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Effects of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadensis* Miller

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Abstract

Physico-chemical modifications promoted by heat treatment and dehydration at different temperatures (30–80 °C) on acemannan, a bioactive polysacharide from aloe vera (*Aloe barbadensis* Miller) parenchyma, were evaluated. Modification of acemannan, a storage polysaccharide, was particularly significant when dehydration was performed above 60 °C. Heating promoted marked changes in the average molecular weight (MW) of the bioactive polysaccharide, increasing from 45 kDa, in fresh aloe, to 75 and 81 kDa, for samples dehydrated at 70 and 80 °C, respectively. This could be attributed to structural modifications, such as deacetylation and losses of galactose-rich side-chains from the mannose backbone. These structural modifications were reflected by the significant changes occurring in the related functional properties, such as swelling, water retention capacity, and fat adsorption capacity, which exhibited a significant decrease as the temperature of dehydration increased. Further, dehydration also promoted significant modification of the main type of cell wall polysaccharides present within the aloe parenchyma tissues. Pectic polysaccharides from the cell wall matrix were affected by heating, probably due to either β-elimination processes or enzyme-catalysed degradation. The influence that these physico-chemical modifications might have on the bioactivity and properties of processed products from *A. barbadensis* Miller needs to be considered.

Keywords: *Aloe barbadensis* Miller; Acemannan; Structural modifications; Dehydration

1. Introduction

Aloe vera (*Aloe barbadensis* Miller) is a member of the *Liliacea*. Aloe leaves are composed of a thick epidermis surrounding the mesophyll, which can be differentiated into collenchyma cells and thinner walled cells forming the parenchyma (filet). The parenchymatous tissue of the inner parenchyma contains over 99% of water. Polysaccharides account for most of the dry matter of the aloe vera parenchyma, with two main types of polymers: acemannan, a storage polysaccharide rich in mannose units which is located within the protoplast of the cells, and a wide variety of polysaccharides forming the cell wall matrix (Femenia, Sánchez, Simal, & Rosselló, 1999b).

Acemannan, commercially known as Carrysin™, is the main bioactive substance found in aloe vera plant (t'Hart, van den Berg, Kuis, van Dijk, & Labadie, 1989; Lee et al., 2001; Reynolds & Dweck, 1999). Acemannan is a linear polysaccharide composed by (1,4)-linked mannosyl residues, with C2 or C3 acetylated and some side-chains formed by galactose units attached to C6 (Manna & McAnalley, 1993; McAnalley, 1993). This storage polysaccharide is located within the protoplast of the parenchymatous cells of the aloe vera parenchyma (Femenia et al., 1999b). Storage glucomannans are not exclusive of the aloe vera plant, since these are commonly found in others members of the *Liliacea* and *Iridacea* families (Brett & Waldron, 1996).

The controversy over the identity of the active substance(s) present in the aloe vera has not yet been settled. It is conceivable that there may be some synergistic action between the polysaccharide base and other components in some of the activities observed (Leung, 1978). However, many examples of cell wall polysaccharides demonstrating pharmacological and physiological activities, without known synergistic help from other components, can be found in the literature. For instance, the anti-tumoural activity of cell wall polysaccharides is widely reported

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(McAnalley, 1993). In particular, Waldron and Selvendran (1992) evaluated the potential of certain pectic substances from edible plant organs as possible sources of anti-tumour polysaccharides.

The potential use of aloe vera products often involves some type of processing, e.g. heating, dehydration. Processing may cause irreversible modifications to the polysaccharides, affecting their original structure, which may promote important changes in the proposed physiological and pharmacological properties of these polymers. At present, very little is known about the effects of processing on either the composition or the structure of the different types of polysaccharides found in aloe vera parenchyma.

Thus, the objective of this investigation was to evaluate the main effects of the air drying temperature on the physico-chemical properties of the main type of polysaccharides, present in aloe vera parenchyma.

2. Experimental

2.1. Material

Fresh whole aloe vera (*A. barbadensis* Miller) leaves obtained from the island of Ibiza (Spain), were used as the raw material in all experiments. The studied leaves, of between 30 and 50 cm of length, corresponded to 4-year old plants. Whole leaves were washed with distilled water to remove dirt from the surface. The spikes, placed along their margins, were removed before slicing the leaf. The epidermis (or skin) was carefully separated from the parenchyma using a scalpel-shaped knife. The filets were extensively washed with distilled water to remove the exudate from their surfaces. Fresh aloe filets were stored no longer than 1 h at 1 °C prior to dehydration.

