Food Chemistry 130 (2012) 937–944

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Arabinan and arabinan-rich pectic polysaccharides from quinoa (*Chenopodium quinoa*) seeds: Structure and gastroprotective activity

Lucimara M.C. Cordeiro [a,*], Vanessa de Fátima Reinhardt [a], Cristiane H. Baggio [b], Maria Fernanda de Paula Werner [b], Ligia Moura Burci [b], Guilherme L. Sassaki [a], Marcello Iacomini [a]

[a] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, CP 19.046, CEP 81.531-980 Curitiba, PR, Brazil
[b] Departamento de Farmacologia, Universidade Federal do Paraná, CEP 81.531-980 Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 9 March 2011
Received in revised form 20 May 2011
Accepted 5 August 2011
Available online 10 August 2011

Keywords:
Arabinan
Arabinan-rich pectic polysaccharides
Chenopodium quinoa
Gastroprotective activity

ABSTRACT

After removal of starch, cell wall polysaccharides of seeds of quinoa were studied. They were extracted successively with water and with aq. 10% KOH. The extracted polysaccharides were fractionated and purified by freeze–thaw treatment and by sequential ultrafiltration through membranes. The purified fractions (PQW, K2-30EM, K1-10RM and K1-30RM) were analyzed by sugar composition, HPSEC, methylation and ^{13}C NMR spectroscopy analysis. The results showed that PQW consisted of a linear arabinan with (1 → 5)-linked α-L-arabinofuranosyl units. Fractions K2-30EM, K1-10RM and K1-30RM were related to rhamnogalacturonan type I with a branched arabinan and galactan side-chains. This arabinan has (1 → 5)-linked α-L-arabinofuranosyl units substituted exclusively in O-3. The main differences between these fractions were their molecular mass and content of Rha and GalA, which probably arise from an increase in their rhamnogalacturonan backbone. A pool of these polysaccharides (arabinan and arabinan-rich pectic polysaccharides) showed gastroprotective activity on ethanol-induced acute gastric lesions in rats.

1. Introduction

Quinoa (*Chenopodium quinoa* Wild) is a pseudocereal of Andean origin and is used principally in the same manner as wheat and rice (Hirose, Fujita, Ishii, & Ueno, 2010). There has been increasing interest in quinoa due to its superior nutritional quality compared to other grains and for not having gluten. Thus, quinoa is an alternative ingredient in the gluten-free diet and can be used by persons who suffer from celiac disease (Alvarez-Jubete, Arendt, & Gallagher, 2010). For these reasons, different studies have been carried out on the chemical composition of the quinoa seeds in the last decade and this pseudocereal has been noted as a new foodstuff in the world (Hirose et al., 2010).

The studies concerning their proteins revealed that it contains a balanced essential amino acid composition, with a high content of essential amino acids, and is thus superior to that of common cereals (Drzewiecki et al., 2003).

Lipid content of quinoa is also higher (between 2 and 3 times) than in common cereals and rich in unsaturated fatty acids, which are desirable from a nutritional point of view (Alvarez-Jubete, Arendt, et al., 2010). Moreover, quinoa contains adequate levels of important micronutrients such as minerals and vitamins and significant amounts of other bioactive components, such as polyphenols, which exerts antioxidative properties (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Hirose et al., 2010).

Concerning the carbohydrates, they consists the major component in quinoa, and varies from 67% to 74% of the dry matter (Jancurová, Minarovicová, & Dandár, 2009). Starch is the only carbohydrate reported and its content varies from 51% to 61%, being stored in the cells of the perisperm. It can be used for specialized industrial applications due to its small granules, high viscosity and good freeze–thaw stability (Watanabe, Peng, Tang, & Mitsunaga, 2007). Moreover, studies have shown that quinoa represent a good source of dietary fiber, with a range from 1.1% to 16.3%, which is much higher than that of rice (0.4%), wheat (2.7%) and corn (1.7%) (Alvarez-Jubete, Arendt, et al., 2010). This is in particular important for celiac disease patients, where the intake of fiber in the gluten-free diet is considered to be inadequate, and thus the incorporation of quinoa seeds in their diets should help alleviate, at least in part, their deficit in fiber intake (Alvarez-Jubete, Arendt, et al., 2010).

The polysaccharides that compose the dietary fiber of quinoa have attracted our attention, and there is no report in the literature about their structures. Polysaccharides have beneficial effects on health and are ubiquitous in plant foods. To better understand

* Corresponding author. Tel.: +55 41 33611655; fax: +55 41 32662042.
E-mail address: lucimaramcc@ufpr.br (L.M.C. Cordeiro).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.08.020

the bio-functionality of polysaccharides scientific elucidation of the structures responsible for the beneficial effect is very important (Yamada, Kiyohara, & Matsumoto, 2003). Thus, in this work the chemical composition, structural features and gastroprotective activity of arabinan and arabinan-rich pectic polysaccharides isolated from the seeds of quinoa (*C. quinoa*) have been described.

2. Materials and methods

2.1. Plant material

Seeds of *C. quinoa* were purchased at local market (QUINUA REAL®).

2.2. General analytical methods

The total lipid quantitation was performed by the method of Bligh and Dyer (1959).

Fractions were carboxy-reduced by the carbodiimide method (Taylor & Conrad, 1972), using $NaBH_4$ as the reducing agent, giving products with the –COOH groups of its uronic acid residues reduced to $–CH_2OH$.

