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Yield and composition of pectin extracted from Tunisian pomegranate peel

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A B S T R A C T

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.

Keywords:
Punica granatum L.
Response surface methodology
Extraction
Pectin

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					
	−1.68	−1	0	+1	+1.68
Factor	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-

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 (5 g/L) were injected and eluted with 0.4 mol/Lsodium 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 tocalibrate the column system. All data are presented as a function of elution time of the samples (ET).

2.5. Statistical analysis

Analysis of pectin samples was performed in duplicate, and pooled standard deviations were calculated for each series of duplicates using the sum of individual variances pondered by the individual degrees of freedom [15]. Statistical analysis was performed using the Statistical Package for the Social Sciences "SPSS" (version17). Duncan test was performed to evaluate the significance of differences between mean values at the level of $P < 0.05$.

The statistical analysis of the dependent variables in the experimental design were carried out using the experimental design software NEMROD-W [11]. Lack of fit, error and model significance were used to judge the adequacy of each model. The fitted model is considered adequate if the variance due to the lack of fit is not significantly different (F-test at the 95% level) from the pure error variance. After obtaining the multifactorial ANOVA, contour plots of the responses "yield" and "galacturonic acid content" were generated.

3. Results and discussion

3.1. Extraction yield

Fifteen experiments were carried out according to the conditions indicated in Table 1. Pectin yields are reported in Table 2. The acid-extracted pectin yield ranged from 6.4 to 11.0 ± 0.2% of the dry weight of the peel with the highest yield obtained at 82 °C, pH 1.6 for 65 min. The yields (64–112 mg/g pomegranate peel) compare favorably with those obtained by Peireira et al. [6] using citric acid and the alcohol insoluble solids (AIS) from pomegranate peel (39–110 mg/g), specially given that AIS represented less than 50% of the pomegranate peel powder in their experiment. Moorthy et al. [7] obtained higher yields using ultrasound assisted extraction and lower pH (the acid used was not specified). Kar and Arslan [16] reported that the extraction process as well as the fruit variety and maturity can affect the quantity and quality of extracted pectin.

The relationship between the extraction yield and the three selected quantitative variables was approximated by the following second order polynomial function (Eq. (2)):

$$Yield = 109.817 + 5.825X_1 + 4.322X_2 - 1.794X_3 - 11.468X_1^2 - 7.363X_2^2 - 9.68X_3^2 - 2.594X_1X_2 - 5.606X_1X_3 - 3.894X_2X_3 \quad (2)$$

Coefficient values and statistical parameters obtained for the model are given in Table 3. The results obtained are then analysed by F-statistical test for analysis of variance (ANOVA) to assess the "goodness of fit". The variance analysis (Table 3) showed significant interactive effect between quantitative variables on the pectin yield. The individual effects of temperature and extraction duration were the most influential parameters.

The result of the ANOVA analysis for the model is shown in Table 4. The fitted model is found to be significant at 95% confidence level by the F-test and the lack of fit is not significant (data not shown). The coefficient of determination, R^2, for pectin extraction yield was 0.978.

3.1.1. Interaction effects of extraction duration and temperature on pectin extraction rate

Both extraction duration and temperature displayed significantly quadratic effects on the yield of pectin (Table 3). As shown in the response surface and contour plots (Fig. 1A), extraction of pectin was affected by changing temperature (from 65.2 °C to 98.8 °C) and duration (from 5 min to 125 min). The extraction yield of pectin first increased rapidly with the increase of both extraction duration and temperature, until 91.3 min (+0.75) and 89.5 °C (+0.75). The total yield of pectin was significantly less at lower temperature and shorter duration. The reason was that the pectin was less dissolved out from the plant cell wall if the extraction temperature was too low and extraction duration was too short. Besides, much higher extraction temperature (above 90 °C) and too long extraction duration (above 91 min) may cause breakdown of pectin molecules as already observed by Chang et al. [17].

3.1.2. Interaction effects of extraction pH and temperature on pectin extraction rate

It is apparent from the isoresponse contours that pH played a great role in the extraction of pectin from pomegranate peel. As shown in Fig. 1B, maximum pectin yield was obtained by soaking the peels in a solution of pH 1.5 (+0.25) to 1.85 (−0.75). However, decline in pectin yield was observed at pH < 1.5 for all temperatures.

