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Extraction and assay of pectic enzymes from Peruvian carrot (*Arracacia xanthorriza* Bancroft.)

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Abstract

Enzyme extraction processes were studied to evaluate the actual activity of pectinesterase (PE) and polygalacturonase (PG) from Peruvian carrot roots. Response surface methodology (RSM) was used to study the effect of NaCl concentration (from 0.5 to 1.5 M), the pH of the homogenate (from 4.0 to 8.0) and the extraction time (from 4 to 52 h) on enzyme activity of the crude extract. The results showed that best conditions for extracting pectic enzymes were 1.0 M NaCl, for 4 h, for both enzymes, whereas pH 7.5 and 4.0 were the appropriate parameters for extracting PE and PG, respectively.

Keywords: Arracacha; Peruvian carrot; Pectic enzymes; Pectinesterase; Polygalacturonase; Enzyme extraction; Response surface methodology

1. Introduction

Roots of Peruvian carrot (*Arracacia xanthorriza* Bancroft.) or "*arracacha*" have a short post-harvest conservation time relative to its long production cycle, from ten to twelve months in the field. After harvest, the roots must be consumed within one week when stored at room temperature. A few days after harvest and before roots actually start to deteriorate, the surface changes by losing brilliance and developing brown spots that are unattractive in market displays. Peruvian carrot is very susceptible to mechanical damage, which causes soft lesions. At the final step, the soft roots are infected by opportunistic parasites, such as *Alternaria* sp., *Erwinia* spp. and *Rhyzoctonia crocorun* (Herman, 2004).

The mechanism of post-harvest deterioration of this tuber is not completely known. Pires, Matos Da Veiga, and Finardi-Filho (2002) identified amylolytic enzymes in the crude extract of Peruvian carrot roots but the low activities of these endogenous enzymes are not the main factor responsible for the lost of root texture during storage (Pires & Finardi-Filho, 2002). A pool of endogenous enzymes, such as the pectinases and even the amylases, could be acting together to promote the deteriorative process of the tuber during the post-harvest-cycle.

Pectinesterase (PE) and polygalacturonase (PG) are well described in higher plants. PE hydrolyses the methyl ester bond of pectin to give pectic acid and methanol (Voragen, Beldman, & Schols, 2001) whereas PG cleaves the α-1,4-glycosidic bound between the anhydrogalacturonic acid units (Benen & Visser, 2003). During fruit and vegetable ripening, PE removes methyl groups from the cell wall pectic constituents, which can then be depolymerized by PG, decreasing the intracellular adhesivity and tissue rigidity (Alonso, Rodríguez, & Canet, 1995). Both enzymes are involved in the softening and senescence process in many fruits and vegetables.

Considering that there are no data about the pectic enzymes in Peruvian carrot roots, the first step, to study the role of these enzymes, was to develop a methodology to extract PE and PG from the roots in order to detect and to preserve their activity.

Different methods have been used to extract PE and PG from fruits and vegetables. Some authors used different pH, extraction times and NaCl concentrations to extract PE and PG. For PE extraction, the pH buffers ranged from 3.0 for tomato (Pressey & Woods, 1992) to

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8.3 for acerola (Assis, Martins, Guaglianoni, & Faria de Oliveira, 2002). The NaCl concentration varied from 0.3 M for grapefruit (Seymour, Preston, Wicker, Lindsay, & Cheng-I-Wei, Marshall, 1991a, 1991b) to 2.0 M for papaya (Fayyaz, Asbi, Ghazali, Che Man, & Jinab, 1993) whereas extraction time ranged from zero for sapote mamey (Ocampo, Lozano, Errasquin, Aparicio, & Ortíz, 2003) to 24 h for banana (Ly-Nguyen, Loey, Fachin, Indrawati, & Hendrickx, 2002a, 2002b), grapefruit (Seymour et al., 1991a, 1991b), guava (Abu-Goukh & Bashir, 2003) and potato (Puri, Solomons, & Kramer, 1982) (see Table 1).

