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Ultrasound-assisted extraction of pectins from grape pomace using citric acid: A response surface methodology approach

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ABSTRACT

An ultrasound-assisted procedure for the extraction of pectins from grape pomace with citric acid as the extracting agent was established. A Box–Behnken design (BBD) was employed to optimize the extraction temperature (X_1: 35–75 °C), extraction time (X_2: 20–60 min) and pH (X_3: 1.0–2.0) to obtain a high yield of pectins with high average molecular weight (MW) and degree of esterification (DE) from grape pomace. Analysis of variance showed that the contribution of a quadratic model was significant for the pectin extraction yield and for pectin MW whereas the DE of pectins was more influenced by a linear model. An optimization study using response surface methodology was performed and 3D response surfaces were plotted from the mathematical model. According to the RSM model, the highest pectin yield (~32.3%) can be achieved when the UAE process is carried out at 75 °C for 60 min using a citric acid solution of pH 2.0. These pectic polysaccharides, composed mainly by galacturonic acid units (<97% of total sugars), have an average MW of 163.9 kDa and a DE of 55.2%. Close agreement between experimental and predicted values was found. These results suggest that ultrasound-assisted extraction could be a good option for the extraction of functional pectins with citric acid from grape pomace at industrial level.

1. Introduction

Recently, there has been an increasing concern for the preservation of the environment and sustainability of resources. Thus, the utilization of natural resources is receiving renewed interest as an alternative to non-renewable resources in material technology (Galanakis, 2013; Liu, Cao, Huang, Cai, & Yao, 2010).

Fruit processing in the food industry gives rise to large amounts of by-products. Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 68 million tons in 2009 (FAOSTAT, 2011). Since about 75–80% of the total amount is used in winemaking, around 12 million tons of grape pomace is produced within a few weeks of the harvest campaign. Grape pomace constitutes the main by-product, and is of great interest to the food industry, since the available carbohydrate fraction can be used to provide dietary fiber and other bioactive compounds (González-Centeno et al., 2010, 2012).

An emerging field of clinical importance is the growing interest in the role of dietary carbohydrates. Previous reports suggest that polysaccharides possess a wide range of pharmacological properties such as anti-tumor and antioxidant, as well as anti-diabetic activity and immunity-modulation. Thus, one of the most characterized bioactive roles for pectin is as an anti-cancer agent (Glinsky & Raz, 2009; Kwon, Qiu, Hashimoto, Yamamoto, & Kimura, 2009; Morris, Gromer, Kirby, Bongaerts, & Patrick Gunning, 2011; Yan et al., 2011). Pectin is also a high-value functional food ingredient widely used as gelling and stabilizing agent. It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants (Willats, Knox, & Mikkelsen, 2006). The pectin is a group of polysaccharides in which the presence of partly methyl-esterified galacturonic acid and, to a lesser extent, rhamnose is a distinctive feature. The molecular weight (MW) and the degree of esterification (DE) of pectic polysaccharides affect the commercial use of pectin as gelling and thickening agents (Atmodjo, Hao, & Mohnen, 2013; Morris, Ralet, Bonnin, Thibault, & Harding, 2010; Morris, Foster & Harding, 2000; Prakash, Sivakumar, Thirugnanasambandham, & Sridhar, 2013).

Industrially, pectins are extracted from apple pomace, sugar beet pulp, and citrus peels using water acidified with a strong mineral acid, notably, nitric, hydrochloric or sulphuric acid (the so-called conventional acid extraction) under pH, temperature, and duration conditions, most often, in the order of 1.4–3, 60–100 °C,

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and 20–360 min, respectively (Min et al., 2011; Prakash Maran et al., 2013a, 2013b). Moreover, pectins produced using low pHs (<2) and high temperatures are, generally, enriched in galacturonic acid (or homogalacturonic building blocks) as a result of substantial hydrolysis of pectin neutral sugars present in rhamnogalacturonic regions. However, the well-known toxicity of these strong mineral acids, and the environmentally unfriendly (corrosive) effluents they generate, are their main drawback. Special treatments, able to remove potentially toxic elements from pectin extracts, are therefore needed for the extracted product to abide by the GRAS (generally recognized as safe) status and to be accepted for consumption (Yapo, 2009).

In connection with the emerging concept of 'Green Chemistry', recent emphasis has been given to nonconventional chemistry based on a combination of chemical and physical treatments. Thus, the processing for pectin isolation is no exception, thereby introducing environment- and human-friendly technology (Galanakis, 2013; Yapo, 2009). Recent studies have reported that citric acid is a good pectin extracting agent with regard to the pectin yield and the main intrinsic parameters (DE and average MW) governing gel formation (Canteri-Schemin, Fertonani, Waszczynskyj, & Wosiacki, 2005; Pinheiro et al., 2008; Yapo, 2009). Furthermore, power ultrasound has a great potential in a wide variety of technological processes. The technique has been used in commercial applications for many years, and over the past two decades, application of ultrasound in chemistry as well as the food and pharmaceutical industries has become an exciting new field of research. In fact, ultrasound has been recognized as an alternative approach to traditional extraction methods (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Ebringerová & Hromádková, 2010; Hromádková, Ebringerová, & Valachovic, 1999; Galanakis, 2013; Rastogi, 2011).

Ultrasound-assisted extraction (UAE) is a process that uses acoustic energy and solvents to extract target compounds from various plant matrices. The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken both of which are significantly affected by ultrasound irradiation (Vinatoru, 2001). Although the application of UAE of different compounds from plant material has been widely published (Ebringerová & Hromádková, 2010; Esclapez, García-Pérez, Mulet, & Cárcel, 2011), few studies have focused on pectin extraction. Increased yields as well as an important reduction in extraction time have been reported as the main advantages of using ultrasounds (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011; Panchev, Kirtchev, & Kratchanov, 1988). To the best of our knowledge, there are no reports of pectin extraction combining the use of ultrasound technology and citric acid.

At present the economical feasibility of an industrial process also requires working in such a way that high extraction efficiency is attained. Many factors have been established to improve the extraction efficacy, such as temperature, time and solvent type. When several factors and their potential interactions may affect a desired response, response surface methodology (RSM) is an effective tool to optimize the process (Nwabueze, 2010). RSM has been very popular for optimization studies in recent years. In fact, RSM has been used to carry out the optimization of pectin extraction process from apple pomace (Shin, Kim, Cho, & Hwang, 2005; Wang et al., 2007), banana peel (Qiu et al., 2010), passion fruit peels (Pinheiro et al., 2008), cacao pod husks (Vriesmann, Teófilo, & Petkowicza, 2011), mangosteen rind (Gan & Latiff, 2011), durian rind (Wai, Alkarkhi, & Easa, 2009), orange peels (Prakash Maran et al., 2013a, 2013b) and Pumpkin (Prakash Maran et al., 2013a, 2013b).

