Optimization of pectin extraction from pomegranate peels by response surface methodology

Paulo Henrique F. Pereira, Túlio Ítalo S. Oliveira[1], Morsyleide F. Rosa[2], Fabio Lima Cavalcante[1], Graham K. Moates, Nikolaus Wellner, Keith W. Waldron, Henriette M. C. Azeredo[2,*]

Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK.

[1] Permanent address: Federal University of Ceara, Chemistry Department, Campus do Pici, Bloco 940, CP 6021, CEP 60455-760, Fortaleza, CE, Brazil, tulioufc@gmail.com (Author Oliveira, T.I.S.), fabiolima2008@hotmail.com (Author Cavalcante, F.L.).

[2] Permanent address: Embrapa Tropical Agroindustry, R. Dra. Sara Mesquita, 2270, CEP 60511-110, Fortaleza, CE, morsyleide.rosa@embrapa.br.

* Corresponding author, henriette.azeredo@embrapa.br.

Abstract

Pectins were extracted from pomegranate peels with citric acid, according to a central composite design with three variables: pH (2 – 4), temperature (70 – 90℃), and extraction time (40 – 150 min). Fourier transform infrared (FTIR) spectroscopy was used to follow changes in material composition during the main steps of pectin extraction, and also to determine the degree of methyl esterification and galacturonic acid content of pectins produced under different conditions. Harsh conditions enhanced the extraction yield and the galacturonic acid contents, but decreased the degree of methoxylation. The optimum extraction conditions, defined as those predicted to result in a yield of galacturonic acid higher than 8g/100g while keeping a minimum degree of methoxylation of 54% were: 88℃, 120 min, pH 2.5. Close agreement was found between experimental and predicted values at the extraction conditions defined as optimum.

Keywords: polysaccharides; biopolymers; response surface methodology; FTIR; pomegranate peel.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits, native to Persia, cultivated in ancient Egypt, Greece and Italy, then spread to several other regions (Ercisli, Agar, Orhan, Yildirim, & Hizarci, 2007). The pomegranate arils are consumed as such or processed into juices and other products, whose consumption has been motivated by their alleged health benefits derived from their high antioxidant capacity (Adhami, Khan, & Mukhtar, 2009; Sturgeon & Ronnenberg, 2010; Viuda-Martos, Fernández-López, & Pérez-Álvarez, 2010). The processing of pomegranate juice results in a pomace consisting of approximately 73 wt% peels (Wang, Pan, Ma, & Atungulu, 2011). Several studies have been focused on extraction of phenolic compounds from pomegranate peels and their active properties (Al-Zoreky, 2009; Kanatt & Chandler, 2010; Pan, Qu, Ma, Atungulu, & McHugh, 2011). A recent study (Moorthy, Maran, Surya, Naganyashree, & Shivamathi, 2015) was published on optimization of ultrasound assisted extraction of pectin from pomegranate peel, although the properties of pectin extracted from different extraction conditions have not been evaluated.

Pectin extraction is most commonly carried out using a dilute mineral acid, usually hydrochloric, sulfuric, or nitric acid, because of their lower price, and their ability to generate pectins enriched in homogalacturonic blocks as a result of significant hydrolysis of neutral sugar-containing rhamnogalacturonic regions at low pH and high temperature (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). However, the main drawback of those mineral acids are their toxicity and the generation of

environmentally unfriendly (corrosive) effluents, requiring special treatments to remove undesirable compounds from the pectin extracts so the final product can receive the GRAS (generally recognised as safe) status (Yapo, 2009). Pectin extraction yields from apple pomace, cocoa husks, and passion fruit peel with citric acid have been reported to be similar to those obtained with hydrochloric acid (Canteri-Schemin, Fertonani, Waszczynskyj, & Wosiacki, 2005; Chan & Choo, 2013; Kliemann et al., 2009). Moreover, organic acids have a lower hydrolyzing capacity than mineral acids (because of their lower dissociation constant), and are therefore expected to cause less de-polymerization of pectins (Kermani et al., 2014).

This study was carried out to evaluate the influence of pH, temperature, and time on pectin extraction from pomegranate peels with citric acid.

2. Materials and Methods

2.1. Chemical composition of pomegranate peel powder and changes with extraction process

Pomegranate peels, provided by Bakkavor Foods Ltd (London, UK), were oven-dried at 60℃ for 24 h, and milled to 0.5 mm in a Retsch Br inkmann ZM-1 centrifugal grinding mill (Retsch GmbH, Haan, Germany).

The ash, extractive, and Klason lignin contents of the pomegranate peel powder were determined according to methods TAPPI T413 OM-93 (1993), TAPPI T204 cm-97 (1997), and TAPPI T222 om-22 (2000), respectively. Hemicellulose and α-

cellulose contents were analyzed as described by TAPPI T203cm-99 (2009), and holocellulose, according to Yokoyama, Kadla, and Chang (2002).