For PG extraction, the pH for enzyme extraction from tomatoes ranged from 3.0 (Ma & Barret, 2001) to 9.0 (Yoshida, Nakagawa, Ogura, & Sato, 1984). The NaCl concentration varied from 0 for banana (Pathak, Mishra, & Sanwal, 2000) to 1.25 M for kiwifruit PG extraction (Wegrzyn & MacRae, 2000). The extraction time ranged from 2 min for papaya (Jiang et al., 2003) to 24 h for guava PG extraction (Abu-Goukh & Bashir, 2003) (see Table 2).

Considering the wide range of pH buffers, NaCl concentration and extraction times found in the literature to obtain PE and PG, we decided to design the experiments by a response surface model (RSM), using different levels of those three parameters. The same procedure to extract the enzymes was adopted for both enzymes. Previous studies used the same methodology to extract PE and PG from the same source. D'Innocenzo and Lajolo (2001) and Jiang et al. (2003) extracted PE and PG from papaya at pH 5.0 for 1 h and 1.0 M NaCl for 2 min, respectively, whereas Labib et al. (1995) extracted both enzymes from mango fruit at pH 6.5, 1.0 M NaCl for 18 h. Guava PE and PG were extracted at pH 8.2, 1.0 M NaCl for 24 h (Abu-Goukh & Bashir, 2003) (see Tables 1 and 2).

The objective of this work was to establish parameters for extraction and assay of PE and PG from Peruvian carrot, verifying the effect of NaCl concentration, pH and time of extraction on enzyme activity.

2. Materials and methods

2.1. Materials

2.1.1. Root material

Peruvian carrot roots (*Arracacia xanthorrhiza* Bancroft.) were purchased from a local market in Sao Paulo (Brazil). Only roots in good shape, free of brown spots or soft texture were used in the assays.

2.1.2. Reagents

Citrus pectin, sodium phosphate (Na_2HPO_4–NaH_2PO_4, 0.1 M), alcohol oxidase from *P. pastoris* (EC 1.1.3.13), 2-4-pentanodione, galacturonic acid, polygalacturonic acid, sodium acetate and BSA were purchased from Sigma Chemical Co. St. Louis. 2-Cyanoacetamide was obtained from Aldrich Chemical Co. Steinhein. All other reagents were analytical grade.

2.2. Methods

2.2.1. Enzyme extraction

After washing and removing the peel, the roots were cut into dices. A sample of 200 g was homogenized with

Table 1
Extraction conditions of PE from fruits and vegetables cited in the literature

Material	NaCl (M)	pH	Extraction time (h)
Acerola (Assis et al., 2002)	0.60	8.3	1
Banana (Ly-Nguyen et al., 2002a, 2002b)	1.00	8.0	24
Carrot (Ly-Nguyen et al., 2002a, 2002b)	1.00	8.0	0.25
Carrot (Tijskens, Waldron, Ng, Ingham, & Dijk, 1997)	1.00	[a]	1
Cherries (Alonso et al., 1995)	1.00	6.0	1
Grapefruit (Seymour et al., 1991a, 1991b)	0.30	8.0	24
Green beans (Laats, Grosdenis, Recourt, Voragen, & Wichers, 1997)	1.00	7.8	2
Guava (Abu-Goukh & Bashir, 2003)	1.00	8.2	24
Kiwifruit (Wegrzyn & MacRae, 1992)	–	5.5	1
Mango (Labib, El-Ashwah, Omran, & Askar, 1995)	1.00	6.5	18
Orange (Hou, 1997)	1.00	4.1	1
Orange (Körner, Zimmermann, & Berk, 1980)	0.25	7.0	2
Papaya (Jiang, Wu, Wu, & Chang, 2003)	1.00	[a]	0.03
Papaya (D'Innocenzo & Lajolo, 2001)	1.00	5.5	1
Papaya (Fayyaz et al., 1993)	2.00	8.0	5
Peach (Javeri & Wicker, 1991)	0.10	7.5	1
Potato (Tijskens et al., 1997)	1.00	[a]	1
Potato (Puri et al., 1982)	1.00	8.0	24
Sapote mamey (Ocampo et al., 2003)	1.50	7.5	0
Tomato (Pressey & Woods, 1992)	0.20	3.0	0.25

[a] The pH extraction was not cited in the article.

