Carbohydrate Polymers 94 (2013) 511–519

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# NMR and rheological study of *Aloe barbadensis* partially acetylated glucomannan

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ARTICLE INFO

*Article history:*
Received 15 September 2012
Received in revised form
20 December 2012
Accepted 13 January 2013
Available online 23 January 2013

*Keywords:*
*Aloe barbadensis*
Partially acetylated glucomannan
Chemical structure
Rheology behavior
*Aloe* polysaccharide
*Aloe* pulp

ABSTRACT

The structural and rheological properties of the *Aloe* extract (AE) and the polysaccharidic fraction (PF) obtained from the leaves pulp of *Aloe barbadensis* Miller were investigated. Structural analyses carried out by composition, methylation analysis and NMR spectroscopy showed that PF is mainly constituted by a partially acetylated 4-linked β-D-glucomannan. The acetyl groups are located at C-2, C-2 and C-3, C-3 and/or C-6. The acetylation pattern of this type of polysaccharide was for the first time established using bidimensional NMR analyses. AE and PF aqueous solutions at 25 °C showed a non-Newtonian behavior (with pseudoplastic characteristics), however PF showed higher apparent viscosity than AE. Dynamic oscillatory analyses showed that both samples, at the same concentration, behaved as a concentrated solution. PF presented higher values of $G'$ compared with those of AE and this behavior could be consequence of its higher content in partially acetylated glucomannan.



## 1. Introduction

Plants from genus Aloe, as *Aloe barbadensis* Miller (*Aloe vera* L.), are adapted to grow in areas of low water availability and are characterized by possessing a large water storage tissue (Burdock, 1997; Ni, Turner, Yates, & Tizard, 2004). The inner central area of the leaves (leaf pulp) contains parenchymal cells that produce a clear slightly viscous (mucilaginous) fluid known as *Aloe* pulp and several other terminologies as for example inner gel, leaf parenchyma (Ni et al., 2004) and inner leaf filet (Williams et al., 2010).

Glucomannans are the mayor polymeric constituents present in *Aloe* pulp and are mainly build up of 4-linked β-D-mannopyranosyl units besides minor amounts of 4-linked β-D-glucopyranosyl units. Differences in the mannose to glucose ratio (2.8–1 to 22.0–1), acetyl groups content (1.1–25%) and position of this substituent along the mannose backbone have been reported (Chow, Williamson, Yates, & Goux, 2005; Gowda, Neelisiddaiah, & Anjaneyalu, 1979; Gowda, 1980; Mandal & Das, 1980b; Manna & McAnalley, 1993; Paulsen, Fagerheim, & Øverbye, 1978; Wozniewski, Blaschek, & Franz, 1990). The presence of side chains of α-galactose (Chow et al., 2005), mannose (Mandal & Das, 1980b), and α-D-galactose and α-L-arabinose residues (Simões, Nunes, Domingues, Coimbra, & Domingues, 2012) as well as differences in molecular weight have also been reported (Gowda et al., 1979; Mandal & Das, 1980b; Reynolds & Dweck, 1999; Wozniewski et al., 1990). Additionally, other polysaccharides have been isolated from *Aloe* pulp such as galactan, arabinan, pectin (Mandal & Das, 1980a, 1980b), acidic arabinogalactan (Wozniewski et al., 1990) and arabinorhamnogalactan (Mabusela, Stephen, & Botha, 1990).

Many biological activities, including anti-viral, anti-bacterial, laxative, protection against radiation, anti-inflammation, antioxidant and immunostimulation have been attributed to this plant, in particular, its polysaccharides (Chun-hui, Chang-hai, Zhi-liang, & Yi, 2007; Ni et al., 2004; Reynolds & Dweck, 1999). *Aloe* extract has been used topically as healing agent for burns, dermal ulcer, wounds and frostbite (Boudreau, Olson, Pogribna, Pogribny, & Beland, 2005; Somboonwong, Jariyapongskul, Thanamittramanee, & Patumraj, 2000; Visuthikosol, Sukwanarat, Chowchuen, Sriuraitana, & Boonpucknavig, 1995).

The rheological properties of the aqueous fluid from the pulp of *A. barbadensis* Miller grown in Negev region of Israel (Yaron, 1993) and Coquimbo, Chile (Opazo-Navarrete, Tabilo-Munizaga, Vega-Gálvez, Miranda, & Pérez-Won, 2012) were investigated. Yaron (1993) proposed that the fresh viscous fluid from *Aloe* presents pseudoplastic behavior, however Newtonian flow properties were obtained after storage of the mucilage at room temperature or incubation at 40 °C for 48 h. Opazo-Navarrete et al. (2012) reported that *Aloe* solution behaves as a solid ($G' > G''$) during storage at 4 °C.

The products obtained from *A. barbadensis* are frequently submitted to different types of processing, e.g. heating or dehydration, which may irreversible modified characteristics of the polysaccharide structures such as degree of acetylation, molecular weight and removal of side chains. These modifications could originate changes

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http://dx.doi.org/10.1016/j.carbpol.2013.01.020

**Table 1**
Yields, chemical analyses and monosaccharide composition of the *Aloe* extract (AE) and polysaccharidic fraction (PF) obtained from *Aloe barbadensis* Miller.

| | Sample[a] | |
|---|---|---|
| | AE | PF |
| Yield (%)[b] | 61.8 | 23.4 |
| Carbohydrate (%)[c] | 79.8 | 79.2 |
| Protein (%)[c] | 5.7 | 1.3 |
| Acetyl (%)[c] | 9.2 | 18.4 |
| Monosaccharides (mol%)[d] | | |
| Rha | 3.5 | tr.[e] |
| Ara | 2.6 | tr. |
| Man | 28.7 | 94.2 |
| Glc | 60.7 | 3.9 |
| UA | 4.5 | 1.9 |

[a] Samples defined in text.
[b] Yield based on freeze-dried leaf pulp.
[c] Carbohydrate, protein and acetyl are given in percentage of samples AE and FP.
[d] Rha corresponds to rhamnose, Ara to arabinose, Man to mannose, Glc to glucose and UA to uronic acid.
[e] Percentages less than 1.5% are considered as traces.

in the biological activities and rheological behavior of these polymers (Femenia, García-Pascual, Simal, & Rosselló, 2003).

The aim of this study was to determine the chemical structure and rheological behavior of the pulp extract and polysaccharidic fraction obtained from the leaves of *A. barbadensis* Miller cultivated in the southern region of Brazil.

## 2. Materials and methods

### 2.1. Isolation of Aloe pulp extract (AE) and the polysaccharidic fraction (PF)

Leaves of *A. barbadensis* (10 kg) were collected from the organic farm Naturama Sucos Integrais do Brasil (Santa Catarina State, southern region of Brazil). After removing the bark and the exudate the inner central area of the leaves (leaf pulp) was crushed and centrifuged at 9800 × *g*, during 30 min, at 25 °C. The resulting supernatant was freeze-dried to afford the crude *Aloe* pulp extract AE. To obtain the PF fraction, the supernatant was submitted to EtOH precipitation (6 vol.) and maintained for 24 h, at 4 °C. The precipitate, obtained by centrifugation, was dissolved with water and dialyzed against distilled water. After a second EtOH precipitation and centrifugation the precipitate was dried in an oven at 40 °C during 24 h and milled (particle size of 200 mesh) to give the polysaccharidic fraction, PF (Table 1).

### 2.2. General procedures

Protein was determined according to the method of Bradford (1976). Total carbohydrate was determined according to the phenol–sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and reducing sugar by the colorimetric method of Miller (1959). Uronic acid content was estimated through the *m*-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991), using glucuronic acid as a standard. Acetyl groups were quantified according to the colorimetric method of Hestrin (1949). The degree of substitution (DS) of the mannose residues by acetyl groups was calculated using the formula reported by Xu et al. (2010).

