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# Preliminary approach to detect amylolytic and pectinolytic activities from maca (*Lepidium meyenii* Walp.)

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*Amylase (AM), pectinesterase (PE) and polygalacturonase (PG) were extracted from maca roots (*Lepidium meyenii *Walp). The response surface model was used to determine the optimum conditions of pH and temperature activity of each enzyme on the crude extract. The highest values of AM activity occurred at pH 6.1 and 33.6 °C, very close to the experimental central point. The PE activity had a maximum activity at pH 6.6 and 49.4 and PG activity showed optimum activity at pH 5.4 and 46 °C.*

**Uniterms**
- Maca
- *Lepidium meyenii* Walp
- Amylase
- Pectinesterase
- Polygalacturonase
- Response surface model

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## INTRODUCTION

Maca (*Lepidium meyenii* Walp.) is a tuber that has one of the highest frost tolerances among other native cultivated plants growing exclusively at high altitudes (3,700-4,500 m) at the Peruvian Andes. No other crop plant, except bitter potatoes and Alpine grasses, gives reliable yields at 4,400 m (Toledo *et al*., 1998). As an ethnical food, the root is the edible part of maca used for human consumption due to its nutritional value, as well as to its exotic characteristic of taste and flavor. It became popular as a botanical extract due to, among other things, claimed energy and fertility function (Tellez *et al.*, 2002). The tuber is rich in carbohydrates (59.0%), proteins (10.2%), fibers (8.5%) and lipids (2.2%), as well as a large amount of amino acids and higher levels of iron and calcium than potatoes (Tellez *et al.*, 2002; Quirós, Cárdenas, 2004). The tuber of this plant has been well appreciated since the pre-Incan times. During the Inca Empire, maca consumption was allowed to the nobility, the clergy, and the privileged classes; it was used as a gift prize to warriors. Nowadays, Andean people consume maca tuber boiled or roasted, as a spice to prepare dishes, soups and also drinks (Piacente *et al.*, 2002).

Although maca composition and compounds have been studied (Tellez *et al.*, 2002; Piacente *et al.*, 2002; Dini, Tenore, Dini, 2002; Sandoval *et al*., 2002; Comas *et al*., 1997), the biochemical activities of their enzymes were not reported yet. In contrast with other roots and tubers like cassava (Campos, de Carvalho, 1990; Matos da Veiga, 2002; Hirose, 1986), potato (Cho, Ahn, 1999; Kahn *et al*., 1981), yam (Oluoha, Ugochukwu, 1995), Peruvian carrot (Pires, Finardi-Filho, 2005; Pires, Veiga, Finardi-Filho, 2002) and sweet-potato (Hagenimana, Vézina, Simard, 1994), far less data are available about the biochemical characteristics of its enzyme activity. During storage, maca roots present loss of humidity and texture, which would be related to the action of endogenous carbohydrases. In addition, a high degree of amylolytic activity was found in preliminary studies, with high value of enzyme activity (around 170 $U_{AM}$) detected at pH 6.1 and 4 °C (unpublished results). The action of pectinolytic and amylolytic enzymes might be connected to the deterioration process of the tuber after harvest.

In this study the response surface methodology (RSM) was applied to determine the optimum pH and temperature of amylase, pectinesterase and polygalacturonase activities of maca roots.

## MATERIAL AND METHODS

### Roots

Roots of maca (*Lepidium meyenii* Walp.) harvested during the year 2003 and stored at –18 °C were used in the present work for enzyme extraction. The roots were purchased at the local market in Peru.

### Chemicals

Citric pectin, sodium phosphate, potato starch, alcohol oxidase from *P. pastoris* (EC 1.1.3.13), 2,4-pentanedione, galacturonic acid, polygalacturonic acid, sodium acetate and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis. 2-Cyanoacetamide was obtained from Aldrich Chemical Co., Steinhein. All other reagents were analytical grade.

