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Compositional and structural modification of the cell wall of cauliflower (*Brassica oleracea* L. var *botrytis*) during tissue development and plant maturation

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Abstract

The purpose of this study was to investigate maturation-related changes in cell-wall polysaccharides in cauliflower (*Brassica oleracea* L.) florets and stem tissues. Alcohol insoluble residues (AIRs) prepared from these were analysed for their carbohydrate composition. The cell walls of the immature tissues contained arabinose- and galactose-rich pectic polysaccharides and the major hemicelluloses consisted of xyloglucans. Maturation was accompanied by a basipetal decrease in the galactose- and arabinose-containing pectic polymers and a decrease in their degree of methyl-esterification. At the same time, there was a considerable increase in (1 → 4)-linked xylose residues and cellulosic glucose, particularly in the lower stem, and an increase in Klason lignin. More precise information on polymer structure was obtained from sequential extraction (with water, cyclohexane-*trans*-1,2-diamine-*N*,*N*,*N*′,*N*′-tetra acetate, Na_2CO_3 and KOH) of the AIRs of upper and lower stem tissues. The cellulose-rich residues remaining contained significant amounts of pectic polysaccharides, a proportion of which was released on neutralization of the residues. The greatest differences between the immature and mature stem cell-wall polymers occurred in the 0.5 M KOH fractions and these were investigated in more detail. Glycosidic linkage analysis showed that the insoluble residues from the immature stem contained much larger amounts of arabinose-rich pectic polysaccharides, and lower amounts of acidic xylans, compared with the mature stem. GC-MS linkage analysis of the polysaccharide fractions recovered from graded EtOH precipitation of the soluble fractions showed a large proportion of the maturation-related increase in (1 → 4)-linked xylose comprised glucuronoxylans closely associated or complexed, with pectic polysaccharides and xyloglucans. The possible relationship between such complexes and the onset of lignification is discussed.

Keywords: *Brassica oleracea*; Cell walls; Maturation; Polysaccharides

1. Introduction

Cell walls are the major component of dietary fibre (Selvendran et al., 1987) and determinant of texture in plant based foods (Van-Buren, 1979). The properties and the composition of the cell wall are related to the tissue type and on the stages of development of the plant organs (Waldron and Selvendran, 1990a,b; 1992). Changes in the plant/organ texture and properties of the fibre are related to changes in the composition and structure of the matrix components. During vegetable maturation, there is continued growth and thickening of the cell walls of many tissues, which may eventually involve lignification and secondary wall formation of vascular and supporting structures, and can account for most of the toughening-related changes in the vegetables, particularly those of the stem and the root origin (Northcote, 1979). Detailed studies on changes in the cell walls of asparagus stems during maturation and storage have suggested that deposition of xylan–pectic-polysaccharide complexes are associated with the initial stages of lignification and toughening of the stem tissues (Waldron and Selvendran, 1990b; 1992). However, there is limited information available on the corresponding changes occurring in the equivalent tissues of dicots. In this study, AIRs from organs of the cauliflower plant exhibiting differing degrees of maturation (florets, upper, middle and lower stems) have been investigated to identify the main classes of polymer which undergo maturation-related changes. Subfractionation of the cell walls was also undertaken to obtain more precise structural information on the interactions between polysaccharides at different stages of maturity. To confirm the possible role of polysaccharide complexes containing xylan during the onset of lignification, two cell wall preparations from upper and lower stem

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sections were examined in greater detail for the component cell wall polymers.

2. Materials and methods

2.1. Plant material

Cauliflower (*Brassica Oleracea* L. var *botrytis*) plants were obtained from Sofalia, Ennezat (France), supplied as mature, market-ready cauliflower plants, variety ''Stella''.

2.2. Organ separation

The plant root system was removed (ground level) and the aerial organs were separated into florets, leaves and stems at room temperature. The leaves were further dissected to give lamina-free midrib regions, and the stems were separated into upper, middle and lower regions to give three parts of approximately equal length. Samples of the upper and lower stem sections were also dissected to isolate the inner parenchymatous tissue (about 4–5 cm in diameter; free from vascular bundles), and the outer stele about 1 cm thick, containing mainly sclerenchymatous tissue and associated vascular bundles. These tissues were prepared by cutting the stem into 1 cm thick discs and excising the required portion with a sharp-nosed scalpel. The separated tissues were cut into small pieces frozen in liquid nitrogen and stored at −30°C until required.

