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

Abril J. Pardo-Rueda[a], *Armando Quintero-Ramos*[a,*], *Diego B. Genovese*[b], *Alejandro Camacho-Dávila*[a], *Armando Zepeda-Rodríguez*[c], *Juan C. Contreras-Esquivel*[d], *A. Patricia Bizarro*[c]

[a] *Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas, Circuito Universitario, s/n, Nuevo Campus, P. Box 31125, Chihuahua, Mexico*

[b] *CONICET-Universidad Nacional del Sur, Planta Piloto de Ingeniería Química, Bahía Blanca, Buenos Aires, Argentina*

[c] *Universidad Nacional Autónoma de México, Facultad de Medicina, Departamento de Biología Celular y Tisular, Ciudad Universitaria, Col. Copilco. C.P. 04510 Delegación de Coyoacan, Mexico DF, Mexico*

[d] *Universidad Autónoma de Coahuila, Facultad de Ciencias Químicas, Blvd. V. Carranza y José Cárdenas Valdés s/n, P. Box. 25280, Saltillo, Coahuila, Mexico*

A B S T R A C T

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.

* *Corresponding author*. Tel.: +52 614 23 66000.
E-mail addresses: aquinter@uach.mx, aqura60@gmail.com (A. Quintero-Ramos).

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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

cultivated plantation of *D. leiophyllum* at the Facultad de Ciencias Agrícolas of the Universidad Autónoma de Chihuahua. Sotol heads were stored at 1 °C at 90% RH for 25 days. They were rinsed and cut into strips: 0.20 m × 0.01 m × 0.005 m for all treatments.

2.2. Carbohydrate extraction treatments

Strips from the sotol heads were subjected to extraction with a 1:10 (w/w) solid/solution ratio at two temperatures (40 and 70 ± 1 °C) for 135 min in four different treatment regimes. Thermal treatment (T), was performed by putting 30 g of sotol strips and 300 mL of distilled water in a 600 mL beaker, which was placed in a water bath at both 40 and 70 °C. During treatment, intermittent manual stirring was applied every 15 min. Sonothermal treatment (ST) was carried out by placing 2 kg of sotol strips on a stainless steel grid (49.53×10^{-2} m l (length) × 99.822×10^{-3} m w (wide) × 72.898×10^{-3} m h (height)) and immersed in 20 L of distilled water in a stainless steel ultrasonic tank (60.96×10^{-2} m (l) × 30.48×10^{-2} m (w) × 40.64×10^{-2} m (h)), with a volume of 60.3 L, insulated with fiberglass with a thickness of 6.35×10^{-2} m. This was equipped with two electrical resistors with a temperature control, as well as an immersible transducer with a radiant surface of 45.72×10^{-2} m (l) by 15.24×10^{-2} m (w) (Branson EB618-25-12). This was adapted to the bottom of the tank and connected to an ultrasonic generator (Branson S8525-12-500W) of variable power from 125 to 500 W. The operating ultrasound frequency was 25 kHz with an ultrasound intensity of 1135 W/m^2 using continuous pulsation. The energy input was controlled by setting the power modulation of the transducer. The ultrasound intensity was determined by the calorimetric method described by Rawson et al. (2011). Pre-enzymatic thermal treatment (PET) was performed according to the methodology described by Ramos de la Peña et al. (2012). Sotol strips (30 g) were placed in 300 mL of a 50 mM buffer solution of sodium acetate (pH of 4.5) containing 50 μL of Pectinex® Ultra SPL, and the temperature was maintained at 40 °C for 6 h in a shaking incubator (Precision Dubnoff Metabolic). After incubation, the suspension (strips-solution) was subjected to the same extraction conditions as the thermal treatment (T) described previously. Pre-enzymatic sonothermal treatment (PEST) consisted in placing sotol strips (2 kg) into 20 L of a 50 mM buffer solution of sodium acetate (pH 4.5) with 10 mL of Pectinex® Ultra SPL, and maintaining a temperature of 40 °C for 6 h in the ultrasonic bath described above. Subsequently, the incubation samples (suspension) were subjected at the same extraction conditions as the sonothermal (ST) treatments (described previously) and at the same temperatures (40 and 70 ± 1 °C). All treatments were carried out in duplicate. The final extracts were filtered, freeze-dried, and stored in hermetic plastic containers at 5 °C until analysis of the contents of fructose, glucose, sucrose and fructans by HPLC. The total content of soluble carbohydrates was obtained from the sum of the individual contents of each sugar.

