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Characterization of pectins extracted from pomegranate peel and their gelling properties

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ABSTRACT

The composition of pomegranate peel, the main by-product during pomegranate processing, and some of the characteristics of the water-soluble pectins were investigated. Four tunisian pomegranate peels were subjected to hot aqueous extractions (86 °C, 80 min, 20 mM nitric acid). Pomegranate peels yielded between 6.8% and 10.1% pectins. The extracted pectins were low methylated and were characterized by the predominance of homogalacturonan regions. Principal component analysis applied on FT-IR spectral data in the region between 4000 and 650 cm^{-1} differentiated the samples according to their degree of methylation. At pH 3, in the presence of 0.7% pectin, all solutions showed a rapid gel formation with $G' > G''$. With decreasing temperature from 90 °C to 10 °C, G' increased to reach a plateau at 10 °C. The variation in the pectin gel formation between varieties was attributed to difference in pectin characteristics particularly the hydrodynamic volume and the neutral sugar content.

1. Introduction

Pectin is a gelling biopolymer originating from plants, and is an essential component in initial cell growth as well as in the ripening process. Pectin is mainly present in the primary cell wall and in the middle lamella of plants, and makes up around 40% (dry matter basis) of the cell wall of fruits and vegetables (Brett & Waldron, 1996). Pectin is also one of the gelling agents added to food products to achieve desired texture or consistency, particularly in jam and jelly manufacturing (May, 1990).

The pectin chain structure mainly consists of α-(1-4)-D-galacturonic acid units forming long homogalacturonic chains interspersed by rhamnogalacturonan sections where rhamnose and galacturonic acid residues alternate. Neutral sugar units are attached to the backbone and concentrated in highly branched "hairy" regions (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Part of the carboxylic groups in the galacturonic chain exists in methyl ester form, and the degree of methylation (DM) divides pectin into two types. In the high-methoxyl (HM) form, more than 50% of the carboxyl groups are methylated, while in the low-methoxyl (LM) form less than 50% are methylated. The degree of methylation is crucial for the gel formation of pectin. Low methoxyl (LM) pectins are often used in low-sugar products due to their gel-forming properties without or with a small amount of sugar and in the presence of Ca^{2+}. At high sucrose concentrations LM pectin tends to pregel (May, 1990). Gel formation of LM pectin occurs over a wide range of pH values, and the efficient Ca^{2+}-binding is an important factor both at low and high pH values. The distribution of free and esterified carboxyl groups in the pectin backbone affects the strength of the Ca^{2+}-binding and thus the functionality of both LM and HM pectin gels (Logfgren, Guillotin, Evenbratt, Schols, & Hermansson, 2005).

The proposed mechanism for LM pectin gelation is based on the so-called egg-box model, with formation of gel networks through ionic cross links with divalent cations, usually calcium. These junction zones are also stabilized by hydrogen bonds and include highly retained water molecules (Braccini & Perez, 2001).

The microstructure and rheology of pectin gels are affected by several parameters, such as sucrose content, pH, temperature, and Ca^{2+} ion concentration.

The gelling ability of pectin depends on its solubility and viscosity, which are a measure of its molecular weight (Rao, 1993). The viscosity depends not only on the concentration of the polymer but also on the molecular weight and shape, pH and ionic strength.

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The higher the molecular weight, the higher is its viscosity and hence, the better is its grade (Rao, 1993).

The major sources of commercial pectins are citrus wastes (pulp and peel), and apple pomace, while some specific products may be extracted from sugar-beet pulp (Arslan & Kar, 1998).

In Tunisia, the total annual production of pomegranate (*Punica granatum* L.) was 71.597 tons in 2010 (Ayed, 2011). In recent years, pomegranate is increasingly consumed as various products such as juices, jams and jellies. In pomegranate juice industry, 1 ton of fresh fruit generates 669 kg by-product containing 78% peel and 22% seeds (Qu et al., 2009). Pomegranate peels, remaining as a valuable by-product, was studied for its antibacterial, antioxidant and anti-allergic activities (Panichayupakaranant, Tewtrakul, & Yuenyongsawad, 2010). However, little attention was devoted to the study of pectin fraction from pomegranate peel.

The objective of this research was to examine the physicochemical characteristics and the gelling properties of pectins extracted from different Tunisian ecotypes of pomegranate peel.

2. Materials and methods

2.1. Plant material

Pomegranate fruits, ecotypes "Acide" (Ac), "Gabsi" (Ga), "Nebli" (Ne) and "Tounsi" (To), were collected from the same oasis at Gabes region (southeast of Tunisia) at mature stage. Fruits were manually peeled then the collected peels were ground into small pieces (particles' size were 1–3 mm) and stored at −12 °C for further physicochemical characterization.

Samples were dried at 50 °C until constant weight, ground and milled through 0.5 and 1.25 mm sieves. Final powders with sizes between 0.5 and 1.25 mm were retained for pectin extraction.

