



# Neutral sugar profile of cell wall polysaccharides of pitaya (*Hylocereus* sp.) fruits

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## ABSTRACT

The composition of cell wall polysaccharides of pitaya (*Hylocereus* sp.) fruit pulp was studied with the aim of designing proper maceration processes for the fruit processing industry. Therefore, the degree of esterification of pectin was determined through a potentiometric titration and the consistency with a Bostwick consistometer. Moreover, the alcohol insoluble residue (AIR) was extracted and subsequently fractionated into water-, oxalate-, acid- and alkali-soluble pectins, hemicellulose and cellulose. Each fraction was analyzed for its neutral sugar composition by gas chromatography. Additionally, the AIR uronic content was measured by the m-hydroxydiphenyl (MHDP) assay. Pectin fractions in the analyzed fruit pulp were composed mainly of arabinose and galactose, while the hemicellulose fraction consists mainly of glucose, xylose and galactose. Considering the limited uronic acid content (32.3%) in the AIR and the high esterification degree of pectin, the high viscous consistency of this fruit pulp might not be highly attributed to pectin fractions.

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## 1. Introduction

Pitaya is native to tropical regions of North, Central and South America (Esquivel, Stintzing, & Carle, 2007). It is a perennial, epiphytic climbing cactus with triangular, fleshy, and jointed green stems. The fruit has red or pink skin covered with bracts or “scales”, and the mesocarp consists of a mucilaginous pulp which contains small digestible seeds (Zee, Yen, & Nishina, 2004). Selected species in this genus have been developed as fruit crops. Among them, *H. undatus* [Haworth] Britton & Rose (red pericarp, white pulp) has been widely planted, while others such as *H. polyrhizus* [(F.A.C. Weber) Britton & Rose] (red pericarp, red-violet pulp) and *H. costaricensis* [(F.A.C. Weber) Britton & Rose] (red pericarp, red pulp) are grown at smaller scale (Mizrahi & Nerd, 1999). Compared to cactus pears, pitayas are devoid of glochids, exhibit an exceptional red-purple hue and feature significantly higher betalain contents (Stintzing, Schieber, & Carle, 2003).

Several studies describe the processing of cactus pears into fruit juices and related products as well as the processing technologies for cactus pads (Moßhammer, Stintzing, & Carle, 2005; Moßhammer, Stintzing, & Carle, 2006; Sáenz, 2000; Sáenz, Sepúlveda, Araya, & Calvo, 1993; Sepúlveda et al., 2007). The hydrocolloid fraction is one of the major factors that complicate cactus pear fruit juice processing, in particular by causing poor fil-

terability. Therefore, removal or degradation of pectic substance has been believed to be a prerequisite to facilitate processing of these fruits into juice products (Moßhammer et al., 2005). More recently, Herbach, Maier, Stintzing, and Carle (2007) adapted the technology developed for cactus pear juice production by Moßhammer et al. (2005, 2006) to pitaya fruit processing. Since pitaya fruit pulp exhibits higher contents of mucilaginous material than cactus pear, some modifications to the process were necessary. Various enzyme preparations mainly composed of pectinolytic activities were tested to achieve optimum degradation of the pectin-like material, thus sufficiently reducing viscosity to allow juice filtration. However, substantial filtering residues, mainly consisting of undegraded mucilage, were retained. As a result, centrifugation was needed before enzymation, affecting the yield of juice production (Moßhammer et al., 2005, 2006). Due to the high viscosity of pitaya pulp its rheology was also studied (Chuah, Ling, Chin, Choong, & Fakhrul-Razi, 2008). Considering that high viscosities have to be reduced to increase juice yield and to improve processing, enzymatic treatment procedures have been also carried out (Nur 'Aliaa, Siti Mazlina, Taip, & Liew Abdullah, 2010). Enzymatic treatment of pitaya pulp resulted in a shift of the color tone, supposedly due to  $\beta$ -glucosidase side activities of the enzymatic preparation (Schweiggert et al., 2009). Enzymation procedures have been conducted without any information related to the cell wall polysaccharide composition of pitaya pulp and, until now, viscosity reduction attempts have not been totally successful. Therefore, to improve the enzymatic maceration during pitaya pulp processing, more detailed

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knowledge on the composition of cell wall polysaccharides is required.