2.3. High-performance liquid chromatography (HPLC) of WSC from D. leiophyllum

Fructans, sucrose, fructose and glucose were analyzed by HPLC with a Waters e2695 instrument (Milford, MA, USA) using a refractive index detector (Waters 2412 IR, Milford, MA, USA) and an Aminex® HPX-87C column (300 mm × 7.8 mm; Biorad, Hercules, CA, USA) with a 9-μm particle size and operation at 65 °C under isocratic conditions. The autosampler temperature was adjusted to 40 °C. Samples and standards (10 μL) were injected and eluted with degassed ultrapure water at a flow rate of 0.3 mL/min. Freeze dried extracts (0.01 g) obtained through the different treatments were re-suspended in 1 mL of HPLC-grade water, sonicated in an ultrasonic bath (Sonorex, RK 255H, Berlin, Germany) at 70 °C for 1 min, and injected to the HPLC. The carbohydrates in the samples were identified by their retention time using the following standards: Inulin ChHP, Dh, At, sucrose, glucose, and fructose. Fig. 1, shows the chromatograms of the extracts and standards. Peak areas from the chromatograms were plotted against known standard concentrations (inulin (2.5–9 mg/mL); sucrose (0.1–1.9 mg/mL); glucose (0.25–1.4 mg/mL); fructose (0.5–1.4 mg/mL)) and fitted by linear regression analysis to generate equations that were used to establish the carbohydrates concentrations in the samples (expressed as mg/$g_{d.m.}$)

2.4. TEM analysis

The modified method for plant-tissue processing described by Ancheta et al. (1996) was used. Samples of sotol treated tissues were taken after the extraction treatments and stored under refrigeration until their preparation for TEM analysis. The samples were fixed for one day in 5% glutaraldehyde in 0.1 M sodium cacodylate, post-fixed with 2% osmium tetroxide solution as a secondary fixative, washed three times in the same buffer, dehydrated in a graded series of acetone, and embedded in Spurrs epoxy resin. The samples were polymerized at 60 °C for 12 h in a standard oven (Shel Lab). Semi-thin cuts of 0.5 μm were obtained, which were analyzed with an Axioskop 2 plus light microscope (Jena, Germany). Then, ultra-thin (80 nm) cuts were obtained, which were analyzed with a ZEISS EM-10C electron microscope at an accelerating voltage of 80 kV, at magnifications of 5000× and 10,000×.

2.5. Analysis of data

Data collected from the WSC samples were statistically analyzed. The effects of the treatments were determined by analysis of variance using Minitab version 16 software (Minitab, 2010), and means were compared by using the Tukey test at a confidence level of 95%.

3. Results and discussion

Fig. 2 shows a significant influence of the solvent temperature on the WSC extraction yield for thermal treatments (T), resulting in 115 mg/$g_{d.m.}$ of WSC at 40 °C, and 482 mg/$g_{d.m.}$ of WSC at 70 °C. These results suggest a strong temperature effect in carbohydrate extraction. This is mainly due to the limited solubility at low temperatures of complex carbohydrates with high molecular weights, such as fructans, which possess β-glycosidic bonds (Chacón, 2006; Kelly, 2008), which lead to moderate solubility in water at room temperature (12% at 25 °C) (Franck, 2002; Roberfroid, 2004). The fructans in the sotol plant, are the predominant carbohydrates among the WSC present (De la Garza-Toledo et al., 2008; Leach and Sobolik, 2010), and their solubility decreases as the temperature decreases. On the other hand, the WSC values obtained for other plants that are usually employed in the commercial production of fructans, such as chicory and dahlia, are around 240 and 350 mg/$g_{d.m.}$, respectively (Mancilla-Margalli and López, 2006). This shows the great potential of the sotol

