Industrial Crops & Products 108 (2017) 355–362

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Extraction, isolation and characterization of inulin from *Agave sisalana* boles

Alexsandra Conceição Apolinário[a], Erika Martins de Carvalho[b], Bolivar Ponciano Goulart de Lima Damasceno[c,d], Paulo César Dantas da Silva[c], Attilio Converti[e,*], Adalberto Pessoa Jr.[a], José Alexsandro da Silva[a,f]

[a] Department of Biochemical and Pharmaceutical Technology, University of São Paulo, Av. Prof. Lineu Prestes, 580 – Bloco 16, Cidade Universitária, 05508-000, São Paulo, Brazil
[b] Institute of Pharmaceutical Technology, Farmanguinhos, Fundação Oswaldo Cruz – Fiocruz, Rua Sizenando Nabuco, 100, 21041-250, Rio de Janeiro, RJ, Brazil
[c] Department of Pharmacy, State University of Paraíba, Brazil
[d] Graduation Program in Pharmaceutical Sciences, State University of Paraíba, Rua Juvêncio Arruda, S/N – Bairro Universitário, 58429-600, Campina Grande, Paraíba, Brazil
[e] Department of Civil, Chemical and Environmental Engineering, Chemical Engineering Pole, Genoa University, Via Opera Pia 15, 16145, Genova, Italy
[f] Department of Agrarian and Exact Sciences, Campus IV – UEPB, Postgraduate Program in Agroindustry Systems, Federal University of Campina Grande, Pombal, Paraíba, Brazil

ARTICLE INFO

Keywords:
Agave sisalana
Sisal
Aqueous extract
Polysaccharide
Inulin

ABSTRACT

Agave sisalana Perrine is a widespread species of the Brazilian Northeast region, where it is exploited only as a source of hard fiber (sisal). Although some other *Agave* species are sources of fructans, there is no study on this issue on *A. sisalana*. This paper aimed at extracting and isolating inulin from aqueous extract of *A. sisalana* boles. After preparation of extracts, crude inulin was precipitated with acetone at low temperature (4 °C). After purification by ion-exchange chromatography, a white powder was obtained by freeze-drying and characterized by X-Ray Diffraction (XRD), thermal analysis, Circular Dichroism (CD) and Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS). Moreover, its polysaccharide structure was confirmed by Fourier Transformed Infrared (FT-IR) spectroscopy and Nuclear Magnetic Resonance (NMR). FT-IR analysis pointed out absorption at 1420 cm^{-1}, corresponding to deformation of CH_2–OH lying on fructose ring, while absorption at 1075 cm^{-1} was assigned to C—O and C—C stretching vibrations of inulin pyranose ring. NMR showed the presence of one signal in the anomeric region at δ 5.4 ppm and others between 3.1 and 4.2 ppm in the ^{1}H spectrum, besides a chemical shift at 104.4 ppm corresponding to the anomeric region of the ^{13}C spectrum of an internal β-fructofuranose unit. XRD highlighted the amorphous state of inulin-rich powder, thermal analysis a glass transition temperature in the range between 50.0 and 55.8 °C, CD a good thermal stability, and MALDI-TOF-MS a prevalence of oligosaccharides with degree of polymerization in the range 5–13. These techniques revealed that *A. sisalana* boles contain inulin with features similar to those extracted from other commercial sources such as *Agave tequilana* or *Agave atrovirens*, which extends the economic importance of this species beyond its simple use as a fiber source.

1. Introduction

The *Agave* genus, belonging to the order Asparagales and to the family Agavaceae, gathers more than 200 monocotyledonous and monocarpic species, which grow mostly in China, Brazil, Mexico, Tanzania, South Africa and Mozambique, although native to North America, with their center of origin in present-day Mexico (Escamilla-Treviño, 2012; Silva and Beltrão, 1999). Many species of *Agave* have been used to produce spirits (e.g., tequila), forage, food, drinks (e.g., pulque), drugs, construction materials, weaving, paper.

