Food Chemistry 215 (2017) 318–325

Contents lists available at ScienceDirect

Food Chemistry

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

Characterization of pectins extracted from pomegranate peel and their gelling properties

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ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form 28 July 2016
Accepted 29 July 2016
Available online 30 July 2016

Keywords:
Punica granatum L.
Extraction
Pectin
Chemical composition
Rheological properties

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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http://dx.doi.org/10.1016/j.foodchem.2016.07.181
0308-8146/

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

intelligent RI detector, and a degasser, and was controlled by ChromNav software (Jasco, Tokyo, Japan). Separations were achieved using two columns in series: a 8.0 mm × 300 mm i.d. OH-pack SB-802 HQ column (Showa Denko Europe, Munich, Germany) and a 300 × 7.8 mm i.d. TSK-Gel PWXL column (Tosohaas, Stuttgart, Germany) at 35 °C and a 40 × 6.0 mm i.d. guard column TSK-Gel PWXL (Tosohaas, Stuttgart, Germany). Solutions (20 μL) of the extracts (0.5%) were injected and eluted with 0.4 mol/L sodium acetate buffer pH 3.5 at 0.8 mL/min. Dextrans T500 and T40 (Pharmacia BioProcess Technology, Uppsala, Sweden) and glucose (Sigma-Aldrich, Deisenhofen, Germany) were used to calibrate the column system. All data are presented as a function of elution time of the samples (ET).

2.6. Viscometry of extracted pectins

Reduced viscosities were determined using 0.125; 0.25; 0.5; 0.75 and 1 g/L pectin solutions prepared in 0.1 mol/L sodium phosphate buffer (pH 7). After dissolution under stirring at ambient temperature for 12 h, the solutions were filtered (0.45 μm). The viscosities of pectin solutions at different concentrations were determined at 20 °C by means of a rheometer (Physica MCR 301: Anton Paar, Germany) using a double gap DG26.7.

The specific viscosity (η_{sp}) of a macromolecular solution is defined by Eq. (1).

$$\eta_{sp} = \frac{(\eta_s - \eta_0)}{\eta_0} \quad (1)$$

The viscosity of the solution is η_s, whereas the viscosity of the solvent is η_0. The reduced viscosity (η_{red}) of a macromolecule in solution is defined by Eq. (2).

$$\eta_{red} = \frac{\eta_{sp}}{C} \quad (2)$$

Here, C is the concentration of the macromolecule.

2.7. Rheological properties of pectin gels

2.7.1. Preparation of pectin gels

For gel preparation at pH 7, 0.116 g of pectin powders were dispersed into 15.21 mL of distilled water and mixed for 120 min at 20 °C, in order to get a complete hydration. The pH was adjusted to 7.1–7.2 with sodium citrate solution. The pectin solutions were heated to 85 °C, upon which 0.0087 g of calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), dissolved in 1 mL of water, was added under magnetic stirring for 5 min. The final pectin concentration was 0.7% (w/v), while the final calcium concentration was 0.04%.

For gel preparation at pH 3, dry pectin powder (0.106 g) was dissolved in 12.2 mL of citrate buffer pH 3 (citric acid 0.45% and sodium citrate 0.1%) and stirred for 120 min at 20 °C. The mixtures were then heated, saccharose was added in 3 portions to a final concentration of 30% and the solution was heated once more until boiling. Afterwards, 0.026 g of calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), dissolved in 1 mL of water, was added with a final concentration of 0.1% and mixed under intense stirring. The solution was boiled again. The final pectin concentration was 0.7%.

2.7.2. Rheological measurements

The applied rheometer was a Physica MCR 301 (Anton Paar, Germany). Oscillation measurements (temperature sweep) of storage modulus G′ and loss modulus G″, in the linear viscoelastic domain, were made using a double gap DG26.7. Pectin gels prepared at pH 7 and pH 3 were transferred on to the pre-heated rheometer at 80 °C and 90 °C, respectively, and cooled to 10 °C with a cooling rate of 2 °C/min. The sample was coated with silicone oil and the cylinder was closed with a special lid in order to avoid evaporation. Dynamic rheological parameter (G′ and G″) were recorded during cooling at a frequency of 1 Hz and deformation amplitude of γ 0.1%.

2.8. Statistical analysis

Experiments were conducted in triplicate and the differences between treatment means were determined by Duncan's procedure at $P < 0.05$ using the SPSS statistics 19. The expressed values are means ± standard deviation of triplicate measurements.

