	4		3		0		0	
4-Xyl	3		5		8		6	
2,4-Xyl	1	***8***	0	***7***	0	***15***	0	***10***
4-Glc	4	***5***	0	***0***	0	***5***	0	***6***

[a] Column aa represents the mole percent of each sugar calculated by using the method of the alditol acetates.

Arabinogalactans II are usually not considered as structural components of the cell wall. Generally they appear linked to hydroxyproline-rich proteins in the so-called arabinogalactan-proteins (AGP). Their function in plants is unknown, although they have been proposed to play a role in some physiological processes such as cell−cell recognition and cell−cell interactions (Fincher and Stone, 1983). They have been also shown to accumulate in response to wounding (Showalter, 1990), as happen with β-glucans (Hayashi, 1989).

Carbonate-Soluble Fraction. All of the polysaccharides included in this fraction bound to the ion-exchange column. The glycosyl composition (Table 5) is characterized by a very high proportion of uronic acids (>60%), suggesting the presence of homogalacturnonans as the main polysaccharides. Methylation analysis suggests also the presence of small amounts of rhamnogalacturonans of type I. In general and in comparison with the phosphate-soluble fractions a high proportion of 5- and 3,5-Ara is observed, as is the absence of 6- and 3,6-Gal and the presence, on the other hand, of 2,4- and 4,6-Gal and different rhamnose residues. Some of these sugars decreased during storage, but the most prominent change is the decrease of uronic acids, which are

Table 6. Glycosyl Composition of the Two Fractions Isolated from the 1 M KOH-Soluble Polysaccharides of the Apex and Base of Fresh and Sotred White Asparagus[a]

	fraction	total sugars (μg/spear)	recovery (%)	glycosyl composition (%)							
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
				Fresh							
apex	neutral	1560	11.0	0	4	4	19	0	41	32	0
	acidic	12543	88.7	2	0	10	15	0	8	0	66
base	neutral	2415	12.0	3	0	5	14	1	58	19	0
	acidic	16308	81.0	3	0	11	21	2	16	4	43
				Stored							
apex	neutral	1272	8.0	0	6	4	29	0	13	48	0
	acidic	11329	74.8	0	0	25	31	0	16	6	22
base	neutral	5472	23.0	0	5	6	27	1	21	41	0
	acidic	18285	77.0	2	0	18	41	0	10	3	23

[a] Recovery is the percentage that each fraction represents in relation with the total sugars loaded in the ion-exchange column

Table 7. Glycosyl Linkage Composition (Mole Percent) of the Acidic Fractions of 1 M KOH-Soluble Polysaccharides of the Apex and Base of Fresh and Stored White Asparagus[a]

	apex				base			
	F	aa	S	aa	F	aa	S	aa
T-Rha	0		0		0		0	
2-Rha	1		3		6		1	
3-Rha	0		0		0		0	
2,4-Rha	0		0		0		0	
total	*1*	***5***	*3*	***3***	*6*	***5***	*1*	***3***
T-Araf	4		4		5		7	
T-Arap	7		13		2		5	
2-Ara	0		0		1		1	
3-Ara	4		10		1		2	
5-Ara	5		3		17		12	
3,5-Ara	3		3		1		1	
total	*23*	***32***	*34*	***27***	*28*	***31***	*27*	***25***
T-Gal	0		2		3		4	
3-Gal	0		0		0		0	
4-Gal	7		18		13		5	
2,4-Gal	0		0		0		0	
3,6-Gal	7		0		11		2	
4,6-Gal	0		0		0		0	
total	*14*	***19***	*20*	***14***	*27*	***21***	*11*	***12***
T-Xyl	4		5		2		5	
4-Xyl	54		37		29		41	
2,4-Xyl	4		2		8		15	
total	62	***44***	44	**52**	39	**34**	60	**57**
4-Glc	0		0		0		0	
total	*0*		*0*	***5***	*0*	***5***	*0*	***3***

[a] Column aa represents the mole percent of each sugar calculated by using the method of the alditol acetates. F, fresh; S, stored.

the components that decreased the most of this group, especially in the basal section of the spear.