The aim of this study is to optimize the ultrasonic assisted extraction of pectins from grape pomace, using citric acid as the extracting agent. For this purpose, the RSM technique was applied considering the extraction temperature, extraction time and pH as variables.

2. Experimental

2.1. Sample

Pomace of the grape variety Cabernet Sauvignon was provided by a winery in Mallorca (Spain). The grape pomace of Cabernet Sauvignon, mainly composed of pressed skins and seeds, had a moisture content of ~62 g/100 g fresh weight. The grape pomace was milled to an average particle size of ~2 mm, with a commercial mill (Braun KSM 2, Mexico City, Mexico), and then vacuum-packed and stored at −20 °C until extractions.

2.2. Ultrasound-assisted extraction (UAE) of pectin from grape pomace

Pectin was extracted using constant ultrasonic power in an ultrasonic bath with internal dimensions of 24.0 cm × 13.7 cm × 15.0 cm and a capacity of 4.25 L (Elmasonic S 40 H, Singen, Germany). The variable conditions were extraction temperature (X_1), extraction time (X_2), and pH (X_3) of the citric acid solution.

To determine the power that was acting on the sample, a characterization of the ultrasonic bath was performed according to the equation (Eq. (1)) proposed by Raso et al. (1999). The calculated power was expressed as power density (Sivakumar and Pandit, 2001).

$$\text{Power Density} = \frac{dT}{dt} c_p m \quad (1)$$

where c_p is the heat capacity of the solvent (in J kg^{-1} K^{-1}) and m is the mass of solvent used (in kg). The solvent used in all experiments was citric acid. Thus, the ultrasonic bath was working at a frequency of 37 kHz, power output of 140 W and power density of 0.05 W mL^{-1}.

The extraction was carried out according to the methods proposed by Canteri-Schemin et al. (2005) and Panchev, Kirtchev, and Kratchanov (1994), with slight modifications. Ten grams of grape pomace were mixed with 100 mL of citric acid solution. The flask was placed in the center of the bath using the conditions of experimental design. The hot acid extract was filtered through a screen of 1 mm mesh, created by two layers of cheesecloth, then the filtrate was cooled down to 4 °C. Following that, the filtrate (containing pectin) was precipitated with ethanol 95% (1:2, v/v) and stirred for 10 min. Next, the mixture was centrifuged at 1750 × g for 30 min. The precipitated pectin was separated by filtration (#40 glass fiber) and rinsed twice with ethanol 95% and acetone. The pectin extracted ultrasonically was dried at room temperature for 12 h. Finally, the resulting material was milled to obtained powdered pectin. The yield was calculated as grams of product obtained per 100 g of grape pomace (fresh weight). Uronic acids were determined by colorimetry (Blumenkrantz & Asboe-Hansen, 1973), using samples hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4. Results showed that uronic acid accounted for 96–99% of the pectin extracted in all samples.

2.3. Determination of the molecular weight

The average molecular weight (MW) of extracted pectins was determined by using high performance size exclusion chromatography connected with a refractive index detector (Agilent 1100 series, CA, USA). Pectin material (3 mg) was dissolved in 50 mM sodium nitrate (3 mL) and filtered through a 5 μm filter. Then,

the samples (0.5 μL) were injected into columns in series (BioSep-SEC-S2000 and S4000, Phenomenex Inc., CA, USA), which have exclusion limits of 3×10^5 and 1.5×10^6 Da, respectively. Sodium nitrate (50 mM) was eluted at 50 °C with a flow rate of 1 mL min^{-1}. To calibrate the column, standard dextrans having MWs of 1185.0, 409.8, 273.0, and 80.9 kDa were used.

2.4. Determination of the degree of esterification (DE) of pectin using Fourier Transform Infrared (FTIR) spectrometry

Samples containing pectin were desiccated prior to FTIR analysis. FTIR spectra of the samples were recorded using a Bruker IFS66 instrument from 800 to 2000 cm^{-1}. The measuring resolution was 3 cm^{-1}. The resultant spectra were smoothed to remove the noise and the second-derivative spectra were obtained using the OMNIC E.S.P. 5.1 software. DE was determined according to Pappas et al. (2004) equations:

$$DE = 124.7R + 2.2013 \quad (2)$$

$$R = \frac{A_{1740}}{[A_{1740} + A_{1630}]} \quad (3)$$

where A_{1740} and A_{1630} were defined as the absorbance intensities of the bands for methyl-esterified and non methyl-esterified carboxyl groups at 1740 cm^{-1} and 1630 cm^{-1}, respectively. These strong mixed peaks are caused by ester and carboxylic vibrations (Manrique & Lajolo, 2002).

2.5. Scanning electron microscopy (SEM)

SEM micrographs of previously untreated grape pomace samples, after heat treatment at 35 °C for 60 min, and after ultrasound treatment during 60 min at 35 °C, were obtained with a Hitachi S-3400N (Japan) scanning electron microscope at an accelerating voltage of 10 kV. Samples were directly observed, without further treatment, under a pressure of 40 Pa.

2.6. Experimental design for optimization

Response surface methodology (RSM) was used to determine the optimum conditions for pectin extraction from grape pomace. A Box–Behnken design (BBD) with three independent variables was employed (Box & Behnken, 1960). The variables used were: extraction temperature (X_1), extraction time (X_2), and pH (X_3). The uncoded and coded values for the three variables can be observed in Table 1. The complete design consisted of 17 experiments including twelve factorial experiments, and five replicates at the center point (Table 2) (Gan & Latiff, 2011). All the experiments were carried out at random, in order to minimize the effect of unexplained variability in the observed responses due to systematic errors.

The variables were coded according to the equation:

$$x = \frac{X_i - X_0}{\Delta X} \quad (4)$$

where x is the coded value, X_i is the corresponding actual value, X_0 is the actual value in the center of the domain, and ΔX is the increment of X_i corresponding to a variation of 1 unit of x. The mathematical model corresponding to the Box–Behnken design is (Eq. (5)):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon \quad (5)$$

where Y is the dependent variable (either pectin yield, average molecular weight or degree of esterification), β_0 is the model constant, β_i, β_{ii} and β_{ij} are the model coefficients and ε is the error. They represent the linear, quadratic and interaction effects of the different variables.

2.7. Statistical analysis

The regression coefficients for linear, quadratic and interaction terms were determined by using multiple linear regression. Statistica software (Statsoft version 7.0, USA) was used for the regression analysis of the experimental data. Student's t-test permitted the checking of the statistical significance of the regression coefficient, and Fischer's F-test determined the second-order model equation at a probability (p) of 0.05. The adequacy of the model was determined by evaluating the lack of fit, the coefficient of determination (R^2), adequate precision (*adeq-precision*) and the F-test value obtained from the analysis of variance (ANOVA) that was generated. The regression coefficients were then used to generate response surfaces. All calculations and graphics were performed using Statistica software (Statsoft version 7.0, USA). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical model.