The examination of the combined effects of temperature and pH showed that the yield increased when increasing temperature and/or decreasing the pH, down to the limit of pH 1.5. These effects were marked for temperatures <92 °C (+1) and low pH levels (2.2 until 1.5). This can be explained by the cleavage of linkages between pectins and other cell wall components, giving a higher yield of extraction [18].

With regard to the effect of pH, El-Nawawi and Shehata [19] reported that acidic conditions contribute to hydrolyze the insoluble pectic constituents into soluble pectin, which increases the pectin recovery. Mayers and Baker [20] reported that yield of pectin increased with decrease in the pH of the extractant and maximum yield of pectin was obtained from lemon albedo with hydrochloric at pH of 1.45. On the other hand, Kim et al. [21] found that temperature was the main parameter influencing the pectin recovery from mandarin. Chen et al. [22] reported that temperature increases the ability of the solvent to solubilize the compounds and reduce the viscosity of the liquid solvent, allowing for better penetration of the solvent into the solid matrix.

3.1.3. Interaction effects of extraction pH and extraction duration on pectin extraction rate

The effect of duration and pH was given in Fig. 1C. Once again, the isoresponse curves showed that a long duration and a low pH of extraction led to higher yields. At 82 °C, the maximum yields (10.8%) were reached at a pH in the range of 1.5–1.85 and extraction duration between 56 and 91 min. A study on pectin extraction from dried okra pod powder has shown that at a fixed temperature of 60 °C and at low pH, the pectin yield improved with increasing extraction duration but decreased after 64 min [22].

3.2. General composition of the extracted pectin

Different conditions of extraction on the same raw material were used, so that the effects of extraction conditions on the composition of pectin could be studied. The average galacturonic acid content (GalA), the total neutral sugars (TNS), the galacturonic acid to rhamnose (GalA/Rha) molar ratio composition, the degree of

Table 2
Experimental conditions of the orthogonal central composite design used for pectin extraction from pomegranate peel, and the corresponding experimental responses.