Table 2
Extraction conditions of PG from fruits and vegetables cited in the literature

Material	NaCl (M)	pH	Extraction time (h)
Banana (Pathak et al., 2000)	0–1.00	7.0	[a]
Guava (Abu-Goukh & Bashir, 2003)	1.00	8.2	24
Kiwifruit (Wegrzyn & MacRae, 1992)	1.25	6.5	0.75
Mango (Labib et al., 1995)	1.00	6.5	18
Strawberry (Nogata, Ohta, & Voragen, 1993)	1.00	6.0	12
Papaya (Jiang et al., 2003)	1.00	[b]	0.03
Papaya (D'Innocenzo & Lajolo, 2001)	1.00	5.5	1
Sapote mamey (Ocampo et al., 2003)	1.00	7.0	3
Tomato (Ma & Barret, 2001)	1.20	3.0	0.5
Tomato (Yoshida et al., 1984)	0.86	9.0	2

[a] Extraction time was not cited in the article.
[b] The pH extraction was no cited in the article.

Table 3
Enzyme extraction conditions for Peruvian carrot roots in scaled and real values[a]

Experiment	Scaled values			Real values		
	X1	X2	X3	NaCl (M)	pH	Extraction time (h)
1	−1.0	1.0	1.0	0.5	8.0	52
2	1.0	1.0	1.0	1.5	8.0	52
3	−1.0	−1.0	1.0	0.5	4.0	52
4	1.0	−1.0	1.0	1.5	4.0	52
5	−1.0	1.0	−1.0	0.5	8.0	4
6	1.0	1.0	−1.0	1.5	8.0	4
7	−1.0	−1.0	−1.0	0.5	4.0	4
8	1.0	−1.0	−1.0	1.5	4.0	4
9	0.0	0.0	0.0	1.0	6.0	28
10	0.0	0.0	0.0	1.0	6.0	28
11	0.0	0.0	0.0	1.0	6.0	28
12	−1.0	0.0	0.0	0.5	6.0	28
13	1.0	0.0	0.0	1.5	6.0	28
14	0.0	−1.0	0.0	1.0	4.0	28
15	0.0	1.0	0.0	1.0	8.0	28
16	0.0	0.0	−1.0	1.0	6.0	4
17	0.0	0.0	1.0	1.0	6.0	52

[a] $x_1 = (\mathrm{NaCl} - 1.0)/0.5$, where NaCl ranged from 0.5 to 1.5 M, $x_2 = (\mathrm{pH} - 6.0)/2.0$, where pH ranged from 4.0 to 8.0 and $x_3 = (t - 28)/24$ where t ranged from 4 to 52 h.

200 ml of NaCl solution at different concentrations, as described in Table 3, in a regular blender for 2 min. The homogenate pH was adjusted to 4.0, 6.0 or 8.0 by adding 3.0 M NaOH or 2.0 M acetic acid. The homogenate was stirred continuously at 4 °C for different times (4, 28 and 52 h). The crude extract was centrifuged at 10,000*g* for 30 min at 4 °C. The supernatant, called enzymatic extract, was used directly as the enzyme source.

2.2.2. Pectinesterase activity

The PE activity was determined by methanol production, assayed by a modification of the method of Klavons and Bennett (1986), as follows: 150 μl of enzymatic extract was added to 100 μl solution containing 100 mM sodium phosphate buffer (Na_2HPO_4–NaH_2PO_4, 0.1 M), pH 6.5, and 0.1% of pectin. The assay mixture was incubated at 25 °C for 15 min in microfuge tubes. The reaction was stopped by heating at 100 °C in a water bath for 3 min and the mixture, cooled to 25 °C, was diluted to 2.0 ml with 20 mM Tris–HCl, pH 7.5, and 1 U of alcohol oxidase was added. After 15 min at 25 °C, 1.0 ml of 20 mM 2-4-pentanodione in 2.0 M ammonium phosphate was added and the reaction mixture placed in a water bath at 60 °C for 15 min. The absorbance was measured at 412 nm against a blank made with the same components but with enzymatic extract previously boiled for 5 min. A calibration curve, using methanol as a standard, was prepared ranging from 0 to 435 nmoles/ml of methanol, considering that the correlation between color development and methanol concentration were linear up to 435 nmoles/ml methanol. One enzyme activity unit was expressed by 1.0 nmol $\mathrm{MeOH} \times \mathrm{mg\ protein}^{-1} \times \mathrm{h}^{-1}$. The pectin used in all experiments was exhaustively dialyzed against water to decrease the high

blank values (Castaldo, Quagliuolo, Servillo, Balestrieri, & Giovane, 1989).