2.3. Alcohol insoluble residue

AIR was obtained as described by Martin-Cabrejas et al. (1994).

2.4. Sequential extraction of AIR

Cell wall polysaccharides were extracted from the AIRs as described by Redgwell and Selvendran (1986). AIR preparation (5 g) was suspended in distilled water (500 ml) and the mixture was stirred (2 h at room temperature), centrifuged (12 000 × g, 20 min) and the supernatant recovered (water extract). The insoluble residue was sequentially extracted with (i) 50 mM *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetate (CDTA, Na salt; 500 ml), pH 6.5 at 20°C for 6 h (CDTA-1 extract); (ii) 50 mM CDTA (500 ml), pH 6.5 at 20°C for 2 h (CDTA-2 extract); (iii) 50 mM Na_2CO_3 + 20 mM $NaBH_4$ (500 ml) at 1°C for 16 h (Na_2CO_3-1 extract); (iv) 50 mM Na_2CO_3 + 20 mM $NaBH_4$ (500 ml) at 20°C for 2 h (Na_2CO_3-2 extract); (v) 0.5 M KOH + 20 mM $NaBH_4$ (500 ml) at 1°C for 2 h (0.5 M KOH extract); (vi) 1 M KOH + 20 mM $NaBH_4$ (500 ml) at 20°C for 2 h (1 M KOH extract); (vii) 4 M KOH + 20 mM $NaBH_4$ (500 ml) at 20°C for 2 h (4 M KOH extract). The alkali extractions were carried out with O_2-free solutions under argon. After each extraction, solubilised polymers were separated from the insoluble residue by centrifugation (CDTA and Na_2CO_3 extracts) or by filtration through G1 glass sinter (KOH extracts). All the extracts were filtered through GF/C and dialysed exhaustively; Na_2CO_3 and KOH extracts were neutralised with acetic acid prior to dialysis. The cellulose-rich residue (CR) remaining after the final alkali extraction (4 M KOH) was suspended in water (250 ml), neutralised with acetic acid and dialysed. The supernatant from the CR was recovered by centrifugation from the final residue (FR). All the extracts collected after dialysis were concentrated and stored as frozen suspensions at −30°C. Aliquots were freeze dried for further analysis.

2.5. Graded precipitation with ethanol

The polymers in the 0.5 M KOH extracts of the florets and stem samples were centrifuged (2000 × g, 20 min), to ensure the removal of insoluble materials. The supernatants were further fractionated using graded ethanol precipitation. The concentration of the alcohol was increased in steps of 20% (final v/v).

Every mixture was stirred at 1°C for 16 h and the precipitate was recovered by centrifugation and the EtOH was removed by evaporation. The fractions were stored at −20°C. A small proportion of each fraction was freeze-dried for sugar and methylation analysis.

2.6. Analysis of carbohydrate composition

Sugars were released from AIR preparations by hydrolysis with 1 M H_2SO_4 for 2.5 h at 100°C, and also after dispersing in 72% H_2SO_4 at room temperature for 3 h to solubilise crystalline cellulose, followed by hydrolysis after dilution to 1 M. Neutral sugars were converted to alditol acetates by the method of Blakeney et al. (1983) and were quantified by GLC. Total uronic acid content was determined calorimetrically from a sample hydrolysed in 1 M H_2SO_4 for 1 h (Blumenkrantz and Asboe-Hansen, 1973).

2.7. Methylation analysis

AIR samples were methylated by the method of Hakamori (1964) as described by Ring and Selvendran (1980). Extracted polysaccharides were methylated by the method of Ciucanu and Kerek (1984) as modified by Needs and Selvendran (1993). Per-methylated alditol acetates (PMAA's) were identified and quantified by GC-MS (Needs and Selvendran, 1993).

