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# Chemical characterization of the mucilage from fruits of *Opuntia ficus indica*

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**Abstract**

Extraction of peeled fruits of *Opuntia ficus indica* afforded with 3.8% yield a mucilage, which contained 23.4% of galacturonic acid. Total hydrolysis of a mucilage and gas–liquid chromatographic analysis of the derived alditol acetates indicated the presence of arabinose, rhamnose, xylose and galactose in the molar ratio 1.0:1.7:2.5:4.1. Gel permeation chromatography on Sepharose CL-4B showed the polysaccharide to be composed of at least five fractions. Treatment with cetrimide allowed the separation of an insoluble fraction (44.3% yield), which contained 28.0% of uronic acid. This fraction contained xylose, rhamnose and galactose in the molar ratio 1.0:2.5:2.8. The fraction soluble in cetrimide (15.6% yield) contained 16.0% uronic acid, and arabinose and galactose in the molar ratio of 1:2.2. It is composed of two main subfractions as shown by gel permeation chromatography. These results indicate that the mucilage from fruits *O. ficus indica* is a complex mixture of polysaccharides, less than 50% corresponding to a pectin-like polysaccharide.


*Keywords:* Opuntia ficus indica; Cactus; Pulp; Mucilage; Arabinogalactans; Rhamnogalacturonan

## 1. Introduction

*Opuntia ficus indica* Mill (Cactaceae) is cultivated in Chile for fruit production. In some countries the young stems (nopalitos) it is also used for human consumption (Sáenz, Corrales, & Aquino, 2002; Rodríguez-Félix, 2002). The production of mucilages, often referred as pectin polysaccharides, is characteristic of members of the Cactaceae family (Nobel, Cacelier, & Andrade, 1992). The chemical composition of *O. ficus indica* mucilage from cladodes (pads), commonly named nopal, has been the subject of various studies. Amin, Awad, and El-Sayed (1970) found that the mucilage was a neutral polysaccharide that contained arabinose, rhamnose, galactose and xylose. On the other hand, Paulsen & Lund (1979) reported that the extract from *O. ficus indica* was a mixture of a neutral glucan, glycoproteins and an acidic polysaccharide composed of L-arabinose, D-galactose, L-rhamnnose, D-xylose and D-galacturonic acid. According to Trachtenberg & Mayer (1981), the mucilage was a polysaccharide, which contained 10% uronic acids, arabinose, galactose, rhamnose and xylose. The composition of peeled nopals mucilage was quite similar, being mainly composed of L-arabinose, D-galactose, D-xylose and 19.4% of uronic acids (Madjdoub, Roudesli, Picton, Le Cerf, Muller and Grisel, 2001). A mucilage with a high content of galacturonic acid (64%) was isolated from fruit peels (Forni, Penci, & Polessello, 1994). McGarvie and Parolis (1981a,b) found that the mucilage of nopals was composed of a family of highly branched polysaccharides. They consisted of a backbone of α-D-galacturonic acid units linked 1→2 to β-L-rhamnose units linked 1→4 with branching on C-4, the branches being oligosaccharides of galactose which carry L-arabinose and D-xylose as substituents. Recently, Habibi, Heyraud, Mahrouz, and Vignon (2004a,b) reported the presence of a pectic polysaccharide from the skin of *O. ficus indica*, with a backbone of →2-α-L-rhamnose residues linked 1→4 to α-D-galacturonic acid carrying α-1→5-L-arabinan and β-1→4-galactan as side chains.

Although, the crude composition of the pulp of *O. ficus indica* fruits was determined in relation to the digestibility of casein (Lamghari El Kossori et al., 2000), little is known about the chemical composition and structural features of its mucilage. The present work was initiated to expand our knowledge of the mucilages from plants of Chilean arid lands. The aim of this work is the chemical characterization of the polysaccharides extracted from peeled fruits of *O. ficus indica*.

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## 2. Materials and methods

Fruits of *O. ficus indica* were collected in a private cultivar in Tiltil (70° 56′ S, 33° 04′W) in the month of November.