2.3. Extraction and purification of polysaccharides

Seeds of quinoa (466.6 g) were milled and then deffated with acetone, in order to remove lipids, pigments and other hydrophobic material. The polysaccharides were extracted from the residue with water at 60 °C for 4 h (8×, 1 l each). The aqueous extracts were obtained by centrifugation (3860*g*, 20 min at 25 °C), joined and concentrated under reduced pressure. The polysaccharides were precipitated with EtOH (3 vol.) and freeze-dried, giving fraction QW. The remaining residue was then extracted twice (1 l each) with aq. 10% KOH, at 100 °C for 4 h and the alkaline extracts were neutralized with acetic acid, dialyzed for 48 h with tap water, concentrated under reduced pressure and freeze-dried, originating fractions QK1 and QK2.

In order to remove starch, fractions QW, QK1 and QK2 were extensively treated with α-amylase (from *Bacillus licheniformis*, Sigma A3403) and dialyzed. Moreover, to remove proteins, they were treated with 10% aqueous trichloroacetic acid and/or Pronase (Roche) and newly dialyzed. Then, a freeze–thaw treatment was applied in these fractions, to give cold-water soluble fractions SQW, SQK1 and SQK2. In this procedure, the sample was frozen and then thaw at room temperature. Insoluble polysaccharides were recovered by centrifugation.

The cold-water soluble polysaccharides were purified by sequential ultrafiltration through membranes (Millipore) with cut-offs of 100 kDa (PLHK04710-Ultracel), 30 kDa (PLTK04710-Ultracel) and 10 kDa (PLGC04710-Ultracel).

The yields were expressed as percentage based on the initial weight of quinoa seeds that were submitted to extraction (466.6 g).

2.4. Sugar composition

Neutral monosaccharide components of the polysaccharides and their ratio were determined by hydrolysis with 2 M TFA for 8 h at 100 °C, followed by conversion to alditol acetates by successive $NaBH_4$ or $NaBD_4$ reduction, and acetylation with Ac_2O-pyridine (1:1, v/v, 1 ml) at room temperature for 14 h, and the resulting alditol acetates extracted with $CHCl_3$. These were analyzed by GC–MS using a Varian model 3300 gas chromatograph linked to a Finnigan Ion-Trap model (ITD 800) mass spectrometer, with He as carrier gas. A capillary column (30 m × 0.25 mm i.d.) of DB-225, hold at 50 °C during injection for 1 min, then programmed at 40 °C/min to 220 °C and hold at this constant temperature for 19.75 min was used for the quantitative analysis.

Uronic acid contents were determined using the m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991).

2.5. Determination of homogeneity of polysaccharides and molecular weight of components

The homogeneity and average molar mass (M_w) of soluble polysaccharides were determined by high performance steric exclusion chromatography (HPSEC), using a differential refractometer (Waters) as detection equipment. Four columns were used in series, with exclusion sizes of 7×10^6 Da (Ultrahydrogel 2000, Waters), 4×10^5 Da (Ultrahydrogel 500, Waters), 8×10^4 Da (Ultrahydrogel 250, Waters) and 5×10^3 Da (Ultrahydrogel 120, Waters). The eluent was 0.1 M aq. $NaNO_2$ containing 200 ppm aq. NaN3 at 0.6 ml/min. The sample, previously filtered through a membrane (0.22 μm, Millipore), was injected (250 μl loop) at a concentration of 1 mg/ml. The specific refractive index increment (*dn/dc*) was determined and the results were processed with software ASTRA provided by the manufacturer (Wyatt Technologies).

2.6. Methylation analysis of polysaccharide

The purified polysaccharides were O-methylated according to the method of Ciucanu and Kerek (1984), using powdered NaOH in DMSO-MeI. The per-O-methylated derivatives were pre-treated with 72% v/v H_2SO_4 for 1 h at 0 °C and then hydrolyzed for 16 h at 100 °C after dilution of the H_2SO_4 to 8%. This was then neutralized with $BaCO_3$ and the resulting mixture of partially O-methylated monosaccharides was successively reduced with $NaBD_4$ and acetylated with Ac_2O-pyridine. The products (partially O-methylated alditol acetates) were examined by capillary GC–MS. A capillary column (30 m × 0.25 mm i.d.) of DB-225, held at 50 °C during injection for 1 min, then programmed at 40 °C/min to 210 °C and held at this temperature for 31 min was used for separation. The partially O-methylated alditol acetates were identified by their typical electron impact breakdown profiles and retention times (Sassaki, Iacomini, & Gorin, 2005).

2.7. Nuclear magnetic resonance (NMR) spectroscopy

^{13}C NMR spectra and DEPT-135 experiment (Distortionless Enhancement by Polarization Transfer) were obtained with a Bruker DRX 400 MHz AVANCE III NMR spectrometer (Bruker Daltonics, Germany), according to standard Bruker procedures. Analyses were performed with a 5 mm inverse gradient probe, at 50 °C, the water soluble samples being dissolved in D_2O and the water-insoluble ones in Me_2SO-d_6. Chemical shifts are expressed as δ PPM, using the resonances of CH_3 groups of acetone internal standard (δ 30.2), or Me_2SO-d_6 (δ 39.7). The spectra were handled using the computer program Topspin® (Bruker) and the assignments were performed using ^{13}C (zgpg) and DEPT-135 (dept135) programs.

2.8. Animals

Experiments were conducted using female Wistar rats (180–250 g), provided by the Federal University of Paraná colony. Animals were kept under standard laboratory conditions (12 h light/dark cycles, temperature 22 ± 2 °C) with food and water provided *ad libitum*. The study was conducted in accordance with the "Principles of Laboratory Animal Care" (NIH Publication 85-23, revised 1985) and approved by local Ethics Committee (CEEA/UFPR; approval number 473).