Applied on FT-IR spectral data, principal component analysis (PCA) usually allows to discriminate contrasted samples and group similar samples according to their physico-chemical properties. The Spectral preprocessing and multivariate data analysis were performed with Matlab 7.5 (Mathworks Inc. Natick, MA) software using SAISIR package (Cordella & Bertrand, 2014). The absorption band around 2400 cm^{-1}, due to carbon dioxide, was discarded. Spectra were systematically pre-treated by standard normal variate correction (SNV).

3. Results and discussion

3.1. Composition of pomegranate peel

The results for physicochemical characteristics of pomegranate peel from different ecotypes are displayed in Table 1. Moisture content in peel ranged from 67.26% in Ac ecotype to 73.23% in Ga ecotype. It can be noticed that the peel of pomegranate of all ecotypes is devoid of fat. The ash content ranged from 3.71 to 4.97% dw. The protein content in (Ga) ecotype is significantly higher than that of other ecotypes (7.13% dw). This value is also higher than that found in other Turkish cultivars, 'Lefon', 'Seedless', 'Kadi', 'Siyah' and 'Koycegiz', whose contents are 3.19; 3.11; 3.06; 2.67 and 2.58% dw, respectively (Hepaksoy, Aksoy, Can, & Ui, 2000).

Total sugars, which account for 30.65–34.83% of the pomegranate peel, consist mainly of soluble fraction (27.33–32.33% dw) which is significantly higher than that insoluble (2.49–3.31% dw). Pomegranate peel contained higher amount of sugars than that of the skin of yellow and purple cultivars of passion fruit: 25 and 28%; respectively (Espiard, 2002). Neutral sugar contents accounted for 29.91–31.00%. These amounts are lower than those found in mango and lime peels: 37 and 41%, repectively (Koubala et al., 2008). Glucose (Glc) (probably of cellulosic origin) was the main neutral sugar in pomegranate peel followed by mannose (Man) and xylose (Xyl). Arabinose (Ara), galactose (Gal), rhamnose (Rha) and fucose (Fuc) were also detected but in lower amounts. Low amounts of uronic acid were detected (<8%), lower than in apple pomace, sugar beet pulp or citrus peels where uronic acids account for 20–30% of the dry weight (DW) (Rinaudo, 1996).

The peel of pomegranate contained considerable contents of fibres ranging from 28.10 to 33.93%, respectively, in cultivars Ga and To. These quantities are much higher than those found in the peels of lemons, oranges and grapefruit; 14, 13.9 and 13.9%; respectively (Gorinstein et al., 2001), linked for a large part to a less hydrated nature of the pomegranate peel. Pomegranate peel could be considered so as a rich source of dietary fibre especially of insoluble fibres which ranged between 27.11 and 32.51% dw.

3.2. FT-IR spectra of pomegranate peel pectins

The FT-IR spectra of pomegranate peel pectins, scanned at wavenumbers ranging from 4000 to 650 cm^{-1} and corrected against the background spectrum of air, are presented in Fig. 1A. These spectra were systematically pre-treated by standard normal

Table 1
Composition of the four selected pomegranate peels.

Parameters	Ac	Ga	Ne	To
Moisture (%)	67.26 ± 0.23^{a}	73.23 ± 0.15^{b}	72.58 ± 0.67^{b}	72.68 ± 0.79^{b}
Proteins (% DW)	3.96 ± 0.49^{a}	7.13 ± 0.53^{c}	5.42 ± 0.01^{b}	5.84 ± 0.38^{b}
Fat (% DW)	–	–	–	–
Total sugar (% DW)	30.65 ± 0.70^{a}	33.58 ± 0.21^{ab}	33.00 ± 2.99^{ab}	34.83 ± 0.79^{b}
Solubles	27.33 ± 0.70^{a}	30.60 ± 0.79^{b}	30.14 ± 2.99^{ab}	32.33 ± 0.21^{b}
Insolubles	3.31 ± 0.07^{ab}	2.98 ± 0.37^{ab}	2.86 ± 0.04^{ab}	2.49 ± 0.44^{a}
Total fibre (% DW)	28.27 ± 0.90^{a}	28.10 ± 1.20^{a}	33.81 ± 0.42^{b}	33.93 ± 0.66^{b}
Insolubles	27.11 ± 0.65^{a}	27.04 ± 0.77^{a}	32.51 ± 0.36^{b}	32.13 ± 0.46^{b}
Solubles	1.16 ± 0.11^{ab}	1.06 ± 0.04^{a}	1.35 ± 0.21^{b}	1.80 ± 0.09^{c}
Ash (% DW)	4.97 ± 0.22^{b}	4.44 ± 0.31^{ab}	3.71 ± 0.37^{a}	4.52 ± 0.75^{ab}
Anhydrouronic acid content (pectin) (% DW)	6.63 ± 0.40^{b}	7.86 ± 0.80^{a}	7.58 ± 0.33^{a}	6.45 ± 0.23^{b}
TNS (% DW)	31.00 ± 0.24^{a}	30.40 ± 1.97^{a}	30.34 ± 0.42^{a}	29.91 ± 0.85^{a}
Rha	0.24 ± 0.00^{c}	0.30 ± 0.01^{a}	0.28 ± 0.00^{b}	0.28 ± 0.02^{a}
Fuc	0.05 ± 0.00^{a}	0.04 ± 0.06^{a}	0.07 ± 0.00^{a}	0.07 ± 0.00^{a}
Ara	1.4 ± 0.07^{d}	1.92 ± 0.05^{a}	1.51 ± 0.10^{c}	1.67 ± 0.05^{b}
Xyl	1.73 ± 0.14^{b}	2.65 ± 0.19^{a}	2.48 ± 0.48^{a}	2.95 ± 0.13^{a}
Man	7.91 ± 0.10^{a}	5.97 ± 0.40^{b}	4.72 ± 0.12^{c}	5.50 ± 0.13^{b}
Gal	0.99 ± 0.01^{a}	1.07 ± 0.13^{a}	0.78 ± 0.01^{b}	0.98 ± 0.04^{a}
Glc	18.67 ± 0.20^{b}	18.44 ± 1.50^{b}	20.49 ± 0.06^{a}	18.44 ± 0.74^{b}

