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The Cuticle of the Cactus *Cereus peruvianus* as a Source of a Homo-α-D-Galacturonan

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ABSTRACT

The waxy pecto-cellulosic cuticle of cladodes of the columnar cactus *Cereus peruvianus* (19% of the whole phytobiomass; dry wt) is a source of an α-D-polygalacturonic or pectic acid (35–40% yield, on a dry wt based on the wax-free pectocellulose layer). Warm EDTA/oxalate or room temperature strong acid/alkali cycles are efficient for pectic acid extraction, since divalent cation (mainly Ca^{2+}) is a barrier to be removed within the native and compact architecture of the cuticle. Despite some molecular dispersion arising from the application of strong mineral acid in the first extraction step, the pectic material appears to be quite homogeneous and, on acid or enzymatic analyses, was shown to contain only D-galacturonic acid as its monomer. *Cereus* cuticle pectate (sodium salt) tends to gel above a concentration of 1%, a useful property that can be more easily obtained by the inclusion of sucrose, light addition of calcium salt, and/or mild acidification.

Index Entries: *Cereus*; pectic acid; cactus cuticle; gel.

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INTRODUCTION

Pectic materials are wide-spread in nature, and fruit pulp is one of their main sources. The most characteristic structural feature of this heterogeneous polysaccharide group is an α-1,4-linked poly-D-galacturonic acid backbone. If the latter occurs simply as such and it is modified solely by esterification of C-6 carboxyls with methanol, the polysaccharide subtype is named pectinic acid. The covalent presence of neutral sugar branches, such as D-galactose, L-rhamnose, and L-arabinose, corresponds to the most frequently found subtype of natural polymers, the pectins (*1*). The simplest structure, the methoxyl-free pectinic acid, is referred to as pectic acid. This latter subtype is seldom found in natural plant sources, exceptions being the bark of *Abies amabilis* (2) and sunflower seed heads (*3*).

The soft parts of the cladode of the columnar cactus *Cereus peruvianus* were recently reported to be an excellent sourcer of a viscous gum, having potential industrial applications, such as water potabilization and cosmetology (*4*). The main byproducts of the isolation of this acidic rhamnogalactan are highly fibrous cellulose (vascular cylinder) and the cladode cuticle (the protective waxy pecto-cellulose external layer).

In order to improve potential commercialization of *Cereus* further, the wax-free cuticle was examined to determine its quantitative and qualitative pectic content.

MATERIALS AND METHODS

Cactus Source and Cuticle Processing

Five wing cladodes (modified stems) of *C. peruvianus* were collected in Maringá, State of Paraná, Brazil. Following a longitudinal cut, the adherent parenchymatous layer of the internal face of each cuticle mantle was mechanically removed with a sharp knife. Residual gum was eliminated by extensive water wash, and cuticles were then air-dried. Dipping the cuticles in warm chloroform removed the waxy external layer and pigments (mainly chlorophyll). For the purpose of weighing of botanical parts, a freshly collected cladode was sliced, lyophilized, carefully dissected with a sharp razor, and the isolated parts weighed. On exhaustive drying, the outermost waxy layer underwent detachment in the form of thin flakes. The commercial sample from orange pectin was obtained from Braspectina (Limeira, Sao Paulo State, Brazil).

Cuticle Polysaccharide Fractionation

Gum- and wax-free cuticles were fractionated in two distinct ways: (1) up to 12 successive 2-h extraction cycles with 2 vol of a mixture of 0.25

g% ethylenediaminetetracetate (EDTA, disodium salt) and 0.25 g% ammonium oxalate dihydrate at 60°C or (2) 2 vol of 6*M* HCl for 30 min with occasional agitation, followed by extensive washing with distilled water and then addition of 4*M* NaOH for pH increase to 7.0–8.0 and the resulting pectate solubilization. The viscous extract was then lightly reacidified and precipitated with HCl as a jelly-like mass (final pH between 4.0 and 5.0) (*5*). Precipitations of both kinds of pectates until completion was made with ethanol (final concentration 70% v/v), and the polymers were finally dried with the pure solvent. The residual solvent was removed at 40°C in a vacuum oven.

Chemical Analyses

The galacturonic acid content of cuticle pectate fractions was estimated colorimetrically with phenyl-phenol reagent (*6*). Methoxyl and *O*-acetyl groups were determined by tritrimetry and with the hydroxylamine reagent, respectively (*7,8*). Cation analysis was carried out by flame photometry in a double-beam CG-AA 7000 ABC apparatus (CG, Sao Paulo State, Brazil).