### 2.3. Monosaccharide composition and methylation analyses

For the monosaccharide composition fractions were hydrolyzed with 2 M trifluoroacetic acid (TFA) for 3 h at 120 °C, followed by sodium borohydride ($NaBH_4$) reduction (Wolfrom & Thompson, 1963a), and acetylation with acetic anhydride for 1 h at 120 °C (Wolfrom & Thompson, 1963b). The resulting alditol acetates were analyzed by GC–MS and identified by their typical electron-impact breakdown profiles and retention times (Jansson, Kenne, Liedgren, Lindberg, & Lönngren, 1976). Methylation analysis was carried out as described by Ciucanu and Kerek (1984). Briefly, the dry sample (15 mg) was dissolved in $Me_2SO$ (1 mL), and powdered NaOH (30 mg) was added. After 30 min under magnetic stirring at 25 °C, $CH_3I$ (0.1 mL) was added, and the reaction was allowed to proceed for 30 min. Except by the $Me_2SO$ addition, the above mentioned process was repeated twice and the reaction was interrupted by addition of water (2 mL) and neutralized with 50% aq. AcOH. The methylation products were dialyzed against distilled water, freeze-dried and submitted to more two steps of methylation in the same way as described above. The resulting partially *O*-methylated alditol acetates were analyzed by GC–MS and identified by their typical electron-impact breakdown profiles and retention times (Jansson et al., 1976).

GC–MS analyses were performed using a Varian 3800 chromatograph connected to a Varian Saturn 2000R ion-trap spectrometer, using He as carrier gas at a flow rate of 1 mL $min^{-1}$. A DB-225 capillary column (30 m × 0.25 mm i.d., J&W) was used applying a linear temperature gradient from 50 to 215 °C at 40 °C $min^{-1}$ for quantitative analysis of alditol acetates and partially *O*-methylated alditol acetates.

### 2.4. Nuclear magnetic resonance spectroscopy (NMR)

NMR spectroscopic analyses were carried out using a Bruker AVANCE™ III 400 NMR spectrometer (Bruker BioSpin Corporation, Rheinstetlen, Germany) equipped with a 5 mm inverse probe, at 65 °C. $^1H$, $^{13}C$ and $^{13}C$-DEPT acquisition parameters were previously reported (Ascêncio, Orsato, França, Duarte, & Noseda, 2006). 2D $^1H$, $^1H$ COSY, and $^1H$, $^{13}C$ HSQC experiments were carried out using the pulse programs supplied with the Bruker manual. Samples were solubilized in $D_2O$ at 30 and 15 mg $mL^{-1}$ for $^{13}C$ and $^1H$, respectively. Chemical shifts (in ppm) are expressed relative to an internal acetone standard at 30.2 and 2.224 ppm for $^{13}C$ and $^1H$ NMR spectra, respectively.

### 2.5. HPSEC/MALLS/RI analyses

High pressure size exclusion chromatography (HPSEC) analyses of AE and PF fractions were carried out on a Waters chromatograph equipped with four Ultrahydrogel columns (2000, 500, 250, 120, molecular weight range from $7 \times 10^6$ to $1 \times 10^2$ Da; Milford, MA, USA) connected in series, and attached to the multidetection system, which consisted of a Waters 2410 differential refractometer (RI) detector (Milford, MA, USA) and a Wyatt Technology Dawn F multiangle laser light scattering (MALLS) detector (Santa Barbara, CA, USA) adapted on-line. The mobile phase consisted of a 0.1 mol $L^{-1}$ $NaNO_2$ solution containing 0.5 g $L^{-1}$ $NaN_3$ at a flow rate of 0.6 mL $min^{-1}$. Samples were solubilized in 0.1 mol $L^{-1}$ $NaNO_2$ solution containing $NaN_3$ (0.5 g $L^{-1}$) overnight under magnetic stirring, at room temperature. The samples (1 mg $mL^{-1}$) previously filtered (0.22 μm, Millipore, Billerica, MA, USA), were injected in the equipment and analyzed at 25 °C. HPSEC–MALLS–RI data were collected and analyzed by a Wyatt Technology ASTRA program. The molar mass (Mw) of PF was determined according to Freitas, Busato, Mitchell, and Silveira (2011). The *dn*/*dc* value for the peak eluted at 38 min, was determined as 0.118 mL $g^{-1}$, using 0.1 M $NaNO_2$ as the solvent at 25 °C.

### 2.6. Preparation of samples for rheological analysis

AE and PF were solubilized in deionized water at concentrations of 0.1, 0.2 and 0.3 g $L^{-1}$ (w/w) under mechanical stirring for 12 h at 25 °C.

### 2.7. Rheological measurements

Rheological measurements were performed using a RheoStress 1 rheometer (Haake GmbH, Germany) coupled to a circulating Haake DC30 bath. All analyses were carried out at 25 °C using a PP35-Ti sensor (plate and plate geometry, diameter 35 mm).

Flow curves in the CR mode (controlled shear rate) were performed from 0.1 to 300 $s^{-1}$, during 300 s, where the coefficient of determination $R^2$ was used as a parameter for the choice of the adopted rheological model (Techawipharat, Suphantharika, & BeMiller, 2008). Mechanical responses of the samples were determined by subjecting them to dynamic frequency sweep (0.01–10 Hz) within the linear viscoelastic region (obtained by strain sweep tests at 1 Hz). The mechanical spectra were characterized by values of $G'$ and $G''$ (Pa) as a function of frequency ($f$). $G'$ is the storage modulus, related to the solid response of the material and $G''$ is the loss modulus, related to the fluid response of the material. "Creep and recovery" tests were carried out for 300 s to creep and 300 s to recovery at 25 °C, at 1 Pa tension. RheoWin 3 Data Manager and ORIGINPRO 8.0 softwares were used to analyze and process the rheological results, respectively. All experiments were performed at least in duplicate and the results are the average values.

## 3. Results and discussion

### 3.1. Yields and chemical analysis of AE and PF fractions

From the leaf pulp of *A. barbadensis* we isolated the crude extract (AE) that after ethanolic precipitations gave rise to the polysaccharidic fraction (PF). Table 1 shows yields, chemical analyses and monosaccharide composition of these fractions. The yields for AE and PF fractions were 61.8 and 23.4%, respectively on a dry weight basis. Based on the fresh leaf pulp AE yield was 0.33%. The *Aloe* pulp from *A. barbadensis* farmed in Texas (USA) (Waller, Pelley, & Strickland, 2004) presented a lower yield (0.11%) compared to that of AE fraction.

The sugar composition of AE showed glucose (60.7 mol%) and mannose (28.7 mol%) as major neutral monosaccharides while for PF the content of glucose decreased (60.7–3.9 mol%) and that of mannose increased (from 28.7 to 94.2 mol%). Thus the PF mannose:glucose molar ratio corresponds to 24:1. Different Man:Glc ratios have been reported for glucomannans isolated from *A. barbadensis*, such as 22:1 (Mandal & Das, 1980b), 19:1–4.5:1 (Gowda et al., 1979), and 15:1 (Chow et al., 2005).

The percentage of total uronic acid content was 4.5 and 1.5% for AE and PF, respectively. The presence of uronic acids together with traces percentages of arabinose and rhamnose are indicative of residual amounts of pectic polysaccharides in the PF fraction as was previously observed in the water-soluble extracts of *A. vera* leaf pulp (Femenia, Sánchez, Simal, & Rosselló, 1999; Ni et al., 2004).

The acetyl content of AE (9.2%) and PF (18.4%) corresponds to a degree of substitution (DS) of 0.38 and 0.84, respectively. The latter value is similar to those reported for the glucomannan isolated from *A. vera* (0.78) (Gowda et al., 1979) and the mannans from *A. saponaria* (0.87) and *A. vanbalenu* (0.81) (Gowda, 1980). Differently, the acetylated glucomannans isolated from *A. plicatilis* (Paulsen et al., 1978) and *A. arborescens* (Wozniewski et al., 1990) presented a relatively lower (0.67) and higher (1.3) DS, respectively.