Ac: Acide, Ga: Gabsi, Ne: Nebli, To: Tounsi, DW: dry weight basis, TNS: Total neutral sugar, Rha: Rhamnose, Fuc: Fucose, Ara: Arabinose, Xyl: Xylose, Man: Mannose, Gal: Galactose, Glc: Glucose. Each value in the table is represented as mean ± SE (n = 3). Significant differences between values in the same row are indicated by different letters ($P < 0.05$).

variate correction (SNV). Typical bands appeared in the spectra, as it is expected for pectin molecules, which prove that the extracted substances are pectins.

The region between 3600 cm^{-1} and 2500 cm^{-1} presents two major peaks. The first, centered at about 3309 cm^{-1}, corresponds to the absorption caused by OH stretching absorption due to inter- and intra- molecular hydrogen bonds. In the case of pectin samples, absorption in the O—H region was due to inter- and intra- molecular hydrogen bonding of the galacturonic acid backbone. The second peak, centered at about 2935 cm^{-1}, refer to C—H absorption, these include CH, CH_2 and CH_3 stretching and bending vibrations.

Below 1500 cm^{-1}, the moderately intense absorption patterns are considered as the "fingerprint" region for pectins and the absorptions cannot unambiguously be assigned to any particular vibration because they correspond to complex interacting vibrating systems. Bands occurring in this region at 994, 1076, 1219 and 1439 cm^{-1} are C—O stretching, C—C stretching; C=O stretching; C—O stretching and asymmetric stretching modes of CH_3, respectively (Szymanska-Chargot & Zdunek, 2013). Adina, Florinela, Abdelmoumen, and Carmen (2010) reported that glucose, fructose and sucrose show intense and characteristic bands in the region between 1200 and 900 cm^{-1} with peaks registered at 1033, 1063 and 995 cm^{-1}, respectively.

The third region between 1800 and 1500 cm^{-1} is of special interest with regards to the evaluation of the degree of methylation, since it allows the observation of infrared absorption by the carboxylic acid and the carboxylic ester groups of the pectin molecules. The examination of this spectral region reveals the existence of two bands centered at 1731 cm^{-1} (C=O, ester carbonyl groups stretching) and 1619 cm^{-1} (COO^- carboxylate ion stretching band) (Szymanska-Chargot & Zdunek, 2013).

To further identify relevant spectral patterns, the spectra were submitted to principal component analysis (PCA). The plot of the scores of Principal Components (PCs) allows discrimination of different groups of samples. The scatter plot of the scores of the two first principal components PC1 and PC2, which together explain 74.5% of the total variability, were used to obtain separation of each group in the 4000–650 cm^{-1} region (Fig. 1B). The scores are scattered along PC1. Along PC1 axis, pectins extracted from different varieties seemed to be grouped according to their DM. The scores of Ac and To (having lower DM) lay on the positive side of PC1 scores, while Ne and Ga (with higher DM) were on the negative side of PC1. Indeed, the load of the PC1 plot vs wavenumber showed that the negative high values of PC1 were obtained for wavenumbers at 1619 cm^{-1} and 1219 cm^{-1} assigned, respectively, to the absorption of the non-esterified carboxyl groups and C—O stretching of the pectin molecules (Fig. 1C). Positive loadings for PC1 cover wavenumber at 994 cm^{-1} assigned to C—O stretching/C—C stretching (Szymanska-Chargot & Zdunek, 2013).