Total sugars were determined by a phenol–sulfuric acid method, using D-galactose as standard (Chaplin, 1986). The content of uronic acids was determined by a 3-hydroxydiphenyl method, using D-galacturonic acid and D-glucurono-6,3-lactone as standards (Filisetti-Cozzi & Carpita, 1991). Microanalysis of nitrogen was performed in Facultad de Química, Pontificia Universidad Católica de Chile. Proteins were determined with Bradford reagent using bovine serum albumin as standard (Boyer, 1993). High performance liquid chromatography (HPLC) analysis was carried out on a Merck-Hitachi L-6000 apparatus equipped with a L-4000 A UV detector employing a Whatman Partisil 10-Sax column (250× 4.6 mm). Elution was carried out with 0.02 M $KH_2PO_4$ (Gacesa, Squirre, & Winterburn, 1983). Gas–liquid chromatography (GLC) analysis of alditol acetates was carried out on a Shimadzu GC-14B gas chromatograph equipped with a flame ionization detector using a fused silica capillary column (15 m×0.25 mm) coated with SP-2330. GLC was performed with an initial hold at 150 °C for 2 min and then ramped at 5 °C/min to 210 °C for 10 min. The helium flow rate was 20 mL/min and the detector and injector temperatures were 220 °C. The identities of all derivatives were determined by comparison with authentic standards. $^1H$ and $^{13}C$ NMR spectra were registered at 70 °C with a Bruker Avance DRX 400 spectrometer operating at 400.13 MHz ($^1H$) and 100.62 MHz ($^{13}C$) using methyl alcohol as internal reference. Optical rotations were measured using a Perkin–Elmer 241 polarimeter.

### 2.1. Extraction

Fruits (12 Kg) of *O. ficus indica* were peeled, blended using an Alexanderwerk screw press and filtered through a cloth. The filtrate was centrifuged (4000×*g*) and the supernatant was concentrated in vacuo and dialysed against distilled water for 48 h. The resulting solution was concentrated in vacuo and poured into ethanol (1:5 v/v). The precipitate was separated by centrifugation, dissolved in distilled water and freeze-dried.

### 2.2. Total hydrolysis of the mucilage

The mucilage (0.020 g) and 90% v/v aqueous formic acid (16 mL) were heated in a sealed tube at 100 °C in an oven for 6 h. The resulting solution was diluted with distilled water (75 mL) and refluxed for 2 h. The solution was then concentrated in vacuo and the excess of acid was removed by repeated co-distillations with distilled water. The residue was dissolved in distilled water (4 mL) and chromatographed on a DEAE Sephadex A-25 column (30×2.5 cm). Elution was carried with distilled water for neutral sugars, and then with 10% v/v aqueous formic acid. It was monitored with the phenol–sulfuric acid reagent (Chaplin, 1986). The fraction eluted with distilled water was concentrated in vacuo, reduced with sodium borohydride and treated with acetic anhydride–pyridine. The alditol acetates were analysed by GLC. The acidic fraction was concentrated in vacuo and the residue was dissolved in distilled water (1 mL) and triethylamine (0.02 mL) was added. After 10 min, the product was analysed by HPLC.

### 2.3. Partial acid hydrolysis of the mucilage

The mucilage (0.100 g) was heated at 90 °C for 2 h with 0.08 M HCl (18 mL), cooled and poured into acetone (50 mL). The precipitate was filtered off, washed with acetone (3× 5 mL), dissolved in distilled water (10 mL) and freeze-dried. An aliquot was hydrolysed as the native mucilage. Optical activity was measured in water.

### 2.4. Saponification of the mucilage

To a 1.5% aqueous solution of the mucilage in water, 0.1 M NaOH was added until the pH had risen to 12. The solution was maintained for 48 h at 4 °C and then, 0.1 M HCl was added to bring the pH back to 5.0. The resulting solution was poured into five volumes of ethanol with vigorous stirring and the precipitate was separated by centrifugation (4000×*g*). The solid was washed thrice with 60% aqueous ethanol and dried.