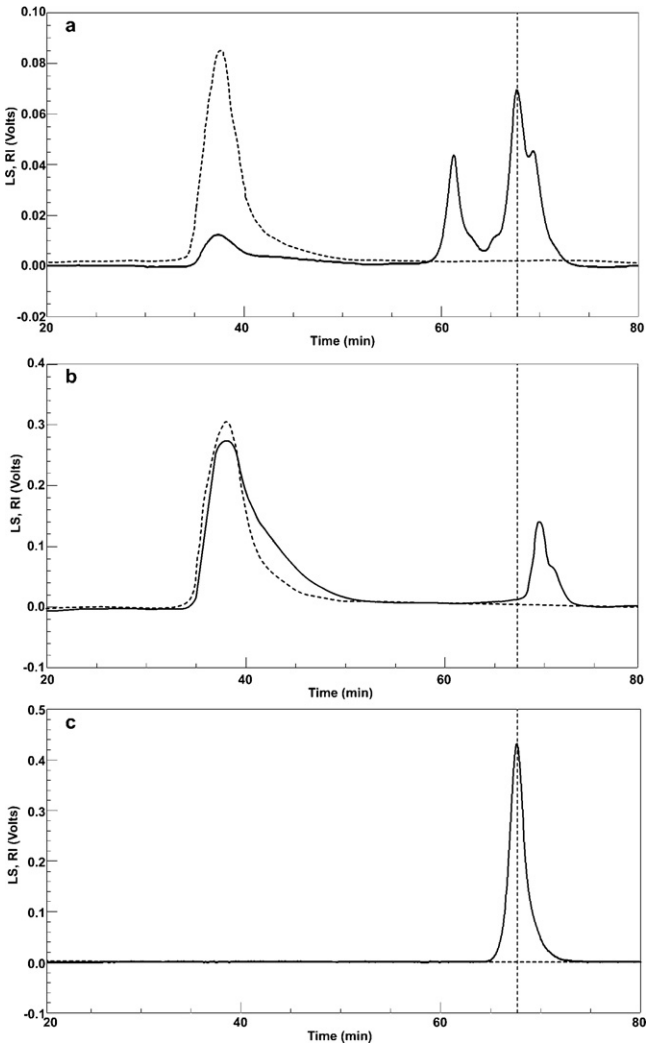


**Fig. 1.** Elution profile of AE (a) and PF (b) fractions from *Aloe barbadensis* and glucose standard (c) obtained by HPSEC–MALLS–RI. MALLS (- - -) and RI (—).

The differences in the chemical composition of glucomannans (Man:Glc ratio and acetyl content) isolated from *Aloe* species were attributed to several factors such as seasonal variations (Femenia et al., 1999; Wang & Strong, 1993), geographic locations (Mandal & Das, 1980a) and/or pulp processing (Femenia et al., 2003; Rodriguez-González et al., 2011) and extraction and purification methods (Alonso-Sande, Teijeiro-Osorio, Remuñán-Lopez, & Alonso, 2009; Gowda et al., 1979).

Methylation analysis of PF showed the presence of 2,3,6-tri-*O*-methylmannose (96%) and 2,3,6-tri-*O*-methylglucose (4.0%) in agreement with the presence of a linear polymer constituted by 4-linked mannopyrannose and glucopyranose residues.

### 3.2. HPSEC/MALLS/RI analyses

Fig. 1 illustrates the elution profiles of AE and PF fractions obtained by HPSEC/MALLS/RI as a function of elution time.

AE fraction from *A. barbadensis* Miller exhibited a multimodal elution profile by HPSEC–MALLS–RI. As observed in Fig. 1a this fraction exhibit heterogeneous and polydisperse profile. AE presents, as shown by the RI detector, three distinct peaks of major intensity at 38, 62 and 68 min. The RI detector main peak eluted at 68 min was assigned to glucose in agreement with the HPSEC profile of this sugar (Fig. 1c) and with the monosaccharide composition and NMR data (see later) of this fraction.

PF sample presents only the peak at 38 min (Fig. 1b) coincidental with that observed in AE (the peak at 70 min = total volume of our chromatographic system corresponds to salts). With MALLS, a large peak was detected at ~38 min for AE and PF, corresponding to a compound with high molecular weight, assigned to the partially acetylated glucomannan. The MW of this polysaccharide was determined as 1.2 MDa.

### 3.3. NMR analyses of AE and PF fractions

The $^{13}$C NMR spectrum of AE (Fig. 2a) showed two major signals in the anomeric region at 92.2 and 96.0 ppm, corresponding to C-1 of α- and β-anomers of glucose reducing units, respectively (Gorin & Mazurek, 1975). These results show the presence of free glucose units in the extract in accordance with the high reducing sugars content (data not shown) determined by the colorimetric method of Miller (1959). Differently, in the $^{13}$C NMR spectrum of PF (Fig. 2b) the aforementioned signals were not observed in agreement with HPSEC/MALLS/RI results (Fig. 1). The $^{1}$H and $^{13}$C NMR spectra of PF were partially assigned using 1D ($^{13}$C DEPT) and 2D ($^{1}$H/$^{1}$H COSY and $^{1}$H/$^{13}$C HSQC) experiments and literature data (Hannuksela & Penhoat, 2004; van Hazendonk, Reinerink, de Waard, & van Dam, 1996).

The PF $^{13}$C NMR spectrum shows an anomeric major signal at 100.2 ppm, attributed to C-1 of β-mannopyranosyl units. In the $^{1}$H NMR spectrum of PF (S1) the presence of anomeric signals at 4.75 and 4.71 ppm are in agreement with β-glycosidic linkage. From the HSQC experiment (Fig. 3), the correlations at 100.2/4.75, 4.71, 70.1/4.10, 71.5/3.79, 76.8/3.82, and 60.6/3.75, 3.88 ppm were assigned to C-1/H-1–C-4/H-4 and C-6/H-6a,b, respectively of the 4-linked β-D-mannopyranosyl units. The C-5 signal at 75.1 ppm shows correlations with H-5 protons at 3.61, 3.54, and 3.46 ppm corresponding to 4-linked β-D-mannopyranosyl units on different chemical environments and/or to acetylated (2- and 2,3-)-4-linked β-D-mannopyranosyl units. These assignments are in good agreement with the values reported for 4-linked β-D-mannopyranosyl units constituent of the glucomannan isolated from fiber flax (van Hazendonk et al., 1996) and the galactoglucomannan from spruce pulp (Hannuksela & Penhoat, 2004). The COSY spectrum of FP showed two sets of H-1/H-2 (4.75/4.10 and 4.71/4.10 ppm), H-2/H-3 (4.10/3.79 ppm), H-5/H-6b (3.61/3.88 ppm) and H-6a/H-6b (3.75/3.88 ppm) correlations. Some of these correlations are shown in Fig. 4.

In the $^{13}$C NMR spectrum of PF the signal of very low intensity at 102.5 ppm (correlated with the H-1 at 4.52 ppm in the HSQC experiment, Fig. 3) indicates the presence of 4-linked β-glucopyranosyl units. HSQC correlations at 73.1/3.36 and 77.1/3.66 ppm were attributed to C-2/H-2 and C-4/H-4, respectively of the aforementioned units (Capek, Alföldi, & Lišková, 2002; Hannuksela & Penhoat, 2004; Sims, Craik, & Bacic, 1997) in agreement with the presence of 4-linked glucopyranose residues as determined by methylation analysis.