2.8. Separation of galacturonic and glucuronic acid by HPLC

Galacturonic and glucuronic acids were separated by HPLC based on the method of De Reuter et al. (1992). Cell wall material (2 mg) was hydrolysed with 2 M TFA (0.5 ml) for 1 h at 121°C, then dried under a stream of argon at 40°C, dispersed in 1 ml of distilled water and

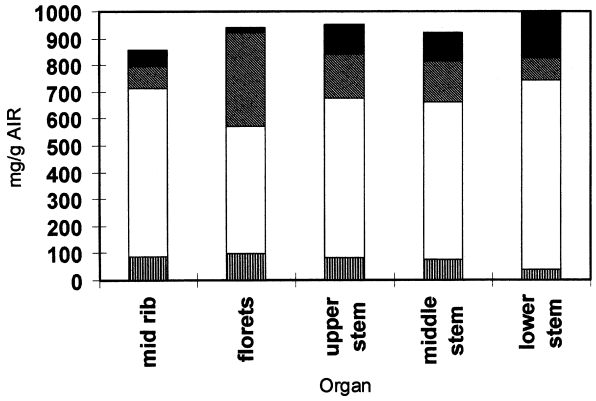

Fig. 1. Overall composition of AIRs from cauliflower organs. Symbols: ▥, moisture; ▧, protein; ■, lignin; □, carbohydrate.

mixed vigorously. Chromatography was performed using a DIONEX Gradient Pump-PED system (Surrey, UK) equipped with a CarboPac PA1 column (4 × 250 mm) in combination with a CarboPac guard column and run at room temperature. Separation was performed with a flow rate of 1 ml/min using three eluents prepared from distilled water and filtered in a Milli-Qplus 185, ultra-pure water system (Millipore UK Ltd., Watford): Eluent A, 0.1 M NaOH prepared from a 50% solution to minimise the carbonate content of the final eluent; Eluent B, 0.6 M sodium acetate (Merck, Darmstadt, Germany) prepared in 0.1 M NaOH; Eluent C, distilled water. The eluents were degassed by flushing with helium. Samples (25 μl) injected were eluted and monitored using a pulsed amperometric mode with a gold working electrode and a combined pH/Ag/AgCl reference electrode (DIONEX, Surrey, UK) to which potentials of E1 0.1 V, E2 0.6 V, and E3 − 0.6 V were applied for the following duration times T1 0.50 s, T2 0.09 s, and T3 0.06 s at a sensitivity of 1–3 μm. Acidic monosaccharides were eluted by mixing 80% of eluent A +20% of eluent B.

Quantification of the samples was made relative to the response factors calculated from the peak areas of the mixed standard solutions of galacturonic acid and glucuronic acid (concentration 0.1 mg/ml for each monosaccharide).

2.9. Protein

Protein was estimated as Kjeldhal nitrogen and multiplied by 6.25.

2.10. Warm-alkali-soluble phenolics and lignin

Alkali-extractable phenolics were measured using the method of Stafford (1960) which was an adaptation of the method of Bondi and Meyer (1948). Lignin was analysed as follows: AIR (100 mg) was dispersed in 72% H_2SO_4, incubated at room temperature for 3 h, and diluted to 1 M H_2SO_4 and heated at 100°C for 2.5 h (Saeman hydrolysis conditions). The acid-insoluble material was recovered quantitatively on a glass sinter (no. 2), washed to neutrality with hot water (90°C) and dried for 24 h at 105°C. The increase in the sinter weight was recorded as Klason lignin.

2.11. Starch

The occurrence of starch in the preparations was tested by staining the AIR with I_2/KI solution, and examined for starch granules by light microscopy.

2.12. Degree of esterification

Degree of esterification was measured as described by Waldron and Selvendran, 1990b.

2.13. Moisture

AIRs were weighed, dried overnight at 60°C in the presence of a desiccant (silica gel), and re-weighed.

3. Results and discussion

3.1. Overall composition

On a fresh weight basis, the florets accounted for 35–40% of the aerial plant and the leaf and stem portions 30–35% and 25–30%, respectively. The AIRs were virtually free of starch and the percentage yield of AIR ranged from approximately 4% in the midrib region to 9.5% in the lower section

Table 1
Carbohydrate analysis using Saeman and 1 M hydrolysis of the different organs/sections of the cauliflower plant: mg anhydrosugar (g. AIR)$^{-1}$

Sugars	Midrib	Florets	Upper stem	Middle stem	Lower stem	Lower stem parenchyma	Lower stem stele
Rhamnose	10.4	8.1	9.7	12.5	8.9	12.7	6.3
Fucose	6.9	4.9	6.3	5.7	3.9	3.9	1.3
Arabinose	49.3	69.5	91.8	71.9	39.0	67.9	21.5
Xylose	43.1	18.5	37.4	34.4	170.7	31.1	175.1
Mannose	20.3	11.9	18.7	16.2	13.5	15.1	9.3
Galactose	31.7	46.1	64.9	47.7	25.1	43.7	16.9
Glucose	244.1	183.8	219.6	217.2	308.8	187.6	319.8
Glucose (1 M)	13.0	54.0	18.0	22.0	11.5	nd	nd
Uronics	225.0	132.9	145.8	178.9	141.5	221.0	126.0
Total (mg/g)	630.8	475.7	594.2	584.5	711.4	582.9	673.7