2.2. Dehydration

Fresh aloe filets were diced to 0.5 mm^3 cubes, and dehydrated in a pilot-scale hot air drier (Simal, Mulet, Tarrazó, & Rosselló, 1996) by convection drying. Temperatures used were 30 °C (30D sample), 40 °C (40D sample), 50 °C (50D sample), 60 °C (60D sample), 70 °C (70D sample) and 80 °C (80D sample). Final moisture content of the dried parenchyma cubes was <1.5 g water/100 g dry matter (d.m.). In addition, an aloe filet was freeze dried (FD sample), and used as a reference.

2.3. Alcohol insoluble residues

Alcohol insoluble residues (AIRs) from fresh, freeze dried and dehydrated aloe vera parenchyma were obtained by immersing in boiling ethanol (final concentration, 85% (v/v) aqueous) as described by Waldron and Selvendran (1990). Prior to further analysis, the AIRs were milled using a laboratory type grain mill and passed through 0.5 mm aperture sieve.

2.4. Isolation and purification of acemannan

Acemannan was initially isolated as described by Femenia et al. (1999b). AIR preparation (200 mg) was suspended in distilled water (200 ml) and stirred for 2 h at room temperature. The supernatant (containing the acemannan) was recovered and extensively dialysed (MW cut-off 10,000–12,000). The water insoluble residue contained most of the cell wall polysaccharides present in the aloe parenchyma.

Further purification of acemannan was carried out through gel permeation chromatography (Femenia, Rigby, Selvendran, & Waldron, 1999a). The elution of dialysed fractions containing acemannan was performed on a column (100 cm × 1 cm) of Sephacryl S-400-HR at a flow rate of 16 ml/h. The fractions were dissolved in 2 ml, 50 mM potassium–phosphate buffer, pH 6.5, containing 0.2 M NaCl. Fractions (2 ml) were collected and aliquots (20 μl) were assayed for carbohydrate by the phenol–sulphuric acid method. The appropriate fractions containing purified acemannan were combined, dialysed, concentrated, and an aliquot was freeze dried for sugar and methylation analysis. The remaining material was stored at −20 °C.

To determine the average molecular weight (MW), the column was calibrated using standard dextrans having MWs of 487, 266, 72, 48.6 and 23.8 kDa.

2.5. Analytical methods

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

Lignin was gravimetrically determined as Klason lignin. AIRs 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.

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

Carbohydrate analysis was performed as in Femenia, Lefebvre, Thebaudin, Robertson, and Bourgeois (1997) for neutral sugars. Sugars were released from residues by acid hydrolysis. Samples (either purified acemannan or water insoluble cell wall polysaccharides from freeze dried and dehydrated aloe filets) were dispersed in 12 M H_2SO_4 for 3 h followed by dilution to 1 M and hydrolysed at 100 °C for 2.5 h (Saeman, Moore, Mitchell, & Millett, 1954). A second sample was hydrolysed only with 1 M sulphuric acid (100 °C for 2.5 h). The cellulose content was estimated by the difference in glucose obtained by Saeman hydrolysis and