Species of the genus *Agave* have been investigated with different aims in mind, mainly for biotechnological and pharmacological applications. These plants are known to accumulate large quantities of sugars and other commercially important substances, hence proving to be a potential raw material for the production of biofuels (Arrizon et al., 2010; Ávila-Fernández et al., 2011) and the recovery of compounds of concern for pharmaceutical and food industries, including excipients (emulsifiers, stabilizers, diluents) among others (Branco et al., 2010;

* Corresponding author.
E-mail addresses: acapolinario@gmail.com (A.C. Apolinário), erikamc@far.fiocruz.br (E.M. de Carvalho), bolivarpgld@pq.cnpq.br (B.P.G. de Lima Damasceno), paullocds@gmail.com (P.C.D. da Silva), converti@unige.it (A. Converti), pessoajr@usp.br (A. Pessoa), alexuepb@yahoo.com.br (J.A. da Silva).

http://dx.doi.org/10.1016/j.indcrop.2017.06.045
Received 24 August 2016; Received in revised form 6 June 2017; Accepted 17 June 2017
Available online 10 July 2017
0926-6690/

Santos et al., 2013).

Sterols, steroidal sapogenins, steroidal alkaloids, alkaloidal amines and flavonoids have been isolated from some *Agave* species (Chen et al., 2011; Debnath et al., 2010), most of which demonstrated to possess anti-inflammatory, antioxidant, antimycotic, antibacterial, antiviral, antituberculosis and gastroprotective activities (Ben Hamissa et al., 2012; Cerqueira et al., 2012; Dunder et al., 2010).

Among the various species of the genus *Agave*, *Agave sisalana* Perrine is a species widely present in the Brazilian Northeast region, where it is exploited only as a source of hard fiber, known worldwide as sisal, of which Brazil is the world's largest exporter. Nonetheless, some studies have demonstrated the potential of *A. sisalana* in different new applications, for example as a source of secondary metabolites and new products of interest for pharmaceutical and food industries (Apolinário et al., 2014a; Ramasamy et al., 2015; Santos et al., 2013).

Crassulacean acid metabolism (CAM) of *A. sisalana* is an evidence that this species can be a source of inulin type-fructans (Apolinário et al., 2014b). CAM plants do in fact produce photosynthetically these fructans that act as osmoprotectants during drought; for this reason, inulin and other fructans are present as storage carbohydrate in more than 30,000 plants species (Espinosa-Andrews and Urias-Silvas, 2012).

Inulin is a water-soluble fructose-based polymer resulting from extended sucrose metabolism, which consists essentially of fructose units linked by β-(2→1) fructosyl-fructose bonds and a terminal glucose one. Its variable degree of polymerization determines its applications and the crop value (Guggisberg et al., 2009). Inulin is also largely used as a prebiotic compound (Oliveira et al., 2011b) and has known beneficial health effects (Barclay et al., 2010).

Based on a previous study showing that aqueous extract of *A. sisalana* boles are rich in sugars (Apolinário et al., 2014a) and taking into account that inulin extraction from this raw material has never been reported before, in this work we extracted crude inulin from *A. sisalana* and performed a first purification by ion-exchange chromatography, with the aim of increasing added value to this crop, whose potential has been explored only a little so far. To this purpose, in order to contain the costs of inulin recovery, we preferred to use the traditional hot (80 °C) extraction technique under agitation, which is the method most widely used to extract inulin from plants (Apolinário et al., 2014b). Moreover, a set of analytical techniques was used to characterize the recovered inulin-rich powder.

2. Materials and methods

2.1. Materials

Six year-old plants of *Agave sisalana* Perrine were collected in Monteiro, PB, Brazil (7°52′40.50" S and 37°07′34.91" W), on January 2013. A voucher was deposited at the Herbarium Manoel de Arruda Câmara (Campina Grande, PB, Brazil) under number 210.

Pure inulin used as a standard was purchased from Orafti® HP (Tienen, Belgium). Fructose, 3,5-dinitrosalicylic acid (DNS) and diethylaminoethyl (DEAE)-cellulose resin were obtained from Sigma-Aldrich® (São Paulo, SP, Brazil). All the other reagents were of analytical grade.