Table 2
Methylation analysis of selected AIRs and extracted polysaccharides t = trace nd = not detected

Linkage types				Relative mole% Upper st.EtOH ppts.		Lower st.EtOH ppts.		
	Up. stem AIR	Mid stem AIR	Low. stem AIR	40–60%	60–80%	40–60%	60–80%	SN
Rhamnose								
1,2	1.30	0.90	0.70	0.11	0.11	0.13	0.23	0.62
1,3				0.08	0.13	0.49	0.67	0.65
1,2,4	1.50	1.30	1.10	0.05	0.03	0.35	0.54	0.72
Fucose								
Terminal	0.80	0.70	0.30	5.46	4.12	0.11	2.87	1.45
Arabinose								
Terminal-f	11.50	10.80	3.90	0.69	2.92	1.29	1.30	2.21
1,2	0.70	0.30	0.50	0.25	0.36	0.26	0.30	0.20
1,3	0.40	0.40	0.40	0.15	0.18	0.20	0.15	0.13
1,5	11.90	11.20	5.40	0.64	0.23	0.45	0.48	1.12
1,2,5	0.00	0.00	0.00	0.31	0.89	0.49	0.80	0.30
1,3,5	4.40	4.90	3.20	0.41	0.00	0.00	0.37	0.32
1,2,3,5	0.00	0.00	0.00	0.00	0.10	1.38	0.05	0.12
Xylose								
Terminal	3.00	2.80	1.50	18.11	11.61	18.52	6.34	6.45
1,2	0.70	0.90	0.70	8.20	11.26	8.40	7.23	4.56
1,4	4.60	5.20	22.70	10.32	17.42	33.18	47.07	57.39
1,2,4	0.50	0.60	1.00	1.41	0.97	1.95	4.31	4.19
1,3,4	0.40	0.70	0.90	0.00	0.00	0.00	0.00	0.00
1,2,3,4	0.00	0.00	0.00	0.08	0.00	0.26	0.09	0.40
Mannose								
1,4	5.60	6.30	4.10	0.75	1.10	0.80	0.00	0.30
1,2,4	2.50	3.70	2.70	0.00	0.00	0.00	0.00	0.32
1,4,6	1.30	2.30	1.30	1.32	1.45	0.24	0.50	0.56
Galactose								
Terminal	0.80	0.90	0.10	3.57	4.23	2.58	2.51	2.89
1,2	t	t	t	5.50	4.85	3.05	2.42	1.27
1,4	5.70	5.80	5.20	1.32	0.00	0.00	0.24	0.00
1,6	0.30	0.30	0.30	0.12	0.18	0.15	0.43	0.21
1,3,6	0.50	0.20	0.10	0.56	0.21	0.39	0.21	0.36
1,4,6	1.20	0.70	0.70	0.60	0.25	0.35	0.71	0.23
Galactitol				0.13	0.55	0.00	0.10	0.20
Glucose								
Terminal	t	t	t	0.15	0.32	0.76	0.91	1.02
1,4	22.90	22.40	23.80	11.74	11.71	7.16	6.22	4.59
1,4,6	8.50	8.10	7.90	28.52	24.41	15.28	12.45	6.35
1,3	0.00	0.00	0.00	0.00	0.09	1.21	0.00	0.00
1,3,4	3.50	3.40	3.60	0.00	0.00	0.00	0.00	0.00
1,2,4	0.60	0.50	0.80	0.00	0.00	0.00	0.00	0.00
Glucitol	6.80	5.10	7.00	0.76	0.33	0.57	0.50	0.87

