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Arabinan-rich polysaccharides isolated and characterized from the endosperm of the seed of *Opuntia ficus-indica* prickly pear fruits

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Abstract

After removal of starch, the reserve storage polysaccharide of the endosperm seed of *Opuntia ficus-indica* fruit was studied. Cell Wall Material (CWM) was extracted successively by boiling water (WSF), hot calcium chelating agent solution (CSF) and cold mild alkaline solution (CASF). All polysaccharides extracted were fractionated by ion-exchange chromatography into five fractions. The resulting major fractions were purified by size-exclusion chromatography and analyzed by sugar composition and glycosyl linkage analyses. The investigations were also supported by ^{1}H and ^{13}C NMR spectroscopy analysis. The results showed that the major fraction of WSF consisted of an arabinan. The backbone contained α-(1→5)-linked arabinofuranosyl residues with high percentage of arabinose units substituted at O-2. The predominant fractions from CSF and CASF were related to rhamnogalacturonan type I which consisted of a disaccharide repeating unit →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→backbone with α-(1→5)-linked arabinan side-chains attached to O-4 of the rhamnosyl residues.

Keywords: *Opuntia ficus-indica*; Prickly pear seed; ^{1}H and ^{13}C NMR spectroscopy; Arabinan; Pectin; Rhamnogalacturonan

1. Introduction

Cactaceaes are a plant family which exhibit *Crassulacean* Acid Metabolism (CAM) and are drought-tolerant. This explains why they are successively cultivated in arid and semi-arid regions. *Opuntia ficus-indica* (OFI) is the most widespread specie of the *Cactaceaes* family. This prickly pear cactus was exploited essentially for its fruits, which can constitute an important and abundant raw material for the food industry in arid regions (Ingelse, Basile, & Schirra, 1997).

The amount of seeds is important as it varies from 20 to 40% dry-weight of the whole fruit, depending on the cultivars (Barbera, Inglese, & La Mantia, 1994). Indeed, seeds of *O. ficus-indica* can play a key role in the valorization of the whole fruit. For that reason, different studies have been carried out on the chemical composition of seeds in the last decade. Their nutritive value was determined by Sawaya, Khalil, and Al-Mohammad (1983), and their reserve proteins have been studied by Uchoa, Souza, Zarate, Gomes-Filho, and Campos (1998). Their main conclusion is that such proteins have an amino acid composition similar to the 2S albumin storage protein family. The oil extracted from the seeds of prickly pear fruit was studied by numerous groups (Barbagallo & Spagna, 1999; Krifa, Villet, Krifa, & Alary, 1993; Sawaya & Khan, 1982). The results of physical and chemical analysis showed that their fatty acid composition is similar to other common edible vegetable oils. They suggested that *O. ficus-indica* seeds presented a good potential source of edible oil for human consumption.

The polysaccharides of the seeds of prickly pear fruit have attracted our attention and in a previous study we isolated several glucuronoxylan from their pericarp, with molar ratios of 4-*O*-methyl-glucuronic acid to xylose varying from 1:12 to 1:65 (Habibi, Mahrouz, & Vignon, 2002).

Arabinans were found in primary cell walls of different parts of plants of many families notably in seeds, fruits

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and roots. They appear either associated with pectic polysaccharides especially rhamnogalacturonan or as 'free' polysaccharides (Capek, Toman, Kardosova, & Rosik, 1983; Cardoso, Silva, & Coimbra, 2002; Churms et al., 1983; Eriksson, Andersson, Westerlund, & Aaman, 1996; Herve du Penhoat, Michon, & Goldberg, 1987; Jiang & Timell, 1972; Joseleau, Chambat, Vignon, & Barnoud, 1977; Larm, Theander, & Aman, 1975; Navarro, Cerezo, & Stortz, 2002; Petkowicz, Sierakowski, Ganter, & Reicher, 1998; Siddiqui & Wood, 1974; Stevens & Selvendran, 1980; Tharanathan, Bhat, Krishna, & Paramahans, 1985; Zawadzki-Baggio, Sierakowski, Correa, & Reicher, 1992). They usually carry a backbone of α-(1→5)-linked L-arabinofuranosyl units, more or less branched by single or multiple stubs of the same kind on positions 2 and/or 3. Various degrees of branching were found; a linear (1→5)-arabinan was found in apple juice (Churms et al., 1983) and in the coat of several legume seeds (Petkowicz, Reicher, Chanzy, Taravel, & Vuong, 2000; Petkowicz et al., 1998; Zawadzki-Baggio et al., 1992), while an almost linear one was found in the hypocotyl cells of *Vigna radiate* (Herve du Penhoat et al., 1987). In early papers, most of the branching was encountered on C-3 (Aspinall & Cottrell, 1971; Cardoso et al., 2002; Siddiqui & Wood, 1974) and more recently from the olive pomace (Cardoso et al., 2002). An arabinan with a low degree of branching mainly on C-2, was found in the roots of the horsebean (Joseleau, Chambat, & Lanvers, 1983).