### Amylase extraction and assay

For AM extraction, a method previously described was used (Pires, Veiga, Finardi-Filho, 2002). After washing, the roots were peeled off and diced. A sample of 25 g was homogenized with 100 mL of 0.2 M phosphate buffer (pH 6.0) in a regular blender. The crude extract was centrifuged at 10,300 x *g* for 30 min at 4 °C. The supernatant was dialyzed against distilled water at 4 °C for 24 h. The sample was stored at -18 °C until its utilization.

The total amylolytic activity was determined according to Street (1974) by measuring starch-iodine complex. Volumes of 100 µL of enzymatic extract and 0.1 M NaCl solution (1:1) were incubated with 200 µL of potato starch 0.1% solution, 500 µL of 0.1 M acetate or phosphate buffer at pH ranging from 5.8 to 7.2 (Table I) and 200 µL of HCl 0.01 M solution, for 15 minutes, at different temperatures from 18 to 46 °C (Table I). The reaction was interrupted by the addition of 400 µL of iodine solution. The volume was completed to 10 mL with distilled water and the absorbance was read at 578 nm. One amylolytic activity unity ($1U_{AM}$) was calculated by the amount of starch (ng) hydrolyzed per minute per milligram of protein.

**TABLE I** – Experimental conditions of maca enzyme activity in coded and real values[a]

| Experiment | Coded values | | Real values | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | AM | | PG | | PE | |
| Treatment | $x_1$ | $x_2$ | pH | T (°C) | pH | T (°C) | pH | T (°C) |
| 1 | -1 | -1 | 6.0 | 22 | 4.5 | 25 | 5.5 | 45 |
| 2 | -1 | 1 | 6.0 | 42 | 4.5 | 45 | 5.5 | 55 |
| 3 | 1 | -1 | 7.0 | 22 | 5.5 | 25 | 7.5 | 45 |
| 4 | 1 | 1 | 7.0 | 42 | 5.5 | 45 | 7.5 | 55 |
| 5 | 0 | 0 | 6.5 | 32 | 5.0 | 35 | 6.5 | 50 |
| 6 | 0 | 0 | 6.5 | 32 | 5.0 | 35 | 6.5 | 50 |
| 7 | 0 | 0 | 6.5 | 32 | 5.0 | 35 | 6.5 | 50 |
| 8 | 1.4142 | 0 | 7.2 | 32 | 5.7 | 35 | 7.9 | 50 |
| 9 | -1.4142 | 0 | 5.8 | 32 | 4.3 | 35 | 5.1 | 50 |
| 10 | 0 | 1.4142 | 6.5 | 46 | 5.0 | 49 | 6.5 | 57 |
| 11 | 0 | -1.4142 | 6.5 | 18 | 5.0 | 21 | 6.5 | 43 |
| 12 | 0 | 0 | 6.5 | 32 | 5.0 | 35 | 6.5 | 50 |
| 13 | 0 | 0 | 6.5 | 32 | 5.0 | 35 | 6.5 | 50 |
| 14 | 0 | 0 | 6.5 | 32 | 5.0 | 35 | 6.5 | 50 |

[a] For the AM activity, $x_1 = (pH - 6.5)/0.5$, where pH ranged from 5.8 to 7.2 and $x_2 = (T - 32)/10$, where T ranged from 18 to 46 °C; For the PG activity, $x_1 = (pH - 5.0)/0.5$, where pH ranged from 4.3 to 5.7, $x_2 = (T - 35)/10$, where T ranged from 21 to 49 °C; for the PE activity, $x_1 = (pH - 6.5)/1.0$, where pH ranged from 5.1 to 7.9, $x_2 = (T - 50)/5$, where T ranged from 43 to 57 °C.

### Pectinesterase and polygalacturonase extraction and assay

PE and PG were extracted according to previous work (Pires, Finardi-Filho, 2005). A sample of 50 g of maca roots was homogenized in 100 mL of NaCl 1.0 M for 2 min, in a regular blender. The homogenate was adjusted to pH 8.5 and 6.0 for PE and PG extraction, respectively, by the addition of 2.0 M NaOH and 2.0 M acetic acid. The homogenate was stirred for 4 h at 4 ºC and then centrifuged at 10,300 x *g* for 30 min at 4 ºC. The supernatant, called crude enzymatic extract, was used directly as enzyme source.

