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Efficient extraction of fructans from sotol plant (*Dasylirion leiophyllum*) enhanced by a combination of enzymatic and sonothermal treatments

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ABSTRACT

The effects of different extraction methods of water-soluble carbohydrates (WSC) from the sotol plant (*Dasylirion leiophyllum*) were investigated. Sotol fragments were extracted at 40 and 70 °C, under thermal treatment (T), pre-enzymatic thermal treatment (PET), sonothermal treatment (ST), and pre-enzymatic sonothermal treatment (PEST) conditions: fructose, glucose, sucrose, and fructans were analyzed by HPLC and the total water soluble carbohydrates was determined. At 70 °C, the highest WSC values (482 mg/$g_{d.m.}$) were obtained, with a fructan proportion of 69%. Pre-enzymatic treatment at 70 °C resulted in a high WSC content with the highest fructans proportion (87%) and lowest contents of RS and sucrose. The effect of the interaction between ultrasound and enzymatic treatments was limited by the high-temperature effect (70 °C), thereby minimizing the extraction. Microscopy analyses showed cell-wall modifications with the ST and PET treatments, which caused an increase in the total soluble sugars. The combination of enzymatic and sonothermal treatments at 70 °C resulted in the extraction of fructans in a higher yield and with less degradation. This circumvents the need for traditional high-energy processes, which could be beneficial for the extraction of WSC such as fructans from sotol or other economically important plants.

Keywords: *Dasylirion leiophyllum*; Carbohydrates; Extraction; Ultrasound; Enzyme; Fructans

1. Introduction

Sotol (*Dasylirion leiophyllum*) is a desert plant (Mancilla-Margalli and López, 2006; De la Garza-Toledo et al., 2008) that grows in the wild, mainly in the north of Mexico and south of the United States. This plant, from the *Nolinaceae* family, is succulent, perennial, and polycarpic (IMPI, 2002; De la Garza-Toledo et al., 2008). *Dasylirion* spp. is used mainly in the production of a traditional distilled alcoholic beverage named sotol (De la Garza-Toledo et al., 2008). The controlled cultivation of *Dasylirion* spp. is important because of its high content of both simple carbohydrates (glucose, fructose, sucrose) and

Abbreviations: T, thermal treatment; PET, pre-enzymatic thermal treatment; ST, sonothermal treatment; PEST, pre-enzymatic sonothermal treatment; WSC, water soluble carbohydrates; RS, reducing sugars; RH, relative humidity; TEM, transmission electron microscopy.

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Nomenclature

kHz	kilohertz
W	watts
mM	millimolar
kV	kilovolts
nm	nanometer

Subscripts

d.m.	dried matter

complex ones such as fructans (Mancilla-Margalli and López, 2006; De la Garza-Toledo et al., 2008; Leach and Sobolik, 2010). Fructans are carbohydrates consisting mostly of fructose units joined through fructosyl–fructose bonds in either linear or branched form. The presence of branched units makes them behave as dietary fiber, so fructans are of importance in the food industry as fat and sugar substitutes or as prebiotics. They are also used in the pharmaceutical industry as excipients for tablets and vaccines (Franck, 2002; Roberfroid, 2004; Kelly, 2008). Fructans are found in some plants in the vacuole along with glucose and fructose (Vijn and Smeekens, 1999). It is thus evident that the breakage of the cell-wall may facilitate the release of these components. However, the breakage of some components of the cell-wall, such as cellulose and hemicellulose, is complicated because these substances provide rigidity to the cell-wall to prevent permeation. In addition, the pectic material that cements the cell wall prevents cell disruption (Raven, 1987; Sterling, 1963; Heredia-Léon et al., 2004). In this regard, some physical methods (maceration, grinding, temperature, ultrasound, and pulsed electric field, among others) may promote cell disruption to release molecules such as fructans without affecting their physical and chemical properties.