### 2.5. Gel permeation chromatography

An aqueous solution of polysaccharide (1 mg/mL) was chromatographed on a Sepharose CL-4B column (100× 1.5 cm). The column was calibrated with 2 mL solutions of Blue Dextran 2000 (4 mg/mL) and with D-glucose (4 mg/mL). Elution was carried out with 0.2 M NaCl and monitored with phenol–sulfuric acid reagent (Chaplin, 1986).

### 2.6. Fractionation with cetrimide

Fractionation was conducted according to Scott (1965). To a 1% solution of the mucilage (500 mL) a 3% solution of cetrimide (*N*-cetyl-*N*,*N*,*N*-trimethyl-ammonium bromide, Merck) (185 mL) was added with stirring, until no more precipitate was formed. The mixture was stirred at 40 °C for 24 h and centrifuged at 4000×*g*. The solid was dissolved in 4M NaCl (250 mL) and poured into five volumes of ethanol. The precipitate was dissolved in distilled water, dialysed against distilled water, concentrated in vacuo and freeze-dried. The supernatant of the treatment with cetrimide was further treated with a 10% w/v solution of NaI until no further precipitation occurred. The precipitate was separated by centrifugation and the supernatant was concentrated in vacuo and freeze-dried.

## 3. Results and discussion

From 12 Kg of fruits of *O. ficus indica* a mucilage (3.8% yield) which contains 93.48% of sugars was obtained. The yield is higher than the value reported for the mucilage extracted from peels of the fruits (Forni et al., 1994) and is

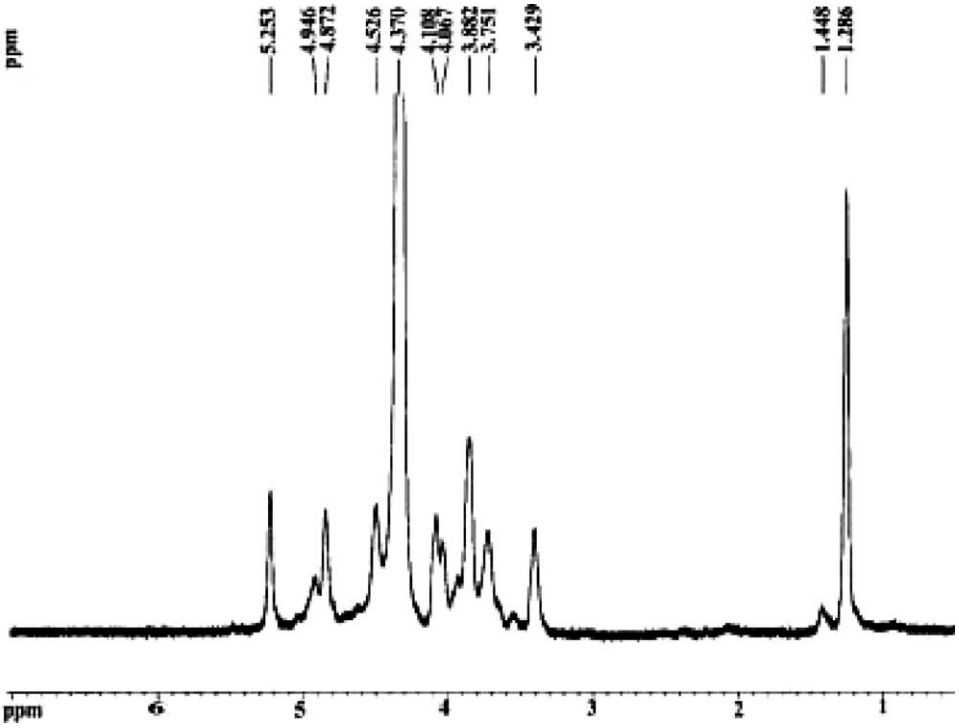


Fig. 1. $^{1}$H NMR spectrum (400.62 MHz) at 70 °C in $D_2O$ of the partially depolymerized polysaccharide from fruits of *Opuntia ficus indica*.