3. Results and discussion

3.1. Ultrasonic assisted extraction of pectins with citric acid

SEM was used to observe the effect of sonication on the physical structure of grape pomace before and after ultrasonic treatment. Fig. 1a and b shows that there were minor differences between fresh and heated samples without sonication. On the contrary, fractural changes in the surface morphology of grape pomace became evident for sonicated samples (Fig. 1c). Therefore, it seems clear that sonication plays an important role in breaking up the vegetal tissue and enhancing extraction yields. Similar results have been reported by Li, Qu, Zhang, and Wang (2012), who compared oil extraction by soxhlet extraction and ultrasound assisted extraction (UAE) on seed powder of *Isatis indigotica*. Further, Maricela, Vinatoru, Paniwnyk, and Mason (2001) reported that ultrasonic treatment enhances the swelling and softening process of cell walls via the hydration of pectinous material from middle lamella, which leads to the break-up of vegetal tissue during sonication.

In this study, the UAE of pectic polysaccharides from grape pomace was combined with the use of citric acid as the extracting agent, which has been reported to be a good option to carry out the extraction of pectins, presenting similar yields to nitric acid and reducing the risk of potential ecological damage (Canteri-Schemin et al., 2005; Yapo, 2009).

Previous studies showed that pH, temperature, and time were the main parameters affecting the yield of pectic extraction from different sources (Gan & Latiff, 2011; Happi-Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008; Masmoudi et al., 2008; Panchev et al., 1994). These parameters also had a significant influence on the intrinsic characteristics of pectins, such as their average molecular weight (MW) (Bagherian et al., 2011; Happi-Emaga et al., 2008; Kurita, Fujiwara, & Yamazaki, 2008; Li et al., 2012) and the degree of esterification (DE) of the acid groups (Faravash & Ashtiani, 2008; Kulkarni & Vijayanand, 2010; Levigne, Ralet, & Thibault, 2002; Masmoudi et al., 2008; Panchev et al., 1994; Pinheiro et al., 2008; Wai, Alkarkhi, & Easa, 2010).

Therefore, effects of extraction temperature (X_1), time (X_2) and pH (X_3) on extraction yield, average MW and DE of pectins were studied during experimentation. The results of 17 runs using a BBD are given in Table 2 that include the design, the observed responses and the predicted values. As can be seen, a close agreement between experimental and predicted values was found.

Table 1
Box–Behnken design with the observed responses and predicted values for yield extraction and DE of UAE of pectin from grape pomace.

Run	Variable levels						Observed (Y_1)			Predicted (Y_0)		
	x_1	(X_1)	x_2	(X_2)	x_3	(X_3)	Yield (%)	Mw (kDa)	DE (%)	Yield (%)	Mw (kDa)	DE (%)
1	1	(75)	0	(40)	−1	(1.0)	27.13	111.00	61.19	27.41	109.63	55.93
2	0	(55)	−1	(20)	1	(2.0)	22.13	205.00	56.41	22.16	203.30	45.70
3	0	(55)	0	(40)	0	(1.5)	10.25	158.00	53.57	9.99	153.82	42.57
4	0	(55)	1	(60)	1	(2.0)	29.00	189.00	52.47	29.12	189.02	54.40
5	−1	(35)	−1	(20)	0	(1.5)	3.19	163.50	29.86	3.43	163.83	35.31
6	−1	(35)	0	(40)	1	(2.0)	14.88	183.00	35.23	14.59	184.36	40.48
7	0	(55)	0	(40)	0	(1.5)	9.63	153.00	39.15	9.26	153.82	42.57
8	0	(55)	0	(40)	0	(1.5)	14.56	152.40	40.60	14.99	153.82	42.57
9	1	(75)	−1	(20)	0	(1.5)	10.69	144.00	37.18	10.52	145.38	44.36
10	0	(55)	0	(40)	0	(1.5)	11.75	152.90	41.30	11.73	153.82	42.57
11	1	(75)	0	(40)	1	(2.0)	29.38	176.00	37.63	29.50	176.31	41.15
12	0	(55)	1	(60)	−1	(1.0)	21.25	132.10	22.79	21.21	133.80	33.50
13	−1	(35)	0	(40)	−1	(1.0)	5.56	138.70	20.09	5.44	138.38	16.57
14	1	(75)	1	(60)	0	(1.5)	22.00	132.60	53.13	21.76	132.26	47.68
15	−1	(35)	1	(60)	0	(1.5)	3.63	152.00	23.89	3.79	150.61	16.71
16	0	(55)	−1	(20)	−1	(1.0)	14.38	145.90	59.40	14.26	145.87	57.47
17	0	(55)	0	(40)	0	(1.5)	13.31	152.80	38.25	13.54	153.82	42.57

Table 2
Analysis of variance for the response surface of yield extraction, MW, and DE of UAE of pectins from grape pomace.

Source	DF	SS	MS	*F*-value	*p*-value	R^2	Adeq. precision
Yield							
Regression	9	28.02	3.11	26.26	0.000	0.971	16.32
Linear	3	16.70	5.56	46.96	0.000		
Quadratic	3	10.24	3.41	28.81	0.000		
Cross-point	3	1.08	0.35	3.03	0.103		
Lack of fit	3	0.39	0.13	1.21	0.414		
MW							
Regression	9	8384.31	931.59	182.08	0.000	0.995	53.99
Linear	3	7369.29	2456.43	480.10	0.000		
Quadratic	3	906.69	302.23	59.07	0.000		
Cross-point	3	108.33	36.11	7.06	0.016		
Lack of fit	3	13.77	4.59	0.83	0.542		
DE							
Regression	9	2039.04	226.56	2.49	0.121	0.762	6.28
Linear	3	959.91	319.97	3.52	0.077		
Quadratic	3	317.75	105.91	1.17	0.388		
Cross-point	3	761.37	253.79	2.79	0.119		
Lack of fit	3	479.27	159.75	4.07	0.104		

Results showed that the yield of extracted pectin ranged from 3.2% to 29.4%. The maximum yield was found under the experimental conditions of $X_1 = 75\,^{\circ}C$, $X_2 = 40$ min and $X_3 = 2.0$. The average MW of those extracted pectins ranged from 111 to 205 kDa. Pectins with the highest MW were extracted under the conditions of $X_1 = 55\,^{\circ}C$, $X_2 = 20$ min and $X_3 = 2.0$. Further, a wide range of degree esterification (DE) values was also found, ranging from 20.1 to 61.2%. The maximum DE value was found at conditions of $X_1 = 75\,^{\circ}C$, $X_2 = 40$ min and $X_3 = 1.0$. Therefore, optimal process conditions were investigated in order to obtain the maximum extraction yield, MW and DE of UAE of pectins from grape pomace with citric acid.

