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Influence of water deficit on the main polysaccharides and the rheological properties of Aloe vera (*Aloe barbadensis* Miller) mucilage

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ABSTRACT

The aim of this study was to evaluate the effect of water deficit on the composition of the main polysaccharides and rheological behaviour from *Aloe vera* (*Aloe barbadensis* Miller) mucilage. In particular, plants with 0 (D0), 40 (D40) and 60% (D60) water deficits were used. Water-soluble polysaccharides were isolated and subjected to carbohydrate and glycosidic linkage analysis. The steady-shear and linear oscillatory flows were studied, not only in fresh but also in reconstituted mucilages. Acemannan and pectic substances were the predominant polysaccharides in the *Aloe vera* mucilage, being the bioactive polymer acemannan the most affected by water deficit. Thus, increasing the water deficit by up to 60% promoted a mannose decrease of 41%, although a significant increase in its average molecular weight, from 54 to 98 kDa, was detected. Interestingly, acemannan did not undergo deacetylation as a consequence of the water deficits applied. All *Aloe vera* mucilages, either fresh or reconstituted, exhibited a shear-thinning flow behaviour ($n < 1$). However, water deficit affected the mechanical properties, changes being more noticeable in the reconstituted mucilages. Thus, the viscosity (η_1) of reconstituted mucilages increased, from 0.12 to 0.28 Pa·s, as water deficit increased, whereas their flow index (n) decreased from 0.57 to 0.47. Further, D40 and D60 reconstituted mucilages exhibited an E_a of 17.4 and 17.6 kJ/mol, respectively, whilst, for D0, E_a was 16.7 kJ/mol. Interestingly, only the D40 reconstituted mucilage showed a crossover point at 7.39 rad/s between viscous and elastic modulus. The understanding of the influence of water deficit on the main physico-chemical characteristics of *Aloe vera* polysaccharides and, in turn, of its effect on the rheology of the mucilages could be a useful tool for the design, development and control of biologically active ingredients based on the *Aloe vera* plant.

1. Introduction

Nowadays there is an ongoing search for new biomaterials that may be used in the development of functional ingredients able to substitute synthetic molecules which are, increasingly, being considered as potentially harmful for human health (Avila-de la Rosa et al., 2015). Different plant materials have been investigated in order to obtain natural components that could be used as these functional ingredients. Within this context, *Aloe barbadensis* Miller (*Aloe vera*) may be considered as a potential and valuable source of gums and hydrocolloids, which are present in the mucilage of *Aloe vera* (Kiran and Rao, 2016). The widespread use of *Aloe vera* mucilage as a source of functional ingredients is mainly due to the considerable number of beneficial properties attributed to this plant (Huseini et al., 2012; Kumar and Tiku, 2016; Pothuraju et al., 2016; Radha and Laxmipriya, 2015).

Aloe vera mucilage is the aqueous extract of the hydroparenchyma cells present in the succulent leaves of *Aloe vera* plant (Javed and Atta-Ur-Rahman, 2014). This mucilage consists of about 98.5%–99.5% water, the remaining solids being composed mainly of polysaccharides (~60% w/w), in particular acemannan and pectic substances (Femenia et al., 1999). Acemannan, considered as the main bioactive component of the *Aloe vera* mucilage, is a storage polymer located within the protoplast of the parenchymatous cells (Femenia et al., 1999). This polysaccharide is mainly composed of partially acetylated mannose units linked by β−(1 → 4) glycosidic bonds (Chokboribal et al., 2015; Chow et al., 2005; Femenia et al., 1999; McAnalley, 1993). On the other hand, pectic substances are the main component of the cell walls, mostly made up of a very high percentage of galacturonic acid units

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(~90–95%), with a low degree of methyl ester substitution (McConaughy et al., 2008; Ni et al., 2004). Interestingly, several studies have shown that the beneficial effects attributed to the *Aloe vera* plant depend to a large extent on the chemical characteristics of these polysaccharides, in particular the molecular weight and the acetylation pattern (Chokboribal et al., 2015; Chow et al., 2005; Im et al., 2005; Kumar and Tiku, 2016; Ni et al., 2004; Salah et al., 2017)

Nevertheless, the geographic location (including soil and climate), growth periods, horticultural conditions and postharvest treatments may play a critical role in determining the compositional and structural features of the main *Aloe vera* polysaccharides, which in turn might result in the modification not only of the functional properties, but also, the beneficial effects of the Aloe plant (Ray and Aswatha, 2013; Ray and Dutta Gupta, 2013; Ray et al., 2015; Rodríguez-González et al., 2012; Salah et al., 2017; Yaron, 1993). And, in particular, it has been observed that the mode of irrigation may have a considerable influence on the mucilage composition, even more so than leaf age or season, affecting mainly the mannose-rich polymers (Silva et al., 2010; Yaron, 1993; Yépez et al., 1999). It is known that the mannose-rich polysaccharides from *Aloe vera* plants act as a food and energy storage, protecting the plant from desiccation when water is scarce or not available (Brett and Waldron, 1990; Femenia et al., 1999; Yaron, 1993).