2.2.3. Polygalacturonase activity

The PG activity was assayed according to Gross (1982) and Honda, Nishimura, Takahashi, Chiba, and Kakehi (1982) with some modifications. The assay of PG activity was based on the hydrolytic release of reducing groups from polygalacturonic acid. Reaction mixtures containing 5 μl of enzyme extract in 45 μl of 37.5 mM Na-acetate (pH 4.4) and 150 μl of the same Na-acetate buffer, with 0.2% of polygalacturonic acid, were incubated at 30 °C for 2 h. For quantifying the released reducing groups, the reaction was stopped by adding 1.0 ml of cold 100 mM borate buffer (pH 9.0), followed by 0.2 ml of 1% 2-cyanoacetamide. The samples were mixed and immersed in a boiling water bath for 10 min. After equilibration at 25 °C, the amount of reducing sugars was measured at $\lambda = 276$ nm against a blank made up with the same components but with enzyme extract previously boiled for 5 min. A calibration curve, using galacturonic acid as a standard, was prepared from 0 to 250 nmoles/ml. One unit of enzymatic activity was expressed by 1.0 nmoles of galacturonic acid produced $\times$ mg of protein$^{-1} \times$ h^{-1}.

2.2.4. Protein quantification

The protein concentration was determined according to the method of Bradford (1976), using bovine serum albumin as a standard.

2.2.5. Experimental design

A central composite face-centred design (CCF) was used with three variables and three replicates at the centre point, for a total of 17 experiments. The three process variables studied included the NaCl concentration (NaCl, M), the pH value of the extraction buffer (pH) and the enzyme extraction time (t, h). Experimental conditions of the central point were NaCl = 1.0 M, pH 6.0 and $t = 28$ h. The experimental number, scaled values and real values are given in Table 3. The scaled values were $x_1 = (\text{NaCl} - 1.0)/0.5$, where NaCl ranged from 0.5 to 1.5 M, $x_2 = (\text{pH} - 6.0)/2.0$, where pH ranged from 4.0 to 8.0 and $x_3 = (t - 28)/24$ where t ranged from 4 to 52 h.

2.2.6. Data analysis

Central composite designs make it possible to approximate the measured data (y_{obs}) using a RSM expressed in scaled variables:

$$y_{\text{obs}} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1x_1 + b_{22}x_2x_2 + b_{33}x_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + e,$$

$$e = y_{\text{obs}} - y_{\text{cal}},$$

where b_0 is a constant; b_1, b_2 and b_3 express the main effect of each process variable, b_{12}, b_{13} and b_{23} show the interaction effect between the variables and b_{11}, b_{22} and b_{33} show the effect of square variables, y_{obs} represents the dependent variable (enzymatic activity) whereas x_1, x_2 and x_3 represent the NaCl (M), pH and the extraction time, respectively. The difference between the experimental data (y_{obs}) and the model (y_{cal}) gives the residual (e). The results were examined by the software Statistica. The RSM were estimated by multiple linear regressions for the 17 experiments in the central composite design. The replicates at the centre point make it possible to estimate the pure error of the analyses, which was used to predict whether the models gave significant lack-of-fit. The reliability of the models was evaluated by calculating the R^2 value.

2.2.7. Optimization of the procedure of PE and PG Peruvian carrot extraction

Considering the results from the 17 experiments described above, the procedure of PE and PG extraction was optimized through eight new experiments using the same method under following conditions: (1) for PG extraction, four new experiments were done. The homogenate was adjusted to pH 3.5, 4.0, 4.5 or 5.0 by adding 2.0 M acetic acid (experiments I–IV, respectively); (2) for PE extraction, four new experiments were done. In this case, the homogenate was adjusted to pH 7.0, 7.5, 8.0 or 8.5 by adding 3.0 M NaOH (experiments V–VIII, respectively). The NaCl concentration and the extraction time were 1.0 M and 4 h for PE and PG extraction.

