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Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis* Miller) plant tissues

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Abstract

A complete chemical characterisation of Aloe vera plant (*Aloe barbadensis Miller*) was carried out from the dissection of the plant whole leaves in filets and skin. In addition, a mucilaginous gel extracted from the filets was also characterised. Extraction with ethanol of lyophilised Aloe fractions (AIRs) allowed to concentrate the major fraction composed of carbohydrates up to 80%. The composition of the main type of polysaccharides present in the Aloe AIRs was determined. Mannose and cellulosic glucose were the major polysaccharide components in all AIRs, significant amounts of pectic polysaccharides were also detected. Sequential extraction of polysaccharides present in Aloe vera plant portions, revealed that two main types of mannose-containing polymers were present in the Aloe vera plant. The polysaccharide detected in the filet and in the gel fractions corresponded to a storage polysaccharide located within the protoplast of the parenchymatous cells. Its structural and compositional features corresponded to the active polysaccharide known as acemannan. On the contrary, in the skin tissue, the mannosyl residues arose from a structural polysaccharide located within the cell wall matrix. Structural and compositional differences between both polymers were confirmed by methylation analysis. The fact that acemannan is a reserve polysaccharide might help to explain most of the compositional variations reported in the literature for Aloe vera carbohydrates. Further, sequential extraction allowed us to identify several pectic polysaccharides, rich in uronic acids, with a composition similar to that of several antitumoral polymers found in different plant tissues. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Aloe barbadensis*; Polysaccharides; Bioactive polymers; Cell walls

1. Introduction

Aloe vera (*Aloe barbadensis* Miller) is a perennial plant with turgid green leaves joined at the stem in a rosette pattern. Aloe leaves are formed by a thick epidermis (skin) covered with cuticles surrounding the mesophyll, which can be differentiated into chlorenchyma cells and thinner walled cells forming the parenchyma (filet). The parenchyma cells contain a transparent mucilaginous jelly which is referred to as Aloe vera gel.

Aloe vera has enjoyed a long history of lay acceptance as possessing curative or healing qualities (Coats and Ahola, 1979; Davis et al., 1994; Visuthikosol et al., 1995) and it has been featured extensively in the area of dermatology, especially for treating radiation-caused skin conditions (Davis et al., 1989; Kaufman et al., 1989). The body of scientific literature documenting medical applications in digestive problems, as a virucidal, bactericidal, and a fungicidal agent and in gynaecological conditions is also extensive (Grindlay and Reynolds, 1986; Shelton, 1991).

According to the literature, the chemical composition of Aloe plants is largely dependant on the species analysed. A prominent feature of Aloe vera filet is its high water content, ranging from 98.5% to 99.5% of fresh matter. More than 60% of the remaining solid being made up of polysaccharides (McAnalley, 1993).

Presently, the controversy over the identity of the active substance(s) in Aloe vera has not been settled. It is therefore important to clearly distinguish between the composition of the different parts which form the Aloe vera plant. There is little information regarding the composition of Aloe skin tissues. In contrast, many studies have reported the presence of polysaccharides as the main component of the filet with minor amounts of various other components. Acemannan, a mannose-containing polysaccharide, has been reported as the main active substance present in Aloe vera filet (McAnalley, 1993; t'Hart et al., 1989). Acemannan, commercially known as Carrysin™, is a lineal polysaccharide composed of (1,4)-linked mannosyl residues, with C2 or

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C3 acetylated and some side chains of mainly galactose attached to C6.

It is probable that there may be some synergistic action between the polysaccharide base and other components in some of the activities observed. However, there are many examples in the literature of polysaccharides exhibiting pharmacological and physiological activities without synergistic help from other components. For example, the antitumour activity of polysaccharides has been widely reported (Kimoto and Watanabe, 1987; Waldron and Selvendran, 1992).

Several workers have tried the separation of the Aloe vera gel carbohydrate polymers into their polysaccharide components. Thus, Gowda et al. (1979) separated the gel polysaccharides into four partially acetylated glucomannans. Mandal and Das (1980a,b) found pectic substances, as the main component of Aloe vera filet, although significant amounts of other polysaccharides such as a galactan, an arabinan and a glucomannan were also detected. According to Leung (1978) the effect of seasonal and cultivar variations may affect the composition of the gel and explain the different results obtained by different experimental workers. In fact, Pierce (1983) showed large differences in the nutritional content of Aloe vera gel as a result of cultivar conditions. McAnalley (1993) attributed the discrepancies over the polysaccharide composition of Aloe vera to chemical purification steps.

Despite the latter studies on isolated polysaccharides, the lack of knowledge of Aloe vera cell walls is clear. None of the earlier studies has focused the problem of such compositional variations taking into consideration structural and functional aspects of the polysaccharides found in the Aloe vera plant. The information on the compositional features of *Aloe barbadensis* Miller is the basis for a better understanding of the biochemical changes which occur in Aloe vera during plant growth and development, and also during processing and storage of Aloe vera derived products.