The production of mucilaginous substances is characteristic of members of the Cactaceae (Nobel, Cavellier, & Andrade, 1992). These substances are distributed in the different parts of the *Opuntia* plants, e.g. cladodes and fruits (peel and pulp) (Sáenz, Sepúlveda, & Matsuhira, 2004). Nutraceutical and functional properties of these mucilaginous substances from *Opuntia* cladodes and fruits have been already reported (Magloire-Feugang, Konarski, Zou, Stintzing, & Zou, 2006; Piga, 2004; Sáenz et al., 2004; Stintzing, Schieber, & Carle, 2001). Several studies on the composition and the properties of hydrocolloids from cladodes, seeds, and skin from cactus pear fruits have been already carried out (Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005; Forni, Penci, & Polesello, 1994; Habibi, Mahrouz, & Vignon, 2003; Madjoub, Roudesli, & Deratani, 2001; Sáenz et al., 2004; Stintzing & Carle, 2005). Recently, the composition of hydrocolloids from the pulp of cactus pear fruit was investigated (Matsuhira, Lillo, Sáenz, Urzúa, & Zárate, 2006); however, little is known about the neutral sugar composition and galacturonic acid present in the mucilage of the pitaya fruit pulp. Therefore, the aim of this work is to study the neutral sugar profile of the alcohol insoluble residue (AIR) extracted from the pulp of pitaya fruits to increase the knowledge in this term in order to enable concentration through more specific enzymatic degradation of the highly viscous polysaccharides.

## 2. Materials and methods

### 2.1. Plant material

Fresh red-fleshed pitaya fruits (*Hylocereus* sp.) were collected in Barranca, Puntarenas, Costa Rica (9°57.566' N; 084°43.217' W). The fresh fruits were cut into halves, peeled manually, and processed in an Oster extractor (Oster 3000). After the separation of the seeds using a 1 mm sieve, the pulp was stored at –80 °C and then freeze-dried. The samples were vacuum-packed in polyethylene bags and stored at –20 °C until analysis.

### 2.2. Pulp consistency and viscosity

The consistency was assessed during 30 s with a Bostwick consistometer at 25 °C according to the experimental procedure described by Brito, Rodríguez, Samaniego, Jaramillo, and Vaillant (2007).

### 2.3. Degree of pectin esterification (DE)

The DE was determined by a potentiometric titration method, after pectin extraction under reflux at 97 °C using a citric acid solution (solute/solvent, 1:50), following the methodology described by Rovaris-Pinheiro et al. (2008).

### 2.4. Alcohol insoluble residues (AIR)

The freeze-dried pulp was ground to a fine powder with a hammer mill. Samples (25 g) were homogenized in boiling aqueous ethanol (250 mL, 80% v/v) using an Ultra-Turrax blender. After boiling for 1 h, insoluble solids were collected in a Büchner funnel. This procedure was repeated five times using 180 mL of ethanol 80% (v/v) until a clear extract was obtained. The residue was stirred overnight in pure acetone (250 mL), passed through a G1 glass sinter filter and air-dried at 50 °C.

### 2.5. Determination of uronic acid (AUA<sub>c</sub>)

The anhydrouronic acid content was quantified colorimetrically (AUA<sub>c</sub>, g/100 g of AIR) using the modified m-hydroxydiphenyl (MHDP) assay (List, Budruß, & Bodtke, 1985) and after a standard calibration curve. For the acid hydrolysis, 20 mg of the AIR were wetted with 200 µL of isopropanol and shaken for 1 h with 5 mL of H<sub>2</sub>SO<sub>4</sub> (72%, w/w) at room temperature. Then, the hydrolysate was transferred to a volumetric flask, made up to 100 mL with de-ionized water and tempered at 20 °C. For serial photometric analyses in triplicate against a sample blank, 250 µL of hydrolysate (or its appropriate aqueous dilution), 250 µL of ionized water and 3 mL of H<sub>2</sub>SO<sub>4</sub> (98%, w/w) with tetraborate (0.0125 mol/L) were mixed, heated (100 °C, 10 min), and cooled in an ice bath for 2 min. Subsequently, 50 µL of MHDP reagent were added (sample blank, 50 µL of NaOH, 0.5%, w/v). Exactly 20 min after the addition of the MHDP, the absorbance of the degassed sample was measured at 520 nm with a Cary 100 spectrophotometer.