	Coded level			Experimental level			Yield (mg/g)	Gal A (mg/g)	Gal A/Rha (molar ratio)	ET (min)	DM (%)	Rha (mg/g)	Fuc (mg/g)	Ara (mg/g)	Xyl (mg/g)	Man (mg/g)	Gal (mg/g)	Glc (mg/g)	TNS (mg/g)
Run	Duration (min)	T (°C)	HNO_3 (mmol/L)	Duration (min)	T (°C)	HNO_3 (mmol/L)													
A1	−1	−1	−1	29	72	11	64	507^{efgh}	58	14.1	46^{bcd}	7^{ab}	1^{a}	47^{e}	7^{abc}	1^{ab}	30^{ab}	192^{de}	285^{g}
A2	−1	−1	1	29	72	58	78	535^{defgh}	56	14.3	45^{dce}	8^{abc}	1^{a}	39^{de}	7^{abcd}	1^{ab}	31^{abcd}	235^{f}	324^{h}
A3	−1	1	−1	29	92	11	82	477^{hi}	54	14.3	36^{ghij}	7^{ab}	1^{a}	39^{de}	6^{ab}	1^{a}	30^{abc}	241^{f}	326^{h}
A4	−1	1	1	29	92	58	84	576^{cde}	61	14.6	39^{efgh}	8^{abc}	1^{a}	23^{c}	6^{ab}	1^{ab}	33^{abcd}	180^{de}	253^{cdef}
A5	1	−1	−1	100	72	11	87	613^{bc}	58	14.2	37^{fghi}	9^{abc}	1^{a}	49^{e}	7^{abcd}	2^{ab}	30^{ab}	187^{de}	285^{g}
A6	1	−1	1	100	72	58	82	553^{cdefg}	58	14.3	43^{def}	8^{abc}	1^{a}	22^{c}	6^{ab}	0^{a}	30^{abc}	179^{de}	246^{cd}
A7	1	1	−1	100	92	11	98	422^{ij}	51	14.7	55^{a}	7^{ab}	0^{a}	22^{c}	5^{a}	0^{a}	32^{abcd}	199^{e}	266^{defg}
A8	1	1	1	100	92	58	74	755^{a}	64	15.2	30^{j}	10^{bc}	0^{a}	2^{a}	8^{bcd}	0^{a}	36^{d}	106^{a}	161^{a}
A9	−1.68	0	0	5	82	25	64	377^{j}	43	14.2	55^{a}	7^{ab}	2^{a}	50^{e}	13^{d}	1^{a}	28^{a}	169^{cd}	271^{fg}
A10	1.68	0	0	125	82	25	91	624^{bc}	59	14.4	33^{hij}	9^{abc}	1^{a}	16^{bc}	8^{bcd}	1^{a}	33^{abcd}	202^{e}	269^{efg}
A11	0	−1.68	0	65	65.2	25	80	557^{cdef}	64	14.2	40^{defg}	7^{ab}	1^{a}	7^{ab}	9^{cd}	0^{a}	32^{abcd}	142^{b}	212^{b}
A12	0	1.68	0	65	98.8	25	99	655^{b}	48	15.0	32^{ij}	12^{c}	7^{b}	30^{cd}	10	6^{b}	30^{abc}	149^{bc}	243^{c}
A13	0	0	−1.68	65	82	6	86	603^{bcd}	51	14.2	40^{defg}	10^{abc}	0^{a}	6^{ab}	7^{abcd}	0^{a}	33^{bcd}	147^{bc}	203^{b}
A14	0	0	1.68	65	82	100	80	754^{a}	91	14.6	33^{hij}	7^{ab}	1^{a}	19^{bc}	6^{ab}	1^{a}	35^{cd}	201^{e}	269^{efg}
A15	0	0	0	65	82	25	107	489^{fghi}	66	14.3	51^{abc}	6^{ab}	1^{a}	25^{c}	5^{a}	2^{ab}	31^{abcd}	189^{de}	259^{cdef}
A16	0	0	0	65	82	25	108	479^{ghi}	73	14.3	51^{abc}	6^{a}	1^{a}	23^{c}	5^{ab}	2^{ab}	31^{abc}	185^{de}	251^{cdef}
A17	0	0	0	65	82	25	110	491^{fghi}	69	14.3	51^{abc}	6^{a}	1^{a}	25^{c}	5^{ab}	2^{ab}	32^{abcd}	191^{de}	262^{cdef}
A18	0	0	0	65	82	25	110	491^{fghi}	73	14.3	52^{ab}	6^{a}	1^{a}	24^{c}	5^{a}	1^{ab}	31^{abcd}	181^{de}	250^{cde}
A19	0	0	0	65	82	25	111	497^{fghi}	65	14.3	52^{ab}	6^{ab}	1^{a}	25^{c}	5^{ab}	2^{ab}	30^{abc}	191^{de}	261^{cdef}
A20	0	0	0	65	82	25	112	486^{fghi}	74	14.3	50^{abc}	6^{a}	1^{a}	23^{c}	5^{a}	2^{ab}	30^{abc}	183^{de}	249^{cde}
B								489			51	6	1	24	5	2	31	187	255
C								35			3	0.8	0.3	7.5	0.8	0.7	2.4	15	14
D								42			2	0.5	0.08	1.85	0.6	0.06	1.3	6	8

T: temperature, HNO_3: nitric acid, Gal A: galacturonic acid, ET: elution time, DM: degree of methylation, Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, TNS: total neutral sugars, B: mean for central point, C: Analytical pooled standard deviation, D: Standard deviation of the mean for central point repliacates. Significant differences between the values in the same column are indicated by different letters a–h ($P < 0.05$).