The PE activity was determined by methanol production as a consequence of pectin hydrolysis, according to Klavons and Bennet (1986) method: 100 µL of enzymatic extract was added to 100 µL solution containing either 100 mM sodium phosphate (0.1 M), or acetate buffer (0.1 M), pH ranging from 5.1 to 7.9 (Table I) and 0.1% of pectin. The reaction mixture was incubated at different temperatures from 43 to 57 ºC, for 15 min (Table I) in microfuge tubes. The reaction was stopped by heating at 100 ºC in a water bath for 3 min and the mixture, cooled to 25 ºC, 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 ºC, 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 ºC for 15 min. The absorbance was measured at 412 nm against a blank made with the same components but with the 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 that limit. One enzyme activity unit was expressed by 1.0 µmol MeOH x mg protein$^{-1}$ x min$^{-1}$.

The assay of PG activity was based on the hydrolytic release of reducing groups from polygalacturonic acid according to Gross (1982) and Honda *et al.* (1982) methods. Reaction mixtures containing 10 µL of enzymatic extract in 30 µL of acetate or phosphate buffer pH ranged from 4.3 to 5.7 (Table I) and 150 µL of the same buffer, with 0.2% of polygalacturonic acid were incubated for 2 h at different temperatures from 21 to 40 ºC (Table I). The reaction was stopped by adding 1.8 mL of 100 mM borate buffer (pH 9.0), followed by 200 µL of 1% 2-cyano-acetamide solution. The samples were mixed and immersed in a boiling water bath for 10 min. After equilibration at 25 ºC, the amount of reducing sugars was measured at 276 nm against a blank made up with the same components but with inactivated enzyme extract, boiled for 5 min. A calibration curve, using galacturonic acid as a standard, was prepared from 0 to 250 µg/mL. One unit of enzymatic activity was expressed by 1.0 µg of galacturonic acid produced x mg of protein$^{-1}$ x h$^{-1}$.

### Protein quantification

The protein concentration was measured by the Bradford (1976) method, using bovine serum albumin as standard.

### Experimental design: RSM and data analysis

The response surface model, the main effects and the interaction among the different factors, each at two different levels, can be simultaneously investigated. The central composite face design (CCFD) was used with two variables, at five levels and six replicates at the central point, for a total of 14 experiments (Barros Neto, Scarminio, Bruns, 1996). The two factors studied included pH ($x_1$) and temperature ($x_2$) of enzyme activity of maca extracts showing the following experimental conditions of the central point: pH = 6.5 and T = 32 ºC for AM activity; pH = 5.0 and T = 35 ºC for PG activity and pH = 6.5 and T = 50 ºC for PE activity of enzyme extract, chosen after preliminary laboratory trails. The experimental index number, scaled values and real values are shown in Table I. The scaled values in which $x_1 = (pH - 6.5)/0.5$, pH ranged from 5.8 to 7.2, $x_2 = (T - 32)/10$, T ranged from 18 to 46 ºC for AM; $x_1 = (pH - 5.0)/0.5$, where pH ranged from 4.3 to 5.7, $x_2 = (T - 35)/10$, T ranged from 21 to 49 ºC for PG activity and $x_1 = (pH - 6.5)/1.0$, pH ranged from 5.1 to 7.9, $x_2 = (T - 50)/5$, T ranged from 43 to 57 ºC for PE activity. Using the Statistical program, SAS version 6.0 (SAS Institute Inc., Cary, NC) for the regression analysis of the experimental data obtained, the following response variables were obtained: $Y_1$ = AM activity ($U_{AM}$), and $Y_2$ = PG activity ($U_{PG}$) and $Y_3$ = PE activity ($U_{PE}$).