Fourier Transform Infrared (FTIR) spectra were collected from the pomegranate peel powder, the alcohol-insoluble residue (AIR), and pectin, in the frequency range of 4000-800 cm^{-1} (128 scans at 2 cm^{-1} resolution) on a Nicolet Magna-IR 860 FTIR spectrometer (Thermo Nicolet, Madison, WI, USA). Samples were placed on a GoldenGate diamond ATR accessory (Specac, Orpington, Kent). The empty crystal was used as reference. The changes in spectra were followed in an attempt to understand any changes which occurred during the pectin extraction process.

2.2. Preparation of the alcohol-insoluble residue (AIR)

The alcohol insoluble residue (AIR) was prepared according to Waldron & Selvendran (1990), with some modifications. 100 g of milled peels were washed three times with ethanol to remove alcohol soluble components (firstly with 600 mL of boiling 70% v/v ethanol solution for 5 min, then with 600 mL of boiling absolute ethanol for 5 min, and the third time with 600 mL of absolute ethanol at room temperature for 5 min), then washed in 200 mL acetone. Between washings, the material was filtered through a 10 µm nylon mesh. The AIR was air dried at room temperature.

2.3. Pectin extraction

The pectin was extracted from the AIR with citric acid solutions (AIR: citric acid solution ratio, 1:20 w/v), according to a central composite design, based on the response surface methodology, with three variables: pH of the citric acid solution,

temperature and extraction time (Table 2). The extraction was carried out in a water bath under stirring (150 rpm). After centrifugation (3000 rpm, 30 min, 10℃), the supernatant was vacuum filtered, added with the same volume of absolute ethanol, and the pH was adjusted to 3.5 (pH of minimum pectin solubility) with KOH. The mixture was stirred for 30 min, left to precipitate at 4℃ for 2 h, and centrifuged (15 min, 4℃, 3500 rpm). The pellet was collected, washed with ethanol 70% (v/v), centrifuged again (20 min, 4℃, 3500 rpm), and dried at room temperature. It was then diluted/stirred in water (Ystral, 20 min), had its pH adjusted to 7, and was again dried and milled to a fine powder by using a basic mill (A10, IKA GmbH, Germany).

2.4. Pectin characterization and statistical analyses

The FTIR spectra (collected as described in section 2.1) were used to determine both the degree of methyl esterification (DM) and the galacturonic acid content (GA) of the pomegranate peel pectin. The DM was determined as described previously (Manrique & Lajolo, 2002, with modifications). Pectin standards with known DM values of 31% (P-9311), 67% (P-9436), and 89% (P-9561) were obtained from Sigma (Steinheim, Germany), their FTIR spectra were recorded (in triplicates), as well as the spectra of four other samples obtained from blends of the pectin standards (with DM values of 40, 49, 60, and 78%). The DM determination was based on the band areas at 1700-1750 cm^{-1} (methyl esterified uronic acids, EUA) and 1600-1630 cm^{-1} (free uronic acids, FUA), calculated by using Origin Pro 9, multiple peak fit function, and Gaussian fitting (OriginLab, Northampton, USA). A calibration curve was obtained by plotting DM against the ratio between the EUA

peak area over the sum of the EUA and FUA peak areas of the standards, and used to determine the DM of the pomegranate peel pectin.

The determination of the galacturonic acid content (GAC) was modified from a method described previously (Monsoor, Kalapathy, & Proctor, 2001). A set of 10 calibration standards was prepared by blending polygalacturonic acid (Fluka, 95% purity) with cellulose (Sigmacell type 20, Sigma) to obtain standards with galacturonic acid contents of 0% (pure cellulose) to 90% (w/w) galaturonic acid. The area above the baseline between 1840 cm^{-1} and 1550 cm^{-1} was used to calculate the total carbonyl peak area (Monsoor, Kalapathy, & Proctor, 2001). A calibration curve was obtained by plotting GAC against the total carbonyl peak area of the standards, and used to determine the GAC of the pectin samples.

The yield of galacturonic acid (YGA) was defined as

$$YGA\ (\%) = \frac{Total\ Yield(\%) \times GAC(\%)}{100}, \quad (1)$$

and was considered as the main response for optimization purposes, since it represented a combination of an economic response (i.e., the total amount of extract) and another related to the pectin purity (i.e., GAC).

Results of total extraction yield (based on AIR), DM, GAC, and YGA were analyzed using the software Minitab® 16 (Minitab Inc., State College, PA, USA). Full quadratic models were fit to the experimental responses by using the DOE (Design of Experiments)/Response Surface analysis, and the regressions were evaluated in terms of their determination coefficients (R^2 values) and the significance of their F values.