2.2. Pectin extraction

Pomegranate peel pectin was extracted with nitric acid solution (HNO_3) (solid–liquid ratio; 1:50; g/mL). The mixture was stirred at 400 rpm (Stirrer Heidolph RZR 20051 electronic, Germany) using optimal extraction conditions obtained in a previous work (80 min, 86 °C and 20 mmol/L nitric acid). The resulting slurries were allowed to cool to room temperature (25 °C) and filtered through cheesecloth. Two volumes of 96% w/w ethanol were added to the filtrate for pectin precipitation and the obtained mixture was kept for 1 h at 4 °C. After centrifugation at 8000 × *g* for 20 min at 10 °C, the pectin precipitate was washed with 50%, 75% and two times with 100% ethanol and centrifuged at 5000*g* for 10 min at 10 °C. Finally, the obtained pectin was dried at 45 °C to a constant weight, ground in a mortar and subjected to further analyses. The gravimetric yield extraction was estimated as the ratio between the weight of the powdered pectin and the weight of the flour raw material (%, w/w), both on a dry basis.

2.3. Physico-chemical characterizations

Proximate composition of the raw material (moisture content, lipid, ash and protein content) was determined according to the method described by the Association of Official Analytical Chemists (AOAC, 1997). Dry matter was determined by drying samples at 105 ± 3 °C to constant weights (AOAC, 1997). The total ash was determined by calcination in muffle furnace at 550°C until constant weight was obtained (AOAC, 1997). The total nitrogen concentration was obtained using Kjeldahl method (AOAC, 1997), and the protein concentration was estimated using a nitrogen conversion factor of 6.25. Total, soluble and insoluble fibre contents were determined according to the AOAC enzymatic-gravimetric method of Prosky, Asp, Schweizer, De Vries, and Fruda (1988). Fat content was determined by Soxhlet extraction with hexane at boiling point of the solvent (AOAC, 1997). Soluble sugars content was determined by the phenol-sulfuric acid method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956) after extraction with ethanol solution 96% (v:v). The insoluble sugars content was determined by the same method after hydrolyse of the insoluble fraction with chloridric acid (30%) at 60 °C for 2 h. The total sugars content is the sum of soluble and insoluble sugar. Individual neutral sugars (NS) were measured as alditol acetates with inositol as an internal standard (i) in the pomegranate peel after prehydrolysis in 250 µL of 72% sulfuric acid (1 h, room temperature) followed by hydrolysis with 1 mol/L sulfuric acid (3 h, 100 °C) and (ii) in the pectins extracts after hydrolysis in 1 mol/L sulfuric acid (3 h, 100 °C). After hydrolysis, they were derivatized to alditol acetates (Englyst & Cummings, 1984). They were injected on a gas chromatography-flame ionization detector HP5890 Series II (Agilent, Inc., Palo Alto, CA) with a capillary column of 30 m × 0.25 mm i.d. coated with DB225 mass spectrometry (MS), having a 0.25 mm film thickness (J&W Scientific, Agilent, Inc.). The conditions for injection were as follows: hydrogen was the carrier gas at 45 cm/s (at 215 °C); the column flow was 1.3 mL/min; the temperature was 250 °C in split mode (ratio 1:25); and the oven temperature was isothermal at 215 °C. The methanol concentration (MeOH) in pectin powders was determined according to Renard and Ginies (2009) by Headspace-GC–MS after saponification. Samples (10 mg) were dissolved or suspended in 3.8 mL of distilled water and then saponified by the addition of 0.8 mL of 1 mol/L KOH containing CD_3OH (1.4 µmol/mL) as an internal standard, and incubated for 2 h at room temperature. For GC, a Shimadzu QP2010 GC–MS was used with a Cp-wax-52cb 30 m × 0.32 mm × 0.5 µm capillary column (Varian, Inc., Palo Alto, USA) equipped with an AOC5000 auto sampler. A sealed vial was placed at 50 °C for 15 min and then 0.5 mL of head-space was injected into the split injector (1:10 ratio). The GC conditions were as follows: helium as gas carrier at 45 cm/s and oven temperature at 40 °C (isothermal). The mass detector conditions were: electronic impact ionization mode (70 eV), temperature of source 200 °C with data collected using SIM for selected ions (*m*/*z* 31; 32; 35) at 5 scans/s. The degree of methylation (DM) was calculated as the molar ratio of methanol to uronic acid.

The uronic acid content (against a galacturonic acid standard) was determined colorimetrically at 520 nm in both peel and pectin powders by the meta-hydroxyl-diphenyl assay according to Blumenkrantz and Asboe-Hansen (1973) after saponification (see the methanol assay).

2.4. FT-IR analysis of extracted pectins

Fourier Transform Infrared (FT-IR) spectra of pectin samples were obtained at room temperature with a Tensor 27 FTIR spectrometer (Bruker Optics, Wissembourg, France) equipped with a single-reflectance horizontal ATR cell (Golden Gate equipped with a diamond crystal, Bruker Optics). The freeze-dried homogenized samples were placed at the surface of the diamond crystal and were pressed with a system press tip flap. The samples were scanned at wavenumbers ranging from 4000 to 650 cm^{-1} and corrected against the background spectrum of air. The spectrum of each sample was obtained by taking the average of 16 scans.

2.5. Hydrodynamic volume of extracted pectins

The hydrodynamic volume distribution of pectin samples were determined using a high pressure size exclusion chromatography (HPSEC) system comprising a Jasco LC-NET II/ADC interface, a Jasco PU-2080 plus intelligent HPLC pump, a Jasco RI-2031 plus