of the stem. Florets, the upper, and middle stem sections each yielded about 5% AIR. The composition of the AIR preparations in terms of moisture, total sugars, protein and lignin are shown in Fig. 1. Carbohydrate was the most abundant component of each AIR. The other main components measured were protein and Klason lignin. Maturation of the stem tissues (florets through to lower stem) resulted in an increase in the levels of carbohydrate and a decrease in protein, the bulk of which will be of intracellular origin—protein content of the cell walls of vegetables is generally about 5% of the dry weight of the cell walls (Selvendran, 1975; Selvendran and O'Neill, 1987). The maturation-related increase in lignin was due to the development of a sub-epidermal ring of vascular stele. The cells of the mature stele had undergone considerable secondary thickening, and stained red in phloroglucinol–HCl, indicative of extensive lignification (results not shown). Cells of the inner (pith) parenchyma were not lignified. The levels of warm-alkali-soluble phenolics in the AIRs ranged from 0.37% in floret AIR through to 2.76% in the lower stem AIR.

3.2. Carbohydrate composition

The results indicate that the pectic polysaccharides (Rha + Ara + Gal + Uronic acids) were an important component of the AIRs contributing around 55% of the

Table 3
Sugar composition of fractions of cell wall material (AIR) from cauliflower upper stem obtained by sequential extraction with aqueous solvents

Fraction	Recovery ($mg.gAIR^{-1}$)	Cell wall sugars (mol%)								
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc (1 M)	Ur.A (GalA) (%)	Total sugar[a] (μg/mg)
H_2O	184.0	0.3	0.0	26.4	3.4	5.6	18.2	12.3	33.9 (93.3)	143.3
CDTA-1	100.8	1.7	0.0	8.4	0.5	0.1	2.4	1.0	85.9 (98.0)	727.7
CDTA-2	15.0	3.7	0.0	12.9	0.9	0.3	1.3	3.8	77.2 (99.3)	367.0
Na_2CO_3-1	117.1	3.8	0.0	20.8	1.9	0.1	4.6	0.9	68.0 (98.1)	673.7
Na_2CO_3-2	22.8	6.4	0.0	35.8	1.8	0.1	6.3	1.4	48.2 (96.8)	618.5
0.5 M KOH	116.9	0.9	2.8	10.4	32.9	2.3	8.1	25.1	17.4 (92.7)	429.8
0.5 M KOH sol	(61.8%)[b]	0.2	4.9	4.0	37.5	3.3	9.9	36.1	4.2	693.9
0.5 M KOH insol	(38.2%)[b]	8.1	2.0	35.7	25.4	0.1	3.7	6.3	18.8	146.8
1M KOH	24.2	0.8	2.4	7.6	27.3	11.6	10.2	29.2	10.9 (98.1)	559.6
4M KOH	20.3	0.8	1.1	8.3	10.3	28.7	11.6	29.2	10.0 (99.3)	747.3
Insol. Residue	193.3	2.5	0.0	14.6	2.3	2.2	4.4	57.9 (8.3)	16.1 (96.6)	831.2
Supt.	(3.6)[c]	6.7	0.0	31.9	3.8	0.5	7.0	3.4	46.8	483.1
Final Resid.	(96.4)[c]	1.7	0.0	7.5	1.4	2.5	3.2	71.3 (7.1)	12.5	865.3

[a] Values are expressed as μg of ''anhydrosugar''/mg dry polymers.
[b] % of 0.5 M KOH extract.
[c] % of insoluble residue.

polysaccharides in the floret tissues, but only 30% in the lower stem (Table 1). Conversely, the proportion of the hemicelluloses increased from around 8% in the florets to around 30% in the lower stem. Allowing for approximately 10% of the cellulosic glucose being hydrolysed in 1 M H_2SO_4 (Selvendran and O'Neill, 1987) then the results indicate that cellulose accounts for between 27% of the cell-wall polysaccharide in the florets to around 42% in the lower stem. In the florets, the relatively high levels of glucose released by 1 M hydrolysis indicates the presence of significant quantities of non-cellulosic glucan which may have been derived from xyloglucan hemicelluloses (refer to methylation analysis described later).