3.3. General composition of pomegranate peel pectins

In our literature review, we have not found previous works reporting on the pectin content of pomegranate peel. Therefore, we compared our data with those available for commonly known sources of pectins.

The extracted pectin yield, at 86 °C, 80 min and at 20 mmol/L nitric acid (pH 1.7), ranged from 6.81 to 10.12% of the dry weight of the peel (Table 2). The highest yield was obtained for the variety Ga ($P < 0.05$). The two commercial sources of pectins (apple pomace and citrus peel) give yields of circa 10–15% and 20–30% on a dry matter basis, respectively (Kanmani, Dhivya, Aravind, & Kumaresan, 2014). Compared to the literature data, the pectin yields were close to those of Kulkarni and Vijayanand (2010) extracted from passion fruit peel (9%) under comparable conditions (90 °C, 60 min, pH2). On the other hand, the values were lower than those reported by Sanchez-Aldana, Aguilar, Nevarez-Moorillon, and Contreras Esquivel (2013) from mexican lime pomace (90 °C, 60 min, 1% citric acid solution) (15%). Previous studies proved that the different extraction methods and the different origins of pectins highly affected the pectin yields (Wang et al., 2015; Müller-Maatsch et al., 2016).

The chemical composition of extracted pectins is shown in Table 2. The uronic acid contents (AUA) of Ac, Ga, Ne and To were 47.71; 47.05; 61.32 and 68.51%, respectively (on a dry-weight basis). Only the extract from To could legally be labeled as pectin (>65% GalA), 1981), however these values were close to those observed from apple pomace pectin (53–75%) (Fertonani et al., 2006).

Another characteristic of pectin is the rhamnose: galacturonic acid ratio which can be used a as surrogate to assess the rhamnogalacturonan to homogalacturonan ratio. The values of the molar ratios of uronic acid to Rha, as shown in Table 2, were high. This