2.2. Preparation of the extract

Plant boles were dried in a forced circulation oven at 40 °C until constant weight and milled. Based on the scientific literature on inulin extraction from plants (Apolinário et al., 2014b), after dilution with distilled water up to 0.143 g mL^{-1}, extraction was performed twice at 80 °C in a thermostatic bath, model SL 155/10 (Solab, Piracicaba, SP, Brazil), for 2 h. Dynamic maceration was allowed by agitation at 1600 rpm using a mechanical stirrer, model 713 (Fisatom, São Paulo, SP, Brazil). To obtain final aqueous extracts, samples were filtered through qualitative paper.

Total carbohydrate content was determined at 480 nm according to the phenolsulphuric acid method described by Dubois et al. (1956), using a UV–vis spectrophotometer, model UV-mini-1240 (Shimadzu, Kyoto, Japan), and a reference curve ($y = 0.0092x + 0.011$; $R^2 = 0.9929$) obtained in triplicate with inulin as a standard. Free reducing sugars were quantified at 540 nm by the dinitrosalicylic acid method described by Miller (1959), using a reference curve ($y = 0.6018x + 0.0481$; $R^2 = 0.9954$) obtained in triplicate with fructose as a standard. The contents of total polyphenols, flavonoids and other components were already reported in a previous study (Apolinário et al., 2014a).

2.3. Isolation and purification of inulin

Polysaccharides of the aqueous extract were precipitated with acetone (1:2 v/v) overnight at 4 °C, centrifuged (Excelsa I 206 BL, Fanem, Guarulhos, SP, Brazil) at 1118*g* for 20 min and submitted to four cycles of resuspension in distilled water at 80 °C and centrifugation under the same conditions as above. The polysaccharide solution was loaded on a 1.5 × 12 cm polypropylene column (Econo-Pac® 732-1010, Bio-Rad, Hercules, CA, USA) packed with 1.0 g of DEAE-cellulose pre-equilibrated with 10 vol of 0.05 mM Tris HCl buffer (pH 7.0).

An aliquot (1.0 mL) of each eluted fraction was collected separately in a 10 mL-test tube. After polysaccharide precipitation with acetone (1:2 v/v) and centrifugation under the same conditions as above, the precipitate was resuspended in distilled water (up to about 0.5 g mL^{-1}), frozen at −80 °C in an ultrafreezer, model MDF-U72 V (Sanyo, Osaka, Japan), and lyophilized at −50 °C by using a freeze drier, model L101 (Liotop, São Carlos, SP, Brazil). Fig. 1 summarizes the steps of production of inulin from *A. sisalana* (INAS) in powder form.

INAS powder diluted with distilled water up to 1000 μg mL^{-1} was submitted to UV–vis scanning in the wavelength range of 200–300 nm to make quality control of the final product. To this purpose, readings were performed in triplicate using 1.0 cm-pathlength quartz cuvettes in the above UV–vis spectrophotometer against water as a blank.

2.4. Fourier Transformed Infrared analysis

The Fourier Transformed Infrared (FT-IR) spectra of INAS were recorded on an IR spectrophotometer, model Vertex 70 interferometer (Bruker Optics, Ettlingen, Germany). After sample (5.0 mg) homogenization with KBr, the resulting mixture was pressed to form tablets, and the spectra were collected in the range 4000–400 cm^{-1} with a resolution of 4 cm^{-1}.

2.5. Nuclear Magnetic Resonance

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra of INAS were recorded on a spectrometer, model Avance 500 (Bruker, Bremen, Germany), at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in D_2O at 30 ± 0.1 °C using 30 pulse (12.5 μs for ^{1}H and 7.0 μs for ^{13}C) and a 5 mm switchable probe. ^{1}H NMR spectra were acquired by 1024 scans with a relaxation delay of 2.0 s, 16 K data points, 8278.1 Hz spectral width using a digital resolution of 0.30 Hz. The ^{13}C ones were acquired by 386,440 scans with 23,980.8 Hz spectral width using a digital resolution of 1.0 Hz and 32 K data points.

2.6. X-Ray Diffraction

X-Ray Diffraction (XRD) patterns of powder samples were recorded at room temperature on a diffractometer, model Miniflex Goniometer (Rigaku, Tokyo, Japan). Diffraction spectra were collected within 2 h in the 2θ range from 10° to 80° with a constant step of 0.04° and a counting time of 1 s per step.