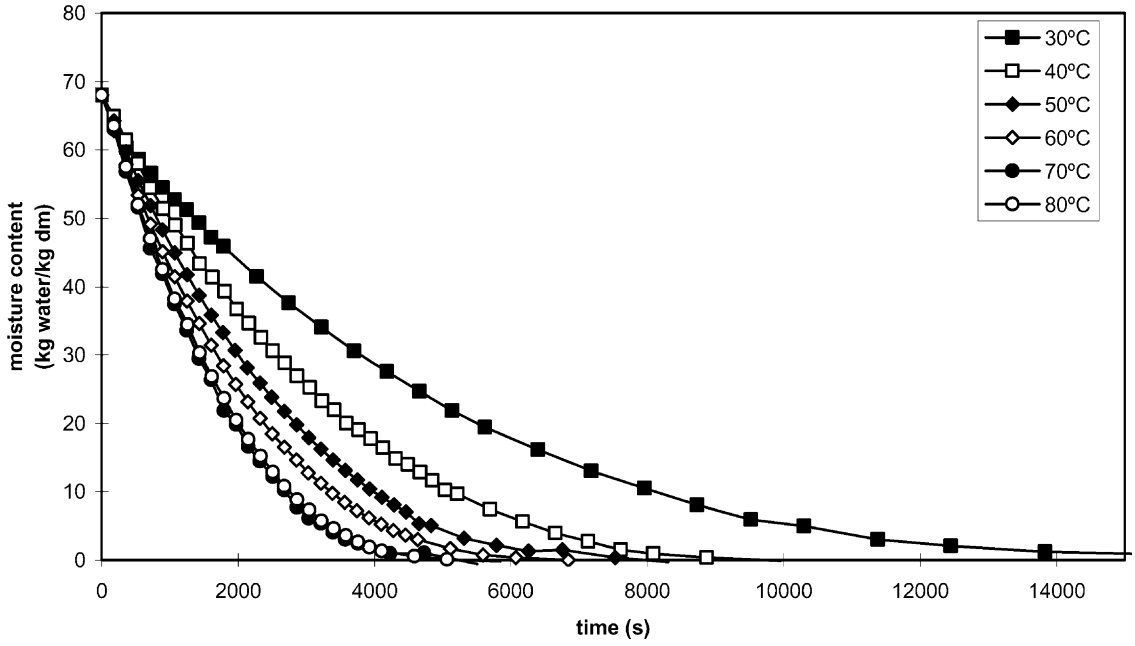

Fig. 1. Drying curves corresponding to the dehydration of aloe vera parenchyma at different temperature.

this milder hydrolysis method. Neutral sugars were derivatised as their alditol acetates and isothermally separated by GC (Selvendran, March, & Ring, 1979) at 220 °C on a 3% OV225 Chromosorb WHP 100/120 mesh column. Uronics acids were determined by colorimetry, as total uronic acid (Blumenkrantz & Asboe-Hansen, 1973), using a sample hydrolysed for 3 h at 20 °C in 12 M H_2SO_4, followed by 1 h at 100 °C in 1 M H_2SO_4.

Methylation analysis of purified acemannan fractions was based on a modified sequential method using sodium hydroxide and methyl iodide (Ciucanu & Kerek, 1984; Needs & Selvendran, 1993). The modifications introduced to improve the overall methylation procedure have been described in detail by Femenia, García-Conesa, Simal, and Rosselló (1998).

2.6. Fourier transformed infrared spectroscopy analysis

Fourier transformed infrared (FTIR) spectra were obtained on a Bruker IFS66 instrument, at a resolution of 3 cm^{-1}, after preparing a KBr disc containing 2 mg of purified acemannan polymer from the freeze dried and dehydrated aloe filets. The single beam traversing each sample was ratioed with the single beam of the corresponding background. Equivalent samples from different experimental runs gave the same spectra in all cases.

2.7. Functional properties

Functional properties included hydration properties (water retention capacity (WRC) and swelling (SW)), and fat adsorption capacity (FAC). WRC and SW were measured using hydrated AIR samples in phosphate buffer (1 M; pH 6.3) to represent pH and buffering conditions of food products. All functional properties were measured on AIR samples from freeze dried and dehydrated aloe filets.

WRC was measured as water retained by the fibre-enriched material (Thibault, Lahaya, & Guillon, 1992). Samples (2.5 g) were suspended (24 h) in phosphate buffer (50 ml) and centrifuged (15,000 rpm; 15 min) with residual solids in the supernatant recovered by filtration (GF/C paper) and recombined with the pellet. The pellet was weighed ($P1$), and dried at 102 °C overnight. After cooling, the dry weight was determined ($P2$) and hence WRC as; $P1 - P2/(P2 - k)$, where $k = a(P1 - P2)$, with $a = 28 \times 10^{-3}$ g salt (phosphate)/ml.

SW was measured as bed volume after equilibration in excess solvent (Kuniak & Marchessault, 1972). Sample (0.05–5.0 g) was weighed into a graduated conical tube with an excess of buffer. The suspension was stirred and after equilibration (16 h) the volume was recorded and expressed as ml/g dry sample.

FAC was measured as oil retention capacity (Caprez, Arrigoni, Amadó, & Neukom, 1986). AIR samples (0.1–1 g) were mixed with sunflower oil (3–30 ml), left overnight at room temperature, centrifuged (15,000 rpm, 10 min), the excess supernatant was decanted, 3–30 ml and FAC expressed as g oil/g dry sample.

2.8. Colour

Colour was measured using a Minolta C.M. 2002 spectrocolorimeter with specular component included, C illuminant, and an observer with an angle of 2°, using CIEL$^*a^*b^*$ co-ordinates (Trouvé, 1991).