3.3. Maturation-related changes in the cell wall composition of the stem

Carbohydrate analysis of the AIR of the upper, middle and lower stem portions revealed a maturation-related increase in xylose and cellulosic glucose, and a decrease in the neutral sugars (arabinose and galactose) of pectic origin, particularly in the lower stem (Table 1). Also the degree of methyl-esterification of pectic polysaccharides decreased basipetally in both the vascular and parenchyma tissues and was lower in the vascular tissues (Femenia et al., 1998). Similar changes in methyl esterification of pectic polysaccharides during maturation have been reported for

Table 4
Sugar composition of fractions of cell wall material (AIR) from cauliflower lower stem obtained by sequential extraction with aqueous solvents

Fraction	Recovery	Cell wall sugars (mol%)								
	($mg.gAIR^{-1}$)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc (1 M)	Ur.A (GalA) (%)	Total sugar[a] (μg/mg)
H_2O	106.2	3.6	0.0	24.5	11.0	4.3	20.3	13.4	22.9 (85.0)	205.9
CDTA-1	82.9	1.8	0.0	8.1	0.9	0.1	2.1	0.5	86.7 (98.9)	886.2
CDTA-2	18.9	0.4	0.0	16.5	2.5	0.4	1.2	6.3	72.7 (99.3)	259.4
Na_2CO_3-1	77.7	4.5	0.0	22.8	2.5	0.1	5.4	2.4	62.3 (98.6)	567.6
Na_2CO_3-2	27.1	6.8	0.0	31.7	7.3	0.1	8.1	2.7	43.4 (92.1)	569.0
0.5M KOH	134.4	1.4	1.4	3.5	67.3	0.6	3.9	10.4	11.4 (84.7)	612.3
0.5M KOH sol	(54.1%)[b]	1.2	2.5	2.3	60.8	0.2	6.1	17.8	9.2	751.3
0.5M KOH insol	(45.9%)[b]	2.4	0.0	4.2	82.5	0.0	0.0	0.0	10.9	219.7
1M KOH	40.6	1.1	0.9	2.2	78.4	1.7	2.6	7.0	6.1 (88.1)	700.7
4M KOH	22.4	1.0	1.1	5.1	44.0	15.6	6.9	18.4	7.8 (99.3)	692.6
Insol. Residue	331.1	1.8	0.0	6.8	5.0	2.2	3.4	66.4 (8.3)	14.5 (96.6)	821.2
Supt.	(2.8)[c]	7.8	0.0	26.5	20.6	0.6	8.6	5.8	31.2	550.7
Final Resid.	(97.2)[c]	1.8	0.0	4.4	5.8	1.8	2.4	72.5 (6.9)	11.5	836.1

[a] Values are expressed as μg of ''anhydrosugar''/mg dry polymers.
[b] % of 0.5 M KOH extract.
[c] % of insoluble residue.

mung bean hypocotyl (Goldberg et al., 1986) and carrot root apex (Liners and Van Cutsem, 1992).

3.4. General linkage analysis

The results of methylation analysis of upper-stem and lower-stem AIRs are shown in Table 2. The recoveries of neutral sugars obtained from methylation analysis and Saeman hydrolysis were in broad agreement. The efficiency of methylation was found to be improved significantly by freeze-milling the AIRs prior to analysis.

The linkages present in the AIR of the upper stem were generally similar to those found in AIRs of the midrib and florets (results not shown) and middle stem tissues (Table 2). All the preparations contained significant amounts of cellulose, as can be inferred from the occurrence of substantial amounts of (1 → 4)-linked glucose residues. They also contained an appreciable amount of (1 → 4,6)-linked glucose residues, which is a characteristic feature of xyloglucans (Aspinall, 1980). All the preparations were rich in pectic polysaccharides as can be inferred from the occurrence of variously linked rhamnosyl, arabinosyl and galactosyl residues. For the midrib and stem sections, the average degree of polymerisation of the arabinose and galactose side chains is about 2–4 units. The small amounts of (1 → 2)- and (1 → 3)-linked arabinose residues detected in the various preparations probably arose from hydroxy-proline-rich cell wall glycoproteins (Lamport and Epstein, 1983; Ryden and Selvendran, 1990b). The maturation-related increase in xylose in the lower stem was due, predominantly, to the increase in (1 → 4)-linked xylose residues, consistent with the synthesis of glucuronoxylans during secondary thickening.

3.5. Sequential extraction of AIR

Cell wall polysaccharides were extracted from the AIRs of the upper and lower stem sections with aqueous solvents. The carbohydrate compositions of the fractions are given in Tables 3 and 4. The percentage contribution of galacturonic acid to total uronic acid of the extracted fractions was obtained from separation of the uronic acids on HPLC (see Section 2).