Physical and Physicochemical Analyses

Polarimetry was carried out with a model PDA 83000 apparatus (ACATEC, Sao Paulo, Brazil) using a 0.1% sodium pectate solution at 25°C. Viscosities were measured in Brookfield model DV-II viscometer with LV-I and IV spindles at 6 rpm and an 8-mL cylindric vessel at 25°C. Solution pH was adjusted to 3.5. Size-exclusion chromatography (SEC; TSK-PW columns) was monitored with differential refractometric index (DRI) and low-angle laser light scattering (LALLS) (Wyatt-Optilab 903, Wyatt Technology, CA, USA; PCLALLS package, LDC Analytical, FL, USA) in order to yield absolute mol-wt distributions, the operation following previously reported general guidelines (*9*).

Pectate Carboxyreduction and Monomer Characterization

Conversion of cuticle sodium pectate (sample CP-A/B) to galactan was performed with three successive cycles of carbodiimide/sodium borohydride (*10*). The neutral galactan was monomerized with 1*M* trifluoroacetic acid (100°C, 6 h). The released monosaccharide was reduced with sodium borohydride, and the resulting alditol peracetylated with acetic anhydride: pyridine 1:1 (room temperature, 24 h followed by 1 h at 90°C) prior to GLC-MS analysis in a QP-2000A apparatus (Shimadzu, Tokyo, Japan) using a capillary CG-FI-547 column (50 m) operated isothermically at 240°C.

Table 1
Cation Composition of *Cereus peruvianus* Native Cuticle

	ppm			
	Na	Mg	Ca	Fe
Nat-CTCL	114	3952	37,704	64
A/B-CTCL	nd	<50	1648	56
CP-A/B	2700[a]	127	444	107

[a]Resulting from the pectate solubilization step with NaOH.
Nat-CTCL = Wax-free native *Cereus* cuticle.
AB-CTCL = HCl-treated wax-free *Cereus* cuticle.
CP-A/B = acid/alkali-extracted *Cereus* cuticle pectate.

Pectate Enzymolysis and Paper Electrophoresis Analysis

Solutions at 10 mg/mL of cuticle pectates and orange peel pectin (Braspectina, Limeira, Sao Paulo) were digested with 1–2 mg of crude enzymes from the gastric juice of the terrestrial snail *Megalobulimus paranaguensis* (*11*) or Novo Pectinex 3XL for 24 h at 40°C. The aqueous ethanol-soluble digest was then electrophoresed on Whatman 1 paper in 5 m*M* $CaCl_2$-containing 10 m*M* pH 9.3 sodium borate buffer (*12*) for 1965 V·h at 16 mA using a tank of carbon tetrachloride as coolant. Neutral and acidic sugars were revealed with silver nitrate/sodium hydroxide (*13*).

RESULTS AND DISCUSSION

Pectin is known as a component of the outermost layer of the cactus cladode (*14*). Solubility properties of pectinous polymers are governed by several factors, such as degrees of methoxylation and *O*-acetylation, occurrence of neutral sugar branching, and carboxyl-counterion contents. These parameters were therefore evaluated both in the wax-free cuticle, acid-treated cuticle, and pectic materials obtained therefrom. As indicated in Table 1, divalent cations are important for the low solubility of pectin "*in situ*", and calcium is, by far, the dominant cation. This divalent cation promotes the ionic interchain crosslinkage (*15*), and the macromolecular aggregate displays reduced solubility. This situation was corroborated by native pectate extractions either with EDTA/oxalate (efficient Ca^{2+} chelators) or HCl/NaOH: the resulting sodium salts presented increased solubility. The reduction of Ca^{2+} content (37,704 ppm for the native cuticle as compared to 444 ppm in the acid/alkali-extracted pectate, sample CP-A/B; Table 1) correlated with the solubility increase and then with the extraction efficiency. Very low degrees of methoxylation and *O*-acetylation

Table 2
Chemical and Polarimetric Analyses of Pectic Materials

	Pectic material[a]		
g%	CP-E/O	CP-A/B	OP-CS
Uronic acid	94.50	96.00	63.50
O-acetyl	0.15	—	0.20
Methoxyl	1.11	0.25	2.70
Methoxyluronate	7.35	1.67	28.80
$[\alpha]^{20}_D$	+222	+220	nd

[a]CP-E/O = EDTA/oxalate-extracted *Cereus* cuticle pectate.
CP-A/B = acid/base-extracted *cereus* cuticle pectate.
OP-CS = orange pectin, commercial sample.