3. Results and Discussion

3.1. Chemical composition of pomegranate peel powder and changes with pectin extraction

Table 1 presents the chemical composition of the pomegranate peel powder (PPP) and the AIR. Alcohol/water soluble extractives constituted more than 50% of the dry weight of the peels. The ethanol extraction to obtain the AIR mainly reduced the contents of these extractives, also removing part of the lignin and minerals (ashes), thus concentrating the polysaccharides, as reported by Gullón, Yáñez, Alonso, & Parajó (2008) for apple pomace.

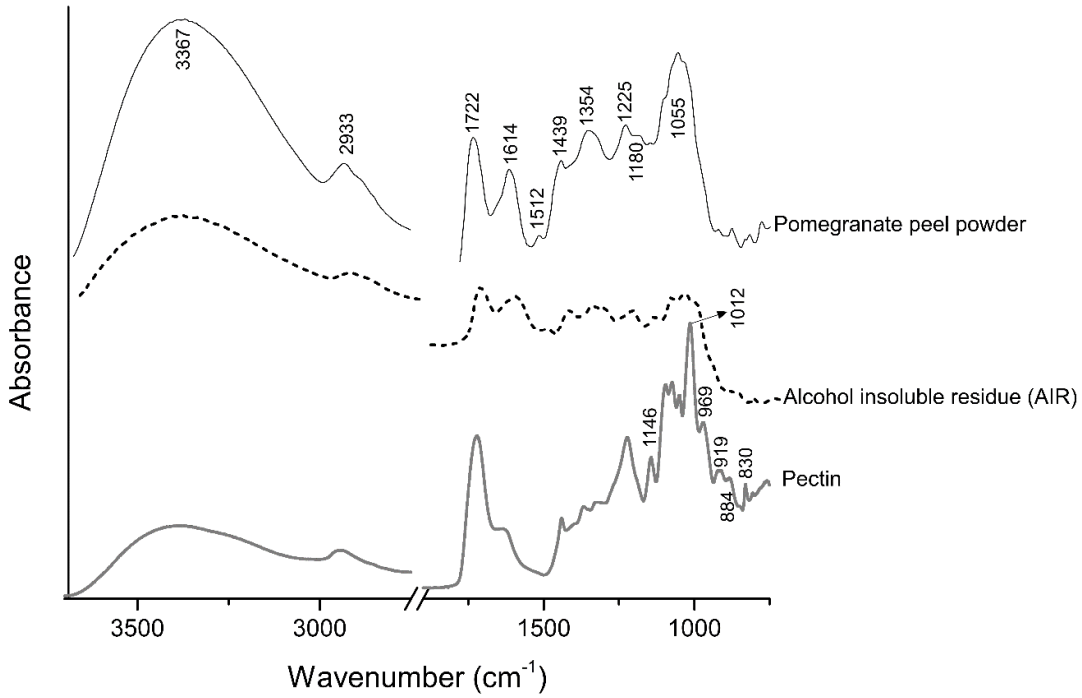
Table 1. Composition of pomegranate peel powder and AIR (on dry matter basis).

Component	Contents (g/100 g)	
	Pomegranate peel powder	AIR
Ash	4.0 ± 0.1	3.4 ± 0.0
Extractives	56.5 ± 1.6	13.0 ± 1.7
Klason lignin	22.1 ± 0.8	19.3 ± 1.6
Holocellulose	16.0 ± 2.2	48.1 ± 3.4
α-cellulose	7.9 ± 1.8	23.6 ± 4.2
Hemicelluloses + pectins	8.1 ± 3.5	24.5 ± 3.9

Values presented are mean ± standard deviation for n = 3.

The FTIR spectra (Figure 1) corroborate the chemical characterization (Table 1), and show some changes which occurred with the pectin isolation process. The band at 1354 cm^{-1} (CH_2 bending), related to the presence of cellulose, decreased with pectin extraction. Some bands related to the presence of lignin decreased or disappeared

146 along the process, such as the one at 1512 cm^{-1} (aromatic skeletal vibration). With
147 lignin removal and pectin isolation, the band at 2933 cm^{-1} (symmetrical C-H
148 stretching) decreased and shifted to a higher frequency. The band around 1614 cm^{-1}
149 in PPP and AIR seems to constitute a superposition of C=O stretching vibration of
150 non-esterified carboxyl groups (pectin) with C=C aromatic skeleton stretching (lignin
151 fractions), and decreases along the process because of lignin removal. The band at
152 1722 cm^{-1} (C=O stretching of methyl esterified galacturonic carboxyl groups) was
153 intensified by the pectin isolation. Some bands are more intense in isolated pectin,
154 such as at 1225 cm^{-1} (-CH_3CO stretching), 1146 cm^{-1} (asymmetric C-O-C stretching
155 vibration, indicating the presence of -O-CH_3), 1012 cm^{-1} (C-O-H deformation) 969
156 cm^{-1} (C-O bending), 919 cm^{-1} (rocking mode of –CH_3), and 884 cm^{-1} (-CCH and –
157 COH bending at the C-6 position). The bands at 1439 cm-1 (–O-CH_3 deformation)
158 and 1354 cm^{-1} (O-H bending) were maintained with the process.