3. Results and discussion

3.1. Enzyme extraction

The extraction procedure for PE and PG were the basis of this study due to the different values cited in the literature for fruits and vegetables (Tables 1 and 2). The RSM was adjusted to 24 h spaced time for the stirring. The best methodologies found for extracting PE and PG will be discussed and described next.

3.2. Pectinesterase extraction and activity

The results of the 17 experiments are shown in Table 4. By the statistical software, the results were evaluated and a complete RSM, including the 17 experiments in the design, was estimated. The achieved mathematical model, expressed in scale variables, was:

Table 4
PE and PG activity of Peruvian carrot enzyme extracts

Experiment	Real values			Enzyme activity	
	NaCl (M)	pH	Extraction time (h)	PE activity (U)	PG activity (U)
1	0.5	8.0	52	11.4	0
2	1.5	8.0	52	5.69	0
3	0.5	4.0	52	0	37.8
4	1.5	4.0	52	0	16.5
5	0.5	8.0	4	0	0
6	1.5	8.0	4	6.36	0
7	0.5	4.0	4	0	20.5
8	1.5	4.0	4	0	17.4
9	1.0	6.0	28	1.02	0
10	1.0	6.0	28	1.10	0
11	1.0	6.0	28	1.26	0
12	0.5	6.0	28	0	3.09
13	1.5	6.0	28	0	0
14	1.0	4.0	28	0	12.5
15	1.0	8.0	28	15.0	6.78
16	1.0	6.0	4	0	1.99
17	1.0	6.0	52	0	17.4
I	1.0	3.5	4	–	6.20
II	1.0	4.0	4	–	67.7
III	1.0	4.5	4	–	48.9
IV	1.0	5.0	4	–	24.5
V	1.0	7.0	4	33.9	–
VI	1.0	7.5	4	39.1	–
VII	1.0	8.0	4	35.1	–
VIII	1.0	8.5	4	23.5	–

$$y_{\text{calc (PE activity (U))}} = 1.65 + 0.07x_1 + 3.84x_2 + 1.07x_3 - 2.04x_1x_1 + 5.45x_2x_2 - 2.04x_3x_3 + 0.09x_1x_2 - 1.50x_1x_3 + 1.34x_2x_3.$$

The pH (x_2) and square terms of pH (x_2^2) of the enzyme extraction significantly influenced PE extraction (Table 5, $p < 0.05$). The estimation of the model gave $R^2 = 0.83$ and $R^2_{adj} = 0.61$. Fig. 1 represents RSM for PE activity as a function of NaCl concentration and extraction time at pH 4.0 (a), 6.0 (b), and 8.0 (c). PG results are shown in Fig. 2.

According to the model, the best conditions for extracting PE were pH 5.26, for 24 h using a 0.97 M NaCl solution. Considering that the best PE activity obtained experimentally was at pH 8.0 (Table 4, experiments 1 and 15, and Fig. 1), we decided to study PE extraction at pH around 8.0 (Table 4, experiments VI–VIII, from 7.5 to 8.5). In this step, we found the highest value of PE activity at pH 7.5, so it was necessary to perform a new experiment at pH 7.0 (Table 4, experiment V). The results showed that the best pH for extracting PE from Peruvian carrot was 7.5 (Fig. 3). Javeri and Wicker (1991) extracted PE from peaches and sapote mamey at the same pH. Potato, papaya, banana, carrot and grapefruit PE were extracted at pH 8.0 (Table 1). Pressey and Woods (1992) extracted PE from tomatoes at pH 3.0, the lowest pH extraction found in the literature; they verified that pH 3.0 yielded PE of relatively high specific activity in the crude extract.

Best results of time for extraction were found after 24 h of mixing the extract. These results were found experimentally and from the RSM. However, we decided to make PE extractions at 4 h of mixing because the model showed that this variable was not significant (Table 5, $p = 0.27$) and at this extraction time we could optimize of time extraction. Results showed that, even when mixing the extract for only 4 h, PE activity was higher than with the other experiments (Table 4, experiments V–VIII). Most studies of PE extraction, found in the literature, obtained the enzyme after 1 or 2 h of extraction (Table 1).