Moreover, the $^{13}$C NMR spectrum of PF (Fig. 2b) presented signals of methyl carbons at 20.2 (major), 20.0 (medium) and 20.5 ppm (minor) and carboxyl carbons (173.8 and 173.0 ppm) of acetyl groups indicating that PF contains an acetylated mannan. The $^{1}$H NMR spectrum (S1) of this fraction presents four signals corresponding to methyl groups at 2.15 (major), 2.10 (medium), 2.19 and 2.18 (minors) ppm indicating that the acetyl groups are located on different positions. The HSQC spectrum (Fig. 3) showed that these methyl protons are correlated with the aforementioned methyl carbons of acetyl groups (20.2/2.15, 2.10, 20.0/2.18, 2.19 and 20.5/2.18, 2.19 ppm).

The HSQC spectrum of PF also showed correlations at 98.9/4.87 (C-1/H-1), 71.5/5.45 and 71.7/5.38 (C-2/H-2), 70.1/4.03 and 70.1/3.97 (C-3/H-3) attributed to 2-acetylated 4-linked β-mannopyranosyl units (Hannuksela & Penhoat, 2004; Teleman, Nordström, Tenkanen, Jacobs, & Dahlman, 2003; van Hazendonk et al., 1996). The presence of *O*-2 acetylated mannose residues on different chemical environments was confirmed by 2D COSY correlations H-1/H-2 (4.87/5.38 ppm) and two sets of H-2/H-3 (5.38/3.97 and 5.45/4.03 ppm). These assignments are in agreement with those reported for partially acetylated polysaccharides such as a glucomannan isolated from *Linum usitatissimum* L. (van Hazendonk et al., 1996) and a galactoglucomannan from spruce wood (Hannuksela & Penhoat, 2004).

In the PF $^{13}$C NMR spectrum the low intensity signal at 99.7 ppm was attributed to C-1 of 3-acetylated 4-linked β-mannopyranosyl units (Hannuksela & Penhoat, 2004; van Hazendonk et al., 1996). Furthermore the HSQC correlations at 68.7/4.18 and 73.6/5.03 ppm were assigned to C-2/H-2 and C-3/H-3, respectively of 3-acetylated mannopyranosyl units and agree with the downfield shift of H-2, H-3 and C-3 as well as to the upfield shift of C-2 when compared with the respective resonances of 4-linked β-mannopyranosyl units (van Hazendonk et al., 1996). For the aforementioned acetylated mannose residues the COSY spectrum (Fig. 4) showed two sets of H-3/H-4 correlations (5.03/4.04 and 5.03/4.08 ppm) probably indicating the different neighboring monosaccharide residues.

In the HSQC spectrum the anomeric correlations at 98.3/4.98 and 98.3/4.97 ppm together with others at 69.7/5.47, 69.7/5.40 (C-2/H-2), 71.9/5.17 (C-3/H-3) and 73.1/4.08 (C-4/H-4) showed that PF contains 2,3-diacetylated mannose residues. The COSY spectrum (Fig. 4) showed H-1/H-2, H-2/H-3 and H-3/H-4 correlations at 4.98/5.47, 5.47/5.17 and 5.17/4.08, respectively. Additional H-1/H-2 and H-2/H-3 correlations at 4.97/5.40 and 5.40/5.17 ppm, respectively were also seen in the COSY spectrum. These data are in accordance with the downfield shift of the geminal H-2 (1.37 to 1.30 ppm) and H-3 protons (1.38 ppm) and the vicinal protons (0.22–0.27 ppm for H-1 and 0.26 ppm for H-4), due to the presence of acetyl groups on both, O-2 and O-3 of 4-linked β-D-mannopyranosyl units.

The $^{13}$C DEPT spectrum (S2) of FP presents an inverted signal at 63.1 ppm ascribed to C-6 of 6-*O*-acetyl mannopyranosyl units. This resonance was shifted downfield (2.5 ppm) in comparison with that of unsubstituted C-6 (60.6 ppm, also DEPT inverted). In the HSQC spectrum the correlations at 63.1/4.28, 4.48 ppm corresponding to C-6/H6a,b confirm that PF also contains mannopyranosyl units C-6 substituted by acetyl groups. The C-6, H-6a and H-6b downfield shifts of the 6-acetylated mannopyranosyl units (2.5, 0.53 and 0.60 ppm, respectively) are in good accordance with those reported for 6-acetylated galactose residues in the disaccharide β-D-Gal*p*-(1→2)-D-Rha obtained from the exopolysaccharide of *Burkholderia cepacia* complex bacteria (2.6 for C-6 and 0.53 ppm for H-6) (Cescutti, Impallomeni, Garozzo, & Rizzo, 2011). For the glycolipids in equine erythrocyte membrane the H-6a and H-6b from O-6 acetylated galactose showed downfield shifts of 0.77 and 0.84 ppm, respectively (Yachida, Tsuchihashi, & Gasa, 1997). The carbon resonances of unsubstituted and acetylated C-6 had relative integrals of 1:0.58 showing that PF presents 37% of the total mannopyranosyl units bearing acetyl groups on O-6. Additionally in the HSQC spectrum of PF the correlation at 72.6/3.66 ppm was attributed to C-5/H-5 of 6-*O*-acetylated mannopyranosyl units. The C-5 upfield shift (2.5 ppm) and H-5 downfield shift (0.05–0.20 ppm) are in agreement with the effect of C-6 acetyl groups on the vicinal carbon and proton (Cescutti et al., 2011). The amount of 38% obtained from the relative integrals of C-5 carbon areas is in agreement with that determined from C-6 carbon areas (37%).

The acetyl groups' location on partially acetylated glucomannans isolated from *A. barbadensis* and *A. plicatilis* have been established based on methylation analysis (Manna & McAnalley, 1993; Paulsen et al., 1978) or as for *A. arborescens* with the additional use of $^{1}$H NMR spectroscopic analysis (Wozniewski et al.,

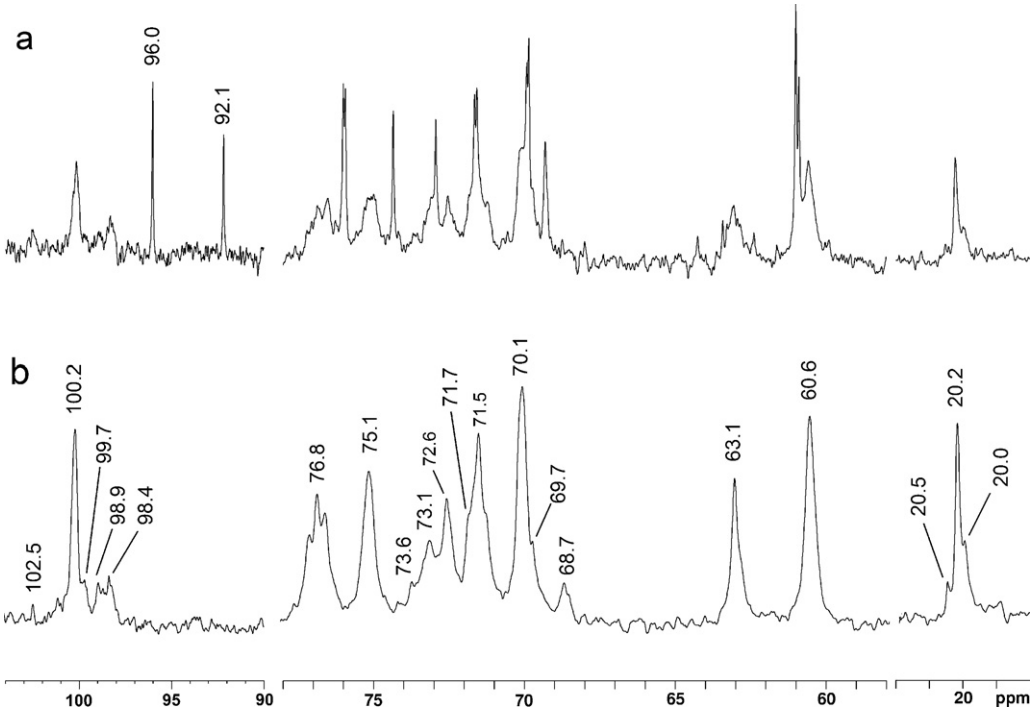


**Fig. 2.** $^{13}$C NMR spectra of AE (a) and PF (b) fractions from *Aloe barbadensis*.

1990). The acetylated polysaccharide from *A. barbadensis* presents acetyl groups on O-2,3 and O-6 of mannose residues in a ~1:1 ratio (Manna & McAnalley, 1993). Methylation analysis and $^{1}$H NMR data showed that the glucomannan obtained from *A. arborescens* presents similar proportions of acetylated mannose residues at positions O-2,3,6, O-2,3 and O-6 besides minor amounts of acetyl substitution at O-3,6 (Wozniewski et al., 1990). Differently the glucomannan isolated from *A. plicatilis* contains mannose and glucose residues substituted by acetyl groups at positions O-2,3,6, O-2,3 and O-3 (Manna & McAnalley, 1993; Paulsen et al., 1978).