159

160 **Figure 1.** FTIR spectra of pomegranate peel powder, alcohol insoluble residue
161 (AIR), and pectin obtained at 88℃, 120 min, pH 2.5.

162

163 **3.3. Characterization of extracted pectins and statistical analyses**

164 The calibration curves generated for the degree of methyl esterification (DM) and
165 galacturonic acid content (GAC) are represented by Equations (2) and (3),
166 respectively, from which the DM and GA values for all treatments were calculated.

167 $$DM = 126.3\left(\frac{A_{EUA}}{A_{EUA}+ A_{FUA}}\right) + 2.493 \quad (R^2 = 0.974) \qquad (2)$$

168 A_{EUA}: area of methyl esterified uronic acids; A_{FUA}: area of free uronic acids.

169 $$GAC(\%) = 0.7934.A_{1550-1840} - 0.1985 \ (R^2 = 0.951)$$ (3)

170 $A_{1550\text{-}1840}$: total carbonyl peak area (1840 cm^{-1} - 1550 cm^{-1}).

171 Table 2 presents the experimental responses, which generated the models
172 represented by the contour plots (Figure 2), and whose regression equations and
173 statistical parameters are presented in Table 3.

174

Table 2. Experimental conditions of pectin extraction from pomegranate peels with citric acid, and corresponding responses. Values in brackets are coded according to the central composite design.

Runs	Temperature (˚C)	Time (min)	pH	TEY (%, w/w)	DM (%)	GAC (%, w/w)	YGA (%, w/w)
1	74 (-1)	62 (-1)	2.4 (-1)	7.02	61.61	74.56	5.23
2	86 (+1)	62 (-1)	2.4 (-1)	9.93	58.35	80.51	8.00
3	74 (-1)	128 (+1)	2.4 (-1)	8.39	56.77	75.90	6.37
4	86 (+1)	128 (+1)	2.4 (-1)	10.95	54.27	81.54	8.93
5	74 (-1)	62 (-1)	3.6 (+1)	3.92	71.45	71.62	2.81
6	86 (+1)	62 (-1)	3.6 (+1)	5.60	67.32	77.37	4.34
7	74 (-1)	128 (+1)	3.6 (+1)	4.52	64.17	73.77	3.34
8	86 (+1)	128 (+1)	3.6 (+1)	6.01	59.52	78.35	4.71
9	70 (-1.68)	95 (0)	3 (0)	4.22	67.48	70.98	3.00
10	90 (+1.68)	95 (0)	3 (0)	7.84	61.67	80.19	6.29
11	80 (0)	40 (-1.68)	3 (0)	4.43	68.47	69.98	3.10
12	80 (0)	150 (+1.68)	3 (0)	6.35	61.21	74.20	4.71
13	80 (0)	95 (0)	2 (-1.68)	11.18	47.18	78.21	8.74
14	80 (0)	95 (0)	4 (+1.68)	4.29	70.72	69.90	3.00
15	80 (0)	95 (0)	3 (0)	5.04	60.19	73.36	3.70
16	80 (0)	95 (0)	3 (0)	5.65	63.85	71.85	4.06
17	80 (0)	95 (0)	3 (0)	4.46	62.39	71.37	3.18

TEY: total extraction yield; DM: degree of methyl esterification; GAC: galacturonic acid content; YGA: yield of galacturonic acid.

