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Yield and composition of pectin extracted from Tunisian pomegranate peel

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ABSTRACT

A central composite design was employed to determine the influence of extraction conditions on production yield and chemical composition of pectin from pomegranate peels. Response surface methodology (RSM) was used to quantify the integral effect of the three processing parameters (extraction duration, temperature and pH) on yield. A second-order polynomial model was developed for predicting the yield of pomegranate peels pectin based on the composite design. Yields ranged from 6.4 to 11.0 ± 0.2%. Optimal temperature, duration and pH value of the extraction were 86 °C, 80 min and 1.7, respectively. The uronic acid and the total neutral sugar content of the extracted pectins ranged from 377 to 755 mg/g and from 161 to 326 mg/g, respectively. Moreover, the degree of methylation varied with the extraction conditions and the extracted pectins were low methylated. On high pressure size exclusion chromatography (HPSEC), the elution pattern of the acid-extracted pectins showed that severe conditions were associated with lower hydrodynamic volume.

1. Introduction

Pectin is a high value functional food ingredient because of its excellent gelling properties and is commonly used in food industry as gelling agent and stabilizer (E440) [1]. It belongs to the group of polysaccharides from higher plants and has several positive effects on human health, including lowering cholesterol and serum glucose levels, reducing cancer and stimulating the immune response [2].

Commercial pectin is essentially extracted from by-products of juice manufacturing, including citrus peels and apple pomace [1]. Many other agricultural by-products such as banana peels [3], sunflower heads [4] and peach pomace [5] were tested for pectin extraction. However, only little attention has so far been paid to the study of pectin from pomegranate peel which is a potential and inexpensive candidate [6,7]. Pomegranate pectins appear to have lower degrees of methylation than classical (apple or citrus) sources [6]; however their molar mass distribution and the proportion of neutral side chains, that also impact functionality, has not been reported.

Pomegranate (*Punica granatum* L.) belongs to the Punicacea family. The cultivation of pomegranate is native to the Middle East and was later known in the Mediterranean. In Tunisia, pomegranate trees have been cultivated since ancient times. The cultivation occupies more than 11.000 ha and extends on all areas, except high level areas. More than 60 local varieties have been denominated (Jbeli, Tounsi, Zehri, Mekki, etc.).

Pomegranate is popularly consumed as fresh fruit or food products (juice, jams and jellies) and is considered as a very interesting fruit "according" to its potential health benefits.

Currently, increases in the production and processing of pomegranate have generated increasing waste, resulting in million tons of pomegranate peel being disposed of every year. In Tunisia and other pomegranate-producing countries, processing this pomegranate waste which constitutes approximately 40% of the whole fruit [8], could provide economic advantages and decrease some of the environmental problems. Because

Abbreviations: RSM, response surface methodology; HPSEC, high pressure size exclusion chromatography; DM, degree of methylation; CCRD, central composite rotational design; MS, mass spectrometry; Gal A, galacturonic acid; ET, elution time; Rha, rhamnose; LM, low methoxyl; HM, high methoxyl; AIS, alcohol insoluble solids; HNO_3, nitric acid; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; TNS, total neutral sugars.

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pomegranate peels are readily available, they could be used to recover value-added compounds such as pectins. It was reported that the efficacity of pectin extraction is affected by different factors, mainly temperature, duration and pH [9]. Response surface methodology (RSM) was proved to be an effective statistical technique for investigating individual and combined effects of processing variables and optimizing the extraction process.

In this context, the main objectives of this work were firstly to optimize, *via* RSM, pectin extraction with nitric acid from pomegranate peel of 'Gabsi' which is the most consumed variety in Tunisia, and secondly to study the composition of the extracted pectins. The optimal conditions were determined for maximum extraction of pectin, thereby enabling the use of pomegranate peel as a source of low methoxyl pectin for the production of low-calorie and dietetic foods, particularly pomegranate-based-food.