(Table 2) were also found for the pectic material extracted from cladodes under mild chelatory conditions, that is, combining EDTA and oxalate, thus presumably preserving the native polysaccaride structure. For comparison purposes, four times more methoxyl was found in orange pectin (sample OP-CS), and this source, as reported previously by other authors (*16*), even after acid extraction (boiling pH 2.2 HCl) still contained 9.5% of methoxyl.

Another interesting feature of the cactus cuticle pectic component was its homogeneous monosaccharide composition, as shown by the unusually high uronyl content, which reached ca. 95% in both mild (CP-E/O) and hardly (CP-A/B) extracted pectates (Table 2). This homopolymeric nature was confirmed in two different ways. The first way was through the carboxy-reduction of the whole polysaccharide material (galacturonan → galactan conversion), which was then subjected to hydrolysis, reduction, peracetilation, and gas chromatography coupled to mass fragmentation by electronic impact, which confirmed galactose as the single monosaccharide unit (Fig. 1A and B), and, hence the absence, at least, of L-rhamnose or L-arabinose branches. Second, since D-galactose (*1*) may be one of the neutral sugar components of true pectins, the cladode pectic material was extensively enzymolyzed with the help of two unrelated enzyme complexes and analyzed by paper electrophoresis showing D-galacturonic acid as the sole component (Fig. 2; lanes 4,5 and 6,7 for acid/base-extracted pectate incubated with snail enzymes and fungal pectinase, respectively; lanes 10,11 and 12,13 for EDTA/oxalate-extracted pectate also incubated with the same respective enzymes). That both crude enzyme preparations bear the galactosidase and rhamnosidase complementary activity enzymes was inferred from the inspection of lanes 15 and 16, where galactose or arabinose, and traces of rhamnose were clearly seen as byproducts from orange pectin digestion. Therefore, cumulative