The use of central composite design allows the dependent variables to be expressed as a polynomial model of the form:

$$y_{obs} = b_o + b_1x_1 + b_2x_2 + b_{11}x_1x_1 + b_{22}x_2x_2 + b_{12}x_1x_2 + e$$

where
$b_0$ = constant;
$b_1$ and $b_2$ = main effect of each process variable (pH and temperature);
$b_{12}$ = interaction effect between the variables;
$b_{11}$ and $b_{22}$ = effect of square variables;
$y_{obs}$ = independent variable (AM, PG or PE activity);
x = factor ($x_1$ = pH and $x_2$ = Temperature);
e = residual error ($e = y_{obs} - y_{calc}$).

## RESULTS

The experimental results of enzyme activity by a complete two-factor five-level factorial experiment design augment with six central points are shown in Table II. Tables III, IV and V represent the ANOVA of AM, PG and PE activity as a function of pH and temperature of enzyme activity of maca extract. The achieved mathematical model, evaluated by the statistical software and expressed in scale variables, was:

$$y_{calc}\,(U_{AM}) = 244.41 - 14.30\,x_1 - 0.08\,x_2 - 8.77\,x_1x_1 - 5.69\,x_2x_2 - 3.16\,x_1x_2$$

$$y_{calc}\,(U_{PG}) = 160.94 + 5.10\,x_1 + 4.91x_2 - 19.73\,x_1x_1 - 8.95\,x_2x_2 + 20.84\,x_1x_2$$

$$y_{calc}\,(U_{PE}) = 35.28 + 2.26\,x_1 - 1.05\,x_2 - 10.49\,x_1x_1 - 4.36\,x_2x_2 - 0.11\,x_1x_2$$

The pH ($x_1$), square terms of pH ($x_1x_1$) and temperature ($x_2x_2$) of the reaction medium influenced significantly the AM activity from the root extracts ($p < 0.05$, see Table III). The estimation of the model gave $R_{AM}^2 = 0.88$ and $R_{AM\ adj}^2 = 0.81$. Since the coefficients of the above equation are all negative, the response surface suggest to have a maximum point. Figure 1 represents the RSM for AM activity as a function of pH and temperature of enzyme activity. The optimal conditions for AM activity occurred at pH 6.1 and 33.6 °C, very close to the central point (see Table II). At this point, the model predicts the highest enzyme activity of 250.38 $U_{AM}$.

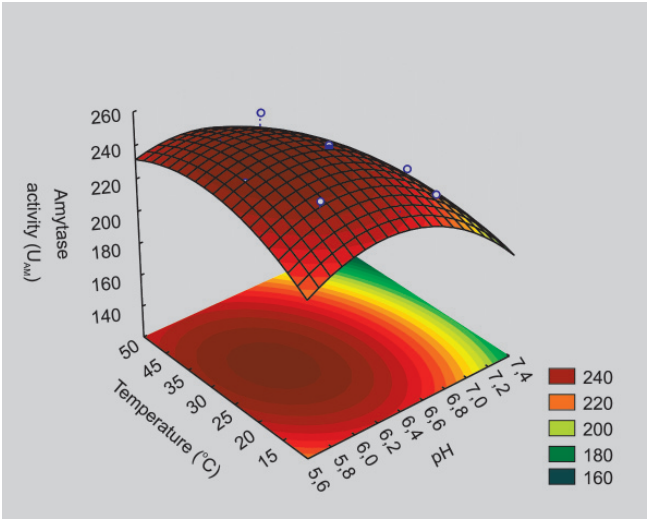


**FIGURE 1 –** RSM for AM activity ($U_{AM}$) as a function of pH and temperature of reaction medium. Experimental conditions at the central point (0.0) were pH = 6.5 and temperature = 32 °C. See Tables I and II for details.

The results of ANOVA for PG activity (Figure 2 and Table II) show that the linear terms ($x_1$ and $x_2$) were not significant (see Table IV). The square terms of pH ($x_1x_1$) and temperature ($x_2x_2$) as well as the interaction of pH and temperature ($x_1x_2$) of the reaction medium influenced PG activity significantly from the root extracts ($p < 0.05$, Table