### 2.6. Sequential extraction of the AIR

AIR samples (800 mg) were mixed with 5 mL of ethanol 96% (v/v). The solution was suspended in 50 mL of double distilled (dd) water and stirred at 40 °C for 30 min. After centrifugation at 6660 × g for 10 min (20 °C), the pellet was resuspended in 50 mL of dd-water, extracted at 40 °C for 1 h under stirring and centrifuged again. The combined supernatants were pooled, and dialysed exhaustively against water for 48 h using dialysis membranes (type 36/32, pore size 25–50 Å, Medicell International London, UK). This water-soluble pectin fraction (WSP) was then freeze-dried. To extract the oxalate-soluble pectin fraction (OXF), ammonium oxalate (0.5% (w/v), 50 mL) was added to the residual pellet and the mixture was stirred 1.5 h at 40 °C. The suspension was centrifuged for 10 min at 6660 × g and the obtained pellet washed twice with 50 mL of distilled water. The OXF fraction was dialysed for two days against water and freeze-dried. For further extraction, the residue was suspended in 50 mL of diluted hydrochloric acid (0.05 mol/L) and stirred for 1 h at 60 °C. The suspension was centrifuged at 6660 × g for 10 min, and the remaining pellet washed twice with 50 mL of distilled water. The supernatants were dialysed under the same conditions mentioned above and freeze-dried to obtain the HCl-soluble pectin (HSP) fraction. The residue was then extracted with 50 mL of aqueous sodium hydroxide (0.05 mol/L) at 5 °C for 3 h. After centrifugation at 6660 × g for 10 min, the pellet was rinsed twice with water. The supernatants were pooled and the pH adjusted to 6.5 with HCl, followed by the treatment according to the previous fractions to produce the NaOH-soluble pectin (OHP) fraction. The final extraction was carried out using 50 mL of aqueous sodium hydroxide (4 mol/L) at 30 °C for 5 h. After centrifugation and washing with water, the supernatants were combined and the pH adjusted to 6.5 to produce the hemicellulose fraction (HC), that was treated as described for the previous fractions. The remaining pellet consisted of insoluble solids, such as lignin and cellulose (C-fraction). This fraction was suspended in 50 mL of distilled water, dialysed and lyophilized.

### 2.7. Hydrolysis of the cell wall fractions

The polysaccharide fractions were hydrolysed with sulfuric acid. For this purpose, 100 µL of isopropanol and 300 µL of H<sub>2</sub>SO<sub>4</sub> (72%, w/w) were added to 30 mg of each fraction in a Pyrex tube and mixed with a glass stirrer. After a reaction time of 1 h at room temperature, the suspension was diluted with 5 mL of N<sub>2</sub>-saturated ultra pure water, and heated at 121 °C for 1 h in a heating block. The hydrolysate was cooled in an ice bath for 10–15 min, and then neutralised with 600 µL of aqueous ammonia (25%). The hydrolysate

**Table 1**  
Characterisation of the AIR of *Hylocereus* fruit pulp<sup>a</sup>.

AIR (g/100 g DM)	AUA (g/100 g AIR)	Neutral sugars (g/100 g AIR)	Pectin esterification degree (%)
5.3 ± 0.4	32.3 ± 1.6	30.3 ± 1.9	80 ± 3

<sup>a</sup> Mean ± SD of *n* = 3 replicate determinations.

was transferred to a volumetric flask and adjusted to 10 mL with distilled water.