Table 1
CIEL$^*a^*b^*$ colour co-ordinates and chrome (ΔC) values for freeze dried (FD) and dehydrated aloe vera parenchyma

	FD	Dehydrated filets					
		30D	40D	50D	60D	70D	80D
L^*	60.7 ± 0.0	58.1 ± 0.2	57.4 ± 0.1	55.9 ± 0.0	57.9 ± 0.1	55.1 ± 0.0	57.1 ± 0.1
a^*	−0.8 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	−0.3 ± 0.1	−0.6 ± 0.1	−0.5 ± 0.1	−0.5 ± 0.0
b^*	2.2 ± 0.0	6.5 ± 0.1	7.0 ± 0.0	6.4 ± 0.0	6.9 ± 0.1	6.2 ± 0.1	7.5 ± 0.0
ΔC[a]	–	3.97 ± 0.1	4.99 ± 0.0	4.77 ± 0.1	4.79 ± 0.2	4.80 ± 0.1	5.12 ± 0.0

[a] Chrome (ΔC) values were calculated as: $\Delta C = [(a^*_{\text{dried sample}} - a^*_{\text{freeze dried}})^2 + (b^*_{\text{dried sample}} - b^*_{\text{freeze dried}})^2]^{1/2}$.

2.9. 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.

3. Results and discussion

3.1. Dehydration of aloe vera parenchyma

Air drying temperature had an important influence on the drying rate (Fig. 1). Thus, at 30 °C over 6000 s were needed to reach a moisture content of 20 g water/g d.m., whereas the same water content could be reached in 3000 s drying at 50 °C, and in only 1800 s when drying at 70 °C.

In general, an increase in the dehydration temperature promotes an increase in the drying rate (Rocha, Lebert, & Marty-Audoin, 1992). However, case-hardening effects have been observed for several vegetable products. This effect hinders the water release and slows down the drying rate, thus, dehydration performed at higher temperature do not promote any further increase in the drying rate (Simal et al., 1996; Torringa, Nijhuis, & Bartlets, 1993).

Case-hardening effects in aloe vera parenchyma were detected when dehydration was performed above 70 °C. As it can be observed in Fig. 1, the dehydration curves obtained for 70D and 80D samples were practically overlapping (Fig. 1) suggesting that even if dehydration had been performed above 80 °C no significant decrease in the drying rate would probably be observed.

Table 2
Chemical characterisation of fresh aloe vera parenchyma AIR (μg/mg AIR)

Protein	151.4 ± 8.7
Lignin	26.1 ± 1.3
Ashes	104.4 ± 6.8
Rhamnose	4.56 ± 0.21
Fucose	4.87 ± 0.15
Arabinose	9.54 ± 0.32
Xylose	25.87 ± 2.11
Mannose	264.43 ± 5.14
Galactose	39.65 ± 1.12
Glucose	156.37 ± 3.15
Glucose (1 M)[a]	(57.32 ± 1.25)
Uronic acids	129.37 ± 2.31
Total carbohydrates	633.7 ± 3.8

[a] Numbers in brackets represent the composition of neutral sugars as determined by 1 M sulphuric acid hydrolysis.

3.2. Browning development during dehydration

Although browning development might not be related to any substantial modification of polysaccharides from aloe vera parenchyma, undoubtedly browning would influence the organoleptic properties of dried filets and would limit their potential applications.

Therefore, this effect was measured through the CIEL$^*a^*b^*$ colour co-ordinates and through the chrome parameter (Table 1). A freeze dried sample of aloe parenchyma which did not show any apparent sign of browning was taken as a reference. A slight browning development was a general feature of all the dehydrated aloe vera filets. The lowest browning development was observed for 30D sample. Slightly higher chrome values were exhibited by 40D, 50D, 60D and 70D samples, although no significant differences ($p > 0.05$) were observed among them. On the contrary, a relatively important increase in browning development was observed for 80D sample.

3.3. Alcohol insoluble residue from aloe vera parenchyma

The bulk of polysaccharides from the aloe parenchyma remained insoluble in 85% ethanol (AIR). On dry matter basis, the AIR represented over 60% of the parenchyma. The protein, lignin, ash and total carbohydrate of the AIR were determined and the results are shown in Table 2. Protein accounted for 15.1% of the AIR, although the bulk of this protein would probably be of intracellular origin since cell wall protein has been shown to account for less than 10% of the dry weight of cell walls (Selvendran & O'Neill, 1987). Ash represented approximately 10.4% of AIR, whereas lignin accounted for less than 3%, indicating the absence of secondary walls in the parenchymatous tissue of the parenchyma. Polysaccharides, accounting for 63% of the AIR, were the predominant fraction of the aloe vera parenchyma.