The concentration of NaCl used to optimize PE extraction was 1.0 M, very similar to 0.97 M, the value obtained from the model. Many workers used the same NaCl concentration to extract PE from other sources (Table 1).

3.3. Polygalacturonase activity and extraction

The results of the 17 experiments are shown in Table 4. A statistical programme evaluated the results.

Table 5
ANOVA of PE activity as a function of NaCl concentration (x_1), pH (x_2) and extraction time (x_3) of different enzyme extracts of Peruvian carrot roots

Effects	Sum of squares	Degrees of freedom	Medium squares	*F*-value	*p*-Value
x_1	0.0490	1	0.0490	0.00612	0.939821
x_1x_1	11.1100	1	11.1100	1.38820	0.277207
x_2	147.2257	1	147.2257	18.39597	0.003615
x_2x_2	79.5412	1	79.5412	9.93874	0.016094
x_3	11.4062	1	11.4062	1.42522	0.271429
x_3x_3	11.1100	1	11.1100	1.38820	0.277207
x_1x_2	0.0612	1	0.0612	0.00765	0.932738
x_1x_3	18.0600	1	18.0600	2.25662	0.176743
x_2x_3	14.2578	1	14.2578	1.78152	0.223739
Error	56.0220	7	8.0031	–	–
Total SS	327.0540	16	–	–	–

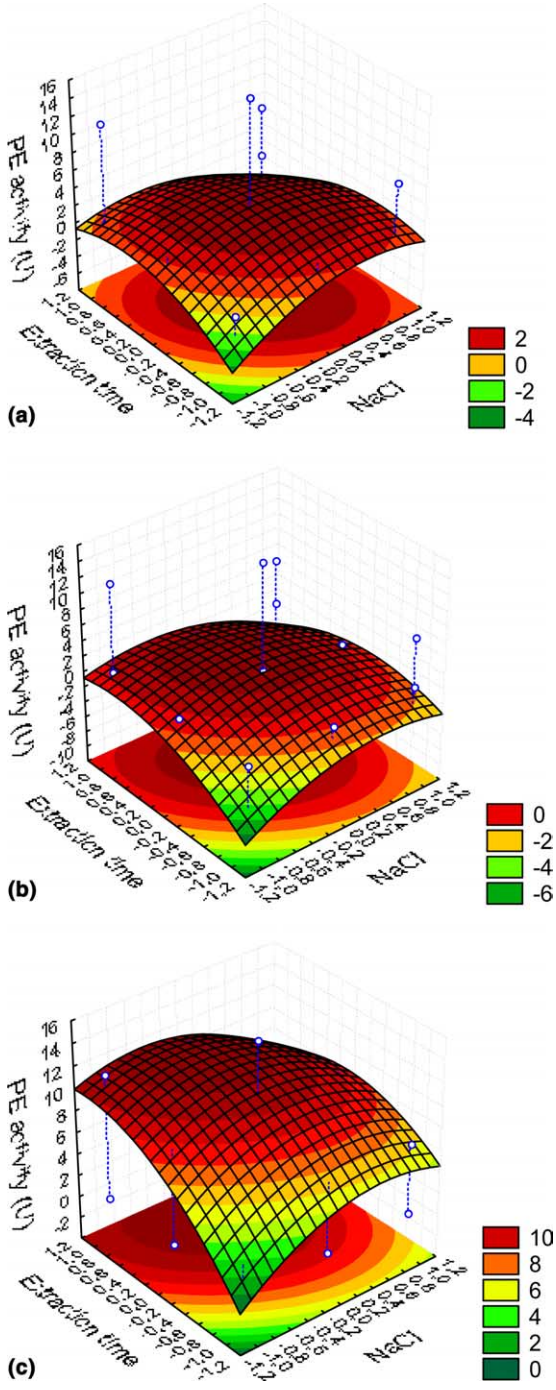

Fig. 1. RSM for PE activity (U) as a function of NaCl concentration and extraction time at pH 4.0 (a), 6.0 (b) and 8.0 (c). Experimental conditions of the central points (0.0) were NaCl = 1.0 M, pH 6.0 and $t = 28$ h. See Table 3 for details.