Within this context, the aim of this investigation was to carry out a complete characterisation of the main type of cell wall polysaccharides present in Aloe vera plant fractions.

2. Experimental

2.1. Plant material and tissue separation

Whole Aloe vera (*Aloe barbadensis* Miller) leaves, supplied by the company Ingeniería Bioenergética S.L. which cultivates Aloe vera plants in the island of Ibiza (Spain), were the starting material for this investigation. The studied leaves, of between 30 and 50 cm length, corresponded to 4-yr old plants. Whole leaves were washed and the spikes, placed along their margins, were removed before slicing the leaf to separate the epidermis or skin from the filet. The skin and filet were washed extensively with distilled water to remove the exudate from their surfaces. Further, the filets were extruded to yield a mucilaginous gel leaving a remaining fibrous fraction which was discarded. The skin, filet and gel constituted the fractions studied in the present work. Fresh aloe leaves were stored for not longer than 24 h at 1°C prior to tissue separation. All separated tissues were frozen in liquid nitrogen and then stored at −20°C until required. All analyses were performed in duplicate on two different lots of Aloe vera leaves.

2.2. Alcohol insoluble residues

Alcohol insoluble residues (AIRs) from Aloe vera fractions were obtained by immersing the frozen samples in boiling ethanol (final concentration 85% (v/v)) as described by Waldron and Selvendran (1990). Prior to further analysis, the AIR was milled using a laboratory type grain mill and passed through a 0.5 mm aperture sieve. All samples were free of starch and were used in subsequent analyses.

2.3. Sequential extraction of cell wall material

Cell wall material (CWM) was extracted following the procedure proposed by Redgwell and Selvendran (1986) for the sequential extraction of CWM from parenchymatous tissues. AIR preparation (5 g) was suspended in distilled water (500 ml) and the mixture was stirred for 2 h at room temperature, centrifuged (12 000 rpm, 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 extracts were filtered through GF/C and dialysed exhaustively; both Na_2CO_3, KOH extracts and the cellulose-rich residue (final residue) were neutralised with acetic acid prior to dialysis. All extracts collected after dialysis were concentrated and stored as frozen suspensions at −30°C, a small amount of each being freeze dried.

2.4. Analytical methods

2.4.1. Moisture

Moisture content was measured by drying samples overnight at 60°C in the presence of silica gel.

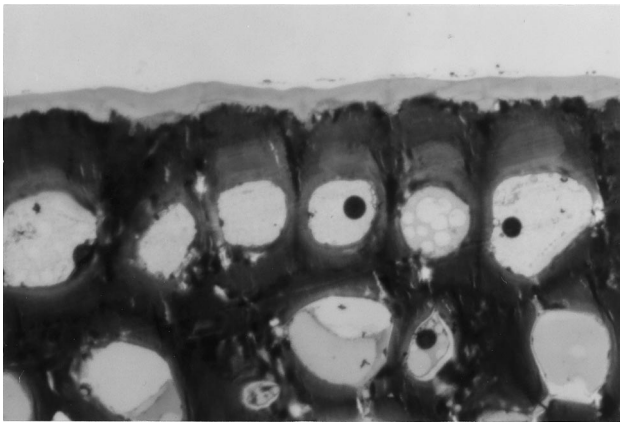

Fig. 1. Section of skin tissue from Aloe vera leaf. Secondary walls stained with toluidine blue can be observed owing to the presence of large amounts of lignin.

2.4.2. Lipids

Total content of lipids was determined gravimetrically by extraction with diethyl ether using a Soxhlet equipment (Cañellas et al., 1992).

2.4.3. Soluble sugars

Soluble sugars were determined according to the method of Wilson et al. (1981) based on the sum of the individual sugar contents obtained by HPLC.

2.4.4. Proteins

The nitrogen content of AIR was measured according to the method of Pearson (1981) using a Tecator Kjeltec autosampler system 1035 analyser. Protein content was estimated by multiplying the nitrogen value by 6.25.

2.4.5. Lignin

Lignin was gravimetrically determined as Klason lignin. Samples were dispersed in 72% H_2SO_4 at room temperature for 3 h then diluted to 1 M H_2SO_4 and heated to 100°C for 2.5 h. Insoluble material was recovered by filtration (sinter no. 2) and washed thoroughly with hot water (90°C) until acid free before drying at 105°C overnight. The residue weight was recorded as Klason lignin.