Otherwise in the present work the pattern of acetylation of the glucomannan from *A. barbadensis* Miller was for the first time established using bidimensional NMR analyses. Therefore the NMR techniques together with chemical analyses demonstrate that PF

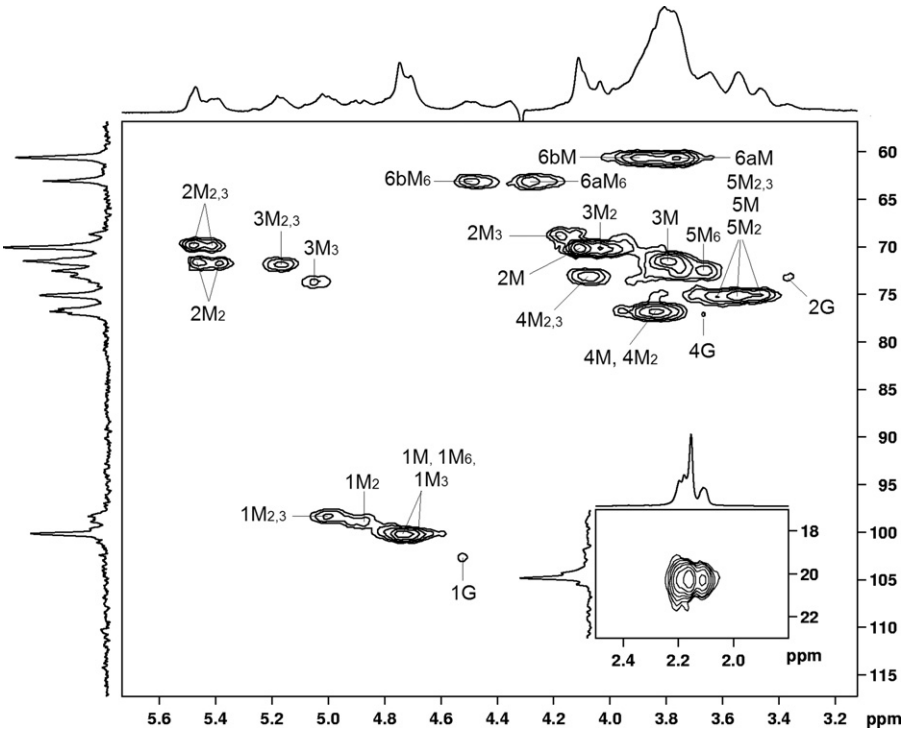


**Fig. 3.** HSQC spectrum of PF. M and G mean 4-linked mannopyranosyl and glucopyranosyl units, respectively. The first number corresponds to the correlated atom position. The acetyl positions are shown in subscript. For example $1M_{2,3}$ corresponds to C-1/H-1 correlation of 4-linked mannopyranosyl units bearing acetyl groups on O-2 and O-3. Insert: methyl correlations of acetyl groups.

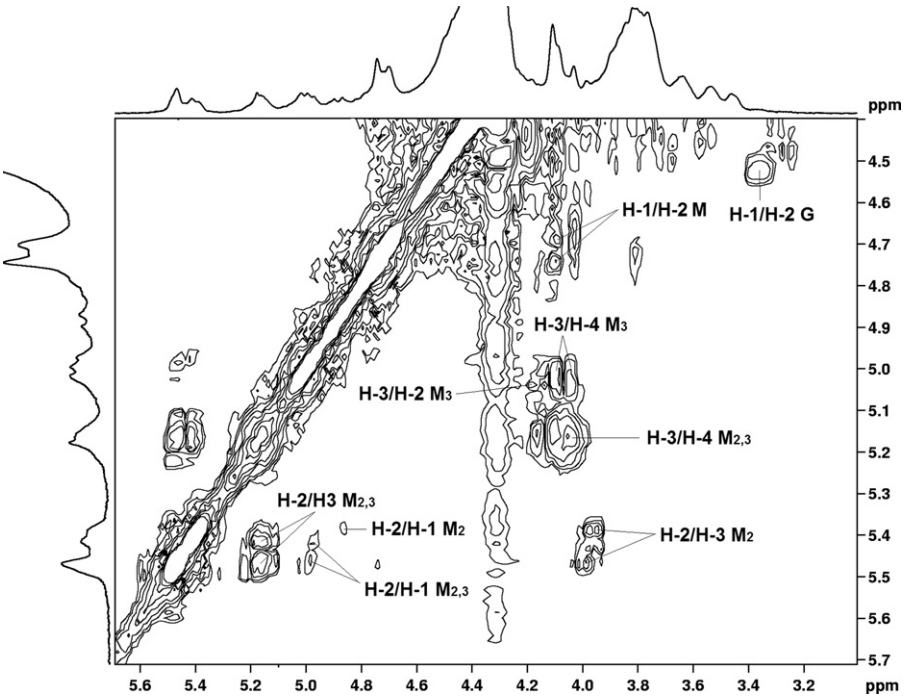


**Fig. 4.** COSY spectrum of PF. M and G mean 4-linked mannopyranosyl and glucopyranosyl units, respectively. The acetyl positions are shown in subscript.

consists of a partially acetylated 4-linked β-D-glucomannan with the acetyl groups located at O-2,3, O-2, O-3, and O-6 of mannopyranosyl units.

### 3.4. Flow behavior of aqueous solution of AE and PF

Together with the chemical structure study of *A. barbadensis* acetylated glucomannan, an additional investigation was performed with AE and PF in order to understand how composition and structure affect their physicochemical and rheological behavior.

The rheological properties of glucomannans from plant origin are still under study (Alonso-Sande et al., 2009; Alvarez-Manceñido, Mariana, & Martínez-Pacheco, 2008; Du, Li, Chen, & Li, 2012; Nishinari & Zhang, 2004; Williams et al., 2010; Yin, Zhang, Huang, & Nishinari, 2008). There are some parameters which affect the rheological behavior of glucomannans, such as acetylation degree, molecular weight, temperature, and polymer concentration. Gelation is favored when molecular weight, concentration or temperature increase and acetylation decreases (Alonso-Sande et al., 2009; Du et al., 2012; Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002).

Aqueous solutions of AE and PF (0.1, 0.2 and 0.3 g $L^{-1}$) were compared through their flow curves (Fig. 5). For both fractions, a non-Newtonian shear-thinning behavior was found, as previously reported for *A. barbadensis* leaf pulp polysaccharides from plants cultivated in Chile (Opazo-Navarrete et al., 2012), CA, USA (Ni et al., 2004) and Israel (Yaron, 1993). PF showed higher viscosity than AE at all the tested shear rates. At 0.01 $s^{-1}$ and concentration of 0.3 g $L^{-1}$, PF had an apparent viscosity of 300.0 mPa s, while AE showed a value of 44.8 mPa s (Fig. 5). For PF, mainly constituted by high molecular weight polysaccharides (as demonstrated in Fig. 1) and consequently subject to greater deformations, its solutions have higher levels of pseudoplasticity than those of AE.