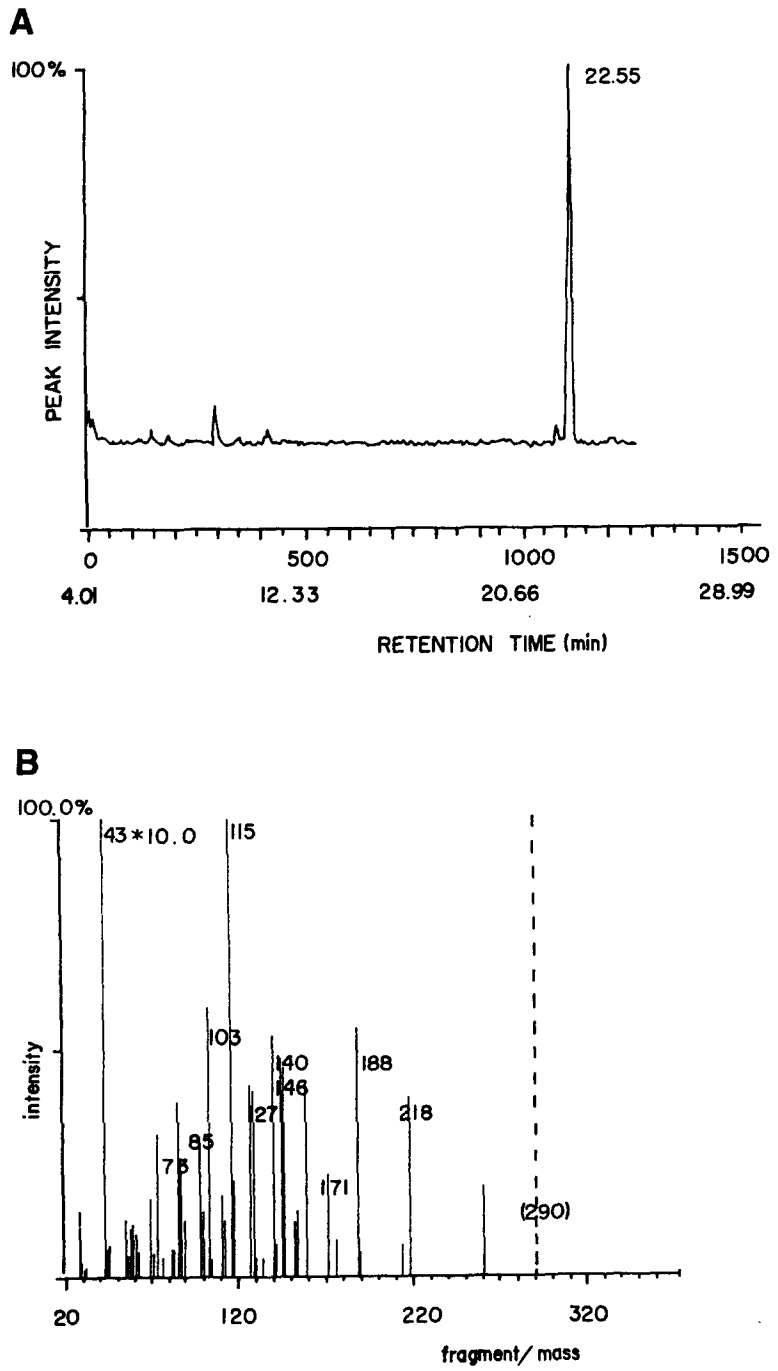

Fig. 1. GLC-MS of carboxy-reduced, hydrolyzed, reduced, and peracetylated *C. peruvianus* cuticle polygalacturonan. A. GLC profile on the capillary CG-IF-547 column. B. MS of the peak with a R_T = 22.55 min.

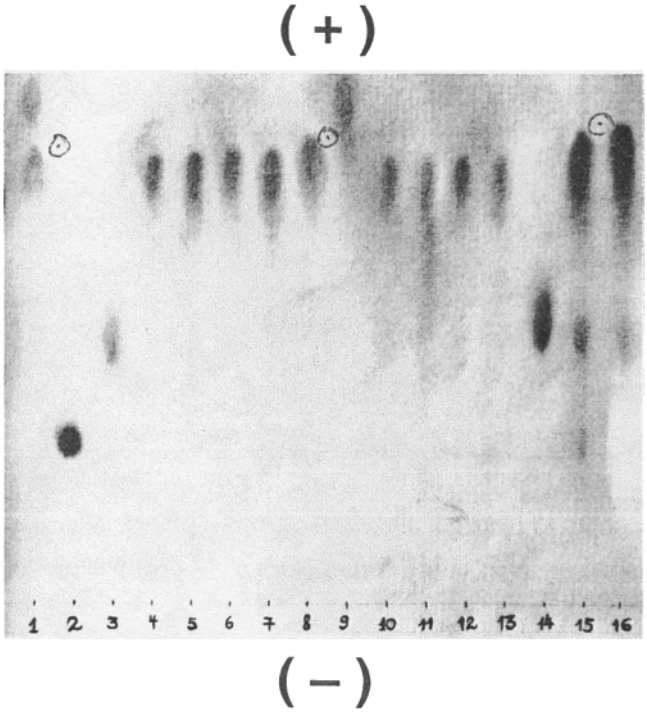

Fig. 2. Paper electrophoretogram of the enzymatic digest of *C. peruvianus* cuticle pectates. Standard: 1 = mixture of D-glucuronic + D-galacturonic acid (in order of increased mobility), 2 = L-rhamnose, 3 = D-galactose, 8 = D-galacturonic acid, 9 = D-glucuronic acid, and 14 = L-arabinose. Samples: 4,5 and 6,7 = CP-A/B pectate digested with snail enzymes or Novo Pectinex, respectively, 10,11 and 12,13 = CP-E/O pectate digested with snail enzymes or Novo Petinex, respectively, and 15 and 16 = orange pectin digested with snail enzymes or Novo Pectinex, respectively. ⊙ = bromphenol marker.

data indicated that pectic acid (mainly as the Ca^{2+} salt; Table 1) is a native structure found in the cladode cuticle. *Cereus* pectate polarimetric measurements with $[\alpha]^{25}_D$ in the +220–222° range are close to the value of +230° observed for orange pectin (*14*). Orange peel may afford 1/3 of the dry weight as pectin. The yield in the case of wax-free cactus cuticle is somewhat higher, being from 35 to 40%. Mostly because of their structural properties, such as lightly methoxylated carboxy-groups, low *O*-acetyl, and native conditions further increased by the strong acid/alkali extraction protocol, the cuticle pectate experienced gelation above the concentration of 1% (Fig. 3). This property was easily enhanced by the inclusion of 20 g% sucrose or 2% citric acid, additions very common in jelly and jam products. Calcium ions (e.g., from the respective chloride; data not shown) also provoked a similar gelling effect on sodium or ammonium pectates.