The presence of the bioactive polymer acemannan could be inferred from the occurrence of large amounts of

Table 3
Carbohydrate composition of the isolated and purified acemannan from aloe vera parenchyma dried at different temperatures (μg sugar/mg dry matter of aloe filets)

Sugar	FD[a]	Dehydrated filets					
		30D	40D	50D	60D	70D	80D
Rhamnose	0.33	0.30	0.35	0.26	0.25	0.29	0.22
Fucose	0.20	0.22	0.20	0.18	0.19	0.21	0.11
Arabinose	0.62	0.36	0.26	0.30	0.22	0.30	0.13
Xilose	1.57	0.82	1.80	1.44	0.82	1.03	0.56
Mannose	220.74	195.92	189.96	191.09	189.55	175.70	164.88
Galactose	12.02	10.04	11.34	10.03	9.38	4.72	3.54
Glucose	28.06	27.85	23.13	25.14	22.83	22.85	21.14
Uronic acid	4.38	3.11	3.57	3.05	2.01	1.91	1.69
Total	267.92	238.62	230.61	231.50	225.25	207.01	192.28

[a] Isolated and purified acemannan from a freeze dried sample of aloe vera parenchyma.

Table 4
Glycosidic linkage analysis and average molecular weights (MW) from purified acemannan-containing fractions from freeze dried and dehydrated aloe samples

	FD[a]	Dehydrated filets					
		30D	40D	50D	60D	70D	80D
Rhamnose							
1,2	0.2	0.1	0.2	0.2	0.1	0.2	0.1
Fucose							
Terminal	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Arabinose							
Terminal-f	0.3	0.3	0.3	0.4	0.4	0.3	0.4
1,5	0.7	0.6	0.7	0.7	0.5	0.6	0.6
1,3,5	0.4	0.4	0.4	0.3	0.4	0.3	0.3
Xylose							
Terminal	0.3	0.2	0.3	0.3	0.3	0.2	0.2
1,4	0.9	0.7	0.8	0.9	0.8	0.8	0.9
Mannose							
Terminal	0.36	0.42	0.33	0.31	0.25	0.28	0.27
1,4	71.42	71.66	72.36	71.49	72.65	74.55	75.89
1,6	0.66	0.58	0.71	0.69	0.73	0.81	0.80
1,3,4	2.14	2.06	2.03	1.94	1.87	1.28	1.12
1,4,6	2.45	2.54	2.33	2.29	1.97	1.63	1.65
1,3,4,6	0.24	0.21	0.20	0.16	0.17	0.11	0.13
Galactose							
Terminal	3.0	2.9	2.6	2.8	1.8	1.7	1.5
1,6	0.3	0.3	0.3	0.2	0.2	0.2	0.2
1,3,4	1.2	1.2	1.2	1.2	0.9	0.6	0.7
1,3,6	1.1	1.1	1.1	1.2	0.8	0.7	0.5
1,4,6	0.5	0.5	0.4	0.3	0.2	0.3	0.3
Glucose							
1,4	10.4	9.5	10.1	9.9	11.0	10.8	10.2
1,3,4	0.5	0.6	0.5	0.5	0.6	0.7	0.5
1,4,6	2.4	2.3	1.7	2.4	2.5	1.7	1.9
Glucitol	0.3	0.2	0.3	0.1	0.2	0.2	0.1
MW (kDa)	45	41	46	48	57	75	81

[a] Isolated and purified acemannan from a freeze dried sample of aloe vera parenchyma.

mannose (Gowda, Neelisiddaiah, & Anjaneyalu, 1979; t'Hart et al., 1989). Although, a significant amount of non glucosic cellulose, that is glucose released using 1 M H_2SO_4, could also be part of the latter polymer (McAnalley, 1993).

Cell wall polysaccharides comprised mainly of pectic substances, cellulose, and also hemicelluloses. Pectic substances were the main type of cell wall polysaccharides present in the aloe vera parenchyma. This was deduced from the presence of large amounts of uronic acids (galacturonic acid) and galactose, and to a minor extent from the occurrence of arabinose and rhamnose which are also characteristic of pectic polysaccharides (Aspinall, 1980).

Cellulose was the second cell wall polymer type in abundance. This fact could be inferred since most of the glucose could only be released using Saeman hydrolysis conditions (Saeman et al., 1954).