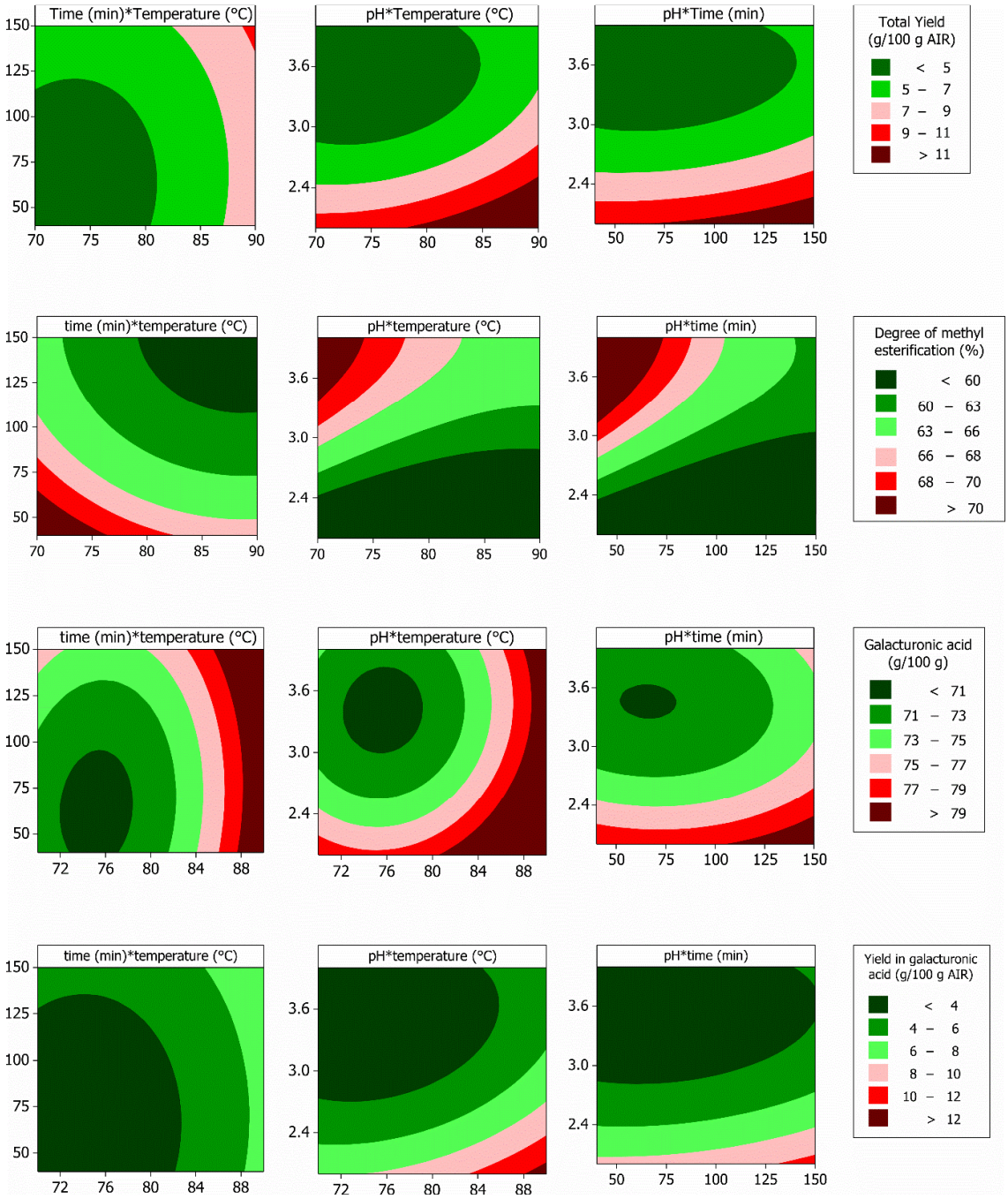


Figure 2. Contour plots representing the models for the experimental responses.

Table 3. Regression coefficients (for the coded values) and statistical parameters of the models.

Terms	TEY		DM		GAC		YGA	
	Coef	**p**	**Coef**	**p**	**Coef**	**p**	**Coef**	**p**
Mean	**5.01**	< 0.01	**62.22**	< 0.01	**72.02**	< 0.01	**3.61**	< 0.01
X_1	**1.08**	< 0.01	**-1.78**	0.04	**2.74**	< 0.01	**1.01**	< 0.01
X_2	**0.48**	0.01	**-2.65**	< 0.01	0.92	0.14	**0.42**	0.01
X_3	**-2.04**	< 0.01	**5.20**	< 0.01	**-1.86**	0.01	**-1.68**	< 0.01
X_1^2	**0.47**	0.02	0.61	0.45	**1.80**	0.02	**0.48**	0.01
X_2^2	0.35	0.15	0.70	0.38	0.56	0.39	0.22	0.16
X_3^2	**1.08**	< 0.01	-1.38	0.11	1.26	0.08	**0.92**	< 0.01
X_1*X_2	-0.07	0.72	0.03	0.97	-0.19	0.81	-0.04	0.80
X_1*X_3	-0.29	0.16	-0.38	0.68	-0.16	0.84	-0.30	0.11
X_2*X_3	-0.17	0.37	-0.77	0.42	0.09	0.90	-0.15	0.41
$\mathbf{R^2}$	98.00		92.60		87.24		97.73	
$\mathbf{F_{regression}}$	38.17		9.73		5.32		33.42	
p	< 0.01		< 0.01		0.02		< 0.01	

TEY: total extraction yield; DM: degree of methyl esterification; GAC: galacturonic acid content; YGA: yield of galacturonic acid; X_1, X_2, and X_3: temperature, time, and pH (respectively), in coded values ranging from -1.68 to 1.68 (according to Table 2). Regression terms in bold are significant ($p<0.05$).