**TABLE II –** Effect of pH and temperature on AM, PG and PE activity of maca enzyme extracts[a]

| Experiment | Coded values | | Experimental values | | |
|---|---|---|---|---|---|
| Treatment | $X_1$ | $X_2$ | $U_{AM}$ | $U_{PG}$ | $U_{PE}$ |
| 1 | -1 | -1 | 247.68 | 135.10 | 15.75 |
| 2 | -1 | 1 | 241.22 | 112.12 | 13.96 |
| 3 | 1 | -1 | 221.27 | 116.24 | 20.75 |
| 4 | 1 | 1 | 202.19 | 176.60 | 18.54 |
| 5 | 0 | 0 | 245.89 | 161.72 | 34.41 |
| 6 | 0 | 0 | 246.79 | 160.80 | 34.99 |
| 7 | 0 | 0 | 244.71 | 161.83 | 34.66 |
| 8 | 1.414 | 0 | 211.44 | 117.01 | 20.50 |
| 9 | -1.414 | 0 | 246.05 | 120.45 | 14.48 |
| 10 | 0 | 1.414 | 241.65 | 140.97 | 28.18 |
| 11 | 0 | -1.414 | 228.15 | 139.58 | 31.31 |
| 12 | 0 | 0 | 243.65 | 160.98 | 37.25 |
| 13 | 0 | 0 | 242.96 | 159.74 | 35.52 |
| 14 | 0 | 0 | 242.45 | 160.57 | 34.87 |

[a] For the AM activity, $x_1 = (pH - 6.5)/0.5$, where pH ranged from 5.8 to 7.2 and $x_2 = (T - 32)/10$, where T ranged from 18 to 46 °C; For the PG activity, $x_1 = (pH - 5.0)/0.5$, where pH ranged from 4.3 to 5.7, $x_2 = (T - 35)/10$, where T ranged from 21 to 49 °C; for the PE activity, $x_1 = (pH - 6.5)/1.0$, where pH ranged from 5.1 to 7.9, $x_2 = (T - 50)/5$, where T ranged from 43 to 57 °C.

**TABLE III** – ANOVA of AM activity as a function of pH ($x_1$) and temperature ($x_2$) of enzyme activity of maca extract

| Effects | Sum of squares | Degrees of freedom | Medium squares | F-value | p-value |
|---|---|---|---|---|---|
| $x_1$ | 1635.553 | 1 | 1635.553 | 40.23120 | 0.000222 |
| $x_1x_1$ | 567.291 | 1 | 567.291 | 13.95418 | 0.005742 |
| $x_2$ | 5.203 | 1 | 5.203 | 0.12798 | 0.729795 |
| $x_2x_2$ | 238.832 | 1 | 238.832 | 5.87478 | 0.041603 |
| $x_1x_2$ | 39.816 | 1 | 39.816 | 0.97939 | 0.351333 |
| Error | 325.231 | 8 | 40.654 | - | - |
| Total SS | 2759.853 | 13 | - | - | - |

**TABLE IV** – ANOVA of PG activity as a function of pH ($x_1$) and temperature ($x_2$) of enzyme activity of maca extract

| Effects | Sum of squares | Degrees of freedom | Medium squares | F-value | p-value |
|---|---|---|---|---|---|
| $x_1$ | 207.661 | 1 | 207.661 | 3.08067 | 0.117304 |
| $x_1x_1$ | 2873.415 | 1 | 2873.415 | 42.62740 | 0.000182 |
| $x_2$ | 193.537 | 1 | 193.537 | 2.87115 | 0.128629 |
| $x_2x_2$ | 591.850 | 1 | 591.850 | 8.78016 | 0.018060 |
| $x_1x_2$ | 1736.389 | 1 | 1736.389 | 25.75950 | 0.000959 |
| Error | 539.262 | 8 | 67.408 | - | - |
| Total SS | 5961.236 | 13 | - | - | - |