Upper stem: Cold water solubilised a significant amount of material (Table 3). This was relatively poor in carbohydrate which was of pectic origin. The bulk of the non-carbohydrate material is likely to consist of co-precipitated intracellular proteins. In contrast, the pectic polysaccharides solubilised by CDTA and Na_2CO_3 contained a greater proportion of galacturonic acid. The relatively poor recovery of carbohydrate from the CDTA-2 fraction was due to incomplete removal of CDTA during the dialysis step (Coimbra et al., 1994) whereas the low recovery of carbohydrate in the Na_2CO_3-2 fraction is most probably due to the presence of intracellular proteins.

The bulk of the material solubilised by alkali was present in the 0.5 M KOH fraction. This fraction was relatively rich in xylose and glucose, and also contained significant amounts of galacturonic acid, arabinose and galactose. The latter sugars would have been derived from highly branched pectic polysaccharides (Selvendran and O'Neill, 1987). As the values for xylose and glucose are comparable, this fraction can be inferred to contain much highly branched xyloglucan. As most of the uronic acid was shown to be galacturonic acid, only very small amounts of acidic xylans are likely to be present.

The composition of the 1 M KOH fraction was comparable with the 0.5 M KOH fraction, but differed from that of the 4 M KOH-soluble fraction. In the 4 M KOH fraction, the ratio of xylose:glucose was 1:3.5, which shows that xyloglucans present were much less branched. Further, significant quantities of mannose were present. Similar observations have been made with other tissues (Redgwell and Selvendran, 1986; Ryden and Selvendran, 1990a,b). The 4 M KOH-insoluble residue also contained much pectic material, a proportion of which could be released on neutralization, leaving a residue richer in cellulose (Table 4). This observation is in agreement with the work of Coimbra et al. (1994).

Lower stem: The carbohydrate compositions of the pectic fractions (water-, CDTA- and Na_2CO_3-soluble; Table 4) were generally comparable to those of the upper stem. However, in keeping with the carbohydrate analysis of the AIRs, they exhibited a higher level of xylose. This was particularly evident in the Na_2CO_3-2 fraction and is comparable with the maturation-related increase in xylose noted in the corresponding Na_2CO_3-2 fraction from asparagus stem (Waldron and Selvendran, 1992). The high level of xylose in the supernatant pectic fraction from the 4 M KOH-insoluble residue was also of interest.

The carbohydrate compositions of the alkali-soluble fractions were different from those of the upper stem. The most notable differences were much lower levels of arabinose and galactose, higher levels of xylose and, in the 0.5 M KOH-soluble polysaccharides, a higher level of glucuronic acid.

The results obtained indicated that a large proportion of the maturation-related changes in the cell-wall polysaccharides occurred in the 0.5 M KOH-soluble components. The polymers present in these extracts were, therefore, further resolved by precipitation with graded ethanol.

3.6. 0.5 M KOH-extracted polymers precipitated on neutralisation

The percentage recovery of carbohydrate from the 0.5 M KOH-extracted, water-insoluble residues (Tables 3 and 4; 0.5 M KOH insoluble) was poor and was probably due to contamination with protein. The insoluble residues from the upper stem (Table 3) contained much higher levels of pectic polysaccharides compared with the same fraction from the lower stem (Table 4). From the ratio of xylose to glucose in the residue from the upper stem, the presence of acidic xylans and a small amount of xyloglucans in close

Table 5
Sugar composition of 0.5 M KOH-extracted fraction from cauliflower upper stem and lower stem AIRs after neutralisation and ethanol precipitation of the soluble fractions

Fraction	Recovery %	Cell wall sugars (mol%)								
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Ur.A (GalA) (%)	Total sugar[a] (μg/mg)
40–60% EtOH										
Upper stem	31.9	0.4	5.9	2.7	38.4	1.8	9.4	37.2	4.3 (90.7)	848.0
Lower stem	23.2	0.7	2.8	1.8	58.4	0.9	5.4	19.6	10.4 (80.6)	892.7
60–80% EtOH										
Upper stem	20.4	0.2	4.3	4.9	42.0	2.5	8.6	29.8	7.7 (83.8)	929.8
Lower stem	20.6	1.1	2.7	1.9	60.7	0.3	5.6	17.6	10.3 (78.7)	931.1
80% EtOH SN										
Upper stem	15.6	1.1	2.4	11.9	21.7	10.7	14.3	26.5	11.5 (82.9)	167.9
Lower stem	23.2	1.9	1.4	2.9	60.2	0.8	5.5	10.9	16.4 (83.9)	636.9

[a] Values are expressed as μg of anhydrosugar/mg dry polymers.

association with pectic polysaccharides can be inferred. The 0.5 M KOH-insoluble residue from the lower stem contained mainly acidic-xylans and a small amount of pectic polysaccharides. These conclusions were confirmed by methylation analysis (results not shown).