1 M KOH-Soluble Fraction. Most of these polysaccharides are of acidic nature, representing in all cases, except in the basal section of the stored asparagus, >85% of the total (Table 6). Good recovery of all sugars was found except for xylose, with a recovery of ~60% on the basal section for both fresh and stored spears.

As was mentioned before, the overall changes for this fraction in the apical and basal sections are very different; whereas in the first one there is a decrease of uronic acids and an increase of arabinose, xylose, and galactose, in the last one the main change was a very important increase of xylose.

In the apical section of the fresh asparagus (Table 7) the xylose is in the form of xylans very poorly substituted, given the ratio of 4-Xyl to 2,4-Xyl. Besides the xylans, the presence of galactose- and arabinose-rich pectic polysaccharides can also be inferred. Galactose is probably in the form of galactans (4-Gal) and arabinogalactan (3,6-Gal), whereas arabinose is in the form of arabinans (3,5-Ara, T-Ara, 5-Ara) and arabinogalactans (3-Ara). It is interesting to note the high proportion of T-Ara in the pyranose configuration, which is very unusual.

Xylose incorporates in the basal section as both neutral and acidic polysaccharides (Table 7); these last ones are basically xylans with a ~25% of substitution (ratio between 4-Xyl and 2,4-Xyl).

4 M KOH-Soluble Fraction. The composition of the different fractions recovered for this group of polysaccharides is shown in Table 8. The recovery for most of the sugars is >90% in all cases except for xylose of the basal section of the stored asparagus, which is recovered only at 48%.

In the fresh spear the majority (>60%) of polysaccharides are recovered in the neutral fraction in both the apical and basal sections. They are polysaccharides rich in glucose and xylose containing moderate amounts of galactose; their glycosyl linkage composition (Table 9) suggests the presence of xyloglucans. The glucose residues of the backbone of these polysaccharides are substituted at ~50% (ratio between 4-Glc and 4,6-Glc) in the apical section and apparently at 75% in the basal one. The acidic fractions are very rich in uronic acids (>50%), arabinose being the main neutral sugar.

Storage induced decreases of the total sugars in the neutral fractions of both the apical and basal sections, suggesting the degradation of xyloglucans because their glycosyl composition and glycosyl linkage composition did not change very much. The total amount of sugars in the acidic fraction of the apical section did not change due to the fact that parallel to the decrease of uronic acids (Table 1) there was an increase of xylose. In the basal section there was a net increase of total sugars due mostly to the very important increase of xylose. Methylation analysis of these fractions (data not shown) reveals a residue distribution similar to that found for the acidic fractions of 1 M KOH-soluble polysaccharides (Table 7). It can, therefore, be concluded that during storage there was degradation of homogalacturonans and xyloglucans from this group of polysaccharides and an important deposition of xylans, mainly in the basal section.

FINAL REMARKS

The results of the present study allow us to determine the main characteristics of polysaccharide turnover in white asparagus during storage.

Table 8. Glycosyl Composition of the Two Fractions Isolated from the 4 M KOH-Soluble Polysaccharides of the Apex and Base of Fresh and Stored White Asparagus[a]

	fraction	total sugars (μg/spear)	recovery (%)	glycosyl composition (%)							
				Ram	Fuc	Ara	Xyl	Man	Gal	Glc	UA
				Fresh							
apex	neutral	11377	72.3	1	6	4	28	8	12	42	0
	acidic	4471	28.4	0	0	16	10	0	6	4	64
base	neutral	15470	64.2	1	0	0	36	8	17	38	0
	acidic	5647	23.0	1	1	22	7	1	8	6	54
				Stored							
apex	neutral	9900	58.8	0	6	6	26	7	11	43	0
	acidic	5971	35.5	5	0	22	23	0	18	3	28
base	neutral	13268	41.2	0	9	5	42	0	8	35	0
	acidic	11006	34.1	8	1	27	27	1	8	6	22

[a] Recovery is the percentage that each fraction represents in relation with the total sugars loaded in the ion-exchange column.