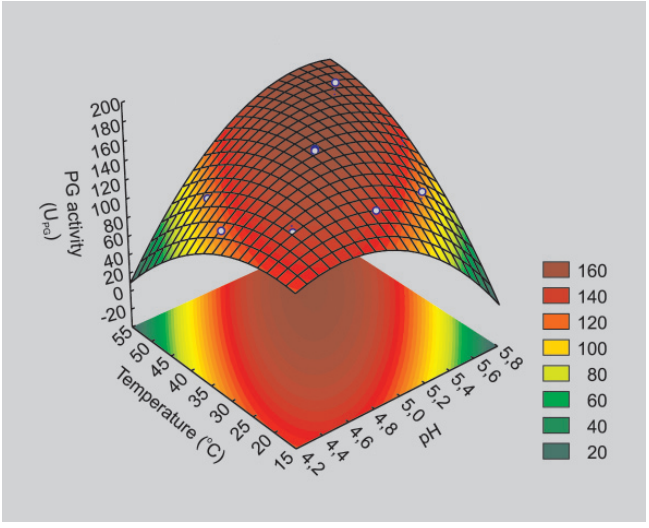


**FIGURE 2** – RSM for PG activity ($U_{PG}$) as a function of pH and temperature of reaction medium. Experimental conditions at the central point (0.0) were pH = 5.0 and temperature = 35 °C. See Tables I and II for details.

IV). The estimation of the model gave $R_{PG}^2 = 0.91$ and $R_{PG\ adj}^2 = 0.85$. The optimum pH and temperature of PG activity occurred at pH 5.4 and 46 °C. The maximum response of PG activity, predicted from the model, was 165.46 UPG.

The PE activity (Figure 3 and Table II) is significantly influenced by the square terms of pH ($x_1x_1$) and temperature ($x_2x_2$) of the reaction medium of the maca enzyme extracts ($p < 0.05$, see Table V). The evaluation of the model showed $R_{PE}^2 = 0.92$ and $R_{PE\ adj}^2 = 0.87$. The best conditions of PE activity occurred at pH 6.6 and 49.4 °C, very close to the central point (see Table II). The highest value of PE activity predicted by the RSM model was 35.47 $U_{PE}$.

## DISCUSSION

The activity of amylolytic and pectinolytic enzymes from maca roots has been studied to understand their deteriorative process. These two groups of enzymes are related to the carbohydrate breakdown and softening during the sprout or the senescence in roots and tubers. Maca presents a high degree of AM activity (170 $U_{AM}$) comparatively to other crops, like cassava 127 $U_{AM}$ (Pascoal, 2005) and Peruvian carrot 225 $U_{AM}$ (Pires, Finardi-Filho, 2005), mainly under low temperature assay. The optimum pH of AM activity from maca extracts, 6.1, was close to optimum pH 6.0 for enzymatic activity from cassava, ichoimo and Peruvian carrot (Table VI). The optimum temperature of AM activity was around 34 °C. This temperature is lower than that in the other sources, which can be explained by the low environmental temperature at cultivation fields and its frost tolerance, as described before.

The most works cited do not include studies on the optimization of pH and temperature conditions of PE activity. When the optimization procedure was included, the optimum temperature was found to be higher as in

**TABLE V –** ANOVA of PE activity as a function of pH ($x_1$) and temperature ($x_2$) of enzyme activity of maca extract

| Effects | Sum of squares | Degrees of freedom | Medium squares | F-value | p-value |
|---|---|---|---|---|---|
| $x_1$ | 40.923 | 1 | 40.923 | 3.78595 | 0.087565 |
| $x_1x_1$ | 812.423 | 1 | 812.423 | 75.16135 | 0.000024 |
| $x_2$ | 8.876 | 1 | 8.876 | 0.82113 | 0.391325 |
| $x_2x_2$ | 140.432 | 1 | 140.432 | 12.99211 | 0.006937 |
| $x_1x_2$ | 0.044 | 1 | 0.044 | 0.00408 | 0.950638 |
| Error | 86.472 | 8 | 10.8091 | - | - |
| Total SS | 1042.649 | 13 | - | - | - |