3.7. 0.5 M KOH-extracted polymers, soluble on neutralisation

The sugar analysis of the soluble 0.5 M KOH-extracted fractions from florets (not shown), upper and lower stem (Tables 4 and 5 respectively; 0.5 M KOH soluble), indicated the predominance of hemicelluloses (xyloglucans and acidic xylans) and small amounts of pectic polysaccharides. This observation was confirmed by methylation analysis (results not shown).

The characteristics of the pectic and hemicellulosic polysaccharides present in these fractions were similar to those found in the insoluble residues above, and the maturation-related increase in acidic xylans was clearly demonstrated. The main difference noted from methylation analysis (not shown) was the presence of larger amounts of highly branched xyloglucans, particularly in the fractions from the immature (upper) stem. These differences in linkage were investigated after precipitation with graded ethanol (below) to a greater extent.

3.8. Graded precipitation with ethanol

The bulk of the polysaccharides from the soluble 0.5 M KOH extracts were precipitated between 40–60% and 60–80% ethanol. The carbohydrate composition is shown in Table 5. This, and the corresponding methylation analysis (Table 2) confirmed that the graded fractions contained heterogeneous mixtures of polysaccharides, including xyloglucans, acidic (glucurono-)xylans and pectic polysaccharides. Maturation resulted in (1) considerable increases in the levels of acidic xylans, as indicated by the increase in (1 → 4)- and (1 → 2,4)-linked xylose; (2) a decrease in xyloglucan hemicelluloses; (3) an increase in branched pectic polysaccharides, as indicated by the increase in (1 → 2,4)-linked rhamnose. Interestingly, maturation was accompanied by an increase in 80% ethanol-soluble polysaccharides (SN, Table 5). This material was rich in xylans, xyloglucans and certain pectic materials (Table 2).

4. General discussion

The results have shown that the maturation of cauliflower stems is accompanied by an increase in the acidic xylans. Fractionation has demonstrated that a large proportion of these are associated closely with xyloglucans and pectic polysaccharides. It is possible that this association reflects the presence of covalently linked polysaccharide complexes which are formed during the cross-linking of the cell wall polymers during maturation-related secondary thickening. Evidence for such cross-linking has already been found, for example the formation of xylan–pectic polysaccharide (-lignin) complexes during maturation of asparagus stems (Waldron and Selvendran, 1992). Further, Coimbra et al. (1994) used highly purified endoxylanases to demonstrate a strong, probably covalent, attachment between xylan and xyloglucan moieties in olive cell walls. The formation of such complexes is likely to have a significant impact on the mechanical properties of the cell walls (Waldron et al., 1997), particularly in the vascular stele which supports the stem tissues. It is possible that the complexes may have a role in acting as initials for lignification, particularly in the middle lamella where lignification is thought to initiate (Waldron and Selvendran, 1992).

5. Conclusions

From these studies the following conclusions can be drawn:

1. Maturation of stem tissues is accompanied by a large increase in (1 → 4)-linked xylose residues and cellulosic

glucose, particularly in the lower stem; and a basipetal increase in Klason lignin.

2. A large proportion of the increase in (1 → 4)-linked xylose occurs in polymers extractable in 0.5 M KOH. Subfractionation of these polymers by graded ethanol precipitation has revealed mixtures of xyloglucans, acidic xylans and pectic polysaccharides
3. From the ratio of (1 → 4)- to (1 → 4,6)-linked glucose residues in the various fractions it can be inferred that a heterogeneous population of xyloglucans exists.
4. Likewise, the pectic polysaccharides and xylans can also be inferred to exhibit heterogeneity.
5. The 0.5 M KOH-soluble fractions precipitated with 40–60% and 60–80% ethanol, and the 80% ethanol supernatant were rich in acidic xylans and contained large amounts of xyloglucans and pectic polysaccharides. The xylan, xyloglucan and pectic components of the above fractions from lower stem cell walls showed major differences compared with the corresponding fractions from the upper stem, and have been selected for further study to examine the possible occurrence of polysaccharide complexes.

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