Moreover, the sugar analysis revealed a great uniformity for the type of pectins obtained in all the extraction procedures. Galacturonic acid was the predominant sugar, accounting for 95–97% of total sugars. Other sugars such as rhamnose, arabinose and galactose were present in small quantities (<2%), suggesting the presence of mainly homogalacturonans within the extracts. The large presence of the latter type of pectic polysaccharides in grape pomace has already been observed by Gonzalez-Centeno et al. (2010). However, it is also possible that the use of power ultrasounds and, in particular, citric acid as the extracting agent, might have contributed to the degradation of pectin ramifications present in RG-I type pectins (Kurita et al., 2008).

3.2. Model fitting

Table 3 presents the results of fitting models to the data. The results of the analysis of variance (ANOVA) indicated that the lack of fit was clearly not significant for the response surface model corresponding to the yield and average MW of extracted pectin, and moderately significant for the model corresponding to DE of pectins. In addition, R^2 was calculated to check the model adequacy. The R^2 values were 0.9712, 0.9957 and 0.7622 for extraction yield, MW and DE, respectively (Table 3). Finally, to make a decision about using the developed model, the adequate precision parameter was also employed. Adequate precision is a measure of the range in predicted response relative to its associated error (Noordin, Venkatesh, Sharif, Elting, & Abdullah, 2004). Values above 4 indicate that the model can be used within the region of operation. Adequate precision values of 16.32 and 53.99 were calculated for the yield and MW responses, respectively; while for the DE model, the adequate precision value was 6.28. The fact that all values were higher than 4, indicated that the models of response surface could be used within the region of operation.

Based on these results, the surface models, for yield, MW and DE of UAE of pectin from grape pomace, were formulated (see Eqs.

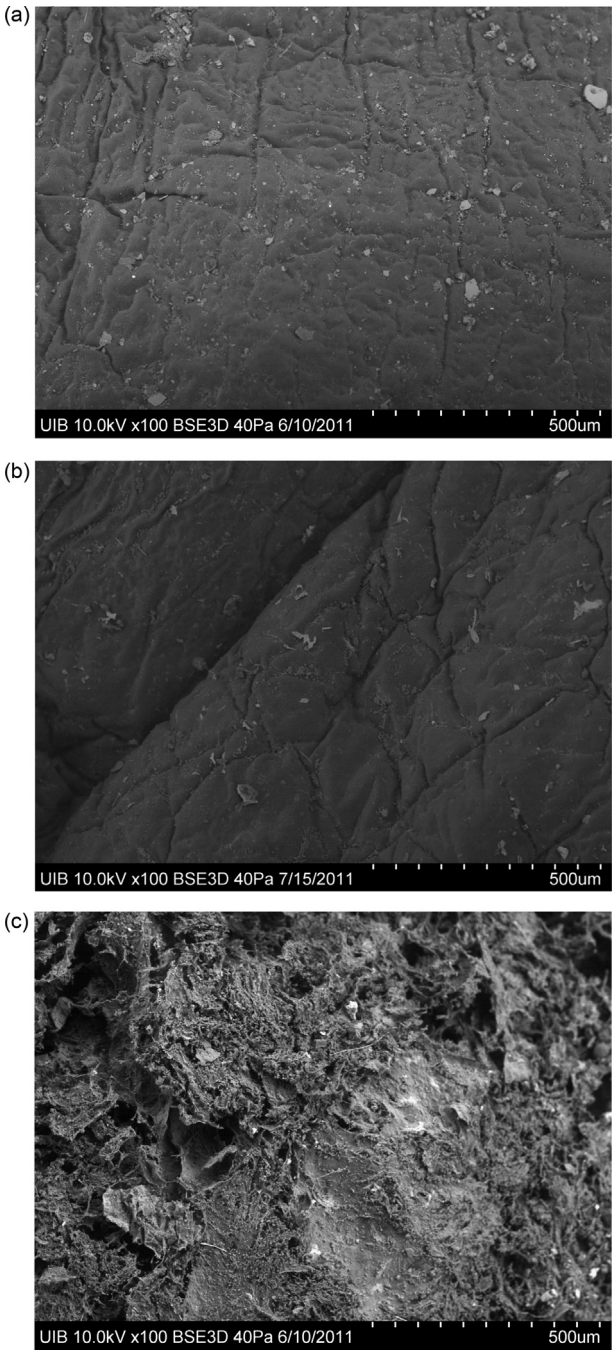

Fig. 1. Scanning electron micrographs of grape pomace from untreated (a), heating at 35 °C during 60 min (b), and heating-sonication combination at 35 °C during 60 min and power density of 0.05 W mL^{-1}.

(6), (7) and (8), respectively).

$$Yield\,(\%) = 11.90 + 7.74x_1 + 3.18x_2 + 3.38x_3 - 2.23x_1^2 + 0.21x_2^2 + 9.57x_3^2 + 2.71x_1x_2 - 1.76x_1x_3 \quad (6)$$

$$MW\,(Da) = 153,820 - 9200x_1 - 6587x_2 + 28,163x_3 - 10,810x_1^2 + 5015x_2^2 + 9165x_3^2 + 25x_1x_2 - 5175x_1x_3 - 550x_2x_3 \quad (7)$$

$$DE\,(\%) = 42.57 + 10.01x_1 - 3.82x_2 + 2.28x_3 + 5.48x_1x_2 - 9.68x_1x_3 + 8.17x_2x_3 \quad (8)$$

On the basis of the results of lack of fit, R^2 and adequate precision tests, it could be inferred that the regression models to predict the effect of temperature, time and pH showed a very good fit for MW, a good fit for pectin yield, and a reasonably good fit for DE of pectins.

The different terms of each of the components that comprise the above models were statistically analyzed in order to observe those that significantly affect the different responses analyzed. For this, the Pareto's diagrams were used as a tool to graphically observe the significance and importance of the various terms. The results for each model are shown in the following sections.

3.2.1. Yield of UAE of pectin from grape pomace

The variables with the largest effect on extraction yield were the linear term of temperature (x_1) and the quadratic term of pH (x_3^2). Other terms, such as the linear terms of extraction time (x_2), pH (x_3), the quadratic term of temperature (x_1^2) and the interaction between temperature and time ($x_1 \times x_2$) were also significant. On the contrary, the quadratic term of time (x_2^2) and the interaction between temperature and pH ($x_1 \times x_3$); and, also time and pH ($x_2 \times x_3$) showed no significant effects on the yield of UAE of pectin from grape pomace (Fig. 2a).