Interestingly, several authors have observed that *Aloe vera* polysaccharides, in particular mannose-rich polysaccharides, play a key role in the rheological behaviour of the mucilage, being responsible for their flow and viscoelastic properties (Campestrini et al., 2013; Lad and Murthy, 2013; Yaron, 1993). This assumption has led to the use of rheological analysis of the mucilage to evaluate the potential modifications on *Aloe vera* polysaccharides promoted by different thermal processes, such as drying (Cervantes-Martínez et al., 2014; Kiran and Rao, 2014; Medina-Torres et al., 2016; Minjares-Fuentes et al., 2016) and evaporation (Swami Hulle et al., 2014) and, also, to study the potential effects on the biological properties associated to the Aloe vera plant.

However, to the best of our knowledge, the information related to the influence of water deficit on the compositional features of *Aloe vera* polysaccharides and, also, on the rheological properties of the *Aloe vera* mucilage, is very limited. Thus, the aim of this study was to evaluate the main effects of water deficit on the compositional and structural features of the main *Aloe vera* polysaccharides, and its influence on the rheological behaviour of the *Aloe vera* mucilage. In particular, the rheological behaviour was evaluated through the analysis of the flow and viscoelastic behaviour of fresh and reconstituted mucilages.

2. Experimental

2.1. Sample

Aloe vera leaves, used as a raw material, were supplied by the "Facultad de Agronomía y Zootecnia (FAZ)" of the Universidad Juárez del Estado de Durango (Gómez Palacio, México). The studied leaves, of 30–40 cm length, corresponded to 2-year old plants.

Aloe vera plants with different water deficits were used in this study. Water deficit was defined as the percentage of water which was not supplied to the plant by irrigation from 100% of evaporated water, during 7 days. Thus, *Aloe vera* plants with water deficits of 40% (D40) and 60% (D60) were selected. In addition, plants without water deficit (D0) were used as a reference.

Aloe vera filets, were separated from the leaves as described by Femenia et al. (1999). Whole leaves were washed and the spikes removed, before slicing the leaf to separate the epidermis or skin from the filet. Then, the filets were cut into small cubes and crushed to extract the mucilages. Fresh *Aloe vera* mucilages (500 g) were used for the rheological determinations, while other batches of ~500 g of mucilages were frozen and lyophilized in a laboratory scale freeze dryer LABCONCO FreeZone Triad Cascade Benchtop (LABCONCO, Kansas city, Missouri, USA) operated at 0.01 mBar with condenser and shelf temperatures of −80 °C and −20 °C, respectively. The lyophilized *Aloe vera* mucilages were packed and stored in anhydrous conditions.

The moisture content of all *Aloe vera* mucilages was determined using a gravimetric technique as described by García-Cruz et al. (2013), and expressed as g water per 100 g fresh weight.

2.2. Alcohol insoluble residues (AIRs)

AIRs from lyophilized *Aloe vera* mucilages were obtained by immersing the samples in boiling ethanol (final concentration 85% (v/v) aqueous) and homogenized with an Ultra Turrax homogenizer T25 Digital (IKA, Staufen, Germany) (13,000 rpm for 1 min), and boiling for 5 min as previously described in Waldron and Selvendran (1990). The mixture was cooled, re-homogenized, filtered through a glass-sintered filter, and re-extracted twice with boiling 85% (v/v) ethanol. Finally, the white residue was washed with absolute ethanol followed by acetone, and allowed to air-dry overnight. Prior to further analysis, the AIRs were milled using a laboratory type grain mill and passed through 0.5 mm aperture sieve.

2.3. Isolation of water-soluble polysaccharides

The isolation of water-soluble polysaccharides was carried out as described by Rodríguez-González et al. (2011) with slight modifications. AIR preparations from each *Aloe vera* mucilage (100 mg) were suspended in distilled water (200 mL) and stirred for 2 h at room temperature. The suspension was then centrifuged at 13,000*g* for 1 h at 20 °C. The supernatant and precipitate, containing water-soluble (WSP) and water insoluble (WIP) polysaccharides, respectively, were collected and lyophilized. The extracts were stored in a desiccator under anhydrous conditions until later analysis.

2.4. Analysis of carbohydrate composition

Carbohydrate analysis was performed as described by Medina-Torres et al. (2016) for neutral sugars. Sugars were released by acid hydrolysis. Approximately 5 mg of *Aloe vera* samples (AIR, WSP and WIP fractions) 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 et al., 1954). A second sample, from AIRs and WIP fractions, was hydrolysed only with 1 M H_2SO_4 (100 °C for 2.5 h). Thus, the cellulose content could be estimated by the difference in glucose obtained by Saeman hydrolysis and this milder hydrolysis method. Neutral sugars were derivatized as their alditol acetates and isothermally separated at 220 °C by GC with a FID detector and equipped with a 30 m column DB-225 (J & W Scientific, Folsom, CA, USA) with i.d. and film thickness of 0.25 mm and 0.15 μm, respectively. Uronic acids were determined by colorimetry, as total uronic acids (Blumenkrantz and 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.

2.5. Methylation analysis

Methylation analysis of WSP samples (containing the acemannan polymer) was based on a modified sequential method using sodium hydroxide and methyl iodide (Ciucanu and Kerek, 1984). The modifications introduced to improve the overall methylation procedure were described in detail by Femenia et al. (1998).

2.6. ^1H Nuclear magnetic resonance (NMR) analysis of Aloe vera

^1H NMR analysis of WSP samples was carried out according to the method proposed by Bozzi et al. (2007). The ^1H NMR spectra at 300.13 MHz were recorded on a Bruker Avance 300 spectrometer, equipped with a 5 mm broadband multinuclear *z*-gradient (BBO)