Besides the influence of molecular mass on viscosity, some authors (Du et al., 2012; Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000; Pippen, McCready, & Owens, 1950; Sengkhamparn et al., 2010; Vriesmann, Amboni, & Petkowicz, 2011) suggested that the acetylation degree of polysaccharides also plays an important role in this rheological parameter by promoting hydrogen bonding and hydrophobic interaction. Du et al. (2012) proposed, by the rheological analysis of konjac acetylated glucomannan, that hydrophobic interaction was weakened while hydrogen bonding strengthened with increasing degree of acetylation. The higher viscosity obtained for PF that presents higher acetyl content (18.4%) when compared to that of AE (9.2%), could suggest the priority of hydrogen bonding and a delay in the gelation process (Huang et al., 2002).

Among the models used to describe the rheological behavior of natural and synthetic polymers, we tested those of Ostwald de Waele (Power Law), Herschel–Bulkley and Carreau-Yasuda for AE and PF samples. The best statistical parameters for adjusting the experimental data were calculated by the Ostwald de Waele model, $\eta = K \cdot \gamma^{n-1}$, where $\eta$ is the apparent viscosity (Pa s), $\gamma$ is the shear rate

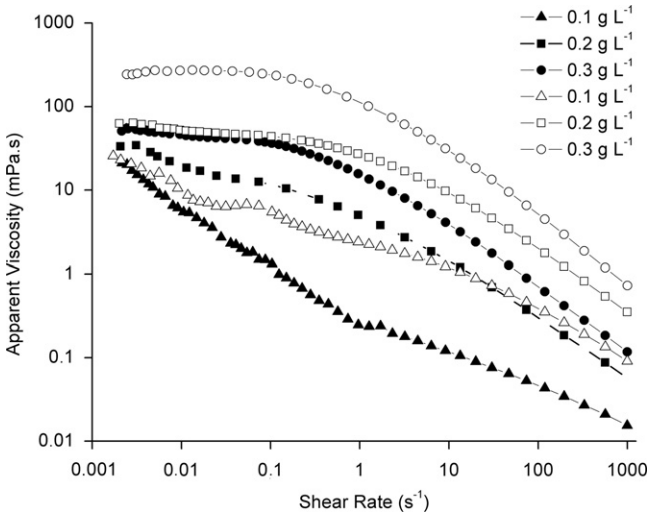


**Fig. 5.** Apparent viscosity as function of shear rate for *Aloe* extract, AE (▲, ■, ●) and polysaccharidic fraction, PF (△, □, ○) in aqueous solutions at concentrations of 0.1, 0.2 and 0.3 g $L^{-1}$, at 25 °C.

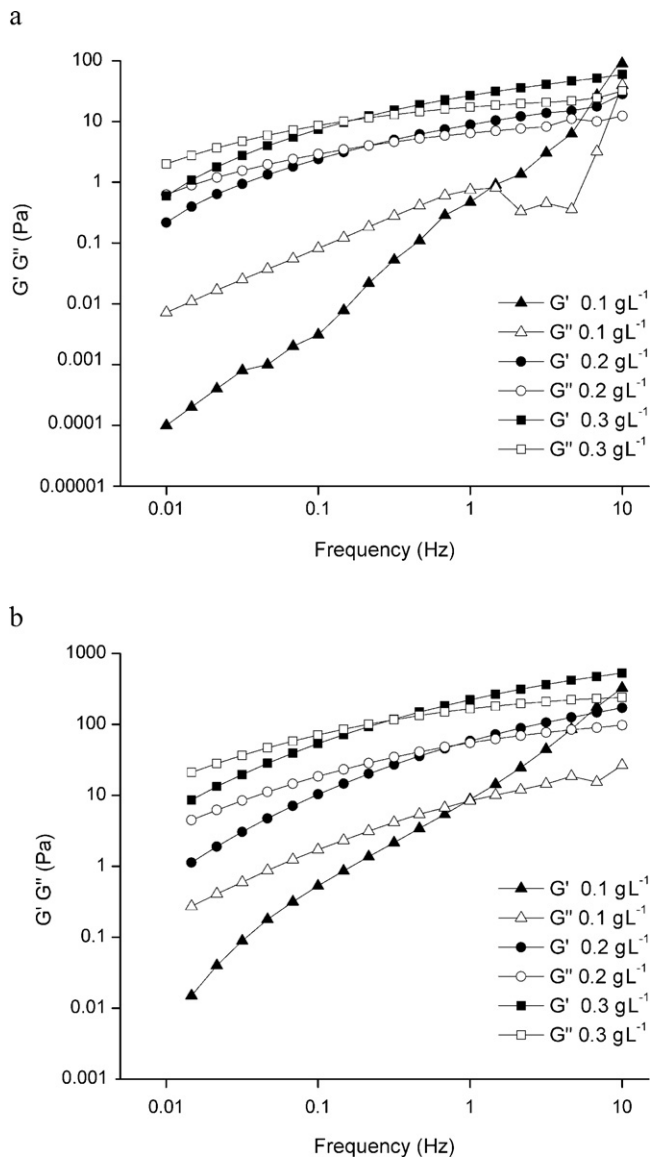


**Fig. 6.** Frequency sweeps of AE (a) and PF (b) in aqueous solutions, at concentrations of 0.1, 0.2 and 0.3 g $L^{-1}$, at 25 °C. $G'$, elastic modulus; $G''$, viscous modulus.

($s^{-1}$), $K$ is the consistency coefficient (Pa $s^n$) and $n$ the flow behavior index (dimensionless). The values of $K$ and $n$ at 0.3 g $L^{-1}$ were 4.15 Pa $s^n$ and 0.68 for AE and 14.17 Pa $s^n$ and 0.56 for PF, respectively. Both samples presented flow behavior index ($n$) values lower than one ($n < 1$) and the consistency coefficient ($K$) was greater than one ($K > 1$) for all the concentrations tested, confirming their shear-thinning behavior. However, it was noted that PF had a lower $n$ value, suggesting a more pseudoplastic behavior.

### 3.5. Viscoelastic behavior of aqueous solution of AE and PF

Polysaccharide solutions are viscoelastic materials that exhibit solid and liquid characteristics simultaneously (Bourbon et al., 2010), where $G'$ and $G''$ moduli refer to elastic and viscous response of a given material, respectively. It is possible to quantify the predominance of the solid or liquid character of a sample through dynamic measurements.

Frequency sweeps of AE and PF fractions (Fig. 6), showed frequency dependence of $G'$ and $G''$. It was found that both samples showed a typical concentrated solution behavior, with $G''$ higher than $G'$ at low frequencies. At 0.3 g $L^{-1}$ concentration they presented similar $G'$ and $G''$ crossover frequencies, 0.2 Hz for AE and 0.3 Hz for PF (Table 2). For both samples, the increase of concentration in the aqueous system caused an increase in the values of crossover frequency.

**Table 2**
The crossover frequency of $G'$ and $G''$ of *Aloe* extract (AE) and polysaccharidic fraction (PF) obtained from *Aloe barbadensis* Miller at different concentrations.

| Sample | Concentration (g $L^{-1}$) | $G' = G''$ (Pa)[a] | Frequency (Hz) |
|---|---|---|---|
| AE | 0.1 | 0.8 | 1.4 |
| | 0.2 | 4.0 | 0.2 |
| | 0.3 | 10.6 | 0.2 |
| PF | 0.1 | 8.2 | 1.0 |
| | 0.2 | 51.1 | 0.8 |
| | 0.3 | 114.4 | 0.3 |

[a] Values obtained in frequency from 0.01 to 10 Hz.