A complete RSM, including the 17 experiments in the design, was estimated. The achieved mathematical model, expressed in scale variables, was:

$$y_{\text{calc (PG activity (U))}} = 2.10 - 2.74x_1 - 9.78x_2 + 3.19x_3 - 2.13x_1x_1 + 5.94x_2x_2 + 6.01x_3x_3 + 3.04x_1x_2 - 2.28x_1x_3 - 2.06x_2x_3.$$

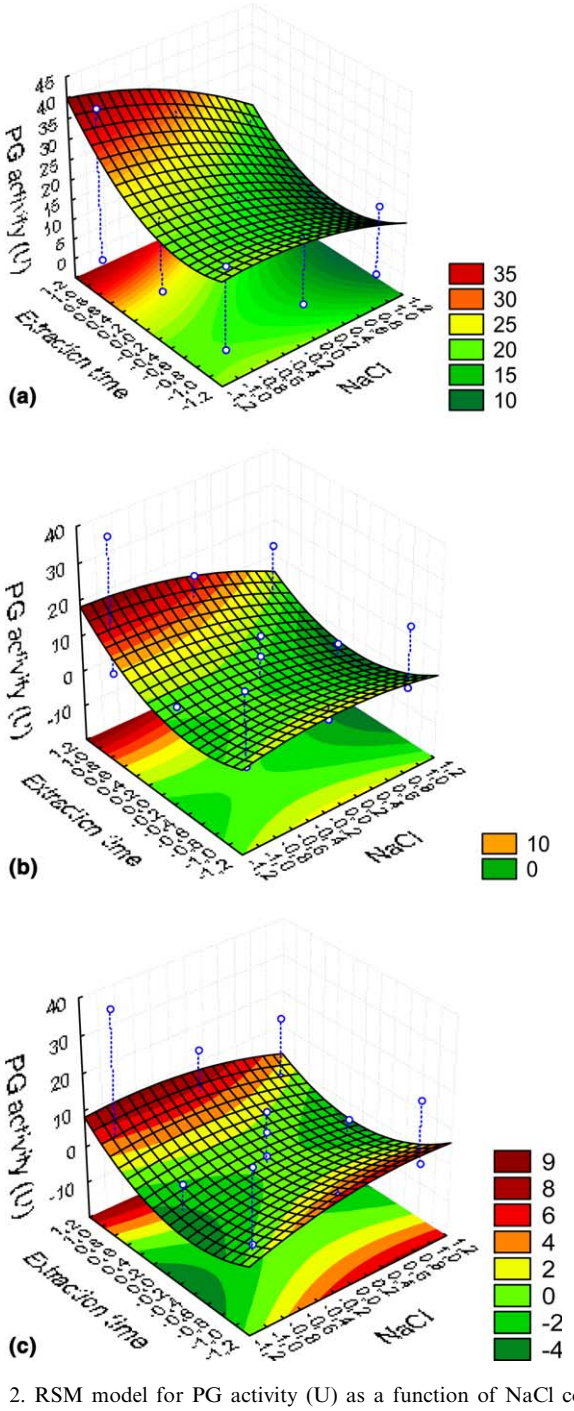

Fig. 2. RSM model for PG activity (U) as a function of NaCl concentration and extraction time at pH 4.0 (a), 6.0 (b) and 8.0 (c). Experimental conditions of the central points (0.0) were NaCl = 1.0 M, pH 6.0 and $t = 28$ h. See Table 3 for details.

The pH (x_2) of the enzyme extraction significantly influenced PG extraction (Table 6, $p < 0.05$). The estimation of the model gave $R_2 = 0.87$ and $R^2_{\text{adj}} = 0.70$. Fig. 2 represents RSM for PG activity as a function of NaCl concentration and extraction time at pH 4.0 (a), 6.0 (b), and 8.0 (c).