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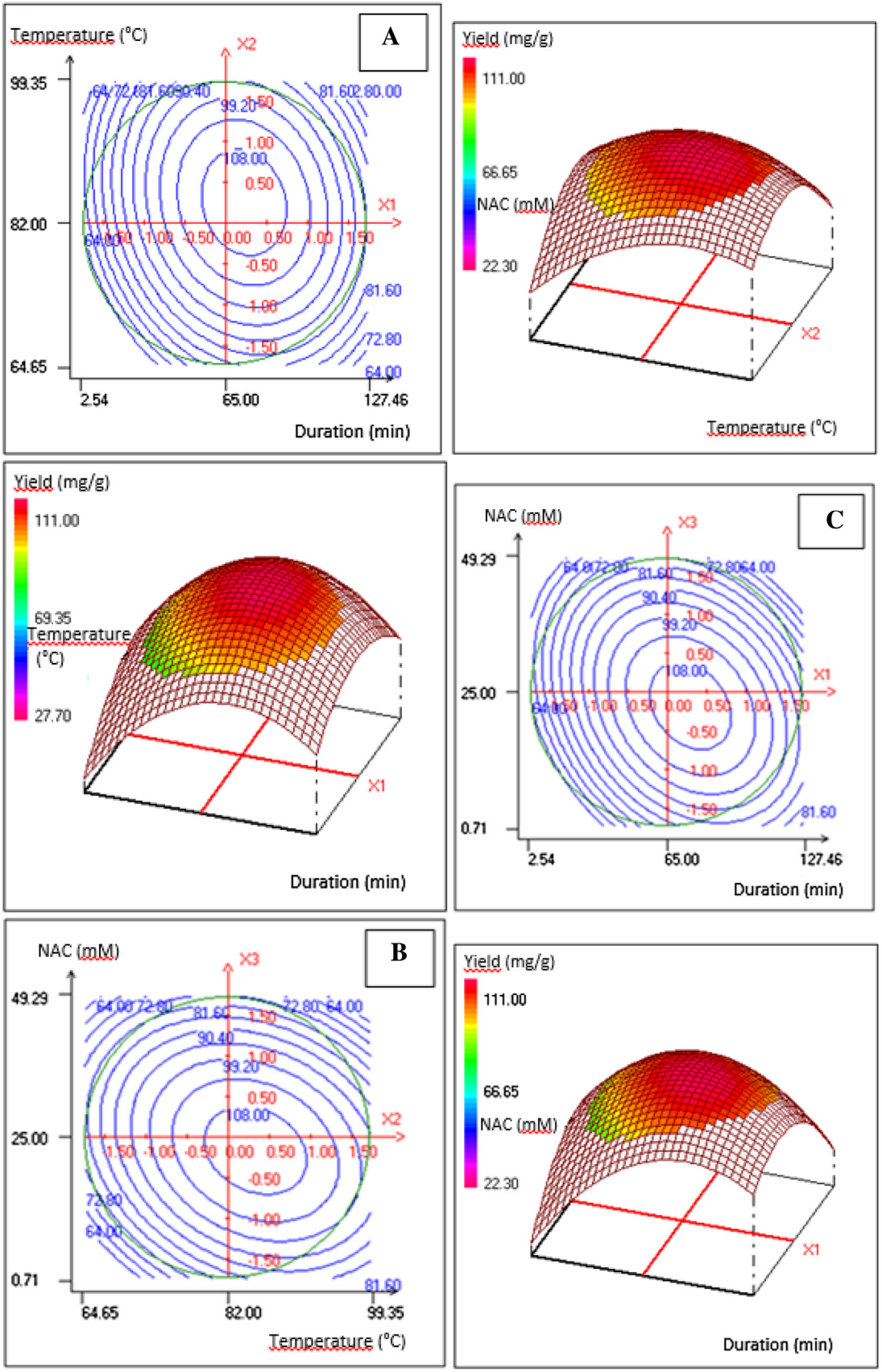

Fig. 1. Contour plots and three-dimensional response surface for the yield of pectin extraction from pomegranate peel. A: effect of duration and temperature at constant HNO_3 concentration (25 mmol/L); B: effect of HNO_3 concentration and temperature for a fixed duration (65 min); C: effect of HNO_3 and duration at constant temperature (82 °C).

Table 3
Coded regression coefficients and statistical parameters obtained for the model built for pectin yield from pomegranate peel.

Coefficient	Coefficient value	Standard deviation	T	P-value
b0	109.817	1.305	84.18	<0.01***
b1	5.825	0.866	6.73	<0.01***
b2	4.322	0.865	4.99	0.0543***
b3	−1.794	0.865	−2.07	6.5
b11	−11.468	0.843	−13.61	<0.01***
b22	−7.363	0.842	−8.74	<0.01***
b33	−9.686	0.842	−11.5	<0.01***
b12	−2.594	1.131	−2.29	4.47*
b13	−5.606	1.131	−4.96	0.0572***
b23	−3.894	1.131	−3.44	0.630**

b0, bj, bjk and bjj, the estimated model coefficients; degree of freedom = 10.
* Significant at the level 95%.
** Significant at the level 99%.
*** Significant at the level 99.9%.

Table 5
Coded regression coefficients and statistical parameters obtained for the model built for galacturonic acid content of pectin extracted from pomegranate peel.

Coefficient	Coefficient value	Standard deviation	t	P-value
b0	491.342	0.984	499.55	<0.01***
b1	40.985	0.653	62.80	<0.01***
b2	19.346	0.653	29.65	<0.01***
b3	52.269	0.653	80.10	<0.01***
b11	−0.124	0.653	−0.19	85.3
b22	22.116	0.653	34.81	<0.01***
b33	56.041	0.653	88.22	<0.01***
b12	0.106	0.853	0.12	90.6
b13	34.886	0.853	40.92	<0.01***
b23	39.819	0.853	46.70	<0.01***

b0, bj, bjk and bjj, the estimated model coefficients; degree of freedom = 5.
*** Significant at the level 99.9%.

methylation (DM) and the elution time (ET) of the main HPSEC peak of pectin samples are listed in Table 2.