Interestingly, Happi-Emaga et al. (2008) and Levigne et al. (2002) reported that pH was the most significant parameter affecting yield of pectins extracted from banana peel and sugar beet, respectively. However, Wai et al. (2009) observed that interactions between temperature and pH, and time and pH were significant for the yield of pectins extracted from durian rind.

3.2.2. Molecular weight

As can be clearly observed in Fig. 2b, the predominant effect on the developed model for the average MW of the extracted pectins corresponded to the linear term of pH (x_3), followed by the linear and quadratic terms of temperature (x_1, x_1^2). Further, the quadratic term of pH (x_3^2), the linear and quadratic terms of extraction time (x_2, x_2^2) and, interestingly, the interaction term between temperature and pH ($x_1 \times x_3$) were also significant ($p < 0.05$).

These results are in agreement with the works of Happi-Emaga et al. (2008) and Levigne et al. (2002), showing the great influence of the pH on the average MW of pectins extracted from banana peels and sugar beet pulp, respectively. Further and more recent has been the finding, by Li et al. (2012), that the most important effects on the average MW of pectins from sugar beet pulp extracted with microwave assistance were promoted by the pH.

Table 3
Predicted and experimental values under optimum conditions on yield extraction and DE of UAE of pectin from grape pomace.

Responses	Process variables			Predicted value	Experimental value[a]
	X_1 (°C)	X_2 (min)	X_3		
Yield	75	60	2.0	34.70%	32.35 ± 1.4%
MW	55	20	2.0	203.70 kDa	207.00 ± 12 kDa
DE	70	20	1.0	62.93%	64.1 ± 3.3%

[a] Mean of triplicate determination.

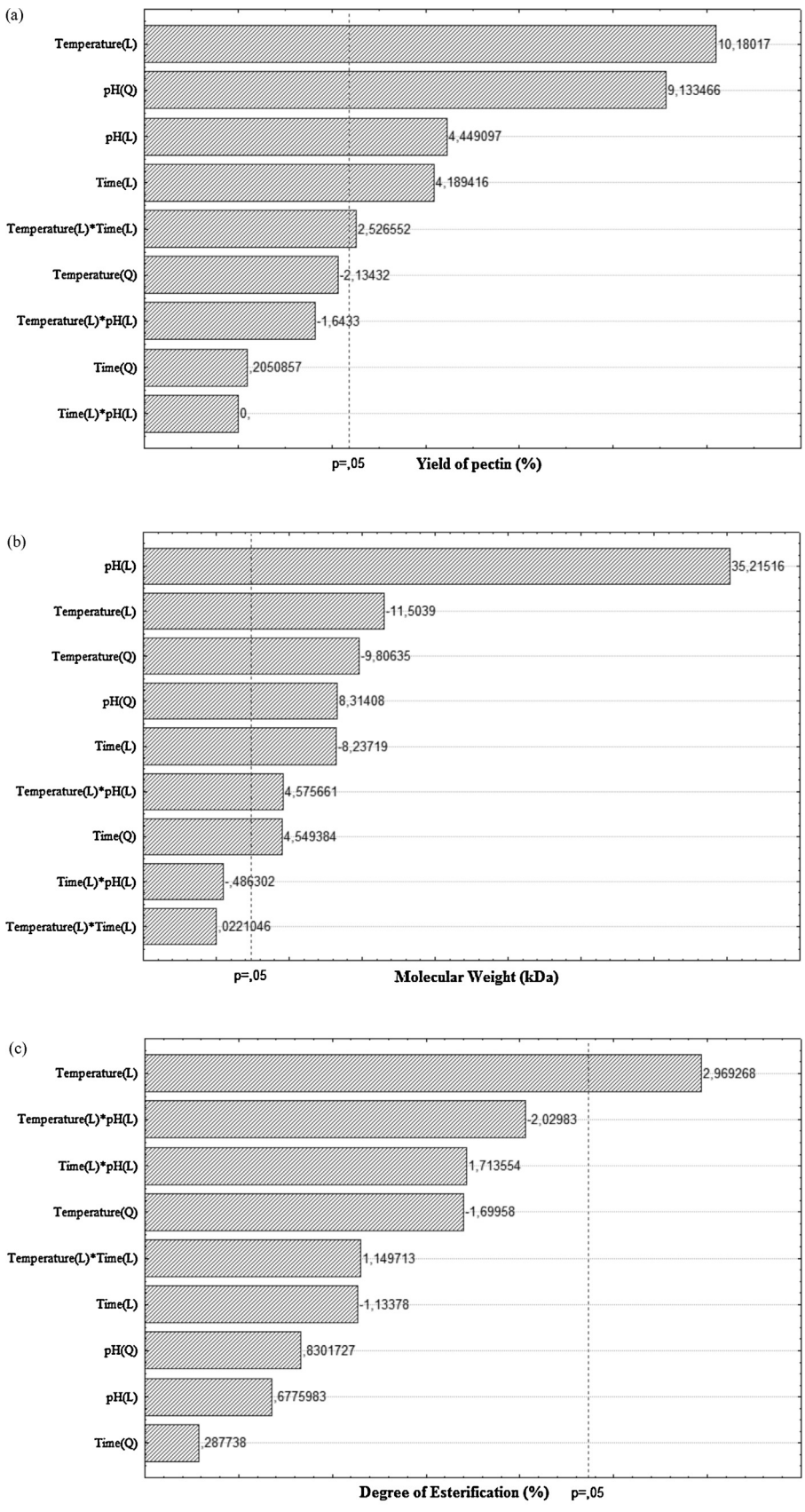

Fig. 2. Pareto's plots showing the significance of the different terms of the quadratic models for the (a) yield, (b) MW, and (c) DE of pectins extracted from grape pomace with ultrasonic assistance.

3.2.3. Degree of esterification

The DE of extracted pectins was severely influenced by the linear term of temperature (x_1), as can be observed in Fig. 2c. In fact, the linear, quadratic and interaction terms of extraction time and pH did not show a significant effect ($p > 0.05$) on the DE of UAE of pectin from grape pomace.

On these results, we are at odds with other workers. Thus, Happi-Emaga et al. (2008) reported that the DE of pectins extracted from banana peels was strongly influenced by not only the temperature but also the extraction time. On the contrary, Levigne et al. (2002) and Wai et al. (2009, 2010) observed that the greatest effect on the DE of pectins obtained from sugar beet pulp and durian rind, respectively, was promoted by time and pH, and to a lesser extent by the temperature.

These important differences could be attributed not only to the inherent complexity of pectic polysaccharides obtained from different vegetable sources, but also to the different experimental conditions applied during the extraction procedure.