### 2.8. Analysis of neutral sugars by gas chromatography

The monosaccharides obtained after the hydrolysis of the different cell wall fractions were separated by gas chromatography with flame ionization detection (GC-FID). For this purpose the neutral sugars were reduced with sodium borohydride and derivatised to their alditol acetates with acetic anhydride in the presence of 1-methylimidazole as catalyst. For the derivatisation, 1 mL of each hydrolysed fraction was mixed with 100 µL of the internal standard (2.0 g/L *myo*-inositol) and 100 µL of ammonia (12 mol/L). Then, 100 µL of freshly prepared sodium borohydride solution (750 mg NaBH<sub>4</sub>, 1.25 mL ammonia (12 mol/L) and 3.75 mL distilled water) were added. After 1 h at 40 °C, 100 µL of glacial acetic acid were added and the solution was allowed to equilibrate to 20 °C. Aliquots of 500 µL were mixed with 500 µL of cold 1-methylimidazole and 5 mL of acetic acid anhydride in a glass tube. After 10 min, 750 µL of absolute ethanol were added. After another 10 min, 5 mL of distilled water and 10 mL of KOH (7.5 mol/L) were added. The upper organic layer was transferred to vials with anhydrous sodium sulfate and stored overnight at –20 °C. Aliquots of 1 µL were injected with a split ratio 1:10. Helium was used as carrier gas at a flow rate of 37.94 cm/s. The GC (Chrompack CP 9001, Chrompack, Middleburg, NL) was fitted with a 30 m × 0.25 mm i.d., 0.15 µm fused silica capillary column (DB-225, J&W Scientific, Folsom, CA, USA) and equipped with a flame ionization detector operated at 230 °C. The oven temperature was kept at 140 °C for 2.5 min, subsequently increased to 200 °C (30 °C/min) and held isothermally for 4.5 min, followed by an increase to 220 °C in 1 min and final isothermal hold at 220 °C for 18 min. The monosaccharides were identified by comparing their retention times with those into alditol acetates derivatised standards of D(–)-ribose, D(+)-galactose, D(+)-glucose-monohydrate, D(+)-xylose, L(+)-arabinose, L(+)-rhamnose-monohydrate (VWR, Darmstadt, Germany), D(+)-mannose (Serva, Heidelberg, Germany), and L(–)-fucose (Sigma–Aldrich, Steinheim, Germany), and quantified using *myo*-inositol (VWR, Darmstadt, Germany) as an internal standard. Data analysis was carried out with the Maestro II 2.4 version.

## 3. Results and discussion

### 3.1. Pulp consistency

A flow of 0.140 ± 0.010 m during 30 s at 25 °C was observed for the pitaya pulp in our study. Perona (2005) found that the consistency of different fruit purees varied between 0.043 and 0.156 m under the same conditions. Not only the high consistency of pitaya pulp affects processing but also the high content of small digestible seeds in the pulp matrix, which are difficult to separate due to the high mucilaginous properties of the pulp, resulting in a very low juice yield (Esquivel et al., 2007).

### 3.2. Alcohol insoluble residues (AIR)

The amount of AIR extracted was 5.3% on dry weight. Since previous studies on the content of AIR of pitaya fruit pulp and its

fractionation are lacking, the results of this work are compared with the data of Matsuiro et al. (2006), who reported a mucilaginous material content of 3.8% for peeled cactus pear fruits, while 27.5% for cactus pear pericarp (Duckstein, 2008).

The uronic acid content, assumed to be mostly galacturonic acid, of the AIR from pitaya pulp as determined spectrophotometrically by the m-hydroxydiphenyl method was 32.3% (Table 1). Even lower contents (23.4%) were measured in peeled cactus pear fruits (Matsuiro et al., 2006). Thus, pitaya fruit pulp may not be considered a good source of pectin, since the minimum galacturonic acid content required for commercial pectins is 65% (FAO/WHO, 2007).

After pectin extraction of the AIR and subsequent titration, the DE was quantified as described by Rovaris-Pinheiro et al. (2008). A high degree of esterification (80 ± 3%) was found for the pectin extracted from pitaya fruit pulp. These findings could indicate that the high viscosity was determined in pitaya fruit pulp may not be mainly attributed to pectin.