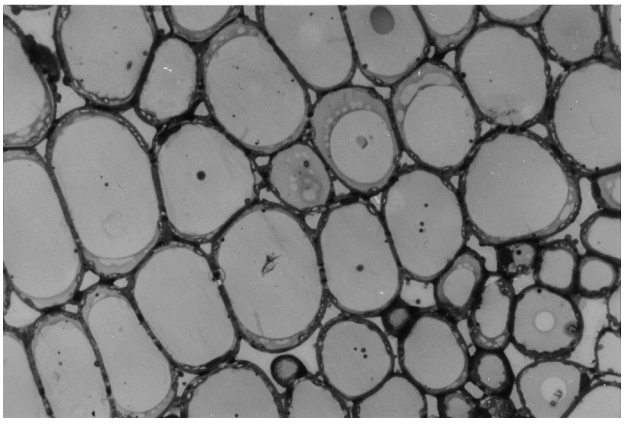

Fig. 2. Parenchymatous cells from Aloe vera filet. The small ‘‘granules attached to the inner surface of the cell walls might contain the bioactive polysaccharide acemannan.

2.4.6. Ashes

Ash contents were determined gravimetrically by overnight-heating at 550°C (AOAC, 1990).

2.4.7. Mineral elements

Simultaneous determination of Ca, Mg, K, Na, Fe, P, Mn, Zn and Cu was carried out by inductively coupled plasma atomic emission spectroscopy (ICP/AES) by means of a calibration curve (Boss and Freeden, 1989).

2.4.8. Starch

The occurrence of starch in the lyophilised fractions was tested for by staining AIR with I_2/KI solution, and examining by light microscopy.

2.4.9. Analysis of carbohydrate composition

Carbohydrate analysis was performed according to Waldron and Selvendran (1990) for neutral sugars. Sugars were released from residues by acidic hydrolysis. AIRs were dispersed in 72% H_2SO_4 for 3 h followed by dilution to 1 M and hydrolysed at 100°C for 2.5 h (Saeman et al., 1954). A 1 M H_2SO_4 hydrolysis (100°C for 2.5 h) was also included to estimate the cellulose content by difference. Neutral sugars were derivatised as their alditol acetates and isothermally separated by GC at 220°C on a 3% OV225 Chromosorb WHP 100/120 mesh column. Uronic acids were colorimetrically determined, as total uronic acid (Blumenkrantz and Asboe-Hansen, 1973), using a sample hydrolysed for 1 h at 100°C in 1 M H_2SO_4.

2.4.10. Degree of esterification of pectic substances

The degree of esterification (DE) of pectic polysaccharides was determined after reducing the samples with sodium borohydride (10 mg/ml) in 50% ethanol overnight (Lurie et al., 1994).

2.4.11. Methylation analysis

The polysaccharides were methylated by a modified sequential method using sodium hydroxide and methyl iodide (Ciucanu and Kerek, 1984; Needs and Selvendran, 1993). The modifications introduced to improve the overall methylation procedure have been described in detail by Femenia et al. (1998a).

2.5. Statistical analysis

Results were analysed by means of a one-way and multifactor analysis of variance, using the LSD test with a 95% confidence interval for the comparison of the test means.

Table 1
Chemical characterisation of lyophilised Aloe vera plant fractions. (Results are expressed as percentages on dry matter basis.)

	Skin	Filet	Gel
Lipids	2.71 ± 0.32	4.21 ± 0.12	5.13 ± 0.23
Proteins	6.33 ± 0.24	7.26 ± 0.33	8.92 ± 0.62
Soluble sugars	11.22 ± 0.73	16.48 ± 0.18	26.81 ± 0.56
Dietary fibres (NSP + lignin)	62.34 ± 1.10	57.64 ± 1.26	35.47 ± 0.62
Ashes	13.46 ± 0.44	15.37 ± 0.32	23.61 ± 0.71
Ca	4.48 ± 0.23	5.34 ± 0.14	3.58 ± 0.42
Mg	0.90 ± 0.12	0.76 ± 0.04	1.22 ± 0.11
Na	1.82 ± 0.09	1.98 ± 0.15	3.66 ± 0.07
K	1.84 ± 0.05	3.06 ± 0.18	4.06 ± 0.21
P	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Fe	0.04 ± 0.01	0.04 ± 0.01	0.10 ± 0.02
Cu	0.02 ± 0.01	0.04 ± 0.00	0.06 ± 0.01
Zn	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00

3. Results and discussion

Aloe vera plant portions, i.e. skin, filet and extruded gel were obtained as described earlier. The thick epidermis accounted for 20%–30% by weight of the whole leaf and it was formed mainly by lignified cell walls as shown in Fig. 1. This contrasted with the big rounded cells with thin primary walls forming the parenchymatous tissue of the filet (Fig. 2), which represented about 65%–80% by weight of the whole Aloe leaf. The Aloe vera gel was mechanically extruded from the filet, the yield of this process being approximately 70% (g gel/100 g filet).