The presence of relatively small amounts of xylose and fucose was indicative of the occurrence of hemicellulosic xyloglucans. Non cellulosic glucose units could have also arisen from the xyloglucan backbone (O'Neill & Selvendran, 1985).

3.4. Effects of dehydration process on the composition of the main type of polysaccharides from aloe vera parenchyma

The most significant changes which affected the main polysaccharides from aloe vera parenchyma tissue during dehydration were evaluated. Thus, the bioactive polymer acemannan was purified and subjected to carbohydrate, glycosidic linkage and FTIR analysis. On the other hand, water insoluble cell wall polysaccharides from aloe vera parenchyma were also subjected to carbohydrate analysis. Purified acemannan and cell wall polymers from a freeze dried (FD) sample of aloe vera parenchyma were used as a reference.

3.4.1. Acemannan

Mannose accounted for over 82% of monomers of the purified acemannan from the FD parenchyma (Table 3). Glucose and galactose were the remaining monomers accounting for 10.5 and 4.5% of acemannan component sugars, respectively.

On dry weight basis, all dehydrated samples exhibited significant changes of the acemannan related sugars in comparison to those of the FD parenchyma (Table 3). However, minor differences were observed among 30D, 40D, 50D and 60D samples of aloe filets after being dehydrated ($p > 0.05$). In the latter samples, mannose losses ranged from 11.3 to 14.1% in comparison to the FD parenchyma composition. Such losses increased to 20.7% when parenchyma was dried at 70 °C (70D), and up to 25.3% of the mannose units when drying was performed at 80 °C (80D). Moreover, a significant decrease of galactose units was detected as temperature of dehydration increased, in particular for 70D and 80D samples ($p < 0.05$).

Table 5
Carbohydrate composition of the cell wall polysaccharides isolated from aloe vera parenchyma dried at different temperatures (μg sugar/mg dry matter of aloe filets)

Sugar	FD[a]	Dehydrated filets					
		30D	40D	50D	60D	70D	80D
Rhamnose	3.24	2.27	3.02	2.81	3.46	2.7	2.81
Fucose	3.08	2.43	3.24	2.92	3.29	2.75	2.54
Arabinose	5.02	4.21	4.75	4.97	4.97	4.81	4.54
Xylose	18.9	15.76	18.79	17.71	17.66	18.14	17.17
Mannose	26.84	25.76	26.19	24.52	23.81	23.44	20.03
Galactose	29.86	28.08	28.03	29.64	29.48	24.49	30.19
Glucose	119.83	108.05	113.35	108.65	115.61	112.91	104.33
Glucose(1 M)[b]	(11.83)	(10.91)	(10.21)	(10.31)	(10.96)	(10.42)	(10.96)
Uronic acid	77.33	52.43	70.02	63.02	55.13	50.44	34.34
Total	282.96	238.95	268.22	254.29	257.69	240.62	216.38

[a] Cell wall polysaccharides isolated from a freeze dried sample of aloe vera parenchyma.
[b] Numbers in brackets represent the composition of neutral sugars as determined by 1 M sulphuric acid hydrolysis.

In order to gain more insight into the latter observations glycosidic linkage analysis was performed on the purified acemannan fractions. The results of methylated polymers are shown in Table 4. Relative sugar mole ratios obtained from alditol acetates and partially methylated alditol acetates were in broad agreement. The presence of only a few types of methylated ethers from each sugar, and the virtual absence of unmethylated monomers in the hydrolysates of the methylated acemannan fractions were indications of a complete methylation.

Methylation analysis revealed important structural differences among the acemmanan polymers from the dehydrated filets. Thus, although (1,4)-linked mannosyl residues were predominant in all samples, important differences may affect to the degree of acetylation and, also, to the abundance of galactose side-chains of purified acemannan polymers from the different aloe samples. The distribution of acetyl groups and galactosyl units along the main chain can have a significant effect on the interactive properties of mannans (Dea & Clark, 1986).

A significant decrease of (1,3,4)-linked mannosyl residues was detected as dehydration temperature increased (Table 4). This might correspond to the deacetylation of the polysaccharide backbone, since acetyl groups have been detected at C3 of mannose units of acemannan (McAnalley, 1993). Moreover, this observation could be confirmed through FTIR analysis of purified acemannan fractions. FTIR spectra (not shown) revealed important decreases of the bands of 1740 and 1250 cm^{-1} which correspond to the C=O and C–O–C stretches of the acetyl groups.