similar to those amounts obtained from nopals by Cárdenas, Higuera-Ciapara and Goycoolea (1997) and by Madjdoub et al. (2001). The uronic acid content determined spectrophotometrically by the m-hydroxydiphenyl method was 23.4%. After saponification of the mucilage, the content of uronic acids increased to 42.3%, which indicates that a considerable proportion of uronic acid was esterified. No proteins were detected by the Bradford method in the mucilage, nitrogen content microanalysis of 0.0% is in accordance with the former results. Total acid hydrolysis of the mucilage and GLC chromatographic analysis of the alditol acetates showed the presence of arabinose, rhamnose, xylose and galactose in the molar ratio 1.0:1.7:2.5:4.1. Analysis by HPLC of the acidic fraction of the hydrolysate identified the uronic acid as galacturonic acid. The native mucilage was submitted to partial acid hydrolysis since its solubility in water is very low for NMR spectroscopy studies. The $^{1}$H NMR spectrum at 70 °C of the partially depolymerized polysaccharide (Fig. 1), which contained 33.5% uronic acid, showed signals at 4.87, 4.52, 4.37, 4.06 and 3.88 ppm which were assigned according to the literature to $H_1$, $H_5$, $H_4$, $H_3$ and $H_2$, respectively, of α-D-galactopyranuronic acid units linked 1→4 (Grasdalen, Bakoy, & Larsen, 1988; Vignon & García-Joldon, 1996). The signal at 5.25 and 1.28 ppm were assigned to the anomeric and methyl protons at position-6, respectively, of α-L-rhamnopyranosyl residues. The signals at 4.10, 3.75 and 3.42 ppm were assigned to $H_2$, $H_5$ and $H_4$, respectively, of rhamnopyranosyl residues. Integration values for the anomeric proton of the galactopyranuronic acid, and of the anomeric and methyl protons of rhamnopyranosyl residues were 1.00, 1.37 and 3.18, respectively. In the $^{13}$C NMR spectrum a signal at 173.46 ppm was assigned to the carbonyl carbon of carboxyl group, nine more well defined signals were assigned to a quite regular repeating unit of a rhamnogalacturonan (Keenan, Belton, Matthew, & Steven, 1985; Vignon & García-Jaldon, 1996). The provisional assignments are given in Table 1. The downfield shift of the signal of $C_2$ (77.95 ppm) of rhamnopyranosyl residues may indicate that it is linked 1→2 as in the mucilage from stems of *O. ficus indica* (McGarvie & Parolis, 1981b).

The presence of two constituent monosaccharides was corroborated by total hydrolysis of the partially depolymerized mucilage. Analysis of the acidic monosaccharide constituents indicates the presence of D-galacturonic acid ($[\alpha]_D^{22^\circ} = +52.0^\circ, c, 0.10$, water; lit. $[\alpha]_D = +50.0^\circ$ for the α anomer) (Stanek, Cerny, Kocourek, & Pacak, 1963). The fraction of neutral sugars was composed of L-rhamnose ($[\alpha]_D^{22^\circ} = +9.3^\circ, c, 0.12$, water; lit. $[\alpha]_D = +8.0^\circ$) (Hough & Richardson, 1967). An aliquot of this fraction was reduced and analysed by GLC as the alditol acetate, co-chromatography with an authentic sample of tetra-*O*-acetyl-L-rhamnitol confirmed the presence of rhamnnose in the partially depolymerized mucilage.

It can be assumed that the treatment with diluted HCl solution produced the debranching of the polysaccharide leaving the backbone of a rhamnogalacturonan-type polysaccharide. In a similar experiment on the mucilage isolated from nopals of *O. ficus indica*, McGarvie and Parolis (1981a) found that the degraded polysaccharide was composed of rhamnose, galacturonic acid and of galactose in low proportion.

