# Polysaccharides from the Cell Walls of Pineapple Fruit

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Cell wall material from pineapple fruit was obtained and sequentially extracted with 50 mM NaOH at 2 °C, 1 M KOH at 2 and 20 °C, and 4 M KOH at 20 °C. From the 4 M KOH-soluble fraction, a "neutral" polysaccharide and an acidic one were isolated by chloride anion exchange chromatography. The "neutral" fraction was composed of xylose, arabinose, glucose, galactose, and minor quantities of mannose. The infrared spectra showed an absorption band at 900 $cm^{-1}$ of $\beta$-linked polysaccharides. The methylation analysis revealed the main glycosidic linkages of the "neutral" polysaccharide: 1,4-linked xylose with branching points in $C_3$ and $C_2$, 1,4-linked glucopyranose with branching points in $C_6$, and terminal glucopyranosyl, arabinofuranosyl, and arabino/xylopyranosyl residues.

**Keywords:** *Pineapple fruit; polysaccharides; cell wall*

## INTRODUCTION

Tropical fruits may contain unusual fiber components with specific physiological properties. The dietary fiber content and composition of pineapple fruit and some other tropical fruits and vegetables were reported by Lund and Smoot (1982). Vidal-Valverde et al. (1982) reported on the content in dietary fiber, cellulose, hemicellulose, lignin, and pectic substances of pineapple and some other Spanish fruits, describing pineapple as very rich in hemicelluloses (41.8%). Voragen et al. (1983) extracted the different polysaccharide fractions from the ethanol-insoluble residue of some fruits and vegetables, pineapple included, determining the sugar composition and the uronic acid content of all these fractions. The hemicellulose fraction was mainly composed of xylose, arabinose, glucose, galactose, mannose, and uronic acid.

Nevertheless, there are no structural data on the different polysaccharidic components of pineapple fruit cell wall material. The aim of this work was to study the hemicellulosic fraction in more detail.

## MATERIALS AND METHODS

**Material.** The pineapple fruits (*Ananas comosus* L. cv. Smooth Cayenne) were purchased at a local market. To have an indication of the physiological maturity of the fruits, the firmness and the soluble solids were measured. Texture evaluation was carried out according to the Kramer Shear test in an Instron 1140 texturometer. Fifty grams of sectioned slices (1.3 cm thick) were laid in the Kramer cell. A force of 200 kg was applied at a crosshead speed of 50 mm/min and a chart speed of 100 mm/min. The mean value for maximum force was calculated and results reported as resistance to shear in Newton per gram of fresh weight. Soluble solids were measured in the exudate from the Kramer cell with an Atago digital refractometer dbx-30 at 20 °C, and results were reported as degrees Brix. The average soluble solids of the fruits was 12.5 °Brix and the average firmness 32.7 N/g. Firm fruits were hand-peeled, cored, sliced, cut into small pieces, packed in polyethylene bags, frozen, and stored at −20 °C until required.

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**Cell Wall Preparation and Extraction Procedure.** Samples were homogenized in a Sorvall Omnimixer with deionized water in an ice bath, to avoid the enzymatic degradation of the material. The homogenate was then filtered through filter paper on a Büchner funnel, and exhaustively washed with cold deionized water, until clear filtrates were obtained, and the residue freeze-dried. Afterward, it was milled in an Osterizer followed by ball milling in a cold room in a Fritsch ball mill at high speed, and for short periods up to 1 h. To control cell breakage, milled samples were observed under the light microscope at regular intervals, until most of the material was broken. The cell wall material (CWM) thus obtained was sieved through a 0.4 mm mesh and stored dessicated at room temperature until required.

The cell wall extraction procedure, based on Rupérez et al. (1985), is summarized in Figure 1. Seven grams of CWM was extracted twice with 300 mL of the NaOH–EDTA solution containing 10 mM $NaBH_4$ for 2 h and, again, for 1 h at 2 °C, to depectinate the material. The residue was then extracted sequentially with 1 M KOH at 2 °C, and with 1 M and 4 M KOH at room temperature. The insoluble residue from the previous treatment was washed with 2 M acetic acid, deionized water, and exhaustively dialyzed against deionized water. The pH of the extracts was adjusted to 5.0 with acetic acid, and they were dialyzed against deionized water. The supernatant solutions (SN) were concentrated under reduced pressure and frozen. A sample from each supernatant solution was freeze-dried for analysis. The preparation, extraction procedure of CWM, and chemical analysis of all samples were repeated twice.