3.1. Overall chemical characterisation

The main feature of all the Aloe plant portions was their high water content. The skin and filet fractions contained approximately 90% and 98% of water, respectively, the extruded gel containing over 99% water.

Lipids, proteins, soluble sugars, fibres, ashes and element minerals present in the Aloe vera lyophilised portions were characterised and the results are shown in Table 1.

On dry matter basis, lipids represented a minor fraction in all samples ranging from 2.71% to 5.13% of the freeze-dried material. Protein was also a minor fraction, ranging from 6.33% for the skin to 8.92% in the gel. The amount of soluble sugars detected differed depending on the Aloe portion. The skin contained 11.22% of soluble sugars, whereas this amount increased to 16.48% in the filet and to 27.81% in the gel, being one of the most important components in this latter fraction. Glucose accounted for over 95% of the soluble sugars in all the Aloe fractions analysed. The ash content was relatively high, in particular in the gel fraction where it accounted for up to 23.61% of the dry matter. Ca, K, Na and Mg were the predominant mineral elements in all the Aloe fractions. In particular, Ca was the main mineral element in all fractions except in the gel where Na and K were detected in higher amounts. Robson et al. (1982) pointed out that the presence of K in different concentrations may regulate the healing properties of the Aloe vera. Other elements such as Fe, Cu, Zn and P were detected in minor amounts.

Table 2
Chemical composition of the AIRs obtained from the different Aloe vera lyophilised fractions. (Results are expressed as percentage of AIR.)

	Skin	Filet	Gel
Carbohydrates	60.34 ± 1.43	79.95 ± 2.12	72.17 ± 1.23
Lignin	19.62 ± 0.97	1.63 ± 0.43	0.34 ± 0.12
Proteins	7.56 ± 0.31	10.12 ± 0.65	15.40 ± 0.87
Ashes	7.12 ± 0.64	7.41 ± 0.54	7.30 ± 0.53
Ca	1.23 ± 0.12	3.12 ± 0.16	3.28 ± 0.22
Mg	0.44 ± 0.02	0.08 ± 0.01	0.26 ± 0.07
Na	0.41 ± 0.07	0.04 ± 0.00	0.03 ± 0.00
K	0.38 ± 0.04	0.11 ± 0.01	0.12 ± 0.01
P	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fe	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Cu	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Zn	0.01 ± 0.00	0.01 ± 0.00	0.06 ± 0.01

3.2. Alcohol insoluble residues

As starch was not detected in any of the Aloe plant portions studied, polysaccharides were precipitated with an 80% ethanol solution. These fractions were denominated as AIRs. The sum of carbohydrates or non-starch polysaccharides (NSP) plus lignin present in the AIRs was taken as the percentage of dietary fibre (DF), which is shown in Table 1.

The percentages of carbohydrates, lignins, proteins, ashes and mineral elements present in the AIRs are shown in Table 2. Protein contamination of the AIRs ranged from 7.56% to 15.40% which can be considered low in relation to other plant tissues where protein is accounted for up to 30%–35% of the AIR preparation (Waldron and Selvendran, 1990; Femenia et al., 1998b). The sum of carbohydrate and lignin present in the AIRs represented between 72% and 82% of the total material precipitated with ethanol. In particular, significant amounts of lignin were detected in the

Table 3
Carbohydrate composition of the polysaccharides present in the AIRs from the different Aloe vera fractions. (Results are expressed as mol%.)

	Skin	Filet	Gel
Rhamnose	2.18 ± 0.07	1.69 ± 0.03	0.84 ± 0.01
Fucose	2.54 ± 0.04	1.94 ± 0.03	0.64 ± 0.01
Arabinose	5.88 ± 0.01	1.92 ± 0.06	1.15 ± 0.02
Xylose	11.72 ± 0.05	2.34 ± 0.03	1.38 ± 0.04
Mannose	30.09 ± 1.01	46.07 ± 1.21	52.81 ± 1.33
Galactose	8.43 ± 0.11	4.97 ± 0.06	3.50 ± 0.04
Glucose[a]	25.10 ± 0.71	27.03 ± 0.54	26.68 ± 0.38
Glucose (1 M)[b]	(2.89 ± 0.07)	(5.95 ± 0.02)	(5.25 ± 0.08)
Uronic acids	14.05 ± 0.21	14.04 ± 0.32	13.00 ± 0.25
DE (%)	21 ± 3	76 ± 2	73 ± 3

[a] Glucose determined using the Saeman hydrolysis conditions.
[b] Glucose determined using 1 M sulphuric acid.

Aloe skin fraction. The presence of secondary lignified walls (see Fig. 1) leads to cross-linking of cell wall polysaccharides and an increase in cell wall strength, which is perceived as a toughening of that tissue.