The total extraction yield (TEY) was favoured by harsh conditions (i.e. higher temperatures, longer times, and lower pH values), as presented in Figure 2 and Table 3. Some previous studies similarly reported the positive effects of harsh extraction conditions on pectin extraction yield from different materials (Happi Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008, from banana peel; Garna et al., 2007, from apple pomace; Masmoudi et al., 2008, from lemon by-product). The TEY

196 was also significantly influenced ($p < 0.05$) by quadratic effects of temperature and
197 pH, which reflects in the presence of a noticeable curvature in the contour plots.

198 However, lower degrees of methyl esterification (DM) resulted from extraction at
199 higher temperatures and times and lower pH values (Figure 2, Table 3),
200 corroborating results reported for pectin extraction from banana peels and durian
201 rinds (Happi Emaga et al., 2008; Wai, Alkarkhi, & Easa, 2010). This is ascribed to
202 the contributing effect of harsher extraction conditions on de-esterification of the
203 polygalacturonic acid chain (Mort, Qiu, & Maness, 1993; Joye & Luzio, 2000).

204 The galacturonic acid content (GAC) was increased by higher temperatures and
205 lower pH values, similarly to previous findings (Gan & Latiff, 2011, for mangosteen
206 rind; Vriesmann, Teófilo, & Petkowicz, 2011, for cacao pod husks). This is a result of
207 substantial hydrolysis of pectin neutral sugars found in rhamnogalacturonic regions
208 (Minjares-Fuentes et al., 2014). Time did not have a significant effect on GAC, but
209 the quadratic effect of temperature was significant ($p < 0.05$), generating an inflection
210 in contour plots at around 75℃ (i.e. GAC tended to decrease from 70℃ to 75℃,
211 then to increase up to 90℃).

212 The yield of galacturonic acid (YGA) was found to be enhanced by higher
213 temperatures and times, and lower pH values (Figure 2, Table 3), which resulted
214 from the positive effects of harsher conditions on both the total extraction yield and
215 the galacturonic acid contents. The quadratic effects of temperature and pH were
216 also significant ($p < 0.05$), generating inflection in contour plots.

217 A region of optimum extraction was defined as the one which produced YGA higher
218 than 8 g/100 g AIR, while keeping DM at a minimum of 54%. This region is

represented as the doubly hatched area (overlaid contour plots) in Figure 3. From that region, a point was chosen (88℃, 120 min, pH 2. 5) in order to evaluate the suitability of the models. The experimental values were found to be in agreement with the predicted ones (Table 4).

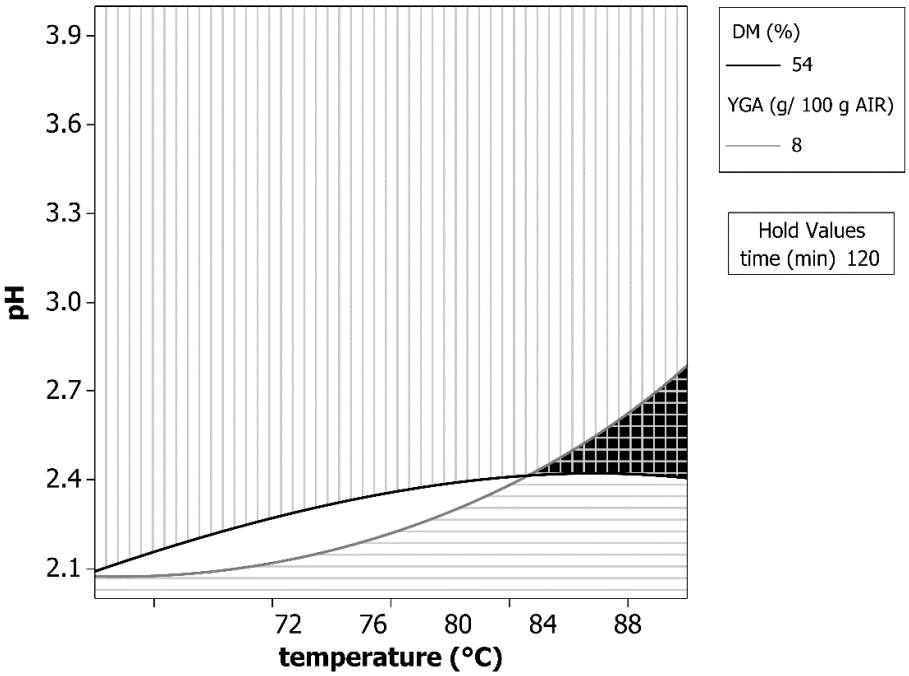


Figure 3. Overlaid contour plots of yield of galacturonic acid (YGA) and degree of methyl esterification (DM), showing the intersection between a DM higher than 54% and a YGA higher than 8 g/100 g AIR.