According to the model, the best conditions for extracting PG were pH 4.40, for 25 h using a 1.0 M NaCl

Table 6
ANOVA of PG activity as a function of NaCl concentration (x_1), pH (x_2) and extraction time (x_3) of different enzyme extracts of Peruvian carrot roots

Effects	Sum of squares	Degrees of freedom	Medium squares	*F*-value	*p*-value
x_1	75.186	1	75.1856	2.14018	0.186886
x_1x_1	12.189	1	12.1892	0.34697	0.574342
x_2	958.875	1	956.8752	27.23772	0.001227
x_2x_2	94.439	1	94.4393	2.68824	0.145099
x_3	101.506	1	101.5060	2.88940	0.132963
x_3x_3	96.679	1	96.6794	2.75201	0.141095
x_1x_2	73.994	1	73.9936	2.10625	0.189993
x_1x_3	41.451	1	41.4505	1.17990	0.313360
x_2x_3	33.908	1	33.9076	0.96519	0.358612
Error	245.914	7	35.1305	–	–
Total SS	1884.438	16	–	–	–

solution. Considering that the best pH extraction obtained experimentally was at pH 4.0 (Table 4, experiments 3 and 7 and Fig. 2), we decided to study PG extraction at pH around 4.0 (Table 4, experiments I–III, starting at 3.5–4.5). In this step, we found the highest value of PG activity at pH 4.0. A new experiment at pH 5.0 was done due to the PG specific activity expressed in nmol of galacturonic acid/mg protein that was affected by the protein solubilization in this pH range. The highest value of PG activity was found at pH 4.5. Results showed that the best pH for extracting PG from Peruvian carrot was 4.0 (Table 4 and Fig. 3). This pH value was not found in the literature extracting PG from other sources, but higher and lower values of pH were used in these different works (Table 2). However, the assay adopted to detect PG activity in this current study was at pH 4.4, very close to 4.0.

The RSM model gave best results of time extraction after 25 h of mixing the extract. As in PE extraction, we decided to make PG extractions at 4 h of mixing because the model showed that this variable was not significant (Table 6, $p = 0.13$) and at this extraction time we could optimize the assay. Results showed that mixing the extract for 4 h gave higher PG activity than the other experiments (Table 4, experiments II–IV). Many studies involving PG extraction used less than 4 h to extract the enzyme (Table 2).

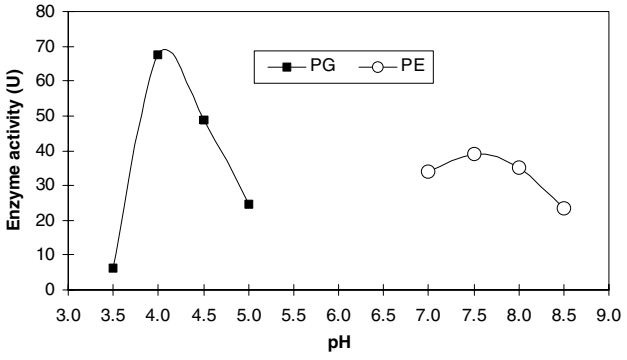

Fig. 3. PE and PG activity of the extracts prepared at pH 3.5–8.5, under the same conditions of NaCl concentration and extraction time.

The concentration of NaCl used to optimize PG extraction was 1.0 M, the same value as given by the RSM model. The results are in accordance with other authors who used the same NaCl concentration to extract PG from different sources (Table 2).

4. Conclusions

The results pointed the small influence of extraction time and NaCl concentration on the extraction of both enzymes, which are easily solubilized after 4 h under stirring at 4 °C, indicating that this variable is not critical. The only significant variable was the pH of extraction, 7.5 and 4.0, for PE and PG extraction, respectively. When extraction of both enzymes (PE and PG) is desired and just one extraction method can be used, the pH of extraction could be adjusted to 7.5, considering that, at pH 8.0, it is feasible to extract PG (Fig. 2(c)). On the other hand, in the acidic range, pH 4.0 or 6.0, the extraction of PE is not recommended (Fig. 1(a) and (b)). These results are an important background for continuing the work on the biochemical characterization of both enzymes during the post-harvest period of Peruvian carrots, involved or not in the deteriorative reactions. Furthermore, there are no data about pectic enzymes from this tuber in the literature.

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