3.2.1. Galacturonic acid content

The average content of galacturonic acid was 529 mg/g. The galacturonic acid content varied from 377 to 755 mg/g (Table 2). The highest values (755 mg/g) were obtained when pectin was extracted at the lowest pH (+1.68) (A14) or at the extreme conditions (A8). Extreme extraction conditions led to better extraction of pectin with higher purity probably by release of neutral sugar side chains as a product of partial acid hydrolysis of pectin [23]. Indeed, the content in both total neutral sugar and arabinose decrease particularly in this sample A8 (161 mg/g and 2 mg/g; respectively). The composition data was also analysed following the experimental design paramters. GalA data fitted a second order polynomial function ($R^2 = 0.837$) as follows (Eq. (3)):

$$GalA = 491.342 + 40.985X_1 + 19.346X_2 + 52.269X_3 - 0.124X_1^2 + 22.116X_2^2 + 56.041X_3^2 + 0.106X_1X_2 + 34.886X_1X_3 + 39.819X_2X_3 \quad (3)$$

Multiple regression coefficients are shown in Table 5. From the results in Table 5, only the quadratic coefficient of extraction duration variable and interaction coefficient between temperature and extraction duration were not significant by F-test at 95% confidence level.

Table 4 shows the results obtained from the analysis of variance (ANOVA). The regression was significant at 95% confidence level by the F-test. The coefficient of determination, R^2, for GalA content was 0.837.

Fig. 2B demonstrates the effect of pH and temperature on galacturonic acid content. The lowest content was obtained when pectin was extracted at temperatures ranging from 68 to 94 °C and at pH 2–1.5. By decreasing pH below 1.5 and/or increasing temperature, the galacturonic acid content was found to increase. The effect of extraction duration over the galacturonic acid is given in Fig. 2A and C. At low pH and for all temperatures, an increase of duration induced an increase of GalA content. This effect was marked for pH below 1.78 (−0.5). The harsher conditions (acidic pH, longer duration, higher temperature) all contributed to higher GalA content in the extracted pectins. As observed above, this could be linked to increased degradation of neutral sugar side-chains during pectin extraction.

Uronic acid contents of A8, A12 and A14 were above the 65% required to be considered pectin according to the FAO (UN Food and Agriculture Organization) and EU (European Union) [24]. This result demonstrated that pectin extraction from pomegranate peel was effective using severe conditions (Extraction duration: 65–100 min; Temperature ≥82 °C and pH: 1–1.6).

3.2.2. Neutral sugars

The total content of neutral sugars varies with the source, the extraction conditions and the subsequent treatments [24]. High glucose contents were obtained systematically, while the main other sugars were galactose (28–36 mg/g) and arabinose (2–50 mg/g) (Table 2). The origin of the glucose was not clear: residual starch, hemicelluloses notably xyloglucans, or sugar might be present in the pomegranate peels.

Except rhamnose, which is 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. The total neutral sugar content calculated as the sum of the individual neutral sugars ranged from 161 to 326 mg/g.

All the extracts were rich in neutral sugars in comparison with those of sugar beet pulp pectin reported in various studies (68–329 mg/g) [9]; (288 mg/g) [25]; (186 mg/g) [26], which is already known for its high neutral sugar content relative to apple or citrus pectins [27].

Generally, use of more severe conditions led to lower amounts of total neutral sugars (161 mg/g versus 285, 324, 326, 285 for A8 versus A1, A2, A3, A5 respectively).

Table 4
Statistical parameters obtained from the ANOVA test performed for the model built for pectin yield and galacturonic acid content (GalA) from pomegranate peel.