**Chemical Analysis.** Neutral sugars were released from CWM and fractions by Saeman hydrolysis and analyzed as their alditol acetates (Selvendran et al., 1979) by gas liquid chromatography (GC) on a 25 m × 0.22 mm, 0.20 $\mu$m film thickness, fused silica WCOT CP-Sil 88 capillary column. Nitrogen (20 psig) was used as the carrier gas on a Perkin-Elmer 8500 gas chromatograph equipped with a hydrogen flame ionization detector (FID). Isothermal oven temperature was 230 °C, sample size, 2 $\mu$L; and split ratio, 1:100. Peaks were identified on the basis of sample coincidence with the relative retention times of the standards, inositol being used as the internal standard.

Uronic acid was determined colorimetrically with *m*-hydroxydiphenyl (Blumenkrantz and Asboe-Hansen, 1973), as modified by Kintner and Van Buren (1982).

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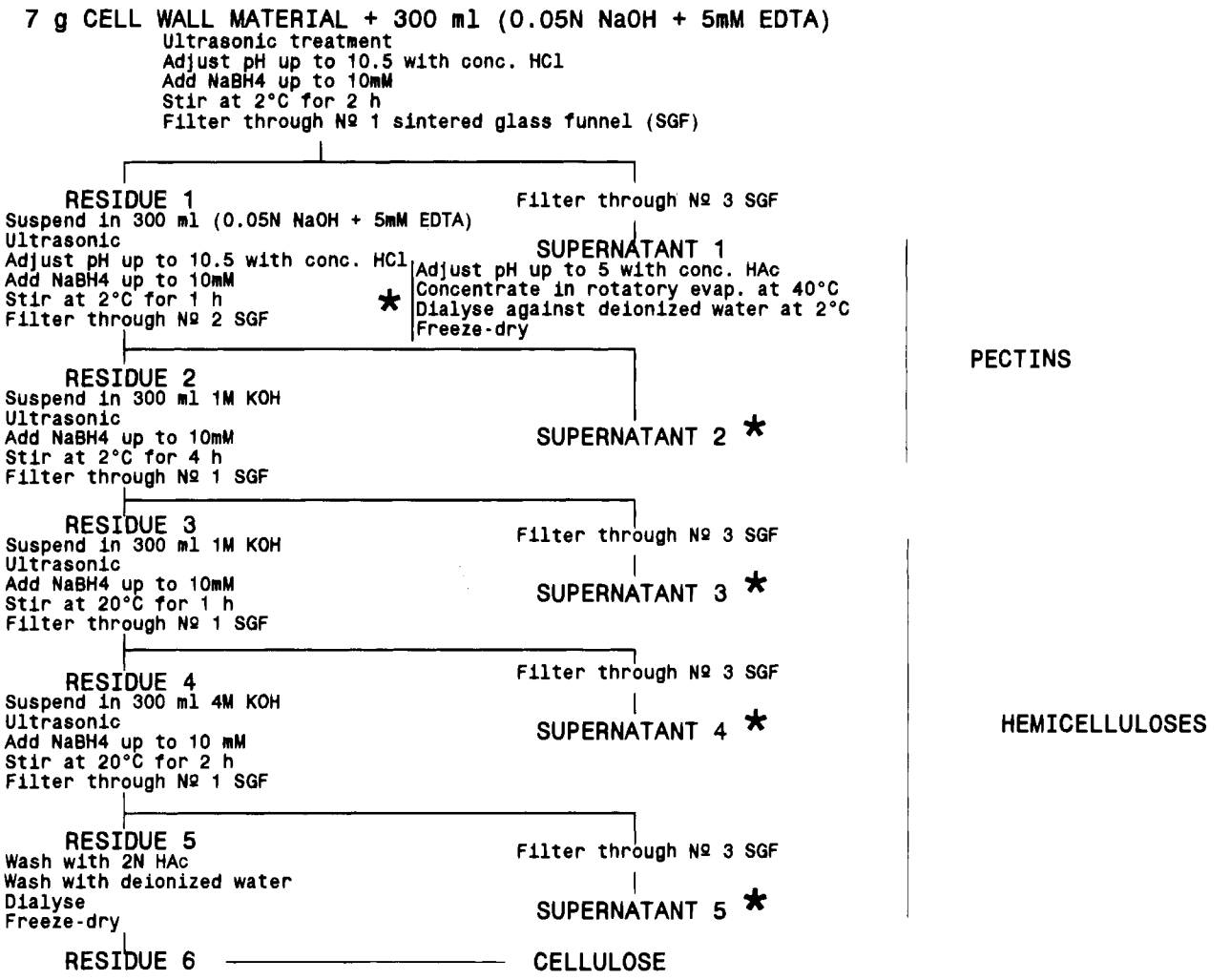