3.3. Interpretation of response surface model and contour plots

The three-dimensional (3D) and two-dimensional (2D) contour plots are the graphic representations of regression models, providing a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. The relationship between independent and dependent variables was illustrated in 3D representation of the response surfaces and 2D contour plots generated by the model for yield, MW and DE of UAE of pectins from grape pomace (Figs. 3–5, respectively), Two variables were depicted in one 3D surface plot while the other variable was kept at level zero.

3.3.1. Yield of UAE of pectin from grape pomace

The 3D and 2D plots for extraction yield as a function of extraction temperature, time and pH are shown in Fig. 3a, b and c. As it can be seen in Fig. 3a, an increase of temperature from 35 to 75 °C (for a 60 min extraction) promoted an increase in the yield of extracted pectins from ~7% to ~28%. Further, an increase in the extraction time from 20 to 60 min (at 75 °C) also promoted a considerable yield increase from ~16% to ~28%. However, at milder temperatures (<55 °C), pectin yield increased moderately with extraction time. This is in general agreement with the studies of Gan and Lattif (2011), Kulkarni and Vijayanand (2010), Panchev et al. (1994), and Wai et al. (2009, 2010) who reported that by increasing either temperature or time of extraction the yield of pectins is maximized, whereas a decrease in any of these parameters promotes a decrease in the pectin yield. Increasing the temperature might favor the extraction of pectic polysaccharides through the disruption of the

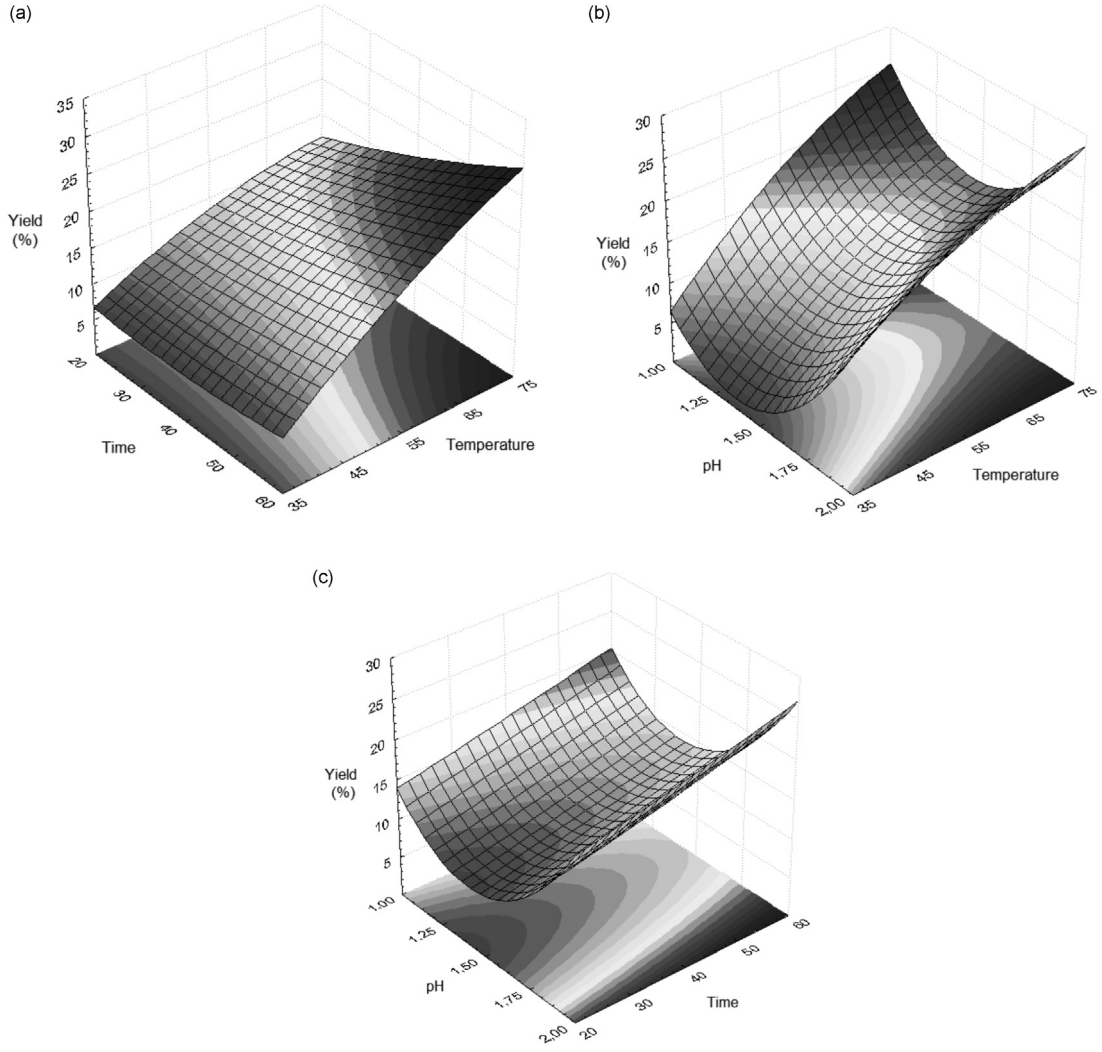

Fig. 3. Response surface and contour plots showing effects temperature, time and pH on yield of UAE of pectin from grape pomace. (a) Time vs Temperature (pH = 1.5). (b) pH vs Temperature (Time = 40 min). (c) Time vs pH (Temperature = 55 °C).

ester linkages and hydrogen bonds (Masmoudi et al., 2008; Renard, Lemeunier, & Thibault, 1995; Wang et al., 2007).

On the other hand, by using citric acid at pH 2.0 and sonication for 40 min, an increase of temperature from 35 to 75 °C, promoted a yield increase from ~7% to ~26%, highlighting the significant effect of the linear term of temperature (Fig. 3b). Moreover, when extracting at 75 °C for 40 min and varying the pH from 1 to 1.5, the extraction yield decreased from ~26% to ~16%, but then by increasing the pH to 2.0 the yield increased up to ~29%, clearly showing the quadratic effect of pH on the pectin extraction process (Fig. 3c). Kalapathy and Proctor (2001) reported a decrease in the yield of extracted pectin from soy hull when the pH decreased. On the contrary, Yapo et al. (2007) and Levigne et al. (2002) observed that the yield of pectin extraction from sugar beet at pH 1.5 was higher than the yield obtained working at pH 2.0.

3.3.2. Molecular weight of UAE of pectin from grape pomace

The 3D and 2D plots representing the effects of temperature, time and pH on the MW of the UAE of pectins are shown in Fig. 4a, b and c. As can be observed in Fig. 4a, pectins with the highest average MW were extracted using shorter times (~20 min) and mild temperatures (~50 °C). Bagherian et al. (2011) also observed a clear decrease in the average MW (from 107 kDa to 82 kDa) of pectins extracted from grapefruit using ultrasounds, when extraction time increased from 20 to 40 min.