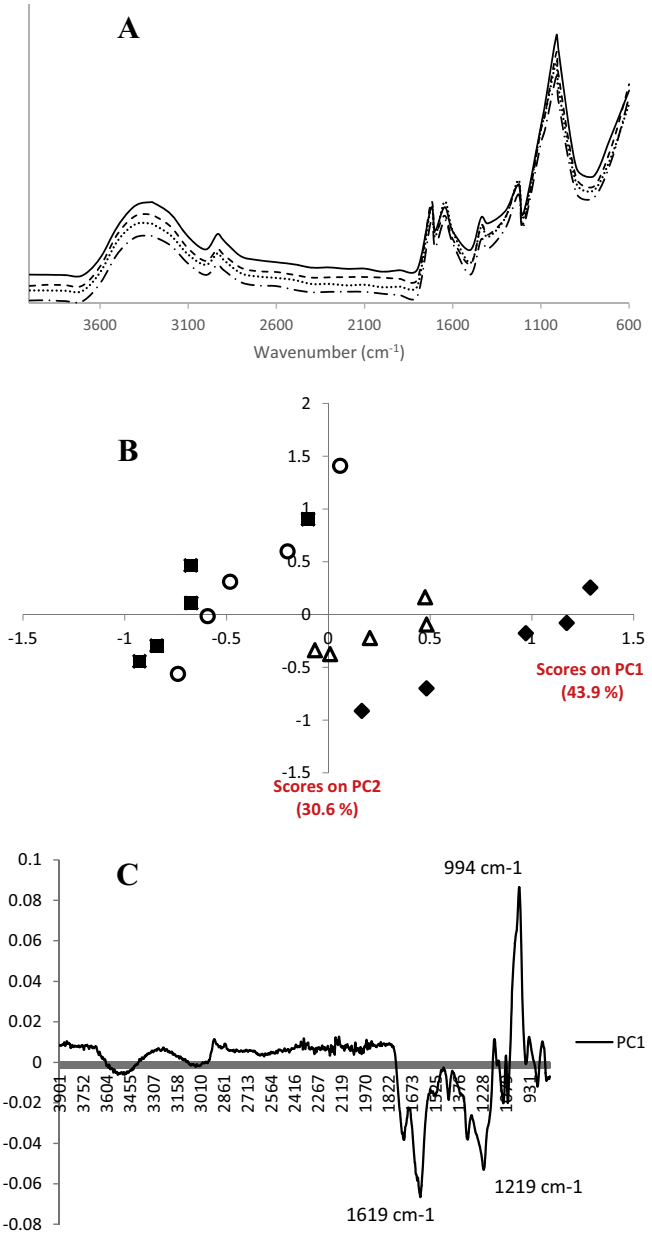

Fig. 1. Analysis of pomegranate peel pectin by FT-IR. (A) FT-IR spectra of pectins from pomegranate peel: Acide (—), Gabsi (–·–·), Nebli (----), Tounsi (––); (B) PCA scores scatter plots of pectin from pomegranate peel FT-IR spectra in the 4000–650 cm^{-1} region: Acide (◆), Gabsi (○), Nebli (■), Tounsi (△); (C) PCA loadings plots (first principal component) of pectin from pomegranate peel FT-IR spectra in the 4000–650 cm^{-1} region.

ratio varies widely across pectin sources, from 3.4 for soybean pectin (Benoit et al., 2012), mostly containing rhamnogalacturonans, to 74 in commercial citrus pectins (Taboada et al., 2010), characterized by the predominance of long homogalacturonan regions. The ratios obtained here for Ac and Ga ecotypes are similar to those reported for murta pectin (66) (Taboada et al., 2010), indicating the predominance of homogalacturonans. On the other hand, this ratio was higher for the pectin extracted from the To variety, suggesting that this pectin contained probably an even lower proportion of rhamnogalacturonic regions.

Furthermore, if the molar ratio of Rha to (Ara and Gal) taken altogether is indicative of the degree of side chain abundance and length, then the Ac variety seemed the most branched product

Table 2
Yield (%), uronic acid (AUA) (%), elution times (ET) (min), partition coefficients (Kav), degree of methylation (DM) (%) and sugar composition (%) of pectins extracted from pomegranate peels.

	Yield	AUA	MR1	MR2	ET	Kav	DM	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	TNS
Ac	8.27 ± 0.25^{b}	47.71 ± 3.50^{c}	63	0.13	15.07	−0.005	34.1 ± 1.9^{b}	0.64 ± 0.04^{a}	0.07 ± 0.01^{b}	1.82 ± 0.01^{c}	0.42 ± 0.01^{b}	0.15 ± 0.01^{a}	3.38 ± 0.07^{a}	23.11 ± 0.31^{a}	29.52 ± 0.33^{a}
Ga	10.12 ± 0.02^{a}	47.05 ± 1.66^{c}	61	0.14	14.46	−0.082	46.1 ± 1.4^{a}	0.65 ± 0.03^{a}	0.07 ± 0.00^{b}	1.88 ± 0.07^{c}	0.31 ± 0.00^{c}	0.14 ± 0.01^{ab}	2.86 ± 0.09^{b}	16.31 ± 0.90^{b}	22.20 ± 1.15^{b}
Ne	6.81 ± 0.41^{c}	61.32 ± 3.12^{b}	77	0.13	14.43	−0.086	41.1 ± 8.0^{a}	0.67 ± 0.03^{a}	0.09 ± 0.00^{a}	2.18 ± 0.01^{b}	0.42 ± 0.01^{b}	0.15 ± 0.01^{a}	2.91 ± 0.02^{b}	5.75 ± 0.08^{c}	12.18 ± 0.10^{c}
To	7.21 ± 0.57^{c}	68.51 ± 2.64^{a}	92	0.11	14.43	−0.086	37.0 ± 3.3^{ab}	0.63 ± 0.08^{a}	0.10 ± 0.01^{a}	2.28 ± 0.05^{a}	0.57 ± 0.07^{a}	0.13 ± 0.01^{b}	3.43 ± 0.10^{a}	5.17 ± 0.03^{d}	12.31 ± 0.09^{c}

Ac: Acide, Ga: Gabsi, Ne: Nebli, To: Tounsi, MR1: molar ratio (AUA/Rha), MR2: molar ratio Rha/(Gal + Ara), Rha: Rhamnose, Fuc: Fucose, Ara: Arabinose, Xyl: Xylose, Man: Mannose, Gal: Galactose, Glc: Glucose, TNS: Total neutral sugar. Significant differences between values in the same column are indicated by different letters ($P < 0.05$).

compared to other varieties, given that it had a low value of GalA/Rha along with a low value of Rha/(Ara + Gal) molar ratios.