Source of variation		Sum of squares	Degrees of freedom	Mean square	Ratio	Significance	R^2
Regression	Pectin yield	4.58E+0003	9	5.09E+0002	49.7837	<0.01***	0.978
	GalA	1.38E+0005	9	1.53E+0004	2632.4398	<0.01***	0.837
Residuals	Pectin yield	1.02E+0002	10	1.02E+0001			
	GalA	2.69E+0004	10	2.69E+0003			
Total	Pectin yield	4.68E+0003	19				
	GalA	1.65E+0005	19				

*** Significant at the level 99.9%.

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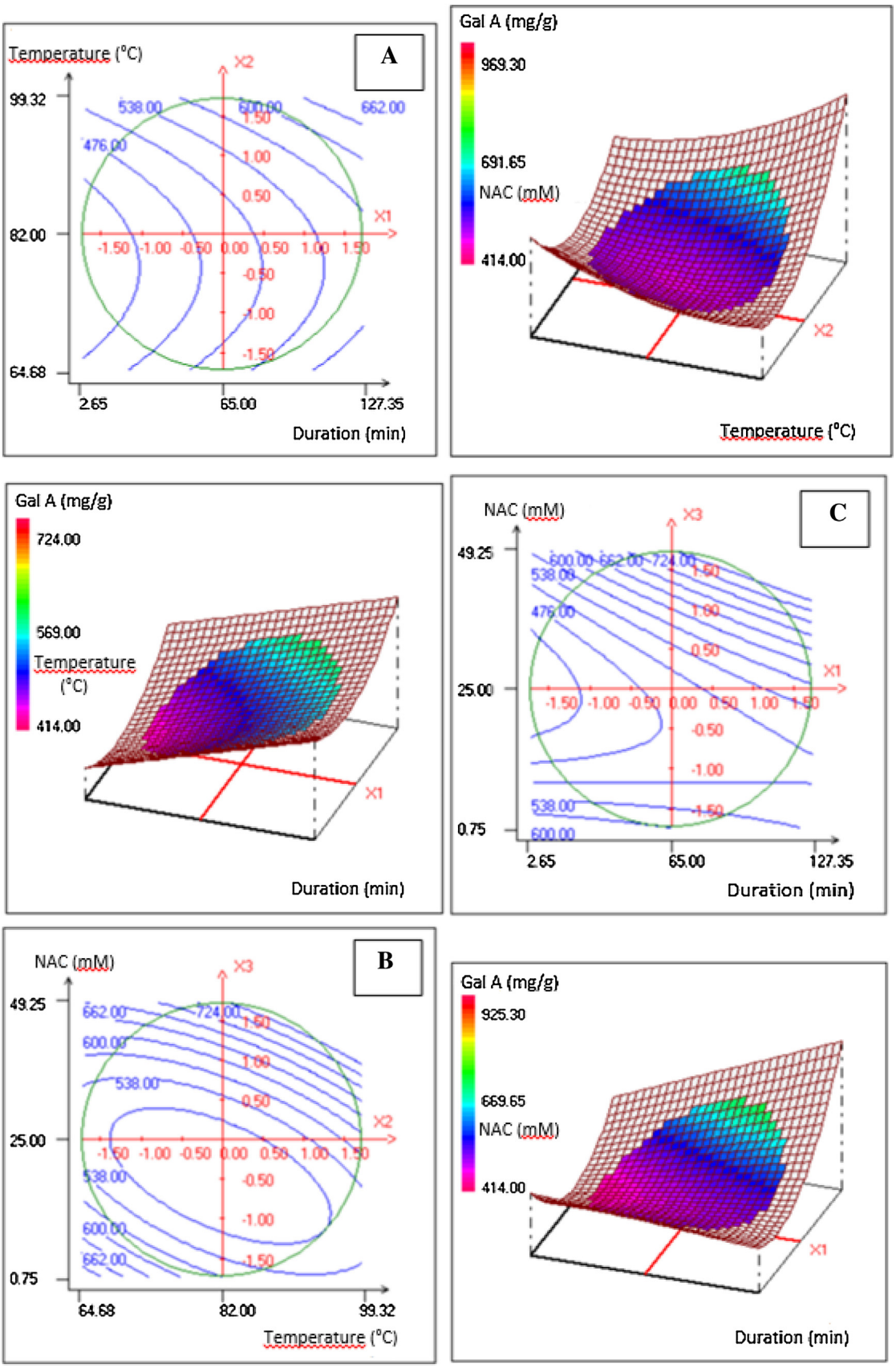

Fig. 2. Contour plots and three-dimensional response surface for the galacturonic acid content in pectins extracted from pomegranate peel. A: effect of duration and temperature at constant HNO_3 concentration (25 mmol/L); B: effect of HNO_3 concentration and temperature for a fixed duration (65 min); C: effect of HNO_3 and duration at constant temperature (82 °C).