Ashes accounted for approximately 7% of the AIRs, representing a small but significant percentage of the AIRs. Most of the mineral elements detected within this fraction were probably associated with polysaccharides. The presence of calcium is of particular interest, this ion has the ability to form calcium bridges between galacturonic acid units of adjacent pectin chains, therefore, it may have a significant influence on the textural characteristics and the porosity of the tissues. The calcium–pectin complex formed acts as an intra-cellular cement giving firmness to the tissues (Alonso et al., 1995). Thus, the fact that the filet contained

Table 4
Yields, carbohydrate composition and total amount of sugars of the different extracts obtained after sequential extraction of polysaccharides from Aloe vera skin, filet and gel portions

Fraction	Yield[a] (%)	Monosaccharides[b] (mol%)								Total sugars (μg/mg)[c]	DE (%)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA		
H_2O											
Skin	40.6	2.1	2.4	2.0	1.4	5.1	10.0	3.1	74.7	260.1	34
Filet	41.5	0.2	0.3	1.0	2.1	73.1	1.7	15.7	5.9	578.7	87
Gel	51.2	0.3	0.2	1.1	0.9	76.8	1.4	12.1	7.2	632.4	81
CDTA-1											
Skin	14.4	1.5	0.6	1.4	0.7	23.5	3.1	8.5	60.8	679.7	11
Filet	14.8	1.5	0.6	2.5	0.7	10.5	4.1	8.5	71.8	679.7	54
Gel	11.3	1.1	0.1	1.0	0.2	13.5	1.2	7.1	75.8	685.2	48
CDTA-2											
Skin	8.3	4.0	1.6	3.8	0.9	35.3	7.1	16.4	31.4	136.7	9
Filet	9.5	0.8	1.1	2.0	2.5	13.6	5.9	44.2	29.9	295.0	38
Gel	10.1	0.9	0.6	1.1	0.9	7.2	5.8	42.1	41.1	281.4	31
Na_2CO_3-1											
Skin	7.3	1.6	1.1	2.0	0.6	2.2	17.5	3.1	71.9	384.8	—
Filet	9.5	2.5	2.6	3.3	0.5	4.6	12.1	5.1	69.4	410.4	—
Gel	5.1	2.7	2.0	3.7	0.6	5.2	7.2	2.4	76.2	523.1	—
Na_2CO_3-2											
Skin	3.6	4.8	4.0	6.5	4.8	4.3	35.1	22.9	17.6	234.7	—
Filet	3.5	4.5	5.5	5.4	6.1	5.6	29.7	26.7	16.5	161.2	—
Gel	2.3	4.3	3.1	6.9	4.3	6.2	30.1	21.2	23.9	154.6	—
0.5 M KOH											
Skin	3.6	1.2	1.6	5.2	40.2	6.2	10.7	29.7	5.3	404.6	—
Filet	3.2	1.3	2.5	7.1	32.5	12.4	5.8	26.8	11.6	173.0	—
Gel	1.4	1.1	5.2	8.7	18.4	13.4	3.4	15.4	33.0	181.7	—
1 M KOH											
Skin	8.4	1.6	3.2	4.1	32.0	13.1	9.2	32.5	4.3	476.4	—
Filet	5.1	1.4	2.7	2.3	21.3	21.6	8.3	36.3	6.3	499.1	—
Gel	1.1	1.3	4.6	3.9	19.5	22.6	7.2	24.2	15.6	476.3	—
4 M KOH											
Skin	3.3	3.8	4.5	7.9	22.5	6.5	19.0	26.4	9.4	109.5	—
Filet	3.0	5.3	3.1	4.7	19.6	10.8	12.0	24.7	19.8	134.4	—
Gel	1.2	2.1	3.8	5.1	23.1	12.4	10.1	21.8	21.6	181.4	—
Final residue											
Skin	10.5	0.6	0.5	2.6	0.6	4.0	12.9	68.0	10.9	845.7	—
Filet	10.0	0.5	0.4	0.7	0.6	5.1	3.4	76.6	12.7	891.4	—
Gel	9.1	0.3	0.1	0.4	0.5	4.3	4.1	71.3	19.0	875.3	—

[a] Yields are expressed as the percentage of AIR material recovered after each step of the fractionantion procedure.
[b] Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannosae, Gal: galactose, Glc: glucose y UA: uronic acids.
[c] Total sugars are expressed as μg of total sugar units per mg of material recovered after each extraction.

higher amounts of Ca than the skin could be owing to pectic substances being the major contributors to the textural aspects exhibited by a soft and fleshy tissue such as the filet, whereas in the skin fraction, the secondary lignified walls could be responsible for most of its textural characteristics.

3.3. Carbohydrate composition

AIR hydrolysis was performed using two procedures: (1) by the Saeman method (Saeman et al., 1954) and (2) a milder hydrolysis using 1 M sulphuric acid. The latter hydrolyses most of the non-cellulosic neutral sugars, whereas the former also hydrolyses cellulosic glucose. In all the samples no significant differences were found between both types of hydrolysis except for the glucose.