Further, the predominance of the linear effect of pH on the MW of pectins was clear from Fig. 4b and c, thus a decrease in the concentration of citric acid (from pH 1 to pH 2) was reflected by a marked increase in the MW, at any temperature used (Fig. 4b) and for any time of extraction tested (Fig. 4c). Similarly, Li et al. (2012) reported a significant increase in the MW (from 60 to 250 kDa) of pectins from sugar beet pulp when the pH increased from 1.0 to 2.0. Happi-Emaga et al. (2008) also reported an increase in the MW (from 120 to 185 kDa) of pectins from banana peels, when the pH increased from 1.5 to 2.0.

Yapo (2009) also observed a marked increase in the average MW (from 218 to 245 kDa) of pectins extracted from the passion fruit (*Passiflora edulis*), when the pH of the citric acid increased from 1.8 to 2.5. This author suggested that the use of citric acid as the extracting agent might reduce the degradation of the homogalacturonan chains.

3.3.3. Degree of esterification of UAE of pectin from grape pomace

The 3D and 2D plots corresponding to the effects of temperature, time and pH on the DE of UAE of pectin from grape pomace are shown in Fig. 5a, b and c.

As can be observed in Fig. 5a, for extractions performed for 60 min with citric acid of pH 1.5, an increase of temperature from 35 to 75 °C promoted an increase of the DE from 20% to 50%,

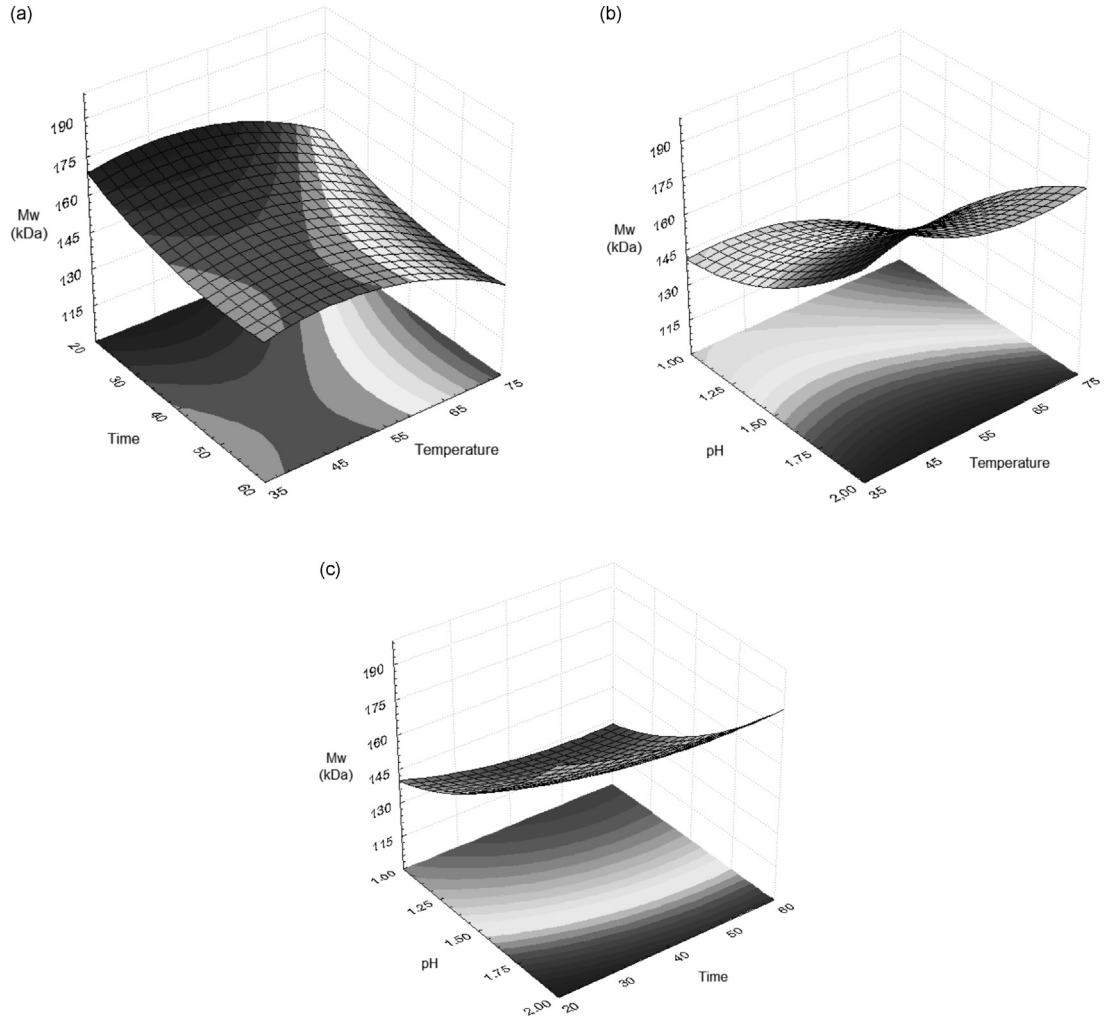

Fig. 4. Response surface and contour plots showing effects temperature, time and pH on MW of UAE of pectin from grape pomace. (a) Time vs Temperature (pH = 1.5). (b) pH vs Temperature (Time = 40 min). (c) Time vs pH (Temperature = 55 °C).