### 3.3. Composition of the AIR sequential fractions

Different pectin fractions, hemicellulose and cellulose were isolated from the AIR and their neutral sugar composition was studied. The pectin fractions of the AIR were fractionated into water-, oxalate-, acid- and alkali-soluble pectins. Pectins with high degree of esterification, which are mainly located in the middle lamella of the cell wall, have little capacity to interact with other components of the cell wall and were thus most likely to be extracted with dd-water, giving the WSP-fraction. The dd-water insoluble residues were extracted with oxalate as a chelating agent (OXF-fraction), thus enhancing the solubility of low and medium methoxylated pectins by complexing bivalent ions from their free carboxylic acid groups. The addition of hot hydrochloric acid to the oxalate insoluble residues releases acid protopectin by cleaving glycosidic bonds (HSP-fraction), while diluted alkali solubilises those pectins that are linked to hemicellulose, cellulose or proteins (OHP-fraction). Hemicelluloses (HC-fraction) are extracted with 4 M NaOH and the remaining alkali-insoluble residues consist predominantly of cellulose and lignin (C-fraction) (Heredia, Jiménez, & Guillén, 1995).

The yields of the AIR fractions resulting from the extraction of fruit cell walls are shown in Table 2. The pectic fractions represented 49.5% of the total cell wall constituents (Table 2). Similar results were reported for cactus pear pulp, where Matsuiro et al. (2006) concluded that the mucilaginous material isolated from peeled fruits of *Opuntia ficus-indica* is a complex mixture of polysaccharides, with less than 50% of them being a pectin-like polymer. According to Goycoolea and Cárdenas (2004) the structure of the mucilaginous material from *Opuntia* spp. pads is composed of two distinct water-soluble fractions, one being low esterified pectin with gelling properties when calcium is added and the other a mucilaginous substance devoid of gelling properties. For cactus pear pericarp Duckstein (2008) found a total of 34.4% pectin related fractions in the AIR.

The water-soluble pectic fraction was the major pectinic fraction of the present study, followed by the oxalate and alkali soluble fractions (Table 2). When compared to peeled cactus pear fruits, Matsuiro et al. (2006) observed a high portion of the galacturonic acid in its de-esterified form. A similar composition was observed for the OXF-fraction of cactus pear pericarp, constituting 19.1% of the AIR, thus being the main pectin fraction of AIR (Duckstein,



**Table 2**Neutral sugar composition of the different pectin fractions from the AIR of *Hylocereus* fruit pulp.

AIR fraction (%)		Composition of neutral sugars (g/100 g DW) <sup>a</sup>							
		Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
WSP	16.4	8.4	tr <sup>b</sup>	0.2	17.9	1.3	0.4	18.9	2.2
OSP	13.4	6.1	tr	0.2	10.3	0.6	0.3	5.9	0.7
HSP	6.6	4.0	tr	tr	19.7	0.7	0.4	15.2	1.9
OHP	13.1	5.2	tr	0.4	20.0	1.4	0.3	11.0	0.8
HC	8.9	1.4	1.0	tr	6.7	13.0	1.8	7.6	15.8
C	26.9	0.3	tr	nd <sup>c</sup>	1.0	0.8	0.5	1.1	9.7
AIR	–	3.3	tr	0.1	9.1	1.9	0.5	7.3	4.7

<sup>a</sup> Results obtained from duplicates, SD < 13%.<sup>b</sup> Traces: <0.1 g/100.<sup>c</sup> Non detectable.

2008). On the other hand, low contents of protopectins (HSP-fraction) representing 6.6% of the AIR were determined in the pitaya fruits (Table 2). Similarly, protopectins extracted from cactus pear pericarp, also showed the lowest value (1.3%) of the pectin fractions (Duckstein, 2008). In pitaya, hemicellulose and cellulose fractions came to 8.9 and 26.9% of the AIR content, respectively (Table 2). As reported by Duckstein (2008), lower proportions of hemicellulose fraction but higher ones for the cellulose fraction were observed for cactus pears pericarp with values amounting to 6.8% and 44.1%, respectively.