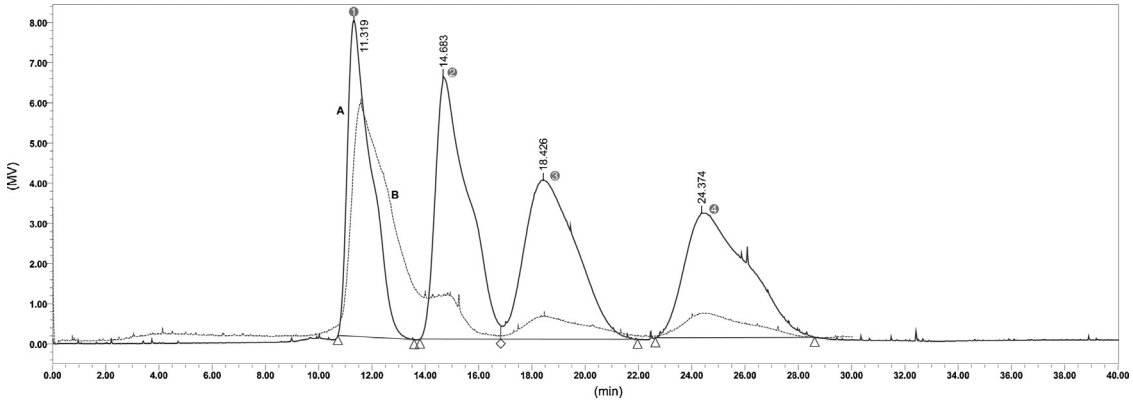

Fig. 1 – Typical chromatogram of WSC obtained from extracts of sotol plant (*D. leiophyllum*) under pre-enzymatic treatment at 40 °C (B) and standard (A). 1: inulin; 2: sucrose; 3: glucose; 4: fructose.

plant as a source of carbohydrates. Previous investigations such as that conducted by Hudson (1910) obtained extracts with 168 mg/$g_{d.m.}$ WSC from *Dasylirion* spp. at 20 °C, while Mancilla-Margalli and López (2006) reported a WSC concentration of 440 mg/g from the extract of wild *Dasylirion* spp. at 80 °C, of which 18% were fructans. These values are consistent with those obtained in this study at low and high temperatures, except in the fructan content, which in this case yielded higher values in the extracts obtained under both thermal conditions, as mentioned above. This is attributed to the characteristics of the raw material. Wild plants are supposed to have a higher fructans contents; however, the extract obtained by Mancilla-Margalli and López (2006) from wild *Dasylirion* spp. contained only 18% fructans. Therefore, other possible factors such as genus, crop type, edge, climate and water stress, and especially operating conditions, should be analyzed (Saengthongpinit and Sajjaanantakul, 2005).