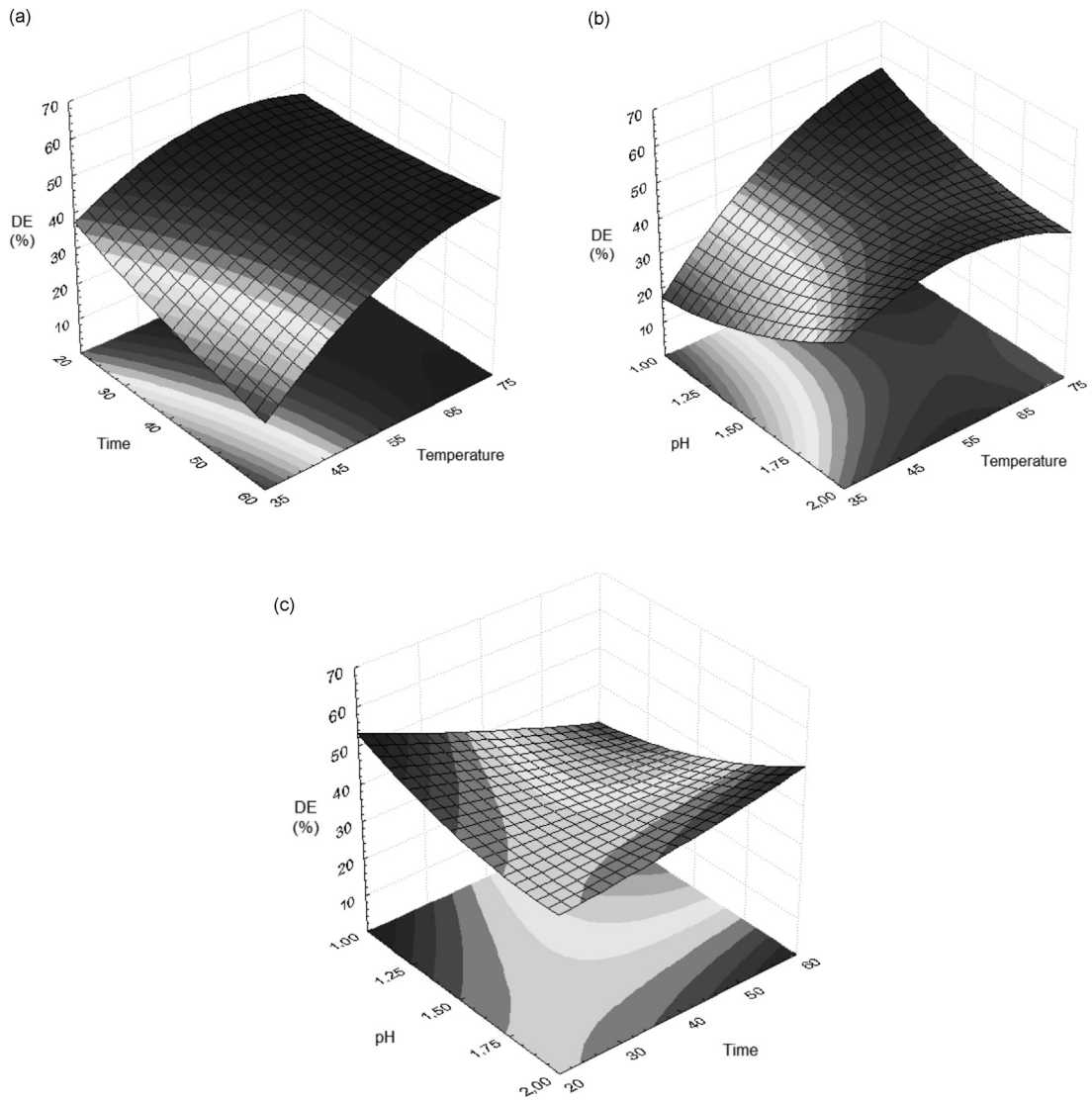

Fig. 5. Response surface and contour plots showing effects temperature, time and pH on DE of UAE of pectin from grape pomace. (a) Time vs Temperature (pH = 1.5). (b) pH vs Temperature (Time = 40 min). (c) Time vs pH (Temperature = 55 °C).

highlighting the significance of the linear effect of temperature. Wai et al. (2010) also observed a significant increase in the DE of durian rind pectins when the extraction temperature increased from 80 and 90 °C. On the contrary, Panchev et al. (1994) observed a clear decrease in the DE of apple pressing when extraction temperatures increased from 40 to 60 °C; and Kulkarni and Vijayanand (2010) did not observe any effect of the temperature (from 70 to 98.7 °C) on the DE of pectins from passion fruit peel.

Fig. 5b indicates that pectins with a relatively high DE (~58%) could be obtained using a low pH and high temperature. However, Levigne et al. (2002) reported that the values of DE of sugar beet pectins extracted with mineral acids (HCl and HNO_3) increased with increasing pH (from pH 1 to pH 2). Furthermore, Fig. 5c, suggested that the extraction of pectins with a high DE could be achieved either using short extraction time and low pH (i.e. 20 min and pH 1.0), or, also, using a higher pH combined with a longer extraction time (i.e. pH 2.0 and 60 min). This would be in agreement with the work of Pinheiro et al. (2008) who reported the great effect of the citric acid concentration (0.086–2.914%, w/v), since pectins from passion fruit peel with the highest DE were obtained using a low-citric acid concentration (0.086%, w/v) and long extraction time (90 min).

3.4. Validation of the developed models

The suitability of the model equations for predicting the maximum response values was tested using the selected optimal conditions. Three confirmation experiments were carried out with yield, MW and DE conditions from the optimization results to validate the developed model. The results of the selected combinations of temperature, extraction time, and pH are given in Table 4, including actual and predicted values of yield, MW and DE. The validation results demonstrated that the developed models were quite accurate in their predictions.

Finally, various experiments were carried out using the parameters that optimize the extraction yield of pectins but without the use of ultrasounds. Thus, the pectin yields (obtained using citric acid at 75 °C, for 60 min and pH 2.0 without ultrasounds) were about 20% lower (25.6 ± 1.2%) than the yields obtained under the same conditions but with ultrasonic assistance (32.4 ± 1.4%). This result is in agreement with the reports that highlight the efficacy of ultrasounds for the extraction of pectins (Bagherian et al., 2011; Panchev et al., 1994). Further, pectins obtained without ultrasonic assistance exhibited similar DE (62.5 ± 2.2%) and similar percentage of GalA units (~96%), although a significantly lower MW (161.5 ± 9 kDa).

4. Conclusions

In this study, RSM was applied to determine the optimal conditions of temperature, extraction time and pH for the UAE of pectins from an important by-product such as grape pomace. Citric acid, instead of mineral acids used in conventional extractions, was used as the extracting agent. The procedure was evaluated in terms of the extraction yield and some basic characteristics of pectins such as the average MW and DE.

The combination of citric acid and ultrasounds showed a high efficiency for the extraction of homogalacturonan-like polysaccharides, accounting for over 95% of extracted pectins for any combination of the conditions tested.

The response values determined exhibited a great variability within the parameter's range analyzed; from 3.2 to 29.4% for the extraction yield, from 110 to 205 kDa for the average MW, and from 20.1 to 62.1% for the DE.

ANOVA and statistical parameters such as LOF, R^2 and *Adeq-precision* indicated that the experimental data corresponding to the extraction yield and average MW showed a good fit to a quadratic model, whereas DE data only exhibited a good fit to a linear model.

The optimal conditions based on individual responses were determined. Results showed that predicted and experimental values were not significantly different.

The yield obtained after UAE of pectins using the optimal conditions (75 °C, 60 min, pH 2.0) was 20% higher than the yield obtained when the extraction was carried out applying the same conditions of temperature, time and pH, but without ultrasonic assistance. In addition, pectins from UAE also exhibited a higher average MW.

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