### 3.4. Monosaccharide compositions of the AIR fractions

The neutral saccharide profile of the AIR from pitaya fruit pulp was dominated by arabinose, glucose and galactose, while fucose and ribose were minor (Table 2). The composition of the neutral sugars of the individual AIR fractions is shown in Table 2. Arabinose and galactose were the major neutral sugars of all pectic fractions, followed by rhamnose. Within the pectic fractions the highest amounts of rhamnose were observed for the water and oxalate soluble pectin. Similar relative amounts of galactose and arabinose were found in the WSP fraction of pitaya fruits (Table 2), whereas galactose was the most abundant sugar in the AIR from *O. ficus-indica* (Matsuhira et al., 2006). Additionally, fucose, ribose, xylose, glucose and mannose were the minor constituents of the pectin fractions of pitaya fruits (Table 2).

The main neutral sugar present in the HC fraction of pitaya was glucose, followed by xylose and galactose. Fucose was present in traces in all fractions, except for the HC-fraction (Table 2), where fucose is a characteristic component of the xyloglucans belonging to the hemicellulose fraction (Heredia et al., 1995). Only trace amounts of ribose were detected in any of the AIR fractions from pitaya fruit (Table 2), while higher contents of rhamnose, arabinose, galactose and glucose in the HC fraction were found compared to those reported for *Opuntia* fruit pericarp (Duckstein, 2008). The high contents of arabinose and galactose may also result from incomplete extraction of arabinogalactans (Kurz, Carle, & Schieber, 2008).

Although glucose was the predominant saccharide of the cellulose fraction (Table 2), low total amounts were detected, which could be a result of a low degree of hydrolysis. Moreover, as described by Heredia et al. (1995), bonds between the cellulose and mannose or xylose may be extremely stable and difficult to cleave even by treatment with mineral acids.

The total saccharide profile of the AIR from pitaya fruit pulp and its individual fractions is depicted on Table 3. Most interestingly, the molar ratio for arabinose, rhamnose, xylose and galactose (1.0:0.4:0.2:0.6) from the AIR of pitaya fruit pulp (Table 3) differed significantly from that of the AIR of cactus pear pulp (1.0:1.7:2.5:4.1) as reported by Matsuhira et al. (2006). The major difference may be due to the higher contents of arabinose com-

**Table 3**

Main neutral saccharide ratio of the different AIR fractions of pitaya fruit pulp.

	Ara:Rha:Xyl:Gal:Man:Glu (molar ratio)
Total AIR	1.0:0.4:0.2:0.6:0.1:0.8
Pectin fraction of AIR	
WSP	1.0:0.4:0.1:0.9:0.0:0.1
OSP	1.0:0.5:0.1:0.5:0.0:0.1
HSP	1.0:0.2:0.0:0.6:0.0:0.1
OHP	1.0:0.2:0.1:0.5:0.0:0.0
HC	1.0:0.2:1.9:0.9:0.2:2.0
C	1.0:0.3:0.7:0.9:0.4:7.7

pared to the other sugars in the AIR of pitaya fruit pulp. According to Matsuhira et al. (2006), arabinose is not present as branch units in cactus pear pulp mucilaginous substance, but constitutes an element of different polysaccharides.

Neutral sugar profiles, obtained by sequential AIR fractionation and characterisation of the pectins, hemicellulose and cellulose fractions, have been used to differentiate fruits belonging to different genera of the Rosaceae (i.e. *Prunus* L. and *Fragaria* L.) and, therefore, were proposed as a fingerprint of fruit authenticity (Fügel, Carle, & Schieber, 2004). As later shown by Kurz et al. (2008) the relative amounts of neutral sugars of the isolated hemicelluloses from fruit species, even belonging to the same genus (*Prunus armeniaca* L. and *Prunus persica* L.), allowed the correct determination of fruit authenticity. Therefore, not astonishingly, *Opuntia* and *Hylocereus* products could equally be distinguished among them and from other raw materials applying this method.

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