The proportion of methoxylated carboxyl groups (degree of methoxylation DM) is very important as it can determine the mechanism of formation of pectin gels, their conformation and rheological properties. Ac has significantly lower DM (34%) compared to that of Ga and Ne varieties with DM of 46% and 41% (Table 2). The DM was inferior to 50% in all samples, indicating that the four extracted pectins were low methylated (LM). Generally such pectins, particularly low methoxyl amidated (LMA) pectin, are preferred for low-calorie product formulas. LM pectins may form gels in the presence of calcium in slightly acidic to neutral pH with or without sugar, contrary to HM pectins which require high sugar content (~65%) and a pH < 3.5 (Thakur, Singh, & Handa, 1997) to form gels by a combination of hydrophobic interactions (at higher temperature) and hydrogen bonds between undissociated carboxyl groups (at lower temperature).

The total neutral sugar (TNS) content in pomegranate peel pectins ranged from 12.18 to 29.52%. This amount was comparable to that of apple pomace extracted at 90 °C and pH 1.5 for 1 h (18.3%) (Garna et al., 2007). The main neutral sugars found in pomegranate peel pectin were Glc (5.17–23.11%), Gal (2.86–3.38%) and Ara (1.82–2.28%) (Table 2). Neutral sugars in this study were comparable to composition previously reported for apple pomace pectin (10.9, 3.2 and 1.1% for Glc, Gal and Ara, repectively) (Garna et al., 2007).

The origin of the glucose in pomegranate peel pectin is not clear: residual starch or sugar might be present in the pomegranate peels. Apart from rhamnose, arabinose and galactose, which are included in the pectin's structure, other neutral sugars, such as fucose, xylose and mannose were present, but in low amounts and were therefore assumed to be "contaminants" from hemicellulosic and sugar materials. Similar findings were reported by Garna et al. (2007): low amounts of xylose and mannose with high glucose content were detected. In addition, the galactose content of all extracts was higher than that of arabinose suggesting the predominance of arabinogalactan side chains.

Pectin extracted from Ac variety was richer in neutral sugars than other cultivars. Compared with the raw peels, pomegranate peel pectins were enriched in rhamnose, galactose and arabinose and impoverished in glucose, xylose and mannose (Tables 1 and 2). This difference in neutral sugar content between the raw peels and the extracted pectins can be attributed to the degradation of some sugars during the extraction at acidic pH. Garna et al. (2007) reported that the neutral sugar side chains of pectins and glycosidic linkages of some polymers (for example the hemicellulose) can undergo a partial hydrolysis during the extraction in free sugars or in small molecular weight compounds that will not be precipitated with the ethanol.

3.4. The hydrodynamic volume distribution of pomegranate peel pectins

The hydrodynamic volume distribution of different samples was determined by HPSEC (Voragen et al., 1995). The elution times (ET) at peak summit and the partition coefficients (Kav) are listed in Table 2, and Fig. 2 shows the HPSEC profiles.

The pectin extracted from Ac ecotype was distinguished by the higher partition coefficient (Kav of −0.005) with the longest elution time (ET = 15.07 min) which reflects the lowest hydrodynamic volume. Whereas this sample had hydrodynamic volume higher than those of dextranes T500 and glucose with elution times (ET) of 15.11 and 23.00 min, respectively. In all ecotypes, the pectin samples showed a relatively high polydispersity, characterized by the presence of a tail of smaller molecules. In addition to the primary effect of degree of polymerization, Fishman, Pfeffer, Barford, and Doner (1984) reported that degree of methylation could affect pectin hydrodynamic volume by affecting the strength of intermolecular ionic repulsions and the character of the attractive forces between chains; however all pomegranate pectins had similar degrees of methylation. The differences observed in the HPSEC patterns was thus likely to reflect different chain lengths of the pectins from the four raw materials.

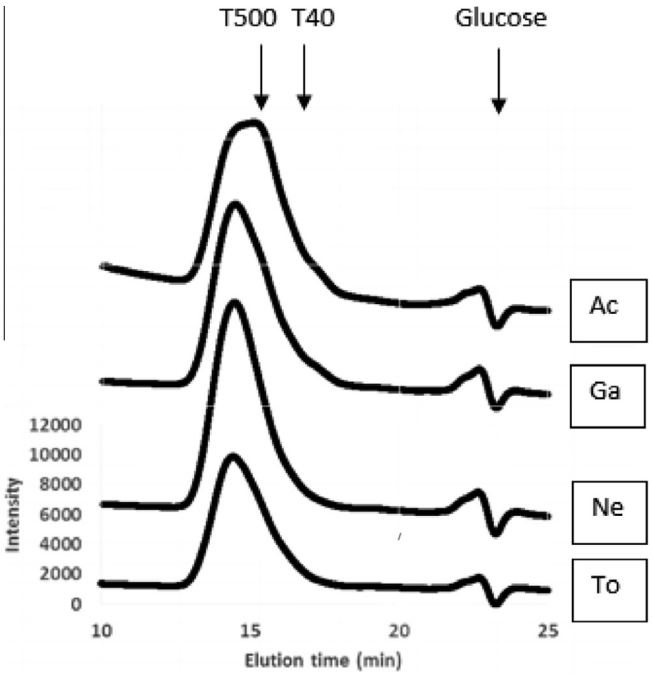

Fig. 2. High-pressure size-exclusion chromatography elution profiles of pectins extracted from pomegranate peel. Ac: Acide ecotype; Ga: Gabsi ecotype; Ne: Nebli ecotype; To: Tounsi ecotype.