Overall carbohydrate analysis of the AIRs revealed that cell walls of the filet and gel were fairly similar in composition and contained mainly mannose-containing polysaccharides, cellulose and pectic polysaccharides whereas the cell walls of the skin contained in addition significant amounts of xylose-containing (hemicellulosic) polysaccharides.

Mannose and glucose were the predominant sugars in all fractions and represented between 55% and 75% of the total monosaccharides determined. Mannose residues from filet and gel tissues had probably arisen from acemannan, the main active component of Aloe vera plant, which has been found in large amounts in these tissues (Fogleman et al., 1992; McAnalley, 1993). However, there is no information about the occurrence of the latter polysaccharide in skin tissues.

The presence of significant amounts of cellulose could be inferred from the fact that most of the glucose could only be released using the Saeman hydrolysis conditions.

The occurrence of significantly larger amounts of pectic polysaccharides in all fractions could be deduced from the presence of uronic acids (galacturonic acid), rhamnose, arabinose and galactose units. Nevertheless, the significant differences in the DE of pectic substances (Table 3) from the skin to those from either the filet or gel fractions suggest a distinct structural arrangement of pectic polysaccharides within the cell wall matrix of the different tissues (Femenia et al., 1998c). Further, the presence of pectic substances should be highlighted as several authors have reported the antitumoral activity exhibited by this type of polysaccharides from other plant sources (Waldron and Selvendran, 1992).

To a minor extent, significant amounts of xylose were detected, especially on those Aloe vera fractions which contained significant amounts of lignin. This may be indicative of the occurrence of hemicellulosic polysaccharides such as xyloglucans and xylans in most of the Aloe portions. The presence of xyloglucans was confirmed by the presence of fucose and glucose released under 1 M sulphuric acid hydrolysis. Thus, the presence of higher amounts of xylose in the skin than in the filet is probably as a result of the presence of acidic xylans deposited on secondary walls which could be responsible for most of the textural differences found between skin and filet tissues.

3.4. Sequential extraction of polysaccharides

The AIR fractions were examined in greater detail to identify the main type of polysaccharides present in the Aloe vera plant portions studied. Thus, the polysaccharides present in the AIRs were sequentially extracted using methods designed to minimise β-eliminative degradation of pectins and thus solubilise the cell wall polymers in as close to their native form as possible. The relative merits of the solvents used have been discussed in several papers (Redgwell and Selvendran, 1986; Ryden and Selvendran, 1990; Martin-Cabrejas et al., 1994).

Data on the amount of polymers solubilised and the carbohydrate composition obtained after performing the sequential extraction on the Aloe skin, filet and gel AIRs is shown in Table 4. As AIRs were the starting material for each extraction other compounds apart from the polysaccharides, e.g. proteins, could precipitate in the different fractions. However, after each step of the fractionation procedure, an exhaustive dialysis of the extracts was carried out, however, not all compounds with low molecular weight could be removed (Waldron and Selvendran, 1990; Coimbra et al., 1994). Both effects could explain the low carbohydrate yields obtained in some of the fractions.

3.4.1. Water extraction

Approximately 41% of the AIR was extracted by water in both the filet and the skin fractions, however, the total amount of sugars detected in these fractions was significantly different ($p < 0.01$); whilst in the filet water extract 58% of the material was of carbohydrate origin, in the skin fraction, this percentage decreased to 26%. However, filet and skin water extracts exhibited very different carbohydrate composition. In the skin water extract, uronic acids were the main type of monosaccharides, whereas in the filet, mannose and glucose were the predominant sugars accounting for 73% and 16%, respectively, of the total carbohydrate. Uronic acids represented only 5.9% of the total sugars in the filet extract. In addition, in the skin fraction, the amount of mannose detected represented only 5% of the total sugars.

As water solubilises polymers which are not cross-linked within the cell wall network, it could be inferred that the mannose-rich polysaccharide(s) detected in the filet and in the gel water extracts, most probably acemannan, did not arise from cell wall polysaccharides. As it has been found in other *liliaceas* (Brett and Waldron, 1996), the mannose-containing polysaccharides could be derived from storage polysaccharides metabolisable by the Aloe plant. On the contrary, the pectic polysaccharides found in the skin water extract had probably arisen from the middle lamella

Table 5
Glycosidic linkage analysis from selected Aloe vera extracts (%mol). Numbers in brackets represent the mol% of neutral sugars as determined by Saeman hydrolysis