The economic importance of fructans has led to the development of several extraction methods, most of which employ hot water (Franck, 2002; Roberfroid, 2004; Ebringerová and Hromádková, 2010). These processes are similar to those used in both beet sugar production (Franck, 2002) and inulin extraction from chicory roots (Franck, 2002; Roberfroid, 2004; Kelly, 2008). Extraction processes using hot water are employed because the elevated temperature increases the solubility of the components, especially that of complex carbohydrates, thus increasing the mass transfer during the extraction. However, these processes consume significant amounts of energy, so more sustainable options must be adopted. Nowadays, there is an increasing trend for applying "green" or sustainable techniques that are more environmentally friendly (Allen and Shonnard, 2001). Alternative techniques such as ultrasound and enzymatically assisted extractions are being investigated, and have been proposed to reduce the use of extraction solvents and processing time, and therefore, decrease the energy consumption (Chemat et al., 2011; Puri et al., 2012).

In ultrasound-assisted extraction, high-intensity sound waves are propagated through the liquid medium, causing the oscillation of its molecules, and creating alternating cycles of compression and rarefaction. During these cycles, pressure changes occur, leading to the breakup of the liquid and the generation of voids or cavities, a phenomenon known as cavitation. Bubbles grow over the period of a few cycles to an equilibrium size and then collapse in succeeding compression cycles, generating energy for chemical and mechanical effects (Mason et al., 2003; O'Donnell et al., 2010). This kind of mechanical effect in raw plant tissue causes disruption to the cell-wall, facilitating both solvent penetration into the cell and the release of biocomponents into the continuous phase (Toma et al., 2001; Lingyun et al., 2006; Ebringerová and Hromádková, 2010). Another technology used widely in maceration or extraction processes, especially of raw materials, is the use of enzymes (Buchert et al., 2005; Landbo et al., 2007; Falkoski et al., 2013). In these processes, enzymes are used to provoke vegetable cell-wall degradation through hydrolysis reactions of the structural polysaccharides, causing an alteration in cell permeability, and thus, leading to the release of biocomponents into the extraction media (Ramos de la Peña et al., 2012; Kashyap et al., 2001; Tadakittisarn et al., 2007; Lee et al., 2006).

The possibility of combining the techniques of ultrasound extraction and enzymatic treatment either to increase the extraction yields or reduce the energy and solvent consumption could be of great benefit for obtaining industrially of important bioproducts such as soybean oil (Kapchie et al., 2008), or for wastewater treatment (Sangave and Pandit, 2006).

In the case of fructans production from *D. leiophyllum*, the combined use of ultrasound extraction with enzymatic pretreatment at different temperatures could increase the yield of fructans and other carbohydrates from the sotol plant. There is a very limited amount of information on the contents of complex carbohydrates from the sotol plant, as well as the variability in their contents according to location, climate and other environmental factors, which has hindered the better use of this natural resource for applications other than alcoholic beverage production. The aim of this study was to determine the effects of ultrasound and enzymatic treatments at different temperatures on the extraction of water-soluble carbohydrates (WSC) and reducing sugars (RS) from *D. leiophyllum*.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Sucrose (assay ≥99.5%) and glucose (assay ≥99.5%) were supplied by Anedra (Buenos Aires, Argentina), and fructose (assay ≥99.5%) was purchased from Mallinckrodt Baker (Center Valley, PA, USA). Standards of inulin from chicory HP (ChHP) (degree of polymerization (DP) ≥10; inulin > 90%), dahlia (Dh) (DP > 10) and *Agave tequilana* (At) (DP > 10 with 85% fructans) were provided by Beneo–Orafti (Tienen, Belgium), Sigma–Aldrich (St. Louis, MO, USA) and Nutri Agaves of Mexico (Ayotlan, México) respectively. Pectinex® Ultra SPL was obtained from Sigma–Aldrich (St. Louis, MO, USA). For the processing of TEM samples, sodium cacodylate, uranyl acetate dehydrate, osmium tetroxide and a Spurr low-viscosity kit were purchased from Electron Microscopy Science (Industry Road Hat field, PA, USA) and glutaraldehyde solution (25%), ethanol and acetone (Lab Grade) were obtained from J.T. Baker (Center Valley, PA, USA). Other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.1.2. Plant material

The *Dasylirion* plants were collected in Northern of Mexico at Delicias, Chihuahua. All plants used in this study were ten years old with an average weight of 18 kg and moisture content of 70%. These were harvested in the winter season from a