Further, as dehydration temperature increased, an important decrease was observed in the ratio of (1,4,6)- to (1,4)-linked mannosyl residues. In addition, lower recoveries of galactosyl residues, in particular (1,3,4)-, (1,3,6)- and terminally linked, were detected. These observations suggest a lower degree of branching, in particular of galactose side-chains, since galactose units attached to C6 of mannose residues have been found in aloe acemannan (McAnalley, 1993).

A notable increase in the average molecular weight (MW) of purified acemannan fractions was observed as dehydration temperature increased (Table 4). Thus, an average MW of 45 kDa was estimated for acemannan from freeze dried filet. Similar values were determined for acemannan from samples dried at either 30, 40 or 50 °C, whereas significant higher values were estimated for acemannan polymers isolated from samples dehydrated at higher temperatures. The losses of galactosyl residues, together with the observed deacetylation process might have contributed to the interaction of different mannose chains by hydrogen bonding. This effect would result in mannose-rich chains of higher MW.

In overall, these chemical modifications may have an important influence on the physiological properties attributed to the bioactive polymer acemannan. Thus, further investigation need to be addressed towards the biological importance of such chemical variations.

3.4.2. Cell wall polysaccharides

On a dry weight basis, dehydrated filets exhibited an overall decrease in the total amount of cell wall sugars in comparison to the FD parenchyma (Table 5). The importance of such decrease depended on the temperature used during dehydration; thus, while less than 5.5% of total cell wall polysaccharides was lost for 40D parenchyma, losses reached over 23% of cell wall sugars for the 80D parenchyma. Interestingly, drying at relatively low temperature, e.g. 30 °C (30D), promoted a similar percentage of sugar losses (approximately 15%) to that obtained when dehydration was performed at 70 °C.

Pectic substances were the most affected type of polysaccharides. Samples 30D and 70D showed a decrease of 32% of total uronic acids content compared to the FD parenchyma; this loss reached 55% for the 80D sample. The

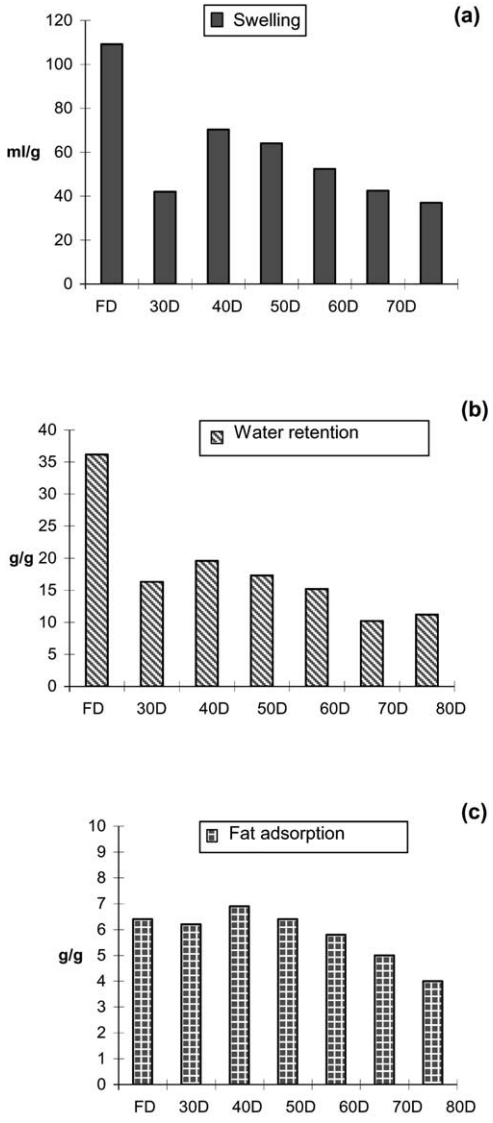

Fig. 2. Functional properties determined for AIR samples from freeze dried and dehydrated aloe vera filets. (a) Swelling (expressed as ml water per g of AIR), (b) water retention capacity (expressed as g water per g of AIR) and (c) fat adsorption capacity (expressed as ml oil per g of AIR).

lowest loss (less than 10%) was observed for dehydration at 40 °C. In all samples, galactose and arabinose were less affected than uronic acids. These results suggested that the pectic backbone underwent severe modifications during dehydration, whereas pectin side-chains were less affected. Degradation of pectic polysaccharides may have occurred due to β-elimination reaction promoted by heating, although temperatures above 50 °C might have also enhanced the activity of pectic polysaccharide degrading enzymes during the dehydration process (Femenia et al., 1997). Modification of pectic polysaccharides from aloe vera might be of especial significance since these type of polymers have been associated with anti-tumoural activity (Femenia et al., 1999b; Waldron & Selvendran, 1992).