Table 4. Predicted and experimental response values at optimum conditions (88℃, 120 min, pH 2.5).

Response	Predicted value	Experimental value*
TEY (g/100 g AIR)	10.68	11.34 ± 1.24
DM (%)	54.90	53.09 ± 2.13
GAC (g/100 g)	82.37	80.95 ± 3.05
YGA (g/100 g AIR)	8.74	9.18 ± 1.09

230 TEY: total extraction yield; DM: degree of methyl esterification; GA: galacturonic acid content; YGA: yield of
231 galacturonic acid. * Mean ± standard deviation for 3 repetitions.

232

233 4. Conclusions

234 Pectins (mostly with high methyl esterification) were extracted from pomegranate
235 peels with citric acid under a range of pH, temperature and extraction time. Harsh
236 conditions (higher temperatures and times, and lower pH values) resulted in higher
237 extraction yields and higher galacturonic acid contents, while decreasing the degree
238 of methyl esterification. Many applications, such as film and gel formation, require
239 pectins with high degree of methoxylation (higher than 50%) and galacturonic acid
240 contents, For these, the optimum conditions of pectin extraction, defined as those
241 which were predicted to produce a yield of galacturonic higher than 8 g/100 g while
242 keeping a minimum degree of methoxylation of 54%, were: 88℃, 120 min, pH 2.5.
243 Under those conditions, the total extraction yield was about 11 g of pectin per 100 g
244 of alcohol insoluble residue. However, other pectin grades (for instance with higher
245 contents of rhamnogalacturonans) can be obtained by selecting the appropriate
246 extraction conditions.

5. Acknowledgements

The authors gratefully acknowledge the financial support of the UK Biotechnology & Biological Sciences Research Council (BBSRC) and the Brazilian Agricultural Research Corporation (Embrapa). Authors also thank the National Counsel of Technological and Scientific Development (CNPq, Brazil) for the Research Productivity Fellowship (304179/2012-4) conceded to H.M.C. Azeredo, and for the scholarships conceded to P.H.F. Pereira (243244/2013-4) and T.I.S. Oliveira (228859/2013-1).

6. References

Adhami, V.M., Khan, N., & Mukhtar, H. (2009). Cancer chemoprevention by pomegranate: laboratory and clinical evidence. *Nutrition and Cancer, 61*, 811-815.

Al-Zoreky, N.S. (2009). Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. *International Journal of Food Microbiology, 134*, 244-248.

Canteri-Schemin, M.H., Fertonani, H.C.R., Waszczynskyj, N., & Wosiacki, G. (2005). Extraction of pectin from apple pomace. *Brazilian Archives of Biology and Technology, 48*, 259–266.

Chan, S.-Y., & Choo, W.-S. (2013). Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. *Food Chemistry, 141*, 3752-3758.

Ercisli, S., Agar, G., Orhan, E., Yildirim, N., & Hizarci, Y. (2007). Interspecific variability of RAPD and fatty acid composition of some pomegranate cultivars

(Punica granatum L.) growing in Southern Anatolia Region in Turkey. *Biochemical Systematics and Ecology, 35*, 764-769.

Gan, C.-Y., & Latiff, A.A. (2011). Extraction of antioxidant pectic-polysaccharide from mangosteen (*Garcinia mangostana*) rind: Optimization using response surface methodology. *Carbohydrate Polymers, 83*, 600-607.

Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K., Legros, H., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield and purity of apple pomace pectin precipitated but not washed by alcohol. *Journal of Food Science, 72*, C1-C9.

Gullón, B., Yáñez, R., Alonso, J.L., & Parajó, J.C. (2008). L-lactic acid production from apple pomace by sequential hydrolysis and fermentation. *Bioresource Technology, 99*, 308-319.

Happi Emaga, T., Ronkart, S.N., Robert, C., Wathelet, B., & Paquot, M. (2008). Characterisation of pectins extracted from banana peels (*Musa AAA*) under different conditions using an experimental design. *Food Chemistry, 108*, 463-471.

Joye, D.D., & Luzio, G.A. (2000). Process for selective extraction of pectins from plant material by differential pH. *Carbohydrate Polymers, 43*, 337–342.