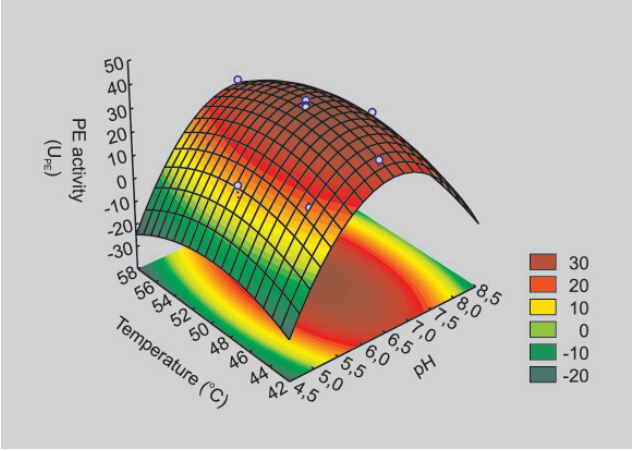


**FIGURE 3 –** RSM for PE activity ($U_{PE}$) as a function of pH and temperature of reaction medium. Experimental conditions at the central point (0.0) were pH = 6.5 and temperature = 50 °C. See Tables I and II for details.

acerola, banana, carrot and orange, which was 90, 64, 48.5 and 60 °C, respectively (Table VII). However, only Ly Nguyen and co-workers (2002a; 2002b) used RSM to achieve the optimum conditions for banana and carrot. At the same set of vegetables, the pH of PE activity ranged from 6.5 to 9.0 (Table VII).

In contrast to AM activity, extensively studied in roots and tubers, pectinolytic enzymes have been described in fruits and vegetables, especially due to their capacity of softening. Most tubers and roots do not present significant loss of texture during the senescence. However, some of them – like maca and Peruvian carrot – present softening during storage, which could be attributed to endogenous or exogenous source of enzymes. Tables VII and VIII show the values of pH and temperature assay for PE and PG activity, respectively, from different fruits and vegetables, found in the literature.

The PE of maca showed optimum activity at pH 6.6 and 49.4 °C according the response surface model. The optimum temperature of PE activity from fruits, roots and other sources ranged from 25 to 90 °C (Table VII). However, most works do not include studies on the optimization of pH and temperature conditions of PE activity. When the optimization procedures were included, the optimum temperatures were found to be higher as in acerola, banana, carrot and orange, which were 90, 64, 48.5 and 60 °C, respectively (Table VII). At the same set of vegetables, the pH of PE activity ranged from 6.5 to 9.0 (Table VII). Although, most of them presented the activity ranging around pH 7.0 to 7.5, the optimum pH value for PE activity from maca was similar to other sources, like apple, pear and Peruvian carrot, which were 6.5.

The maximum PG activity from maca occurred at pH 5.4 and 46 °C according to the response surface model. The PG activity from maca showed higher assay temperature compared to fruits and vegetable (Table VIII). There was no study with RSM or single assay to optimize PG activity to support the values of pH and temperature mentioned (Table VIII), so the results are not absolute by the lack of better data.

**TABLE VI –** Assay conditions of pH and temperature (T, °C) of AM activity from different sources found in the literature

| Material | pH | T (°C) |
|---|---|---|
| Cassava (Matos da Veiga, 2002) | 6.0 | 60 |
| Ichoimo (Arai, Kawabata, Taniguchi, 1991) | 6.0 | 55 |
| Peruvian carrot (Pires *et al*., 2002) | 6.0 | 50 |
| Sweet potato (Hagenimana *et al*., 1994) | 5.8-6.4 and 5.3-5.8 [a] | 71.5 and 53 [b] |
| Yam (Takase, Ogata, Ito, 1990) | 5.0 | 60 |

[a]Optimum pH of range α- and β-amylases, respectively. [b]Optimum temperature for α- and β-amylases, respectively