Table 9. Glycosyl Linkage Composition (Mole Percent) of the Neutral Fractions of 4 M KOH-Soluble Polysaccharides of the Apex and Base of Fresh and Stored White Asparagus[a]

	punta				base			
	F	aa	S	aa	F	aa	S	aa
T-Fuc	4		6		6		3	
total	4	***7***	6	**7**	6	**0**	3	**7**
T-Araf	0		2		4		2	
total	0	***5***	2	**7**	4	**0**	2	**5**
T-Gal	4		5		4		4	
4-Gal	25		7		1		16	
total	*30*	***12***	*12*	***10***	*5*	***13***	*20*	***10***
T-Xyl	15		24		26		12	
2-Xyl	8		12		15		22	
4-Xyl	0		0		0		2	
2,4-Xyl	0		1		1		4	
total	23	***32***	36	**29**	42	**34**	41	**35**
4-Glc	22		15		11		16	
4,6-Glc	21		29		32		18	
total	*43*	***38***	*44*	***41***	*44*	***39***	*33*	***42***

[a] Column aa represents the mole percent of each sugar calculated by using the method of the alditol acetates. F, fresh; S, stored.

Cell wall bound polyuronides, most likely in the form of homogalacturonans, are lost from both the apical and basal sections of the spear and mostly from weak alkali-soluble polysaccharide fractions (carbonate-soluble and 1 M KOH-soluble fractions). The same has been observed during the softening of fruits (Fisher and Bennet, 1991); however, whereas during fruit softening the loss of cell wall bound polyuronides is parallel to an increase of soluble ones, in the case of asparagus no increase of soluble polyuronides could be detected, suggesting that polyuronides are degraded to very small oligomeric material.

In the apical section of the spear galactose is lost from the cellulose residue and partially recovered in the form of galactans in the 1 M KOH-soluble fraction, whereas in the basal section galactose is lost from the carbonate-soluble and 1 M KOH-soluble fractions and in higher amounts from the cellulose residue. Galactan metabolism is also a characteristic of fruit softening (Gross, 1989), and in some cases it has been shown that galactose is lost from polysaccharides not associated with pectic ones. In the case of asparagus the situation seems similar because at least the galactose lost from the cellulose residue of the basal section is independent of uronide solubilization because no uronic acids are lost from this residue. As happens with the polyuronides, in the basal section no soluble galactans are recovered, suggesting extensive degradation of these polysaccharides.

Cellulose degradation in the apical section and synthesis in the basal one can be inferred from the changes in the glycosyl composition of the cellulose residue. Xyloglucan incorporation in the neutral fraction of the 1 M KOH-soluble fraction of the apex can also be suggested together with degradation of these polysaccharides in the 4 M KOH-soluble fraction of the basal section.

Quantitatively the most important change that takes place in the overall composition of asparagus cell wall is the incorporation of xylans in the basal section of the spear. These polysaccharides are incorporated as KOH-soluble polysaccharides and mostly as acidic polymers; whether they are or not associated with pectic polysaccharides is a question that cannot be addressed on the basis of the results of the present study.

Waldron and Selvendran (1992) have proposed the presence of complexes of xylans−pectic polysaccharides in green asparagus and suggested that these complexes could provide the initial carbohydrates for the onset of lignification of cell walls. From this point of view these complexes could be an important key for the postharvest control of asparagus toughening and therefore merit further research.

In general terms the same kind of polysaccharides are implicated in green asparagus cell wall turnover (Waldron and Selvendran, 1992), although, as was pointed out before (Rodríguez et al., 1999), the process in white asparagus seems to be much more intense, which is in accordance with the higher rate of toughening of white asparagus as compared to the green (Lipton, 1990).

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LITERATURE CITED

Bartley, I. M.; Knee, M. The chemical of textural changes in fruit during storage. *Food Chem*. **1982**, *9*, 47−58.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem*. **1973**, *54*, 484−489.

Carpita, N. C.; Shea, A. M. Linkage structure of carbohydrates by gas chromatography−mass spectrometry of partially methylated alditol acetates. In *Analysis of Carbohydrates*

by GLC and MS; Biermann, C. J., McGinnis, G. D., Eds.; CRC Press: Boca Raton, FL, 1989; pp 157−216.