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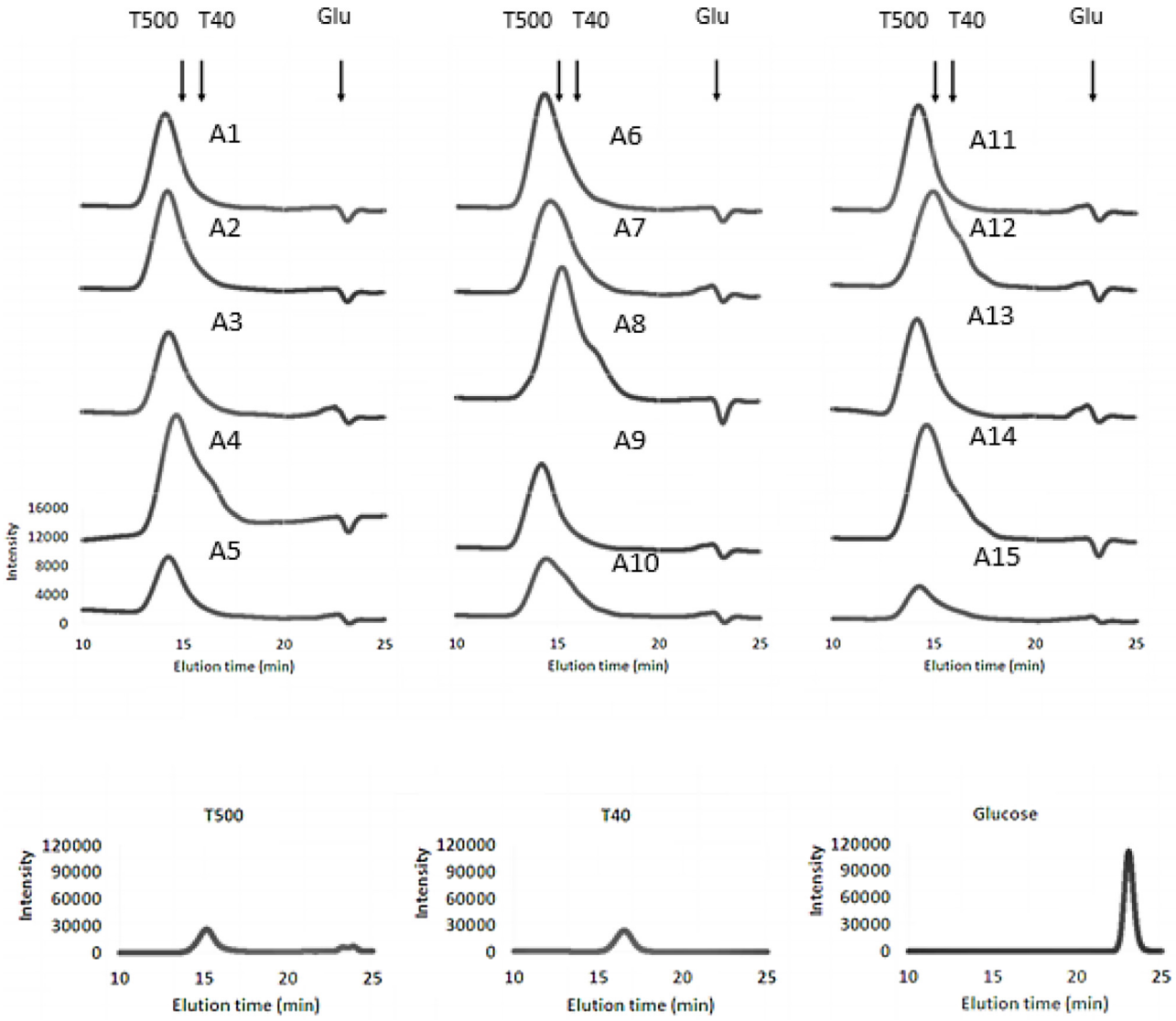

Fig. 3. High-pressure size-exclusion chromatography elution profiles of pectins extracted from pomegranate peel. The numbers correspond to the conditions of extraction as given in Table 2. HPSEC was carried out on combined OH-pack SB-802 HQ and TSK-Gel PWXL column using 0.4 mol/L Na acetate pH 3.8 as eluent. The arrows designate the elution times for dextrans T500 and T40. All samples were spiked with glucose.

Hence, under more extreme conditions the extraction of the rhamnogalacturonan backbone was enhanced while neutral sugar side-chains could be partially degraded. Specificity of extraction was also improved, with a relative decrease of the proportion of hemicellulose ballast in the extract.