**Figure 1.** Extraction procedure of pineapple fruit cell wall material.

**Table 1. Monosaccharide Composition of Pineapple Fruit Cell Wall Material after Sequential Extraction with Alkali**

| fraction[a] | yield[b] (wt %) | monosaccharide composition[c] (wt %) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ara | Xyl | Man | Gal | Glc | UA |
| CWM | 100.0 | 11.1 | 16.4 | 1.3 | 3.9 | 24.3 | 10.3 |
| SN 1 | 6.7 | 3.5 | 4.0 | 0.9 | 2.4 | 2.2 | 19.0 |
| SN 2 | 1.5 | 5.4 | 5.4 | 1.0 | 3.7 | 11.0 | 9.9 |
| SN 3 | 8.8 | 7.6 | 18.7 | 2.4 | 3.9 | 11.8 | 11.3 |
| SN 4 | 5.2 | 12.0 | 12.8 | 1.4 | 1.7 | 6.0 | 6.6 |
| SN 5 | 5.8 | 22.6 | 26.7 | 3.1 | 8.2 | 12.9 | 10.4 |
| residue | 42.7 | 9.3 | 12.5 | 2.0 | 4.8 | 36.3 | 12.8 |

[a] Key: CWM, cell wall material; SN, supernatant. [b] Recovery after CWM sequential extraction. [c] After Saeman hydrolysis. Deoxyhexose was present in trace amounts in all fractions. Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid. All values are the average of duplicate determinations.

**Infrared Spectra.** Infrared spectra (IR) were obtained with a Perkin-Elmer 1420 infrared spectrophotometer, using KBr disks.

**Purification of the Polysaccharide.** A solution of a portion (100 mg) of SN 5, the 4 M KOH-soluble material in 10 mM potassium phosphate buffer (25 mL), was eluted from a column (14 × 0.8 cm) of DEAE-Sephadex ($Cl^-$ form), initially with the potassium phosphate buffer (50 mL), and then with this buffer in a linear gradient of NaCl (0 → 1 M, 100 mL), and finally with 1 M NaCl (20 mL) in potassium phosphate buffer. Fractions (1 mL) were collected and monitored for carbohydrate by reaction with phenol sulfuric acid (Dubois et al., 1956). Appropriate fractions were combined, dialyzed, and concentrated to a small volume and freeze-dried.

**Methylation Analysis.** The "neutral" fraction from DEAE-Sephadex was methylated by the Hakomori's method (Hakomori, 1964) as modified by Jansson et al. (1976), remethylated at 80 °C with methyl iodide and silver oxide as a catalyst according to Purdie and Irvine (1903), and then converted into partially methylated alditol acetates (PMAA), which were separated by GC on OV-225 (Ring and Selvendran, 1978) and SP-2340 (Rupérez and Leal, 1981) packed columns, and examined by GC–mass spectrometry on a 30 m × 0.25 mm, 0.25 μm film thickness, fused silica SPB-1 capillary column, with a temperature program from 160 °C (1 min hold) to 220 °C, and a temperature increase of 2 °C/min. Helium (10 psi) was used as the carrier gas. The mass spectra (MS) were obtained by electron impact in a Perkin-Elmer Ion Trap Detector, coupled to a Perkin-Elmer Sigma 3B dual FID gas chromatograph. Both injection port and transfer line were programmed at 270 °C. PMAAs quantitation was done by using the ion current areas from the GC-MS analysis on SPB-1, since peak separation was much better in the capillary than in the OV-225 packed column.