	Filet		Gel		Skin			
	(water)		(water)		(CDTA-1)		(CDTA-2)	
Rhamnose								
1,2	0.2	(0.2)	0.3	(0.3)	3.7	(3.8)	5.2	(5.8)
Fucose								
Terminal	0.1	(0.3)	0.1	(0.2)	1.2	(1.5)	1.7	(2.3)
Arabinose								
Terminal-f	0.3		0.2		0.4		0.7	
1,5	0.7	(1.1)	0.6	(1.2)	1.9	(3.6)	2.3	(5.5)
1,3,5	0.4		0.5		1.3		1.5	
1,2,3,5	0.1		0.1		0.3		0.1	
Xylose								
Terminal	0.3		0.2		0.4		0.7	
1,4	0.9	(2.2)	0.7	(1.0)	0.9	(1.8)	0.7	(1.3)
1,2,4	0.1		0.1		0.3		0.1	
Mannose								
Terminal	0.36		0.33		0.11		0.09	
1,4	70.22		76.93		52.07		46.86	
1,6	1.66	(77.7)	1.23	(81.6)	0.34	(59.9)	0.27	(51.7)
1,3,4	1.64		1.70		—		—	
1,4,6	1.45		1.63		4.23		3.57	
1,3,4,6	0.24		0.27		0.46		0.29	
Galactose								
Terminal	1.0		0.7		4.2		6.1	
1,4	—		—		—		0.3	
1,6	0.3	(1.8)	0.2	(1.5)	1.5	(7.9)	2.6	(10.3)
1,3,4	0.2		0.1		0.7		1.0	
1,3,6	0.1		—		0.5		0.6	
1,4,6	0.1		0.1		0.4		0.5	
Glucose								
1,4	12.4		10.8		16.7		18.2	
1,3,4	0.5	(16.7)	0.3	(12.9)	0.9	(21.7)	0.6	(23.9)
1,4,6	3.4		2.5		3.7		3.9	
Glucitol	0.3		0.2		0.4		0.3	

region as it has been observed in other plant tissues (Selvendran and Ryden, 1990).

3.4.2. CDTA extracts

All CDTA-1 extracts exhibited relatively high yields ranging from 10.1% to 14.8%. The yields of CDTA-2 extractions were also significant, although in each of the Aloe fractions, the amount of carbohydrates present was significantly smaller than the corresponding amount detected in the CDTA-1 fraction. Pectic polysaccharides were the main type of polymers present in all CDTA fractions. In general, CDTA-1 fractions contained pectic polysaccharides less ramified than those extracted by CDTA-2 solution. The most relevant feature of CDTA extracts was the presence of significant amounts of mannose in the skin fraction suggesting that the mannose-containing polysaccharides present in this fraction corresponded to structural cell wall polymers as CDTA was required for its solubilisation.

3.4.3. Na_2CO_3 extracts

In general, sodium carbonate extracts exhibited lower recoveries than previous (CDTA or water) fractions, Na_2CO_3-1 yields ranged from 5.1% for the gel to 9.5% for the filet, whereas none of the yields of the Na_2CO_3-2 extracts was higher than 4%. No significant differences were found ($p < 0.05$) in the carbohydrate compositions of the Na_2CO_3 extracts obtained from the different Aloe plant fractions. Na_2CO_3-1 extracts contained mainly slightly ramified pectic polysaccharides, galactose being the main component of the pectic side chains, whereas Na_2CO_3-2 extracts solubilised highly ramified pectic polysaccharides. In addition, a significant amount of glucose was also detected in the latter extracts.

3.4.4. Alkali extracts (0.5, 1 and 4 M KOH)

All alkali extracts exhibited low recoveries. However, the amount of carbohydrates detected in several 0.5 M and in all 4 M KOH extracts were also relatively low. This could be owing to the presence of co-precipitated protein. The most relevant aspect in terms of composition was the presence of xylose and glucose, indicating the presence of hemicellulosic polysaccharides such as xyloglucans and xylans. Most of the xylosyl residues was released after 0.5 and 1 M KOH extractions. To a lesser extent pectic polysaccharides were also present in all the alkali extracts, pectic–xylan–xyloglucan complexes have been identified in other tissues capable of undergoing secondary thickening (Coimbra et al., 1995; Femenia et al., 1999).

3.4.5. α-cellulose residue

The remaining materials after all the steps of the fractionation procedure, referred to as final residues, ranged from 9.1% for the Aloe gel to 10.5% for the skin. Carbohydrate recoveries were very high in all the Aloe fractions, cellulosic glucose being the predominant sugar detected in these fractions. Pectic polysaccharides were also found in most of the final residues, suggesting that there are inter- or intramolecular cross-links between pectic polysaccharides which are resistant to the chemical reagents used during the sequential extraction, and which cause the physical enmeshing of the pectic polysaccharides with the cellulose microfibrils. Moreover, all the Aloe fractions contained between 4% and 7.5% of mannose in these final residues which could be owing to the presence of mannans or glucomannans probably cross-linked with the cellulose.