Dische, Z. Color reactions of carbohydrates. In *Methods in Carbohydrate Chemistry*; Whistler, R., Wolfran, M., Eds.; Academic Press: New York, 1962; pp 477−512.

Englyst, H. N.; Cumming, J. H. Simplified method for the measurement of total nonstarch polysaccharides by gas-chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937−942.

Fernández-Bolaños, J.; Rodríguez, R.; Guillén, R.; Jiménez, A.; Heredia, A. Activity of cell wall-associated enzymes in ripening olive fruit. *Physiol. Plant.* **1995**, *93*, 651−658.

Fincher, G. B.; Stone, B. A. Arabinogalactan-proteins: structure, biosynthesis and function. *Annu. Rev. Plant Physiol.* **1983**, *34*, 47−70.

Fischer, R. L.; Bennett, A. B. Role of cell wall hydrolases in fruit ripening. *Annu. Rev. Plant Physiol.* **1991**, *42*, 675−703.

Gross, K. Cell wall dynamics. In *Biotechnology and Food Quality*; King, S., Bills, D. D., Eds.; 1989; pp 143−158.

Hakomori, S. A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *J. Biochem.* **1964**, *65*, 205−208.

Hayashi, T. Measuring β-glucan deposition in plant cell walls. In *Modern Methods of Plant Analysis. New Series*; Linskens, E., Jackson, A., Eds.; Springer-Verlag: New York, 1989; pp 138−159.

Heredia, A.; Guillén, R.; Jiménez, A.; Fernández-Bolaños, J. Olive fruit glycosidades: factors affecting their extraction. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 561−565.

Jiménez, A.; Guillén, R.; Fernández-Bolaños, J.; Heredia, A. Cell wall composition of olives. *J. Food Sci.* **1994**, *59*, 1192−1196, 1201.

King, G. A.; O'Donoghue, E. M. Unravelling senescence: New opportunities for delaying the inevitable in harvested fruit and vegetables. *Trends Food Sci. Technol.* **1995**, *6*, 385−389.

Kvernheim, A. Methylation analysis of polysaccharides with butyllithium in dimethyl sulfoxide. *Acta Chem. Scand. Ser. B: Org. Chem. Biochem.* **1987**, *41*, 150−152.

Labavitch, J. M. Cell wall turnover in plant development. *Annu. Rev. Plant Physiol.* **1981**, *32*, 385−406.

Lipton, W. J. Postharvest biology of fresh asparagus. *Hortic. Rev.* **1990**, *127*, 111−127.

O'Neill, M.; Albersheim, P.; Darvill, A. The pectic polysaccharides of primary cell walls. In *Methods in Plant Biochemistry. 2. Carbohydrates*; Dey, P. M., Ed.; Academic Press: London, U.K., 1990; pp 415−442.

Rodríguez, R.; Jiménez, A.; Guillén, R.; Heredia, A.; Fernández-Bolaños, J. Postharvest changes in white asparagus cell wall during refrigerated storage. *J. Agric. Food Chem.* **1999**, *47*, in press.

Sánchez-Romero, C.; Guillén, R.; Heredia, A.; Jiménez, A.; Fernández-Bolaños, J. Degradation of pectic polysaccharides in pickled green olives. *J. Food Prot.* **1998**, *61*, 78−86.

Showalter, A. M. Structure and function of plant cell wall proteins. *Plant Cell* **1993**, 9−23.

Van Buren, J. P. The chemistry of texture in fruits and vegetables. *J. Texture Stud.* **1979**, *10*, 1−23.

Waldron, K. W.; Selvendran, R. R. Cell wall changes in immature asparagus stem tissue after excision. *Phytochemistry* **1992**, *31*, 1931−1940.

York, W. S.; Darvill, A. G.; McNeil, M.; Stevenson, T. T.; Albersheim, P. Isolation and characterization of plant cell walls and cell wall components. In *Methods in Enzymology 118. Plant Molecular Biology*; Weissbach, A., Weissbach, H., Eds.; Academic Press: New York, 1986; pp 3−40.

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