## RESULTS AND DISCUSSION

**Extraction of Cell Wall Material.** The yield (dry weight %) of the different CWM fractions and their monosaccharide composition are shown in Table 1. Fractions SN 1 and SN 2 (8.2%) were extracted with dilute alkali at 2 °C to minimize their degradation by β-elimination and hydrolysis of labile glycosidic linkages. The residue from the previous treatments was extracted with 1 and 4 M alkali (19.8%, comprising fractions SN 3, SN 4, and SN 5), to leave an alkali-

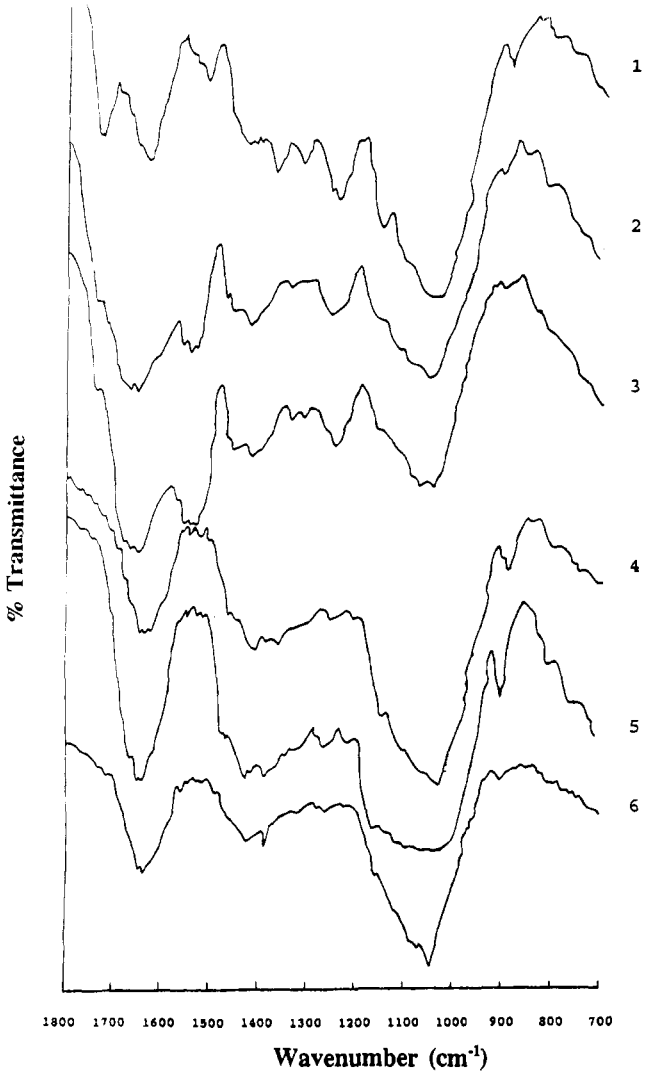


**Figure 2.** Infrared spectra from cell wall material and the fractions obtained after its sequential extraction: (1) CWM; (2) SN 1; (3) SN 2; (4) SN 3; (5) SN 4; (6) SN 5.

insoluble material (42.7%). All extracted fractions plus the insoluble residue represented approximately 71% of CWM, due to losses along the extraction procedure.

Voragen et al. (1983) followed a similar procedure to obtain pectin, hemicellulose, and cellulose fractions from the ethanol-insoluble residue of several vegetables and fruits, including pineapple. The sugar composition of the pineapple fruit CWM (Table 1) is very similar to the ethanol-insoluble residue reported by the previously mentioned workers.

Hemicellulosic fractions and residue showed different amounts of uronic acid, this could be due either to the fact that in some cases hemicelluloses may contain residues of uronic acid (Haard, 1985), or because part of the uronide material was left in the pellet after the NaOH/EDTA treatment, thus pectic substances would have been released all along the extraction procedure. Voragen et al. (1983) also detected uronic acids in the hemicellulose and cellulose fractions from pineapple fruit.