**TABLE VII –** Assay conditions of pH and temperature (T, °C) of PE activity from different sources found in the literature

| Material | pH | T (°C) |
|---|---|---|
| Acerola (Assis *et al*., 2002) | 9.0 | 90 |
| Apple (Castaldo *et al*., 1989) | 6.5 | 25 |
| Banana (Ly-Nguyen *et al*., 2002a) | 7.0 | 64 |
| Carrot (Tijskens *et al*., 1997) | [c] | 25 |
| Carrot (Ly-Nguyen *et al*., 2002b) | 7.4 | 48.5 |
| Grapefruit (Seymour *et al*., 1991) | 7.0 | 25 |
| Green beans (Laats *et al*., 1997) | 7.5 | 25 |
| Kiwifruit (Wegrzy, Macrae, 1992) | 7.5 | [b] |
| Mango (Abu-Sarra, Abu-Goukh, 1992; Labib *et al*., 1995) | 7.0 | 30 |
| Orange (Korner, Zimmermann, Berk, 1980; Versteeg *et al*., 1980) | 7.5 | 60 |
| Papaya (Fayyaz *et al*., 1993) | 7.0 | 30 |
| Peach (Javeri, Wicker, 1991) | 8.0 | 60 |
| Pear (Nagel, Patterson, 1967) | 6.5-7.4[a] | 30 |
| Peruvian carrot (Pires *et al*., 2005) | 6.5 | 25 |
| Potato (Puri, Solomons, Kramer, 1982) | 7.5 | 55 |
| Potato (Tijskens *et al*., 1997) | [c] | 25 |
| Sapote mamey (Arenas-Ocampo *et al*., 2003) | 7.5 | 25 |

[a] PE activity was measured in an automatic titrimeter that maintained the pH at a constant value within the range of 6.5 to 7.4. [b] The temperature of PE activity was not cited in the paper. [c] The pH of PE activity was not cited in the paper.

**TABLE VIII –** Assay conditions of pH and temperature (T, °C) of PG activity from different sources found in the literature

| Material | pH | T (°C) |
|---|---|---|
| Banana (Pathak, Sanwal, 1998) | 3.3-4.3 [a] | 37 |
| Mango (Abu-Sarra *et al*., 1992) | 4.5 | 37 |
| Pear (Nagel *et al*., 1967) | 4.7 | 30 |
| Peruvian carrot (Pires *et al*., 2005) | 4.4 | 30 |
| Sapote mamey (Arenas-Ocampo *et al*., 2003) | 4.0 | [b] |
| Strawberry (Nogata, Ohta, Voragen, 1993) | 5.5 | 37 |
| Tomato (Pressey, 1986; Jackman, Gibson, Stanley, 1995; Yoshida *et al*., 1984) | 4.5 | 37 |

[a] Optimum pH was found to be 3.3, 3.7 and 4.3 for three multiple forms of PG from banana. [b] The temperature of PG activity was not cited in the paper.

The RSM is a statistical-mathematical method, which uses quantitative data in an experimental design to determine, and, simultaneously, solve multivariable equations in order to optimize experiments, processes and products. However, it is important to quote that, in the present study, the model reproduces optimal conditions of enzyme activity under the circumstances assayed. Although the RSM presents few experiments in comparison to the conventional method of enzyme characterization, many previous assays are always necessary when the enzyme source is unknown in order to achieve the optimization process.

As mentioned before, few data are available about enzyme activity on the roots of maca to understand its deteriorative process. Nevertheless, the results in this work on the definition of optimal conditions of pH and temperature of AM, PE and PG enzyme activity can represent an important step concerning the knowledge of the roots deterioration, once the enzymes can be related to the post-harvest senescence process of the flavored roots of maca.

## RESUMO

**Ensaios preliminares de detecção de atividades amilolítica e pectinolítica em maca (*Lepidium meyenii* Walp.)**

O objetivo do presente estudo foi determinar valores ideais de pH e temperatura para avaliar as atividades amilolítica (AM), pectinesterásica (PE) e poligalacturonásica (PG) em

raízes de maca (*Lepidium meyenii* Walp.). Foi utilizado o modelo de superfície de resposta para atingir valores confiáveis de atividades enzimáticas em extratos brutos. Os valores máximos de atividade AM ocorreram em pH 6,1 a 33,6 °C, muito próximos do ponto central dos experimentos. Para as atividades de PE e PG, o valores ótimos foram atingidos em pHs 6,6 e 5,4, a 49,4 e 46 °C, respectivamente.

*UNITERMOS: Maca. Lepidium meyenii. Amilase. Pectinesterase. Poligalacturonase. Metodologia de superfície de resposta.*

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