Cellulose, the second type of cell wall polysaccharide in abundance, was not significantly lost during drying. This fact is in agreement with cellulose being the most resistant polymer forming the cell wall matrix (Franz & Blaschek, 1990).

Hemicelluloses, probably xyloglucans, underwent slight losses during drying in most of the samples, as indicated by small losses in xylose and, also, in non cellulosic glucose. Sample 30D exhibited the highest losses of xylose and fucose.

3.5. Effects of dehydration process on functional properties of aloe parenchyma AIRs

Functional properties are related to the chemical structure of the plant polysaccharides (Fleury & Lahaye, 1991; Robertson, 1987). Dehydration may alter the physico-chemical properties of the original products, modifying their functional properties (Thibault et al., 1992). Therefore, in order to evaluate possible changes in the structural arrangement of either storage or cell wall polysaccharides from aloe vera parenchyma, hydration-related properties such as swelling (SW) and water retention capacity (WRC), and fat adsorption capacity (FAC) were measured.

AIR from lyophilised aloe vera parenchyma was taken as a reference, since freeze drying preserves cell wall matrix structure, whereas heat drying may promote a breakdown of the cell wall polysaccharides network (Cohen & Yang, 1995) or produce dehydrated material which will not rehydrate. However, it should also be taken into consideration that during the freeze drying process, the removal of ice crystals retains a matrix of honeycomb-type structure which tends to rehydrate rapidly and more completely (Cohen & Yang, 1995; Holloway & Greig, 1984). This fact may explain the higher SW and WRC values exhibited by the freeze dried filets in comparison to the dehydrated samples (Fig. 2a and b).

Nevertheless, important differences either for SW and/or WRC were observed among the different dehydrated samples. While SW values underwent a dramatic increase from 30D to 40D sample, a gradual decrease was detected from the 50D to 80D dehydrated parenchyma.

WRC values were significantly lower than values detected for SW. However, WRC exhibited a similar trend to SW, a marked increase from 30D to 40D samples, followed by a decrease up to 70D sample.

Fleury and Lahaye (1991) observed a similar increase from 30 to 40 °C of the SW and WRC properties of a fibrous fraction of some algae. Furthermore, drying performed at 75 °C caused a significant decrease of hydration properties of cauliflower fibre in comparison to the same properties when cauliflower fibre was dehydrated at 40 °C (Femenia et al., 1997). In general, these results are in agreement with those observed for aloe vera dehydrated filets.

Interestingly, most of dehydrated samples exhibited similar FAC values than those determined for FD sample (Fig. 2c). Nevertheless, FAC values detected for dried samples followed a similar trend to hydration properties. It

has been reported that the presence of lignin might play some role in the oil adsorption (Thibault et al., 1992), but the FAC values measured for aloe vera parenchyma do not support such hypothesis.

It should be pointed out that the functional properties exhibited, not only by FD sample but also by aloe parenchyma dehydrated at temperatures between 40 and 60 °C, were significantly higher than the maximum values reported for fruit and vegetables (Thibault et al., 1992). Thus, the high affinity of aloe vera parenchyma to retain water and oil may explain its widespread use in cosmetics. Moreover, the capacity to bind organic molecules might play an important role in the reported capacity of aloe vera to lower the levels of cholesterol, carcinogens and other toxic compounds.

4. Conclusions

The importance of the physico-chemical modifications detected in dehydrated aloe vera parenchyma depended on the temperature used during the drying process. Regarding the chemical composition, the bioactive polysaccharide acemannan underwent similar losses of mannosyl residues when dehydration was performed between 30 and 60 °C, above the latter temperature, losses increased significantly. Important losses of galactosyl and acetyl residues were also detected through methylation analysis, these effects could be responsible for the increase in the average molecular weight of acemannan. Further, the structural modifications detected on the acemannan polymers could be responsible for the important changes observed in the functional properties of dehydrated filets. Deacetylation and reduction of galactosyl residues might have influenced the interaction between mannose chains affecting their binding capacity. The physico-chemical alterations of the main type of polysaccharides from aloe vera parenchyma observed during dehydration may have important implications on the physiological activities attributed to the aloe vera plant. Therefore, further studies on the biological significance of these modifications are needed.

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