All fractions contained deoxyhexose (rhamnose or fucose) in trace amounts, and xylose, arabinose, glucose, galactose, mannose, and uronic acid in different percentages (Table 1). SN 1 was enriched in uronic acid (19%) as expected. Except for SN 5 and residue, the acid hydrolysis recovery was low, although the Saeman acid hydrolysis conditions employed were strongly recommended (Selvendran et al., 1979).

Voragen et al. (1983) pointed out the extremely low content of uronic acid in the pectic fraction from pineapple fruit and remarked that the sugar composition of this fraction differed greatly from that of the other fruits analyzed, reporting xylose, arabinose, galactose and glucose as the main sugars in the hemicellulose fractions.

**Infrared Spectra.** Data from IR spectra of CWM and fractions are shown in Figure 2. All of them showed an absorption band at 900 $cm^{-1}$ of $\beta$-polysaccharides. SN 1 and SN 2 showed in addition bands at 1550 and 1650 $cm^{-1}$ of proteins, which could be partly of intracellular origin. CWM, SN 1, and SN 2 fractions showed also an absorption band at 1730 $cm^{-1}$ due to uronic acids. Orr (1954) reported the presence of this band in free carboxylic acids and its absence in the salt forms.

**Purification of the Polysaccharide and Methylation Analysis.** The 4 M KOH-soluble fraction (SN 5) that represented 5.8% of the CWM was purified on DEAE-Sephadex (Figure 3). A "neutral" material (60%),

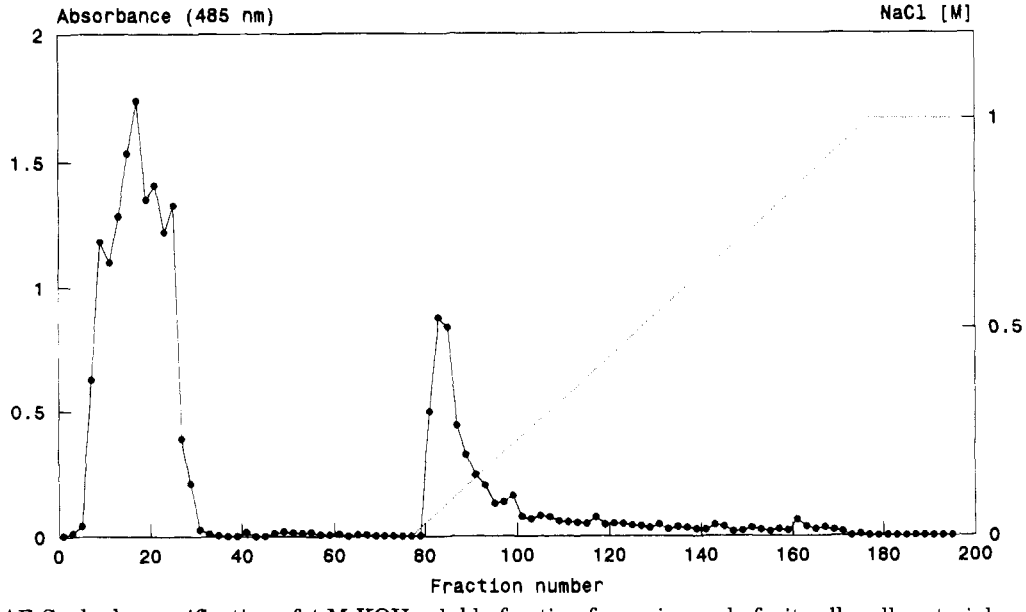


**Figure 3.** DEAE-Sephadex purification of 4 M KOH-soluble fraction from pineapple fruit cell wall material.

**Table 2. Partially Methylated Alditol Acetates Derived from the Pineapple Fruit "Neutral" Fraction of SN 5 Obtained on DEAE-Sephadex**