The sonothermal treatment (ST) had a significant effect on the WSC extraction (Fig. 2). Sonothermal treatment promotes WSC extraction at 40 °C, giving higher values (148.61 mg/$g_{d.m.}$) than the thermal treatment at the same temperature (115.50 mg/$g_{d.m.}$). A significant increase in the content of simple carbohydrates such as glucose and fructose was also observed (Fig. 2). In sonothermal treatment at 70 °C, the extraction of WSC was greater (361.60 mg/$g_{d.m.}$) than with ST at 40 °C (148.61 mg/$g_{d.m.}$), but lower than with the thermal treatment (481.83 mg/$g_{d.m.}$) at the same temperature (Fig. 2). It is observed (Fig. 2) that at 70 °C, the fructans proportion was higher for the sonothermal (72.47%) than for the pure thermal treatment (68.94%). Furthermore, with the ST70 treatment, lower amounts of simple sugars were released. The observed changes with sonothermal treatment at low temperature (40 °C) can be attributed to the degradation effect of the ultrasound on the cell-wall maximizing the release of WSC. This can be ascribed to diverse factors such as the chemical breakage of complex polysaccharides induced by ultrasound. A similar effect during vegetable sonication was reported by Toma et al. (2001). Upon sonothermal treatment at 70 °C, as seen in Fig. 3, the cell walls were damaged, and modifications to the plasmatic membrane can be observed. This could be due to the release of fructans, which is found almost entirely in the vacuole along with fructose and glucose (Vijn and Smeekens, 1999). Notwithstanding the physical alteration produced on the tissue by the ultrasound treatment, this did not contribute significantly to the WSC extraction compared with the thermal treatment, possibly because of the counteracting effect of the temperature over the sonication effect. As the solvent temperature increases, the vapour pressure rises, and more of this vapour fills the cavitation bubbles, causing less violent collapses, and thus, lowering the intensity of the sonication effect (Mason, 2000; Santos et al., 2009).

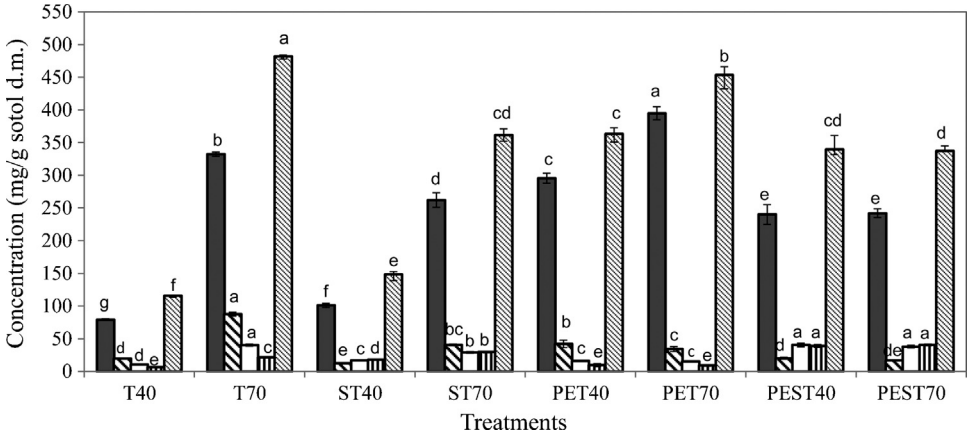

Fig. 2 – Sugar profile obtained from sotol plant extracted under different conditions: PET, pre-enzymatic treatment; T, thermal treatment; ST, sonothermal treatment; PEST, pre-enzymatic sonothermal treatment. Fructans; sucrose; glucose; fructose; WSC. Numbers in the treatment code represent the extraction temperature (40 or 70 °C). Bars represent the standard deviation. For each carbohydrate, mean values with different letters for the different treatments indicate a significant difference according to the Tukey test ($P < 0.05$).

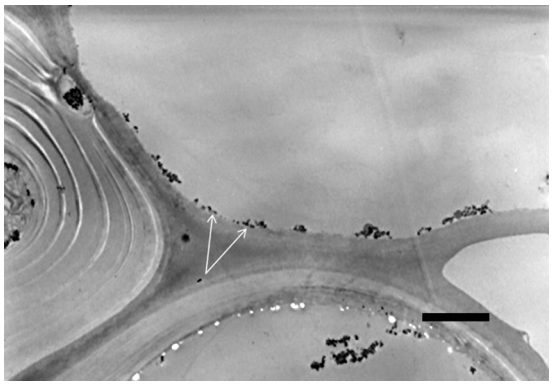

Fig. 3 – Transmission electron microscopy (TEM) image of transverse section of tissue from *D. leiophyllum* subjected to extraction by sonothermal treatment at 70 °C. Contiguous, epidermal cells from *D. leiophyllum* showing holes adjacent to the plasmatic membrane and/or inner wall due to the ultrasound effect (arrows). Magnification: 5000×. Bar = 2 μm.