Kanatt, S.R., & Chandler, R. (2010). Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. *International Journal of Food Science and Technology, 45*, 216-222.

Kermani, Z.J., Shpigelman, A., Kyomugasho, C., Van Buggenhout, S., Ramezani, M., Van Loey, A., & Hendrickx, M.E. (2014). The impact of extraction with a chelating agent under acidic conditions on the cell wall polymers of mango peel. *Food Chemistry, 161*, 199-207.

Kliemann, E., Simas, K.N., Amante, E.R., Prudêncio, E.S., Teófilo, R.F., Ferreira, M.M.C., & Amboni, R.D.M.C. (2009). Optimisation of pectin acid extraction from passion fruit peel (*Passiflora edulis* flavicarpa) using response surface methodology. *International Journal of Food Science & Technology, 44*, 476-483.

Manrique, G.D., & Lajolo, F.M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology, 25*, 99-107.

Masmoudi, M., Besbes, S., Chaabouni, M., Robert, C., Paquot, M., Blecker, C., & Attia, H. (2008). Optimization of pectin extraction from lemon by-product with acidified date juice using response surface methodology. *Carbohydrate Polymers, 74*, 185-192.

Minjares-Fuentes, R., Femenia, A., Garau, M.C., Meza-Velázquez, J.A., Simal, S., & Rosselló, C. (2014). Ultrasound-assisted extraction of pectins from grape pomace using citric acid: A response surface methodology approach. *Carbohydrate Polymers, 106*, 179-189.

Monsoor, M.A., Kalapathy, U., & Proctor, A. (2001). Determination of polygalacturonic acid content in pectin extracts by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 74*, 233-238.

309 Moorthy, I.G., Maran, J.P., Surya, S.M., Naganyashree, S., & Shivamathi, C.S.
310 (2015). Response surface optimization of ultrasound assisted extraction of pectin
311 from pomegranate peel. *International Journal of Biological Macromolecules, 72*,
312 1323-1328.

313 Mort, A.J., Qiu, F., & Maness, N.O. (1993). Determination of the pattern of methyl
314 esterification in pectin. Distribution of contiguous nonesterified residues.
315 *Carbohydrate Research, 247*, 21–35.

316 Pan, Z., Qu, W., Ma, H., Atungulu, G.G., & McHugh, T.H. (2011). Continuous and
317 pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel.
318 *Ultrasonics Sonochemistry, 18*, 1249-1257.

319 Sturgeon, S.R., & Ronnenberg, A.G. (2010). Pomegranate and breast cancer:
320 possible mechanisms of prevention. *Nutrition Reviews, 68*, 122-128.

321 TAPPI. (1993). *T413 om-93. Ash in wood, pulp, paper and paperboard: combustion*
322 *at 900ºC*.

323 TAPPI. (1997). *T204 om-97. Solvent extractives of wood and pulp.*

324 TAPPI. (2000). *T 222 om-02. Acid-insoluble lignin in wood and pulp*.

325 TAPPI. (2009). *T 203 cm-99. Alpha-, beta- and gamma-cellulose in pulp*.

326 Viuda-Martos, M., Fernández-López, J., & Pérez-Álvarez, J.A. (2010). Pomegranate
327 and its many functional components as related to human health: a review.
328 *Comprehensive Reviews in Food Science and Food Safety, 9*, 635-654.

Vriesmann, L.C., Teófilo, R.F., & Petkowicz, C.L.O. (2011). Optimization of nitric acid-mediated extraction of pectin from cacao pod husks (*Theobroma cacao* L.) using response surface methodology. *Carbohydrate Polymers, 84,* 1230-1236.

Wai, W.W., Alkarkhi, A.F.M., & Easa, A.M. Effect of extraction conditions on yield and degree of esterification of durian rind pectin: An experimental design. *Food and Bioproducts Processing, 88*, 209-214.

Waldron, K.W., & Selvendran, R.R. (1990). Composition of the cell walls of different asparagus (*Asparagus officinalis*) tissues. *Physiologia Plantarum, 80*, 568–575.

Wang, Z., Pan, Z., Ma, H., & Atungulu, G.G. (2011). Extract of phenolics from pomegranate peels. *The Open Food Science Journal, 5*, 17-25.

Yapo, B.M. (2009). Lemon juice improves the extractability and quality characteristics of pectin from yellow passion fruit by-product as compared with commercial citric acid extractant. *Bioresource Technology, 100*, 3147-3151.

Yapo, B.M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry, 100*, 1356–1364.

Yokoyama, T., Kadla, J.F., Chang, H.-M. (2002). Microanalytical method for the characterization of fiber components and morphology of woody plants. *Journal of Agricultural and Food Chemistry, 50*, 1040–1044.

Figure 2 (gray-scale version)