| PMAA | $RR_t$[a] | linkage type | MS[b] area (%) | parent sugar | from MS[c] (%) | by direct[d] analysis (%) |
|---|---|---|---|---|---|---|
| 2,3-$Me_2$Ara | 0.85 | →5)-Araf-(1→ | 9.7 | Ara | 20.7 | 26.6 |
| 2,4-$Me_2$Ara | 0.76 | →3)-Ara*p*-(1→ | 6.7 | | | |
| 2,3,5-$Me_3$Ara | 0.60 | Ara*f*-(1→ | 3.3 | | | |
| 2,3,4-$Me_3$Ara/Xyl | 0.68 | Ara/Xyl*p*-(1→ | 1.0 | | | |
| 2-MeXyl | 1.04 | →3,4)-Xyl*p*-(1→ | 32.9 | Xyl | 39.7 | 36.6 |
| 3-MeXyl | 1.04 | →2,4)-Xyl*p*-(1→ | 6.8 | | | |
| 2,3,6-$Me_3$Man | 1.18 | →4)-Man*p*-(1→ | 5.0 | Man | 5.0 | 4.4 |
| 2,3,6-$Me_3$Gal | 1.19 | →4)-Gal*p*-(1→ | 10.4 | Gal | 16.5 | 12.3 |
| 2,4,6-$Me_3$Gal | 1.22 | →3)-Gal*p*-(1→ | 6.1 | | | |
| 2,3-$Me_2$Glc | 1.48 | →4,6)-Glc*p*-(1→ | 14.4 | Glc | 17.9 | 20.1 |
| 2,3,4,6-$Me_4$Glc[e] | 1.00 | Glc*p*-(1→ | 3.5 | | | |

[a] Retention time relative to 2,3,4,6-$Me_4$-Glc on SPB-1. [b] Area percentage on SPB-1. [c] Total yield of the same parent sugar by summing individual PMAAs. [d] Sugar analysis of neutral fraction as dry weight % on CP-SIL 88. [e] 2,3,4,6-$Me_4$-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, etc. Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose. All values are the average of duplicate determinations.

which eluted with the phosphate buffer, and an acidic one (24%), eluting with the NaCl gradient, were separated.

Further analysis focused on the "neutral" fraction obtained on DEAE-Sephadex which contained the purified hemicellulosic material. Table 2 shows the results of sugar composition (by direct analysis as alditol acetates), and the methylation analysis data of this fraction (as PMAAs). For comparison with results from MS, the values from direct sugar analysis were adjusted to 100% without considering uronic acid. The results obtained by the methylation analysis were in reasonable accordance with the direct analysis of the parent sugars. The main glycosidic linkages were (1 → 4)-linked xylose with branch points at $C_3$ (32.9%) and at $C_2$ (6.8%), (1 → 4)-linked glucose with branch points at $C_6$ (14.4%), and end groups of glucopyranose, arabinofuranose, and arabino/xylopyranose. In addition to the above linkages, it was also found arabinofuranose (1 → 5)-linked, and a high percentage of galactopyranose (1 → 3)- and (1 → 4)-linked. We have detected traces of 2-linked galactose in pineapple CWM, a very unusual structural feature, although previously reported in onion cell wall polysaccharides (Redgwell and Selvendran, 1986).

As reported previously (Stevens and Selvendran, 1984; Rupérez et al., 1985), there were discrepancies between branch points and end groups, although the IR spectra of the remethylated samples showed negligible absorption for hydroxyl. This could be due to the selective loss of the most volatile methyl ethers during hydrolysis and methylation, corresponding to terminal residues (Siddiqui and Emery, 1990). Nevertheless, undermethylation cannot be ruled out.

The 4 M KOH-soluble neutral fraction from pineapple fruit CWM, containing hemicelluloses, it probably composed of a complex mixture of polysaccharides as inferred from the PMAAs formed. This is in agreement with the fact that hemicelluloses are described as a heterogeneous group of polysaccharides (xylans, arabinogalactans, glucomannans, etc.) that contain numerous kinds of hexose and pentose sugars, and in some cases residues of uronic acids (Haard, 1985). The excess of xylose over glucose in most CWM fractions, suggested that the majority of xylose is not of xyloglucan origin. Methylation analysis confirmed this: the major PMAA was 2-methylxylitol, suggesting the presence of arabinoxylans in the neutral fraction of SN 5.

## ABBREVIATIONS USED

Ara, arabinose; CWM, cell wall material; FID, flame ionization detector; Gal, galactose; GC, gas–liquid chromatography; Glc, glucose; IR, infrared; Man, mannose; MS, mass spectra; PMAA, partially methylated alditol acetate; SN, supernatant; SPB-1, Supelco bonded methyl silicone; Xyl, xylose; UA, uronic acid.

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