The extraction of WSC, fructans and sucrose in the PET treatments was affected significantly by temperature, while no significant changes in sugar extraction were found upon PEST treatment at both temperatures. PET gave the best fructans yields at both temperatures, in the range 81–87%, compared with all the other treatments, and led to a proportional decrease in the reducing sugars (25.77 and 24.36 mg/$g_{d.m.}$). PET70 gave the highest concentration of fructans (394.82 mg/$g_{d.m.}$) and the best yields, representing 87% of WSC with this treatment (Fig. 2).

The increase in temperature minimizes the cavitation effect (Grönroos, 2010) during ultrasound application, and did not lead to an improvement in the extraction in PEST70, while PEST40 did not improve the sugar extraction, perhaps because ultrasound may inhibit enzyme activity during treatment (O'Donnell et al., 2010). With PET treatment, the fructan and WSC extraction was greater at 70 °C than at 40 °C. Some reports (Chacón, 2006; Kelly, 2008; Franck, 2002; Roberfroid, 2004) stated that complex carbohydrates such as fructans presented limited or moderate solubility at low temperatures. Therefore, an increase in temperature to 70 °C increased the sugar solubility, and led to an increase in the efficiency of the fructans and WSC extraction.

However, PEST treatment at 40 °C did not improve the extraction compared with PEST at 70 °C. The enzymatic treatment effect on the extraction of WSC and fructans was evident at low temperatures, with PET40 and PEST40 treatments achieving high WSC extractions of 363.5 and 339.6 mg/$g_{d.m.}$, respectively, although there was no significant difference between them. However, the fructans content was significantly higher for the PET40 treatment (295.50 mg/$g_{d.m.}$) than with PEST40 (240.02 mg/$g_{d.m.}$) or the other treatments at 40 °C (T40 and ST40). The PEST40 treatment showed significant increases in glucose and fructose, while the fructans decreased compared with the PET40 treatment. This can be attributed to the degradation effect due to ultrasound, which causes the cleavage of glycosidic bonds of both structural and branched polysaccharides (Ebringerová and Hromádková, 2010) such as pectin, cellulose, or hemicelluloses contained in the cell wall. Treatment at 70 °C with pre-enzymatic incubation resulted in higher contents of fructans in all treatments. It also resulted in a significantly higher WSC content than that obtained through the combined enzymatic and sonothermal extraction (PEST70), which did not potentiate the extraction of WSC and fructans (Fig. 2). This was explained above, and has been attributed to the thermal inactivation of the enzyme and further inactivation of the pectinase by the ultrasound treatment during the extraction process, as reported by other authors (Fachin et al., 2004; Tiwari et al., 2009; O'Donnell et al., 2010; Gonzalez and Rosso, 2011; Duan and Kasper, 2011). The PEST70 treatment showed a significant increase in the fructose and glucose contents compared with PET70. This result is mainly because plant tissue is previously subjected to an enzymatic pre-treatment that acts on the pectic cell-wall material. Although PEST treatments show low WSC extractions, they may contribute to the release of simple sugars like fructose and glucose derived from the rupture of pectic material contained in the cellular wall, increasing their content.

Thus, at 40 °C, enzyme activity played an important role in the extraction, which is attributed to the presence of the pectinases contained in the enzyme cocktail Pectinex Ultra SPL used in the pre-incubation process at 40 °C (Novozymes, 2007), which was the most suitable temperature for adequate stimulation of the enzyme activity. However, an increase in solvent temperature to 70 °C resulted in thermal inhibition of the enzyme, minimizing its effect during the extraction (Fachin et al., 2004; Gonzalez and Rosso, 2011).