In this work we describe the chemical composition and structural features of arabinan-rich polysaccharides isolated from the endosperm of the seeds of prickly pear fruit.

2. Experimental

2.1. Materials

Fresh mature prickly pear fruits of *O. ficus-indica* (OFI) were collected from the experimental station plantation located in the vicinity of Marrakech (Morocco). The harvested fruits were washed, carefully hand-peeled and the pulp was mixed for a few minutes in a mixer grinder. The seeds were recovered from the resulting pulp juice by straining through metallic strainer and cleaned by several washings in distilled water. After drying, they were cracked in an analytical grinder for a few minutes and the endosperm was recovered after sieving on 60 mesh sieve.

2.2. Analytical methods

Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen method (Blumenkrantz & Asboe-Hansen, 1973). Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by GLC as their corresponding alditol acetates, using a Packard and Becker 417 instrument coupled to a Hewlett-Packard 3380 A integrator. Glass columns (3 mm×2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh), or 3% OV 17 on the same support were used. The carboxyl groups of the D-galactosyluronic acid were reduced according to the method of Taylor and Conrad (1972). The carboxyl-reduced and the neutral samples were methylated twice by the Hakomori procedure, as described by Jansson, Kenne, Liedgren, Lindberg, and Lonngren (1976). The partially methylated carbohydrates were then converted into their alditol acetates by successive treatments with $NaBH_4$ and pyridine-Ac_2O and analyzed on a fused-silica widebore column (30 m×0.53 m) half bonded with SP-2380. Peak identification was based on retention times using partially methylated alditol acetates standard and confirmed by GLC by using a SP 2380 capillary column (0.32 mm) coupled to a Nermag R1010C mass spectrometer. Peak areas were corrected by using the molar response factors according to Sweet, Shapiro, and Albersheim (1975).

2.3. Preparation of cell wall material (CWM)

Fats, waxes and oils were removed from endosperm powder by refluxing in a Soxhlet apparatus during 24 h with 38:62 toluene–EtOH. The endosperm powder was suspended in water (water to pulp ratio of 20:1) and ground in a warring blender apparatus for 10 min. The slurry was then poured on 0.25 mm sieve and washed with water to remove the starch granules. The residual starch content was removed by treatment with pancreatic α-amylase in order to prepare CWM. The sample was suspended in 0.2 M NaOAc buffer solution (pH 6.1) containing a 1% α-amylase in order to make 1 mg amylase for 10 mg of sample. The mixture was incubated at 37 °C for 36 h. The suspension was centrifuged and the supernatant was removed. The resulting residue was washed exhaustively with water and freeze-dried to give an amylose-free sample, CWM (52% of dry matter).

2.4. Isolation of polysaccharides

Polysaccharides were sequentially extracted from CWM by water (2×2 h at 100 °C) and aqueous solution of calcium chelator agent 0.5% EDTA (2×2 h at 100 °C) and finally by 0.5 M NaOH aqueous solution (2×1 h at 20 °C). The alkaline extract was neutralized by acetic acid solution 20% at pH≈5–6. The different extracts were precipitated with EtOH (4 vol) resulting, in a water-soluble fraction (WSF), a chelating soluble fraction (CSF) and a cold alkaline soluble fraction (CASF). The extraction scheme is given in Fig. 2.

2.5. Ion-exchange chromatography

The different fractions (WSF and CSF) were partially esterified, and were saponified with 0.1 M NaOH (overnight, N_2, 4 °C) in order to hydrolyze the acetyl and methyl esters. The solution was then acidified to pH 4–5 by addition