3.5. Pectin viscosity

Fig. 3 contains typical Huggins plots of reduced viscosity (η_{red}) against pectin concentration. All samples of pectin exhibited a pseudo-hyperbolic pattern with a continual increase in molecular volume with dilution. This data is consistent with earlier work (Fishman, Gillespie, Sondey, & Barford, 1989) which showed that the increase in size with decreasing pectin concentration is a sequential process: as the concentration of pectin increased, smaller sized species appeared. Increasing size with dilution could be attributed to the formation of large spherical networks or to the polyelectrolyte expansion due to an increase in charge-charge repulsions along the chain.

3.6. Rheological study

Gel formation was monitored in two steps. First, the temperature was decreased linearly from 90 °C to 10 °C at 2 °C/min, then, this temperature was maintained for 30 min. Variations of elastic modulus (G′) and viscous modulus (G″) as a function of temperature are shown in Fig. 4A for LM pectins of varieties Ac, Ga, Ne and To at pH 3, in the presence of 0.7% pectin (w/w) and 0.1% $CaCl_2$. Fig. 4A revealed the formation of a cross-linked network, which was characterized by the temperature independence of G′ and G″ on cooling. As a matter of fact, during cooling from 90 °C to 10 °C, no crossover between the storage modulus (G′) and the loss modulus (G″) occured. It is worth noting that all gels show a rapid gel formation with G′ > G″, which reveals a typically gel-like

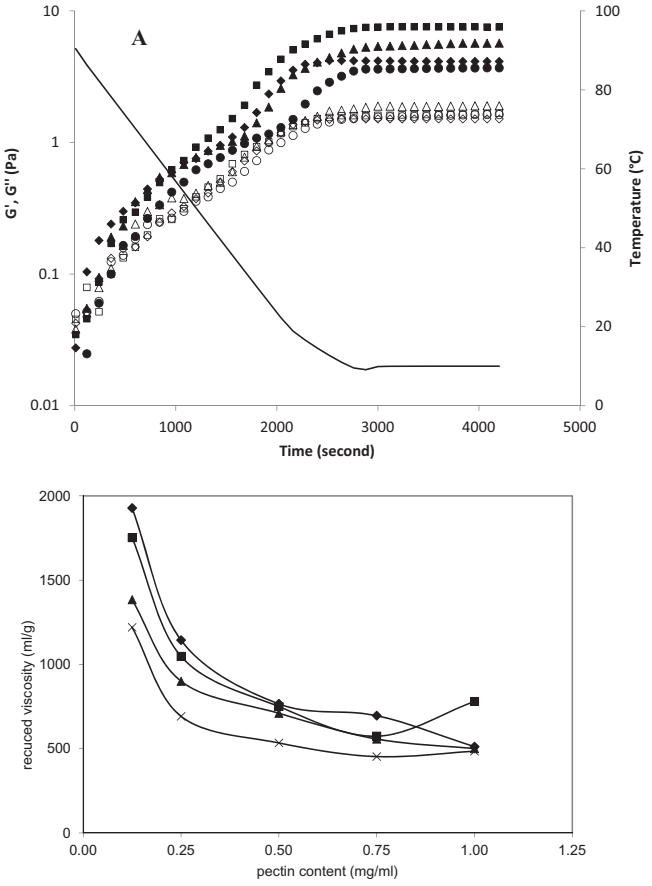

Fig. 3. Huggins plot of pectins in 0.1 mol/L sodium phosphate buffer (pH7). Acide (×), Gabsi (▲), Nebli (■), Tounsi (◆).