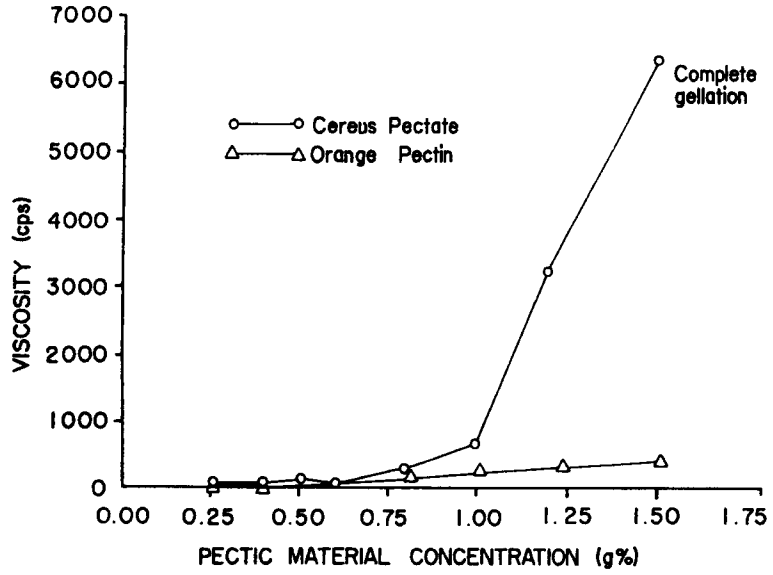

Fig. 3. Viscosimetric measurements. A = CP-A/B pectate. B = commercial sample of orange pectin.

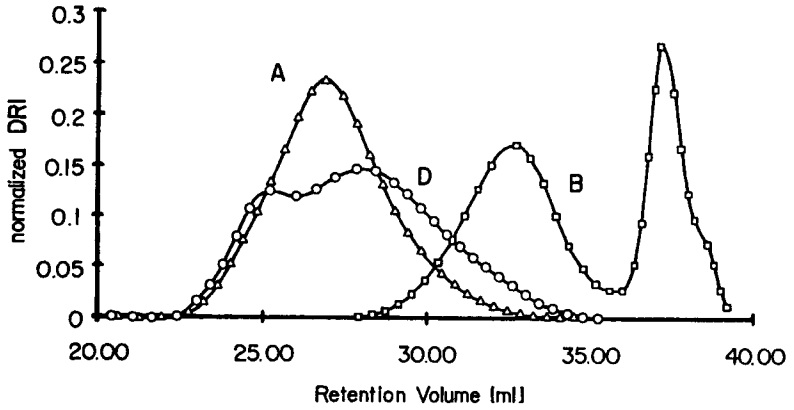

Fig. 4. SEC separation of pectates with absolute mol-wt distribution from DRI-LALLS-data. A = CP-A/B pectate (acid/base extraction), B = CP-E/O pectate (EDTA/oxalate extraction), and D = OP-A/B orange peel pectin (acid/base extraction).

Heterogeneity of polysaccharide structure in terms of variability of monosaccharide units is one known restriction for accurate determination of molecular weight. Having determined the absence of neutral branching units in *Cereus* cuticle pectate, this circumstances stimulated the absolute determination of molecular characteristics by means of double-monitored SEC. Detection of universal mass by DRI yielded SEC-eluograms, which indicated a significant difference of main peak molecular weight for samples CP-A/B (curve A) and OP-A/B (curve D) compared to sample CP-E/O (curve B) because of their position on the SEG-retention axis (Fig. 4).

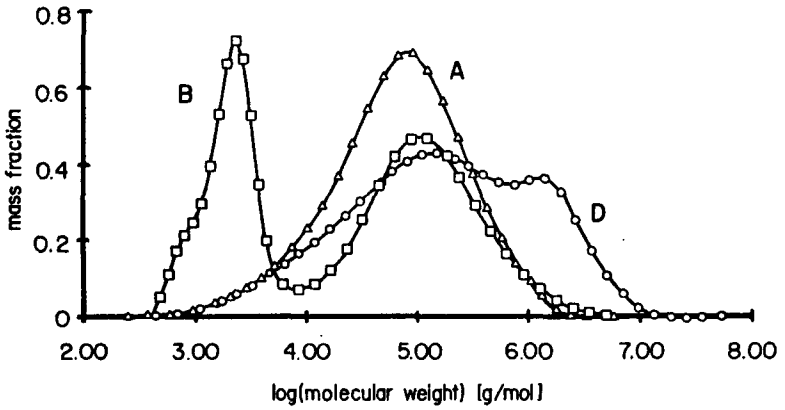

Fig. 5. Absolute mol-wt distribution from SEC-DRI/LALLS data (abbreviations as in Fig. 4).