2. Materials and methods

2.1. Plant material

Pomegranate fruits from the "Gabsi" cultivar were collected from an oasis at Gabes region (southeast of Tunisia). Fruits were manually peeled then the collected peels were cut into small pieces, oven dried at 50 °C (WTB binder-78532 TUTTLINGEN, Germany) and ground (particles' size between 0.5 mm and 1.25 mm) to conduct pectin's extraction described in Section 2.2.

2.2. Pectin extraction

A total of 2 g pomegranate peel was stirred at 400 rpm (Stirrer Heidolph RZR 20051 electronic, Germany) in 100 mL of the HNO_3 solution (solid–liquid ratio; 1:50; g/mL) using the extraction conditions established by the experimental designs.

The resulting slurries were allowed to cool to room temperature (25° C) and filtered through cheesecloth. For pectin precipitation, two volumes of 96% w/w ethanol were added to the filtrate. The obtained mixture was kept for 1 h at 4 °C. Then, pectin gels were centrifuged at 8000*g* for 20 min at 10 °C. To remove the mono and disaccharides, 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 oven dried at 45° C to a constant weight, and ground in a mortar.

The gravimetric yield was estimated as the ratio between the weight of the powdered pectin and the weight of the flour raw material (%, g/g), both on a dry basis. The experiments to determine the effect of extraction duration, temperature and acid concentration followed the experimental design described as follows.

2.3. Experimental design

A response surface methodology (five levels, three variable central composite rotational design [CCRD]) was used to optimize pectin extraction. The design comprised eight points of a factorial design, six axial points at a distance $\alpha = \pm 1.68$ from the centre, and a centre point. In order to estimate pure error variance, six replications were performed at the centre point. The number of experiment required is given by the expression 2^k ($2^3 = 8$ points) + $2 \times k$ ($2 \times 3 = 6$ axial points) + 6 centre points (6 replications) [10].

The ranges and the central point values of the three independent variables were based on the results of a preliminary study. The independent factors studied were extraction duration (5 min–125 min), temperature (65.2–98.8 °C) and nitric acid concentration (6–100 mmol/L). The variables and their levels, with both the coded values and the real values used in this study, are shown in Table 1. The experiments were performed randomly to avoid systematic errors.

Table 1
Experimental domain of the central orthogonal composite design used for pectin extraction from pomegranate peel.

Coded levels					
Factor	−1.68	−1	0	+1	+1.68
	Experimental levels				
X1: Extraction duration (min)	5	29	65	100	125
X2: Temperature (°C)	65.2	72	82	92	98.8
X3: Nitric acid concentration (mmol/L)	6	11	25	58	100
Equivalent pH	2.2	1.96	1.6	1.24	1

A regression analysis was done to fit the tendency to second order polynomial model as shown in Eq. (1).

$$y = b_0 + b_1X_1 + b_{11}X_1^2 + b_2X_2 + b_{22}X_2^2 + b_3X_3 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where y is the response variable, b_0, b_1, b_2 and b_3 are the regression coefficients of variables for individual effects, b_{11}, b_{22} and b_{33} are quadratic effects and b_{12}, b_{13} and b_{23} are interactive effects. X_1, X_2 and X_3 are independent variables.

The obtained response values were used to estimate the model coefficients bj by the least square method using the experimental design software NEMROD-W [11].

2.4. Pectin characterization

Neutral sugars were measured as alditol acetates after hydrolysis in 1 mL of 1 mol/L sulfuric acid (3 h, 100 °C) with inositol as an internal standard. After hydrolysis, they were derivatized to alditol acetates [12]. They were injected on a gas chromatography-flame ionization detector HP5890 Series II (Agilent, Inc.,PaloAlto, 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 galacturonic acid content (GalA) was determined by a *meta*-hydroxyl-diphenyl assay according to Blumenkrantz and Asboe-Hansen [13]. The methanol concentration (MeOH) was determined according to Renard and Ginies [14] 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 galacturonic acid.

The hydrodynamic volume distribution of polysaccharides was 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 solftware (Jasco,Tokyo, Japan). Separations were achieved using two columns in series: a 8.0 mm × 300 mmi.d. OH-