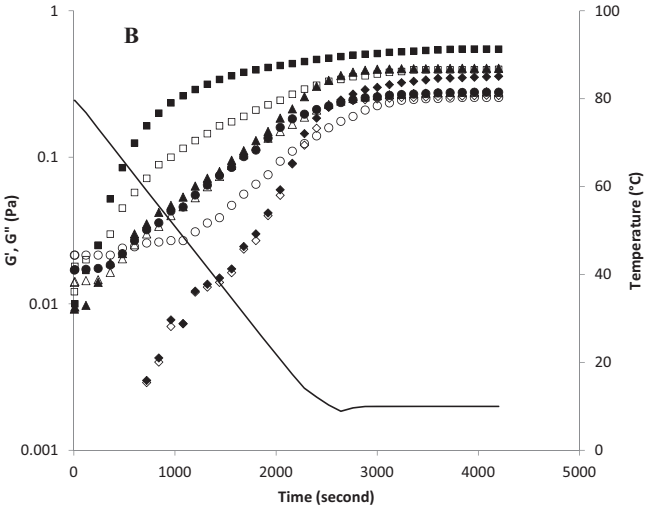

Fig. 4. Semi-logarithmic plots of G′ and G″ vs time obtained at constant frequency (1 Hz) during controlled cooling (2 °C/min) of pectin gels at pH3 (A) and pH 7 (B). G′ Ac: (◆), G″ Ac: (◇), G′ Ga: (▲), G″ Ga: (△), G′ Ne: (●), G″ Ne: (○), G′ To: (■), G″ To: (□), Temperature: (-).

structure, but the development in G′ during the measurement period varied with the variety, reaching the plateau at 10 °C.

For all samples G′ increased with decreasing temperature during cooling from 90 °C to 10 °C, however the To pectin exhibit a significantly higher storage modulus than those of other varieties at the same pectin concentration with greater separation of G′ and G″. Thus, the gel To was characterized by a denser network with more interconnected chains. This behavior could be attributed to the combination of the low neutral sugar content, the low DM and the high galacturonic acid content of pectin extracted from this ecotype (Tounsi). This could be explained by the fact that sugar side chains in the pectin molecule hinder gel formation by preventing pectins' aggregation (Smidsrod & Haug, 1971). The presence of methyl groups prevents the formation of junction zones in the interjunction segments of molecules, making them more flexible (Thakur et al., 1997). Moreover, the molecules with an increased number of charged groups and lower degree of methoxylation are straighter than esterified ones, and hence more likely to form a Ca^{2+} bridge. For the galacturonic acid contribution, the same effect was reported by Rinaudo (1996) who reported that the elastic modulus is directly proportional to the galacturonic acid yield.

The comparison between the different ecotypes showed a variation in the gel formation which could be attributed to difference in pomegranate peel pectin characteristics such as the hydrodynamic volume and the neutral sugar content. Indeed, the strength of the gels obtained for Ac, Ga and To increased with increase in the hydrodynamic volume. Thus, a decrease in the elastic modulus (G′) can be observed when the degradation of sample was important. These findings were in agreement with the results reported by Kim, Rao, and Smit (1978) for low-ester pectin.

G′ was also significantly influenced by the neutral sugar content. As shown in Fig. 4A and Table 2, the storage modulus G′ was lower in the presence of higher amount of neutral sugar. The presence of side branches significantly affects gelling property. This can be explained by the fact that neutral side chains in the pectin molecule hinder gel formation by preventing pectins' aggregation (Thakur et al., 1997).

With increasing the pH to 7 (Fig. 4B), gels exhibited behavior different to those of pH 3. At pH 7, in the presence of 0.7% pectin and 0.04% $CaCl_2$ (3.6 mmol/L), all pectins formed gels with G′ > G″ at 10 °C, i.e. a typical gel-like structure, however, the shape of the mechanical spectra was that of a weak gel. Gigli, Garnier, and Piazza (2009) also reported that the gel made at similar conditions (DM of pectin 23%; pH7; 1% pectin and 3 mmol/L of $CaCl_2$, was a weak gel with G′ ∼ 1 Pa.

4. Conclusions

In the present study, the four tunisian pomegranate peels studied (Acide, Gabsi, Nebli and Tounsi) appeared relatively low in pectins, as revealed by their low content in uronic acids. The recovered pectins were low methylated (LM) and characterized by the predominance of homogalacturonan regions. The rheological study showed that the extracted pectins present good characteristics to be exploited industrially for their gelling properties in presence of calcium. Indeed, at pH 3 and pH 7 all pectin solutions (0.7% pectin, 30% sucrose) formed gels with G′ > G″ reaching the plateau at 10 °C, i.e. a typical gel-like structure. We are currently investigating the effect of substitution of commercial pectin by that extracted from pomegranate peel on pomegranate jam characteristics. The final product could be enriched with fibre extracted from pomegranate peel.

Acknowledgements

The authors thank Mrs L. Touloumet for assistance with analytical methods. This work was financed by the Ministry of Higher Education and Scientific Research – Tunisia to Mouna Abid for an internship at UMR408 Sécurité et Qualité des Produits d'Origine Végétale and UMR Ingénierie Procédés Aliments.

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