The crossover frequency provides a good indication of the viscoelastic behavior of a given material. The lower the crossover value is, greater is the elastic contribution (Ramachandran, Chen, & Etzler, 1999). $G'$ values at the crossover frequency for AE and PF, show a more elastic behavior at higher concentrations (0.3 g $L^{-1}$, Table 2). For PF fraction, $G'$ values were about 10 times higher at the same concentration than those determined for AE, indicating a higher viscoelastic characteristic of the former. Comparing the $G'$ values at the crossover frequency of PF aqueous solution at 0.3 g $L^{-1}$ (114.4 Pa at 0.3 Hz) and konjac partially acetylated glucomannan at 1 wt% (~3 Pa at 3 Hz) (Du et al., 2012), it can be inferred that PF even at a much lower concentration presented higher viscoelastic properties than konjac glucomannan.

PF contains a major high molecular weight polysaccharide, a partially acetylated glucomannan, stabilized by a mixture of hydrophobic interactions and hydrogen bonding between the polysaccharide chains. This feature is in agreement with the fact that the dispersions showed a typical concentrated solution behavior. The aforementioned interactions are related with the molecular structure and the presence of high acetyl content that determines the non-Newtonian flow behavior, as well as the creep analysis results (S3), where the characteristic of viscoelastic fluid is marked.

The study of Ni et al. (2004) found in the polysaccharidic fraction obtained from the leaf pulp of *A. barbadensis*, a glucomannan with percentages of 63 and 13% of mannose and glucose, respectively. The authors suggest that the glucomannan is the responsible for the viscoelastic property, in agreement with the rheological behavior observed for PF, a mannose-rich partially acetylated glucomannan.

## 4. Conclusions

The structural and rheological properties of the *Aloe* extract (AE) and the polysaccharidic fraction (PF) obtained from the leaf pulp of *A. barbadensis* Miller were investigated. Chemical, spectroscopic and HPSEC–MALLS–RI analyses carried out on the polysaccharidic fraction demonstrate that *A. barbadensis* produces a partially acetylated 4-linked β-D-glucomannan of high molecular weight. The acetyl groups are positioned at O-2 and O-3, O-2, O-3, and/or O-6 of mannopyranosyl units. The acetylation pattern of this type of polysaccharide was for the first time established using bidimensional NMR analyses. On the other hand the AE extract is composed by a high amount of free glucose (~61%) besides minor amounts of the aforementioned acetylated polysaccharide.

This study confirmed that the rheological response of AE and PF aqueous fractions, at 25 °C showed a pseudoplastic behavior in all tested concentrations, and the dynamic oscillatory assay demonstrated that both samples behaved as a typical concentrated solution. For PF, $G'$ values were about 10 times higher for the same concentration than those of AE, indicating higher viscoelastic

characteristic of the former. The elastic contribution after crossover obtained for PF at higher values of $G'$ compared to AE can be given by the higher content of the partially acetylated glucomannan present in PF.

## Acknowledgments

The authors thank the Brazilian agencies, CNPq, Fundação Araucária (PRONEX-Carboidratos), Rede Nanoglicobiotec-MCT/CNPq for financial support and Prof. Maria Rita Sierakowski for the use of the rheometer. J.L.S., M.E.D. and M.D.N. are Research Members of the National Research Council of Brazil (CNPq). L.H.C. acknowledges a doctoral scholarship from CNPq.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.01.020.

## References

Alonso-Sande, M., Teijeiro-Osorio, D., Remuñán-Lopez, C., & Alonso, M. J. (2009). Glucomannan, a promising polysaccharide for biopharmaceutical purposes. *European Journal of Pharmaceutics and Biopharmaceutics*, *72*, 543–562.

Alvarez-Manceñido, F., Mariana, L., & Martínez-Pacheco, R. (2008). Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*, 573–581.

Ascêncio, S. D., Orsato, A., França, R. A., Duarte, M. E. R., & Noseda, M. D. (2006). Complete $^{1}H$ and $^{13}C$ assignment of digeneaside, a low-molecular-mass carbohydrate produced by red seaweeds. *Carbohydrate Research*, *341*, 677–682.

Boudreau, M. D., Olson, G. R., Pogribna, M., Pogribny, I., & Beland, F. A. (2005). Consumption of *Aloe barbadensis* Miller (*Aloe vera*) induces hyperplasia in the colon of F-344 rats and B6C3F1 mice. *The FASEB Journal*, *19*, A450–A451.

Bourbon, A. I., Pinheiro, A. C., Ribeiro, C., Miranda, C., Maia, J. M., Teixeira, J. A., et al. (2010). Characterization of galactomannans extracted from seeds of *Gleditsia triacanthos* and *Sophora japonica* through shear and extensional rheology: Comparison with guar gum and locust bean gum. *Food Hydrocolloids*, *24*, 184–192.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.

Burdock, G. A. (1997). *Aloe extract (Aloe spp.). Encyclopedia of food and color additives* Boca Raton, FL: CRC Press., pp. 105–106

Capek, P., Alföldi, J., & Lišková, D. (2002). An acetylated galactoglucomannan from *Picea abiest* L. Karst. *Carbohydrate Research*, *337*, 1033–1037.

Cescutti, P., Impallomeni, G., Garozzo, D., & Rizzo, R. (2011). *O*-acetyl localization on Cepacian, the principal exopolisaccharide of *Burkhlderia cepacia* complex bacteria. *Carbohydrate Research*, *346*, 2905–2912.

Chow, J. T. N., Williamson, D. A., Yates, K. M., & Goux, W. J. (2005). Chemical characterization of the immunomodulating polysaccharide of *Aloe vera* L. *Carbohydrate Research*, *340*, 1131–1142.

Chun-hui, L., Chang-hai, W., Zhi-liang, X., & Yi, W. (2007). Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. *Process Biochemistry*, *42*, 961–970.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Du, X., Li, J., Chen, J., & Li, B. (2012). Effect of degree of deacetylation on physicochemical and gelation properties of konjac glucomannan. *Food Research International*, *46*, 270–278.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Femenia, A., García-Pascual, P., Simal, S., & Rosselló, C. (2003). Effects of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadensis* Miller. *Carbohydrate Polymers*, *51*, 397–405.

Femenia, A., Sánchez, E. S., Simal, S., & Rosselló, C. (1999). Compositional features of polysaccharides from *Aloe vera* (*Aloe barbadensis* Miller) plant tissues. *Carbohydrate Polymers*, *39*, 109–117.

Freitas, R. A., Busato, A. P., Mitchell, D. A., & Silveira, J. L. M. (2011). Degalactosylation of xyloglucan: Effect on aggregation and conformation, as determined by time dependent static light scattering, HPSEC–MALLS and viscosimetry. *Carbohydrate Polymers*, *83*, 1636–1642.

Filisetti-Cozzi, T. M. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, *197*, 157–162.

Gorin, P. A. J., & Mazurek, M. (1975). Further studies on the assignment of signals in $^{13}C$ magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry*, *53*, 1212–1222.

Gowda, D. C. (1980). Structural studies of polysaccharides from *Aloe saponaria* and *Aloe vanbalenii*. *Carbohydrate Research*, *83*, 402–405.

Gowda, D. C., Neelisiddaiah, B., & Anjaneyalu, Y. V. (1979). Structural studies of polysaccharides from *Aloe vera*. *Carbohydrate Research*, *72*, 201–205.

Hannuksela, T., & Penhoat, C. H. (2004). NMR structural determination of dissolved *O*-acetylated galactoglucomannan isolated from spruce thermomechanical pulp. *Carbohydrate Research*, *339*, 301–312.

Hestrin, S. (1949). The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. *Journal of Biological Chemistry*, *180*, 249–261.