On the other hand, the galactose content of most of the extracts was higher than that of arabinose which decreased with harsher extraction conditions (A8) suggesting either the predominance of arabinogalactan side chains and/or a higher degradation of arabinan side chains, probably due to the fact that arabinofuranosyl linkages are the most acid-labile [28].

The Gal A/Rha molar ratios ranging from 43 to 91 were high, though lower than those obtained for banana peels (210–402) [29] and for lemon by-product with acidified date juice (53–149) [30] (Table 2). This shows that the studied pectin samples contained probably a higher proportion of rhamnogalacturonic regions. This could be attributed to the pH values used for the pectin extraction (pH 1–2.2 versus pH 3.4 and 2.8 for pomegranate peel and lemon by-product, respectively).

Levigne et al. [31] reported that extraction at lower pH values led to higher amounts of pectins with more rhamnogalacturonan regions. Linkages between uronic acids or between uronic acids and rhamnose were reported as the most resistant, whereas arabinofuranosyl linkages are the most labile to acid hydrolysis [26].

3.2.3. *The hydrodynamic volume distribution*

In this work, the hydrodynamic volume distribution was determined by HPSEC [27] to compare the extracted samples. The elution times (ET) of the pectin samples and the partition coefficients (Kav) are listed in Table 2, and Fig. 3 shows the HPSEC profiles.

As shown in Fig. 3, temperature had a strong effect on the hydrodynamic volume distribution, in interaction with pH. Samples extracted at 82 °C with an extended extraction duration (+1.68) (A10) or at the lowest pH (pH = 1) (+1.68) (A14) presented a profile of elution with a shift to the right, due to increased cleavage of pectins. This effect was not noted at 72 °C even though the pH and duration were fixed at their extreme values. The extraction at 92 °C and pH 1.24 (A4, A8) also resulted a cleavage of pectin. At the extreme temperature (98.8 °C) pectins were cleaved (A12) resulting in a low hydrodynamic volume.

The runs with the lowest elution times (A1, A9 and A13), indicating the highest hydrodynamic volumes, corresponded to pectin

extracted at mild conditions. The lowest yield was obtained for A9. According to Scabio et al. [32], pectin with a less modified molecular mass profile can be obtained with extraction conditions giving low yields. In all trials, the mild extraction conditions were not always associated with high hydrodynamic volume, but severe conditions resulted in low hydrodynamic volumes (A8, A12, A14, A4 and A7). The longest elution time (ET = 15.2 min) with the highest partition coefficient (Kav of +0.008) which reflects the lowest hydrodynamic volume was detected in pectin extracted in the most severe conditions (A8). Whereas the lowest elution time (ET = 14.1 min) with the lowest partition coefficient (Kav of −0.134) was detected in pectin extracted in the mildest conditions (A1).

Pectin was probably partially degraded into smaller molecules during the extraction by the extreme extraction conditions. The decrease of the hydrodynamic volume could be attributed to the degradation of the side sugars chains and the hydrolysis of galacturonic acid chains.

3.2.4. Degree of methylation

Generally, pectin is characterised by its degree of methylation which is a major factor determining pectin functionality [27].

The pectin had a DM ranging between 30% and 55% (Table 2). The pectin was low methoxyl (LM, DM < 50) in all samples except A7, A9 and centre point. The lowest DMs were obtained for the pectins extracted at the extreme extraction conditions (A8, A10, A12 and A14) probably because harsher conditions of temperature, duration and pH increased the de-esterification of polygalacturonic chain [33]. A negative correlation between yield and DM was observed in citric acid extracts from pomegranate peel [6]. In commercial applications, LM pectins are less common than high methoxyl (HM) pectins. However, in recent times, there is more interest in the manufacturing of LM pectins due to their gelling characteristic that is suitable for the production of low-calorie and dietetic foods.

4. Conclusion

Pomegranate peel has the potential to be a source of low-methoxyl pectin which could be used as gelling agent in low-sugar jams for example. By using the response surface, the optimal conditions considering the yield were obtained graphically in order to reach the highest yield. The optimal conditions were 86 °C, 80 min and pH 1.7, respectively, providing a yield of 11.03%. Degree of methylation, uronic acid and total neutral sugar contents as well as hydrodynamic volume were all markedly affected by the extraction conditions. Higher purities (estimated by the GalA content) but lower hydrodynamic volumes and degrees of methylation were obtained in harsher conditions. Further work needs to be carried out to investigate the gelation conditions of this naturally low methoxyl pectin.

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