However, gel permeation chromatography on Sepharose CL-4B (Fig. 2) of the mucilage showed that it was heterogeneous. Treatment with cetrimide afforded an insoluble fraction (IF) in 44.3% yield and a soluble fraction (SF) in

Table 1
Assignment of signals in the $^{13}C$ NMR spectrum of the partially hydrolysed polysaccharide from fruits of *Opuntia ficus indica*

| Types of unit | Chemical shifts ($\delta$, ppm) | | | | | |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | C-5 | C6 |
| $\alpha$-D-galactopyranuronic | 100.39 | 70.06 | 70.96 | 78.90 | 71.95 | 173.46 |
| $\alpha$-L-rhamnopyranosyl | 99.16 | 77.95 | 70.96 | 73.28 | 70.20 | 17.85 |

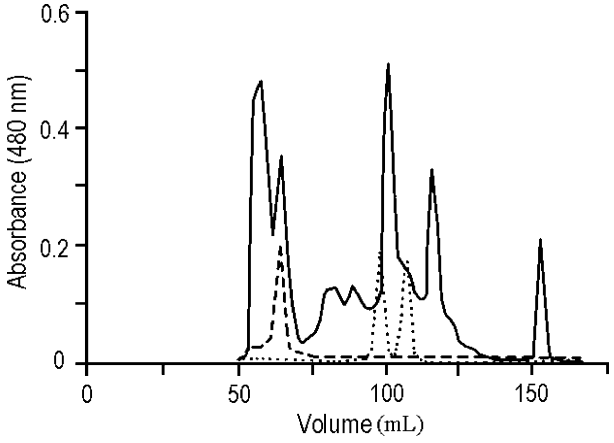


Fig. 2. Gel permeation chromatography on Sepharose CL-4B of the mucilage (—) from fruits of *Opuntia ficus indica*, IF (– –) (fraction insoluble in cetrimide) and SF (…) (fraction soluble in cetrimide).

15.6% yield. Analysis by gel permeation chromatography showed that IF contains a main fraction whereas SF is composed of two fractions (Fig. 2). Fraction IF contains 28.0% of galacturonic acid and the neutral sugars xylose, rhamnose and galactose in the molar ratio 1.0:2.5:2.8. Its $^1H$ NMR spectrum is not well resolved and presented in the anomeric region five signals which indicate the presence of a highly branched polymer. A broad singlet at 5.42 ppm was assigned to $H_1$ of $\alpha$-L-rhamnopyranosyl units. The soluble fraction, SF, still contains uronic acids (16.0%) and the neutral sugars, arabinose and galactose in the molar ratio 1.0:2.2. From *Opuntia dillenii* fruits, Srivastava & Pande (1974) isolated an arabinogalactan which by methylation analysis showed to be composed of 1→4 linked galactopyranosyl residues, approximately half of which carry arabinofuranosyl residues at *O*-3. Brillouet, Williams, Will, Müller and Pellerin (1996) isolated from apple juice an arabinogalactan with low proportions of uronic acids and proteins, its structure differs from that of the polysaccharide from *O. dillenii*. Methylation analysis showed that galactosyl residues linked 1→3 constituted the main chain with substitution at position 6 by galactose oligosaccharides with terminal arabinofuranosyl residues. Oosterveld, Voragen and Schols (2002) found in hop pectins an arabinogalactan fraction with a similar structure but it contained 13% of protein. From the skin of *O. ficus indica* fruits, a neutral arabinogalactan having a backbone of 1→4 linked β-D-galactopyranosyl residues branched at *O*−3 by L-arabinofuranosyl units was obtained (Habibi, Mahrouz, Marais, & Vignon, 2004a,b). Recently, Habibi, Mahrouz, and Vignon (2005) reported the presence of arabinan-rich polysaccharides in endosperm of the seed of *O. ficus-indica* fruits. The major polysaccharide was composed of $\alpha$-1→5-arabinofuranosyl residues substituted at O-2.

In conclusion, the results obtained in this work indicate that the mucilage isolated from peeled fruits of *O. ficus indica* is a complex mixture of polysaccharides, less than 50% of which corresponds to a pectin-like polymer. Arabinose is not present as ramification but constitutes different polysaccharides.

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