Huang, L., Takahashi, R., Kobayashi, S., Kawase, T., & Nishinari, K. (2002). Gelation behavior of native and acetylated konjac glucomannan. *Biomacromolecules*, *3*, 1296–1303.

Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B., & Lönngren, L. (1976). A practical guide to the methylation analysis of carbohydrates. *Chemical Communication*, *8*, 1–74.

Mabusela, W. T., Stephen, A. M., & Botha, M. C. (1990). Carbohydrates polymers from *Aloe ferox* leaves. *Phytochemistry*, *29*, 3355–3558.

Manna, S., & McAnalley, B. (1993). Determination of the position of the *O*-acetyl group in a β-(1→4)-mannan (acemannan) from *Aloe barbadensis* Miller. *Carbohydrate Research*, *241*, 317–319.

Mandal, G., & Das, A. (1980a). Structure of the D-galactan isolated from *Aloe barbadensis* Miller. *Carbohydrate Research*, *86*, 247–257.

Mandal, G., & Das, A. (1980b). Structure of the glucomannan isolated from *Aloe barbadensis* Miller. *Carbohydrate Research*, *87*, 249–256.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*, 426–428.

Ni, Y., Turner, D., Yates, K. M., & Tizard, I. (2004). Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *International Immunopharmacology*, *4*, 1745–1755.

Nishinari, K., & Zhang, H. (2004). Recent advance in the understanding of heat set gelling polysaccharides. *Trends in Food Science Technology*, *15*, 305–312.

Oosterveld, A., Beldman, G., Searle-van Leeuwen, M. J. F., & Voragen, A. G. J. (2000). Effect of enzymatic deacetylation on gelation of sugar beet pectin in the presence of calcium. *Carbohydrate Polymers*, *43*, 249–256.

Opazo-Navarrete, M., Tabilo-Munizaga, G., Vega-Gálvez, A., Miranda, M., & Pérez-Won, M. (2012). Effects of high hydrostatic pressure (HHP) on the rheological properties of *Aloe vera* suspensions (*Aloe barbadensis* Miller). *Innovative Food Science and Emerging Technologies*, http://dx.doi.org/10.1016/j.ifset.2012.06.006

Paulsen, B. S., Fagerheim, E., & Øverbye, E. (1978). Structural studies of the polysaccharide from *Aloe plicatilis* Miller. *Carbohydrate Research*, *60*, 345–351.

Pippen, E. L., McCready, R. M., & Owens, H. S. (1950). Gelation properties of partially acetylated pectins. *Journal of American Chemistry Society*, *72*, 813–816.

Ramachandran, S., Chen, S., & Etzler, F. (1999). Rheological characterization of hydroxypropylcellulose gels. *Drug Development and Industrial Pharmacy*, *25*, 153–161.

Reynolds, T., & Dweck, A. C. (1999). *Aloe vera* leaf gel: A review update. *Journal of Ethnopharmacology*, *68*, 3–37.

Rodriguez-González, V. M., Femenia, A., González-Laredo, R. F., Rocha-Guzmán, N. E., Gallegos-Infante, J. A., Candelas-Cadillo, M. G., et al. (2011). Effects of pasteurization on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadensis* Miller. *Carbohydrate Polymers*, *86*, 1675–1683.

Sengkhamparn, N., Sagis, L. M. C., de Vries, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2010). Physicochemical properties of pectins from okra (*Abelmoschus esculentus* (L.) Moench). *Food Hydrocolloids*, *24*, 35–41.

Simões, J., Nunes, F., Domingues, M., Coimbra, P., & Domingues, M. A. M. R. (2012). Mass spectrometry characterization of an *Aloe vera* mannan presenting immunostimulatory activity. *Carbohydrate Polymers*, *90*, 229–236.

Sims, I. M., Craik, D. J., & Bacic, A. (1997). Structural characterization of galactoglucomannan secreted by suspension-cultured cells of *Nicotiana plumbaginifolia*. *Carbohydrate Research*, *303*, 79–92.

Somboonwong, J., Jariyapongskul, A., Thanamittramanee, S., & Patumraj, S. (2000). Therapeutic effects of *Aloe vera* on cutaneous micro-circulation and wound healing in second degree burn model in rats. *Journal of the Medical Association of Thailand*, *83*, 417–425.

Techawipharat, J., Suphantharika, M., & BeMiller, J. N. (2008). Effects of cellulose derivatives and carrageenans on the pasting, paste, and gel properties of rice starches. *Carbohydrate Polymers*, *73*, 417–426.

Teleman, A., Nordström, M., Tenkanen, M., Jacobs, A., & Dahlman, O. (2003). Isolation and characterization of *O*-acetylated glucomannans from aspen and birch wood. *Carbohydrate Research*, *338*, 525–534.

van Hazendonk, J. M., Reinerink, E. J. M., de Waard, P., & van Dam, J. E. G. (1996). Structural analysis of acetylated hemicellulose polysaccharides from fibre flax (*Linum usitatissimum* L.). *Carbohydrate Research*, *291*, 141–154.

Visuthikosol, V., Sukwanarat, Y., Chowchuen, B., Sriuraitana, S., & Boonpucknavig, V. (1995). Effect of *Aloe vera* gel to healing of burn wound a clinical and histological study. *Journal of the Medical Association of Thailand*, *78*, 403–409.

Vriesmann, L. C., Amboni, R. D. M. C., & Petkowicz, C. L. O. (2011). Cacao pod husks (*Theobroma cacao* L.): Composition and hot-water-soluble pectins. *Industrial Crops and Products*, *34*, 1173–1181.

Wang, Y. T., & Strong, K. J. (1993). Monitoring physical and chemical properties of freshly harvested field-grown *Aloe vera* leaves. A preliminary report. *Phytotherapy Research*, *7*, S1–S4.

Waller, T. A., Pelley, R. P., & Strickland, F. M. (2004). Industrial processing and quality control of *Aloe barbadensis* (Aloe vera) gel. In *Aloes—The genus Aloe*. Boca Raton: CRC press.

Williams, L. D., Burdock, G. A., Shin, E., Kim, S., Jo, T. H., Jones, K. N., et al. (2010). Safety studies conducted on a proprietary high-purity *Aloe vera* inner leaf fillet preparation, Qmatrix®. *Regulatory Toxicology and Pharmacology*, *57*, 90–98.

Wolfrom, M. L., & Thompson, A. (1963a). Reduction with sodium borohydride. In R. L. Whistler, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (pp. 65–68). New York: Academic Press.

Wolfrom, M. L., & Thompson, A. (1963b). Acetylation. In R. L. Whistler, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (pp. 211–215). New York: Academic Press.

Wozniewski, T., Blaschek, W., & Franz, G. (1990). Isolation and structural analysis of a glucomannan from the leaves of *Aloe arborescens* var. Miller. *Carbohydrate Research*, *198*, 387–391.

Xu, C., Leppänen, A. S., Eklund, P., Holmlund, P., Sjöholm, R., Sundberg, K., et al. (2010). Acetylation and characterization of spruce (*Picea abies*) galactomannans. *Carbohydrate Research*, *345*, 810–816.

Yachida, Y., Tsuchihashi, K., & Gasa, S. (1997). Novel di-*O*-acetylated GM3s from equine erythrocytes, one containing 4,9-di-*O*-acetyl-*N*-glycolylneuraminic acid and another containing 4-*O*-acetyl-*N*-glycolylneuraminic acid and 6-*O*-acetyl-D-galactose. *Carbohydrate Research*, *298*, 201–212.

Yaron, A. (1993). Characterization of *Aloe vera* gel before and after autodegradation, and stabilization of the natural fresh gel. *Phytotherapy Research*, *7*, S11–S13.

Yin, W. C., Zhang, H. B., Huang, L., & Nishinari, K. (2008). Effects of the lyotropic series salts on the gelation of konjac glucomannan in aqueous solutions. *Carbohydrate Polymers*, *74*, 68–78.