Apart from water and CDTA pectins, the remaining pectic substances which were present in sodium carbonate and alkali extracts, and also in the final residues contained a large amount of uronic acids, thus, neutral pectic polysaccharides were not found as separate entities from the galacturonic acid-containing polysaccharides in any of the extracts. Pectic polysaccharides are easily degradable when extracted under certain conditions, for example, extraction with hot water or hot oxalate causes β-eliminative degradation releasing fragments rich in neutral sugars

derived from the side chains attached to the main pectic backbone. Thus, the D-galactan isolated by Mandal and Das (1980a) from *Aloe barbadensis* Miller filet might have arisen from the degradation of pectic polysaccharides because hot water extraction was used. The same effect has been observed in other plant tissues (Wood and Siddiqui, 1971; Ring and Selvendran, 1981). By using modern non-degradative techniques which minimise β-eliminative degradation, neutral pectic polysaccharides have not been found as separate entities from the galacturonic acid-containing polysaccharides.

3.5. Glycosidic linkage of selected mannose-containing aloe fractions

Glycosidic linkage analysis was performed on selected Aloe vera fractions, namely CDTA-1 and CDTA-2 skin fractions, water soluble filet fraction and water soluble gel fraction. Relative sugar mole ratios obtained from alditol acetates and partially methylated alditol acetates were in good agreement, as can be observed in Table 5.

Methylation analysis revealed important structural differences between the mannose-containing polymers detected in the skin and those found either in the filet or gel fractions. Despite this (1,4)-linked mannosyl were the predominant residues in both types of polymers, significant differences were found in the average molecular weight (MW), the degree of acetylation and, also, in the abundance of side chains.

From the ratio (1,4)-, (1,4,6)- and (1,3,6)-linked residues to terminally linked mannosyl units, an approximate MW could be determined for mannose-rich polymers. Thus, an MW of 80–90 kDa was estimated for the mannose-rich polymers of the skin fraction, whereas the mannose-containing polymers from the filet and gel showed an average MW of, approximately, 30–40 kDa.

The presence of (1,3,4)-linked mannosyl residues were detected in the filet and gel but not in the skin fractions. This may correspond to the acetylation of the polysaccharide backbone, as acetyl groups have been detected at C3 of mannose units of the active component acemannan (McAnalley, 1993).

Side chains attached at C6 occurred in both type of polymers. However, mannans from the filet exhibited a lower degree of branching as indicated by the ratio of (1,4,6)- to (1,4)-linked residues. Galactose units attached to C6 of mannose residues have been found in Aloe acemannan. The galactose side chains might prevent crystallisation of the galactomannans and make the polysaccharides hydrophilic.

4. Conclusions

Sequential extraction of polysaccharides has demonstrated that the mannosyl residues detected by carbohydrate analysis of Aloe vera fractions, namely skin, filet and gel, arose from different types of polysaccharides. Thus, in the filet, mannose was the main component of the storage polysaccharide acemanan, whereas in the skin, most of the mannose corresponded to structural polysaccharide(s) located within the cell wall.

Therefore, most of the important fluctuations in the polysaccharide composition of Aloe vera filet found in the literature (Gowda et al., 1979; Mandal and Das, 1980a,b; Pierce, 1983) could be explained by the fact that most of the mannosyl residues arise from a reserve polysaccharide. This would also explain the significant influence of seasonal and cultivar variations on the amount and composition of mannose-containing polysaccharide present within the cells.

The considerable similarity in the composition of the AIR sequential extractions of the gel and the filet indicates that the gel contains significant quantities of cell wall material. Hence, the extrusion treatment does not provide much separation. Therefore, the main composition of the filet AIR is actually gel material.

From the carbohydrate composition of water, CDTA and sodium carbonate extracts of the different Aloe samples, it was observed that the composition of certain fractions was fairly similar to the composition of other polysaccharides with reported antitumoral activity. For example, the carbohydrate compositions of the Na_2CO_3-1 fractions from Aloe skin and filet are fairly similar to the polysaccharide identified by Kimoto and Watanabe (1987) with composition Ara (7): Man (7): Gal (26): Glc (11): GalA (60) in *Pinus parviflora*. The water extract from Aloe skin and the CDTA-1 extract from Aloe filet also showed features similar to that of the latter polysaccharide. Further, the mannose-containing-polysaccharide detected in the filet and gel water fractions, identified as the bioactive polysaccharide acemannan, is similar to the bioactive polymer found in yeast (*Candida utilis*) which exhibited growth inhibition effects against Sarcoma 180 solid tumour in mice (Kumano, 1972). These findings would support the supposed antitumoral activity exhibited by Aloe vera.

Overall, this investigation has provided a better understanding of the main compositional and structural features of Aloe vera cell walls, and also of the origin of a non-cell wall polysaccharide such as the bioactive polymer acemannan.

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