As described previously, the enzyme cocktail Pectinex Ultra SPL has the ability to degrade the cell-wall making it thinner. Fig. 4 shows the middle lamella width after a sonothermal treatment at 70 °C (Fig. 4a) compared with that after pre-enzymatic treatment at 70 °C (PET70) (Fig. 4b). It is observed that the PET70 treatment caused a significant reduction in the thickness of the cell-wall. This could be attributed to an enzymatic action developed by the enzymes contained in the cocktail acting on pectin, hemicelluloses, and other

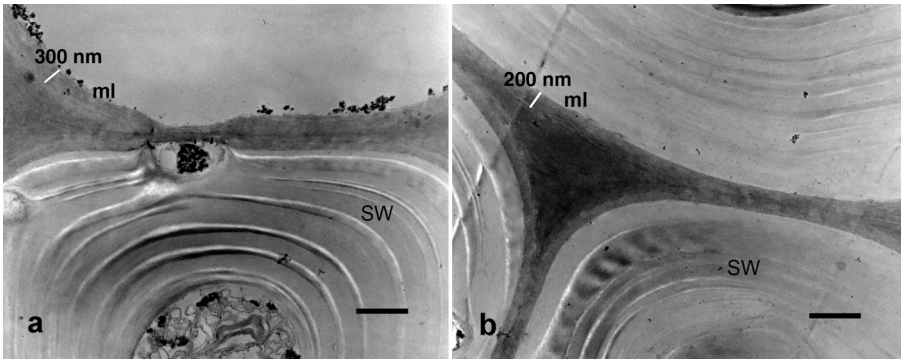

Fig. 4 – TEM micrographs of transverse sections of epidermal cell from *D. leiophyllum* subjected to extraction by ST70 treatment (a) and PET70 treatment (b). sw, secondary wall, ml, middle lamella. Magnification: 10,000×. Bar = 1 μm.

polysaccharides, thereby leading to permeability modification and damage to the walls of some cells. The epidermal cell section over the secondary wall (sw) from samples of ST70 treatments showed cornered cells with marked bands and less dense material when compared to those subjected to enzymatic treatments. High-electron-density material from the stain that appears deposited in the cell corner of samples (dark section) from PET70 (Fig. 4b) could be the result of the cell-wall degradation by the enzymes, which modifies the cellular permeability, promoting the degradation of some structural polysaccharides such as pectin, and thus, increasing the extraction. Similar findings that the degradation of structural polysaccharides such as pectin by pectinase leads to an increase in the free water of the system, causing the greater release of sugars and other components, have been reported (Ramos de la Peña et al., 2012; Kashyap et al., 2001; Tadakittisarn et al., 2007; Lee et al., 2006). Moreover the physical degradation effect of the ultrasound in PEST treatments may promote cell disruption and/or the detachment of some reducing sugars from the cell-wall (Toma et al., 2001; Lingyun et al., 2006; Ebringerová and Hromádková, 2010; Zheng and Bhatty, 1998; Whitaker et al., 2003; Puri et al., 2012), thus contributing to the increase in the content of simple sugars in the final extracts through the interaction of both extraction techniques.

4. Conclusions

The extraction of WSC from sotol plant was increased significantly by the thermal treatment at 70 °C; this treatment led to the highest WSC value of all the treatment methods investigated. The pre-enzymatic treatment at the same temperature gave rise to a high WSC content with the highest fructan proportion and the lowest content of RS and sucrose, suggesting that this combination of processes results in fructans of higher purity. The high temperatures used during the extraction counteracted both the ultrasonic and enzymatic effects, minimizing the extraction of these treatments. Microscopy analysis showed alterations in cellular permeability and some damage to the cell-wall of the sotol plant with both ultrasound and enzymatic treatments, causing a major release of carbohydrates in the extracts.

Despite the effect of high temperatures on the enzymatic activity, such a combination could present some advantages regarding the traditional thermal extraction of fructans from *Agavaceae* plants. Additional studies are required for evaluation of the optimum process conditions and energy consumption to allow the implementation of this process in industrial production.

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