Table 3
Pectate Mol-Wt Estimation by SEC-DRI/LALLS[a]

Sample	Mn, g/mol	Mol wt, g/mol
CP-A/B	20,000	150,000
CP-E/O	12,000	140,000
OP-A/B[b]	26,000	715,000

[a]Mn and mol wt values calculated from the retention volumes of Fig. 5 and mol-wt log of Fig. 5 after SEC column calibration with standard mol-wt polymers.
[b]OP-A/B = orange pectin extracted with strong acid/alkali schedule. Other abbreviations are as in the previous tables.

Additional inclusion of simultaneous monitored low-angle laser light scattering (LALLS) data corrected this first assumption and shifted the main contributing components to almost identical values close to log (M) = 5 (100,000 g/mol) (Fig. 5). Although weight average molecular weights for CP-A/B and CP-E/O (Table 3) differed only slightly, mol-wt distribution (Fig. 5) showed that CP-E/O is constituted by two populations, whereas CP-A/B is composed of a single (broad distributed) population. Sample OP-A/B, on the other hand, has to be assumed to consist of high- and midrange mol-wt fractions. Quite differently than the initially expected high similarity in terms of molecular weight from DRI-eluograms of Fig. 4 for samples CP-A/B and OP-A/B (both in turn different from CP-E/O), absolute molecular weight distributions from SEC-DRI/LALLS (Fig. 5) showed that all of them had a midrange domain of molecular weight in common. Sample CP-E/O additionally contained a low-mol-wt population and sample OP-A/B a high-mol-wt population.

Table 4
Gravimetric Balance for Anatomical Parts of *Cereus* Cladodes[a]

Anatomical part	% dry wt
Cuticle	19.9
Wax	1.3
Pectocellulose	18.6
Pectate	7.0
Cellulose	11.6
Gummy parenchyma	69.3
Reserve (aqueous)	59.2
Medular	10.1
Cellulosic vascular cylinder	10.8

[a]Rounded figures from duplicate (top and bottom zones of grown-up cladode; losses [<2%] during dissection of lyophilized cladode were not considered).

The fresh cactus phytobiomass gave rise to about 10–11% of dry matter. The gravimetric balance of the dissected botanical parts is shown in Table 4. Gum arising from the parenchyma (including the medular zone) accounted for most of the dry mass (average, 70%) and, owing to rheological properties (high viscosity), it is the most valuable product obtainable from the whole cactus. The cuticle is the next dominant fraction, corresponding to 1/5 of the dry mass and can be further separated into a high-density wax and pectocellulose in a ratio of 6:94. The pectocellulose subfraction itself is composed of 35–40% of pectic acid and 65–60% of cellulose. Hence, in approximate quantities, 1 t of fresh cactus (about 100 kg of dry matter) can provide about 7 kg of pectate. Considering that 1 ha of 3–4 y-cultivated fertiled soil (1–2 m spacing between individual plants; 5000–10,000 individual plants; average of 3 cladode columns/plant) may furnish 150–300 t of fresh cactus (1/10 of this as dry phytobiomass), the pectate yield could be 1.05–2.8 t/ha/year, along with a higher yield for gum and lesser yields for the other byproducts, such as fibrous and nonfibrous cellulose, and wax.

Owing to the low methoxyl content and gelling properties, *Cereus* cuticle pectate may deserve several industrial uses, such as in jams, jellies, gelled milk, yogurts, desserts, and so forth.

CONCLUSIONS

All features presently found on examination of the pectic material from the *C. peruvianus* cuticle, namely, high-yield, homopolymeric galacturonic backbone, and almost nonmethoxylated carboxyl contents heavily

charged with calcium ions, clearly indicated the highly important contribution of this pectate fraction to the compact and impermeable cuticular barrier of the cactus cladode. Taking into account that the cactus gum concentrated in the aqueous reserve parenchyma has potential industrial applications (*4*), any use for the cuticle pectate byproduct, as indicated by the present research, may raise the value of investigating *Cereus*.

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